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Chemistry of Singlet Oxygen. XXI. Kinetics of Bilirubin Photooxygenation[1]

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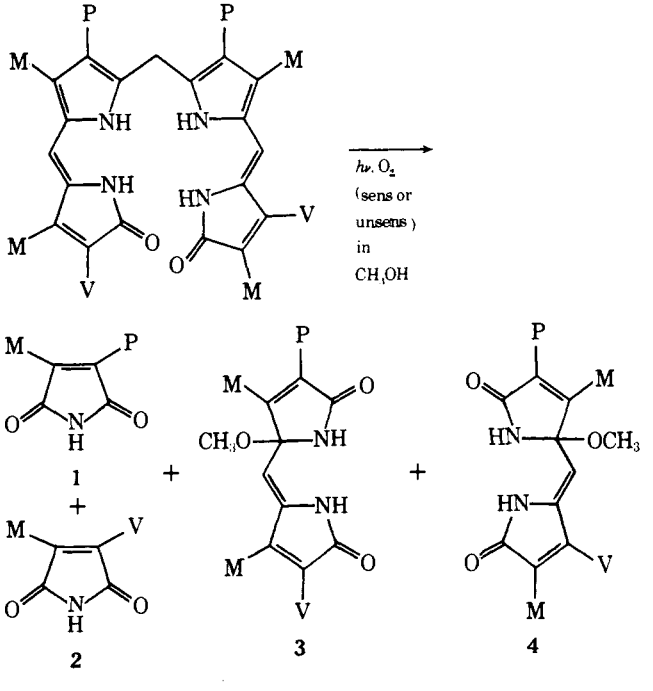
Abstract: The photooxidation of bilirubin in chloroform has been studied kinetically by several independent techniques. The rate of total consumption of singlet oxygen ($k_Q + k_R$) is 2.5×10^9 M^{-1} sec^{-1}. The value for reaction alone (k_R) is 0.4×10^9 M^{-1} sec^{-1}. The value for k_R is significantly higher than that determined by Matheson and Lee (in Freon 113); reasons for the discrepancy are discussed. The large value of k_R is consistent with the rapid disappearance of bilirubin during phototherapy of neonatal jaundice and may explain why side effects of this treatment are rare.

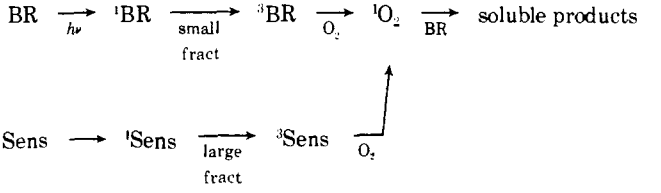
A common problem among newborn, especially premature, infants is jaundice; untreated, brain damage may result. The cause of the jaundice is insufficient activity of a hepatic system which effects the conversion of the lipid-soluble yellow pigment, bilirubin (BR), to its water-soluble glucuronic acid conjugate. The resulting excess BR deposits in the skin and brain of the infant. The common treatment for neonatal jaundice is irradiation of the infant with light in the wavelengths absorbed by BR (centered at 450 nm). Irradiation bleaches the BR in the skin, apparently by a photooxidative mechanism, and seems to prevent brain damage.[2-4]

The photooxidation of BR in vitro has been studied by several groups. The products (**1-4**) are those which might



be expected from singlet oxygen attack.[5,6] The products apparently derive from cleavage of enamine double bonds and by 1,4 addition of oxygen to pyrrole rings. Both processes have ample precedent in singlet oxygen chemistry.

Singlet oxygen, formed by BR as sensitizer, has been shown to be the reactive intermediate in the destruction in vitro.[7,8] Singlet oxygen sensitizers increase the rate of BR disappearance, and singlet oxygen quenchers inhibit it. The photooxidation rate increases five-fold in CD_3OD compared with CH_3OH,[7] which is evidence for the intermediacy of singlet oxygen, which has a longer lifetime in deuterated than in protiated solvents. BR can also photosensitize the oxidation of singlet oxygen acceptors but is a comparatively poor sensitizer compared with common sensitizing dyes.[7]



Because systems which generate singlet oxygen have been shown to produce a variety of damage (called photodynamic) in numerous organisms,[9] one might anticipate some photodynamic damage to infants during phototherapy, although bilirubin is quite rapidly degraded. Only a few reports have appeared: Odell and coworkers reported photolytic hemolysis of blood samples to which bilirubin had been added and suggested that this hemolysis was responsible for an anemia which they believe is associated with phototherapy.[10] Odell had earlier reported photodynamic damage to serum albumin sensitized by bilirubin.[11]

Because of its medical importance and chemical interest, a careful mechanistic study of the photooxygenation of BR is overdue. BR might be expected to be highly reactive toward singlet oxygen as a result of its high electron density.

Recently, several types of singlet oxygen acceptors have been shown both to react with singlet oxygen and to quench it without reaction. Sulfides were the first class of substrate for which this behavior was identified;[12] more recently, certain phenols (in particular α-tocopherol)[13] and indenes[14] have been found to have similar behavior. We therefore thought it important to test for this behavior with BR as

well. This study shows that bilirubin is one of the most reactive known acceptors for singlet oxygen; it also quenches singlet oxygen at a rate somewhat faster than it reacts.

After this work was nearly completed, two papers by Matheson, Lee, and coworkers appeared, in which the reaction of BR[15] and diphenylisobenzofuran[16] (DPBF) with singlet oxygen was reported. In these papers, singlet oxygen was generated by laser irradiation of oxygen at high pressures in Freon 113 and D_2O solutions. They reported that both BR and DPBF undergo reaction with singlet oxygen, but DPBF quenches it far more rapidly than it reacts. Their results, which are quantitatively at variance with earlier work by others and with our results reported in this paper, will be examined more closely in the Discussion section.

Experimental Section

Materials. Bilirubin (BR) was obtained from Dr. A. F. McDonagh (UCSF); these highly purified samples gave results which were indistinguishable from samples obtained from Koch-Light Ltd. Diphenylisobenzofuran (DPBF) was purchased from Aldrich. Tetramethylethylene was purified by distillation and its purity checked by GLC. Methylene Blue (MB) was from Merck. Rose Bengal was purified by Dr. S.-Y. Wong.

General Conditions and Controls. All solutions were equilibrated with air. In numerous kinetic runs, a sample maintained in the dark showed no measurable change in absorption at 450 nm during the course of a run (≤6 hr); a 10^{-5} M solution of BR in $CHCl_3$ kept for 5.5 days under air still retained 96% of its absorbance at 450 nm and had no major change in absorption features. Most photooxidation runs were carried out to only a few percent conversion of bilirubin at most (fluorescence technique and most competition runs); the disappearance runs were carried out to no more than 15% conversion in most cases. The presence or absence of photolyzing light (absorbed only by Methylene Blue) did not cause any change in the emission intensity of DPBF.

BR-Inhibited Reaction of DPBF (Fluorescence Technique). $CHCl_3$ solutions of BR ($2\text{–}60 \times 10^{-6}$ M), DPBF (4×10^{-6} M), and MB (2.5×10^{-6} M) were photolyzed in the apparatus previously described.[13a] The photolyzing light (absorbed only by MB) was a Sylvania 500 Q/C1 tungsten-halogen lamp, operated at 25 V with a Corning 3-68 filter (cutoff 540 nm). Fluorescence of DPBF was excited by a 200-W Xe-Hg lamp (Hanovia), powered by a Schoeffel LPS 251 power supply through a Jarrell-Ash Model 82-410 monochromator at 395 nm (0.25-mm slit). The fluorescence of DBPF was detected at 515 nm (1.00-nm slit) by a 1P-28 photomultiplier in a Heath EU-703-31 photometric readout system.

Direct Disappearance of BR. $CHCl_3$ solutions of BR ($1.59\text{–}32.5 \times 10^{-6}$ M) containing 2.5×10^{-6} M Methylene Blue were photolyzed in a merry-go-round with a 650 W Sylvania DWY tungsten-halogen lamp operated at 10–20 V. The temperature was controlled at 23° with a water bath. A 3% $K_2Cr_2O_7$ solution was used as a filter (cutoff 550 nm). The concentrations of BR before and after photolysis were measured with a Beckman DU spectrophotometer at 450 nm.

Competition—BR and DPBF. Competition experiments were carried out using DPBF (4×10^{-6} M), MB (2.5×10^{-6} M), and BR (10^{-5} M). The apparatus described in the first section was used to photolyze the solutions; the loss of DPBF was measured by the change in its fluorescence and the change in BR concentration by the change in its absorbance at 475 nm with a Beckman DU spectrometer.

Competition—BR and TME. $CHCl_3$ solutions of TME ($0.2\text{–}7.14 \times 10^{-2}$ M), MB (2.5×10^{-6} M), and BR ($0.37\text{–}3 \times 10^{-5}$ M) were photolyzed in a merry-go-round, as in the second section; BR concentrations were measured by absorbance, as above. The solutions were then reduced with excess $NaBH_4$ in $CHCl_3$–CH_3OH at 0° and stored overnight. The product of TME photooxygenation, 2,3-dimethyl-3-buten-2-ol, was analyzed with a Perkin-Elmer 800 gas chromatograph with an 8 ft × ⅛ in. UCON-WS column (70°) with *tert*-amyl alcohol solution as external standard. The peaks were integrated with an Infotronics CR5-208 autointegrator.

Actinometry.[17] A 0.15 M solution (2.00 ml) of $K_3Fe(C_2O_4)_3$ in H_2O was irradiated in a 1-cm quartz cell with the lamp and monochromator (546 nm, slit 0.25 mm) described in the first section for 2 hr. To the photolyzed solution, 2.00 ml of 0.1% by weight 1,10-phenanthroline solution and 1.00 ml of HOAc–NaOAc buffer were added, and the mixture was allowed to stand 1 hr. The Fe^{2+} complex generated was determined by absorbance (Beckman DU) at 510 nm (ϵ 1.11×10^4 M^{-1} cm^{-1}).

Because of the insensitivity of ferrioxalate at 546 nm, it was felt that the ferrioxalate results should be checked using the Reinecke's salt actinometer described by Wegner and Adamson.[17b] Reinecke's salt was prepared by the method of ref. 17b from the commercially available ammonium salt. Irradiations were carried out through a combination of Corning CS3-68 and $CaCl_2$–$CuCl_2$–H_2O[17a] filters which has a sharp bandpass (½ band width 80 nm) at 550 nm, followed by the monochromator and cell system described above. Irradiated actinometer solutions were diluted and $Fe(NO_3)_3$ and $HClO_4$ added to make concentrations 0.5 and 1.0 M, respectively. The uv of the resulting complex was measured at 450 nm.

DPBF Photolysis. Solutions of DPBF in methanol–chloroform (1:9, v/v) were prepared containing 4×10^{-5} M Rose Bengal, which absorbs 98.5% of the light under these conditions. These solutions were irradiated in a 1-cm cell; loss of DPBF was measured at 410 nm.

BR Photolysis. Solutions of BR were photolyzed in the same way as described for DPBF. BR concentration was measured at 450 nm; Rose Bengal in the concentrations used has negligible absorbance at this wavelength.

Results and Discussion

The work of McDonagh[8] and Bonnett and Stewart[7] had shown that the sensitized photooxygenation of bilirubin (BR) is cleanly a singlet oxygen reaction, i.e., no type 1 oxygenation by interaction of sensitizer with BR is involved. For this reason, this point was not separately investigated. Nevertheless, the kinetic results reported in this section, in particular the competition experiments, are consistent only with a singlet oxygen mechanism and serve to confirm the results of McDonagh and of Bonnett and Stewart.

Because of the importance of the substrate and the disagreements with the work of Matheson, Lee, and coworkers,[15,16] BR photooxygenation was studied kinetically by several different techniques to obtain independent values of rate constants. If BR can either react with (k_R) or quench (k_Q) singlet oxygen, in the presence of a second acceptor, F (reaction rate k_F), the kinetic scheme is as shown below, where k_d is the rate of decay of $^1O_2$.

$$F + {}^1O_2 \xrightarrow[k_F]{} FO_2$$

$$BR + {}^1O_2 \xrightarrow[k_R]{} \text{products (reaction)}$$

$$BR + {}^1O_2 \xrightarrow[k_Q]{} BR + {}^3O_2 \text{ (quenching)}$$

$$O_2 \xrightarrow[k_d]{} {}^3O_2$$

Fluorescence Technique. In the technique developed by Young et al.,[18] F is fluorescent and is present in extremely low concentration so that its disappearance is cleanly first order. (Under the reaction conditions $k_F[F]$ is always <20% of k_d and, even at 10^{-5} M BR, is only about 7% of the total denominator even at the beginning of the reaction where its concentration is highest.) The fluorescence of F is used to monitor its disappearance, and inhibition of the reaction by other acceptors can then be easily studied. The steady state treatment then gives, where K is the rate of formation of singlet oxygen:[14,18]

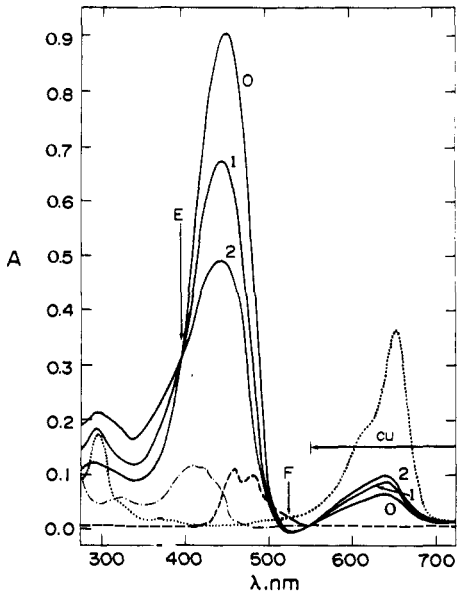


Figure 1. Absorption spectra of (—) bilirubin in $CHCl_3$ before (0) and during (1, 2) photolysis to substantial conversion; (···) 2.5×10^{-6} *M* Methylene Blue; (—·—) 4×10^{-6} *M* DPBF. (- - -) Emission spectrum of 4×10^{-6} *M* DPBF; (E) fluorescence excitation wavelength; (F) fluorescence monitoring wavelength; (CU) wavelength of light admitted by cut-off filter for photolysis.

$$\frac{-d[F]}{dt} = K\left(\frac{k_F[F]}{k_F[F] + k_R[BR] + k_Q[BR] + k_d}\right) \approx K\left(\frac{k_F[F]}{k_R[BR] + k_Q[BR] + k_d}\right) \quad (1)$$

Under conditions where BR is not appreciably photooxidized, this technique gives excellent ($R > 0.999$) linear plots of ln (fluorescence) vs. time whose slopes (S) are given by

$$S = K\left(\frac{k_F}{k_R[BR] + k_Q[BR] + k_d}\right) \quad (2)$$

and a plot of S_0/S_{BR} (slopes in absence and presence of BR) vs. BR has a slope = $(k_R + k_Q)/k_d$. This technique gives the sum $(k_R + k_Q)$ only, and the values thus obtained are independent of the reactivity of F.

However, this technique proved somewhat difficult to use with bilirubin. Because of the high reactivity of BR, it is necessary to use the most reactive possible acceptor F; only diphenylisobenzofuran (DPBF) is sufficiently reactive. Unfortunately, the absorption of DPBF is overlapped by the strong absorption of BR in the entire region, and the emission spectrum is also partly overlapped. The problem was solved by exciting the DPBF fluorescence at 395 nm, where BR has an isosbestic point, and monitoring the fluorescence at 515 nm, where, although the fluorescence of DPBF is quite weak, neither BR nor the sensitizer, Methylene Blue, has appreciable absorption. A photooxidation product (λ_{max} 640 nm), presumably biliverdin,[19-21] (λ_{max} ~650) does not absorb an appreciable fraction of the light (>540 nm) used to irradiate the sensitizer under the conditions of the kinetic experiments. Figure 1 shows the absorption and emission spectra of the species involved.

Experiments were carried out using BR (2 to 60×10^{-5} *M*), DPBF, and Methylene Blue in chloroform. A sample plot of S_0/S_{BR} vs. BR is shown in Figure 2; the excellent linearity of the plot shows that the overlapping absorptions did not cause serious difficulty, and also that the bilirubin concentration did not change appreciably under the conditions of the kinetic runs. The value of $k_d/(k_R + k_Q)$ obtained in this way is $6.7 \pm 0.4 \times 10^{-6}$ *M*.

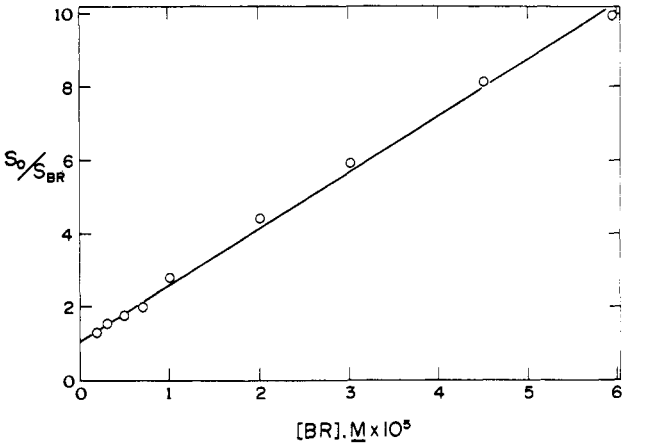


Figure 2. Plot of S_0/S_{BR} (slope in absence and presence of BR) vs. BR concentration.

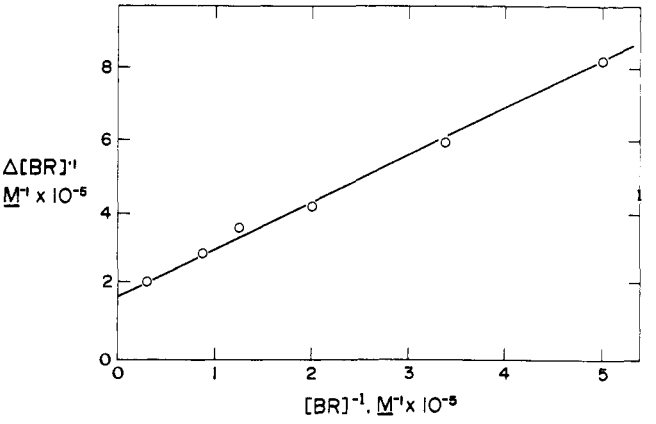


Figure 3. Plot of $\Delta[BR]^{-1}$ vs. $[BR]^{-1}$.

Bilirubin Direct Disappearance Technique. In the absence of a second singlet oxygen acceptor, the rate of disappearance of BR is (where K is again the rate of singlet oxygen formation):

$$\frac{d[BR]}{dt} = K\left(\frac{k_R[BR]}{(k_R + k_Q)[BR] + k_d}\right) \quad (3)$$

and

$$\Delta[BR]^{-1} = (K\Delta t)^{-1}\left(\frac{k_R + k_Q}{k_R} + \frac{k_d}{k_R}[BR^{-1}]\right) \quad (4)$$

A plot of $\Delta[BR]^{-1}$ vs. $[BR]^{-1}$ will give a linear plot (if $\Delta BR \ll BR$). The ratio of slope to intercept of such a plot is $k_d/(k_R + k_Q)$ and again yields $k_R + k_Q$ only. The absolute value of $K\Delta t$ is needed to evaluate k_R and k_Q separately; this is discussed in a subsequent section.

Solutions of BR ($1.6\text{-}32 \times 10^{-6}$ *M*) in $CHCl_3$ were photolyzed, and the loss was determined by the change in absorbance at 450 nm. Methylene Blue was the sensitizer, irradiated with wavelengths >550 nm, where bilirubin is transparent. A sample plot is shown in Figure 3; the value of $k_d/(k_R + k_Q)$ obtained in these experiments is $7.7 \pm 3 \times 10^{-6}$ *M*, in reasonable agreement with the value previously determined.

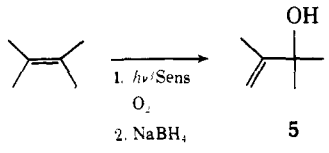
Competition with DPBF. In order to obtain an absolute value for k_R, which is important because only k_R would correspond to the actual destruction of BR during phototherapy, competition experiments were carried out. In these

experiments, loss of BR and DPBF (in $CHCl_3$) are independently monitored. The equation of Higgins et al.[22] for the reaction of two competing substrates is shown below. This equation gives k_R for BR relative to k_R for DPBF, independent of whether one or both substrates also quench singlet oxygen.

$$\frac{k_R^{BR}}{k_R^{DBPF}} = \frac{[\log (BR_f/BR_0)]}{[\log (DBPF_f/DBPF_0)]}$$

The amount of bilirubin lost at the time when 50% of the DPBF had disappeared (by fluorescence, as above) was determined by measuring the uv absorbance of bilirubin at 475 nm. The ratio k_R^{BF}/k_R^{DPBF} measured in this way is 0.30 ± 0.02.

Competition with Tetramethylethylene. As a second, independent, measure of k_R, a set of competition experiments were carried out using tetramethylethylene (TME) as acceptor in $CHCl_3$. Loss of BR was again determined by changes in uv absorbance. Oxidation of TME was measured by reduction of the solutions and measurement of the product, 2,3-dimethylbut-3-en-2-ol (**5**), by gas chromatography.



The value of k_R^{BR}/k_R^{TME} determined in this way was 9.0 ± 1.4.

Absolute Quantum Yield Measurements. From eq 4, the intercept of plots of $\Delta[BR]^{-1}$ vs. $[BR]^{-1}$ is $[K\Delta t k_R/(k_R + k_Q)]^{-1}$. Thus if $K\Delta t$, the amount of singlet oxygen formed during the irradiation is known, the ratio of k_R to k_Q can be determined directly. In the expression above, $K = I_a \cdot \phi_{isc} f^1O_2$, where I_a is the rate of absorption of light by the sensitizer in mol quanta sec^{-1}, ϕ_{isc} is the triplet yield of the sensitizer, and f^1O_2 is the yield of singlet oxygen from triplet dye. From many previous studies,[23-25] $f^1O_2 = 1.0$ for dyes in oxygenated organic solvents in the absence of triplet quenchers. The rate of production of light by the lamp was determined by conventional ferrioxalate actinometry.[17] Unfortunately, ferrioxalate is not decomposed at wavelengths absorbed by Methylene Blue; for this reason, Rose Bengal had to be used. Because of the insensitivity of ferrioxalate at 546 nm, some actinometry was also carried out using Reinecke's salt;[17b] the values so obtained were in good agreement with the ferrioxalate values. Rose Bengal is not soluble in chloroform: however, it is sufficiently soluble in chloroform-methanol (9:1, v/v) to permit experimentation. Solutions of DPBF and, in a separate series, of bilirubin, with Rose Bengal in this solvent were irradiated for a known period of time at 546 nm with the calibrated lamp; Rose Bengal absorbs 98.5% of the incident light in the solutions at this wavelength. Plots of $(\Delta[\text{Acceptor}]/I_a\Delta t)^{-1}$ vs. $[\text{Acceptor}]^{-1}$ were made; the intercepts of these plots are $[\phi_{isc}k_R/(k_R + k_Q)]^{-1}$: the reciprocal of the value thus determined for DPBF was 0.66, and that for BR was 0.11. The ratio of slope to intercept of these plots is $k_d/(k_R + k_Q)$; for DPBF, the value determined in this way is 7.9×10^{-5} M, and that for BR was 3.1×10^{-5} M. (These values were not directly comparable to those previously determined because of the change in solvent and, thus, k_d; this point is discussed further subsequently.)

Rates of Reaction and Quenching. Table I summarizes the results obtained in the present investigation. In order to convert the ratios in Table I to absolute rates, it is necessary to substitute absolute values for the decay rate of singlet oxygen (k_d) and reaction rates (k_R) for TME and DPBF.

Table I. Kinetic Parameters Determined for Bilirubin (BR) and Diphenylisobenzofuran (DPBF)

Substrate[a]	Technique	Parameter	Value
BR	Fluorescence	$k_d/(k_R + k_Q)$	$6.7 \pm 0.4 \times 10^{-6}$ M
BR	Disappearance	$k_d/(k_R + k_Q)$	$7.7 \pm 3 \times 10^{-6}$ M
BR[b]	Disappearance	$k_d/(k_R + k_Q)$	$3.1 \pm 0.6 \times 10^{-5}$ M
BR	DPBF Competition	k_R^{BR}/k_R^{DBPF}	0.30 ± 0.02
BR	TME Competition	k_R^{BR}/k_R^{TME}	9.0 ± 1.4
BR[b]	Actinometry[c]	$\phi_{isc}k_R/(k_R + k_Q)$	0.11 ± 0.01
DPBF[b]	Actinometry[c]	$\phi_{isc}k_R/(k_R + k_Q)$	0.66 ± 0.19
DPBF[b]	Disappearance	$k_d/(k_R + k_Q)$	$7.9 \pm 1.8 \times 10^{-5}$ M[b]

[a] In $CHCl_3$, except as noted. [b] In $CHCl_3$–10% CH_3OH (v/v). [c] Average of three runs by ferrioxalate (ref 17a) and one by Reinecke's salt (ref 17b) actinometry.

The value of k_d in $CHCl_3$ (1.67×10^4 sec^{-1}) has been reported by Merkel and Kearns.[23] The value of k_d in $CHCl_3$–10% CH_3OH is not known but can be estimated [from the known values in $CHCl_3$ and CH_3OH (1.4×10^5 sec^{-1})[26] and the mole fractions of each solvent in the mixture] to be 3.9×10^4 sec^{-1}.[29] The values of $k_Q + k_R$ calculated with these k_d's are listed in Table II.

Values of k_R can be obtained by two methods. The competition experiments with DPBF and TME give ratios of k_R values. However, it is necessary before substituting literature values of k_R for DPBF and TME to establish that they do not contain a k_Q component since this possibility was not considered when the values were originally measured. Several lines of evidence show that neither substrates quenches appreciably.

First, large numbers of independently measured rates, determined by procedures which would actually give $k_Q + k_R$, are available. Relative rates calculated from these values are in excellent agreement with relative rates (k_R) determined in competition experiments. Thus, if any of the common substrates such as olefins, furans, or aromatic componds have a k_Q component, all do, and in the same proportion, which seems unlikely. Table III lists a few representative values, of the many available. Also, in a detailed study, the intercept of kinetic plots of the type of Figure 3 was identical for diphenylanthracene and 2-methyl-2-pentene;[12] this can only be the case if the ratio of k_Q to k_R is the same for both substrates.

More direct evidence is available. Merkel and Kearns showed that the amount of DPBF consumed in a laser flash experiment was equal to the amount of singlet oxygen removed, and thus no quenching was occurring.[23] They also showed the amount of singlet oxygen produced to be equal to the amount of triplet dye formed.

In order to be certain that no quenching is occurring, it is necessary to do actinometry, to show that light absorbed appears as product and is not wasted. Gollnick showed the quantum yield of triplet formation (ϕ_{isc}) for Rose Bengal in methanol is 0.76, and that this is equal to the quantum yield of singlet oxygen formation and the limiting quantum yield of dimethylfuran disappearance so that there is no quenching with this acceptor.[24] Dimethylfuran has been correlated with TME by relative k_R values,[22] which confirms that no quenching occurs with TME in solution. Thus neither TME nor DPBF quenches singlet oxygen appreciably, and literature k_R values can be used. The values used were 4.8×10^7 M^{-1} sec^{-1} for TME in CH_3OH (calculated from data in ref 18 and 22) and 7×10^8 M^{-1} sec^{-1} for DPBF in C_6H_6, from ref 30. Since it has been shown that k_R values of aromatic and olefinic acceptors do not vary appreciably with solvent,[28,31-33] the data are available to calculate k_R for bilirubin in $CHCl_3$, and the values are listed in Table II. As a

Table II. Rates of Reaction (k_R) and Quenching (k_Q) Calculated for Bilirubin

Technique	Parameter	Value × 10^9, M^{-1} sec^{-1}
Fluorescence[a]	$k_R + k_Q$	2.5
Disappearance[a]	$k_R + k_Q$	2.2
Disappearance[b]	$k_R + k_Q$	1.3
DBPF Competition[a]	k_R[c]	0.21
TME Competition[a]	k_R[d]	0.43
Actinometry[b]	k_R[e]	0.43

[a] In $CHCl_3$ ($k_d = 10^4$ sec^{-3}). [b] In $CHCl_3$–10% CH_3OH ($k_d \approx 3.9 \times 10^4$ sec^{-1}. [c] Using $k_R^{DPBF} = 7 \times 10^8 M^{-1}$ sec^{-1} (in C_6H_6, ref 30). [d] Using $k_R^{TME} = 4.8 \times 10^7 M^{-1}$ sec^{-1} (in CH_3OH, ref 18 and 22). [e] Using the fluorescence value of $k_R + k_Q$ and the value of $\phi_{isc}(k_R + k_Q)/k_R$, with $\phi_{isc} = 0.66$ (see text).

Table III. Selected Values of Literature Rate Values (in CH_3OH–*t*-BuOH, 1:1)

Compd	$k_R + k_Q$ (relative to TME)[a]	k_R (relative to TME)[b]
TME	(1.00)	(1.00)
2-Methyl-2-pentene	0.019	0.019
cis-4-Methyl-2-pentene	0.00029	0.00027

[a] Calculated from data in ref 18. [b] Calculated from data in ref 22.

check, the relative reactivity, k_R^{DPBF}/k_R^{TME} calculated from the above values is 14.5, whereas the value calculated from $(k_R^{BR}/k_R^{TME})/(k_R^{BR}/k_R^{DPBF})$ determined in this work is 30, in fair agreement considering the number of independent measurements in different solvents involved.

The actinometry provides an excellent check on the rate constants for both BR and DPBF. In Table I, the value of $\phi_{isc}k_R/(k_R + k_Q)$ for DPBF in $CHCl_3$–10% CH_3OH is 0.66 ± 0.19.

Since ϕ_{isc} for Rose Bengal in CH_3OH is 0.76,[24] it is reasonable to conclude that ϕ_{isc} is ~0.66 and $k_R/(k_Q + k_R)$ is ~1.0 in the mixed solvent, i.e., $k_R \gg k_Q$, as concluded previously. Data available show ϕ_{isc} for Rose Bengal does not vary appreciably in several solvents.[32] In any case, since ϕ_{isc} cannot exceed 1.0, $k_R/(k_R + k_Q)$ must be at least 0.66, and k_Q is small. From the value of $k_d/(k_R + k_Q)$ in Table I, $k_R + k_Q$ ($\simeq k_R$) for DPBF is calculated to be 5×10^8 M^{-1} sec^{-1}, in good agreement with the value 7×10^8 M^{-1} sec^{-1} given by Stevens.[30]

The value of $k_R + k_Q$ for BR in $CHCl_3$–10% CH_3OH is calculated from the actinometric runs to be 1.3×10^9 sec^{-1} in fair agreement with the value in $CHCl_3$.

Substituting $\phi_{isc} = 0.66$ into the expression for BR in Table I gives $k_R/(k_Q + k_R) = 0.17$. Using this number, with the value 2.5×10^9 M^{-1} sec^{-1} for $k_R + k_Q$ from Table II, gives $k_R = 0.43 \times 10^9$ M^{-1} sec^{-1} for BR, in excellent agreement with the values obtained by the other techniques.

We conclude (1) DPBF does not quench singlet oxygen without reaction, in agreement with the results of Merkel and Kearns[23] and (2) singlet oxygen quenching by BR accounts for about 83% of the total removal of singlet oxygen, with reaction accounting for the rest.

A recent note by Merkel and Kearns[34] has reviewed the arguments for lack of DPBF quenching and, in agreement with the present work, they conclude that DPBF reacts essentially without physical quenching (i.e., $k_Q \leq 0.1\ k_R$).

Matheson, Lee, and coworkers have very recently reported a study of the reaction of both DBPF[16] and BR[15] with $^1O_2$, generated by direct excitation of oxygen at high pressures in Freon 113. They report $k_R = 1.1 \pm 0.6 \times 10^8$ M^{-1} sec^{-1} and $k_R + k_Q = 10 \pm 2 \times 10^8$ M^{-1} sec^{-1} for DPBF, and $k_R = 1.0 \times 10^7$ M^{-1} sec^{-1} for bilirubin. Both values of k_R are considerably lower than our values. The reason for this discrepancy is not entirely clear. It is possible that the differences are due to a large (and unprecedented) solvent effect, causing the reaction rates to differ by a factor of 10 in Freon 113 and $CHCl_3$. However, the low rates of Matheson and Lee may result from a low value of the rate of quenching of $^1\Delta_gO_2$ by $^3\Sigma_g^-O_2$ since their k_R values are measured relative to this rate. Their value, also determined by a laser technique, is 2.7×10^3 M^{-1} sec^{-1} in Freon 113; Stevens and Perez have reported a value for the oxygen quenching of $6 \pm 4 \times 10^4$ M^{-1} sec^{-1} in benzene.[25c] This discrepancy may also reflect a solvent effect, or it may be caused by more fundamental experimental problems. The values of Matheson and Lee are dependent on their ability to measure the amount of $^1O_2$ produced accurately; this requires knowledge of several experimental parameters, such as cell cross-sectional area, absorbance of the oxygen, and intensity of the laser pulse.

A referee argues that the difference between the present results and those of Matheson and Lee et al. may be caused by the fact that singlet oxygen may not be the intermediate in photosensitized oxygenations because there are discrepancies between estimates of rate constants between various groups of workers. In fact, solution photosensitized rate constants with a variety of different sensitizers are remarkably consistent (see references cited in this paper), as are values for rate constants with the product of the NaOCl–H_2O_2 reaction;[22] only the values of Matheson and Lee seem to deviate consistently from those of other workers, not only for DPBF and bilirubin, but also for β-carotene.[16] An additional possible reason for the discrepancy which has recently occurred to us is that excitation of oxygen through the dimol absorption produces singlet oxygen in the direct neighborhood of (or possibly even weakly bound to) a second oxygen molecule. The lifetime of this species may not be easily compared with that of free singlet oxygen in solution. Among other complications, excitation is transferred rapidly from one oxygen molecule to another, at least in the gas phase.[35]

The very large rate of reaction of bilirubin with $^1O_2$ probably explains the fact that phototherapy of neonatal jaundice is effective. Bilirubin produces singlet oxygen on irradiation but is almost certainly the most reactive local substrate in the lipid environment in which it is localized. Since it is so reactive, it is destroyed preferentially to other potential substrates.

Recently, riboflavine-sensitized photooxidation of bilirubin was reported.[36] On the basis of these studies, the authors suggested that riboflavine or other sensitizers might be used clinically for the treatment of jaundice. Since riboflavine or other sensitizers would not be consumed, this treatment would surely result in severe photodynamic side effects.

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Rhodium(I) Catalysis in Olefin Photoreactions

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Abstract: The photorearrangement (254 nm) of 1,5-cyclooctadiene (**1**) in the presence of rhodium(I) chloride to give 1,4-cyclooctadiene (**4**) is shown by deuterium labeling to involve an intramolecular [1,3] shift of hydrogen. A rate-determining cleavage of an allylic C–H bond is indicated by a deuterium isotope effect, $k_H/k_D = 1.55 \pm 0.03$ for the $1 \rightarrow 4$ rearrangement. The acyclic 1,5-diene, 3,3-dimethyl-1,5-hexadiene (**8**), rearranges in the presence of rhodium(I) chloride upon uv irradiation (254 nm) to give *cis*-3,3-dimethyl-1,4-hexadiene (**10**) and the trans isomer **11** in a 1:4 ratio, respectively. This observation supports a mechanism for the photorearrangement of olefins catalyzed by rhodium(I) involving a initial photodissociation of one of two rhodium(I) coordinated carbon–carbon double bonds. This results in an increase in the coordinative unsaturation of rhodium(I) and enhances the proclivity of this d_8 metal atom toward oxidative addition of an allylic C–H bond. A η^3-allylrhodium hydride intermediate then gives rearranged olefin by reductive elimination. Lastly, a novel photochemical, rhodium(I) catalyzed hydrogen transfer is reported which gives cyclooctene (**7**) from cyclooctadienes under unprecedentedly mild conditions.

The effort directed toward discovering and understanding organic photochemical reactions which require transition metals as catalysts[2a] has been meager compared with the extensive work on catalyzed thermal reactions.[2b-f] Metal carbonyl complexes are often active catalysts for photoreactions of olefins.[2a,3] The primary process in such reactions appears generally to be photodissociation of a carbonyl ligand to generate coordinatively unsaturated intermediates which catalyze transformations of the olefin substrates in subsequent dark reactions.[4] Metal carbonyl catalysis is thus dominated by the photochemistry of the M–CO bond. It has been suggested that photochemical pathways are operative in olefin rearrangements in olefin–metal carbonyl systems.[3] However, nothing is known about the mechanisms involved since studies of these pathways are beclouded by the dominant thermal pathways.

$$M(CO)_n \xrightarrow{h\nu} M(CO)_{n-1} + CO$$

Some transition metal complexes which do not contain carbonyl groups are also known to catalyze olefin photorearrangement.[2] Even simple salts of copper(I) and rhodium(I) catalyze olefin photoreactions.[6-8] These reactions are especially interesting since the salts form isolable olefin complexes, and since the olefin–metal interaction almost certainly plays a key role in the photochemical process. Thermal pathways are not expected to be important, and these reactions should be relatively simple and especially well suited for mechanistic study.

Recent studies[5] have elucidated the mechanism of olefin photodimerization catalyzed by copper salts.[6] Photodimeri-