servations apply only to Freon 113; the rate constant may be higher in other solvents. There is some evidence that β-carotene dimerization can occur in some vents leading to an apparent lowering of the quenching rate constant,[22] but we have no evidence for carotene dimerization occurring in Freon 113 at the typical experimental concentrations used.

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Reaction Rate of Bilirubin with Singlet Oxygen ($^1\Delta_g$) and Its Strong Enhancement by Added Base

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Abstract: The oxidation of bilirubin by singlet oxygen produced by direct laser irradiation of the oxygen $^1\Delta_g + 1v \leftarrow {}^3\Sigma_g^-$ electronic transition has been studied in Freon solution and in D_2O. In Freon 113 the bilirubin chemical reaction rate constant is 1.0×10^7 l. mol^{-1} sec^{-1} and in D_2O at pD <7 within experimental error the same, 1.5×10^7 l. mol^{-1} sec^{-1}. At higher pD (>8) the reaction rate increases to 3×10^9 l. mol^{-1} sec^{-1}. This rate constant is about 30 times higher than our recently revised value for 1,3-diphenylisobenzofuran, hitherto thought to be the most rapidly reacting singlet oxygen acceptor.

A considerable literature on singlet oxygen reactivity has been built up over the least few years.[1-3] Some of the reactions studied may be of biological significance, a particular case being the high quenching efficiency of singlet oxygen by β-carotenes.[4-6] Another biological molecule worthy of study for its singlet oxygen reactivity is the bile pigment bilirubin. The mechanism of the photooxidation of bilirubin is of some importance because of the phototherapy treatment used for the condition of neonatal jaundice.[7]

There have been indications[8-10] that the *in vivo* reaction may be a self-sensitized photooxidation, and there is ample evidence that bilirubin reacts with singlet oxygen produced in organic solution by external sensitizers such as Methylene Blue.[8]

The singlet oxygen reaction of bilirubin has been studied in this laboratory where the singlet oxygen was produced directly in solution by irradiation of the dissolved oxygen $^1\Delta_g + 1v \leftarrow {}^3\Sigma_g^-$ electronic absorption band with the 1.06 μm output of a Nd-YAG laser.[11] This method avoids interference with a possible competing reaction such as the acceptor with the triplet sensitizer.

Experimental Section

The apparatus was as described in the preceding paper.[12] Bilirubin (crystalline) was from the J. T. Baker Chemical Co., Phillipsburg, N. J., and was used as supplied. Freon 113 (1,1,2-trichlorotrifluoroethane) spectrograde was supplied by Matheson Coleman and Bell, East Rutherford, N. J. D_2O, 99.8%, was supplied by the Columbia Organic Chemicals Co., Columbia, S. C. Medical grade oxygen was used in all experiments.

Results

The bilirubin reaction was studied by measurement of the pseudo-first-order decay of bilirubin absorbance at the absorption maximum 435 nm D_2O and 450 nm Freon, which occurred on irradiation of oxygenated bilirubin solutions in Freon 113 and heavy water.

Bilirubin may be made water soluble as its dianion by dissolving in water containing 2 equiv of base per equivalent of bilirubin. The laser irradiation technique is difficult to use in light water since it absorbs considerably at 1.06 μm and solvent heating results. D_2O has an absorption of only 0.018 A cm^{-1} at 1.06 μm and has the added benefit that the $O_2(^1\Delta_g)$ is longer lived in D_2O than in H_2O.[5,13]

Studies of bilirubin photooxidation are confused by

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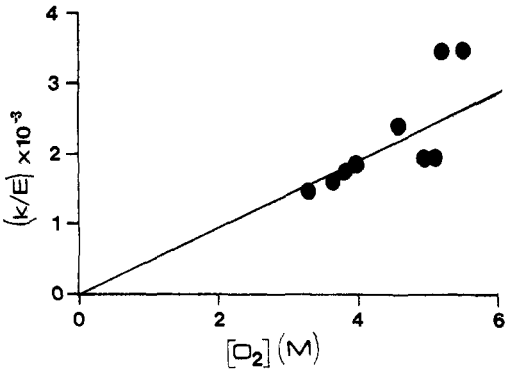

Figure 1. Bilirubin in Freon 113. Variation of k with E and $[O_2]$.

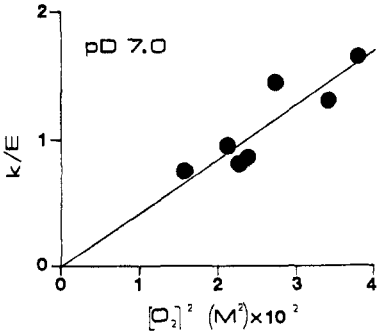

Figure 2. Bilirubin in D_2O at pD 7.0. Variation of k with E and $[O_2]^2$.

the number of reactions which are present. These are summarized by the following. (a) Reaction of bilirubin solutions on standing to give the green pigment biliverdin.[14] This does not seem particularly sensitive to light and oxygen but is slowed down considerably by storing the solutions in the dark below room temperature. (b) Reaction of bilirubin solutions containing dissolved oxygen in the dark to form colorless products. (c) Reaction of bilirubin solutions containing dissolved oxygen with light to form colorless products. (d) Reaction of bilirubin with singlet oxygen to give colorless products.

Fortunately reaction a is too slow to interfere with singlet oxygen reactions over the normal experimental time scale, and reactions b and c may be readily separated from reaction d by the following blank technique. Prior to laser irradiation the decay of the bilirubin absorbance is observed in the apparatus spectrophotometer, and this decay is subtracted from that subsequently observed when the laser is switched on.

Reactions b and c are very much slower in Freon 113 than in D_2O. The singlet oxygen reaction d is the only one to be described in detail in this paper.

Laser irradiation of oxygen generates $O_2(^1\Delta_g)$ in a bimolecular process[15]

$$O_2 + O_2 \xrightarrow[\sigma E]{1.06\ \mu m} O_2(^1\Delta_g) + O_2$$

where E is the laser intensity ($N h\nu$ sec^{-1}) and σ the absorption cross section at a wavelength of 1.06 μm.

There are three loss processes given by the reactions

$$O_2(^1\Delta_g) + B \xrightarrow{k_B} BO_2$$

$$O_2(^1\Delta_g) + O_2 \xrightarrow{k_{O_2}} O_2 + O_2$$

$$O_2(^1\Delta_g) \xrightarrow{k_S} O_2$$

The first is a chemical reaction with bilirubin (B) to form colorless products BO_2, the second is quenching by ground state oxygen which is the dominant process in Freon 113 since $k_{O_2}[O_2] \gg k_S$, and the third is quenching by solvent (S) at a pseudo-first-order rate k_S which dominates in D_2O since $k_S \gg k_{O_2}[O_2]$.

Applying the stationary state assumption for the concentration of $O_2(^1\Delta_g)$ making the usual approximation that $k_{O_2}[O_2] > k_B[B] + k_S$ since $[B] \sim 10^{-6}$ M, it may be shown[12] in Freon 113

$$k = \sigma E[O_2]k_B/ak_{O_2} \quad (1)$$

and in D_2O

$$k = \sigma E[O_2]^2k_B/ak_S \quad (2)$$

where k is the observed pseudo-first-order rate of disappearance of bilirubin absorbance of the absorption maximum, and a is the solution cross-sectional area in decimeters squared. The quantity σ/a is essential to calibration of the apparatus and its derivation was described in the preceding paper.[12]

A further assumption implicit in the D_2O experiments is that the oxygen $^1\Delta_g + 1v \leftarrow {}^3\Sigma_g^-$ electronic absorption is not perturbed in any way by the solvent. This information is not easily experimentally accessible due to the low solubility of oxygen in water and the D_2O absorbance of 0.018 A cm^{-1} which makes the use of long path length optical cells impracticable.

Rearrangement of (1) and (2) gives

$$k/E = \sigma[O_2]k_B/ak_{O_2} \quad (1a)$$

$$k/E = \sigma[O_2]^2k_B/ak_S \quad (2a)$$

predicting the normalized reaction rate is a linear function of $[O_2]$ in Freon 113 and depends on $[O_2]^2$ in D_2O.

Reaction in Freon 113. A plot corresponding to eq 1a is shown in Figure 1. These experiments were carried out at room temperature and the oxygen concentrations were derived from spectrophotometric measurements.[15]

Results in D_2O. A line corresponding to eq 2a is shown for bilirubin in D_2O at pD 7 in Figure 2. These experiments were carried out at room temperature and the oxygen concentrations derived by assumption of attainment of equilibrium between the gaseous and liquid phases and application of Henry's law.[16] The absorption cross section σ (1.06 μm) was assumed to be the same in D_2O as in Freon since, as mentioned above, it was too low to be measured. The lack of a direct measurement of oxygen concentrations for the data shown may account for the large scatter evident on this line and that of Figure 3 compared with the data in Figure 1.

A similar line corresponding to eq 2a is shown for bilirubin in D_2O at pD 10 in Figure 3. Note the difference in vertical scales between Figures 2 and 3.

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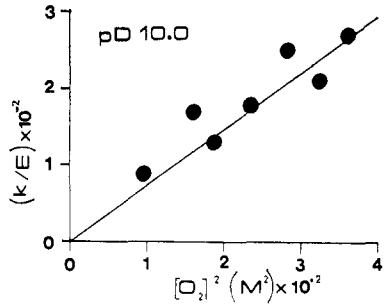

Figure 3. Bilirubin in D_2O at pD 10.0. Variation of k with E and $[O_2]^2$.

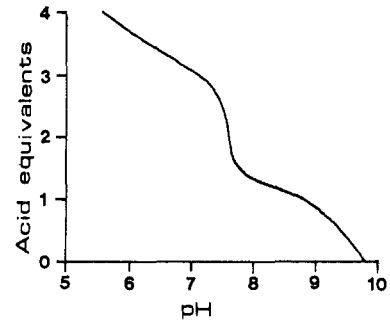

Figure 5. pH titration for bilirubin in H_2O.

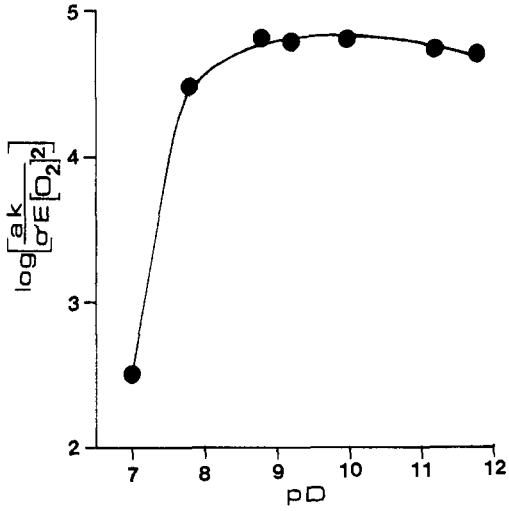

Figure 4. Bilirubin in D_2O. Variation of $ak/\sigma E[O_2]^2 = k_B/k_S$ as a function of pD.

The marked pD effect is shown more strikingly in Figure 4 where $\log \{ak/\sigma E[O_2]^2\} = \log (k_B/k_S)$ is shown as a function of pD. Each point corresponds typically to an average of five experimental rate determinations.

A pH titration of millimolar bilirubin in H_2O is shown for comparison in Figure 5. (Insolubility of bilirubin < pH 7 distorts this.)

Discussion

The data of Figure 1 yield a slope of $4.76 \times 10^2 = \sigma k_B/ak_{O_2}$ and substitution of $\sigma/a = 0.135$ l. mol^{-2} appropriate to the cell used, and $k_{O_2} = 2.7 \times 10^3$ l. mol^{-1} sec^{-1} from the preceding paper[12] yields for the Freon 113 results $k_B = 1.0 \pm 0.4 \times 10^7$ l. mol^{-1} sec^{-1}.

The data of Figure 2 for bilirubin in D_2O at pD 7 show moderate linearity and agreement with the kinetic scheme and may be similarly analyzed to yield a value for k_B in D_2O: slope $= 42.5 = \sigma k_B/Ak_S$, and using the k_S value of 5×10^4 sec^{-1},[5] $k_B = 1.5 \pm 0.4 \times 10^7$ l. mol^{-1} sec^{-1}. This value is identical with the Freon 113 value within experimental error and has a number of consequences. (1) The same bilirubin species is reacting with singlet oxygen in Freon and in neutral D_2O. (2) That being the case, there is excellent consistency between results based on the k_{O_2} value obtained in this laboratory and the k_S value for D_2O reported by Merkel and Kearns.[5] (3) The oxygen absorption cross section σ does not seem to change much from Freon 113 up to 5 M to D_2O up to 0.2 M, which suggests that D_2O cannot perturb the oxygen $^1\Delta_g \leftarrow {}^3\Sigma_g^-$ electronic transition to any significant degree.

The data of Figure 3 are for bilirubin in D_2O at pD 10 and the line shows similar linearity to that of Figure 2. The slope ratio of 180 shows the reaction rate increases sharply with pD. The reaction rate pD curve (Figure 4) shows the same effect and the rate ratio between the maximum and pD 7 is 200. This is most simply interpreted as the formation of an anionic bilirubin species with a pK of around 7.5 which reacts 200 times faster with singlet oxygen. This is supported by the pH titration curve for bilirubin (Figure 5). The high pD rate constant is $3 \pm 0.7 \times 10^9$ l. mol^{-1} sec^{-1} which is the most rapid acceptor rate constant known for singlet oxygen.

An alternative explanation of the rate enhancement with increasing pD is that $^1\Delta_g$ oxygen in basic solution forms some different species with a longer lifetime than $^1\Delta_g$ oxygen. This appears to be rather unlikely as the results of some preliminary experiments, where the enzyme superoxide dismutase has been studied as an aqueous singlet oxygen quencher, show that the quenching rate constant is not a function of pD.[17] Since the data when worked up yield $k_Q[Q]/k_S$[18], k_S cannot be changing with pD. If a more stable species were being formed, k_S would have to appear to change by a factor of 200 between pD 7 and 10 to completely account for the data.

The self-photolysis reaction of bilirubin, where oxygenated aqueous solutions of bilirubin are irradiated in the bilirubin visible absorption band, does not show this pD effect.[19] This, in addition to the fact that the self-photolysis reaction is solvent insensitive, suggests that freely diffusing singlet oxygen is not present in the self-photolysis reaction.

The pH of human blood serum is normally at 7.4.[20] The marked pH sensitivity of the reaction rate of bilirubin with singlet oxygen (Figure 4) could be of physiological significance.

However, it does appear that bilirubin anion, with the highest known acceptor rate constant for singlet oxygen,

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will be of value in studying singlet oxygen interactions in 100% aqueous systems, where 1,3-diphenylisobenzofuran cannot be used because of insolubility and dimerization problems.[5]

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Quenching by Hydrogen Bromide of the Norrish Type II Process in the Photolysis of 2-Pentanone. Chemical Trapping of a Triplet 1,4 Biradical in the Gas Phase

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Abstract: The effect of hydrogen bromide on the gas phase 2-pentanone photochemistry (3130 Å, 35–194°) is reported. The Norrish type II primary process (intramolecular elimination to ethene and acetone) and the Norrish type I process (simple bond fission) are both significantly reduced by hydrogen bromide. The dominant type I process is shown to be reaction to acetyl and *n*-propyl radicals. Quenching by HBr is explained in terms of chemical trapping of the 2-pentanol 2,5-triplet biradical intermediate (T_R) formed *via* the six-center intramolecular H-abstraction reaction which is known to precede type II product formation in the uninhibited system. A lifetime for T_R of $10^{-4.9}$ sec at the mean reaction temperature (388°K) is calculated from the data. An extended primary process mechanism involving singlet and triplet biradical states as well as singlet and triplet ketone states is proposed. Available data are shown to be consistent with the mechanism and are used to obtain reasonable rate constant and rate constant ratio values. An estimate of the equilibrium constant for the singlet to triplet biradical exchange reaction of $K_{S_R \rightarrow T_R} = 1.1 \times 10^3$ (25°) is deduced. Relevance of the rate constants obtained for spin inversion between triplet and singlet biradicals to recent interpretations of spin correlation effects is discussed.

The photolysis of 2-pentanone is a complex and interesting system since, as shown below, three different primary processes are known to occur: Norrish type I, simple bond fission; Norrish type II, intramolecular elimination; and isomerization to a cyclobutanol derivative (designated here as process III).

2-Pentanone primary processes

$$\begin{aligned} CH_3COC_3H_7 + h\nu &\xrightarrow{I_a} C_3H_7\cdot + CH_3CO\cdot \\ &\xrightarrow{I_b} C_3H_7CO\cdot + CH_3\cdot \\ &\xrightarrow{II} C_2H_4 + CH_3COCH_3 \\ &\xrightarrow{III} CH_2(CH_2)C(CH_3)OH \end{aligned} \qquad (I)$$

A wealth of quantum yield data exists for all three primary processes over a range of experimental variables (T, λ, and P) in both the gas phase and in solution.[1-7] The influence of various triplet state quenchers on the primary process quantum yields has also been rather extensively investigated.

The most important of these observations, including those of the present study, are summarized and referenced in Table I. Although the many observations on this system have prompted numerous interpretations dealing with specific aspects of mechanism, no detailed mechanistic scheme addressing and correlating data on all three processes has ever been presented.

With the modest intention of obtaining gas phase kinetic parameters for the type II process (*i.e.*, A_{II} and E_{II}) originating from the 2-P triplet state and of determining the lifetime of that state, we investigated the 2-P photochemistry at 3130-Å exciting radiation in the presence of varied amounts of hydrogen bromide over the temperature range 35–194°. Since prior results indicated that most of the type II process occurred *via* the triplet state of 2-P,[1-5] and since hydrogen bromide has been demonstrated to be a very efficient trapping agent for the acetone triplet,[8] it seemed reasonable that a study of the temperature dependence of the type II quenching effected by HBr would lead to the desired information. As anticipated, type II quenching was observed; however, the nature of the quenching process was not as expected. Our results indicate that in addition to upper singlet and triplet ketone states both singlet and triplet 1,4 biradicals are very important photochemical reaction intermediates and that hydrogen bromide quenches the type II reaction by trapping the triplet biradical and not the triplet ketone.

We have correlated our HBr-quenching data with the existing wealth of quantum yield data for the 2-P photochemical system and propose here a compre-

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