Conference On Singlet Molecular Oxygen

84

JANUARY 4-7, 19**84**
CLEARWATER, BEACH, FLORIDA

A

PROGRAM
and
ABSTRACTS

N00014-84-C-0032

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FINAL REPORT

on

CONFERENCE ON SINGLET MOLECULAR OXYGEN (COSMO 84)
(Report #18-2705-124F)

by

BRIAN STEVENS

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to

OFFICE OF NAVAL RESEARCH CODE:612B:PMW

800 NORTH QUINCY STREET

ARLINGTON, VA 22217

Security Classification - Unclassified

Number of Pages - 4

Distribution: -

> Associate Director for Life Science Programs ONR (1)
> ACO, ONR, Georgia Institute of Technology (1)
> Defense Technical Information Center (1)

Award Amount - $5000

Duration - 1-1-84 to 6-30-84

Contract # N00014-84-C-0032

Signature   *Brian Stevens*
_____
Brian Stevens

Date: September 24, 1984

CONFERENCE ON SINGLET MOLECULAR OXYGEN
COSMO 84
January 3-7th, 1984
Clearwater Beach, Florida

Some 80 scientists from 12 countries attended the Conference at which 47 papers were presented in the following sessions:

Sources and Yields (B. Stevens)
Relaxation and Quenching (M. A. J. Rodgers)
Reaction Mechanisms (C. S. Foote)
Biological/Biomedical Implications (R. W. Murray)
Gas Phase and Atmospheric Manifestations (R. P. Wayne)
Synthetic and Other Applications (A. P. Schaap)

The sessions organizers indicated, together with A. M. Trozzolo, formed the planning committee.

Sponsors included:

The Office of Naval Research
The National Science Foundation
The American Society for Photobiology
The Inter-American Photochemical Society
Jacobs-Fendig, Inc.
Mr. Robert Dressler
The University of South Florida

The proceedings will be published in a dedicated issue (3 volumes) of the Journal of Photochemistry later this year.

The Conference objective was to promote interdisciplinary awareness and communication by assembling research workers in such diverse fields as photochemistry, photophysics, synthetic chemistry, photobiology, photomedicine, laser and atmospheric physics, to present their recent findings with singlet molecular oxygen as the common theme. This was prompted by several recent developments, notably the direct observation of $O_2{}^1\Delta_g$ in emission at 1.27μ which now provides solvent-dependent life-times, reaction or quenching rate constants, and sensitized yields directly, the trapping of zwitterionic intermediates and the role of catalysts in electron trans-fer peroxidation, the feasibility of a singlet oxygen-iodine chemical laser, direct observation of $O_2{}^1\Delta_g$ in enzymic processes, the use of endoperoxides as actinometers, the phototherapy of malignant tumors, and prospects for $O_2{}^1\Delta_g$ as a solar energy storage intermediate. If attendance on the final morning (which equalled that at the opening session) is a criterion of success, then any failure to meet the stated objectives was not the fault of the participants. Since COSMO 84 does not represent the continuation of a Conference series, e.g. on Oxygen radicals (Pinawa 1977, Texas 1980, Munich 1983), on Polymers (Stockholm 1976) or the role of singlet oxygen in environmental sciences (New York 1970) more objective criteria of its success will be provided by the reception of the Conference Proceedings and the planning of COSMO 8?

ii

SPONSORSHIP

OF THE

CONFERENCE ON SINGLET MOLECULAR OXYGEN

COSMO 84

by

THE OFFICE OF NAVAL RESEARCH
(Division of Biological Sciences)

THE NATIONAL SCIENCE FOUNDATION
(Biophysics and Atmospheric Science Programs)

THE INTERAMERICAN PHOTOCHEMICAL SOCIETY

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N00014-84 C-0032

These Abstracts are not to be considered as publications
of the work described and should not be cited.

CONFERENCE AND PROGRAM NOTES

1. ALL SESSIONS will be HELD in the BIG SURF BALL ROOM.

2. REGISTRATION will take place at the RECEPTION on TUESDAY
   EVENING and in the HOTEL LOBBY throughout the CONFERENCE.

3. SPEAKERS are asked to give their SLIDES to one of the
   PROJECTIONISTS before the appropriate session and their
   MANUSCRIPTS to the REGISTRATION DESK before they leave.

4. In order to avoid parallel and poster sessions it has
   been necessary to schedule MORNING, AFTERNOON and
   EVENING SESSIONS on WEDNESDAY.

5. Since the attendance of some PROGRAMMED SPEAKERS is
   UNCERTAIN at the time of 'going to press', there is NO
   TIMETABLE. COFFEE, LUNCH and (on WEDNESDAY) DINNER BREAKS
   will be announced by the SESSION CHAIRMAN.

6. LOCAL INFORMATION may be obtained at the HILTON REGISTRATION
   DESK or the LOBBY REGISTRATION DESK. A LINDO'S TOUR DESK is
   located in the HILTON LOBBY and a LINDO'S AGENT has been
   invited to the RECEPTION on TUESDAY.

7. Please note that in order to BOARD the COACHES on THURSDAY
   EVENING at 6:30 PM you will need a TICKET for the LUAU
   DINNER.

8. You are reminded that the WEATHER can be COOL and WET on
   the BEACH in JANUARY.

PROGRAM

7 - 10 PM   <u>RECEPTION AND REGISTRATION</u>

                          Poolside (weather permitting) or Crown Ballroom

WEDNESDAY MORNING/AFTERNOON   JANUARY 4
Big Surf Ballroom

8:25 AM   WELCOMING REMARKS AND ANNOUNCEMENTS

8:30 AM   <u>SESSION on SOURCES and YIELDS</u>

                          B. STEVENS - Chairman

A. SINGH   S5   'Possible Formation of Singlet Oxygen from Vibration-
                          ally-Excited Water'

J.R. KANOFSKY   K1   'Singlet Oxygen Production by Lactoperoxidase:
                          Halide Dependence and Quantitation of Yield'

D.F. EVANS   E2   'Studies on Singlet Oxygen in Aqueous Solution'

G.P. GURINOVICH   G6   'Mechanism of Singlet Oxygen Luminescence Sensi-
                          tization by Polyatomic Molecules'

A.A. GORMAN   G2   'The Efficiency of Triplet Energy Transfer to
                          $O_2(^3\Sigma_g^-)$: Sensitizer and Medium Dependence'

C. TANIELIAN   T1   'Production and Quenching of Singlet Oxygen by
                          the Sensitizer in Dye-Sensitized Photooxygenations'

              <u>SESSON on RELAXATION and QUENCHING</u>
            M.A.J. Rodgers and P.R. Ogilby - Chairmen

M.A.J. RODGERS   R2   'The Relaxation of $O_2\,^1\Delta_g$ in Condensed Systems'

M. MAIER   M1   'Relaxation of the $^1\Delta_g$ State in Pure Liquid
                          Oxygen and in Liquid Mixtures of $^{16}O_2$ and $^{18}O_2$'

J.G. PARKER   P1   'Optical Determination of the Rates of Formation
                          and Decay of $O_2\,^1\Delta_g$ in H_2O, D_2O and Other Solvents'

B. STEVENS   S7   'Preferential Solvation of $O_2\,^1\Delta_g$ in Mixed Solvents'

B.M. DZHAGAROV   D2   'Singlet Oxygen Luminescence'

A.A. KRASNOVSKY   K3   'Photosensitized Luminescence of Singlet Oxygen in
                          Solutions: Mechanism of Quenching by Solvents and
                          Biologically Important Compounds'

F. WILKINSON   W5   'The Mechanism of Qhenching of Singlet Oxygen by
                          Molecular Iodine'

WEDNESDAY AFTERNOON/EVENING

SESSION ON REACTION MECHANISMS
C.S. FOOTE - Chairman

W. ANDO	W1	'Stereochemical Studies on the Mechanism of Dioxetane and Epoxide Formation from Hindered Olefins'
A.P. SCHAAP	S3	'Nucleophilic Oxygen - Atomic Transfer from a Perepoxide Intermediate to Sulfoxides'
G.W. GRIFFIN	G3	'Sensitized Photooxidation of Methyl Substituted Cyclobutenes'
A.P. SCHAAP	S2	'Cosensitized Electron - Transfer Photo-oxygenation'
P.B. GANDHI	G1	'Dye-sensitized Photo-oxygenation of Isoniazid by Singlet Molecular Oxygen'
J.S. CONNOLLY	C1	'Effects of Solvent on the Rate of Bacterio-chlorophyll $\underline{a}$ Photooxidation'
I. FORSSKAHL	F3	'Aspects of Photosensitized Lignin Oxygenation'
A.A. FRIMER	F4	'The effect of Strain on the Rearrangements of Primary Singlet Oxygen Products'
G.D. MENDENHALL	M2	'Structure and Kinetics of the Uncatalyzed Decomposition of Trialkyl Phosphite Ozonides'

SESSION ON BIOLOGICAL/BIOMEDICAL IMPLICATIONS

8:30 AM   R.W. MURRAY - Chairman

D.G. WHITTEN   W4   'Self-Sensitized Photooxidation of Protoporphyrin IX and
                          Related Porphyrins in Erythrocyte Ghosts and Microemulsions:
                          A Novel Photooxidation Pathway'

L.I. GROSSWEINER   G4   'Reactions of Singlet Oxygen with Liposomes'

R.B. CUNDALL   C2   'Oxidation Photosensitized by 2-Chlorothioxanthone:
                          The Role of Singlet Oxygen'

   BREAK   L.I. GROSSWEINER - Chairman

M.K. LOGANI   L2   't-Butylated Hydroxytoluene Sensitized Photooxidation of
                          the Fatty Acids: Inhibition by Diazabicyclooctane and Role
                          of Singlet Oxygen'

N. DURAN   D1   'Singlet Oxygen Generation from the Peroxidase-Catalysed
                          Aerobic Oxidation of an Activated - CH_2 - Substrate'

C.S. FOOTE   F2   'Singlet Oxygen Production from Photodynamic Sensitizers'

   LUNCH   L.L. SMITH - Chairman

H. SINGH   S6   'Singlet Oxygen and Ribosomes: Inactivation and Sites of
                          Damage'

M.J. PEAK   P3   'Reactive Oxygen Species in the Biological Effects of
                          Solar Ultraviolet Radiation'

S. ZIGMAN   Z1   'Active Oxygen Chemistry of Ocular Tissues'

   BREAK   A.M. TROZZOLO - Chairman

W. NULTSCH   N1   'Possible Role of Singlet Oxygen in the Control of the
                          Phototactic Reaction Sign of the Cyanobacterium
                          Anabaena Vaniabilis'

A.U. KHAN   K2   '$O_2(^1\Delta_g)$ Emission from Enzyme Reactions'

R.W. MURRAY   M3   'Singlet Oxygen Oxidation of Substituted Thiobenzamides'

6:30 PM   COACHES LEAVE HILTON FOR TIKI GARDENS

8:30 AM   <u>SESSION ON GAS PHASE AND ATMOSPHERIC MANIFESTATIONS</u>

R.P. WAYNE - Chairman

R.P. WAYNE   W2   'Laboratory Kinetics and Spectroscopy Applied
                           to Airglow Studies'

E.J. LLEWELLYN   L1   'Singlet Molecular Oxygen in Planetary
                               Atmospheres'

E.A. OGRYZLO   O1   'The Yield of $O_2(b^1\Sigma_g^+)$ in Oxygen Atom Re-
                             combination'

P. BORRELL   B2   'High Temperature Kinetic and Spectroscopic
                           Studies of Singlet Oxygen'

J.G. PARKER   P2   'Optical Determination of $O_2(^1\Delta_g)$ Quenching
                           Rates and Relative Emission Intensities in High
                           Pressure Oxygen Gas Using Pulsed Laser Excitation
                           at 10640 Å'

D.J. BOGAN   B1   'Chemiluminescent Reactions of Organic Molecules
                           Forming or Consuming Singlet Oxygen'

E.H. FINK   F1   '$O_2(^1\Delta_g)$ Sensitized Chemiluminescence of
                         $a^1\Delta - X^3\Sigma^-$ and $b^1\Sigma^+ - X^3\Sigma^-$ Transitions of Group
                         V-VII Diatomic Molecules'

W.C. EISENBERG   E1   'Generation and Reactions of Singlet Delta Oxygen at
                               Ambient Atmospheric Conditions'

R.F. HEIDNER, III   H1   'Behaviour of Singlet Oxygen in the Oxygen-
                                 Iodine Transfer Laser'

P.L. HOUSTON   H2   'Kinetics of the Chemical Oxygen-Iodine Laser

P.D. WHITEFIELD   W3   '$O_2(^1\Delta_g)$ as an Energy Transfer Partner for
                               Chemical Laser Pumping ($O_2\ ^1\Delta$ + IF)'

SATURDAY MORNING   JANUARY 7

8:30 AM   SESSION ON SYNTHETIC AND OTHER APPLICATIONS

A.P. SCHAAP - Chairman

H.H. WASSERMAN   W1   'Recent Applications of Singlet Oxygen Reactions
                        in Synthesis'

H.-D. BRAUER   B3   'Photoperoxidation of Aromatic Compounds and
                        Wavelength - Dependent Photochemistry of the
                        Obtained Peroxides'

R. SCHMIDT   S4   'Self-Sensitized Photooxidation of Aromatic
                        Compounds and Photocycloreversion of Endoperoxides.
                        Applications in Chemical Actinometry'

J.E. GUILLET   G5   'Prospects for Solar Synthesis. I. A New Method
                        for Singlet Oxygen Reactions Using Natural Sun-
                        light'

I. SAITO   S1   'On the Use of Water Soluble $^1O_2$ Sources for
                        Mechanistic Study and for Oxygenation of
                        Biological Systems'

J.F. RABEK   R1   'Synergistic and Antagonistic Effects of
                        Quenching Singlet Oxygen in a Polymer Matrix'

C.W. JEFFORD   J1   'Construction of 1,2,4-Trioxane Rings by
                        Photooxygenation of Enol Ethers'

CLOSING REMARKS

Stereochemical Studies on the Mechanism of
Dioxetane and Epoxide Formations from Hindered Olefins

Wataru Ando, Yoshio Kabe and Toshikazu Takata
Department of Chemistry, University of Tsukuba
Sakura-mura, Ibaraki 305, Japan
Katsuhiko Ueno
Research Institute for Polymers and Textile
Yatabe-cho, Ibaraki 305, Japan

Considerable interest is focused on reaction of sterically hindered olefins such as adamantylidene adamantane (1), since oxygenation of 1 gives the remarkably stable dioxetane sometimes with accompanying formation of epoxides. Such observation has

1   2-anti   2-syn
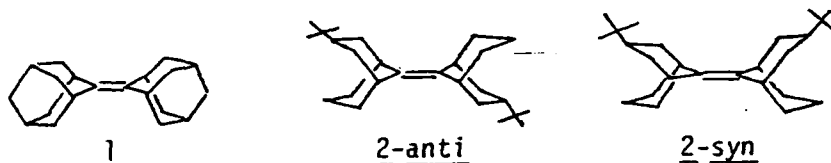
enlivened the controversy that deoxygenaiton of perepoxide occurs or not. More recently, it has been shown that cation radical of 1 uniquely reacts with triplet oxygen to yield dioxetane by chain reaction. In spite of the continuous interests concerning with hindered olefins, no intrinsic stereochemical study has been done yet. Then, we synthesized two stereoisomeric di-tert-butyl (bicyclo[3,3,1]non-9-ylidene)s (2-anti and 2-syn). Assignments of the stereochemistry followed from the X-ray crystal analysis of 2-anti (Fig. 1). The first objective is on the mechanism of dioxetane formation. Either TPP (tetraphenylporphine) or DCA
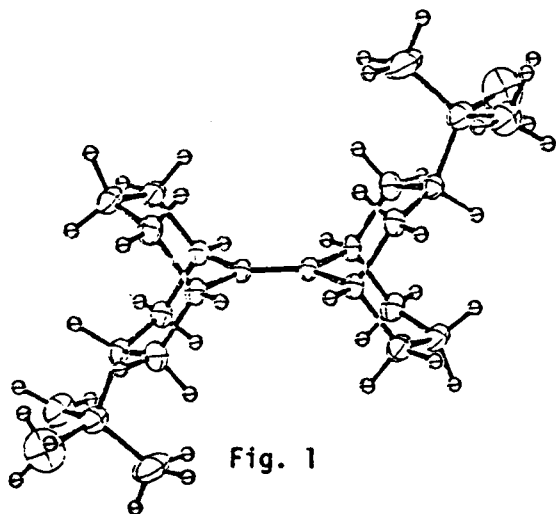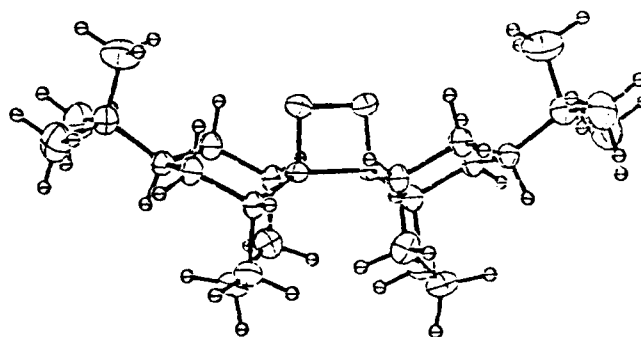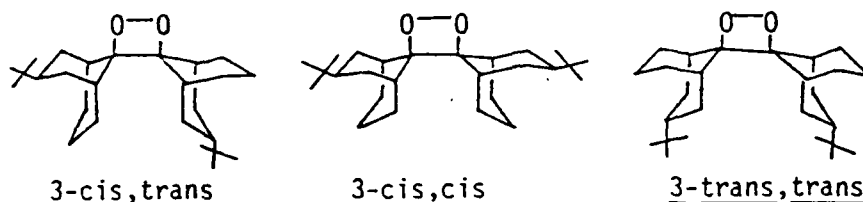

Fig. 1   Fig. 2

(9,10-dicyanoanthracene)-sensitized photooxygenation of 2-anti
gave stereospecifically single dioxetane 3-cis,trans. Similarly,
photooxygenation of 2-syn by either TPP or DCA gave two dioxetanes,
3-cis,cis and 3-trans,trans, respectively. Structures of all
dioxetanes (3) isolated were easily assigned by spectroscopies

3-cis,trans   3-cis,cis   3-trans,trans

and X-ray crystal analysis of 3-cis,cis (Fig. 2). In contrast,
the electrode-catalysed oxygenation of each isomeric olefin
gave mixture of all three dioxetanes. These results suggested that
the concerted cycloaddition of singlet oxygen occurred in dye-
sensitized oxygenation, and singlet oxygen might also be involved
in the case of DCA. In the electrode-catalysed oxygenation,
cation radical is seriously anticipated.

Second set of experiments is concerned with photoepoxidation.
Both Rose bengal and TPP-sensitized photooxygenations of 2 in the
presence of methyl phenyl sulfoxide gave rise to the formation
of epxoides (4) with retention of stereochemistry. These result:

4-cis,trans   4-cis,cis   4-trans,trans
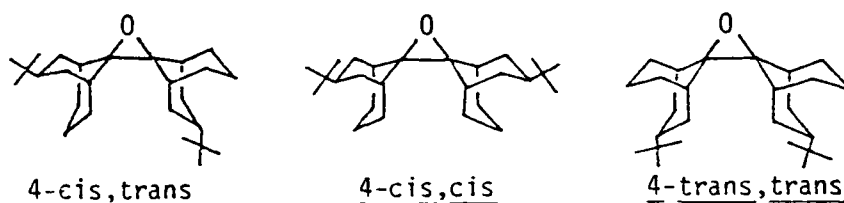
might confirm the deoxygenaiton of perepoxide. Meanwhile, biacetyl-
sensitized photoepoxidation has been kown to be non-stereospecific.
However, the photoepoxidation of 2 was completely stereospecific.
Peracid formed by hydrogen abstraction of acylperoxy radical
may be actual oxidizing species.

Chemiluminescent Reactions of Organic Molecules Forming or Consuming Singlet Oxygen

Denis J. Bogan
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This paper will present and discuss gas phase chemiluminescence studies of two reactions, each proceeding through a long lived vibrationally excited peroxide intermediate. These are; (1) Reaction of O_2 ($^1\Delta_g$) with olefin giving electronically excited carbonyl product via a dioxetane intermediate, and (2) Reaction of two (non-tertiary) alkyl peroxy radicals by Russell's mechanism giving O_2 ($^1\Sigma_g$) via a dialkyl tetraoxide intermediate. Reaction coordinate diagrams for typical examples of (1) and (2) are shown below.
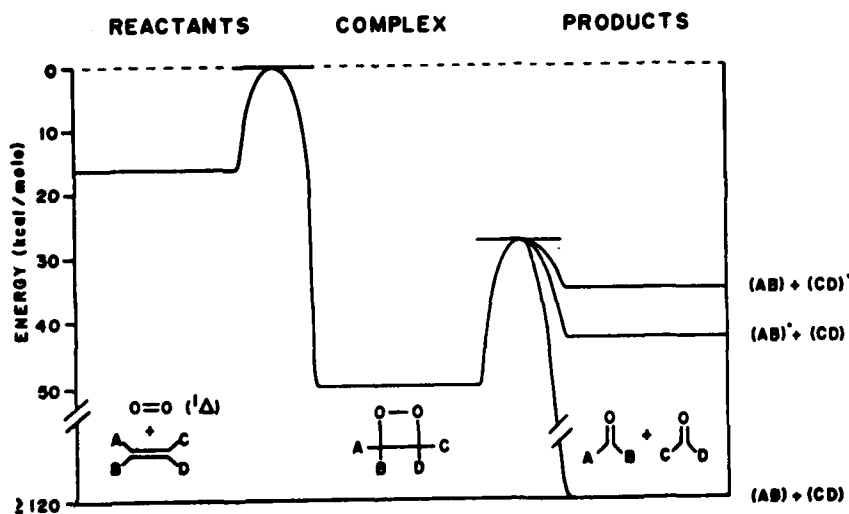
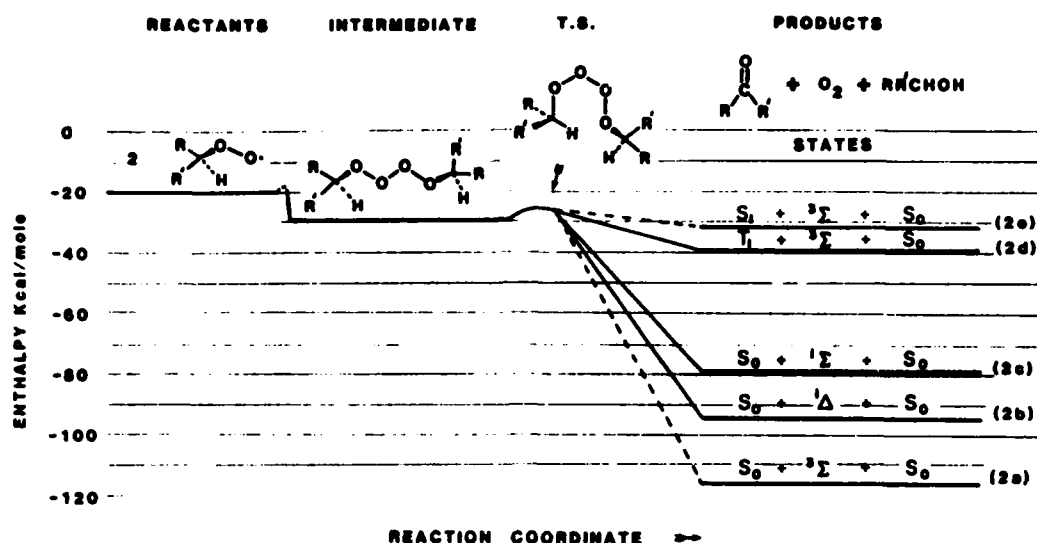Figure 1. Schematic Reaction Coordinate Diagram for Reaction (1)

Figure 2. Schematic Reaction Coordinate Diagram for Reaction (2) Enthalpy scale is for the ispropyl peroxy system.

We have studied many examples of reaction (1) in a flow system where O_2 ($^1\Delta$) was formed by 2450 MHz discharge of O_2 doped with a trace of Hg vapor to recombine O atoms. Extensive data on product energy partitioning have been obtained from chemiluminescence spectra.

Examples of reaction (2) have also been studied in the flow system. Trapping and analysis were performed for all products of reacting mixtures typically consisting of; 98% O_2, less than 1/2% O (^3P) and/or Cl (^2P) atoms, and 1 to 1.5% RH, at temperatures (200 - 450 $^\circ$C) chosen to insure the loss of alkoxy radicals by unimolecular fragmentation. The presence of O_2 ($^1\Sigma$) product was confirmed by the spectrum of O_2 ($^1\Sigma \rightarrow {}^3\Sigma$) chemiluminescence in the isopropyl peroxy reaction. This experiment made $iPrO_2{}^\cdot$ via reaction of H with dimethyl ketene in the presence of a large excess of O_2. The combined results of our studies of reaction (2) are interpreted as proof of the occurence of Russell's mechanism in the gas phase.

For presentation at COSMO '84

High Temperature Studies of Singlet Molecular Oxygen

BY

Peter Borrell, Patricia M. Borrell, D.S. Richards and Razmik Boodaghians

Department of Chemistry, Keele University, Staffordshire, England.

The aim of the work to be presented is to study the collisional quenching reactions of $O_2(a^1\Delta_g)$ and $O_2(b^1\Sigma_g^+)$ over as wide a temperature range as possible with a variety of quenching partners. The paper reviews the experimental technique and gives an account of the principal results obtained for both species including some new results for quenching by hydrogen.

The method used is a combined discharge flow/shock tube technique in which singlet oxygen is generated in a microwave discharge and then heated in a glass shock tube. The factors which contribute to the reliability of the method for obtaining results between 600 and 1800 K will be discussed.

For the quenching of $O_2(a^1\Delta_g)$ there are two classes of quencher: those for which there is an Arrhenius dependence of rate constant on temperature with an activation energy between 15 and 20 kJ mol^{-1}, HCl, NO and SO_2, and those for which the change is not measurable in our system ($E < 8$ kJ mol^{-1}).

There are again two classes of quencher for $O_2(b^1\Sigma_g^+)$; poor quenchers at room temperature, N_2, O_2, SO_2 for which the rate constant increases with temperature; and more efficient quenchers, HCl, NO, NH_3, CO_2, N_2O for which the rate constant is nearly independent of temperature or actually decreases above 1000 K.

The present theoretical approaches do not appear to be capable of explaining these results in a quantitative way.

PHOTOPEROXIDATION OF AROMATIC COMPOUNDS AND WAVELENGTH-DEPENDENT PHOTOCHEMISTRY OF THE OBTAINED ENDOPEROXIDES

H.-D. Brauer, A. Acs, W. Drews, R. Gabriel, S. Ghaeni

K. Schaffner, W. Trost and R. Schmidt

Institute for Physical and Theoretical Chemistry,

University of Frankfurt, Robert-Mayer-Str. 11,

D 6000 Frankfurt/Main, F.R.G.

A.   Photoperoxidation of Aromatic Compounds

A.1. Generation of $^1O_2$

It is well known that $^1O_2$ in its $^1\Delta_g$-state is the reactive intermediate in the sensitized and self-sensitized photoperoxidation of aromatic compounds leading to endoperoxide formation. $^1O_2$ can be generated by both the lowest excited singlet and triplet states of the aromatic compounds, if the singlet-triplet energy separation E_{ST} and the triplet energies E_T, respectively, exceed the excitation energy of O_2 $^1\Delta_g$ at 7990 cm^{-1}. This was established for the first time for the case of rubrene by measurements of the photoperoxidation quantum yield as a function of the rubrene concentration and of the dissolved oxygen concentration in six solvents [1].

Recently, we have measured the photoperoxidation of a series of

aromatic compounds and evaluated our results in the way de-
scribed in [1]. From these measurements one can classify the
investigated compounds into two groups with respect to oxygen
quenching of the S_1-state.

I.   Oxygen quenching of the S_1-state of the compounds results
     exclusively in the formation of the lowest triplet state.

II. Oxygen quenching of the S_1-state of the compounds may
     lead either to the formation of the lowest triplet state or
     to the formation of the singlet ground state.

Within both groups one can further distinguish two types of
compounds with respect to $^1O_2$ generation. Thus the follow-
ing subdivision is useful:

I.1. Each compound molecule in the S_1-state which is quenched
      by oxygen generates two molecules of $^1O_2$. This is the
      case for rubrene [1] heterocoerdianthrone [2], 9.10-di-
      methylanthracene [3] and benzodixanthene [3].

I.2. Each compound molecule in S_1-state which is quenched by
      oxygen generates only one molecule of $^1O_2$. This is
      the case for tetracene [3] and anthradichromene [3].

II.1. Each compound molecule in the S_1-state which, upon oxy-

gen quenching, passes to the T_1-state generates two mo-
cules of $^1O_2$. This is the case for 9.10- diphenylanthra-
cene [4] and 1.4-dimethoxy-9.10-diphenylanthracene [3]

II.2. Each compound molecule in the S_1-state which, upon oxy-
gen quenching, passes to the triplet manifold generates
only one molecule of $^1O_2$. This is the case for meso-
diphenylhelianthrene [3].

A.2. Wavelength-Dependent Photoperoxidation of Meso-diphenyl-
helianthrene [3]

Generally, the self-sensitized photoperoxidation of aromatic
compounds is independent of the excitation wavelength. As a
rule the formation rate of the endoperoxide FR decreases with
increasing reaction time, since at a given oxygen concentration
the substrate concentration decreases. Meso-diphenylhelian-
threne is an exception from this rule.
Excitation with light in the wavelength range between 475 - 610
nm results in behavior similar to that of other aromatic com-
pounds. However, during irradiation at $\lambda_{exc} \leq 450$ nm FR ini-
tially increases remarkably with time reaching a maximum and
decreases then continuously. This behavior can be explained
taking into account that the formed endoperoxide absorbs stron-
ger than meso-diphenylhelianthrene at the excitation wavelength

and is also able to produce $^1O_2$.

B. Wavelength-Dependent Photochemistry of Endoperoxides of Aromatic Compounds

On the basis of orbital and state correlations diagramms, Kearns and Khan predicted a novel type of photoreaction for the photolysis of the endoperoxides of cyclopentadiene and of aromatic compounds, respectively: an adiabatic cycloreversion originating from an upper singlet state. Up to now such a type of photoreaction has never been observed in solution.

The results of our investigations on the photolysis of the endoperoxide of heterocoerdianthrone confirm for the first time the predictions made by Kearns and Khan [5].

Furthermore, systematic investigations of other endoperoxides lead to the conclusion that the adiabatic cycloreversion is a general phenomenon of the photochemistry of endoperoxides [3,6, 7].

Two different reactions are observed during the photolysis of the endoperoxides:

1. Excitation of the S_1-band causes an irreversible decomposition of the endoperoxides.

2. Excitation of the S_2-band leads to an adiabatic photocleavage of the endoperoxides into hydrocarbon and $^1O_2$.

Some of the systems: endoperoxide $\underset{h\nu'}{\overset{h\nu,\Delta}{\rightleftharpoons}}$ hydrocarbon + O_2 are highly reversibele photochromic systems, which are of interest for practical applications [8,9]

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[3] upublished data

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Effects of Solvent on the Rate of Bacteriochlorophyll a Photooxidation

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Solar Energy Research Institute
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Early investigations of the photostability of isolated bacteriochlorophyll a (BChl a) in organic media revealed significant solvent effects on the rate of pigment photooxidation. Thus, Smith and Calvin[1] reported a ~40-fold increase in the photodegradation rate of BChl a in acetone compared to the rate in diethyl ether. Recently, accurate values for the lifetimes (τ_Δ) of singlet oxygen ($O_2\ ^1\Delta_g$) have become available[2], but these fail to account quantitatively for the observed solvent effect on photooxidation rate. Connolly et al.[3] estimated that the lowest triplet state of BChl a lies significantly lower than $O_2\ ^1\Delta_g$ (7882 cm^{-1}) in polar or moderately polar solvents, and suggested that O_2 may quench ^{3}BChl a by an electron-transfer process which presumably would lead to degradation of the pigment. That $O_2\ ^1\Delta_g$ is involved in photooxidation of BChl a appears certain, however, based on the cumulative evidence concerning photooxidation of other synthetic and natural magnesium porphyrins.[4-7] Moreover, Krasnovsky reported that BChl a is both a moderately effective sensitizer and a potent quencher of $O_2\ ^1\Delta_g$ luminescence.[8]

The mechanistic aspects of BChl a photooxidation appear complicated based on the number (>5) of isolable photoproducts.[9] However, the spectrophoto-metric progress of the reaction in most solvents appears simple, exhibiting clear and well-defined isosbestic points that are preserved essentially to reaction completion, which indicates that the stoichiometry, however complex, remains constant.

We have carried out comparative studies of direct and rubrene-sensitized photooxidation of BChl a in nine solvents spanning a moderately wide range of dielectric constant (~2 to 37), O_2-solubility (~1-4 mM), τ_Δ (~16-100 μs) and solvent nucleophilicity; the last property affects the coordination state and the central Mg-ion of BChl a[10] and the fluorescence properties of the pigment.[11] The relative rates of pigment photodegradation show parallel trends in both the direct and sensitized experiments. However, there is no consistent correlation of initial photooxidation rates (in the direct irradiations) or relative degradation rates (compared to rubrene disappearance in the sensitized experiments) with either solvent polarity or τ_Δ. The only clear trend appears to be with the coordination number of BChl a magnesium. Thus, the rates are significantly slower when the central Mg is hexacoordinat-ed (pyridine, p-dioxane, tetrahydrofuran, 2-methyltetrahydrofuran) compared to pentacoordinated Mg-species (acetonitrile, dichloromethane, benzene and acetone). It may be significant in this respect that the calculated fluorescence quantum yields[11] are higher when the Mg-coordination number is six, presumably reflecting lower intersystem crossing efficiencies. The sole exception to this trend is diethyl ether (pentacoordinated Mg) in which BChl a is remarkably stable.

A possible explanation of our results is that the triplet energy (E_T) of BChl $\underline{a}$ lies close to the $^1\Delta_g$ state of O_2[12] but varies with solvent.[13] Thus, in hexacoordinating solvents and in ether, E_T may be less than 7882 cm^{-1}, but in pentacoordinating solvents (except ether) $E_T > 7882$ cm^{-1}. Experiments are currently in progress to verify this hypothesis; these include inhibition of direct photooxidation of BChl $\underline{a}$ by DABCO and β-carotene and laser flash photolysis experiments to determine the rates of quenching of ^{3}BChl $\underline{a}$ by oxygen, β-carotene,[3] tetracene and pentacene.

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OXIDATION PHOTOSENSITISED BY 2-CHLOROTHIOXANTHONE; THE ROLE OF SINGLET OXYGEN

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2-Chlorothioxanthone (CTX) is a well known photoinitiator for the UV curing of polymer coatings. Its photoreactions are strongly inhibited by molecular oxygen which suggests that it might be an efficient photosensitiser by a singlet oxygen mechanism. CTX is practically insoluble in water but it can be incorporated into lipid vesicles and detergent micelles. Another interesting feature of this sensitiser is that its fluorescence is sensitive to the nature of the solvent. Thus, the fluorescence yield in non polar solvents is very low ($\phi_f < 0.02$) whereas, in alcohols, an intense blue fluorescence is observed. This fluorescence is further enhanced by addition of water. It was hoped that CTX would perform the dual role of photosensitiser and fluorescence probe when incorporated into lipid vesicles and detergent micelles.

The photosensitised-oxidation experiments were carried out using a Rank Brothers oxygen electrode. The CTX was excited by light in the range ⁓ 380 to ⁓430 nm.

When CTX was solubilised in SDS micelles the solution exhibited an intense blue fluorescence. This indicates that the photosensitiser is not buried in the hydrophobic core of the micelles, but protrudes into the aqueous environment where hydrogen bonding to water molecules leads to fluorescence enhancement.

On addition of methionine to the CTX/SDS solution
photosensitised absorption of oxygen occurred which, in the
early stages, was linear. Addition of 1,4-diazabicyclo
[2.2.2] octane (DABCO) quenched the oxygen uptake as did
azide ion, consistent with a singlet oxygen-mediated
oxidation of the methionine.

CTX was incorporated into the membranes of dipalmitoyl
phosphatidylcholine (DPPC) vesicles. When an aqueous suspension
of the vesicles was irradiated no oxygen absorption occurred.
When vesicles were prepared from egg-yolk phosphatidyl
choline rapid oxygen uptake occurred. In mixed vesicles,
composed of DPPC and egg-yolk PC, the rate of photosensitised
oxygen absorption increased with increasing concentration of
egg-yolk PC.

The oxygen-uptake versus time traces for the CTX-
photosensitised oxidation of egg PC differed significantly
from those obtained for the CTX-photosensitised oxidation of
methionine. After a period of 3 - 4 minutes, over which time
the rate gradually increased, a high rate of oxygen uptake
was observed which was almost linear - indicating the
attainment of a steady state. When the light source was cut
off, oxygen uptake continued in the dark, although at a much
slower rate. The CTX-Photosensitised oxidation of egg yolk
PC was inhibited by azide ion indicating the involvement of
singlet oxygen. The rate of oxygen absorption was also reduced
by the addition of p-methoxyphenol. This indicates that
free radicals, as well as singlet oxygen, are involved in
the CTX-photosensitised oxidation of egg-yolk PC vesicles.

SINGLET OXYGEN GENERATION FROM THE PEROXIDASE-CATALYZED AEROBIC OXIDATION OF AN ACTIVATED - CH_2-SUBSTRATE

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There is strong evidence that electronically excited products as well as those that give bioluminescence are present in biochemical systems[1-3]. Indirect evidence for this are: energy transfer processes, quenching, kinetics and comparison with photochemical processes[4,5], and direct one is emission spectra[6,7]. Meanswhile peroxidase catalyzed aerobic oxidation of substrates such as indole-3-acetic acid[8], 2-methylpropanal[5], propanal[6], acetoacetate[9] and many others generates triplet ketone in its excited states[1,2], but there are few cases where the important emitting species seems to be singlet oxygen[7,10,11].

Although we are aware of the limitation of biological systems in applying the techniques which have been developed using pure organic solvents[12], methods which are nicely discussed by Foote[13], we want to communicate a systematic study on the detection of singlet oxygen generation in peroxidase catalyzed oxidation of malonaldehyde (MA).

Correlation in O_2 consumption, product formation and Photon emission:

In the MA/MRP/O_2 system the oxygen consumption was much faster than light emission and product formation; this indicates accumulation of an intermediate, a fact which in turn suggests than the excited species may be formed outside the enzyme.

Conformational effect on the MA/HRP/O_2 Systems:

The variation of the spectra and its reactivity towards MA of solutions of HRP in dimethylsulfoxide has been studied. A broad pattern of changes was observed in the CD spectra of peroxidase, specially in the 400 nm region. These variation were correlated with conformational changes leading to a displacement of aromatic side chains in the heme environment[14]. Data has shown that the MA/HRP/O_2 system generates singlet oxygen probably outside the enzyme, i.e.,

in the bulk solutions. The actual data indicates that singlet oxygen product-
ion and its emission is completely insensitive to peroxidase conformational
modifications. This is in agreement with the observation that the excited
species in this system are formed outside the enzyme.

Photon emission: quenchers and enhancers:

The emission measured originated from dimol singlet oxygen was not affected
by SOD, catalase, benzoate, bicarbonate, DB AS, but the emission was quenched
by DOPA, histidine, methionine, tryptophan, guanosine and bilirrubin, all
excellent singlet oxygen quenchers. A small enhancement of the emission was
observed in the presence of 10 mM DABCO. Also enhancement (4 fold) of the
emission was observed in the presence of 10 μM eosin and D_2O (5 fold higher
than in H_2O). The effect of guanosine and histidine upon the emission of the
$MA/HRP/O_2$ reaction was studied at concentration that did not influence the ra-
te of oxygen consumption.

Products distribution:

Cadet and Teoule[17] reported the guanosine specifically react with singlet
oxygen. In fact, using $(5,8-{}^{14}C)$ deoxyguanosine in the $MA/HRP/O_2$ system we
detected N(2-deoxy-D-erythro-pentosyl) urea and N^1(2-deoxy-β-erythro-pentofu-
ranosyl)N^2-formylurea.

Emission spectrum:

In this system the intensity of the light was so intense, that it was possible
to run the spectrum in a conventional fluorimeter in H_2O or in D_2O. The princi-
pal emission peak in just above 700 nm and ocassionally a smaller peak was
observed at 630 nm.

Kinetic studies:

Constancy of intercept in the plot of $(I_o-I_{A,Q})^{-1}$ versus $(A)^{-1}$ at different
(Q) is diagnostic for singlet oxygen quenching and implies that A and Q
compete for a common intermediate. The quenching of histidine oxidation by
guanosine in the $MA/HRP/O_2$ system gave constancy in the intercept. The ra-
tio of reactivity of histidine to guanosine was ~5 in the photodynamic
processes[16]; and in the ratio from Stern-Volmer plot of the enzymatic sys-
tem gave a value of 4.3. A similar value was obtained from this steady state
technique.

In conclusion, we suggeste that the presence and role of singlet oxygen
($^1\Delta g\ ^1\Delta g$) in the horseradish peroxidase-substrate sytem through photoenergized
process can be ascertained in terms of combined data from photon emission,
emission spectra, inhibition by quenchers, products analyses, D_2O and DABCO
effects, energy transfer to xanthenic dyes and kinetics analyses. This enzy-
matic system was proved to be very efficient for producing changes in riboso-
mes[11], plasmid DNA[18] and in t-RNA[19].

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Singlet Oxygen Luminescence

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A method is reported for the measurement of singlet molecular oxygen ($^1\Delta g$) lifetime in the solutions. The method is based on the pulsed laser excitation of the photosensitizing molecule and the $^1\Delta g \rightarrow {}^3\Sigma_g^-$ luminescence registration with the help of the photodetector sensitive in the near IR spectral region. The developed method opens the way for studies of proper physico-chemical characteristics of singlet oxygen in different conditions, makes it possible to detect its presence and clarify its role in different photosensitization and oxidation processes.

Using this method or, as the need arose, also the method of laser nanosecond flash-photolysis, the following investigations have been made:

(i) Lifetimes and relative luminescence yields of singlet oxygen in a variety of solvents have been measured.

(ii) The mechanism of quenching the triplet states of compound organic molecules by molecular oxygen has been investigated, special emphasis being placed on tetrapyrrole molecules of different classes.

(iii) A method has been proposed for measuring relative quantum yield of the intersystem crossing based on the comparison of singlet oxygen luminescence intensities, all other thing being equal. This method was used for the investigation of porphyrins and metalloporphyrins in order to study the intramolecular energetics parameters and select effective singlet oxygen sensitizers.

GAS PHASE GENERATION OF SINGLET OXYGEN
AT ATMOSPHERIC PRESSURE BY DIRECT LASER EXCITATION

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The generation of singlet oxygen in the gas phase at atmospheric pressure was investigated using direct laser excitation. A Holobeam 255 Nd. YAG laser was used in the experiments. The output of the laser at 1065 nm lies within the O_2 $^3\Sigma g$ (v=0) → O_2 $^1\Delta g$ (v=1) absorption band of the oxygen molecule at 1064 nm. Although the transition of ground state oxygen to the electronically excited singlet state is a "forbidden" process, we have previously shown that this transition does occur using simulated sunlight.[1]

Evidence for the formation of singlet oxygen by direct laser excitation was achieved in chemical trapping experiments with tetramethylethylene. These experiments were performed using a 12" by 1" OD pyrex tube having 1/8" quartz windows at each end. The tube containing the trapping agent and air or oxygen was placed in the beam of the laser during an experiment with the laser operating in a continuous mode. Control experiments involved no irradiation period. The production of detectable quantities of singlet oxygen was demonstrated in the conversion of tetramethylethylene to its known singlet oxygen product, 2,3-dimethyl-3-hydroperoxy-1-butene. No hydroperoxide was observed in the control experiments which were identical except there was no irradiation.

Further evidence for atmospheric gas phase generation of singlet delta oxygen by direct laser excitation was obtained by monitoring its characteristic 1.27 mμ emission using a germanium photodiode. In the second series of experiments, the laser was operated in a pulse mode. The laser beam was focused into an integrating sphere (excitation chamber) consisting of a 50 ml Pyrex

glass bulb with two 1/4" OD Kovar glass tubes attached at right angles. The
surface cf the integrating sphere was mirrored to permit many reflections of
the laser beam. The optical detector was positioned at 90° with respect to
the laser beam, and a light shutter was installed between the detector and the
integrating sphere. The light shutter was open only when the laser was shut
off. The laser was operated at full power for a period of 15 seconds, and was
automatically shut off when the shutter was opened. The signal was led directly
into an oscilloscope and data were collected only when oxygen was present in
the integrating sphere. When nitrogen was used instead on oxygen or air, no
1.27 mμ emission was observed. The deactivation of singlet oxygen followed a
first order decay with a mean value of k_r=8.04 sec^{-1} and a mean lifetime τ
of 0.124 seconds.

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STUDIES ON SINGLET OXYGEN IN AQUEOUS SOLUTION

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The caesium and sodium salts of anthracene 9,10 bis ethane sulphonate (AES) are readily soluble in water over a wide range of pH, and react with $^1O_2$ to give the endoperoxide as the sole detectable product. The reaction can be followed both from the U.V. absorption of AES and by HPLC of the endoperoxide. The trapping efficiency of AES for $^1O_2$ has been measured as a function of concentration. AES does not react with O_2^- or HO_2, but acts as a trap for radicals such as $.OH$. The products, however, can readily be distinguished from the endoperoxide by HPLC.

AES has been used to study quantitatively the production of singlet oxygen in a variety of reactions occurring in aqueous solution.

(1) Two electron oxidation of H_2O_2.

The reaction of H_2O_2 with N chlorosuccinimide and Chloramine T produces $^1O_2$ with $\sim 100\%$ efficiency. With Chloramine T, the reactive species is dichloramine T produced by disproportionation.

Both iodoxybenzene and iodosobenzene sulphonic acid react with H_2O_2 to give oxygen, of which $\sim 45\%$ only is in the singlet state. This may be due to spin-orbit coupling involving the heavy iodine atom. With IO_4^- and H_2O_2, the yields of $^1O_2$ are irreproducable, and slightly lower, indicating a concomitant free radical reaction.

(2) Decomposition of peroxyacids at their pK. When sufficient precautions to avoid catalysis by transition metal ions are taken, the decomposition of peroxymonophthalic, peroxyacetic and peroxymono sulphuric acids give $^1O_2$ in essentially quantitative yields. The reported reaction of H_2O_2 with peroxyacid anions does not produce $^1O_2$, and in the absence of metal catalysis is very slow indeed, if it occurs at all.

(3) Catalyzed decomposition of H_2O_2.

$O_2(a^1\Delta_g)$ SENSITIZED CHEMILUMINESCENCE OF $a^1\Delta \rightarrow X^3\Sigma^-$ AND $b^1\Sigma^+ \rightarrow X^3\Sigma^-$ TRANSITIONS OF GROUP VI-VI AND GROUP V-VII DIATOMIC MOLECULES

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Abstract

Systematic studies of the near infrared emissions from the $b^1\Sigma^+$ and $a^1\Delta$ states of group VI-VI and group V-VII diatomic molecules in chemiluminescence systems, following the excimer laser photolysis of suitable parent compounds, and by Raman-shifted dye laser fluorescence (LIF) are reported. The $b \rightarrow X$ band systems have been observed for 25 molecules of the two groups mostly by studying the $O_2(a^1\Delta_g)$ sensitized chemiluminescence in fast flow systems. For SO also the (0,0) band of the $a \rightarrow X$ transition has been detected. Using a Fourier-Transform-IR-Spectrometer, rotationally resolved spectra of the $b \rightarrow X$ bands of S_2 and SeS were measured. Time resolved studies of the $b \rightarrow X$ emissions by excimer laser photolysis and LIF yielded preliminary results for the radiative lifetimes and the quenching rate constants of the b states of SO, SeO, PCl, PBr, and SbCl. These studies as well as Stern-Volmer type measurements in flow systems strongly indicate that the quenching of the b states in many cases proceeds by near-resonant $E \rightarrow V$ energy transfer. As will be shown, a wealth of other energy transfer and energy pooling processes is operative in the chemiluminescence and photolysis systems.

SINGLET OXYGEN PRODUCTION FROM PHOTODYNAMIC SENSITIZERS

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There is a wide range of naturally occuring photodynamically active compounds from plant sources. Examples include the fungal pigment cercosporin and a variety of plant polyacetylene derivatives. These compounds probably serve to protect the synthesizing organism from animal (mammal or insect) browsing, or, in the case of cercosporin, to promote the attack of the cercospora fungus on the target cell. Another interesting compound is 4-thiouridine, a nucleoside found in transfer RNA of certain wild-type e. coli strains. This nucleoside appears to be the sensitizer in an oxygen-sensitive near-UV photodynamic single strand breakage of DNA in these organisms.

Using the 1268 nm emission from singlet oxygen, it is relatively simple to quantitate the production of singlet oxygen from many sensitizers. The sensitizer is excited by a short pulse from a Nd:YAG laser, and the weak luminescence from singlet oxygen is detected in a time resolved system. The intensity of the luminescence is compared with that produced from a sensitizer with a known singlet oxygen quantum yield. These quantum yields are checked by measuring the quantum yield of photooxidation of typical singlet oxygen acceptors; the results of the

control experiments agree well with those of the luminescence experiments in all cases so far studied. Using this technique, cercosporin, terthienyl, and 4-thiouridine have all been found to produce singlet oxygen in solution in substantial yields.

The efficient production of singlet oxygen from these sensitizers is a necessary but not sufficient condition for the intermediacy of singlet oxygen in the photodynamic damage sensitized by these compounds. That is, singlet oxygen production could be only a side reaction accompanying the actual photodynamic processes. The strong protective effect of singlet oxygen quenchers against damage mediated by cercosporin and terthienyl makes it nearly certain that singlet oxygen is the active species with these pigments. However, further work will be required for the 4-thiouridine case, since the effects of inhibitors do not seem to be totally explicable on the basis of a pure singlet oxygen mechanism.

ASPECTS OF PHOTOSENSITIZED LIGNIN OXYGENATION

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Substantial knowledge has been accumulated of the chromophoric structures in native lignin and in modified degraded lignin. These structures, acting as sensitizers, together with light and oxygen are directly or indirectly responsible for reactions leading ultimately to photo-degradation of the starting material. This is often displayed by the darkening or the yellowing of wood specimen and industrial articles made of wood, such as high yield masses and different paper products. It has been of interest for the economical and ecological development to reduce the use of bleaching chemicals and to prevent re-yellowing of the products.

Earlier studies have reveiled the emphasis of oxygen in photochemical lignin degradation and that the process involves oxygenation pathways.

This work is a contribution to the understanding of the mechanism of the yellowing process and clearly shows the complexity of the reaction.

The results are derived from model experiments with tert-butylguaiacylcarbinol sensitized by aromatic α-carbonyl triplets and by compounds containing ring-conjugated double bonds. The rates of disappearance of the reactants were determined by gas chromatography and the formation of peroxides were measured by iodometric titration. The kinetics of the reactions were also studied in the presence of "so called singlet oxygen quenchers and free radical scavengers.

The results indicate that the mechanism for photo-oxygenation is very complicated with many pathways operating simultaneously, one of which is attributed to a reaction

involving singlet oxygen in addition to the known
hydrogen abstraction process. Radical reactions including
thermo- or photochemically instable peroxides are also
of significance, particularly during prolonged irradiations.

The relative importance of these pathways is discussed
and the course of the reaction depends largely on the
conditions, such as the concentrations, the properties of
the reactants and the reaction time. This means that one
mechanism can dominate in one system, whereas another is of
major importance in another one.

THE EFFECT OF STRAIN ON THE REARRANGEMENTS

OF PRIMARY SINGLET OXYGEN PRODUCTS

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We have argued[1] previously that $^1O_2$ is relatively insensitive to strain consideration either present in the starting material or developing in the product. More important factors seem to be the ground state geometry of the olefin, the $C_\alpha - H_{allylic}$ interatomic distance and the ionization potential of the double bond. Strain does, however, play a crucial and fascinating role in the secondary rearrangements of primary $^1O_2$ products. The plethora of products obtained can be easily rationalized in terms of intriguing variations on the "Hock-cleavage" theme.[2] These include ring rather than vinyl group migration and divinyl ether formation in competition with hemiacetal production yielding oxetanes, oxetenes and alkylidene oxetenes as intermediates.

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DYE-SENSITIZID PHOTO-OXYGENATION OF ISONIAZID BY SINGLET MOLECULAR OXYGEN

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A B S T R A C T

The present paper deals with the formation of a dicarbonyl cyclic compound from the isoniazid, by the dye-sensitized photo-oxygenation method. The formation of product has been explained by both Gollnick's "type I" and "type II" reaction mechanisims. The participation of singlet oxygen has been confirmed by the use of different dyes and quenchers.

The structure of the product has been proposed on the basis of electronic absorption, infrared, nmr and mass spectural data.

The yield of product in different conditions has been also determined.

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The Efficiency of Triplet Energy Transfer to $O_2(^3\Sigma_g^-)$:
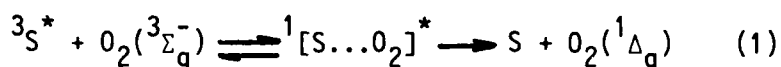
Sensitizer and Medium Dependence

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The efficiency of formation of $O_2(^1\Delta_g)$ in the process whereby electronically excited triplet states are quenched by $O_2(^3\Sigma_g^-)$ has been a matter of some debate. The two spin-allowed processes are represented by channels 1 and 2 which proceed *via* the singlet and triplet manifolds

$$^3S^* + O_2(^3\Sigma_g^-) \rightleftharpoons {}^1[S...O_2]^* \longrightarrow S + O_2(^1\Delta_g) \qquad (1)$$

$$^3S^* + O_2(^3\Sigma_g^-) \rightleftharpoons {}^3[S...O_2]^* \longrightarrow S + O_2(^3\Sigma_g^-) \qquad (2)$$

$$^3S^* + O_2(^3\Sigma_g^-) \rightleftharpoons {}^5[S...O_2]^* \qquad (3)$$
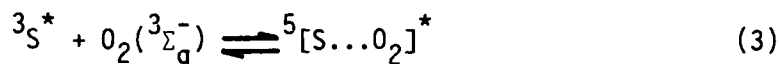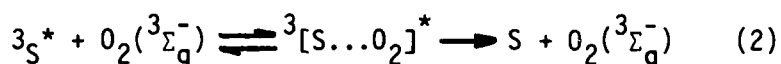
respectively of the encounter complex. Quenching *via* the quintet manifold is forbidden. Rate constants for oxygen quenching of triplet states do not *generally* exceed ~$1/9$th of the diffusion controlled value and it was naturally concluded that quenching is restricted to the singlet manifold and that each quenching act leads to a molecule of $O_2(^1\Delta_g)$.[1] The validity of this conclusion has been seriously questioned as a result of pulse radiolysis[2] and pulsed laser[3] studies which in turn have themselves been the subjects of criticism based on steady-state experiments.[4]

- 2 -

In the work to be described a series of sensitizers in aerated media have been subjected to pulsed laser excitation at 355 nm. In each case the sensitizer triplet yield and the $O_2(^1\Delta_g)$ emission intensity have been monitored by kinetic absorption and kinetic emission spectroscopy respectively. The significance of these data will be discussed.

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SENSITIZED PHOTOOXIDATION OF METHYL SUBSTITUTED CYCLOBUTENES

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The series of methyl-substituted 1,2-diphenylcyclobutenes 1a-d were subjected to photooxidation in the presence of sensitizers including methylene blue (MB) and rose bengal (RB).
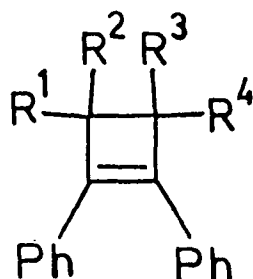
1a $R^1=R^2=R^3=R^4=Me$

1b $R^1=R^2=R^3=Me$, $R^4=H$

1c $R^1=R^3=Me$, $R^2=R^4=H$ (trans)

1d $R^1=Me$, $R^2=R^3=R^4=H$

Sensitized photooxidations of 1a with MB and RB lead to formation of the ring-contracted product 6 presumably by initial "4+2" addition of singlet oxygen to the styryl diene moiety of the substrate.

When the electron-transfer sensitizer 9,10-dicyanoanthracene is used, the reactions of 1 take a markedly different course. For example, 1a is rapidly photooxidized in CH_3CN to give the spectrum of pr   ~ts 2-5 through a mechanism in which superoxide radical anion is implicat   Thus tetramethylcyclobutene 1a may be employed to differentiate between photo-oxygenations involving singlet oxygen and superoxide ion. The selectivity exhibited by this substrate has been used to probe the identity of the cytotoxic agent responsible for the dramatic cancer cures observed with hematoporphyrin derivative (HPD) as a phototherapeutic agent. Our prelimi-nary data using HPD as a sensitizer in vitro supports Dougherty's contention that singlet oxygen is involved although his conclusion was based on trapping experiments with 1,3-diphenylisobenzofuran, an assay which must now be considered questionable.
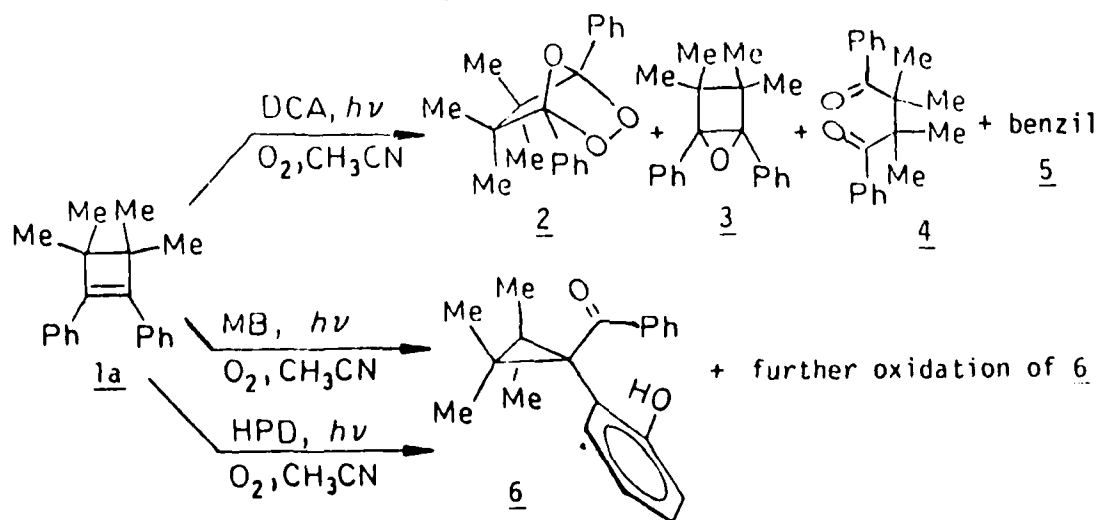
Reactions of Singlet Oxygen with Liposomes

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Liposomes have been used as model membranes for the investigation of
chemical and biological processes photosensitized by natural and synthetic
pigments. Work in this laboratory has been done with egg phosphatidyl-
choline (EPC) liposomes photosensitized by methylene blue (MB) [1,2],
riboflavin (RF)/EDTA [1], hematoporphyrin (HP) [3], the constituents of hemato-
porphyrin derivative (HPD) [4-6], and 8-methoxypsoralen (8-MOP) [7]. Damage
sensitized by these agents, incorporated in the liposomes and added to the
external aqueous phase, has been assayed by membrane lysis, marker release,
and lipid peroxidation. The involvement of singlet oxygen ($^1O_2$) was demon-
strated for MB, HPD, dilute HP and 8-MOP; aggregated HP photosensitized via
an anaerobic, Type I mechanism; superoxide (O_2^-) was implicated for RF/EDTA.
Dipalmitoylphosphatidylcholine liposomes were not sensitized by these agents,
implicating the unsaturated fatty acids as the site of $^1O_2$ and O_2^- attack.
Liposome damage was inhibited by water soluble acceptors, azide ion, DABCO,
and human serum albumin, indicating that $^1O_2$ generated in the liposomes
diffused into the external medium prior to reacting with the membrane. The
efficiency was estimated by generating $^1O_2$ in the gas phase and bubbling into
the liposome suspension. The analysis based on large target diffusion kinetics
indicates that 3000 $^1O_2$ interactions at the liposome surface were required for
lysis of 1.5 μm EPC liposomes [8]. The diffusion of HP and HPD into sonicated
EPC liposomes leads to a large red shift of the Soret bands and enhanced
fluorescence efficiency, indicative of monomerization. Visible light irradiation
of the dyed liposomes induced lipid peroxidation, with positive mechanistic
tests for $^1O_2$. The occccurrence of these effects with the tumor localizing
and photosensitizing constituent of HPD strongly suggests that the in situ
tumor sensitizer in photoradiation therapy is a monomerized, fluorescent
constituent of HPD localized in a lipophilic environment that generates $^1O_2$
when excited by visible radiation.

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PROSPECTS FOR SOLAR SYNTHESIS. I. A NEW METHOD FOR SINGLET OXYGEN REACTIONS USING NATURAL SUNLIGHT. <u>J. E. Guillet</u>, J. Sherren, H. M. Gharapetian and K. MacInnis. Department of Chemistry, University of Toronto, Toronto, Canada M5S 1A1.

The study of organic photochemistry has provided a variety of interesting synthetic routes to novel organic compounds and intermediates. Unfortunately, the high cost of using electrically-driven lamps to provide the necessary photon energy has made photochemical steps impractical in most commercial applications. We wish to report a new method of carrying out organic photochemical reactions using sunlight as the photon source, which is both inexpensive and easily scaled up for commercial synthesis.

In this procedure, the organic reagent is absorbed in transparent beads of crosslinked ethylene-vinyl acetate copolymer. These beads float readily on water, and can be exposed to natural sunlight on the surface of a small pond or pool. After exposure, the beads are collected, washed with water, and dried in air. The products and unreacted starting material are removed by selective extraction with suitable solvent mixtures, after which the beads can be recycled through the process. The photoreaction can be carried out either batchwise or in a continuous process. In the dry form the beads can be moved by airveyor, while during exposure they are most conveniently moved by adjusting the flow of water through the pond-reactor.

A number of classical photochemical reactions have been studied in these systems, including photoisomerizations, the Norrish type II reaction in various ketones, and the addition of singlet oxygen to olefinic double bonds. The latter process is of particular interest, because it is sensitized by compounds absorbing visible light near the maximum intensity of the solar spectrum. Furthermore, the beads have a relatively high permeability to oxygen from the air.

In the present work the rates of addition of singlet oxygen to squalene were measured. Rose Bengal was used as the sensitizer. The resulting allylic hydroperoxide was estimated by the triphenyl phosphine method. Conversions of up to 10% were obtained after four days exposure to sunlight. The results of singlet oxygen additions were compared with those for the type II reaction of aromatic ketones.

Mechanism of Singlet Oxygen Luminescence
Sensitization by Polyatomic Molecules

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Of the various methods of singlet oxygen ($^1O_2$) generation, the formation of $^1O_2$ by dye- and pigment sensitization is of particular interest both for studying different quenching mechanisms of organic molecule excited (singlet and triplet) states and for understanding chemical, biological and medical light-initiated processes involving oxygen.

This paper presents a thorough consideration of mechanisms of quenching the excited states of the sensitizer's molecules by oxygen resulting in the formation of $^1O_2(^1\Delta_g)$, primary attention being focused on utilizing the $^1O_2(^1\Delta_g)$ own luminescence for dealing with the following problems:

- oxygen quenching of sensitizer fluorescence with different $E(S_1-T_1)$ energy intervals;

- quenching of the sensitizer molecules' triplet states;

- intermolecular energy transfer involving oxygen;

- $^1O_2(^1\Delta_g)$ luminescence resulting from interaction of oxygen molecules with excited molecules of the sensitizer;

- photosensitized $^1O_2(^1\Delta_g)$ reactions in solutions.

BEHAVIOR OF SINGLET OXYGEN IN THE
# OXYGEN-IODINE TRANSFER LASER

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ABSTRACT

$O_2(^1\Delta)$ supplies all the energy required to produce the laser medium in the chemical oxygen-iodine (COIL) laser. Since $O_2(^1\Delta)$ can be made by the chemical reaction between Cl_2 and basic hydrogen peroxide, this near-infrared laser ($\lambda = 1.315 \ \mu m$) is one of the very few all-chemical laser devices.

It is necessary to produce high densities of $O_2(^1\Delta)$, however, it is equally important that one produce high ratios of $O_2(^1\Delta)/O_2(^3\Sigma)$. The electronic state equilibrium

$$O_2(^1\Delta) + I \ \langle\text{---}\rangle \ O_2(^3\Sigma) + I^* \qquad (1)$$
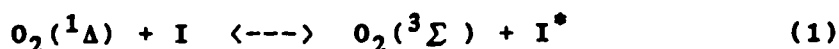
$$K_{eq} = 2.9 \ (T = 295K)$$

will support gain on the $I(^2P_{1/2} \text{--}\rangle \ ^2P_{3/2})$ transition ($I^*/I > 0.5$) only if $O_2(^1\Delta)/O_2(^3\Sigma) > 0.17$. Thus, the loss processes for $O_2(^1\Delta)$ are critical for the energetic efficiency of this laser. This talk will review the loss processes associated with the presence of atomic and molecular iodine. These may be summarized as follows:

(1) I_2 dissociation by $O_2(^1\Delta)$

(2) I^* pumping by $O_2(^1\Delta)$

(3) Quenching of $O_2(^1\Delta)$ by I-atom related processes

These processes will be considered in the context of the conventional cw COIL laser system. In addition, recent experimental work on the kinetics of a premixed, pulsed version of this promising laser system will be presented.

Laser Kinetic Studies of I($^2P_{1/2}$) and $O_2(^1\Delta_g)$

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Multiphoton ionization has been used to detect sensitively $O_2(^1\Delta)$, both in a microwave discharge and from the 266-nm photolysis of O_3. The spectra showed several vibrational levels for each of two Rydberg transitions in the 260-245 nm region as well as a valence band near 340 nm. None of the $O_2(^1\Delta)$ states has previously been reported. Their assignment is currently in progress.

The equilibrium between excited iodine atoms and oxygen has been examined by monitoring the time-dependent I*$\left[\equiv I(^2P_{1/2})\right]$ concentration following creation of this species by pulsed laser photolysis of iodine-containing precursors. A double exponential decay is observed which reflects a fast approach to equilibrium followed by a slower relaxation of the equilbrated mixture. Data obtained with the I* precursors HI and CH_3I show that the rate constant for the reaction I* + O_2 → I + O_2 is insignificant compared to that for the reaction I* + O_2 → I + $O_2(^1\Delta)$. Data obtained from the precursors i-C_3F_7I, n-C_3F_7I, C_2H_5I, and CF_3I suggest that $O_2(^1\Delta)$ is rapidly relaxed by the precursor itself, by the precursor radical, or by some product formed in a reaction between the precursor radical and oxygen. The rate constant for the process I* + O_2 → I + $O_2(^1\Delta)$ has been found to be $(8.8 \pm 0.9) \times 10^5$ sec^{-1} $torr^{-1}$, while that for the process I* + O_2 → I + O_2 is $(0.3 \pm 1.3) \times 10^5$ sec^{-1} $torr^{-1}$.

Electronic-to-vibrational energy transfer from I*(5 $^2P_{1/2}$) to $I_2(25<v<43)$ has been observed. I* was created by pulsed laser photolysis of either I_2/Ar mixtures at 475 nm or $CF_3I/I_2/Ar$ mixtures at 266 nm, while the resulting vibrational distribution of I_2 was monitored by laser induced fluorescence on the $I_2(B \leftarrow X)$ transition. The experimental results are consistent with a nascent I_2 product distribution which is inverted, with a substantial fraction of the I_2 molecules formed in v>30. Roughly 2% of the I* deactivations result in $I_2(v=40)$. The rate constants for vibrational relaxation of $I_2(v=40)$ by argon, helium, and I_2 at room temperature are $(7.3 \pm 0.3) \times 10^5$ sec^{-1} $Torr^{-1}$, $(1.0 \pm 0.2) \times 10^6$ sec^{-1} $Torr^{-1}$, and $(1.8 \pm 0.4) \times 10^6$ sec^{-1} $Torr^{-1}$, respectively. These results have important implications for the mechanism of I_2 dissociation in the chemical oxygen/iodine laser. A chain branching mechanism consisting of the steps I* + I_2 → I + $I_2(20<v<40)$, $I_2(20<v<40)$ + $O_2(^1\Delta)$ → 2I + O_2, and $O_2(^1\Delta)$ + I → O_2 + I* may be responsible for the dissociation.

Construction of 1,2,4-Trioxane rings by Photo-oxygenation of Enol Ethers

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Enol ethers which lack the option of forming hydroperoxides react readily with singlet oxygen to give 1,2-dioxetanes by a mechanism which could involve charge-transfer complexes, radical ion pairs, biradical or zwitterionic speices. We report that dioxetane ring formation can be intercepted when aldehydes are used as solvents. They are efficiently trapped undergoing conversion to 1,2,4-trioxanes, thereby providing proof for an intermediate zwitterion.

In other cases, 1,2-dioxetanes and 1,4-endoperoxides, products typically obtained by photo-oxygenation, can be opened by acid catalysis to give the corresponding stable β-hydroperoxy cations which are either sufficiently long-lived or reactive enough towards carbonyl compounds to give trioxanes. The mechanistic and synthetic aspects of these new reactions will be discussed.

In view of the paucity of structural information on 1,2,4-trioxanes, illustrations of conformational features will be given in the light of recently obtained X-ray analytical and NMR data.

SINGLET OXYGEN PRODUCTION BY LACTOPEROXIDASE:
HALIDE DEPENDENCE AND QUANTITATION OF YIELD

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Preliminary studies in this laboratory have demonstrated singlet oxygen (SO) production in the lactoperoxidase (LPO)-H_2O_2-Br^- system using 1268 nm chemiluminescence (CL) (JBC 258: 5991, 1983). Further details about the technique and the LPO-H_2O_2-halide systems are now reported. For the study of biochemical systems, 1268 nm CL is both more sensitive and more selective than dimole CL. The current CL spectrometer can measure SO production rates of 30 nM/min (flow system study using the H_2O_2 + NaOCl reaction as the SO source). In systems where D_2O solvent can be used, this decreases to 1 nM/min. In contrast, the sensitivity of dimole CL (cooled RCA 8852 photomultiplier using single photon counting) is 1000 nM/min. Of more significance is the empirical observation that CL at 1268 nm from sources other than SO is rare, while biologic oxidations often produce CL at visible and near infrared wavelengths including the dimole bands. For example, HOBr, an intermediate in the LPO-H_2O_2-Br^- system, reacts with albumin to produce CL with a broad spectral distribution overlying the dimole bands, but no detectable CL at 1268 nm. However, there is no reason to think that 1268 nm CL is totally specific for SO in complex systems, so additional supporting evidence to confirm SO production should be obtained. This includes (1) spectral analysis, (2) a large isotope effect in D_2O of about 30 and (3) inhibition of CL with appropriate SO quenchers and traps (Quenching is not highly specific for SO, but the failure to quench is strong evidence against SO.).

Under optimal conditions (0.1 mg/ml LPO, 3 mM NaBr, pH 4.5 and 1 mM H_2O_2) the LPO-H_2O_2-Br^- system stoichiometrically produces SO by the mechanism

$$H_2O_2 + H^+ + Br^- \xrightarrow{\text{LPO}} HOBr + H_2O$$

$$H_2O_2 + HOBr \longrightarrow H^+ + Br^- + H_2O + O_2\,(^1\Delta_g)$$
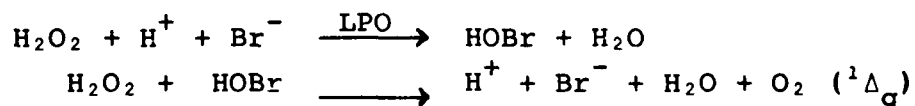
The bromine containing species HOBr, Br_2 and Br_3^- are in rapid equilibrium, so it is not possible to determine which of these is the initial product of the LPO catalyzed oxidation of Br^-. While oxidized bromine species are important reaction intermediates, they are quantitatively minor final system products.

Kanofsky-2

LPO does not produce SO with either Cl⁻ or I⁻ as a substrate. Cl⁻ is not oxidized. Past studies have shown that I⁻ is quantitatively converted to I_2 (Anal Biochem 42: 195, 1971).

ENZYME GENERATION OF SINGLET ($^1\Delta_g$) MOLECULAR OXYGEN:

NEAR IR SOLUTION EMISISON SPECTROSCOPY

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ABSTRACT

Enzyme production of singlet ($^1\Delta_g$) molecular oxygen in a dark reaction is revealed via near IR emission spectroscopy in solution. Singlet molecular oxygen emission [(0,0) $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ at 1.28 micron] is observed in the enzyme systems (1,2): chloroperoxidase/H_2O_2/Cl^- and lactoperoxidase/H_2O_2/Br^-. In the catalase/H_2O_2 system a strong emission at 1.64 micron with bandwidth of 753 cm^{-1} is observed, tentatively interpreted as arising from perturbed $^1\Delta_g$ state of molecular oxygen.

The development of near IR solution emission spectroscopy has enabled several other seminal observations: (i) the generation of O_2 ($^1\Delta_g$) in the disproportionation of the superoxide anion, O_2^- (3); (ii) quenching of O_2 ($^1\Delta_g$) by vitamin C in aqueous solution (4); and (iii) solvent perturbed O_2 ($^1\Delta_g$) emission (5).

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PHOTOSENSITIZED LUMINESCENCE OF SINGLET OXYGEN IN SOLUTIONS: MECHANISMS OF QUENCHING BY SOLVENTS AND BIOLOGICALLY IMPORTANT COMPOUNDS.

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The photosensitized luminescence of singlet oxygen in pigment solutions discovered in our experiments several years ago / I / is now intensively used as a tool for investigation of $^1\Delta_g$ state of molecular oxygen. In many cases this tool yields more reliable data than methods based on the use of $^1\Delta_g$ acceptors. The present paper summarizes the results of our measurements of the rate constants of $^1\Delta_g$ quenching (K_q) made with the aid of the oxygen luminescence / I,2 and refs. therein /.

It has been found that K_q values of n-alkanes (pentane-dodecane) increase with the increase of the number of hydrogen atoms (n_H) in alkane molecules. The K_q/n_H ratio is constant (~ 450 $M^{-1}s^{-1}$). It can be regarded as an activity of a single CH group. For n-alcohols (methanol-decanol) and saturated organic acids (acetic-behenic) this ratio declines with the increase of n_H. Analysis leads to conclusion that the activities of CH and OH groups are different, OH groups being more active by an order of magnitude. Using the experimental values of K_q it is shown that $^1\Delta_g$ lifetimes(τ_Δ) and probabilities of light emission by the $^1\Delta_g$ state (p_r) in all the solvents studied are well approximated by the following equations:

$$\tau_\Delta = I/(K_q[s]); \quad p_r = I/(K_q \cdot \tau_r \cdot [s])$$

[s] is the molar concentration of solvents; τ_r is the radiative lifetime of $^1\Delta_g$ equal to 4 s.

The energy transfer from $^1\Delta_g$ to vibrational sublevels of organic molecules is the most probable mechanism of $^1\Delta_g$ quenching by the mentioned compounds. The same mechanism determines the activities of saturated fatty acids and lipids, while its contribution to the overall quenching effect of unsaturated fatty acids varies from 40 to 45 depending on the number of double bonds in fatty acid molecules. Chemical oxygenation of these molecules appears to play a more important role in their interaction with $^1\Delta_g$.

Chlorophylls and porphyrins were found to be efficient physical quenchers of $^I\Delta_g$. Their activities depend on the macrocycle structure as well as on the presence and nature of the central metal atom. Two mechanisms appear to determine the quenching effect of these compounds - energy transfer from $^I\Delta_g$ to low lying electronic levels of quenchers or formation of exciplexes with charge transfer between $^I\Delta_g$ and porphyrins. The first mechanism is probably valid for Fe-, Co-, Ni- and Mn-porphyrins, the second one - for metal free porphyrins, their complexes with Mg, Zn, Cd, Al, and Sn, chlorophylls and pheophytins. The rate constants of chemical quenching of singlet oxygen (K_{ox}) were measured for the second group of compounds. The data suggest that chemical quenching is also a consequence of exciplex formation.

K_q and K_{ox} values of carotenoids have been measured and investigated. The data suggest that formation of the exciplexes fully provides for the activity of vitamin A and retinal and for not less than 10% of activities of β-carotene and zeaxantin.

The information obtained allow for a new interpretation of some concepts relevant to functioning of the quenchers in living cells. In particular, chlorophylls can be regarded not only as singlet oxygen photogenerators but also as photosynthetic membrane protectors.

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SINGLET MOLECULAR OXYGEN IN PLANETARY ATMOSPHERES

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Airglow emissions from singlet molecular oxygen have been observed in the atmospheres of Earth, Mars and Venus although the emission intensities of the different systems are very different. Recent observations are reviewed and the vibrational development of the different emission systems discussed in some detail. A model which can describe this vibrational development is also presented.

The excitation mechanisms for the different emissions are considered and from a detailed examination of the volume emission profiles in the Earth's atmosphere it is shown that the emissions arising from the low lying singlet states are probably excited by energy transfer from some precursor. The required reaction rates are determined and it is suggested that the oxygen recombination reaction

$$O + O + M \longrightarrow O_2 + M$$

leads to a preferential population of one electronic state. From a comparison between the Earth and Cytherean airglow it is proposed that the precursor, and the preferred state, is $O_2(c \, {}^1\Sigma_u^-)$ and that the transfer rates are vibrational level dependent. An attempt to use a vibrational level independent scheme is also described and the resulting rate constants for the quenching of the precursor are determined. These nature of these values is to provide direct support for the laboratory measurements of Ogryzlo et al (1984).

E.A. Ogryzlo, Y.Q. Shen and P.T. Wassell, paper presented at COSMO 84.

t-Butylated hydroxytoluene sensitized photooxidation of the fatty acids:
Inhibition by diazabicyclooctane and role of singlet oxygen.

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Envrironmental ultraviolet radiation, primarily of solar origin is
generally conceded to be the primary etiologic agent for common forms of human
skin cancer. In recent years, there has been accumulating evidence that
antioxidants such as BHT, BHA, glutathione, etc. provide a considerable pro-
tection against photocarcinogenesis. The inhibitory action of antioxidants is
believed to act through free radical quenching, thus preventing lipid peroxi-
dation of cellular membranes. However, photooxidation of lipids in biomem-
branes is believed to occur through photosensitization via Type I or Type II
mechanisms which are believed to be not affected by common antioxidants used
to prevent 'dark oxidation'. The inhibitory effect of these antioxidants on
photocarcinogenesis may thus be operating through some other mechanisms than
by simple free radical quenching. In order to determine whether or not the
phenolic antioxidants can prevent the photooxidation of lipids, we have
studied the in vitro effect of some commonly used antioxidants on photooxida-
tion of unsaturated fatty acids. Irradiation (wavelength > 320 nm) of methyl
linoleate with unfiltered black light (10 x 20 watt) showed less than 1% con-
sumption. A marked increase in the photooxidation (18%) of methyl linoleate
was observed in presence of BHT. Similarly, consumption of methyl linolenate
and arachidonate was much lower with black light alone (15.5 and 19.8%
respectively) in comparison to when BHT was present (57.9 and 67.6%
respectively). Conversely, BHA and tocopherol acetate did not show any
significant effect on the photooxidation. Enhanced photoxidation by BHT was
shown to involve $^1O_2$ by characterization of the lipid hydroperoxides formed
and by the effect of singlet oxygen quenchers. It was further shown that a
diet supplemented with BHT and some other antioxidants enhanced photocar-
cinogenesis instead of reducing it. Whether this enhancement occurs through
$^1O_2$ is under further investigation.

Relaxation of the $^1\Delta_g$ state in pure liquid oxygen and in liquid mixtures of $^{16}O_2$ and $^{18}O_2$.

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We investigated the relaxation of the first excited electronic $^1\Delta_g$ state and the vibrational levels of the electronic ground state $^3\Sigma_g^-$ of oxygen in the liquid phase. The experiments were done in natural O_2, $^{18}O_2$, and $^{16}O_2$-$^{18}O_2$ mixtures. The relaxation rate constants of the electronic states and the vibrational states were determined from time-resolved measurements of the fluorescence light and from time-delayed spontaneous anti-Stokes Raman scattering, respectively.

The following results were obtained:

i) The relaxation rate constants of the $^1\Delta_g$ state in liquid natural O_2 and $^{18}O_2$ are 2.2×10^4 s^{-1} and about 100 s^{-1}, respectively, at 77 K.[1,2] The relaxation rate is determined by intersystem crossing to higher vibrational levels of the electronic ground state

$$^1\Delta_g(v'=0) + {}^3\Sigma_g^-(v'=0) \rightarrow {}^3\Sigma_g^-(v) + {}^3\Sigma_g^-(5-v) + \Delta E_v, \quad (v=0-5).$$
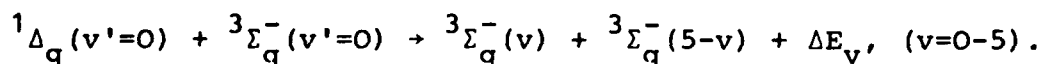
Calculations of the transition probability using the theory of Schwartz, Slawsky, and Herzfeld[3] (SSH) modified to include attractive forces[4] show, that the collisional deactivation of the $^1\Delta_g$ state to the vibrational levels

- 2 -

v=2 and 3 of the electronic ground state should be do-
minant. The higher vibrational levels relax by rapid
vibrational-vibrational energy exchange. In liquid na-
tural O_2 the energy accumulates in the $^3\Sigma_g^-(v=1)$ state
and is transferred slowly ($\sim$ ms) to translational motion.

ii) At high $^1\Delta_g$ population additional relaxation channels
cause a non-exponential decay of the $^1\Delta_g$ state.[2] The
energy pooling reaction to the second excited electro-
nic $^1\Sigma_g^+$ state of O_2 is an important decay channel.

iii) In $^{16}O_2$-$^{18}O_2$ mixtures there is a nonlinear dependence
of the $^1\Delta_g$ relaxation rate constant k on $^{16}O_2$ concen-
tration. For an explanation of the experimental results
we discuss the following points: changes of the transi-
tion probability in the mixtures compared to the pure
liquids, which are calculated from the SSH theory,[3,4]
and the concentration dependence of the collision fre-
quencies in the liquid mixtures.

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Structure and Kinetics of the Uncatalyzed Decomposition of Trialkyl Phosphite Ozonides. <u>G.D. Mendenhall</u>, R.F. Kessick, and M. Dobrzelewski, Department of Chemistry and Chemical Engineering, Michigan Technological University, Houghton, MI 49931.

Activation parameters for uncatalyzed loss of oxygen from phosphite ozonide solutions (equation (1)) were measured for a number of different

$$(RO)_3P + O_3 \longrightarrow (RO)_3PO_3 \longrightarrow (RO)_3PO + {}^1O_2 \qquad (1)$$
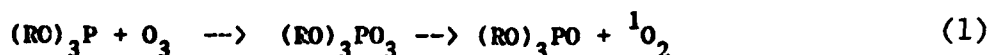
phosphites in several solvents. Triphenyl phosphite ozonide (TPPO) in toluene, chlorobenzene, and acetonitrile showed Ea (kcal/mole) = 19.4-20.1, and log (A/sec) = 13.5-13.6, whereas in dichloromethane the rate constants were not very reproducable and depended on the precautions followed to exclude moisture from the ozonide preparations. Activation parameters for TPPO in dichloromethane reported in the literature, or derived from reported rate constants vary from log(A/sec) = 8.1-11.9 and Ea (kcal/mole) = 14.4-17.2. Other phosphite ozonides show activation parameters which range approximately between the extremes given above for TPPO, even in solvents other than dichloromethane. We suggest that the decompositions with relatively high Ea and log A proceed through a singlet biradical intermediate, and those with low and variable activation parameters involve a zwitterion or an induced decomposition. The greater stability of cyclic and bicyclic ozonides is suggested to result in part from O-O bond strengthening in the cyclic cases, analogous to O-H bond strengthening in certain bicyclic hydroxylamines. An earlier theory involving O-O bond cleavage in the ozonide and pseudorotation of the resulting open-chain intermediate is discussed. A molecular weight determination and the 31P-NMR spectrum of trimethylolphosphite ozonide are consistent with a monomeric, non-equilibrating structure.

Singlet Oxygen Oxidation of Substituted Thiobenzamides. Robert W. Murray
and Sudhir K. Agarwal, Department of Chemistry, University of Missouri-
St. Louis, St. Louis, Missouri 63121.

Thiobenzamides are believed to require metabolic activation in order
to express their observed hepatotoxicity. Hanzlik and coworkers have
shown that relative toxicity, using several indices of toxicity in vivo,
can be correlated with thiobenzamide substituent Hammett σ values in a
manner which is consistent with an electrophilic biooxidation process.
They have also found a similar $\rho\sigma$ correlation when using substitute
thiobenzamides and H_2O_2 as the oxidant. During the course of our studies
on the use of carbonyl oxides as models for the flavin-assisted mono-
oxygenases we have had reason to study the reactions of substituted
thiobenzamides with singlet oxygen. The relative rates of these reactions
have been found to fit a Hammett $\rho\sigma$ correlation with $\rho = -0.39$ indicative
of an electrophilic oxidation process.

POSSIBLE ROLE OF SINGLET OXYGEN IN THE CONTROL OF THE PHOTOTACTIC REACTION
SIGN OF THE CYANOBACTERIUM ANABAENA VARIABILIS.

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Marburg, FRG.

In earlier papers (Nultsch et al., Arch.Microbiol. 134, 33-37, 1983; Nultsch
and Wenderoth, Z.Pflanzenphysiol. 111, 1-7, 1983) it has been shown that the
phototactic reaction sign of the cyanobacterium Anabaena variabilis at high
fluence rates is reversed from negative (movement away from the light source)
to positive (movement toward the light source) by addition of 10^{-3} M sodium
azide. Moreover, photobleaching is also prevented by 10^{-3} M azide (Nultsch et
al., Z.Pflanzenphysiol. 111, 327-332, 1983). Based on these findings the
hypothesis has been proposed that the phototactic reaction sign reversal gene-
rator of Anabaena is controlled by the level of an active oxygen species,
probably singlet oxygen, which is quenched by azide.
In order to confirm this hypothesis, the effects of other quenching agents
have been studied. $5 \cdot 10^{-3}$ M DABCO (= diazabicyclo-2,2,2-octane) reverses the
reaction sign from negative to slightly positive, but is cytotoxic at higher
concentrations. L-Histidin inhibits negative phototaxis at concentrations
$< 5 \cdot 10^{-4}$ M, whereas positive phototaxis is not influenced. On the contrary
$2 \cdot 10^{-2}$ KJ inhibits both positive and negative phototaxis. This could be due
to a quenching of the photoreceptor triplet state.
Experiments with phosphate buffer of different pH demonstrate that the transi-
tion point from positive to negative phototaxis is shifted from 2.7 Wm^{-2} at
pH 7 to 10 Wm^{-2} at pH 6, and that at pH 5.5 only positive responses occur
even at 50 Wm^{-2}. At lower pH values movement ceases because of the decrease
of the proton motive force. If imidazole buffer (pH 6,0) is used, negative
phototaxis is suppressed the more the higher the imidazole concentration is.
Above $2,5 \cdot 10^{-4}$ M positive reactions occur at 13,5 Wm^{-2}.
These results as well as some experiments being in progress support the hypo-
thesis that the singlet oxygen level regulates the phototactic reaction sign,
probably via a reaction sign reversal generator, this way enabling the
Anabaena filaments to escape dangerous light conditions by negative phototaxis.

The Yield of $O_2(b^1\Sigma_g^+)$ in Oxygen Atom Recombination

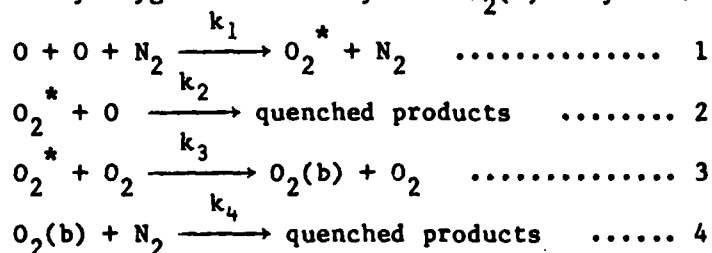E.A. Ogryzlo, Y.Q. Shen and P.T. Wassell

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Earlier laboratory studies of the yield of $O_2(b^1\Sigma_g^+)$ in the recombination of oxygen atoms in the gas phase resulted in values which were too small to account for the observed emission from $O_2(b)$ in the atmospheric night airglow. We have reinvestigated this process in a fast flow system using the N+NO reaction as a source of oxygen atoms. The rate of formation of $O_2(b)$ along the observation tube was monitored by following the 762 nm emission from this state.

In the absence of any added O_2, the production of $O_2(b)$ in the stream of oxygen atoms was found to occur even more slowly than indicated by earlier work. When O_2 was added to the system a dramatic increase in the $O_2(b)$ production rate was observed. We have measured the kinetic dependencies of the emission intensity when a steady state concentration of $O_2(b)$ is reached. These results indicate that the process cannot be described by the simple termolecular reaction:

$$O + O + O_2 \longrightarrow O_2(b) + O_2$$
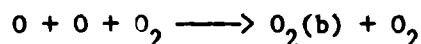
The observations are, however, consistent with the mechanism described by reactions 1-4, in which recombination initially occurs into a precurser (O_2^*) which is quenched by oxygen atoms and yields $O_2(b)$ only on collision with O_2.

$$O + O + N_2 \xrightarrow{k_1} O_2^* + N_2 \quad \cdots\cdots\cdots\cdots \quad 1$$

$$O_2^* + O \xrightarrow{k_2} \text{quenched products} \quad \cdots\cdots\cdots \quad 2$$

$$O_2^* + O_2 \xrightarrow{k_3} O_2(b) + O_2 \quad \cdots\cdots\cdots\cdots \quad 3$$

$$O_2(b) + N_2 \xrightarrow{k_4} \text{quenched products} \quad \cdots\cdots \quad 4$$

When the O_2 concentration is large reaction 1 is rate controlling and k_1 can be calculated from a measurement of the steady state $O_2(b)$ concentration and an independent measurement of k_4. The value which we obtain for k_1 $(1.6(\pm0.2)\times10^{-34} \text{ cm}^6 \text{ molec}^{-2} \text{ s}^{-1})$ is confirmed by separate measurements of the rise time for this state.

The identity of O_2^* remains uncertain. Our results indicate that it cannot be identified with the lower vibrational levels of $O_2(A)$, $O_2(A')$ or $O_2(c)$ since the ratio k_2/k_3 for O_2^* is 3.6±0.7 which is much too small for these states.

Optical Determination of the Rates of Formation and Decay of $O_2(^1\Delta_g)$
in H_2O, D_2O and Other Solvents

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Abstract

Recent experimental investigation, using dissolved photosensitizers subjected to pulsed laser radiation, has permitted accurate determination of the quenching rates of $O_2(^1\Delta_g)$ in a wide variety of solvents. Of particular importance in this application is the use of remote optical sensing to monitor the temporal behavior of the resultant $O_2(^1\Delta_g)$ concentration produced.[1-3] Such optical sensing involves detection of the relatively weak emission accompanying the collisionally induced radiative transition to the $^3\Sigma_g{}^-$ ground electronic state. One distinct advantage inherent in this method, in addition to the fact that it obviates problems associated with alternatives such as chemical acceptors, is that a positive identification of the emitting species as $O_2(^1\Delta_g)$ is possible through determination of the emission spectrum.

Another important advantage of remote optical monitoring is that $O_2(^1\Delta_g)$ concentrations may be determined in a heterogeneous medium,[4] one of the most important applications in this regard being the interface separating a lipid and aqueous phase. It is clear that rather significant $O_2(^1\Delta_g)$ concentration gradients at this interface must occur due to the fact that in general lifetimes in H_2O are approximately an order of magnitude shorter than in a hydrocarbon environment. Recent studies[5] have shown that the quenching rate for a given solvent depends strongly on the presence of bound H atoms and in particular on the exact nature of the binding, i.e., C-H or O-H type.

The relatively long decay times in hydrocarbon solvents provide a particularly favorable case for optical monitoring. Thus, for example, the lifetime of $O_2(^1\Delta_g)$ in benzene has been established as lying in the range 30-35 μsec. Addition of OH groups causes a systematic lowering of lifetimes, e.g., 15 μsec for ethanol and 48 μsec for ethylene glycol.[4] Earlier measurements of the lifetimes in H_2O have ranged from 2-7 μsec. Factors tending to complicate lifetime measurements in this important case are twofold: (1) relatively long (2-3 μsec) times required for transfer of energy from the sensitizer triplet, and (2) interference due to the presence of an infrared component of the sensitizer fluorescence. Separation of the latter from the $O_2(^1\Delta_g)$ emission places a premium on fast detector response time. Earlier measurements indicated that germanium photodiodes used as detectors were inadequate in this regard. Recent measurements, however, using an InGaAs device have yielded much more satisfactory results.[4]

The problem of determination of the lifetime of $O_2(^1\Delta_g)$ in H_2O in addition to requiring detectors capable of rapid response also requires use of a sensitizer capable of providing a high $O_2(^1\Delta_g)$ yield and exhibiting high resistance to subsequent oxidation. Evaluation of a series of water soluble synthetic porphyrins led to the selection of tetra(4-N-methylpyridil) porphin as being the optimum sensitizer. This sensitizer, soluble in H_2O over a wide range of

pH, proved extremely resistant to degradation even when incident laser pulse energies of 100 millijoules were used, ten times the level used normally. Possibly this inertness is due to an absence of photoionization processes.[6]

A detailed series of measurements was carried in H_2O using a T4MPP sensitizer for air-saturated, oxygen-enriched and oxygen-depleted samples, for low, medium and high pulse energies. No systematic dependence of decay time on laser pulse energy was observed; however, a definite shortening of the formation time in air-saturated solutions was observed with increasing laser power. Due to the fact that the triplet transfer times and $O_2(^1\Delta_g)$ decay times were very close to the same value for the case of air saturation, it was impossible to carry out an accurate determination of the respective time constants. Thus, determination of $O_2(^1\Delta_g)$ decay times necessitated the use of oxygen-enriched samples with correspondingly reduced triplet energy transfer times. Statistical averaging of the decay times for 14 independent runs gave the value $\tau_D = 3.21 \pm .12$ μsec. In all these runs the sensitizer concentration was fixed at 1×10^{-4}M. Similar runs with the concentration reduced to 5×10^{-5}M produced results in agreement with this value. Using this value for the $O_2(^1\Delta_g)$ decay time, triplet transfer times were extracted from the formation times characterizing the air-saturation cases. Analysis of the data gives $\tau_T = 2.43 \pm .18$ μsec.

Similar runs were carried out using the same sensitizer in D_2O. In this case the $O_2(^1\Delta_g)$ decay time is greatly lengthened while the triplet transfer time is essentially unchanged thus permitting the use of air saturated solutions. Analysis of the data yielded $\tau_D = 68$ μsec and $\tau_T = 2.1$ μsec. Thus solvent deuteration gives rise to an increase in the $O_2(^1\Delta_g)$ decay time of more than an order of magnitude. It is interesting to point out that earlier measurements using another water-soluble synthetic porphyrin tetra(4-sulfonato-phenyl)porphine gave for D_2O an initial value of 55 μsec, however, this value steadily decreased with increasing length of exposure to the laser radiation. This sensitizer degradation is possibly due to its known ability to photoionize[6] coupled with the relatively long $O_2(^1\Delta_g)$ lifetime. No such effects were observed using the T4MPP sensitizer.

Other solvents investigated were: ethylene glycol, characterized by increased viscosity and correspondingly lengthened triplet transfer times; 1-octanol with similar behavior, however with a significantly longer $O_2(^1\Delta_g)$ decay time; glacial acetic acid and formamide.

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Optical Determination of $O_2(^1\Delta_g)$ Quenching Rates and Relative
Emission Intensities in High Pressure Oxygen Gas Using
Pulsed Laser Excitation at 1.064 μ

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Abstract

Singlet molecular oxygen in the $v' = 1$ vibrational level of the $^1\Delta_g$
electronic state was generated in high pressure oxygen gas by means of pulsed
laser radiation at a wavelength of 1.064 μ. The resulting $O_2(^1\Delta_g)$ population
was monitored optically, using a remotely positioned germanium photodiode in
combination with appropriate filtering to record the subsequent infrared
emission. Pressures ranged from approximately 40 to 95 atm. Measurements
were carried out both in the gas phase and also with the oxygen dissolved in a
freon solvent.

The observed time dependence of this infrared emission in the gas phase
is well described by an exponential with an average time constant $p_o\tau$ = .0221
sec-atm. Runs were also made in which a sizable helium addition was used to
investigate possible effects due to an increased vibrational quenching rate on
the overall $O_2(^1\Delta_g)$ deactivation process. A slight increase in the time
constant to the value .0250 sec-atm was found, comparing favorably with the
value .0244 sec-atom obtained from earlier measurements on a similar mixture
using a photoacoustic technique.[1] Lifetimes obtained by others using infrared
monitoring techniques, in which significantly different methods of $O_2(^1\Delta_g)$
generation were used, lie in the range from .0179 to .0259 sec-atm, encom-
passing the above values.

A series of runs was devoted to a determination of the dependence of
emission intensity on oxygen pressure, with peak intensity being recorded as
the pressure was increased sequentially from an initial value of 47.9 atm to a
final value of 80.9 atm. A plot of the logarithm of relative emission inten-
sity I_R versus logarithm of pressure p_o indicated that $I_R = Kp_o^3$. It is well
known that the optical absorption at 1.064 μ leading to the formation of
$O_2(^1\Delta_g)$ varies quadratically with pressure and the presence of the additional
factor thus implies that the emission rate must depend linearly on p_o in
accord with experimental results obtained at lower pressure. The emission
process at these pressures is therefore collisionally induced. Calculation,
using the empirical dependence of radiative lifetime on gas pressure esta-
blished by Badger et al.[2] at lower pressures, indicates that in the pressure
interval from 50 to 100 atm, this lifetime should range from 10 to 20 seconds.

Recent data indicates $O_2(^1\Delta_g)$ radiative lifetimes in various solvents to
actually be independent of the particular solvent and to be equal to a value
of approximately 4 seconds.[3] The suggestion is therefore that for solvents
exhibiting high oxygen solubility, such as the freons, sequential pressuriza-
tion to levels approaching 100 atm from lesser values ought to span an
important range, at the lower end of which emission due to $O_2(^1\Delta_g)$-solvent
collisions is dominant, to the upper end where $O_2(^1\Delta_g)$-oxygen collisions

control. At lower pressures, therefore, a quadratic dependence of emission intensity on pressure is expected, while at higher pressures transition to a cubic dependence should be observed.

Thus a series of runs was carried out in which the cylindrical observation section of a high pressure cell was filled with Freon-113 (1,1,2-trichlorotrifluoroethane) and the overlying volume carefully pressurized with oxygen. The laser was positioned so that its beam axis coincided with that of the observation section of the cell. With the laser incident pulse energy fixed at 40 millijoules, $O_2(^1\Delta_g)$ decay curves were obtained at time intervals of one hour subsequent to pressurization. Corresponding decay times and amplitudes were obtained from each run. These times were observed to decrease steadily as oxygen diffused to the center of the cell, with an increase in the amplitude factor.

Equilibrium was attained after a period of eight hours. The dependence of the reciprocal equilibrium time constants on oxygen pressure was linear, with a slope consistent with the gas phase data and an intercept of 1.30 $(millisec)^{-1}$ corresponding to a time constant of 770 μsec, i.e., the deactivation time for $O_2(^1\Delta_g)$ infinitely dilute in the Freon-113 solvent. This value is substantially smaller than that obtained for this solvent under air-saturated conditions.[4]

Dissolved oxygen levels were determined as a function of time from the sequential values of decay times obtained during the approach to equilibrium. Using a simplified model of the cell geometry, axial oxygen levels were calculated as a function of time from a one-dimensional diffusion equation. Comparison of results of these calculations with the experimental data yielded a diffusion coefficient $D = 5.8(-5)$ cm^2/sec.

The dependence of emission intensity on oxygen pressure exhibited a significant departure from a cubic dependence at the lowest pressures indicating the dominance of $O_2(^1\Delta_g)$-solvent collisions in this region. However, the onset of the $O_2(^1\Delta_g)$-oxygen collision controlled regime occurs at lower values of pressure than one would predict from lifetime calculations based on Ref. 2.

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Reactive Oxygen Species in the Biological Effects of Solar Ultraviolet Radiation.

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Ultraviolet action spectra for the killing of Escherichia coli by ultraviolet (UV) and visible radiations in the presence and absence of ground state oxygen were prepared. No oxygen enhancement ratios (OERs) were obtained at wavelengths shorter than 320 nm. Above this wavelength, we observed OER values that increased with increasing wavelength up to a value of about eight at 405 nm (1). Our hypothesis of the mechanism whereby molecular oxygen participates in the lethal effects of these longer wavelengths is that the radiation reacts with an endogenous photosensitizer, which in turn excites ground state oxygen into one or more reactive oxygen species, which then kill the cell. It is generally believed that it is damage to DNA that is the critical cause of this cell killing (2). We have identified one of the cellular sensitizers involved in these lethal events in E. coli as 4-thiouridine (s^4U), a rare base found in tRNA. This nucleoside is responsible for 20 to 50% of the lethal events caused by 334 nm UV radiation (3).

In order to avoid problems with permeability barriers in the cells, we used purified genetically active DNA from B. subtilis in our studies. We measured its genetic activity and molecular weight after treatment with near-UV radiation and a variety of added modifying compounds (quenchers and sensitizers). The following results were obtained. Experiments in which the purified DNA was irradiated anoxically by monochromatic near-UV radiation (> 320 nm) showed that oxygen plays a role in the loss of DNA genetic activity and also in the induction of DNA single-strand breaks (SSBs). Any of the quenchers--histidine, diazobicyclo[2.2.2]octane (DABCO), aminoethylisothiouronium bromide hydrobromide (AET), benzoate, azide, and glycerol--added during irradiation were shown to protect the DNA against near-UV radiation damage (see 2 for review). Replacement of H_2O with D_2O during the irradiation doubled the rate of induction of SSBs in the DNA. Our UV action spectra for the modifying effects (histidine, AET, DABCO, glycerol) all show maximum protection near 350 nm. At 300 nm and below, no protective effect is shown. In other experiments, a number of putative sensitizers were added to the DNA during irradiation. These compounds were the free nucleosides s^4U and 5-methylaminomethyl-2-thiouridine, the free base 2-thiouracil (s^2Ura), the nucleotide coenzyme riboflavine, and the oxidized and reduced forms of nicotinamide adenine dinucleotide. All these compounds increased the induction of SSBs in DNA by monochromatic 334 nm UV radiation (4). As s^2Ura was the most effective photosensitizer for induction of the SSBs, it was selected for further study. Maximal enhancement by s^2Ura of SSB induction was shown to occur when s^2Ura was present at a concentration of approximately 0.001 M. Anoxia, histidine, DABCO, benzoate and glycerol when present separately during irradiation in the presence of 0.001 M s^2Ura were all shown to decreased the number of SSBs caused by s^2Ura and 334 nm UV radiation. Studies were performed of the concentration dependence of the protection by DABCO and by benzoate against the SSBs caused by 334 nm radiation in the

presence of 0.001 M s^2Ura. The sigmoidal shapes of these curves showed maximal protection by DABCO and benzoate at a concentration of 0.1 M against SSBs caused by 334 nm radiation in the presence of s^2Ura and no protection below a concentration of 0.001 M. When H_2O was replaced by D_2O during irradiation of the DNA by 334 nm radiation in the presence of s^2Ura, the rate of induction of SSBs was about doubled. Finally, superoxide dismutase reduced by about 20% the rate of induction of SSBs in the DNA by 334 nm radiation in the presence of 0.001 M s^2Ura.

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SYNERGESTIC AND ANTAGONISTIC EFFECTS OF QUENCHING SINGLET OXYGEN IN A POLYMER MATRIX

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A deterioration of many polymeric materials (rubbers,plastics, fibers and biopolymers) occurs under sunlight irradiation in photooxidation processes (light ageing processes) in which a singlet oxygen $^1O_2$ may play a considerable role [1]. The most susceptible polymers towards $^1O_2$ oxidation are polydienes [2-4]. The reaction of $^1O_2$ with polybutadiene (PB) leads to the formation of allylic hydroperoxide according to the ene-type mechanism.

Various compounds have been reported to quench $^1O_2$ in the solution or polymer matrix. These compounds include caretenoids [5], hindered piperidines [6],phenols [7],etc. However,all of these compounds are either inefficient quenchers or are themselves unstable and subject to photooxidation.

In this paper we present results of the synergestic and antagonistic effects of quenching of $^1O_2$ by the mixture of hindered phenols and hindered piperidines in a PB matrixes and in solution.

It is generally accepted that the presence of carbonyl groups (internal and/or external impurities) in a polymer matrix can be responsible for the generation of $^1O_2$ (energy transfer mechanism). Considering a fact that triplet-excited benzophenone can produce $^1O_2$ [8] we have studied its reaction with PB. Hindered phenols beside their reactions with $^1O_2$ may also react with triplet-benzophenone to yield fuchsone derivatives. Addition of hindered phenoles decreases sensitized photooxidation of PB. A mechanism of these reactions is discussed in a detail.

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"The Relaxation of $O_2(^1\Delta_g)$ in Condensed Systems"

This paper will serve largely as a brief introductory statement for the session on "Relaxation and Quenching." The major focus will be on the non-radiative decay of $O_2(^1\Delta_g)$ in liquid systems as determined through measurements of the radiative process $^3\Sigma_g^- \longleftarrow {}^1\Delta_g$ that result in luminescence at 1.27 μm. Time resolution of this luminescence to the submicrosecond regime has been achieved by the use of high intensity laser pulses, fast photodetectors and high bandwidth amplifiers. This has led to rapid, straightforward and precise measurement of $O_2(^1\Delta_g)$ lifetime values (τ_Δ) in a variety of solvent systems. Recent work has shown that bimolecular quenching constants for solvent quenching exhibit regular variations throughout homologous series of n-alkyl compounds and additivity parameters can be evaluated. This leads to the possibility that τ_Δ values can be predicted from a consideration of molecular structure. Other data will be presented on the non-radiative decay of $O_2(^1\Delta_g)$ in micro-heterogeneous liquid systems and water-swollen polymers.

ON THE USE OF WATER-SOLUBLE $^1O_2$ SOURCE FOR MECHANISTIC STUDY AND FOR OXYGENATION OF BIOLOGICAL SYSTEMS

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We observed the formation of $O_2^{\cdot-}$ from the reaction of electron-rich substrates, e.g., N,N-dimethylanilines and $^1O_2$ generated from a water-soluble endoperoxide 1 in aqueous media.[1,2] We suggested that the substrates with oxidation potentials less than 0.5 V vs. SCE are capable of undergoing one electron-transfer to $^1O_2$ to generate $O_2^{\cdot-}$ in polar aqueous solvents.[2] In order to know the generality of $O_2^{\cdot-}$ formation, we have examined the reaction of thioanisoles with $^1O_2$ in aqueous solvents. p-Hydroxythioanisole ($E_{1/2}$ = 0.48 V vs. SCE) was readily oxidized to the corresponding sulfoxide at 35 °C in the presence of 1 in aqueous methanol. In this reaction the formation of $O_2^{\cdot-}$ was confirmed by the assay utilizing a combination of NBT and SOD. Addition of increasing amounts of NBT reduced the yield of sulfoxide with the enhancement of NBT reduction. The result suggests that $O_2^{\cdot-}$ is directly involved in the sulfoxidation.

Other example for the use of the water-soluble $^1O_2$ source is the oxidative cleavage of DNA. $^1O_2$ is known to selectively oxidize guanine base among nucleic acid bases. We have synthesized a new intercalator (2) possessing $^1O_2$ source 1 at the side chain. Incubation of a 5'-[^{32}P]-end-labeled DNA fragment with 2 at 35 °C in phosphate buffer (pH 7) followed by piperidine treatment gave rise to the efficient cleavage of the DNA chain selectively at guanine residues.

Usefulness of polymer-bound $^1O_2$ source in oxygenation of organic substrates will also be discussed.
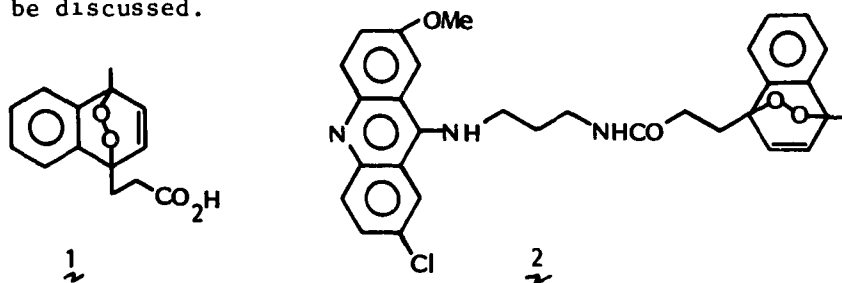
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COSENSITIZED ELECTRON-TRANSFER PHOTOOXYGENATION

*A. Paul Schaap,[*] Shahabuddin Siddiqui,
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9,10-Dicyanoanthracene (DCA) and biphenyl (BP) cosensitize the electron-transfer photooxygenation of epoxides in oxygen-saturated acetonitrile to form ozonides. These epoxides do not measurably quench the fluorescence of DCA and are, therefore, relatively unreactive under standard photooxygenation conditions with only DCA as sensitizer. However, a dramatic increase in the rate of reaction is observed upon the addition of BP as a non-light-absorbing cosensitizer. An investigation of the stereochemistry of the reaction with *cis*- and *trans*-2,3-diphenyloxiranes has shown that both epoxides are converted exclusively to the *cis*-ozonide. This striking stereochemical result is consistent with a proposed mechanism involving singlet oxygen addition to an intermediate carbonyl ylide.
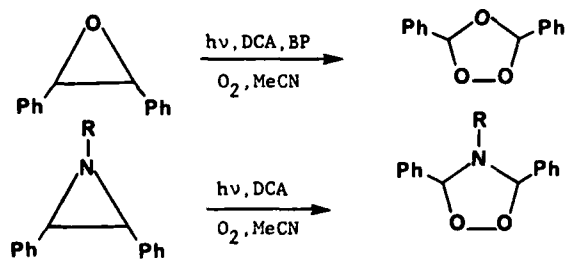
DCA-sensitized photooxygenation of aziridines provides the corresponding 1,2,4-dioxazolidines. The addition of biphenyl as a cosensitizer is not required in this case as the more easily oxidized aziridines efficiently quench the fluorescence of DCA. The stereochemistry of the product is a function of R. The *cis* isomer is virtually the exclusive product when R=H. However, the *trans* isomer is the major product when R=PhCH$_2$. A mechanism involving the addition of singlet oxygen to intermediate azomethine ylides is proposed.

NUCLEOPHILIC OXYGEN-ATOM TRANSFER FROM A PEREPOXIDE INTERMEDIATE TO SULFOXIDES

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Gary R. Faler, and Steven R. Villasenor

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Singlet oxygen reacts with adamantylideneadamantane to form an unusually stable 1,2-dioxetane. We have recently obtained evidence for a trappable intermediate in this 1,2-cycloaddition reaction of $^1O_2$. This species, which we propose is the perepoxide shown below, can be quantitatively trapped by nucleophilic oxygen-atom transfer to sulfoxides to yield sulfones and the epoxide. The ratio of epoxide to dioxetane is found to be a function of the concentration of the sulfoxide.

Additional evidence for the proposed mechanism is provided by a study of substituent effects on the trapping reaction. A series of competition experiments with substituted-phenyl methyl sulfoxides gave a Hammett plot with a ρ value of +0.52. These results are consistent with the proposed nucleophilic character of the intermediate. Solvent effects on this reaction have also been investigated.
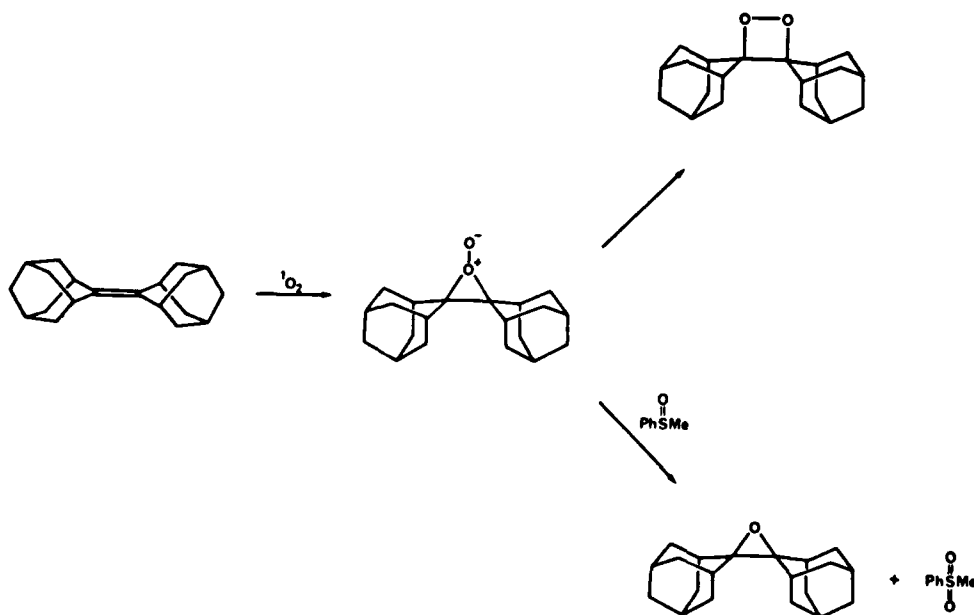
SELF-SENSITIZED PHOTOOXIDATION OF AROMATIC COMPOUNDS AND PHOTOCYCLOREVERSION OF ENDOPEROXIDES. APPLICATIONS IN CHEMICAL ACTINOMETRY.

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The self-sensitized photooxidation of aromatic compounds (HC) leading with quantum yield Q_2 to the formation of thermally stable endoperoxides (HCPO) is a photoreversible process. Via adiabatic photocycloreversion the endoperoxides can be split into the parent aromatic compound and singlet oxygen during short wavelength irradiation[1,2]. Therefore, the HCPO/HC + O_2 systems are in principle photochromic. However, irreversible rearrangement reactions (quantum yield: Q_{dec}) of the endoperoxides generally compete with cycloreversion, thus reducing the number of possible photochromic cycles[3].

HCPO
hν (uv), Q_1 ⇌ HC + O_2
hν (vis) Q_2
hν (uv), Q_{dec}
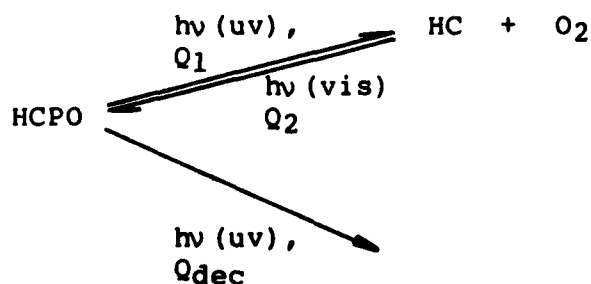
Due to the large shift in wavelength of absorption, which accompanies the photooxidation and the photocycloreversion, the HCPO/HC + O_2 systems are in principle well suited as convenient chemical actinometers, in which the absorbed radiation is determined spectrophotometrically. However, a photochemical system has to meet a number of additional important requirements in order to be a useful chemical actinometer. These are: exact knowledge of the relevant photochemical reactions; reproducibility; almost wavelength independent actinometric factors; high sensitivity; high accuracy; and simplicity in the evaluation of the data.

It will be demonstrated that the self-sensitized photooxidation of mesodiphenylhelianthrene (MDH) fulfils these requirements. Above all MDH is well suited as actinometric material
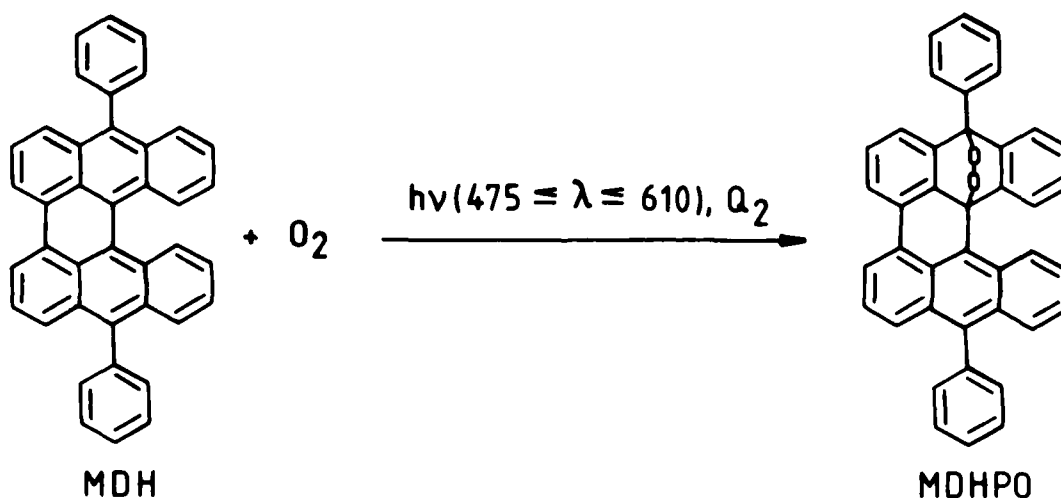
because the quantum yield of photooxidation Q_2 depends neither on the irradiation wavelength nor on the concentrations of MDH and O_2 in the ranges used for actinometry.

The highly reversible photochromic system consisting of hetero-
coerdianthrone (HCD) and its endoperoxide (HCDPO) is excellent-
ly suitable for reusable actinometry in the uv-region. During

$$hv(248 \leq \lambda \leq 334), Q_1$$
$$hv(\lambda \sim 435), Q_2$$

$+ O_2$

HCDPO   HCD
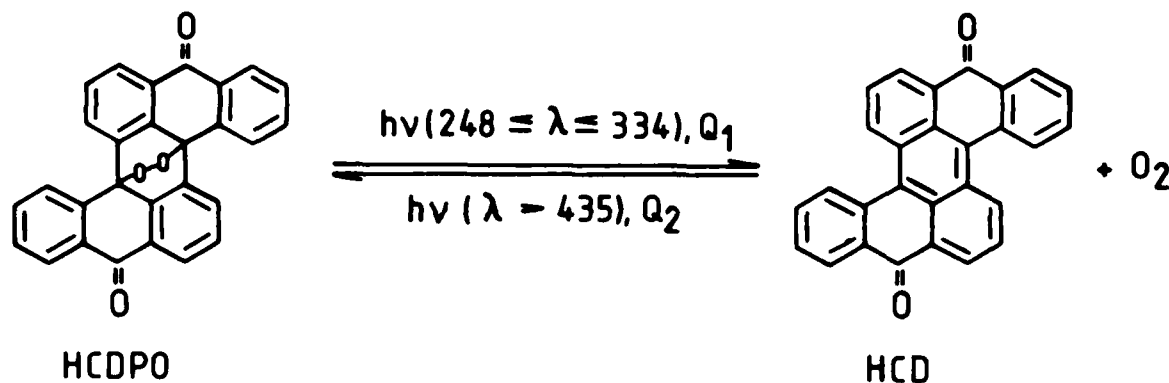
each actinometric measurement the original colourless solution
turns red due to the formation of HCD. Subsequently, the solu-
tion can be decolourized completely by using a cut off filter
and sun light, thus being ready again for the next experiment.
In this way it is possible to do 100 actinometric measurements
with the same solution without loss in accuracy.

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POSSIBLE FORMATION OF SINGLET OXYGEN FROM
VIBRATIONALLY EXCITED WATER

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Solubility of oxygen in H_2O and D_2O is similar and is lower than that in Freon-113 by a factor of about 40. In a 5 cm cell in solutions in Freon-113 (~13MPa), absorption bands of oxygen are clearly observed in the wavelength range 370-1300 nm. However, in solution in water at that pressure only the absorption band at 760 nm is observed.

In the literature solute decomposition has been attributed to singlet oxygen for solutions of O_2 in D_2O (~15MPa) on irradiation at 1056 nm, with a Nd-Yag laser. However, under these conditions absorption of light by D_2O would be about two orders of magnitude greater than that by oxygen. This raises the question, how is singlet oxygen formed in solutions of O_2 in D_2O (~15MPa)?

Solutions of 1,3-diphenylisobenzofuran (DPBF, ~1 x 10^{-5} mol dm^{-3}) in H_2O and D_2O (containing 3% acetonitrile), under 13 MPa oxygen, were partially bleached on exposure to repeated pulses from a Nd-Yag laser. The bleaching was greater in the solution in H_2O. Similar bleaching was also seen when DPBF solutions (open to air) were exposed to light at 990 nm from a 1600 W Xe-arc lamp. Decomposition of solutions of dimethylfuran (1 x 10^{-3} mol dm^{-3}) in H_2O and D_2O (open to air) was also seen on exposure to light, λ >850 nm, from a Xe-arc lamp, but the decomposition appeared to be greater in the solutions in D_2O.

The results suggest that one or both of the following mechanisms contribute to solute decomposition in aqueous solutions irradiated at λ>850 nm: (i) transfer of vibrational energy from water to oxygen to produce singlet oxygen; and (ii) formation of vibrationally excited solute molecules that react with oxygen.

SINGLET OXYGEN AND RIBOSOMES: INACTIVATION AND

SITES OF DAMAGE

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Singlet oxygen has been assigned a significant role in photosensitized processes in biologically important molecules. We have studied the role of singlet oxygen in ribosomes using a number of dyes, psoralens and ketones. With many dyes, evidence has been obtained that singlet oxygen is the inactivation species. However, all of the inactivation seen with some dyes (e.g., acridine orange, methylene blue, etc.) cannot be attributed to singlet oxygen, indicating that other mechanisms are involved. Furthermore, preliminary data with some dyes indicate inactivation in the absence of oxygen (e.g., acridine orange, acridine flavine, etc.). Some dyes (e.g., rhodamine B and ethidium bromide), which reportedly do not cause cell inactivation, cause some inactivation of ribosomes. Inactivation by psoralens is mainly through singlet oxygen. However, singlet oxygen plays a minor role in ketone photosensitized systems, where free radical reactions seem to dominate. Singlet oxygen also appears to be of negligible importance to the inactivation of ribosomes by ionizing radiation.

Analyses of damage to the macromolecules within the ribosomes indicate no chain breaks in ribosomal RNA on photosensitization with the dyes, psoralens or ketones. However, with some dyes, damage to ribosomal proteins is seen by gel electrophoresis.

PREFERENTIAL SOLVATION OF $O_2{}^1\Delta_g$ IN MIXED SOLVENTS

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A cage displacement model[1], appropriate to the description of luminescence decay constants ($1/\tau$) at high quencher concentrations where diffusion along concentration gradients is not a quenching prerequisite, is applied to mixed solvent quenching of $O_2{}^1\Delta_g$. The dependence of $1/\tau$ on mole fractions Y_A and Y_B of quenching cosolvents A and B in the solvent cage, is expressed by

$$\frac{1}{\tau} = \frac{1}{\tau_A} + \frac{Y_B\nu_A}{Y_B\nu_A + Y_A\nu_B}\left\{\frac{1}{\tau_B} - \frac{1}{\tau_A}\right\} \qquad\qquad \text{I}$$

where τ_i is the lifetime, and ν_i the displacement frequency, of $O_2{}^1\Delta_g$ in pure solvent i. Y_A, Y_B are related[2] to mole fractions X_A, X_B in the bulk solvent by

$$Y_A/X_A = (Y_B/X_B)\,\exp\gamma_A \qquad\qquad \text{II}$$

and insofar as ν_i may be expressed in terms of viscosity η_i and molar volume V_i by[1,3]

$$\nu_i\eta_iV_i = RT \qquad\qquad \text{III}$$

eq I rearranges to

$$f(\tau) = (\frac{1}{\tau_B} - \frac{1}{\tau})/(\frac{1}{\tau} - \frac{1}{\tau_A}) = \frac{X_A\eta_AV_A}{X_B\eta_BV_B}\,\exp\gamma_A = f(X)\exp\gamma_A \quad \text{IV}$$

The index γ_A of preferential solvation of $O_2{}^1\Delta_g$ by solvent A in the presence of B, accessible from the experimental quantities $f(\tau)$ and $f(X)$, is found to be essentially zero for a number of polar and non-polar quenching cosolvents; this implies that solvent quenching of $O_2{}^1\Delta_g$ is not determined by different stabilities of solvent - $O_2{}^1\Delta_g$ complexes.

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PRODUCTION AND QUENCHING OF SINGLET OXYGEN BY THE SENSITIZER IN THE DYE-SENSITIZED PHOTOOXYGENATIONS

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Many dyes are commonly used as singlet oxygen sensitizers (S) in photooxygenation reactions. Nevertheless, there remain some significant gaps in our quantitative knowledge of such fundamental parameters as quantum yield of O_2 ($^1\Delta$) production γ_Δ and rate constant k_Q of quenching of singlet oxygen by S. It is particularly important to realize that there are some situations in which the dye molecules themselves may cause quenching of $O_2(^1\Delta)$ since neglecting this fact led to erroneous results in the determination of the lifetimes of singlet oxygen and of the rate constant for its reaction with a substrate. This communication reports the measurement of γ_Δ and k_Q for various dyes in various solvents saturated with oxygen. For example, it is shown that :

(i) the quantum yield γ_Δ is 0.50, 0.79 and 0.60 for methylene blue and rose bengal in methanol and chlorophyll a in benzene respectively

(ii) the rate constant of quenching of $O_2(^1\Delta)$ by rose bengal in acetonitrile is $k_Q \simeq 5\times10^7$ $M^{-1}s^{-1}$.

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The reactions of singlet oxygen with oxazoles have provided a novel method for protecting carboxyl groups which may then be regenerated in an activated form. Recent use of this procedure in the synthesis of dipeptides will be discussed.
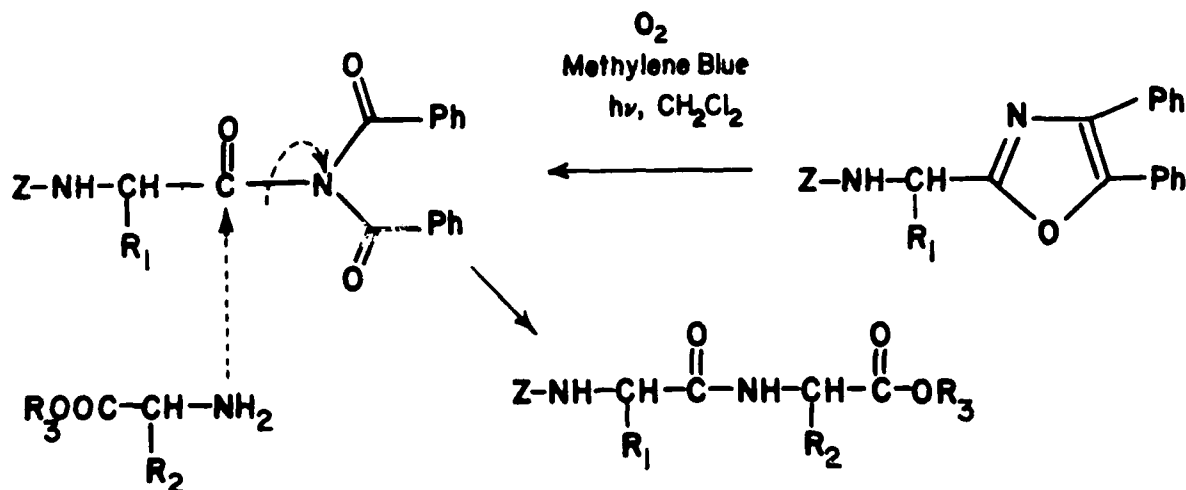
A singlet oxygen reaction which has potential use in synthesis is the oxidation of β-dicarbonyl derivatives leading to vicinal tricarbonyl systems. Thus, while enols of β-diketones show only sluggish reactivity or no reaction with singlet oxygen, the presence of fluoride ion in the reaction medium promotes rapid uptake of singlet oxygen leading to 1,2,3-tricarbonyl products or their hydrates. This reaction most probably results from electron-enrichment in the double bond through hydrogen bonding. Another method for forming vicinal tricarbonyls from β-diketones involves the formation of α-enamines followed by singlet oxygen oxidative cleavage. Synthetic applications will be discussed.
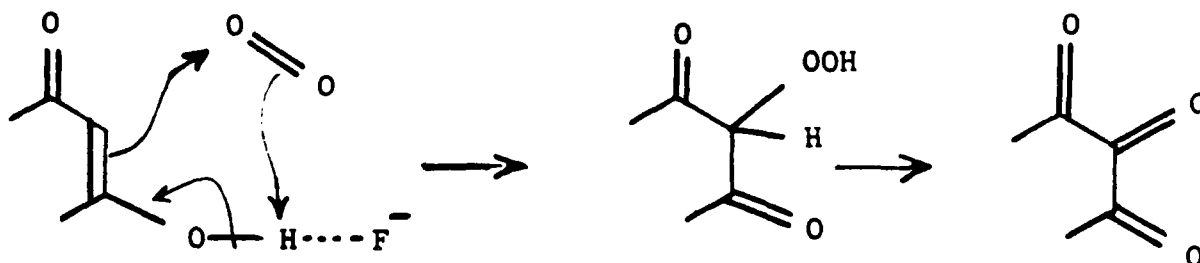
Laboratory Kinetics and Spectroscopy Applied to Airglow Studies

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This survey of excitation processes for singlet oxygen in planetary atmospheres is intended to provide an introduction to the following papers which discuss in more detail laboratory investigations and field measurements. The three singlet states, a $^1\Delta_g$, b $^1\Sigma_g^+$ and c $^1\Sigma_u^-$ contribute to features in the airglows of Venus, Earth and Mars, the most intense feature being in each case the dayglow of the O_2(a $^1\Delta_g$ – X $^3\Sigma_g^-$) 'Infrared Atmospheric Band'. Ozone photolysis is the daytime source of $O_2(^1\Delta_g)$ on Earth and Mars, but is unlikely to be on Venus. Some remaining problems about the ozone photolytic source are reviewed, and possible excitation mechanisms on Venus are considered. O_2(b $^1\Sigma_g^+$) is excited during the day in the Terrestrial atmosphere by a combination of resonance scattering and of energy transfer from $O(^1D)$ to O_2. The state is unimportant on either Venus or Mars because of its sensitivity to quenching by CO_2, the major atmospheric constituent.

Nightglow emissions from $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g^+)$ are much harder to explain than the daytime phenomena, because they must be excited from chemical energy stored in species, such as H, O or O_3, that persist at night. Of the many mechanisms that have been proposed, none has been substantiated explicitly by laboratory experiments, and the hypothetical sources are frequently judged by their ability to match atmospheric measurements of airglow both for absolute intensity and for altitude profile. We consider here the additional factor of the plausibility of the mechanisms.

A particularly important question is whether recombination of ground state atomic oxygen

$$O(^3P) + O(^3P) \xrightarrow{M} O_2^{(*)} \tag{1}$$
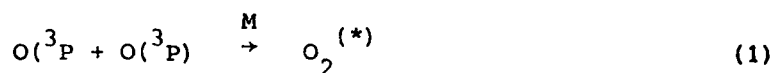
populates the several molecular states, with which the atoms correlate, statistically, or whether some states (perhaps even the ground state) are formed preferentially. Several developments, of which we shall hear more in later lectures, have focussed interest on this problem. For a start, the discovery that $O_2(^1\Delta_g)$ is formed by the recombination of O on pyrex walls has indirectly made it practically certain that the atomic oxygen 'green line' in the airglow is excited by a two-step 'Barth' mechanism. Thus some state $O_2^{\#}$ excited in reaction (1) itself reacts with O

$$O + O_2^{\#} \rightarrow O(^1S) + O_2 \tag{2}$$
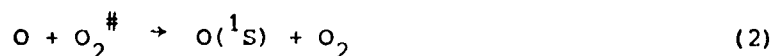
Such mechanisms can, at least on paper, explain the intensities, peak emission altitudes, and layer thicknesses of $O(^1S)$, $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g^+)$ emissions,

although the states #, † and †† of O_2 have not yet been identified. Experimental confirmation of a reaction corresponding to process (4) will be presented later in this meeting.

In the context of these ideas, recent advances in our understanding of the c $^1\Sigma_u^-$ state of O_2, both in atmospheres and in the laboratory, may be relevant. Following the identification of the 'Herzberg II' $O_2(c\ ^1\Sigma_u^- - X^3\Sigma_g^-)$ system in the Venusian airglow, laboratory investigations showed that the c $^1\Sigma_u^-$ state is formed in the O atom recombination. At first, it appeared that CO_2 was a <u>necessary</u> third body M for the appearance of the state, but it then emerged that $O_2(c\ ^1\Sigma_u^-)$ was formed, but in reduced yield, with other third bodies. Clever kinetic studies, in skilled hands, have indicated that O atoms themselves quench $O_2(c\ ^1\Sigma_u^-)$, and also that the peculiar influence of CO_2 may possibly arise from its ability to funnel most of the recombining pairs of atoms to the c $^1\Sigma_u^-$ state. The existence of the laboratory source of $O_2(c\ ^1\Sigma_u^-)$ has made it possible to obtain high resolution spectra of the Herzberg II system. In turn, comparison of these spectra with those of the Earth's airglow have revealed that $O_2\ c^1\Sigma_u^-$ is indeed present in our atmosphere. Two differences distinguish Earth's Herzberg II emission from that of Venus : it is about 27 times weaker, but it does exhibit transitions from vibrationally excited levels, with the peak emission corresponding to v' = 7. The relative paucity of CO_2 in the Earth's atmosphere can explain both observations, if CO_2 is efficient in promoting population of the electronic state as well as in vibrational quenching. More subtle arguments further show that the relative intensities of the Herzberg II system on Venus and Earth, and the virtual absence on Mars, can be related to the concentrations of CO_2, N_2 or O_2 compared to that of O in the atmospheres of the three planets.

Finally, we are forced to ask whether the states of O_2 designated as #, †, †† actually correspond to $O_2(c\ ^1\Sigma_u^-)$ possessing differing degrees of vibrational excitation. Energy requirements are satisfied for reaction (2) for $O_2(c\ ^1\Sigma_u^-)$ with v' greater than ~2 or 3. Reactions (3) and (4) are thermally acccessible for v' = 0. Alternative states for $O_2^\#$, such as the A $^2\Sigma_u^-$ or A' $^3\Delta_u$, are made less likely by the weakness of $O(^1S)$ emission in the Venusian airglow. The v' = 0 levels of c, A, and A' states are all populated in the Venusian atmosphere, but only that of the c state lies <u>below</u> the $O(^1S)$ excitation energy and thus has an activation energy that would make the process slow at the altitude of peak emission. On the other hand, the new evidence about reaction (4) suggests that, even though an energy exchange process excite $O_2(\ b^1\Sigma_g^+)$, none of the states c $^1\Sigma_u^-$, A $^3\Sigma_u^-$ or A' $^3\Delta_u$ can be involve ., at least in their lower vibrational levels.

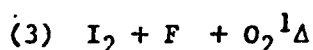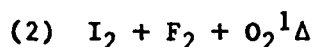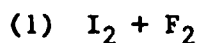# IF $B^3\pi(0^+)$ Pumping by Energy Transfer from $O_2{}^1\Delta_g$ to $IFX^1\Sigma^+$

A Potential Chemical Laser Pumping Scheme.*

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Studies have been made of the IF (B-X) chemiluminescence resulting from the mixing of three sets of reagents:

(1) $I_2 + F_2$

(2) $I_2 + F_2 + O_2{}^1\Delta$

(3) $I_2 + F + O_2{}^1\Delta$

Simultaneous mixing of the third set of reagents produced the most intense IF (B-X) chemiluminescence. In all cases the chemiluminescence indicated that rapid rotational thermalization occurs. When $O_2{}^1\Delta_g$ was present, however, an extremely non-thermal vibrational population in the IF $B^3\pi(0)^+$ was observed.

Indirect evidence suggests that sequential pumping of ground state IF(X) molecules produces the observed chemiluminescence. A possible reaction scheme which describes a sequential pumping mechanism will be presented.

The photon yield measured for the chemiluminescence arising from the third set of reagents is small, $\sim 0.3\%$. This yield can be explained largely by the low densities of $O_2{}^1\Delta_g$ used in these studies. The $I_2 + F + O_2{}^1\Delta_g$

system may hold promise as an efficient chemical excitation source for IF (B), particularly if higher densities of $O_2{}^1\Delta_g$ can be produced and utilized.

Finally, collisional ladder climbing has been observed when the B states of the analogous interhalogens BrF and BrCl have been excited.[1,2] Ladder climbing from vibrational levels close to the predissociation limit result in non-radiative loss to predissociation inhibiting the establishment of a population inversion. No measurements have been published to date on ladder climbing in the IF(B) state, but inferences of its existence from the chemiluminescence data will be discussed.

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SELF-SENSITIZED PHOTOOXIDATION OF PROTOPORPHYRIN IX AND RELATED PORPHYRINS IN ERYTHROCYTE GHOSTS AND MICROEMULSIONS: A NOVEL PHOTOOXIDATION PATHWAY

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Departments of Chemistry

University of North Carolina, Chapel Hill, NC 27514

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ABSTRACT: Previous studies have shown that protoporphyrin IX can sensitize its own photooxidation by paths involving primarily singlet oxygen and to a lesser extent superoxide (via excited state electron transfer). These reactions involve reaction of the vinyl groups of the protoporphyrin to yield porphyrins with modified side chains. Quantum efficiencies are generally low and the product distribution is somewhat solvent dependent. In recent work we have examined the photooxidation of protoporphyrin IX, mesoporphyrin IX and hematoporphyrin IX in erythrocyte "ghosts", a natural membrane system containing saturated lipids, unsaturated lipids and a full complement of membrane proteins. We observe that all three porphyrins are rapidly photooxidized in the "ghosts" and that the products produced from protoporphyrin IX do not include the "usual" singlet oxygen products. We have been able to model the kinetic behavior observed in the natural membrane systems by using an oil/water microemulsion as a solvent medium and adding various amino acids that are easily oxidized. In particular we have investigated the behavior of methionine and a number of related thioether derivatives. The results of this study suggest that the porphyrins sensitize singlet oxygen efficiently but that the

singlet oxygen is rapidly scavenged by substrates such as methionine and other amino acids. The oxygenated amino acids can subsequently act as agents to efficiently oxidize the porphyrins by attacking the porphyrin ring directly. Thus, although singlet oxygen is clearly indicated to be involved in these reactions, the actual agent in the photooxidation of the porphyrins appears to be an intermediate occuring subsequent to its consumption.

THE MECHANISM OF QUENCHING OF
SINGLET OXYGEN BY MOLECULAR IODINE

by

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The various methods for determining singlet oxygen quenching constants are reviewed in Reference 1 which includes a compilation of quenching constants by 690 compounds. Although quenching of singlet oxygen by some alkyl iodides and the iodide ion has been studied[1] quenching by molecular iodine has not been reported previously despite the fact that I_2 quenches excited singlet[2] and excited triplet states[3] of aromatic compounds with high efficiency. We have used a nanosecond pulsed ruby laser to sensitize the production of single oxygen using anthracene and other organic triplet states as singlet oxygen sensitizers and determined the pseudo first-order decay constant of singlet oxygen k_D from a kinetic analysis of the disappearance of a small proportion of a fixed concentration of the singlet oxygen acceptor diphenyl-isobenzofuran, DPBF. In the presence of various amounts of quencher

$$k_D = k_1 + k_R[DPBF]_{AV} + k_Q[Q]$$

where k_1 is the decay constant for singlet oxygen in the absence of DPBF and any quencher Q and k_R and k_Q are the second-order reaction and quenching constants. Thus a plot of k_D versus [Q] is often linear and k_Q can be obtained from the slope (see refs. 1 and 4). In the case of iodine as a potential quencher the plot of k_D versus [Q] is not linear although the initial slope gives a k_Q value of $1.4 \times 10^9 dm^3 mol^{-1} s^{-1}$. The mechanism for quenching and the reasons for the non-linear plot of k_D versus $[I_2]$ will be discussed.

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ACTIVE OXYGEN CHEMISTRY OF OCULAR TISSUES- S. Zigman

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Because they must receive and absorb much radiant energy to perform their visual functions, ocular tissues must also be capable of protecting themselves against free radicals and oxidation products that would destroy them. While only low levels and limited wavelengths of light are required for vision, excessive quantities and visually useless light enter the eye and is absorbed by various tissues whose function is to deliver the high quanta necessary for vision to occur. Besides these considerations, it is important to note that the levels of oxygen vary temendously in these tissues, with very low levels being present in the ocular lens and very high levels in the retina.

Data on the spectral quality and quantities of radiant energy interacting with various ocular tissues, their oxygen content, and enzymes and anti-oxidants present to scavenge or protect against photooxidative damage needs to be collated in order to assess the potential hazard to these tissues relative to oxygen exicted states and radicals. Such data will be provided in our presentation. Besides this information chemopathological conditions have been observed in the ocular tissues (ie: cataract; retinal degeneration) that have been ascribed to imbalances in the above-mentioned system. These will be described and the potential that oxygen excited states and free radicals have an important role in the damges stated will be discussed.

This talk will introduce practical situations relating eye health to the handling of oxygen products induced by radiant energy and metabolism.

MacInnis, K.	G5	Siddiqui, S.	S2
Maier, M.	M1	Singh, A.	S5
Marinelli, W.	H2	Singh, H.	S6
Marsh, K.L.	C1	Snelson, A.	E1
Matsuura, T.	S1	Stanbro, W.D.	P1
McDade, I.C.	L1	Stevens, B.	S7
Mendenhall, G.D.	M2	Sugiyama, H.	S1
Merritt, J.	S6		
Murray, R.W.	E1, M3	Takata, T.	A1
		Tanielian, C.	T1
Nultsch, W.	N1	Taylor, K.	E1
		Trost, W.	B3
Ogryzlo, E.A.	O1		
		Ueno, K.	A1
Parker, J.G.	P1, P2	Umrigar, P.P.	G3
Peak, J.G.	P3	Upton, M.W.	E2
Peak, M.J.	P3		
Prasad, G.	S2	Villasenor, S.R.	S3
Rabek, J.F.	R1	Wassell, P.T.	O1
Ranby, B.	R1	Wasserman, H.H.	W1
Richards, D.S.	B2	Wayne, R.P.	W2
Rodgers, M.A.J.	G2,R2,S7	Whitefield, P.D.	W3
Russell, V.P.	C2	Whitten, D.G.	W4
		Wiesenfeld, J.R.	H2
Saito, I.	S1	Wildt, J.	F1
Salokhiddinov, K.I.	D2	Wilkinson, F.	W5
Schaap, A.P.	S2, S3	Winter, R.	F1
Schaffner, K.	B3		
Schmidt, R.	B3, S4	Young, A.T.	H2
Schuchart, H.	N1		
Shen, Y.Q.	O1	Zabel, F.	F1
Sherren, J.	G5	Zigman, S.	Z1

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