This study was undertaken to determine whether 4-benzylamino-7-nitrobenz-2-oxa-1,3-diazole (BBD) generates the first excited singlet state of molecular oxygen in liquid solution. The problem was first approached using the sensitized photooxidation method of singlet oxygen detection with 1,3-cyclohexadiene (CHD) as acceptor. A reaction between BBD and the acceptor rendered this method of singlet oxygen detection useless.

In a second approach, the rate of light-induced oxygen consumption for solutions having various components was measured. The results showed that for a given monolefin acceptor the solutions with BBD as sensitizer consumed oxygen about 25 times slower than solutions containing the known singlet oxygen sensitizer rose bengal. This suggests that either BBD does not generate singlet oxygen or that it does so about 25 times less efficiently than rose bengal. Both possibilities are discussed.

Further insight into the photochemical reaction of BBD and CHD was obtained by carrying out a quantity preparation of this reaction. Two major products were isolated by tlc. Evidence for a \( \text{4}_s + \text{4}_s \) photochemically allowed cycloaddition reaction was obtained leading to hypothesized structures for the two products.
SINGLET OXYGEN GENERATION VIA IRRADIATION OF 4-BENZYLAMINO-7-NITROBENZ-2-OXA-1,3-DIAZOLE

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by
Robert Langworthy Crouch
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SINGLET OXYGEN GENERATION VIA IRRADIATION OF
4-BENZYLAMINO-7-NITROBENZ-2-OXA-1,3-DIAZOLE

by
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS</td>
<td>29</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>52</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>67</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>68</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>72</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Photosensitized effects in the presence and absence of molecular oxygen.</td>
<td>10</td>
</tr>
<tr>
<td>II.</td>
<td>Decrease in absorbance at selected wavelengths for the photoreaction of BBD with various acceptors.</td>
<td>34</td>
</tr>
<tr>
<td>III.</td>
<td>Thermal reactions of BBD.</td>
<td>37</td>
</tr>
<tr>
<td>IV.</td>
<td>Thin layer chromatograph data for the photoreaction products of BBD plus CHD.</td>
<td>38</td>
</tr>
<tr>
<td>V.</td>
<td>Rate of oxygen consumption for various combinations of reactants.</td>
<td>45</td>
</tr>
<tr>
<td>VI.</td>
<td>Ratio of fluorescence intensity at selected wavelengths to intensity at fluorescence maximum.</td>
<td>48</td>
</tr>
<tr>
<td>VII.</td>
<td>Effect of increased rose bengal concentration on emission.</td>
<td>49</td>
</tr>
<tr>
<td>VIII.</td>
<td>Comparison of the literature values of the infrared absorption wavenumbers of DBO with those obtained herein.</td>
<td>55</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Molecular orbital diagrams for the ground and first excited singlet electronic states of molecular oxygen.</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>Common electronic transitions in organic molecules.</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Schematic diagram of photochemical reactor.</td>
<td>19</td>
</tr>
<tr>
<td>4.</td>
<td>Infrared spectrum of the product of the sensitized photooxidation of 1,3-cyclohexadiene.</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>Proton magnetic resonance spectrum of the product of the sensitized photooxidation of 1,3-cyclohexadiene.</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>Visible-ultraviolet absorption spectrum of the ( R_f = 0.00 ) component from the reaction of BBD plus CHD.</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>Visible-ultraviolet absorption spectrum of the ( R_f = 0.65 ) component from the reaction of BBD plus CHD.</td>
<td>41</td>
</tr>
<tr>
<td>8.</td>
<td>Infrared spectrum of the BBD plus CHD photoreaction product having ( R_f = 0.65 ).</td>
<td>42</td>
</tr>
<tr>
<td>9.</td>
<td>Typical trace of oxygen consumption rate for rose bengal sensitizer.</td>
<td>46</td>
</tr>
<tr>
<td>10.</td>
<td>Proton assignment for DBO.</td>
<td>53</td>
</tr>
<tr>
<td>11.</td>
<td>Proton magnetic resonance spectrum of 2-norbornene.</td>
<td>54</td>
</tr>
<tr>
<td>12.</td>
<td>Infrared spectrum of ascaridole.</td>
<td>57</td>
</tr>
<tr>
<td>13.</td>
<td>Infrared spectrum of DBO.</td>
<td>58</td>
</tr>
<tr>
<td>14.</td>
<td>Infrared spectrum of BBD.</td>
<td>73</td>
</tr>
<tr>
<td>15.</td>
<td>Proton magnetic resonance spectrum of NBD-Cl.</td>
<td>75</td>
</tr>
<tr>
<td>16.</td>
<td>Infrared spectrum of NBD-Cl.</td>
<td>76</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>17.</td>
<td>Infrared spectrum of the photochemical decomposition product of BBD.</td>
<td>78</td>
</tr>
<tr>
<td>18.</td>
<td>Infrared spectrum of CHD.</td>
<td>80</td>
</tr>
<tr>
<td>19.</td>
<td>Proton magnetic resonance spectrum of CHD.</td>
<td>82</td>
</tr>
<tr>
<td>20.</td>
<td>Infrared spectrum of mineral oil used as solvent in other infrared spectra.</td>
<td>83</td>
</tr>
</tbody>
</table>
INTRODUCTION

The question of singlet oxygen generation by 4-benzylamino-7-nitrobenz-2-oxa-1,3-diazole (I), also known as BBD, is not only of theoretical importance but also may have application as a drug treatment. This is due to the unusual protein binding capabilities of BBD and its p-methoxy analog (II). It is thought that if a compound could be obtained that generates highly reactive singlet oxygen and binds to proteins as well, then it might be possible to selectively denature proteins in vivo. This would be a novel and potentially effective treatment of cancer and other diseases caused by malfunctions or excesses at the molecular level. The initial step in using BBD for such treatment is to determine whether it does generate singlet oxygen.

Singlet oxygen is the first electronically excited state of molecular oxygen (Figure 1). During the past decade, it has been increasingly recognized as an important chemical entity. Whereas ground state oxygen is essentially inert toward biological materials, singlet oxygen is quite
Figure 1. Molecular orbital diagrams for the ground and first excited singlet electronic states of molecular oxygen. Levels shown are $n = 2$. Note that the two highest energy electrons have the same spin and occupy separate anti-bonding orbitals in the ground state. In singlet oxygen these have paired spins and occupy the same orbital.
reactive and toxic to living things. (37, 42, 58) Models of the enzyme systems that protect organisms from other toxic oxygen species, such as peroxides and superoxide anion, are currently being revised to include mechanisms for the detoxification of singlet oxygen. (24) In contrast, other researchers have postulated that singlet oxygen may be a necessary intermediate in certain biosynthetic reactions. (52) Singlet oxygen has been shown to be the species responsible for the chalking of paints containing ZnO and TiO₂ pigments (53) and it has become a useful tool in synthetic organic chemistry. (63, 13)

Singlet oxygen can be formed by direct optical (10) or thermal (60) excitation or as a product of certain chemical reactions (39, 47, 48). Perhaps the most interesting way it is generated is by interaction with certain organic compounds. This process is called sensitized photooxidation and the overall reaction is illustrated in Scheme 1. When a solution of the sensitizer compound,

\[
\text{Sens + photon} \rightarrow \text{Sens}^* \\
\text{Sens}^* + O_2 \rightarrow \text{Sens} + O_2^* \quad (\text{Eq. 2}) \\
\text{Acceptor + O}_2^* \rightarrow \text{Oxidized Acceptor} \quad (\text{Eq. 3})
\]

Scheme 1.

oxygen and a suitable acceptor is irradiated with visible light, the sensitizer molecule absorbs a photon and is excited to a higher energy electronic state. The excited
sensitizer then interacts with a ground state oxygen molecule in a way that allows transfer of the energy to oxygen. Thus formed, the singlet oxygen molecule reacts with an acceptor molecule. In order to differentiate this type of oxidation from those occurring with ground state oxygen, such as free radical oxidations, the acceptor must react specifically with singlet state oxygen. Identification of the oxidized acceptor may then indicate that singlet oxygen is the reactive intermediate.

The possibility that singlet oxygen might be involved in sensitized photooxidation was suggested by Kautsky. (35) He showed that significant oxidation of acceptor molecules occurred even when acceptor and sensitizer were physically separated by adsorption onto different sets of silica gel particles. He concluded that the process involved some metastable species capable of migrating in the gas phase from sensitizer to acceptor. Since no oxidation was observed when oxygen was excluded from the system, he postulated that singlet oxygen was the oxidizing agent. In spite of these experiments, the Kautsky mechanism was rejected by many noted scientists of the day. (65)

From spectroscopic studies carried out in the early 1960's, Khan and Kasha determined that singlet oxygen was produced from the reaction of hydrogen peroxide with metal hypochlorites (Eq. 4). (34, 39, 40, 41) They observed emission bands at 630 nm and 477 nm which were subsequently
described as resulting from a bimolecular collision of gas phase singlet oxygen molecules. (2, 3) Browne and Ogryzlo measured the single molecule emission at 1270 nm using a refined technique. (10, 11) It was clear from these studies that the NaOCl-H$_2$O$_2$ reaction was a good source of singlet oxygen.

The important link between the chemical generation of singlet oxygen and sensitized photooxidation was provided by Foote and coworkers. (18-23) They found that the products from the reaction of singlet oxygen generated from the NaOCl-H$_2$O$_2$ system with various acceptors were identical to the products obtained using sensitized photooxidation (Scheme 2). At about the same time, Corey and Taylor showed that oxygen molecules excited in a radiofrequency (rf)

\[
\text{NaOCl} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2^* + \text{Cl}^- + \text{Na}^+ + \text{H}_2\text{O} \quad \text{(Eq. 4)}
\]

discharge reacted with various substituted anthracenes and olefins to yield the same products as sensitized photooxidation. (13) Based on these studies it was concluded
that singlet oxygen was probably the reactive intermediate. After thirty years, Kautsky's mechanism has been accepted as the essential process of sensitized photooxidation. (37)

Detection of singlet oxygen in the gas phase is easily accomplished using spectroscopic techniques. (37) Problems arise when these methods are applied to singlet oxygen dissolved in liquids. The possible decomposition pathways of singlet oxygen are illustrated in Scheme 3.

\[
\begin{align*}
2O_2^* & \longrightarrow 2O_2 + \text{photon} \quad \text{(Eq. 6)} \\
O_2^* & \longrightarrow O_2 \quad \text{(Eq. 7)} \\
O_2^* + \text{Acceptor} & \longrightarrow \text{Oxidized Acceptor} \quad \text{(Eq. 8)}
\end{align*}
\]

Scheme 3.

Equation 6 represents a bimolecular collision of singlet oxygen molecules leading to emission. Equation 7 is a radiationless deactivation process and Equation 8 is reaction. The problem of differentiating these has been approached and it has been generally agreed that, in liquids, Equations 7 and 8 predominate to the exclusion of 6. (26, 29, 37) Since Equation 6 is a bimolecular process, it will depend heavily on the concentration of singlet oxygen. At oxygen concentrations normally obtainable in solution, radiationless deactivation (Eq. 7) would be expected to occur before two singlet oxygen molecules could diffuse and collide. Evidence has been obtained supporting
Recently, Nakano and coworkers have reported a direct spectroscopic observation of singlet oxygen emission from solution. (49, 50) A time integrated photon counting system was used that was claimed to be much more sensitive than the spectroscopic methods used previously.

The usual method of detecting singlet oxygen in solution is by the acceptor method described above. Singlet oxygen reacts with mono-olefins by a mechanism analogous to the "ene" reaction of Alder to form allylic hydroperoxides (Eq. 9). (1) A peroxepoxide intermediate (III) has been

\[
\begin{align*}
\text{C} &= \text{C} \quad + \quad \text{O} \\
\text{CH}_3 &= \text{CH}_2
\end{align*}
\quad \text{Eq. 9}
\]

suggested for this reaction (37) but recent evidence has refuted this (32). The hydroperoxide could also be produced as a result of free radical oxidation of the olefin as in Scheme 4. Although Foote (21) has presented several

\[
\begin{align*}
\text{C} &= \text{C} \quad + \quad \text{O} \quad \text{O} \\
\text{CH}_3 &= \text{CH}_2
\end{align*}
\quad \text{Eq. 10}
\]

(III)
arguments that strongly favor the intermediacy of singlet oxygen, the free radical mechanism cannot be completely ruled out. The use of mono-olefins as acceptors thus does not yield conclusive evidence for the intermediacy of

\[
\begin{align*}
\ce{C==C(CH3) & \rightarrow h\nu} & \ce{C==C(CH2) + H+} \quad \text{(Eq. 11)} \\
\left[ \ce{C==C(\hat{CH2} \leftrightarrow C==C)} \right] + \ce{\dot{O}O} & \rightarrow \ce{C==C(CH2O-O)} \quad \text{(Eq. 12)} \\
\ce{C==C(CH2O-O) + H+} & \rightarrow \ce{C==C(CH2O0H)} \quad \text{(Eq. 13)}
\end{align*}
\]

Scheme 4.

singlet oxygen and their use is not recommended. (20, 37)
The reaction of singlet oxygen with cyclic and heterocyclic conjugated dienes yields products with structures analogous to (IV). This reaction is a Diels-Alder addition of oxygen

\[
\ce{C==C + \hat{O}} \rightarrow \ce{C==C(CH2O0H)} \quad \text{(Eq. 14)}
\]

(IV)

to the diene and appears to be unique to singlet state
oxygen. (18, 19, 26) A number of researchers have reported that certain acceptors react with singlet oxygen to form dioxetane intermediates (V). (4, 22, 30, 32) This mechanism also appears to be unique to singlet state oxygen.

![Chemical structure](attachment:structure.png)

The biological phenomenon known as photodynamic action is intimately tied to sensitized photooxidation. Photodynamic action was first described by Raab who noted that while there was no effect on paramecia in a solution of eosin dye in the dark, placing the mixture in light resulted in the rapid death of the organisms. (54) Subsequent experiments showed that oxygen was required for death to occur. A large number of systems of this type have since been discovered, a summary of which is presented in Table I. In addition to these laboratory experiments, there are examples of naturally occurring photodynamic action. (6) The reaction is usually brought about by ingestion of plant components that are subsequently converted to photosensitizing materials in the small intestine. These then enter the bloodstream and are carried throughout the body. Exposure of the individual to sunlight results in a histamine reaction that produces a sunburn-like rash or
Table I. Photosensitized effects in the presence and absence of molecular oxygen.\(^a\)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Effect for (O_2) Present</th>
<th>Effect for (O_2) Absent</th>
<th>Sensitizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (B. proteus)</td>
<td>killed</td>
<td>none</td>
<td>Rose Bengal Methylene Blue</td>
</tr>
<tr>
<td>Erythrocytes (mammalian)</td>
<td>hemolyzed</td>
<td>none</td>
<td>Rose Bengal Methylene Blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Eosin Hematoporphyrin Chlorophyll</td>
</tr>
<tr>
<td>Blood clot</td>
<td>clotting prevented</td>
<td>normal clotting</td>
<td>Methylene Blue</td>
</tr>
<tr>
<td>Skeletal muscle (frog)</td>
<td>stimulation to contraction</td>
<td>none</td>
<td>Rose Bengal</td>
</tr>
</tbody>
</table>

\(^a\)Reference 6.
hives. These effects have been described in human as well as laboratory animals and livestock. A photodynamic response in many individuals is induced by ingestion of tetracycline antibiotics. (12)

Many studies have been made of the effects of photodynamic action on proteins, amino acids and nucleic acids. It is now agreed that sensitized photooxidations of biological materials proceed either by singlet oxygen or free radical pathways. (33) As expected, free radical sensitized photooxidations are inhibited by ground state oxygen. (33) Although photodynamic action does not always involve singlet oxygen (57), studies of the photodynamic oxidation of amino acids (51, 17) and pyrroles (45) have shown that singlet oxygen is the effective oxidizing agent in many cases. Other researchers have demonstrated the involvement of singlet oxygen in the induction of genetic changes (43, 68), suggesting that a reaction occurs with nucleic acids. Wasserman has reported that a complex mixture of products is formed from the reaction of singlet oxygen with histidine. (63) Other amino acids that appear to be susceptible to singlet oxygen oxidation are tyrosine, methionine, cysteine and tryptophan. (58)

It is postulated that photodynamic inactivation of proteins likely occurs from the reaction of singlet oxygen with the side chains of amino acids. (57) Perhaps the most striking demonstration of this effect is to be found in the
work of Dougherty and coworkers. (15, 64) Laboratory mice possessing subcutaneous solid tumors of measured size were injected with fluorescein dye and placed under moderately intense light for a short time. Irradiation was repeated at regular intervals over a period of days. The animals were then sacrificed and the extent of the tumors was measured. In all cases, the treatment resulted in dramatic, though not complete, remission of the tumors. The advantage of using fluorescein as sensitizer was that this material was known to be preferentially incorporated into tumors. Irradiation then induced photodynamic action only in the tumor and not in healthy tissue. Subsequent experiments showed that singlet oxygen was the effective cytotoxic agent. (64)

The compound 4-benzylamino-7-nitrobenz-2-oxa-1,3-diazole (BBD) (I) and its p'-methoxy analog (II) have been shown to bind specifically to the hydrophobic sites in proteins. (38) The synthesis of these compounds provides a convenient method of preparing highly fluorescent materials that may bind to proteins at sites governed by the group para on the benzylamino portion of the molecule. It is thought that if a substance could be found that generates singlet oxygen and binds selectively to proteins as well, then it might be possible to selectively denature proteins in vivo. This would be a novel and perhaps effective treatment of cancer and other diseases caused by malfunctions at
The role of the sensitizer in sensitized photooxidation is especially important to the present work. Beyond the point that the mechanism of energy transfer from excited sensitizer to oxygen is undoubtedly collisional in nature, little is known of the actual process. A number of possible mechanisms have been discussed by Kearns. (37)

The electronic states available to sensitizers under usual conditions are illustrated in Figure 2. Absorption of a photon usually excites the molecule to a singlet state. This is because absorption to the triplet state involves a change in the spin of an electron, which is a theoretically forbidden process. Absorption to the triplet does occur, but only rarely. From Figure 2 it is evident that absorption may occur to an excited vibrational state. Relaxation from excited vibrational states is a thermal process and does not emit light. For a molecule having a singlet ground state, as in Figure 2, fluorescence is defined as a transition from the lowest excited singlet \( S_1 \) to the ground state \( S_0 \) and phosphorescence is a transition from the lowest triplet \( T_1 \) to the ground state. Both of these processes occur with emission of a photon and may occur from or to excited vibrational levels. Competing with fluorescence is intersystem crossing, a spin forbidden process by which the \( S_1 \) state is converted to the \( T_1 \) state. Intersystem crossing is the main pathway which forms the \( T_1 \)
Figure 2. Common electronic transitions in organic molecules. $S_0$ is the singlet ground state. $S_1$, $S_2$ and $S_3$ are excited singlet states and $T_1$, $T_2$ and $T_3$ are excited triplet states. IX is intersystem crossing and R is vibrational relaxation. Adapted from Figure 1.1 in Reference 46.
state under usual conditions. Note that the net energy of the molecule is unchanged by intersystem crossing and that this process occurs without emission or absorption of light.

Molecular oxygen is known to efficiently quench both the excited singlet and excited triplet states of sensitizers. (56, 36, 27, 62) Quenching involves the transference of the sensitizer energy to the quencher, in this case oxygen, instead of releasing energy in the form of light or thermal radiation. In separate experiments, Foote (20) and Gollnick and Schenk (26) have shown that singlet oxygen can be generated by oxygen quenching of sensitizer excited triplet states and possibly by quenching of excited singlets. Other researchers have shown that the oxygen quenching of excited singlet states does not produce singlet oxygen. (56, 59) This conflict can be resolved, according to Kearns, by considering the possibility that oxygen quenching of singlets results in the enhancement of intersystem crossing. (37) The triplet state thus formed would then be capable of generating singlet oxygen.

The high fluorescence exhibited by BBD and its analogs is evidence that electronic transitions in these molecules are frequent. The emission wavelength for these compounds lies near 550 nm. This corresponds to about 50 kcal/mol and is more than the 22.5 kcal/mol of energy needed to excite oxygen to the singlet state. (33) This property of BBD, along with its protein binding capabilities, led
to its choice as the subject of this work.

In summary, the purpose of this research is to determine whether BBD is capable of generating singlet oxygen in liquid solution. This is an important initial step in establishing the effectiveness of this class of compounds for the possible selective denaturation of proteins in vivo.
Instruments. Absorbance spectra were obtained using a Beckman DK-2A recording spectrophotometer. Infrared spectra were taken on a Beckman IR-8 and were calibrated with polystyrene. A Varian T-60A proton magnetic resonance spectrometer was used for pmr spectra. Chemical shifts are given in parts per million (ppm) from (CH₃)₄Si reference. Studies of singlet oxygen emission from the liquid phase were carried out using an Aminco-Bowman spectrofluorometer equipped with a ratio photometer and a Sargent Model SRG-GC strip chart recorder. All emission and excitation spectra are uncorrected. Dissolved oxygen was measured with a Yellow Springs Instruments Model 53 Oxygen Monitor equipped with a polarographic membrane electrode. Melting points were taken on either a Fisher-Johns hotstage or a Thomas-Hoover capillary apparatus and are uncorrected.

Materials. Nitrogen and oxygen were used as obtained from Air Products Co. Indicator grade rose bengal and 1,3-cyclohexadiene, 99%, were purchased from Aldrich Chemical Co. All solvents were reagent grade or were purified by distillation immediately prior to use. Student prepared 2-methyl-2-butene was dried and distilled: the fraction boiling at 37.5-38°C was retained for use. Authentic BBD was purchased from Pierce Chemical Co.: mp 207.5-208.5°, lit. 207-208°; ir (KBr pellet) 3290, 3100, 1583, 1488, 1294, 1258, 1220, 1180, 1124, 1050, 1010, 908,
850, 812, 757, and 704 cm$^{-1}$. (Actual spectra are presented in the Appendix.)

The method of Boulton et al., (7) was used for the preparation of 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl): mp 96.2-98°, lit. 96.5-97°; ir (KBr pellet) 3110, 1620, 1510, 1451, 1368, 1328, 1051, 1007, 967, 896, 864, 810, 777, 735 and 680 cm$^{-1}$. One of the intermediates of this synthesis, 4-chlorobenz-2-oxa-1,3-diazole, mp 78.9-81.2°, lit. 83-84°, was retained for use in the reactivity study with benzylamine.

**Thin Layer Chromatography.** All tlc's were eluted on either Eastman No. 6060 prepared silica gel plates with fluorescent indicator or Eastman No. 6065 cellulose with fluorescent indicator. Reagent grade solvents were used for elution. Spots were visualized under both short and long wave ultraviolet light.

**Photochemical Apparatus.** A simple photochemical reactor was devised as illustrated in Figure 3. The reaction chamber was Pyrex for filtering out ultraviolet light which might cause photodissociation or other unwanted processes. The chamber volume was 50 ml. To prevent overheating, the reaction mixture was kept at 0°C or lower by circulating water from a salt-ice slurry bath through the jacket surrounding the reaction chamber. Oxygen was constantly passed through the solution from a gas dispersion tube. The lamp was a 600 watt Smith-Victor tungsten-halogen
Figure 3. Schematic diagram of photochemical reactor.
type and was kept 13 cm from the reaction mixture. The mixture was stirred constantly.

**Photosensitized Oxygenation of 1,3-Cyclohexadiene Using Rose Bengal as Sensitizer.** Solutions which were 1.2 x 10^{-4} M in rose bengal and 0.01 M in 1,3-cyclohexadiene (CHD) were irradiated for 15 min. Five such solutions were combined and evaporated below room temperature. The resulting red syrup was taken up in ether and a red precipitate was filtered. After drying (MgSO_4) and filtering again, the ether was evaporated below room temperature. The resulting reddish-yellow semisolid was distilled at 60° and 1.3 torr yielding a pale yellow waxy material in a small amount of yellow liquid. Both phases were investigated and found to have essentially the same spectral properties (compare Figures 4 and 13): ir (film) 1370, 1215, 1165, 1060, 965, 920, 710 and 670 cm^{-1}; pmr (CCl_4) 1.75 (4,m), 4.50 (2, broad), 6.60 (2, overlapping doublets). The literature values for the pmr spectrum of 2,3-dioxabicyclo[2.2.2.]oct-5-ene (DBO) are 1.74 (multiplet), 4.53 (broad), 6.57 (overlapping doublets) in the ratio of 2:1:1. (21) The spectral data obtained herein support the assigned structure.

**Photosensitized Oxygenation of 1,3-Cyclohexadiene Using BBD as Sensitizer.** The method used was strictly analogous to that described for rose bengal. Irradiation was accompanied by rapid bleaching of BBD color and fluorescence. Evaporation of solvent yielded a slight amount
of yellowish-brown material adhering to the walls of the flask. The characteristic odor of DBO was not detectable in either the stripped solvent or the residue. Melting point of the residue: 79-93°.

In an attempt to determine the fate of CHD in this reaction, the methanol solvent was collected during the evaporation process. Mixing this with either bromine in CCl₄ or permanganate resulted in no perceptible change in the solutions.

**Photochemical Reactions of BBD.** Solutions which were 0.01 M in CHD and 1.2 x 10⁻⁴ M in BBD were irradiated for 15 minutes, 30 minutes or 35 minutes. No attempt was made to isolate any products. Instead, absorbance spectra of diluted aliquots of the solutions were taken before and after irradiation. A decrease in absorbance at λ_max of BBD was indicative of a reaction which consumed BBD. Since any products and their molar absorptivities were unknown, this procedure yielded only semiquantitative data about the extent of reaction. Besides CHD, cyclohexene and furan were used as acceptors. The effect of nitrogen and oxygen on these reactions was determined by comparing the decreases in absorbance of the systems with gas absent to those with gas present. To determine whether BBD was reacting with singlet oxygen, BBD was used as an acceptor in the rose bengal sensitized system. The effect of oxygen on this reaction was determined as above.
The possibility of a self-sensitized photooxidation of BBD was explored by irradiating a $1.2 \times 10^{-4}$ M solution of BBD for 35 min. Gaseous oxygen was passed through the solution during irradiation. Absorbance spectra were taken before and after irradiation as described above. Evaporation of solvent yielded a slight amount of yellowish-brown material adhering to the walls of the flask: ir (KBr pellet) 3290, 3095, 1581, 1485, 1293, 1255, 1215, 1180, 1120, 1050, 1008, 905, 848, 810, 753 and 702 cm$^{-1}$ (Fig. 17 in Appendix).

Thermal Reactions of BBD. Solutions were prepared by adding 1 ml aliquots of acceptor to 100 ml $1.2 \times 10^{-4}$ M BBD solution. After taking absorbance spectra of conveniently diluted aliquots of these solutions, the flasks were stoppered and placed in the dark at ambient temperature for a period of weeks. Absorbance spectra were taken at the end of the reaction period and the same dilutions were used as at the outset. This procedure was also used for a solution containing 60 mg rose bengal in 100 ml of $1.2 \times 10^{-4}$ M BBD.

Identification of BBD Plus CHD Reaction Products. To 50 ml methanol was added 0.0379 g BBD (0.14 mmol) and the mixture was stirred for 18 hrs to effect dissolution. A large excess of CHD, 0.33 g (4.1 mmol), was added. Because of the increased reactant concentrations, the irradiation time was increased to one hour so that the reaction
might go more nearly to completion. After one hour of irradiation, the solvent was evaporated and the residue was stored in the dark at 0°C. Separation of the residue was accomplished by tlc using CHCl₃, benzene, CCl₄, ether, chlorobenzene and ethyl acetate as eluents. The best separation of the major components was obtained with CHCl₃. A large quantity of the product mixture was separated using this solvent. The separated components were scraped from the plate and weighed. Each silica-product mixture thus obtained was dissolved in methanol and filtered. All methanol solutions of each component were combined and diluted to a final volume of 10 ml. Absorbance spectra were taken of each component after diluting the solutions as necessary. Reference solutions were prepared by scraping an equal weight of tlc silica from the plate, dissolving it in an equal volume of methanol, filtering and diluting to the same final volume as the sample.

The $R_f$ values of the two major components were calculated for each solvent used. In each solvent, one component always had $R_f = 0$ while the other major component had an $R_f$ value that varied with the solvent used. The infrared spectrum of the component with variable $R_f$ was taken: (film) 3032, 2950, 2880, 1640, 1555, 1455, 1395, 1355, 1305, 1050, 980, 875, 780 and 703 cm⁻¹ (cf. Figure 8).

**Oxygen Depletion Studies.** To a sample chamber immersed in the Yellow Springs Instruments Standard Bath
Assembly was added 15 ml of $1.2 \times 10^{-4}$ M sensitizer solution. The solution was brought to 0°C or lower by circulating water from a salt-ice slurry bath through the assembly. The solution was saturated with gas that had first been passed through absolute methanol. Gas flow was then stopped, the sample chamber sealed, the recorder started and the lamp turned on. When a stable reading was obtained, acceptor was added to make the solution 0.2 M. Reaction was allowed to continue until the rate of oxygen consumption could be easily measured. This was generally about 12 minutes. The acceptors used were 2-methyl-2-butene (amylene) and cyclohexene. Because of the known photoreaction between BBD and CHD and the fact that the products of this reaction were unknown, CHD was not used as an acceptor in oxygen depletion studies. Instead, these experiments were carried out using rose bengal and CHD to allow comparison with a known singlet oxygen generating system.

The response of the polarographic membrane electrode used to measure oxygen concentrations is extremely sensitive to changes in temperature. Reproducible traces were difficult to obtain in this experiment because the water bath used for cooling the reactor was not well insulated. The temperature of the reactor was recorded manually at intervals and the rates of oxygen consumption were measured over the same time period and temperature range. The
temperature range used was -6° to -4°C. Rates of consumption were determined by taking the slopes of the traces obtained and are expressed as change in percent oxygen saturation per minute. The normalized rates of oxygen consumption were then determined by subtracting the rate before acceptor addition from the rate after addition.

Spectral Observation of Singlet Oxygen Emission.
The cuvettes were cleaned by boiling in concentrated HNO₃ and a gas tight seal was obtained for each as follows: the rounded portion of a rubber medicine dropper bulb was cut off; a rubber septum was pressed into the base of the bulb and the assembly was fitted into the cuvet with the septum outermost; rubber cement was then applied to the top of the cuvet to effect the seal. Introduction of sensitizer solutions and saturation with gas was accomplished with hypodermic needles. Six cuvettes were used. Two were filled with BDD solution, two were filled with rose bengal solution and two were filled with methanol blank solution. Sensitizer solutions were 1.2 x 10⁻⁴ M. One cuvet of each solution was saturated with oxygen and the other with nitrogen. To avoid evaporation and subsequent concentration changes in sensitizer solutions during saturation, gases were passed through absolute methanol in a trap between the gas tank and the cuvet. All solutions were prepared with methanol that had been purified by distillation from NaOH pellets. Emission spectra for all six solutions were obtained at
excitation wavelengths of 470 nm and 594 nm. These are the wavelengths of maximum excitation for BBD and rose bengal, respectively.

To compensate for differences in base line in different spectra, the data were reduced by taking the ratio of the emission intensity at selected wavelengths to the intensity at the emission maximum:

\[
\text{relative intensity} = \frac{I}{I_{\text{max}}}
\]

The wavelengths selected were those at which singlet oxygen is known to have emission, namely 480 nm, 520 nm, 580 nm, 633 nm and 703 nm. Had any emission other than that of the sensitizer been observed in any of the spectra, other wavelengths would have been chosen for measurement.

The effect of increased sensitizer concentrations on the emission spectra was determined using the above procedure for rose bengal solutions that were \(2.5 \times 10^{-4}\) M and \(1.3 \times 10^{-3}\) M. These concentrations are approximately two and ten times that used in the sensitized photooxidation experiments, respectively. This experiment was not done for BBD.

**Synthesis of BBD.** The method of Kenner and Aboderin was used. (38) To 80 ml ethyl acetate containing 4.0 mmol NBD-Cl was added dropwise with stirring 80 ml ethyl acetate containing 4.7 mmol benzylamine. Benzylamine was purified by vacuum distillation prior to use. When addition was
complete, the mixture was stirred at ambient temperature for two hours. The resulting dark green liquid was extracted once with 10 ml water and dried (MgSO₄). The solution was decanted. The residue was washed three times with 25 ml portions of fresh solvent. All ethyl acetate solutions were then combined and the solvent was removed by vacuum evaporation. The resulting dark green solid was recrystallized from absolute ethanol. The residue from hot filtration had a melting point of 185-195°C when dried. The filtrate was allowed to stand in ice water for 45 min, then filtered and the residue was dried. This yielded yellowish-green crystals, mp 170-205°C. Both products were subjected to column chromatography on neutral alumina with ethyl acetate as eluent. Evaporation of each solution gave a small portion of dark green crystals each melting at 207.5-208.5°C when dried.

The residue from the hot filtration step was extracted with ethyl ether in a Soxhlet apparatus until no green fluorescence was observed in the extractate. The process took about four days. The dried residue had a melting point greater than 270°C whereas the material remaining after evaporation of ether melted 106-169°C.

Reaction of 4-Chlorobenz-2-oxa-1,3-diazole with Benzyllamine. To 30 ml ethyl acetate was added 0.23 g 4-chlorobenz-2-oxa-1,3-diazole and 1 ml purified benzyllamine. The mixture was swirled to insure homogeneity. The
container was sealed and allowed to stand at ambient temperature in the dark for three months. The appearance of the solution was noted at intervals. After three months, the mixture was refluxed for 20 hours in an attempt to induce reaction. The green fluorescent color typical of the NBD-Cl-amine adduct was not observed and the initial appearance of the solution did not change at any time during the procedure.
RESULTS

The photosensitized oxidation of 1,3-cyclohexadiene using rose bengal as sensitizer resulted in the formation of 2,3-dioxabicyclo[2.2.2]oct-5-ene (DBO). The infrared (Fig. 4) and pmr (Fig. 5) spectra obtained herein for the product of this reaction correspond to the data obtained by Foote et al. (21) An analysis of this spectral data is presented in the Discussion section. The predicted product for this reaction is DBO, and its isolation is considered strong evidence for the intermediacy of singlet oxygen.

No DBO was isolable from the product mixture when BBD was used as sensitizer. The reaction was accompanied by rapid bleaching of BBD color and fluorescence suggesting that BBD undergoes some reaction. The possibilities are that BBD generates singlet oxygen which immediately reacts with BBD (a self-sensitized photooxidation) or that the singlet oxygen reacts to form DBO which then reacts with BBD. It is also possible that singlet oxygen is not involved at all. The reactions that might then occur would be either a photochemical decomposition of BBD, a photochemical reaction between CHD and BBD, or an oxidation involving ground state oxygen.

Knowing the fate of CHD would provide evidence for which of the above reactions was occurring. To determine whether CHD was removed with the methanol solvent during vacuum evaporation, the methanol was collected and subjected
Figure 4. Infrared spectrum of the product of the sensitized photooxidation of 1,3-cyclohexadiene.
Figure 5. Proton magnetic resonance spectrum of the product of the sensitized photooxidation of 1,3-cyclohexadiene.
to qualitative tests for alkenes. Although great care was taken in collecting the solvent, both the Baeyer and bromine/CCl₄ tests were negative. This suggests that either the CHD was quantitatively consumed in some reaction, possibly with BBD, or that it was too volatile to be collected by the method used. Since CHD was present in excess, the latter is more likely. The possibility that CHD is photochemically polymerized is ruled out because this reaction was not observed in the rose bengal system. Also, any polymer would be easily identifiable in the product mixture because it should be not very soluble in methanol.

The gross features of the photochemical reaction of BBD were explored by taking the absorbance spectra of solutions of various combinations of reactants before and after irradiation. A significant decrease in absorbance at λ_max of BBD would indicate a reaction consuming BBD. This method was only semi-quantitative because the molar absorptivities of the products were not known and their concentrations could not be calculated. However, general statements may be made regarding whether or not certain systems react.

From Table II it is apparent that there may be a slight photodecomposition of BBD which is unaffected by oxygen. The possibility that BBD is generating singlet oxygen which immediately reacts with BBD is thus ruled out. Reaction does occur between BBD and CHD and between BBD and furan. Neither of these are measurably affected by oxygen.
Table II. Decrease in absorbance at selected wavelengths for the photoreaction of BBD with various acceptors.

<table>
<thead>
<tr>
<th>Components</th>
<th>t</th>
<th>Decrease in Absorbance at (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>35</td>
<td>0.04 0.01</td>
</tr>
<tr>
<td>O₂</td>
<td>35</td>
<td>0.03 0.01</td>
</tr>
<tr>
<td>CHD</td>
<td>15</td>
<td>0.02 0.00</td>
</tr>
<tr>
<td>CHD</td>
<td>30</td>
<td>0.52 0.15</td>
</tr>
<tr>
<td>CHD + N₂</td>
<td>15</td>
<td>0.50 0.13</td>
</tr>
<tr>
<td>CHD</td>
<td>15</td>
<td>0.10 0.02</td>
</tr>
<tr>
<td>Furan</td>
<td>15</td>
<td>0.40 0.07</td>
</tr>
<tr>
<td>Furan + O₂</td>
<td>15</td>
<td>0.40 0.08</td>
</tr>
<tr>
<td>Cyclohexene</td>
<td>15</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>15</td>
<td>0.03 0.02 0.25 0.12</td>
</tr>
<tr>
<td>Rose Bengal + O₂</td>
<td>15</td>
<td>0.00 0.00 0.01 0.00</td>
</tr>
</tbody>
</table>

a Gases indicated as components were passed continually through the reaction mixture and are present in saturation quantities. All other runs were run exposed to atmosphere.

b Irradiation time in minutes.

c Wavelengths of maximum absorbance: 463, 330 for BBD; 560, 520 for Rose Bengal.

d Concentration of CHD equal to concentration of BBD.
Excluding oxygen by passing nitrogen through the reaction mixture does not affect the reaction of BBD with CHD. Since oxygen is necessary to make DBO, this means that DBO is probably not the species reacting with BBD. When a solution equimolar in BBD and CHD is irradiated over the same time period as the reaction with excess CHD, the decrease in absorbance at \( \lambda_{\text{max}} \) of BBD is much less. The rate of the photochemical reaction of BBD plus CHD thus appears to depend on the concentration of CHD. It may be concluded that the reaction observed is very likely a photochemical process involving BBD and CHD.

The similar decrease in absorbance in the BBD-furan system suggests that these compounds also react photochemically. The fact that no measurable reaction occurs with cyclohexene is evidence that conjugation in the acceptor is needed for reaction to occur.

In an attempt to determine whether BBD reacts with singlet oxygen, the rose bengal system was used with BBD as acceptor. Interestingly, there appears to be a photochemical reaction between BBD and rose bengal which is inhibited by oxygen. This suggests a free radical process occurring here, since such reactions are known to be inhibited by oxygen. Since singlet oxygen is probably formed in this system, the lack of any observable decrease in BBD absorption is further evidence against the possibility of a self-sensitized photooxidation of BBD.
Thermal reactions of BBD with both CHD and cyclohexene were also carried out and the results are collected in Table III. The purpose of this experiment was to provide evidence for or against a cycloaddition process. If CHD undergoes a photochemically allowed $4_s + 4_s$ cycloaddition with BBD, then cyclohexene should react with BBD in a thermally allowed $4_s + 2_s$ cycloaddition. (67) The measured decrease in absorbance of 0.02 at 463 nm corresponds to a decrease in BBD concentration of less than 1%. ($\epsilon_{\text{BBD}} = 2.0 \times 10^4$ l/mol·cm) Thus, there is no significant thermal reaction of BBD with either cyclohexene or CHD under the conditions used. The same conclusion must be drawn for the thermal reaction of BBD plus rose bengal.

To determine the product distribution of the reaction of BBD with CHD, the reaction was run again using higher concentrations of both reactants. The products were separated by tlc using a number of solvents. The $R_f$ values of the various products are collected in Table IV. This reaction yields a complex mixture of products. Since the irradiation time had to be increased to one hour in this experiment, the possibility of secondary reactions was increased. Using trichloromethane gave the best separation of the two major components, so this solvent was used to separate a larger quantity of the product mixture. Visible-ulv spectra were taken of each major component. Figure 5 is the absorbance spectrum of the $R_f = 0.00$ component and
Table III. Thermal reactions of BBD. Decrease in absorbance at selected wavelengths for various components.

<table>
<thead>
<tr>
<th>Components</th>
<th>( t )</th>
<th>463</th>
<th>330</th>
<th>560</th>
<th>520</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>44</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclohexene(xs)</td>
<td>44</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD(xs)</td>
<td>35</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>31</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\( a \) For concentrations see Methods section.

\( b \) Time between absorbance measurements in days.

\( c \) Wavelengths of maximum absorbance: 463, 330 for BBD; 560, 520 for Rose Bengal.
Table IV. Thin layer chromatograph data for the photoreaction products of BBD plus CHD.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( R_f )</th>
<th>Component or Appearance of Spot\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl\textsubscript{3}</td>
<td>0.00</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>II</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td>0.00</td>
<td>I &amp; II</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>Faint</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>Quenches Fluorescence</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.00</td>
<td>I &amp; II</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>Quenches Fluorescence</td>
</tr>
<tr>
<td></td>
<td>0.77</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
<td>Faint</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>0.00</td>
<td>I &amp; II</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>Faint</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>Faint</td>
</tr>
<tr>
<td></td>
<td>0.62</td>
<td>Faint</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.00</td>
<td>I &amp; II</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>Faint</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>Quenches Fluorescence</td>
</tr>
<tr>
<td>Ether</td>
<td>0.00</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>0.76</td>
<td>BBD</td>
</tr>
<tr>
<td></td>
<td>0.88</td>
<td>II</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Silica-gel adsorbant.

\textsuperscript{b}Components designated I and II were consistently the two most intensely colored and are numbered in increasing order of \( R_f \). Both I and II appeared as yellowish-brown spots. BBD and CHD were used as standards.
Figure 6 is the spectrum of the $R_f = 0.65$ component. In both spectra, intense absorption occurs at shorter wavelengths than the absorption of BBD. If the products are derived from BBD, this indicates a loss of conjugation in the BBD molecule during reaction.

Additional separations were attempted on silica gel using pentane and glacial acetic acid as solvents. Pentane eluted nothing and acetic acid eluted everything. A 1:1 mixture of CHCl$_3$-CCl$_4$ yielded no better separation than each solvent alone.

The infrared spectrum of the product component with $R_f = 0.65$ is presented in Figure 8.

The acceptor method of demonstrating singlet oxygen generation cannot be used because of the photochemical reactions of BBD that occur. If BBD acts as a sensitizer to produce singlet oxygen, it was thought that oxygen consumption by the system could be monitored. This was done using an oxygen sensitive membrane electrode. To prevent any BBD plus acceptor reactions in this experiment, mono-olefins known to react with singlet oxygen were used as acceptors. Several reaction mixtures were monitored for oxygen consumption and their rates compared. The results are presented in Table V.

It should be pointed out that inferring a reaction mechanism from a reaction rate is not possible. In this experiment, evidence for the amount of singlet oxygen, if
Figure 6. Visible-ultraviolet absorption spectrum of the $R_f = 0.00$ component from the reaction of BBD plus CHD. Eluted on silica gel with trichloromethane.
Figure 7. Visible-ultraviolet absorption spectrum of the R_f = 0.65 component from the reaction of BBD plus CHD. Eluted on silica gel with trichloromethane.
Figure 8. Infrared spectrum of the BBD plus CHD photoreaction product having $R_f = 0.65$. Trichloromethane solvent and silica gel adsorbant were used.
any, produced by BBD was sought. This may be a measure of the efficiency of BBD as a singlet oxygen sensitizer.

It is apparent from Table V\textsuperscript{1} that the BBD system consumes oxygen at a rate significantly higher than the methanol blank. Similarly, the rate of consumption by the rose bengal system is about 25 times that of the BBD system. This suggests that if BBD does generate singlet oxygen it does so less efficiently than rose bengal by a factor of about 25. It is also possible that the oxygen consumption observed in the BBD system is due to a process not involving singlet oxygen. A typical trace from the oxygen consumption studies is presented in Figure 9. The linearity of these plots suggests that the reactions are first order with respect to oxygen. Since acceptor concentrations were always large compared to oxygen, the kinetics actually would be pseudo first order. This is in agreement with the proposed reaction sequence for sensitized photooxidations involving singlet oxygen (Scheme 1.).

Because of the difference in oxygen consumption rate between the BBD-amylene system and the methanol blank, it was deemed necessary to devise another method that might

\textsuperscript{1}Note added in proof. The reduction method used for oxygen consumption data may not be valid. To obtain the actual measured rates add \(5.62 \times 10^{-2}\) \% \(\text{O}_2\) sat/min for amylene acceptor and \(2.75 \times 10^{-2}\) \% \(\text{O}_2\) sat/min for cyclohexene acceptor. This does not change the conclusions derived from the data.
Table V. Rate of oxygen consumption for various combinations of reactants.

<table>
<thead>
<tr>
<th>Components</th>
<th>Rate(^a) (%sat/min)</th>
<th>Acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH + O(_2)</td>
<td>6.53 \times 10^{-2}</td>
<td>Amylene</td>
</tr>
<tr>
<td>BBD + O(_2)</td>
<td>7.47 \times 10^{-1}</td>
<td>Amylene</td>
</tr>
<tr>
<td></td>
<td>1.20 \times 10^{-2}</td>
<td>Cyclohexene</td>
</tr>
<tr>
<td>Rose Bengal + O(_2)</td>
<td>17.8</td>
<td>Amylene</td>
</tr>
<tr>
<td></td>
<td>3.08 \times 10^{-1}</td>
<td>Cyclohexene</td>
</tr>
<tr>
<td></td>
<td>32.2</td>
<td>CHD</td>
</tr>
</tbody>
</table>

\(^a\)Average of two measurements except for BBD + O\(_2\) (Amylene acceptor) which is an average of four measurements.
Figure 9. Typical trace of oxygen consumption rate for rose bengal sensitizer.
resolve the question of singlet oxygen generation by BBD. Since, to the knowledge of the author, direct observation of singlet oxygen emission from methanol-sensitizer solutions using a fluorescence spectrometer had not been accomplished, it was decided that this might be a productive course to follow.

Table VI contains the results of this experiment. The emission wavelengths chosen for measurement were those for the gas phase emission of singlet oxygen. This was done because of the absence of any well-defined emission peaks in the spectra other than those due to fluorescence of the sensitizer. Oxygen is known to quench the fluorescence of many compounds, but at the sensitizer concentrations used here this effect should be negligible. (27) The spectrum of each sensitizer solution when saturated with oxygen is essentially the same as when saturated with nitrogen. No singlet oxygen emission is evident.

Increasing the sensitizer concentration by a factor of two had the effect of enhancing emission at 580 nm in the oxygen plus rose bengal system, as shown in Table VII. Emission at the other wavelengths studied was not increased. When the sensitizer concentration was increased to ten times the amount used initially, no increased emission was observed at any wavelength studied. This suggests that there is a range of sensitizer concentrations near $2.5 \times 10^{-4}$ M that is particularly effective in generating singlet oxygen
Table VI. Ratio of fluorescence intensity at selected wavelengths to intensity at fluorescence maximum.\(^a\)

<table>
<thead>
<tr>
<th>System</th>
<th>(\lambda_{ex})</th>
<th>480</th>
<th>520</th>
<th>580</th>
<th>633</th>
<th>703</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB + O(_2)</td>
<td>RB</td>
<td>0.05±0.02</td>
<td>0.05±0.02</td>
<td>0.22±0.03</td>
<td>0.46±0.01</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>RB + N(_2)</td>
<td>RB</td>
<td>0.07±0.04</td>
<td>0.07±0.03</td>
<td>0.18±0.01</td>
<td>0.49±0.05</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>BBD + O(_2)(^d)</td>
<td>RB</td>
<td>0.73±0.16</td>
<td>0.78±0.13</td>
<td>1.69±0.49</td>
<td>0.73±0.16</td>
<td>0.73±0.18</td>
</tr>
<tr>
<td>BBD + N(_2)(^d)</td>
<td>RB</td>
<td>0.68±0.14</td>
<td>0.74±0.09</td>
<td>2.16±0.46</td>
<td>0.69±0.07</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>RB + O(_2)</td>
<td>BBD</td>
<td>0.58±0.01</td>
<td>0.57±0.01</td>
<td>0.80±0.02</td>
<td>0.74±0.02</td>
<td>0.56±0.01</td>
</tr>
<tr>
<td>RB + N(_2)</td>
<td>BBD</td>
<td>0.62±0.03</td>
<td>0.59±0.02</td>
<td>0.81±0.04</td>
<td>0.78±0.03</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>BBD + O(_2)</td>
<td>BBD</td>
<td>0.04±0.00</td>
<td>0.43±0.03</td>
<td>0.61±0.01</td>
<td>0.11±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>BBD + N(_2)</td>
<td>BBD</td>
<td>0.04±0.00</td>
<td>0.43±0.02</td>
<td>0.60±0.01</td>
<td>0.11±0.00</td>
<td>0.04±0.00</td>
</tr>
</tbody>
</table>

\(^a\)Fluorescence maxima: BBD = 550 nm, RB = 610 nm.

\(^b\)Excitation wavelengths: RB = 560 nm, BBD = 463 nm.

\(^c\)Presented as average intensity ratio ± standard deviation.

\(^d\)Average of four measurements. All others are average of three measurements. Wavelengths chosen were those for which singlet oxygen is known to have emission. The only emission discernable in these spectra were those due to the sensitizer.
Table VII. Effect of increased rose bengal concentration on emission. Ratio of fluorescence intensity at selected wavelengths to intensity at fluorescence maximum.\(^a\)

<table>
<thead>
<tr>
<th>System</th>
<th>(c)</th>
<th>520</th>
<th>580</th>
<th>633</th>
<th>703</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{RB} + \text{O}_2)</td>
<td>2x</td>
<td>0.09±0.03</td>
<td>0.10±0.01</td>
<td>0.59±0.02</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>(\text{RB} + \text{N}_2)</td>
<td>2x</td>
<td>0.04±0.03</td>
<td>0.04±0.02</td>
<td>0.63±0.02</td>
<td>0.05±0.03</td>
</tr>
<tr>
<td>(\text{RB} + \text{O}_2)</td>
<td>10x</td>
<td>0.09±0.03</td>
<td>0.28±0.03</td>
<td>0.63±0.04</td>
<td>0.09±0.04</td>
</tr>
<tr>
<td>(\text{RB} + \text{N}_2)</td>
<td>10x</td>
<td>0.08±0.01</td>
<td>0.29±0.00</td>
<td>0.70±0.06</td>
<td>0.11±0.00</td>
</tr>
</tbody>
</table>

\(^a\)Fluorescence maximum of \(\text{RB} = 610\) nm. Excitation wavelength of rose bengal was used for each (560 nm).

\(^b\)Concentration of rose bengal (\(x = 1.25 \times 10^{-4} \text{ M}\)).

\(^c\)Presented as average intensity ratio \(\pm\) standard deviation. Each data point is an average of two measurements.
under the conditions used. The effect of increased concentration of BBD on the emission from that system was not determined because the results of the oxygen consumption studies indicated that if BBD produces singlet oxygen at all, it does so about 25 times less efficiently than rose bengal. Such a small amount of singlet oxygen would not be observable using this method.

The initial stages of this study were involved with the synthesis of BBD. This line of work was abandoned when the compound became commercially available, since the question to be answered was that of singlet oxygen generation. The results of this work are presented here as a guide for future workers.

Difficulty was encountered in the synthesis of BBD using the method of Kenner and Aboderin (38). The experiment proceeded as expected until purification of the product was necessary. Recrystallization from absolute ethanol, as suggested by Kenner and Aboderin, was not effective. Purification by column chromatography was successful but the recovery was unacceptably low. During the photochemical experiments it was observed that commercially obtained BBD had a solubility of about 0.8 g/l in methanol at room temperature. Once BBD was dissolved in boiling solvent for recrystallization, it stayed in solution even upon cooling. These problems with the method have been corroborated by others. (9, 61) The synthesis was not pursued further for
the reasons described above. A preliminary experiment was conducted to determine whether the postulated substitution reaction between the chlorobenzoxadiazole system and benzylamine would occur if there were no nitro group present. Characteristically, 4-amino adducts of the benzoxadiazole are highly colored. The lack of any color change upon addition of benzylamine, after standing for three months and after refluxing the mixture for 20 hours suggests that either no reaction occurred or that the product formed was colorless in solution.
DISCUSSION

The isolation of DBO as a product of the rose bengal sensitized photooxidation is strong evidence that singlet oxygen is the reactive intermediate. Although Spikes (58) has noted that there are many dye-sensitized photooxidation reactions that do not involve singlet oxygen, the studies of Foote and coworkers (18-23, 29) and others (5, 8, 14, 16, 64) have shown that rose bengal does form singlet oxygen through sensitization. Rose bengal is currently one of the most widely used sensitizers for making singlet oxygen.

The pmr and ir spectral data obtained support the conclusion that DBO is formed from the rose bengal sensitized photooxidation. The pmr spectrum (Fig. 4) shows a symmetrical multiplet centered at about 1.15 δ. This is assigned to the methylene protons labeled a in Figure 10. The haystack at 4.50 δ is assigned to the bridgehead protons (b) and the apparent triplet at 6.60 δ is assigned to the vinylic protons (c). Foote and coworkers have suggested that the 6.60 δ absorption is actually an overlapping pair of doublets. (21) Close inspection indicates that this may be true, however, obtaining the 100 MHz spectrum should resolve this question.
Figure 10. Proton assignment for DBO.

An analog of DBO is 2-norbornene, the 60 MHz pmr spectrum of which is given in Figure 11. The similarity of the two spectra is obvious. The main difference, other than the absorption ratios, is the position of the bridgehead and vinyl proton absorption. In the DBO spectrum these occur farther downfield. This is presumably due to the higher electronegativity of the oxygen bridge relative to the methylene bridge resulting in greater deshielding of the vinyl and bridgehead protons in DBO. The similarity of the DBO and 2-norbornene spectra is strong evidence that the structure assigned to DBO is correct.

Table VIII is a comparison of the infrared absorptions for DBO obtained in this study with those obtained by Foote et al. (21) The close correlation indicates that the two products are the same.

Further insight into the structure of the product presumed to be DBO may be obtained by comparing its infrared spectrum (Fig. 4) with that of 1-methyl-4-isopropyl-2,3-dioxabicyclo[2.2.2]oct-5-ene (ascaridole), which appears as
Figure 11. Proton magnetic resonance spectrum of 2-norbornene. Reference 55.
Table VIII. Comparison of the literature values of the infrared absorption wavenumbers of DBO with those obtained herein.

<table>
<thead>
<tr>
<th>Literature Values (cm$^{-1}$)$^a$</th>
<th>Experimental Values (cm$^{-1}$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>685</td>
<td>670</td>
</tr>
<tr>
<td>725</td>
<td>710</td>
</tr>
<tr>
<td>926</td>
<td>920</td>
</tr>
<tr>
<td>962</td>
<td>965</td>
</tr>
<tr>
<td>1053</td>
<td>1060</td>
</tr>
<tr>
<td>1163</td>
<td>1165</td>
</tr>
<tr>
<td>1212</td>
<td>1215</td>
</tr>
<tr>
<td>1374</td>
<td>1370</td>
</tr>
</tbody>
</table>

$^a$Reference 21.

$^b$Taken from Fig. 13. Solvent absorptions not included.
Figure 12. The absorption near 3000 cm\(^{-1}\) in Figure 4 suggests the presence of methyl and/or methylene groups, since this absorption is generally due to C-H stretching vibrations. Assuming the suggested structure of DBO to be correct, absorption due to the carbon-carbon double bond should be observed at about 1650 cm\(^{-1}\). However, since this absorption is not observed in the ascaridole spectrum, its absence supports the assigned structure. The strong absorption at about 700 cm\(^{-1}\) in both spectra can be attributed to the out-of-plane olefinic C-H bending vibration. (This absorption is not due to aromatic C-H out-of-plane bending because of the lack of aromatic proton absorption in the pmr spectrum.)

Thus, both the pmr and infrared spectra obtained for the product of the rose bengal sensitized photooxidation of 1,3-cyclohexadiene indicate that DBO is the product.

When the photochemical generation of singlet oxygen was attempted using BBD as sensitizer, an apparent reaction occurred between BBD and CHD. This reaction was quite unexpected and rendered the acceptor method of singlet oxygen detection useless. The reaction occurring is most likely a \((4_s + 4_s)\) photochemically allowed cycloaddition. (67) There are two sites on the BBD molecule that are susceptible to this type of reaction. Addition of CHD to the benzoaxadiazole system on carbons 4 and 7 would yield compound VI. If CHD adds to the oxadiazole nitrogens, compound VII would be formed. That there are two likely products from the
Figure 12. Infrared spectrum of ascaridole. Reference 55.
cycloaddition and that only two major products were actually found is support for this type of reaction. The occurrence of a single polymerization reaction is ruled out since two products were formed. However one or both of the cycloaddition products may polymerize or further react in other ways to yield the complex product distribution observed (Table IV). Since one product was easily eluted by a number of solvents in the tlc studies, it is likely that this product is not formed by polymerization. Also, a polymerization would probably yield a number of chains having different lengths. These should be differentiable by tlc.

The observation of only two major products supports a simple cycloaddition occurring in at least one of the reactions. The product that was not eluted in the tlc experiments may well be a polymer or a combination of polymers.

The ultraviolet spectra of the major products from the BBD-CHD photoreaction provide further support for a cycloaddition. From Figures 6 and 7 it is evident that a larger amount of conjugation has been lost in forming the
product in Figure 6. Because loss of conjugation results in a hypsochromic shift in absorption, Figure 6 is the spectrum of a compound with less conjugation. This might correspond with compound VI. Since Figure 6 is the absorbance of the product with $R_f = 0.00$, it is possible that this product is a polymer and does not have the molecular structure of VI. Polymerization would reduce the effective chromophores in the product causing a further blue shift in absorption.

If one neglects the effect of the oxadiazole ring on the absorption of VII, the molecule becomes an analog of p-nitroaniline. Having the $-\text{NO}_2$ and $-\text{NHR}$ groups para on a benzene ring shifts the primary benzene absorption by 171 nm to longer wavelength. (31) The resulting absorption occurs at 375 nm for p-nitroaniline in absolute ethanol. The absorption maximum in Figure 7 occurs at about 340 nm. (The effect of using methanol instead of ethanol shifts the wavelength by about 3 nm. (31) This effect can be neglected because it is beyond the resolution of the instrument at the sensitivity used.) The effect of amine groups at the 2,3-positions of p-nitroaniline would probably further shift the primary absorption to the red. For maximum delocalization of the nitrogen lone-pair electrons and concomittant maximum red shift in absorption to occur, the lone pair electrons must be in the same plane as the ring. (31) As a result of the cycloaddition to form VII, the
nitrogen atoms of the oxadiazole ring are sp$^3$ hybridized and the lone-pair orbitals are no longer in the same plane as the benzene ring. The result would be that a compound having the structure of VII would have a wavelength of maximum absorption between the $\lambda_{\text{max}}$ of BBD and that of a 2,3-amine disubstituted p-nitroaniline. Moreover, based on the relative amounts of conjugation in the three compounds, VII would have its absorption maximum nearer to the absorption maximum of the disubstituted aniline. Thus, it is at least possible that compound VII could give rise to the absorption in Figure 7.

Any conclusion about the actual structures of the products of the photoreaction of BBD with CHD based on ultraviolet absorption is speculative. If the cycloaddition products are indeed formed, structure VII probably corresponds with the absorbance spectrum in Figure 7 and VI probably corresponds with Figure 6.

The fact that no thermal reaction was observed between cyclohexene and BBD (Table III) does not necessarily violate the Woodward-Hoffman prediction. The activation energy may not have been available at ambient temperature. Heating the reaction mixture might well have induced the thermal reaction.

The data from the oxygen consumption studies suggest that when BBD is used as sensitizer, the system consumes oxygen at least 25 times slower than rose bengal. This
suggests that BBD may produce singlet oxygen, but approximately 25 times less efficiently than rose bengal. However, because of the low rate of oxygen uptake with BBD as sensitizer, it is possible that singlet oxygen is not involved at all in this system. The increase in rate of uptake on going from BBD to rose bengal as sensitizer is a factor of 26 for cyclohexene and a factor of 24 for amylene. This may indicate a change in the mechanism of oxygen uptake with a change in sensitizer, since the change of rate for both acceptors is similar. This is in agreement with the work of Kopecky and Reich (44) and of Wilson (66) who showed that the rate of reaction of various acceptors with singlet oxygen, i.e. the rate constant for Equation 3, does not depend on sensitizer. Since the acceptor concentrations were equal in the present work, the rates of oxygen consumption should have been the same for both the BBD and rose bengal sensitized reactions if singlet oxygen was generated in both cases. Because of the difference in rate in the BBD sensitized reaction, it is apparent that BBD did not generate singlet oxygen.

A basis for the lack of singlet oxygen generation by BBD is provided by the work of Snelling (56) and Stevens and Algar (59). They concluded that singlet oxygen results from the quenching of sensitizer triplet states but not from quenching of the singlet. Thus, the efficiency of singlet oxygen production for a given sensitizer should depend on
the intensity of the phosphorescence transition. Since BBD is highly fluorescent, the phosphorescence transition should be weak in this compound. The predominance of the fluorescence transition may be the primary reason that BBD does not generate singlet oxygen.

Returning to the oxygen consumption studies, the difference in rate between the blank and the BBD-amylene system suggests that BBD is sensitizing an oxygenation not involving singlet oxygen. This is supported by the data in Table II for the BBD-rose bengal reaction. Since the extent of reaction of rose bengal is reduced by adding oxygen, a known free radical inhibitor, BBD is apparently sensitizing a free radical oxidation. As mentioned earlier, many dye-sensitized photooxidation reactions do not involve singlet oxygen. The results of the present work suggest that BBD does not generate singlet oxygen but may sensitize photo-oxidation by some other mechanism.

The relative reactivities of various acceptors toward singlet oxygen have been reported by Higgens et al. (29) It was found that amylene was about 32 times more reactive than cyclohexene. From the oxygen uptake studies conducted herein, the relative reactivity of amylene to cyclohexene is about 58. The difference in these two values may be attributable to differences in temperature and acceptor concentration between the two studies. Also, the effect of different acceptors on the sensitivity of the
oxygen electrode is unknown. Since the two relative reactivities differ by less than a factor of 2, this latter effect may be overshadowed by temperature and concentration differences.

The results of the direct singlet oxygen emission studies are in agreement with those reported by others in that at sensitizer concentrations usually employed (1.2 x 10^{-4} M no emission is detectable. (29, 37) Increasing sensitizer concentration should result in an increase in the amount of singlet state oxygen in a given system. When the concentration of rose bengal was increased by a factor of 2, the emission from the solution at 580 nm, a known emission energy of singlet oxygen, was enhanced. This transition of gaseous oxygen has been assigned to a collision of one singlet oxygen molecule in the \( \nu = 1 \) vibrational state and another singlet oxygen molecule in the \( \nu = 0 \) vibrational state. (25) If this were the case here, peaks at 633 nm and 520 nm, corresponding to the bimolecular collisions of the \( \nu = 0 \) and \( \nu = 1 \) states, respectively, should have been observed also. It appears that there is no satisfactory explanation for the increased emission at 580 nm for a rose bengal concentration of 2.4 x 10^{-4} M.

Further increasing the rose bengal concentration to ten times the original concentration yielded spectra showing no enhanced emission. This may be due to concentration quenching of the sensitizer triplet state, or sensitizer
quenching of singlet oxygen. Alternatively, it may be that in any liquid phase system singlet oxygen is quenched by solvent too rapidly for the bimolecular emission to be observed. (37)
SUMMARY

The evidence obtained herein suggests that BBD does not generate singlet oxygen at a rate comparable to known sensitizers. Since other studies have shown that either a given sensitizer generates singlet oxygen or it does not, BBD may not generate singlet oxygen at all. The rate of oxygen depletion using the method described should be measured for various sensitizers. The results of such an experiment should allow a firmer statement to be made as to whether or not BBD generates singlet oxygen.

The method of identifying singlet oxygen intermediacy by oxygen depletion might be developed into an analytical tool provided all sensitizers that generate singlet oxygen do so with the same efficiency. This is suggested as an area of future study.

The structures of the products of the hypothesized cycloaddition of CHD and BBD, if known, would provide evidence for the true mechanism of this reaction. A structural determination was not attempted here because of the low recoveries and the possibility of interfering side reactions inherent in the method. An analysis of the products is necessary before this data may be presented in the literature.
LITERATURE CITED


61. N. Valentino, Pfaltz and Bauer, Inc., Stamford, CN, personal communication.
Figure 14. Infrared spectrum of BBD.
Figure 14. (continued)
Figure 15. Proton magnetic resonance spectrum of NBD-Cl. Sweep offset 30 Hz.
Figure 16. Infrared spectrum of NBD-C1.
Figure 16. (continued)
Figure 17. Infrared spectrum of the photochemical decomposition product of BBD.
Figure 18. Proton magnetic resonance spectrum of CHD.
Figure 20. Infrared spectrum of mineral oil used as solvent in other infrared spectra.