Singlet Molecular Oxygen

By A. A. Gorman
CHEMISTRY DEPARTMENT, UNIVERSITY OF MANCHESTER
MANCHESTER M13 9PL U.K.
and M. A. J. Rodgers
CENTER FOR FAST KINETIC RESEARCH
UNIVERSITY OF TEXAS AT AUSTIN
AUSTIN, TEXAS 78712, U.S.A.

1 Introduction

In 1928 an article in *Nature* appeared which presented an interpretation of the atmospheric oxygen absorption bands and established the electronic levels of the oxygen molecule.[1] Atmospheric spectroscopy allowed measurements of the energy differences between the ground state ($^3\Sigma_g^-$) and the low-lying $^1\Delta_g$ and $^1\Sigma_g^+$ states.[2] With a single notable exception the $^1\Delta_g$ state remained within the province of spectroscopy until the early 1960's, at which time it attracted the attention of solution-phase chemists and biologists; a phenomenal literature on the subject has since arisen, by far the major part since 1965. Singlet oxygen is now believed to be involved in many dye-sensitized photo-oxidations of organic systems and in the quenching of electronically excited molecules. It has been invoked as an intermediate in phenomena such as chemiluminescence, photodynamic action, photocarcinogenicity, and decomposition of O_2-rich compounds. In this review we attempt to present a glimpse of the main features of the accumulated research and to indicate where the future lies. Many excellent reviews[3-14] have appeared over the years which discuss the several aspects of the physics, chemistry, and biology of this intriguing metastable entity.

Of the sixteen electrons in the O_2 molecule, twelve have valence implications:

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[1] R. S. Mulliken, *Nature*, 1928, **122**, 505.
[2] W. H. J. Childs and R. Mecke, *Z. Physik*, 1931, **68**, 344.
[3] C. S. Foote, *Acc. Chem. Res.*, 1968, **1**, 104.
[4] K. Gollnick, *Adv. Photochem.*, 1968, 6, 1.
[5] J. W. Hastings and T. Wilson, *Photophysiology*, 1970, **5**, 49.
[6] R. P. Wayne, *Adv. Photochem.*, 1969, **7**, 311.
[7] M. Kasha and A. U. Khan, *Ann. N.Y. Acad. Sci.*, 1970, **171**, 5.
[8] D. R. Kearns, *Chem. Rev.*, 1971, **71**, 395.
[9] W. Adam, *Chem. Z.*, 1975, 99, 142.
[10] 'Singlet Molecular Oxygen', ed. A. P. Schaap, Dowden, Hutchinson and Ross, Stroudsburg, Pennsylvania, 1976.
[11] B. Stevens, *Acc. Chem. Res.*, 1973, 6, 90.
[12] C. S. Foote, in 'Free Radicals in Biology', Volume 11, ed. W. A. Pryor, Academic Press, New York, 1976.
[13] K. Gollnick, in 'Radiation Research: Biomedical, Chemical and Physical Perspectives', ed. O. F. Nygaard, H. I. Adler and W. Sinclair, Academic Press, New York, 1975.
[14] 'Singlet Oxygen', ed. H. H. Wasserman and R. W. Murray, Academic Press, New York, 1979.

the inner four (*KK*) probably retain essentially atomic character. According to MO theory the remaining twelve occupy the lowest available bonding and antibonding orbitals. The $2\sigma_g$ (bonding) and $2\sigma_u$ (antibonding) pair are each doubly occupied and contribute only weak interactions. The $2p_z$ atomic orbitals from each atom give rise to a $3\sigma_g$ bonding pair which is doubly occupied, gives O_2 its bond, and defines the molecular axis. The doubly degenerate $1\pi_u$ bonding pairs are both doubly occupied, leaving two electrons for the $1\pi_g$ antibonding pair. Strong interactions are contributed by the $3\sigma_g$, and $1\pi_u$ and $1\pi_g$ electrons; thus O_2 has one σ-bond, two π-bonds, and one π-antibond, *i.e.* two electon-pair bonds, one σ and one π. The distribution of the two electrons in the $1\pi_g$ orbitals determines the overall symmetry, angular momentum, and spin characteristics of O_2. The combination of two indistinguishable electrons with two orbitals results in six electronic sub-states having different electron distributions, energies, and magnetic properties. There are three degenerate $^3\Sigma_g^-$ states, two equal-energy $^1\Delta_g$ states, and a unique $^1\Sigma_g^+$ state. The $^1\Delta_g$ and $^1\Sigma_g^+$ states are 0.98 eV and 1.63 eV above the $^3\Sigma_g^-$ ground state respectively. The three states have almost identical binding energies and dissociate at a common energy limit (*ca.* 5 eV). The transitions $^1\Delta_g \leftarrow {}^3\Sigma_g^-$ and $^1\Sigma_g^+ \leftarrow {}^3\Sigma_g^-$ are highly forbidden and correspond to very weak absorption coefficients and long upper-state lifetimes at zero pressure. A full and lucid description of the electronic structure of oxygen has appeared recently.[15]

The upsurge in interest in the chemistry of $O_2(^1\Delta_g)$ stemmed from studies of the orange-red luminescence emitted from mixed hydrogen peroxide–sodium hypochlorite solutions. Originally observed in 1927[16] it was rediscovered in 1960,[17] shown to consist of two red bands, and incorrectly assigned to a solvent-shifted $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition in 1963.[18] Comparison of the spectra of emissions from the H_2O_2-OCl^- system and from radiofrequency discharges in O_2 gas led[19] to assignment of the chemiluminescence peaks to transitions from an O_4 species. It is now accepted that the weak bands at 634 and 703 nm giving rise to the glow arise from the simultaneous transitions[20] of equations (1) and (2), the energy difference corresponding to the vibrational spacing in ground-

$$O_2(^1\Delta_g) + O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-) + O_2(^3\Sigma_g^-) + h\nu \text{ (634 nm)} \quad (1)$$

$$O_2(^1\Delta_g) + O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-) + O_2(^3\Sigma_g^-) + h\nu \text{ (703 nm)} \quad (2)$$

state O_2. Simultaneous transitions can occur as a consequence of collisions between excited molecules and can lead to 'pooling' of the excitation energy into a single photon. Such processes are only usually observed when the forbiddenness of the single-molecule transition is so large that sufficient collisions between excited states can occur. The formation of the molecular pair state $(^1\Delta_g)\,(^1\Delta_g)$ of overall singlet multipicity is able to correlate with the singlet component of

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[15] M. Kasha and D. E. Brabham, Ref. 14, Chapter 1.
[16] L. Mallet, *C.R. Hebd. Seances Acad. Sci.*, 1927, **185**, 352.
[17] H. H. Seliger, *Anal. Biochem.*, 1960, **1**, 60.
[18] A. U. Khan and M. Kasha, *J. Chem. Phys.*, 1963, 39, 2105.
[19] S. J. Arnold, E. A. Ogryzlo, and H. Witzke, *J. Chem. Phys.*, 1964, **40**, 1769.
[20] A. U. Khan and M. Kasha, *J. Am. Chem. Soc.*, 1970, **92**, 3293.

the $(^3\Sigma_g^-)(^3\Sigma_g^-)$ ground-state pair which has singlet, triplet, and quintuplet contributions. Energy pooling *via* simultaneous transitions has been observed[21] for all the possible $^1\Delta_g$, $^1\Sigma_g^+$, $^3\Sigma_g^-$ combinations leading to photons of wavelengths as short as 380 nm. Only recently have singlet oxygen luminescences been observed as a result of photosensitization in the liquid phase. The long radiative lifetime and the efficient deactivation processes which occur in liquids lead to very low quantum yields of luminescence. Nevertheless, high-intensity light sources, sensitive photodetectors, and noise-discrimination techniques have led to detection of the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ transition at 1268 nm in chlorocarbon and fluorocarbon solvents where lifetimes approach 10^{-3} seconds.[22–24] A Russian group[23] has reported time resolution of the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ emission (1270 nm) in CCl_4 solution.

During the early 1960's the attention of organic chemists was drawn towards singlet oxygen. In a series of contiguous communications in 1964 it was shown that organic substrates were oxidized by (i) the peroxide–hypochlorite reaction,[25] (ii) dye-photosensitization,[26] and (iii) oxygen submitted to electrodeless discharge,[27] in such a way as to allow the conclusion that $O_2(^1\Delta_g)$ was the common reactive species. Eventually it became established that many dye-sensitized photo-oxidations proceed *via* an $O_2(^1\Delta_g)$ intermediate[3] and it transpired that the mechanistic conclusions made in the early 1930's[28,29] and largely ignored until the mid-1960's concerning the intervention of excited O_2 molecules in dye photo-oxidations were revived and substantiated.

At this time considerable evidence exists that $O_2(^1\Delta_g)$ is a reactive intermediate in photo-oxidation reactions. $O_2(^1\Sigma_g^+)$ is, however, more elusive in condensed phase. It has been estimated[30] that in solution $^1\Sigma_g^+$ is relaxed to $^1\Delta_g$ in *ca.* 10^{-9} s by collisional quenching interactions and although it is well characterized as a gas-phase intermediate[31] its solution properties are uncertain. The remainder of this review concerns $^1\Delta_g$ only. An excellent reprint volume which collects together the benchmark papers in this subject has been published.[10]

2 Methods of Generation

Three methods of production of $O_2(^1\Delta_g)$ have been important for performing quantitative measurements of its reactivity: peroxide decomposition, high-frequency discharge, and energy transfer. In addition singlet oxygen formation has been invoked in a miscellany of chemical and biological systems.[32]

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[21] K. Furukawa, E. W. Gray, and E. A. Ogryzlo, *Ann. N.Y. Acad. Sci.*, 1970, **171**, 175.
[22] A. A. Kraznovsky, *Photochem. Photobiol.*, 1979, **29**, 29.
[23] I. M. Byteva and G. R. Gurinovitch, *J. Lumin*, 1979, **21**, 17.
[24] A. U. Khan and M. Kasha, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6047.
[25] C. S. Foote and S. Wexler, *J. Am. Chem. Soc.*, 1964, **86**, 3879.
[26] C. S. Foote and S. Wexler, *J. Am. Chem. Soc.*, 1964, **86**, 3880.
[27] E. J. Corey and W. C. Taylor, *J. Am. Chem. Soc.*, 1964, **86**, 3881.
[28] H. Kautsky and H. de Bruijn, *Naturwissenschaften*, 1931, **19**, 1043.
[29] H. Kautsky, H. de Bruijn, R. Neuwirth, and W. Baumeister, *Chem. Ber.*, 1933, 66, 1588.
[30] S. J. Arnold, M. Kubo, and E. A. Ogryzlo, *Adv. Chem. Ser.*, 1968, No. 77, p. 133.
[31] E. A. Ogryzlo, in Ref. 14, Chapter 2.
[32] R. W. Murray, in Ref. 14, Chapter 3.

A. Peroxide Decomposition.—The H_2O_2–OCl^- system in water has already been mentioned. The reagent can be generated either by adding aqueous OCl^- solution to an alkaline solution of H_2O_2 or by bubbling Cl_2 gas into alkaline hydrogen peroxide. The reaction proceeds according to equation (3). Spectroscopic evidence shows that both $^1\Delta_g$ and $^1\Sigma_g{}^+$ states are generated and in

$$OCl^- + H_2O_2 \rightarrow Cl^- + H_2O + O_2 \qquad (3)$$

methanolic solutions the efficiency of $^1\Delta_g$ production approaches 80%.[32] Using ^{18}O-enriched H_2O_2 or OCl^- it was demonstrated that the O_2 is derived wholly from H_2O_2 and not from OCl^- or water.[33] A variation on this method uses bromine and alkaline H_2O_2.[34]

In aqueous alkaline solution organic peracids decompose with evolution of oxygen, some of which was proposed to be in the $^1\Delta_g$ state.[34] More recent work[35] casts doubt on whether $^1\Delta_g$ production is a significant channel.

The proposal,[36] based on trapping experiments, that the oxidation (by Ce^{4+} ions) of s-butylhydroperoxide gives rise to $O_2(^1\Delta_g)$ has been confirmed by spectroscopic measurements.[37] A discussion of whether various transition-metal–oxygen complexes (*e.g.* potassium perchromate) release $O_2(^1\Delta_g)$ on decomposition has recently been published.[32]

Endoperoxides of aromatic compounds formed by the interaction of the substrate with singlet oxygen have been shown to re-form $O_2(^1\Delta_g)$ when heated. Substrates such as rubrene, 9,10-diphenylanthracene,[38] and 2,5-diphenylfuran[39] have been shown to behave in this way. A similar scheme has been proposed for 1,3-diphenylisobenzofuran.[40]

Organic phosphites interact with ozone to yield adducts which are stable at low temperatures but break down on warming to phosphate and molecular oxygen. Multiplicity considerations and experimental observations led to the conclusion that singlet molecular oxygen was a product of this decomposition.[41] A detailed account of phosphite–ozone reaction is contained in a recent singlet oxygen review volume.[32]

The question as to whether the superoxide ion dismutes to yield $O_2(^1\Delta_g)$ has been the subject of much discussion. The proposition was initially made based on a work-up of products from the decomposition of KO_2 in DMSO containing 2,5-dimethylfuran.[42] Luminescence observations of the decomposing KO_2–DMSO system, however, showed only a weak glow[43] when small amounts of

[33] A. E. Cahill and H. Taube, *J. Am. Chem. Soc.*, 1952, **74**, 2312.

[34] E. McKeown and W. A. Waters, *J. Chem. Soc.* (*B*), 1966, 1040.

[35] R. F. Boyer, C. T. Lindstrom, B. Darby, and M. Hylarides, *Tetrahedron Lett.*, 1975, 4111.

[36] J. A. Howard and K. U. Ingold, *J. Am. Chem. Soc.*, 1968, **90**, 1056.

[37] M. Nakano, K. Takayama, Y. Shimizu, Y. Tsuji, H. Inaba, and T. Migita, *J. Am. Chem. Soc.*, 1976, **98**, 1974.

[38] H. H. Wasserman, J. R. Scheffer, and J. L. Cooper, *J. Am. Chem. Soc.*, 1972, **94**, 4991.

[39] A. M. Trozzolo and S. R. Fahrenholtz, *Ann. N.Y. Acad. Sci.*, 1970, **171**, 61.

[40] A. Singh, N.R. McIntyre, and G. W. Koroll, *Photochem. Photobiol.*, 1978, **28**, 595.

[41] R. W. Murray and M. Kaplan, *J. Am. Chem. Soc.*, 1968, **90**, 537.

[42] A. U. Khan, *Science*, 1970, **168**, 476.

[43] R. Nilsson and D. R. Kearns, *J. Phys. Chem.*, 1974, **78**, 1681.

water were present and no oxidation of singlet-oxygen-reactive substrates was found. On the other hand, electrolytically produced $O_2\cdot^-$ converted 1,3-diphenylisobenzofuran into dibenzoylbenzene.[44] The production of $O_2\cdot^-$ in the xanthine–xanthine oxidase system[45] is accompanied by luminescence which has been proposed as arising from $O_2(^1\Delta_g)$.[46] Other work on this enzyme system led to the suggestion that hydroxyl radicals produced in a biological cycle similar to the Haber–Willstätter reaction, equation (4), can oxidize $O_2\cdot^-$ to $O_2(^1\Delta_g)$.[47] The lack of inactivation of the enzymes lysozyme and ribonuclease under

$$O_2\cdot^- + H_2O_2 \rightarrow O_2 + OH^- + \cdot OH \qquad (4)$$

γ-radiolysis conditions[48] where $HO_2\cdot$ (pH 3.1) or $O_2\cdot^-$ (pH 7.6) are the only radical species present, strongly suggests that $O_2(^1\Delta_g)$ is not formed from $HO_2\cdot$ or $O_2\cdot^-$. This is supported by recent observations of the decomposition of tetramethylammonium superoxide in DMSO in the presence of cholesterol supported on microbeads.[49] From measurements of the yields of cholesterol oxidation products (specific for singlet oxygen[50]) it was concluded that no more than 0.2% of $O_2(^1\Delta_g)$ was produced even when corrected for quenching by $O_2\cdot^-$.

B. Electrical Discharge.—Electrical discharges have been used as a means of generating metastable molecules in the gas phase since the turn of the century. The development of high-power radio and microwave generators has allowed elimination of electrodes in the gas stream since such radiation can penetrate glass walls. The most commonly used generators are microwave (2450 MHz), the power from which can be localized and directed *via* waveguides. Gaseous oxygen excited in such a way yields $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g^+)$, which are detected by their light emission. A system like this can be used for measuring the rate parameters for reaction of excited oxygen states with gas-phase substrates, or in a preparative manner by exposing the gas stream to a stirred condensed-phase reactor containing substrates.[31]

C. Energy Transfer.—*Singlet States.* Oxygen has long been known to quench the fluorescence of many organic molecules with diffusion-controlled rate constants, but the exact mechanism of this quenching remains controversial. The interaction of an organic molecule (^{1}M) in its first excited state (^{1}M*) with molecular oxygen ($^3\Sigma_g^-$) produces a collision complex of triplet multiplicity, designated by $^3(M\text{–}O_2)^*$. The decay of $^3(M\text{–}O_2)^*$ to ^{1}M and $O_2(^3\Sigma_g^-)$ is regarded as unlikely, owing to the large amount of electronic energy to be dissipated by the complex and the small Franck–Condon factor.[51] Decay of

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[44] E. A. Mayeda and A. J. Bard, *J. Am. Chem. Soc.*, 1974, **96**, 4023.
[45] I. Fridovich, *J. Biol. Chem.*, 1970, **245**, 4053.
[46] J. Stauff and H. Wolf, *Z. Naturforsch., Teil B*, 1964, **19**, 87.
[47] R. M. Arneson, *Arch. Biochem. Biophys.*, 1970, **136**, 352.
[48] G. E. Barlow, R. H. Bisby, and R. B. Cundall, *Radiat. Phys. Chem.*, 1979, **13**, 73.
[49] C. S. Foote, F. C. Shook, and R. A. Abakerli, *J. Am. Chem. Soc.*, 1980, **102**, 2504.
[50] M. J. Kulig and L. L. Smith, *J. Org. Chem.*, 1973, **38**, 3639.
[51] A. U. Khan and M. Kasha, *Ann. N.Y. Acad. Sci.*, 1970, **171**, 5.

$^3(M-O_2)^*$ to $^3M^*$ and $O_2(^3\Sigma_g^-)$ is considered to be more probable. The dissociation of $^3(M-O_2)^*$ to $^3M^*$ and $O_2(^1\Delta_g)$ is a spin-allowed process, and debate has surrounded the ability of this process to complete with the overall deactivation to yield $^3M^*$ and ground-state oxygen. In order for $O_2(^1\Delta_g)$ to be formed in the energy-transfer step, the $^1M^* \rightarrow ^3M^*$ gap of the organic molecule must exceed 94.5 kJ mol^{-1}, the energy of $^1\Delta_g$. Since rate constants for oxygen quenching of $^1M^*$ emission have been shown to be diffusion controlled regardless of the size of this energy gap for aromatic species, it has been concluded that energy transfer to O_2 is not necessary for efficient quenching.[52]

In studies of self-sensitized photoperoxidations, it was found that the products of $^1M^*$ quenching were $^3M^*$ and $O_2(^3\Sigma_g^-)$ rather than $^3M^*$ and $O_2(^1\Delta_g)$ for species M with high intersystem crossing yields.[53] However, subsequent studies[54,55] involving the strongly fluorescent hydrocarbon rubrene suggest that $O_2(^1\Delta_g)$ may indeed be produced as a consequence of oxygen quenching of $^1M^*$. The experimental result for rubrene (which has also been observed for several other aromatics with high Φ_F[56]) was that singlet oxygen was produced with a quantum yield in excess of unity; this phenomenon can be accounted for by processes (5) and (6).

$$^1M^* + O_2(^3\Sigma_g^-) \rightarrow {}^3M^* + O_2(^1\Delta_g) \qquad (5)$$

$$^3M^* + O_2(^3\Sigma_g^-) \rightarrow {}^1M + O_2(^1\Delta_g) \qquad (6)$$

Therefore, it appears likely that certain aromatic singlet states may sensitize singlet oxygen formation, particularly if the fluorescence quantum yield is high. Another report[57] has provided evidence that singlet excimers of pyrene and naphthalene sensitize the formation of singlet oxygen.

Triplet States. Tripet states are also quenched by molecular oxygen, but rate constants for this process have values that are often an order of magnitude below those for quenching of singlet states. A factor of one-ninth can be explained on the basis of spin statistics.[8] A collision complex of the triplet aromatic ($^3M^*$) with ground-state oxygen can be formed with singlet, triplet, or quintet multiplicity, *i.e.* with only a one in nine probability of singlet formation. Only the singlet complex can yield singlet oxygen directly; the triplet complex dissociates to give ground-state products and the quintet can only return to $^3M^*$ and $O_2(^3\Sigma_g^-)$. The observed limiting value of one-ninth of the diffusion-controlled rate constant thus suggests that quenching occurs only *via* the singlet complex.

Several cases have been reported of quenching rate constants exceeding one-ninth of the diffusion-controlled rate constant,[58] particularly for amines. These results have been explained in terms of an intermediate charge-transfer

[52] C. S. Parmenter and J. D. Rau, *J. Chem. Phys.*, 1969, **51**, 2242.
[53] B. Stevens and B. E. Algar, *J. Phys. Chem.*, 1968, **72**, 3468.
[54] B. Stevens and J. A. Ors, *J. Phys. Chem.*, 1976, **80**, 2164.
[55] K. C. Wu and A. M. Trozzolo, *J. Phys. Chem.*, 1979, **83**, 2823.
[56] K. C. Wu and A. M. Trozzolo, *J Phys. Chem.*, 1979, **83**, 3180.
[57] D. M. Shold, *J. Photochem.*, 1978, **8**, 39.
[58] A. Garner and F. Wilkinson, *Chem. Phys. Lett.*, 1977, **45**, 432.

complex with an energy $E(^1M^*) < E_{CT} < E(^3M^*)$. This mechanism allows for enhanced oxygen quenching rates by the processes of intersystem crossing between singlet and triplet charge-transfer complexes and/or deactivation of the triplet collision complex to ground-state products *via* a CT state. Since this scheme results in deactivation of $^3M^*$ without production of $O_2(^1\Delta_g)$, the quantum yield of singlet oxygen will be reduced from unity (as observed for aromatic hydrocarbons[59]) to as little as one-quarter if statistical proportions of triplet complex decay to ground-state products. Measurements of the fraction of sensitizer triplet states quenched by $O_2(^3\Sigma_g^-)$ that lead to $O_2(^1\Delta_g)$ in benzene solution have shown a strong dependence on sensitizer.[60]

Excited-state sensitizers for singlet oxygen generation *via* energy transfer can be produced by light sources (continuous or pulsed) or by electron beams. The former directly excite the sensitizer states; the latter populate solvent excited states which can rapidly transfer their energy to sensitizer molecules.[60] For quantitative studies of the kinetic properties of $O_2(^1\Delta_g)$ in its reactions with added substrates the photosensitization technique has been the most widely and profitably used.

3 Determination of Singlet Oxygen Lifetime and Reactivity Parameters

As described in Section 2 the most common means of production of $O_2(^1\Delta_g)$ involves transfer of electronic energy from a sensitizer triplet state. This method has been used for virtually all kinetic and much product analysis work, the H_2O_2–OCl^- system having frequently been employed as supporting evidence for the intermediacy of $O_2(^1\Delta_g)$. The sequence of photochemical events leading to the formation of this species and the channels for its subsequent decay are summarized in equations (7)—(14) for a sensitizer S, a substrate A, which reacts exclusively to give an adduct AO_2, and a second quenching species Q. The determination of the rate constants for the last three steps involving $O_2(^1\Delta_g)$, for a variety of media and a host of reactants, and the significance of such rate constants with respect to mechanism, have been the subject of a vast body of research which has appeared over the past fifteen years.

$$\left.\begin{array}{lll} S & \xrightarrow{h\nu} & ^1S^* \\ ^1S^* & \longrightarrow & S \\ ^1S^* & \longrightarrow & ^3S^* \end{array}\right\} \Phi_T \qquad \begin{array}{r} (7) \\ (8) \\ (9) \end{array}$$

$$^3S^* \xrightarrow{k_s} S \tag{10}$$

$$^3S^* + O_2(^3\Sigma_g^-) \xrightarrow{k_{et}} S + O_2(^1\Delta_g) \tag{11}$$

$$O_2(^1\Delta_g) \xrightarrow{k_d} O_2(^3\Sigma_g^-) \tag{12}$$

$$O_2(^1\Delta_g) + A \xrightarrow{k_A} AO_2 \tag{13}$$

$$O_2(^1\Delta_g) + Q \xrightarrow{k_Q} \text{loss of } O_2(^1\Delta_g) \tag{14}$$

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[59] B. E. Algar and B. Stevens, *J. Phys. Chem.*, 1970, **74**, 3029.

[60] A. A. Gorman, G. Lovering, and M. A. J. Rodgers, *J. Am. Chem. Soc.*, 1978, **100**, 4527.

A. Steady State Techniques.—In the absence of a quencher Q the quantum yield for production of AO_2 is given by equation (15) where Φ_T is the triplet yield on

$$\Phi_{AO_2} = \Phi_T \left[\frac{k_{et}[O_2]}{k_S + k_{et}[O_2]}\right]\left[\frac{k_A[A]}{k_d + k_A[A]}\right] \tag{15}$$

excitation of S. Experiments are most commonly carried out at constant oxygen concentration by bubbling and thus equation (16) will hold, where C is a constant.

$$\Phi_{AO_2}^{-1} = C(1 + k_d/k_A[A]) \tag{16}$$

Slope/intercept ratios for plots of $\Phi_{AO_2}^{-1}$ *vs.* $[A]^{-1}$ yield the ratios k_d/k_A, the so called β-values. In the presence of Q, equation (14), the relationship of equation (17)

$$\Phi_{AO_2}^{-1} = C\left(1 + \frac{k_d + k_q[Q]}{k_A[A]}\right) \tag{17}$$

will hold and values of k_d/k_A and k_q/k_A are readily abstracted from slope/intercept treatments for varying [Q].[61] If two substrates are known to react with $O_2(^1\Delta_g)$ to give adducts, direct measurement of competitive rates of product formation, or less preferably loss of substrate, will yield relative rate constants for reaction.[62–64] Thus, steady-state techniques only allow determination of rate constants for reaction of a species with $O_2(^1\Delta_g)$ relative to the rate constant for natural decay of the latter under the conditions of the particular experiment. Nevertheless a whole body of pioneering work based on the above approach made a significant advance towards an understanding of the factors which influence the reactivity of $O_2(^1\Delta_g)$ towards organic/biological substrates. A distinct disadvantage concerned the fact that conclusions concerning solvent effects on reactivity were jeopardized by lack of knowledge of the variation of $O_2(^1\Delta_g)$ lifetime, *i.e.* of k_d [equation (12)], with solvent. Solvent effects were therefore generally studied on the basis of product distributions rather than of rate constants. Thus the determination of $O_2(^1\Delta_g)$ lifetimes using time-resolved techniques represented a major advance in $O_2(^1\Delta_g)$ research.

B. Time Resolved Techniques.—Early work had indicated that the $O_2(^1\Delta_g)$ lifetime in common organic solvents was longer than 10 μs[65] and, it was generally assumed, relatively insensitive to solvent variation. This was shown not to be the case by experiments in which pulsed laser excitation of sensitizer molecules allowed rapid production of $O_2(^1\Delta_g)$ *via* channels (7)—(11). The subsequent decay of this species could be witnessed by use of a suitable monitor.

Diphenylisobenzofuran [DPBF (1)] absorbs intensely at 415 nm and reacts rapidly with $O_2(^1\Delta_g)$ according to equation (18) to give a colourless intermediate (2) which collapses to products.[66,67] The time-resolved bleaching of DPBF

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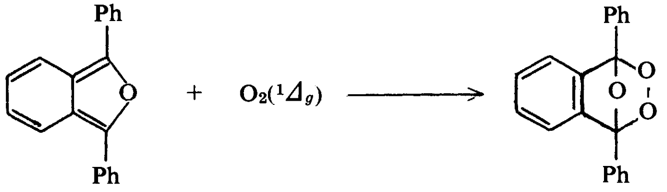
[61] C. S. Foote, in Ref. 14, Chapter 5.
[62] K. R. Kopecky and K. J. Reich, *Can J. Chem.*, 1965, **43**, 2265.
[63] T. Wilson, *J. Am. Chem. Soc.*, 1966, **88**, 2898.
[64] R. Higgins, C. S. Foote, and H. Cheng, *Adv. Chem. Ser.*, 1968, No. 77, p. 102.
[65] C. S. Foote and R. W. Denny, *J. Am. Chem. Soc.*, 1968, **90**, 6233.
[66] M. P. Stevens, F. Nahavandi, and F. Razmara, *Tetrahedron Lett.*, 1973, 301.
[67] R. W. Murray and D. P. Higley, *J. Am. Chem. Soc.*, 1974, 96, 3330.



DPBF (1) (2) (18)

has been monitored at 415 nm after production of $O_2(^1\Delta_g)$ *via* ruby-laser excitation of Methylene Blue as sensitizer.[68–71] It can be easily shown that, for a solution in which the initial $O_2(^1\Delta_g)$ concentration is small compared with that of DPBF and of any quencher Q, the rate of loss of DPBF is given by equation (19) and a plot of $\ln(D_t - D_\infty)$ against time will be linear with a slope k' given

$$\frac{-\mathrm{d[DPBF]}}{\mathrm{d}t} = k_\mathrm{A}[\mathrm{DPBF}]\,[O_2(^1\Delta_g)]_0 \exp\{-(k_\mathrm{d} + k_\mathrm{A}[\mathrm{DPBF}] + k_\mathrm{q}[\mathrm{Q}])t\} \qquad (19)$$

by equation (20) where D_t and D_∞ are respectively optical densities of DPBF at

$$k' = k_\mathrm{d} + k_\mathrm{A}\,[\mathrm{DPBF}] + k_\mathrm{q}[\mathrm{Q}] \qquad (20)$$

time t and at completion of the quenching event. The rate parameters k_d, k_A, and k_q can be evaluated from plots of k' against (i) DPBF concentration (in the absence of quencher) for k_d and k_A and (ii) quencher concentration (at fixed DPBF concentration) for k_q. It was shown[71] that the $O_2(^1\Delta_g)$ decay was independent of the sensitizer employed and this was confirmed in a later time-resolved study in which the pulse radiolysis technique was used to produce the sensitizer triplet states.[60]

It is generally accepted that carotenoid pigments play a role in the protection of biological systems and that their mode of action may well be $O_2(^1\Delta_g)$ quenching.[65,72] The laser flash technique has been used to show that β-carotene quenches $O_2(^1\Delta_g)$ *via* collisional energy transfer [equation (21)] and that subsequent decay of triplet β-carotene proceeds *via* channels (22) and (23).[73] At sufficiently high carotene concentrations the carotene triplet formation is rate

$$O_2(^1\Delta_g) + \beta\text{-car} \xrightarrow{k_t} O_2(^3\Sigma_g^-) + {}^3\beta\text{-car}^* \qquad (21)$$

$${}^3\beta\text{-car}^* + O_2(^3\Sigma_g^-) \longrightarrow \beta\text{-car} + O_2(^3\Sigma_g^-) \qquad (22)$$

$${}^3\beta\text{-car}^* \longrightarrow \beta\text{-car} \qquad (23)$$

limiting and therefore the rate constant for decay of ${}^3\beta$-car*, k', follows the decay of $O_2(^1\Delta_g)$ according to equation (24) in the presence of added quencher,

$$k' = k_\mathrm{d} + k_\mathrm{t}\,[\beta\text{-car}] + k_\mathrm{q}\,[\mathrm{Q}] \qquad (24)$$

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[68] P. B. Merkel and D. R. Kearns, *Chem. Phys. Lett.*, 1971, **12**, 120.
[69] P. B. Merkel and D. R Kearns, *J. Am. Chem. Soc.*, 1972, 94, 1029.
[70] P. B. Merkel and D. R. Kearns, *J. Am. Chem. Soc.*, 1972, **94**, 7244.
[71] D. R. Adams and F. Wilkinson, *J. Chem. Soc.*, *Faraday Trans. 2*, 1972, **68**, 586.
[72] C. S. Foote, Y. C. Chang and R. W. Denny, *J. Am. Chem. Soc.*, 1970, **92**, 5216.
[73] A. Farmilo and F. Wilkinson, *Photochem. Photobiol.*, 1973, **18**, 447.

Q.[73,74] Extraction of the rate constants k_d, k_s, and k_q is as for DPBF bleaching. It turns out that β-carotene is a diffusion-controlled quencher of $O_2(^1\Delta_g)$, $k_t = 1.3 \times 10^{10}$ l mol^{-1} s^{-1},[75] and this places its triplet energy at least slightly below that of $O_2(^1\Delta_g)$ (94.5 kJ mol^{-1}; *cf.* ref. 76). The same has since been shown to be the case for a wide range of carotenoid substances.[77,78]

A pulse technique for $O_2(^1\Delta_g)$ production using direct excitation with a Nd–YAG laser has been developed.[79] Rate constants determined, usually for Freon-113 as solvent, are usually significantly lower than other reported values.

The above work allowed determination of the absolute rate constants for interaction of $O_2(^1\Delta_g)$ with any molecule of interest which was soluble in organic solvents. In addition, determination of the $O_2(^1\Delta_g)$ lifetime, τ_Δ, for specific solvents allowed all previously determined relative rate constants derived from β-values or competitive experiments to be placed on an absolute basis.

C. Differentiation Between Physical and Chemical Quenching.—The quenching rate constants determined as described above may represent either a process which removes quencher (chemical quenching), one which leads to oxygen and quencher ground states (physical quenching), or a combination of both. The separation of the respective contributions generally involves experiments in which the amount of quencher actually lost is compared with the extent of removal of a substance known to quench $O_2(^1\Delta_g)$ exclusively *via* chemical reaction. The nature of particular quenching processes will be discussed in Section 4.

D. Solvent Effects on τ_Δ.—The lifetime of $O_2(^1\Delta_g)$ is now known for a whole range of solvents.[80] Values vary over a wide range from a few microseconds for water to a millisecond in perfluorinated hydrocarbons. In addition a significant deuterium isotope effect is observed, τ_Δ usually, although not always, being appreciably longer in the deuteriated medium. It has been shown[70] that the intensity of the solvent i.r. absorption near 7880, 6280, and 4700 cm^{-1}, resonant with the $0' \rightarrow 0$, $0' \rightarrow 1$, and $0' \rightarrow 2$ bands for the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ transition, correlates well with the rate constant for natural decay of $O_2(^1\Delta_g)$. In effect the electronic energy of the excited oxygen molecule is converted into solvent vibrational energy. In the 7880 and 6280 cm^{-1} region the i.r. overtone bands of common solvents correspond to C—H and O—H vibrations. Thus *C*- and *O*-deuteriation normally lowers absorption intensity in this region, resulting in larger values of τ_Δ. Of particular importance was the fact that, in agreement with theory, τ_Δ

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[74] A. Garner and F. Wilkinson, in Ref. 75, p. 48.

[75] 'Singlet Oxygen Reactions with Organic Compounds and Polymers', ed. B. Ranby and J. F. Rabek, Wiley, N.Y., 1978.

[76] W. G. Herkstroeter, *J. Am. Chem. Soc.*, 1975, 97, 4161.

[77] F. Wilkinson and W. T. Ho, *Spectrosc. Lett.*, 1978, **11**, 455.

[78] M. A. J. Rodgers and A L. Bates, *Photochem. Photobiol.*, 1980, **31**, 533.

[79] I. B. C. Matheson, B. C. Lee, B. S. Yamanashi, and M. L. Wolbarsht, *J. Am. Chem. Soc.*, 1974, **96**, 3343.

[80] D. R. Kearns, in Ref. 14, Chapter 4.

was found to be ten times larger in D_2O than in H_2O.[81] It has recently been shown[82,83] that τ_Δ in D_2O is in fact 53 ± 3 μs, longer than that originally determined (20 μs) and larger than that in H_2O (4 μs) by a factor of 13, in agreement with an earlier ratio based on steady-state-derived β-values.[84] Nevertheless, the D_2O/H_2O lifetime difference previously observed[81] has led to a powerful means of identification of $O_2(^1\Delta_g)$ in photobiological processes (Section 5A).

Owing to expanding interest in the biological aspects of $O_2(^1\Delta_g)$ reactivity the emphasis has shifted towards experimentation in aqueous systems and aqueous systems containing hydrophobic regions. A new water-soluble monitor for $O_2(^1\Delta_g)$, 9,10-anthracenedipropionic acid has been introduced[85] which allows τ_Δ and reactivity measurements for water-soluble quenchers.[83] Several papers have appeared concerning the reactivity of $O_2(^1\Delta_g)$ in aqueous micellar systems.[86-89,82] Generally these systems are D_2O based because of the more favourable τ_Δ value and in such situations $O_2(^1\Delta_g)$ behaves kinetically as if in pure D_2O. Lipid-soluble substrates within the micelles exhibit rate constants for reaction with $O_2(^1\Delta_g)$ that are typical of such reactions in organic media.

E. Solvent Effects on Reactivity of $O_2(^1\Delta_g)$ with Organic Substrates.—In general rate constants for quenching of $O_2(^1\Delta_g)$ by organic substrates are insensitive to solvent and this has been a key factor in mechanistic discussions. Individual effects or the lack of them will be commented upon in the following section.

4 Mechanism of Reaction of $O_2(^1\Delta_g)$ with Organic Substrates

It has been mentioned that the quenching of $O_2(^1\Delta_g)$ may be physical or chemical in nature. Two physical mechanisms, energy-transfer and charge-transfer quenching, have been well characterized and are discussed in the sections on carotenes, amines, and phenols. For many compounds with a π-system which reacts chemically with $O_2(^1\Delta_g)$ physical quenching is probably unimportant although in the majority of cases this is not established.

A. Carotenoids and Other Energy-transfer Quenchers.—As already noted, carotenoids are particularly efficient quenchers of $O_2(^1\Delta_g)$. In several cases it has been clearly demonstrated that the mechanism involves essentially diffusion-controlled energy transfer as witnessed by the time resolved monitoring of carotenoid triplet formation.[77,78] Clearly such a mechanism operates because

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[81] P. B. Merkel, R. Nilsson, and D. R. Kearns, *J. Am. Chem. Soc.*, 1972, **94**, 1030.

[82] B. A. Lindig and M. A. J. Rodgers, *J. Phys. Chem* , 1979, **83**, 1683.

[83] B. A. Lindig, M. A. J. Rodgers, and A. P. Schaap, *J. Am. Chem. Soc.*, 1980, **102**, 5590.

[84] C. S. Foote, in Ref. 75, p. 135.

[85] A. P. Schaap, A. L. Thayer, K. A. Zaklika, and P. C. Valenti, *J. Am. Chem. Soc.*, 1979, **101**, 4016.

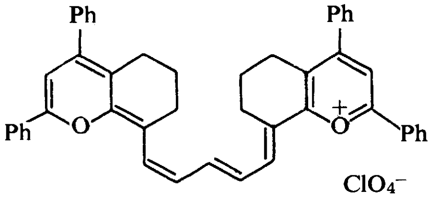
[86] A. A. Gorman, G. Lovering, and M. A. J. Rodgers, *Photochem. Photobiol.*, 1976, **23**, 299.

[87] A. A. Gorman and M. A. J. Rodgers, *Chem. Phys. Lett.*, 1978, **55**, 52.

[88] I. B. C. Matheson, A. D. King, and J. Lee, *Chem. Phys. Lett.*, 1978, **55**, 55.

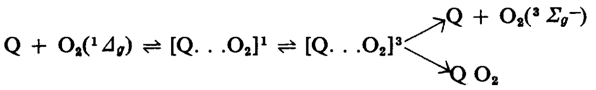
[89] I. B. C. Matheson and R. Massoudi, *J. Am. Chem. Soc.*, 1980, **102**, 1942.

these extended conjugated π-systems exhibit triplet energies close to or below that of $O_2(^1\Delta_g)$. Other compounds with extended chromophores, *e.g.* (3),[69] certain dyes,[70,76,90] and metal complexes[73,91,92] are diffusion-controlled quenchers but direct evidence for energy transfer has not been obtained.



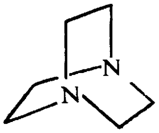
(3)

B. Amines.—Species that quench $O_2(^1\Delta_g)$ *via* a charge-transfer process generally react chemically as well, to an extent depending on individual structure and reaction conditions. Amines typify such behaviour and the primary process is envisaged as formation of a complex between the electron-donating quencher and the electron-deficient oxygen species as shown in equation (25).[93–97] The fact that quenching rate constants correlate with amine ionization potentials[94,95] supports such a mechanism. The subsequent partitioning of the triplet complex



$$Q + O_2(^1\Delta_g) \rightleftharpoons [Q\ldots O_2]^1 \rightleftharpoons [Q\ldots O_2]^3 \begin{array}{l} \nearrow Q + O_2(^3\Sigma_g^-) \\ \searrow QO_2 \end{array} \qquad (25)$$

determines the ratio of physical to chemical quenching. Formation of oxidation products requires an abstractable hydrogen α to nitrogen, *N*-methyl groups being particularly susceptible.[98–100] Otherwise only physical quenching is observed. This is the case for diazabicyclo-octane [DABCO (4)], chemical

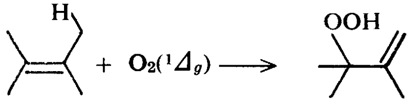


(4)

[90] W. F. Smith, W. G. Herkstroeter, and K. L. Eddy, *J. Am. Chem. Soc.*, 1975, **97**, 2764.
[91] F. Wilkinson, in Ref. 75, p. 27.
[92] D. J. Carlsson, T. Suprunchuk, and D. M. Wiles, *Can. J. Chem.*, 1974, **52**, 3728.
[93] C. Ouannes and T. Wilson, *J. Am. Chem. Soc.*, 1968, **90**, 6258.
[94] K. Furukawa and E. A. Ogryzlo, *J. Photochem.*, 1972, **1**, 163.
[95] R. H. Young and R. L. Martin, *J. Am. Chem. Soc.*, 1972, **94**, 5183.
[96] R. H. Young and D. R. Brewer, in Ref. 75, p. 36.
[97] E. A. Ogryzlo, in Ref. 75, p. 17.
[98] J E. Lindner, H. J Kuhn, and K. Gollnick, *Tetrahedron Lett.* 1972, 1705.
[99] M. H. Fisch, J. C. Gramain, and J. A. Olesen, *Chem. Commun.*, 1971, 663.
[100] D. Bellus, H. Lind, and J. F. Wyatt, *Chem. Commun.*, 1972, 1199.

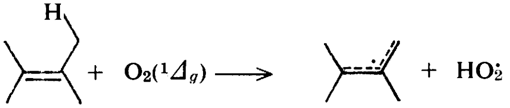
reaction presumably being forbidden on steric grounds. Inhibition of reaction DABCO is commonly used as a means of identifying $O_2(^1\Delta_g)$ intermediacy.

C. Reaction with Simple Olefins: The Ene Reaction and Dioxetan Formation.—*The Ene Reaction.* The process whereby olefins possessing an allylic hydrogen react with $O_2(^1\Delta_g)$ to form allylic hydroperoxides [equation (26)] has been a



(26)

subject of major interest and controversy for 15 years.[3,4,8,101] Despite an increasing rate of appearance of papers, both experimental and theoretical in nature, no definitive mechanistic conclusions have been drawn. Early experiments[102–104] demonstrated that the double bond migrates, thus eliminating the simple H-abstraction process of equation (27). At the present moment discussion



(27)

centres around four of the mechanisms outlined in equation (28), which involve either a concerted six-centre transition state typical of the classical ene reaction (a), a perepoxide or closely related intermediate (b and c) or a biradical intermediate (d).

General points concerning the reactivity of simple olefins towards $O_2(^1\Delta_g)$ are as follows. Quenching rate constants, which correspond to chemical reaction only, are relatively low, 10^3—10^7 l mol^{-1} s^{-1} [101] compared with 10^6—10^9 l mol^{-1} s^{-1} for amines.[61] Some correlation between rate constant logarithms and ionization potentials,[105] together with data from Hammet plots,[106] indicates the electrophilic character of $O_2(^1\Delta_g)$ in these reactions. Solvent effects are minimal. Activation energies for reactions in solution have only been reported in one paper,[107] which contains discrepancies concerning rate constant data. Nevertheless it is clear that activation energies are very low (0—21 kJ mol^{-1}) and 8—17 kJ mol^{-1} lower than for corresponding gas-phase reactions.[31] Thus, in solution these processes exhibit low enthalpies of activation and highly negative entropies of activation. This, coupled with a high overall exotherm-

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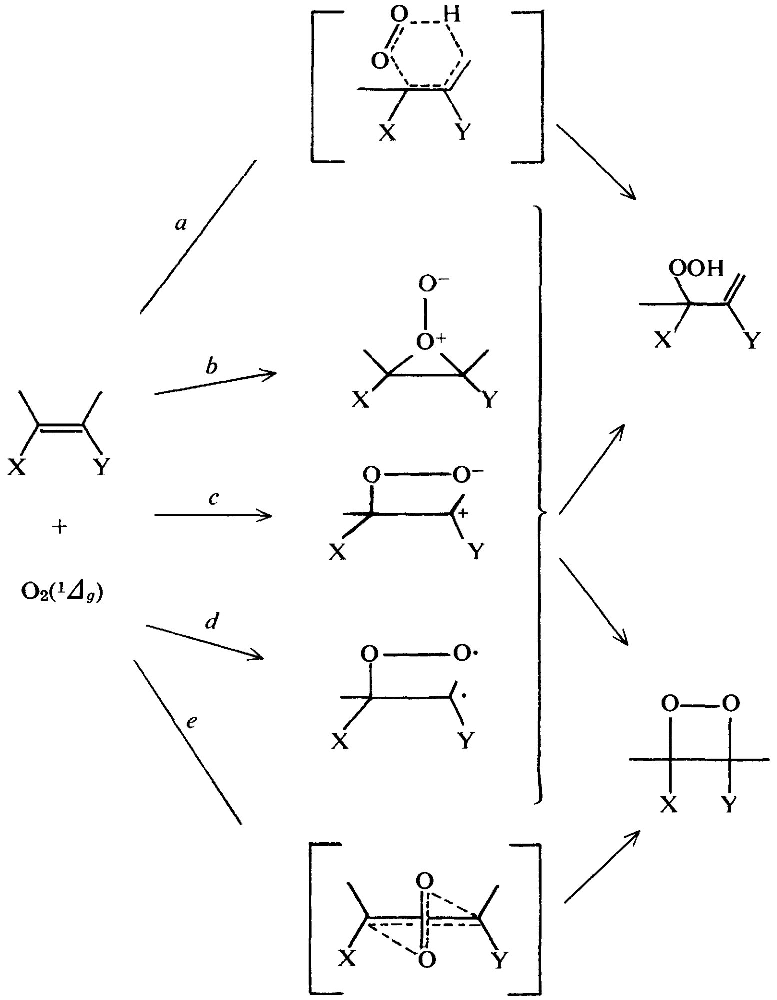
[101] K. Gollnick and H. J. Kuhn, in Ref. 14, Chapter 8.
[102] C. S. Foote, R. Wexler, and W. Ando, *Tetrahedron Lett.*, 1965, 4111.
[103] K. Gollnick and G. O. Schenck, *Pure Appl. Chem.*, 1964, 9, 507.
[104] A. Nickon and J. F. Bagli, *J. Am. Chem. Soc.*, 1961, **83**, 1498.
[105] D. R. Kearns, *J. Am. Chem. Soc.*, 1969, 91, 6554.
[106] C. S. Foote and R W. Denny, *J. Am. Chem. Soc.*, 1971, 93, 5162.
[107] E. Koch, *Tetrahedron*, 1968, **24**, 6295.



(28)

icity,[101] points to a highly ordered rate-determining transition state with little change in substrate structure. The fact that isotope effects are either very small or absent[108–112] is therefore not surprising. It would be impossible to cover the vast amount of discussion and argument presented over the years on the basis of stereochemical and electronic effects. It suffices to say that experimenta-

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[108] K. R. Kopecky and J. H. Vander Sande, *Can. J. Chem.*, 1972, **50**, 4034.

[109] A. Nickon, V. T. Chaung, P. J. L. Daniels, R. W. Denny, J. B. DiGiogio, J. Tsunetsugu, H. G. Vilhuber, and E. Werstiuk, *J. Am. Chem. Soc.*, 1972, 94, 5517.

[110] L. M. Stephenson, D. E. McClure, and P. K. Sysak, *J. Am. Chem. Soc.*, 1973, **95**, 7888.

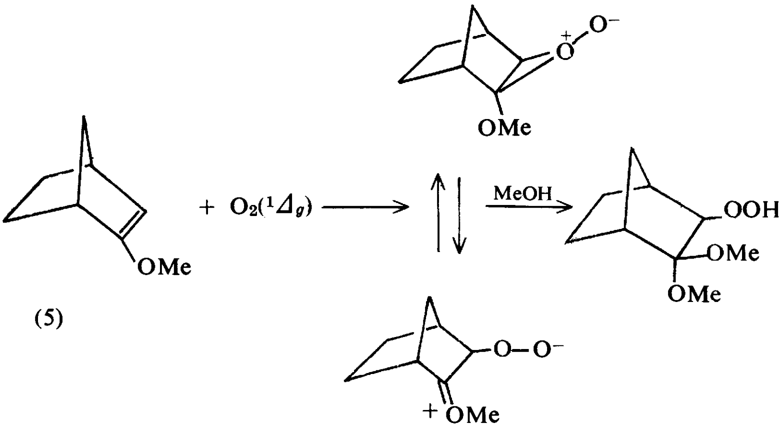
[111] M. B. Grdina, M. Orfanopoulos, and L. M. Stephenson, *J. Am. Chem. Soc.*, 1979, **101**, 3111.

[112] M. Orfanopoulos and L. M. Stephenson, *J. Am. Chem. Soc.*, 1980, **102**, 1417.

tion has not allowed definitive mechanistic conclusions to be drawn and the reader is referred to a comprehensive compilation of data.[101]

The advent of theoretical calculations in this area has in no way decreased mechanistic controversy. Orbital correlation diagrams,[8] HOMO–LUMO analysis,[113] and both CNDO/2 CI[114] and MINDO/3[115] calculations favour initial perepoxide formation. In contrast an *ab initio*/thermochemical approach[116] is claimed to rule out the perepoxide on energetic grounds and favour the biradical intermediate of equation (28d) with varying zwitterionic character dependent on solvent and substituents. Whereas others[3,8] have ruled out such an intermediate on the basis of the absence of Markovnikov directing effects it was reasoned that the least-substituted biradicals of the type shown in equation (28d) will be the most stable.[116] However, thermochemical and kinetic arguments against irreversible biradical formation,[117] which is necessary to explain the lack of olefin *cis–trans* isomerization, were not countered and it was emphasized that it was impossible to distinguish between the biradical and concerted mechanisms.

Of particular recent interest is work on 2-methoxynorbornene (5) in which products incorporating the solvent, methanol, clearly appear to provide



(5)

(29)

evidence in favour of a zwitterionic intermediate, perepoxide or open form as shown in equation (29).[118] The substrate cannot, in this case, participate in allylic hydroperoxide formation for steric reasons and the authors' claim that this result supports calculations[114,115] in favour of perepoxide intermediacy in general does not seem justified.

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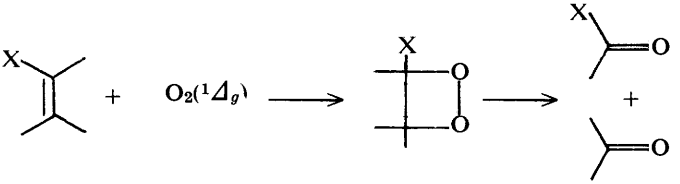
[113] S. Inagaki, H. Fujimoto, and K. Fukui, *Chem. Lett.*, 1976, 749.
[114] S. Inagaki and K. Fukui, *J. Am. Chem. Soc.*, 1975, **97**, 7480.
[115] M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, 1975, **97**, 3978.
[116] L. B. Harding and W. A. Goddard, *J. Am. Chem. Soc.*, 1980, **102**, 439.
[117] F. A. Litt and A. Nickon, *Adv. Chem. Ser.*, 1968, No. 77, p. 118.
[118] C. W. Jefford and C. G. Rimbault, *J. Am. Chem. Soc.*, 1978, **100**, 6437.

Finally it has been suggested[119] that the reaction of $O_2(^1\Delta_g)$ with olefins involves *irreversible* formation of a π- or charge-transfer complex, the geometry of which, and therefore the stereochemical aspects of subsequent decay processes, is governed by frontier orbital interactions. However, whether the low rate constants for reaction of $O_2(^1\Delta_g)$ with olefins, compared with other substrates, are compatible with *irreversible* complex formation is a matter of conjecture.

Dioxetan Formation. Olefins in which the double bond possesses an electron-donating heteroatom, generally N, O, or S, react with $O_2(^1\Delta_g)$ with overall cleavage of the double bond according to equation (30) and it is known that



(30)

reaction proceeds *via* a dioxetan, the exact origin of which is as much a matter of controversy as that of the allylic hydroperoxide. Activated substrates undergoing dioxetan formation quench $O_2(^1\Delta_g)$ with rate constants somewhat higher generally than for the simple olefins although no clear-cut correlation between rate constants and ionization potentials exists.[120] As for the ene process, activation energies are very low[107] and solvent effects are minimal.[121,122] The two processes are clearly closely related and indeed may compete where an allylic hydrogen is available.[123,124] Discussion concerning the mechanism of dioxetan formation centres around the intermediates of equation (28), which could be common to both ene and dioxetan reactions, and the discrete concerted four-centre process of equation (28e). A concerted $_\pi 2_s + _\pi 2_s$ cycloaddition is forbidden but would become allowed when the olefin HOMO is higher in energy than the $O_2(^1\Delta_g)$ LUMO.[8,125] However, both the $_\pi 2_a + _\pi 2_s$ addition, which experiment [123,126] shows must be antarafacial with respect to oxygen as shown in equation (28e), and perepoxide formation are allowed.[8] In contrast to previously mentioned calculations on simple olefins[114,115] the MINDO/3 treatment of vinylamine and 2,3-dihydropyran[115] reactivity towards $O_2(^1\Delta_g)$ indicates rate-limiting formation of an open zwitterion as in equation (28c). This may rearrange to dioxetan or perepoxide, the latter of which may participate in the ene reaction, if this is sterically possible. The *ab initio*/thermochemical approach,[116] which suggests the

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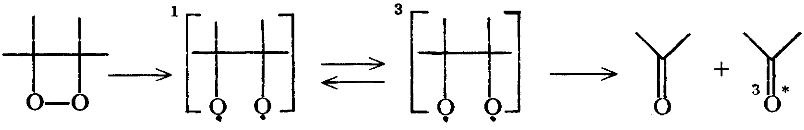
[119] L. M. Stephenson, *Tetrahedron Lett.*, 1980, **21**, 1005.
[120] G. R. Faler, Ph.D. Dissertation, Wayne State University, Detroit, Michigan, 1977.
[121] A. P. Schaap, Ph.D. Dissertation, Harvard University, Cambridge, Massachusetts, 1970.
[122] C. S. Foote, A. A. Dzakpasu, and J. W.-P. Lin, *Tetrahedron Lett.*, 1975, 1247.
[123] P. D. Bartlett and A. P. Schaap, *J. Am. Chem. Soc.*, 1970, **92**, 3223.
[124] A. P. Schaap and P. D. Bartlett, *J Am Chem. Soc.* 1970, **92**, 6055.
[125] C. S. Foote, *Pure Appl. Chem.*, 1971, **27**, 635.
[126] G. Rio and J. Berthelot, *Bull. Soc. Chim. Fr.*, 1971, 3555.

biradical as an intermediate common to both ene and dioxetan reactions as in equation (28d), has already been discussed.

Several dioxetans have now been isolated[127-131] using low-temperature techniques. All decompose with the emission of light at elevated temperatures according to equation (30). This supports the original proposal that breakdown of a dioxetan in a concerted symmetry-controlled process should give one ground-state carbonyl fragment and one electronically excited singlet state.[132] However, considerable work on such processes has now shown that by far the major excited product is the carbonyl triplet state.[133] A proposal [134] that the forbidden spin inversion is an integral part of the concerted cleavage has received support from MINDO/3 calculations[135] which suggest that, for the parent dioxetan, this process is favoured over that involving an intermediate biradicaloid, which is in turn better than the standard cycloreversion to give singlet states, one electronically excited.

In contrast the activation parameters for dioxetan decompositions are in excellent agreement with thermochemical calculations based on the biradical mechanism.[136-139] Intersystem crossing would then take place within the biradical as shown in equation (31). Additional support for this mechanism comes from CNDO/2[140,141] and CNDO/S[142] calculations. The preparation, properties, and reactivities of dioxetans have been comprehensively reviewed.[143]



(31)

D. 1,4-Addition to π-systems: Endoperoxide Formation.—The earliest report

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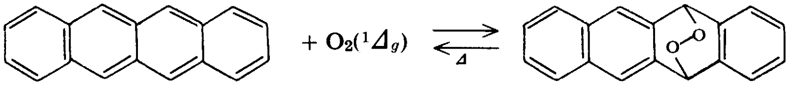
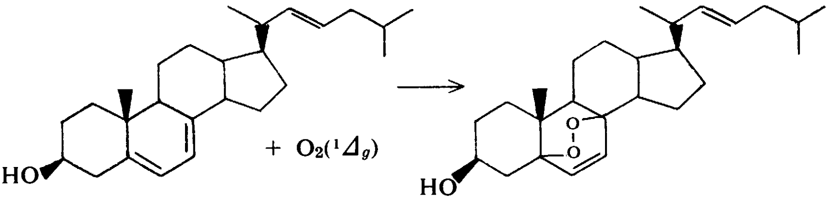
[127] S. Mazur and C. S. Foote, *J. Am. Chem. Soc.*, 1970, **92**, 3225.
[128] A. P. Schaap, *Tetrahedron Lett.*, 1971, 1757.
[129] T. Wilson, D. E. Golan, M. S. Harris, and A. L. Baumstark, *J. Am. Chem. Soc.*, 1976, **98**, 1086.
[130] K. A. Zaklika, P. A. Burns, and A. P. Schaap, *J. Am. Chem. Soc.*, 1978, **100**, 318.
[131] K. A. Zaklika, A. L. Thayer, and A. P. Schaap, *J. Am. Chem. Soc.*, 1978, **100**, 4916.
[132] F. McCapra, *Chem. Commun.*, 1968, 155.
[133] A. P. Schaap and K. A. Zaklika, in Ref. 14, Chapter 4.
[134] N. J. Turro and P. Lechtken, *J. Am. Chem. Soc.*, 1973, **95**, 264.
[135] M. J. S. Dewar and S. Kirschner, *J. Am. Chem. Soc.*, 1974, 96, 7578.
[136] H. E. O'Neal and W. H. Richardson, *J. Am. Chem. Soc.*, 1970, 92, 6553.
[137] W. H. Richardson, M. B. Yelvington, and H. E. O'Neal, *J. Am. Chem. Soc.*, 1972, 94, 1619.
[138] W. H. Richardson, F. C. Montgomery, M. B. Yelvington, and H. E. O'Neal, *J. Am. Chem. Soc.*, 1974, 96, 7525.
[139] K. R. Kopecky, J. E. Filby, C. Mumford, P. A. Lockwood, and J.-Y. Ding, *Can. J. Chem.*, 1975, **53**, 1103.
[140] E. M. Eveleth and G. Felar, *Chem. Phys. Lett.*, 1973, **22**, 499.
[141] G. Barnett, *Can. J. Chem.*, 1974, **52**, 3837.
[142] D. R. Roberts, *Chem. Commun.*, 1974, 683.
[143] P. D. Bartlett and M. E. Landis, in Ref. 14, Chapter 7.

of photo-oxidation[144] was the result of 1,4-addition of $O_2(^1\Delta_g)$ to a conjugated π-system, that of naphthacene as shown in equation (32), although the structure of the endoperoxide was not established until much later.[145] The first such product actually isolated was that formed by dye-sensitized addition of oxygen to the plant hormone ergosterol according to equation (33),[146,147] and subsequent work extended these results to simple 1,3-dienes.[145] It is now well documented that such reactions typically take place between $O_2(^1\Delta_g)$ and conjugated π-systems in general, acyclic, cyclic, aromatic, heteroaromatic, *etc.* The initially formed endoperoxides, either spontaneously or at elevated temperatures, may undergo fragmentation back to reactants as indicated by equation (32) or rearrange to products formed by homolytic fission of the O—O bond. Such products are often epoxides or further rearrangement products thereof. Fragmentation to give back $O_2(^1\Delta_g)$ is most typical of polyaromatic substrates, in particular those with phenyl groups attached to the 1- and 4-positions of the diene unit. For instance, the thermal decomposition of 9,10-diphenylanthracene 9,10-peroxide in benzene, has been utilized as a high-yield chemical source of $O_2(^1\Delta_g)$.[148] Transfer of the latter species from 2,5-diphenylfuran endoperoxide has also been demonstrated.[39]



(32)



(33)

The endoperoxides were previously accepted[8,149] as the products of a symmetry-allowed cycloaddition process of the Diels–Alder type, equation (34a). The latter reaction is itself now the subject of considerable controversy,[150] said to proceed *via* a highly unsymmetrical biradicaloid species according to

[144] M. Fritzche, *C.R. Hebd. Seances Acad. Sci.*, 1867, **64**, 1035.

[145] K. Gollnick and G. O. Schenck, in '1,4-Cycloaddition Reactions', ed. J. Hamer, Academic Press, New York, 1967.

[146] A. Windaus and J. Brunken, *Liebigs Ann. Chem.*, 1928, **460**, 225.

[147] W. Bergman and M. J. McLean, *Chem. Rev.*, 1941, **28**, 367.

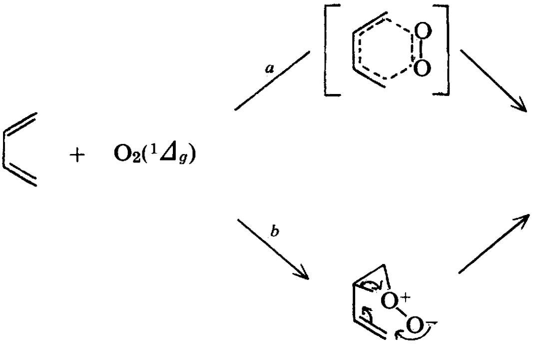
[148] H. H. Wasserman and D. L. Larsen, *J. Chem. Soc., Chem. Commun.*, 1972, 253.

[149] A. U. Kahn and D. R. Kearns, *Adv. Chem. Ser.*, 1968, No. 77, p. 143.

[150] P. Caramella, K. N. Houk, and H. C. Domelsmith, *J. Am. Chem. Soc.*, 1977, **99**, 4511.

MINDO/3[151] and *via* a synchronous reaction according to two *ab initio* calculations.[152,153] Recent MINDO/3 data[154] suggest that the reaction of butadiene with $O_2(^1\Delta_g)$ yields the endoperoxide *via* a perepoxide, equation (34b).

It has recently been shown[155] that the reactions of $O_2(^1\Delta_g)$ with a series of furans in toluene exhibit $\Delta H^{\ddagger}$ values of zero and $\Delta S^{\ddagger}$ values of -80 to -122 J K^{-1} mol^{-1}. The highly negative entropies of activation are typical of Diels–Alder reactions[156] and this fact, together with the absence of significant solvent effects,[157] appears to favour the concerted process. However, as for the ene- and dioxetan-forming reactions one cannot exclude perepoxide formation *via* an early transition state.



(34)

E. Phenols.—In contrast to the polyaromatics, phenols quench $O_2(^1\Delta_g)$ *via* a combination of physical and chemical processes. It has been shown that for a series of 2,4,6-trisubstituted phenols the quenching rate constant logarithms are a linear function of the phenol half-wave oxidation potentials[158] and that both phenols and the corresponding ethers fit the same plot.[159] This together with the direct observation of a phenoxy-radical led to the mechanistic proposal summarized in equation (35).[158] The partitioning of the phenoxy radical between steps a and b determines the make up of the overall quenching. Although this mechanism appears of general applicability it has been suggested that in the case of highly activated phenols 1,4-cycloaddition to give the endoperoxide is the primary step as in equation (36).[160] The fastest phenolic quencher of $O_2(^1\Delta_g)$ is α-tocopherol [Vitamin E (6)], which plays an important biological role in the

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[151] M. J. S. Dewar, A. C. Griffin, and S. Kirschner, *J. Am. Chem. Soc.*, 1974, **96**, 6225.

[152] R. E. Townsend, G. Ramunni, G. Segal, W. J. Hehre, and L. Salem, *J. Am. Chem. Soc.*, 1976, **98**, 2190.

[153] L. A. Burke, G. Leroy, and M. Sana, *Theor. Chim. Acta*, 1975, **40**, 313.

[154] M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, 1977, 99, 2338.

[155] A. A. Gorman, G. Lovering, and M. A. J. Rodgers, *J. Am. Chem. Soc.*, 1979, **101**, 3050.

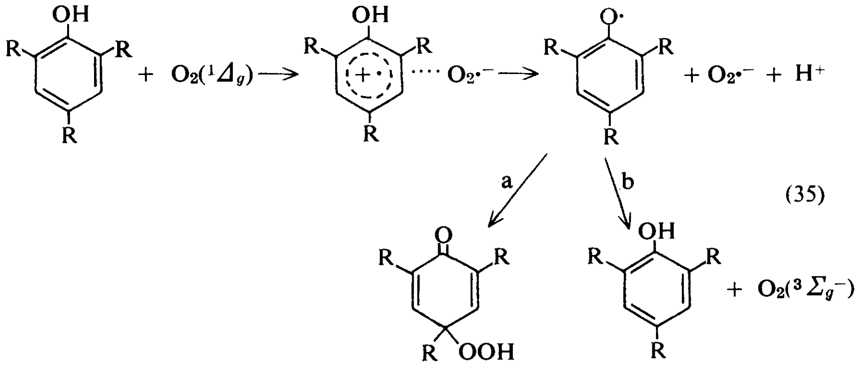
[156] A. Wasserman, 'Diels–Alder Reactions', Elsevier, Amsterdam, 1965.

[157] R. H. Young, K. Wehrly, and R. L. Martin, *J. Am. Chem. Soc.*, 1971, **93**, 5774.

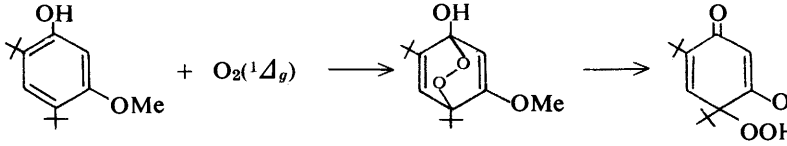
[158] M. J. Thomas and C. S. Foote, *Photochem. Photobiol.*, 1978, **27**, 683.

[159] I. Saito, M. Imuta, and T. Matsuura, *Tetrahedron*, 1972, **28**, 5307.

[160] I. Saito, N. Yoshimura, T. Arai, A. Nishinaga, and T. Matsuura, *Tetrahedron*, 1972, **28**, 5131.



(35)



(36)

protection of tissue and lipids from oxidation damage. Physical quenching predominates over chemical reaction by factors of 13.5,[161] 100,[162] and 120[163] in methanol, benzene, and pyridine respectively. The products of chemical reaction on proflavin-sensitized oxidation in methanol are summarized in equation (37).[164]

F. Heterocycles.—The importance of $O_2(^1\Delta_g)$ with respect to biological damage has stimulated research into the reactivity of this species with heterocycles of every type. The majority of work has been based on product analysis and generally the extent of physical as opposed to chemical quenching is unknown. It would appear, however, that the simpler heterocycles are predominantly chemical quenchers and this has been shown to be true for DPBF (1)[60,165] and some furans. [155,166] In the majority of cases the products of chemical reactions may be rationalized in terms of initial formation of an endoperoxide (Section 4D), otherwise of a dioxetan or corresponding open zwitterion (Section 4C). Isolated products are usually the result of rearrangement involving cleavage of the O—O bond, often with participation of an alcoholic solvent. Products may be isolated which correspond stoicheiometrically to two molecules of substrate and one molecule of oxygen. These possibly result from either the presence of water

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[161] C. S. Foote, T.-Y. Ching, and G. G. Geller, *Photochem. Photobiol.*, 1974, **20**, 511.

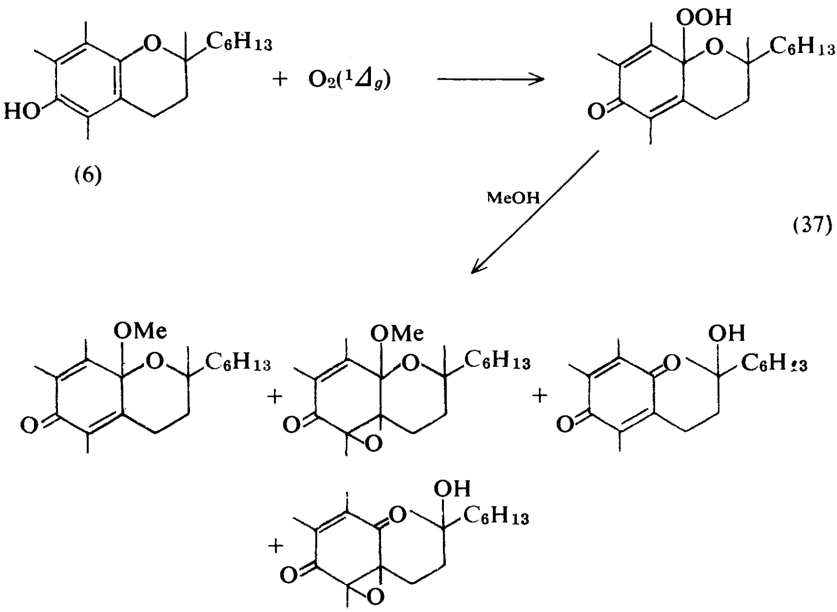
[162] B. Stevens, R. D. Small, and S. R. Perez, *Photochem. Photobiol.*, 1974, **20**, 515.

[163] S. R. Fahrenholz, F. H. Doleiden, A. M. Trozzolo, and A. A. Lamola, *Photochem. Photobiol.*, 1974, **20**, 505.

[164] G. W. Grams, E. Eskins, and G. E. Inglett, *J. Am. Chem. Soc.*, 1972, **94**, 886.

[165] P. B. Merkel and D. R. Kearns, *J. Am. Chem. Soc.*, 1975, **97**, 462.

[166] Y. Usui and K. Kamogawa, *Photochem. Photobiol.*, 1974, **19**, 245.



(6)

(37)

in the solvent, effectively replacing —O—C—OOH by —O—C—OH,[167] by reaction of the initial adduct or some rearranged form thereof with a second substrate molecule, or by reaction of two molecules of adduct with concomitant loss of $O_2(^1\Delta_g)$.[67] Such overall behaviour is typified by aryl-substituted furans, several endoperoxides of which have been isolated and their reactivity examined.[39,168–170] Equation (38) exemplifies the product dependence on reaction conditions.[168,171]

Much of the interest in the reactivity of pyrroles towards $O_2(^1\Delta_g)$ stems from the observation that neonatal jaundice may be treated by exposure of the infant to visible light.[172] It is thought that such phototherapy depends on the ability of $O_2(^1\Delta_g)$ to degrade the pigment bilirubin (7) to water-soluble products which may be excreted.[173] The products of sensitized photo-oxidation of bilirubin in methanol are biliverdin (8) and the fragmentation products (9)—(12).[174–176] Both bilirubin and biliverdin are efficient quenchers of $O_2(^1\Delta_g)$, about

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[167] C. S. Foote, M. T. Wuesthoff, S. Wexler, I. G. Burstain, R. Denny, G. O. Schenck, and K. J. Schulte-Elte, *Tetrahedron*, 1967, **23**, 2583.

[168] R. E. Lutz, W. J. Welstead, R. G. Bass, and J. I. Dak, *J. Org. Chem.*, 1962, **27**, 1111.

[169] C. Dufraise, G. Rio, and A. Ranjon, *C.R. Hebd. Seances Acad. Sci. Ser. C*, 1967, **264**, 516.

[170] C. Dufraise and S. Ecary, *C.R. Hebd. Seances Acad. Sci.*, 1946, **223**, 735.

[171] H. H. Wasserman and A. Liberles, *J. Am. Chem. Soc.*, 1960, **82**, 2086.

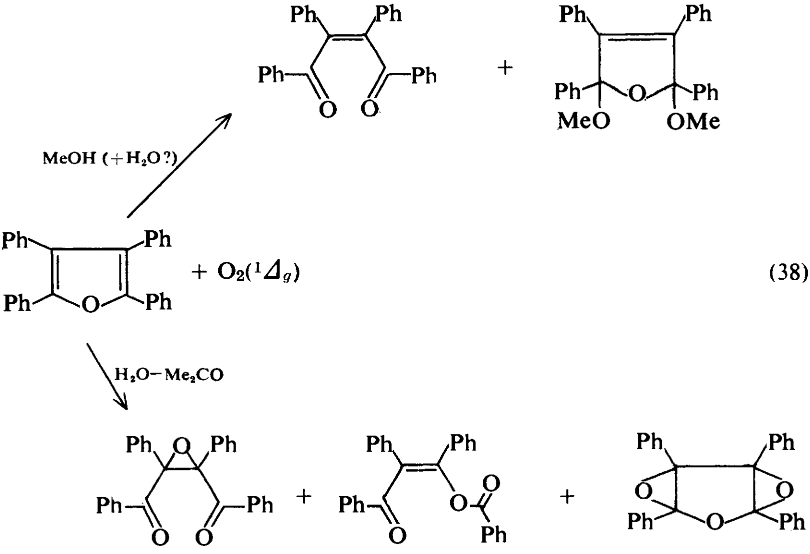
[172] R. J. Cremer, P. W. Perriman, and D. H. Richards, *Lancet*, 1958, **1**, 1094.

[173] R. Schmid, *N. Engl. J. Med.*, 1971, **285**, 520.

[174] D. A. Lightner, D. C. Crandall, S. Gertler, and G. B. Quistad, *FEBS Lett.*, 1973, **30**, 309.

[175] A. F. McDonagh, *Biochem. Biophys. Res. Commun.*, 1971, **44**, 1306.

[176] R. Bonnet and J. C. M. Stewart, *J. Chem. Soc., Perkin Trans. 1*, 1975, 224.



(38)

an order of magnitude down on diffusion control. Whereas chemical reaction makes a ten percent contribution in the case of bilirubin it is much less significant for biliverdin.[177,178] The latter is a product of bilirubin oxidation but does not lie on the principal degradative pathway. Compounds (9) and (10) result from cleavage of dioxetans formed by $O_2(^1\Delta_g)$ addition to the double bonds exocyclic to rings A and D whereas (11) and (12) are the consequences of endoperoxide formation at rings B and C respectively. The latter reaction is strictly analogous to the dealkylation process observed during similar treatment of a simple tetra-substituted pyrrole as shown in equation (39).[179]

The reactivity of indoles towards $O_2(^1\Delta_g)$ is essentially characteristic of electron-rich olefins (Section 4C), the principal products at room temperature being those resulting from dioxetan fragmentation as in equation (40a). Much recent work,[180–182] in which alcohol and amine trapping products have been formed at low temperature, has been claimed to establish the primary formation of the open zwitterionic species of equation (40b) and therefore to support previously mentioned MINDO/3 calculations[115] (Section 4C). However, in

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[177] C. S. Foote and T.-Y. Ching, *J. Am. Chem. Soc.*, 1975, **97**, 6209.

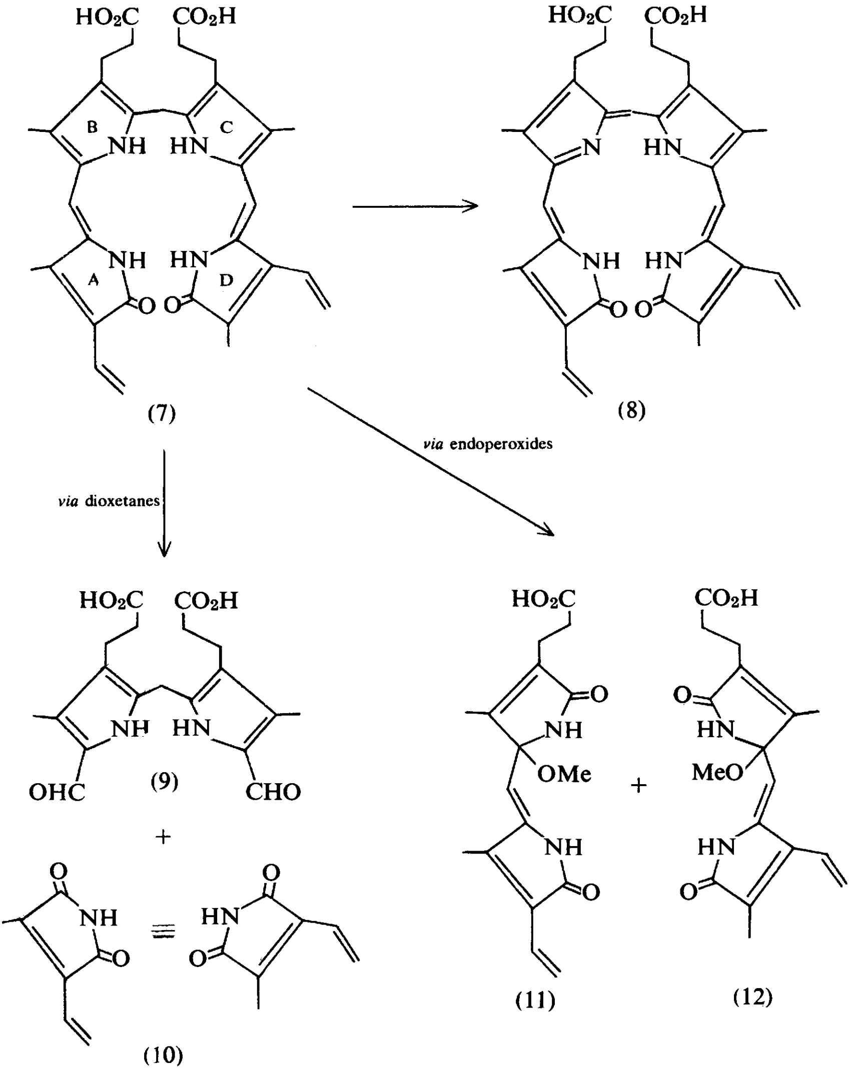
[178] B. Stevens and R. D. Small, *Photochem. Photobiol.*, 1976, **23**, 33.

[179] D. A. Lightner and G. B. Quistad, *Angew. Chem. Int. Ed. Eng.*, 1972, **11**, 215.

[180] I. Saito, M. Imuta, Y. Takahashi, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc.*, 1977, **99**, 2005.

[181] I. Saito, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc.*, 1979, **101**, 7332.

[182] M. Nakagawa, S. Kato, S. Kataoka, and T. Hino, *J. Am. Chem. Soc.*, 1979, **101**, 3136.


HO₂C
CO₂H
B
C
NH
HN
N
A
D
O
(7)
(8)
via endoperoxides
via dioxetanes
OHC
CHO
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OMe
MeO
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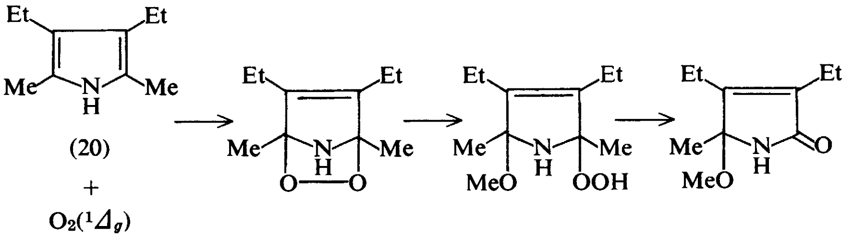
Et
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O₂(¹Δg)
O
MeO
OOH

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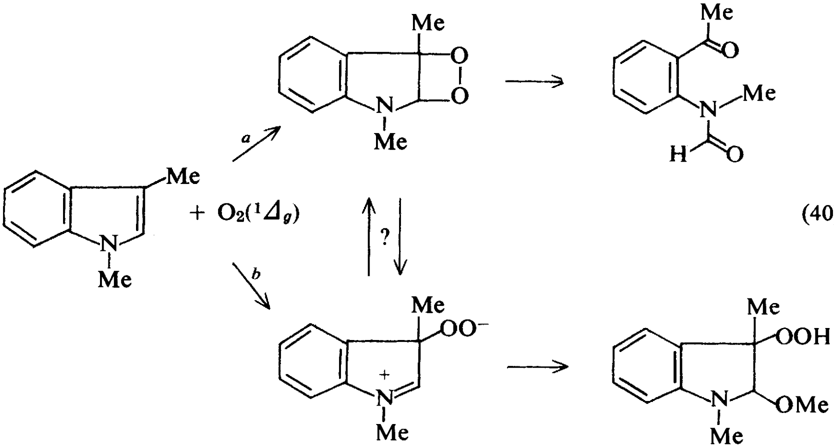
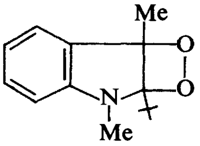


(40)

the authors' opinion no evidence presented eliminates a situation in which the primary step is concerted formation of a dioxetan, the retrocyclization of which no longer competes with solvent-induced processes at low temperature. The n.m.r. characterization of dioxetan (13), formed by photo-oxidation of 2-t-butyl-1,3-dimethylindole at −78 °C in $CFCl_3$[183] certainly does not argue against such a possibility. It has recently been shown[155] that the reactions of a series of indoles with $O_2(^1\Delta_g)$ in toluene exhibit $\Delta H^{\ddagger}$ values of zero and $\Delta S^{\ddagger}$ values in the range -97 to -143 J K^{-1} mol^{-1}, certainly in agreement with the highly ordered transition-state requirement of concerted dioxetan formation.



(13)

The examples referred to in this section have been chosen to typify $O_2(^1\Delta_g)$ reactivity towards heterocycles. A recent excellent review[184] provides a much more detailed survey.

5 Biochemical and Biological Significance of Singlet Oxygen

Oxidation processes are of paramount importance in cellular systems. Many aspects of metabolic change rely on oxidation steps for their proper functions; other oxidation events induced by exogenous or adventitious initiators can be severely damaging to organisms. With the experimental realization that $O_2(^1\Delta_g)$

[183] I. Saito, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc.*, 1979, **101**, 4757.

[184] H. H. Wasserman and B. H. Lipshutz, in Ref. 14, Chapter 9.

has a significant lifetime and is capable of undergoing a number of oxidative reactions with the chemical components of biological assemblies, many observers of biological change have attempted to rationalize their data in terms of singlet oxygen mechanisms. It is possible to categorize the effects under two major headings:

(1) Oxidations induced by the presence of light, a sensitizer (extrinsic or intrinsic), and oxygen—Photodynamic Effects.

(2) Naturally occurring metabolic processes that proceed *via* oxidation without assistance from radiant energy.

These will be considered in order.

A. Photodynamic Effects.—An enormous volume of published research is concerned with these effects both at the biochemical and biological level. Excellent recent reviews are available.[12,185–187] Requirements are the presence of a photosensitizer, light, and oxygen, all of which are, of course, important for the photogeneration of singlet oxygen. These three requirements are not a sufficient criterion of singlet oxygen intervention since the excited sensitizer molecule may react directly with the substrate (atom abstraction, electron transfer, *etc.*) subsequent to which O_2 (ground state) can react with the modified substrate to produce long-term change. Such mechanisms are referred to as Type I; mechanisms involving $O_2(^1\Delta_g)$ as a demonstrated intermediate are Type II. Additional criteria have been developed for demonstrating Type II behaviour. These usually are (*a*) enhancement of the effect in a D_2O-based system and (*b*) prevention of the effect in the presence of recognized singlet oxygen quenchers, azide ions, DABCO *etc.* (see Sections 3D and 4B).

Photo-oxidations have been studied at every level from small molecules in aqueous solutions to the sensitization of multicellular animals to damage or death. A comprehensive review[186] covers most recorded work on a variety of systems and rather than attempt to cover the multitude of published data here it is better to concentrate on a few examples that have been especially well documented.

Erythropoietic protoporphyria (EPP) is a disorder which is brought on in susceptible patients by exposure to sunlight and is characterized by swelling, erythema, and lesions. The photosensitivity of EPP patients can be reduced by oral administration of β-carotene.[188] The red blood cells of EPP patients contain large amounts of free protoporphyrin [a visible-light chromophore which efficiently sensitizes $O_2(^1\Delta_g)$]. These cells are haemolysed upon irradiation[189,190] with visible light *via* photo-oxidation of membrane components.

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[185] N. I. Krinsky, in 'The Survival of Vegetative Organisms', ed. T. G. R. Gray and J. R. Postgate, Cambridge University Press, London, 1976, pp. 209—239.

[186] J. D. Spikes, in 'The Science of Photobiology', ed. K. C. Smith, Plenum Press, New York, 1977, Chapter 4.

[187] J. D. Spikes and R. Straight, in 'Oxygen and Oxy-Radicals in Chemistry and Biology', ed. M. A. J. Rodgers and E. L. Powers, Academic Press, New York, 1981.

[188] M. M. Mathews-Roth, M. A. Pathak, T. B. Fitzpatrick, L. C. Harber, and E. H. Kass, *N. Engl. J. Med.*, 1970, **282**, 1231.

[189] L. C. Harber, A. S. Fleischer, and R. L. Baer, *J. Am. Med. Assoc.*, 1964, **189**, 191.

[190] B. D. Goldstein and L. C. Harber, *J. Clin. Invest.*, 1972, **51**, 892.

Incubation of EPP red cells with Vitamin E (α-tocopherol)[191] and derivatives[190] affords protection from haemolysis. Further, it has been shown[192] that 3β-hydroxy-5α-hydroperoxy-Δ^6-cholestene, the product of $O_2(^1\Delta_g)$ attack on cholesterol,[50] is produced on irradiation of aqueous suspensions of normal red blood cell ghosts incorporating protoporphyrin. It has been concluded that photo-oxidation of cholesterol, initiated by $O_2(^1\Delta_g)$, leads eventually to breakdown of erythrocyte membranes in EPP patients. These conclusions are substantiated by observations that membrane damage to egg-lecithin liposomes has been shown to arise from singlet oxygen processes.[193] Similar processes appear to be involved in damage to membrane lipids in certain viruses.[194] Inactivation and genetic changes in *Saccharomyces cervisiae* have been shown[195,196] to be photosensitized by xanthene, thiazene, and acridine dyes. These effects, which have been attributed to $O_2(^1\Delta_g)$ initiation are D_2O-enhanced and quenched by azide.[195,196]

At the biochemical level there is clear evidence that some amino-acids, particularly histidine, methionine, tyrosine, and tryptophan, are photo-oxidized *via* a singlet oxygen mechanism.[12,197] Leading on from this the deactivation of the enzymes alcohol dehydrogenase and trypsin under photodynamic conditions is enhanced in D_2O and quenched by NaN_3.[198] Again the fairly clear conclusion can be reached that $O_2(^1\Delta_g)$ is involved. Similarly the photodynamic oxidation of tryptophan residues in several enzymes (lysozyme, papain) has been attributed to singlet oxygen reaction.[199–201]

In all biological photo-oxidations examined so far in which $O_2(^1\Delta_g)$ has been invoked as a precursor to lethal or sub-lethal damage, nowhere has its intervention been directly demonstrated. All the evidence relies on the correlation of oxidation products with those of established singlet oxygen reactions, on the effects of D_2O and small molecule quenchers, and on the identification of specific reaction products from added reactive substrates. It is to be hoped that continued research effort in biological and model systems will close the gap and offset the need for the long extrapolation from chemical properties to biological effect.

B. Metabolic Events where $O_2(^1\Delta_g)$ is Invoked.—The microbicidal activity of phagocytes and the action of some oxidase enzymes have been examined for any possible role played by singlet oxygen. A thorough review of these aspects

[191] G. D. Ludwig, D. Bilheimer, and L. Iverson, *Clin. Res* , 1967, **15**, 284.

[192] A. A. Lamola, T. Yamane, and A. M. Trozzolo, *Science*, 1973, **179**, 1131.

[193] S. M. Anderson, N. I. Krinsky, M. J. Stone, and D. C. Clagett, *Photochem. Photobiol.*, 1974, **20**, 65.

[194] W. Snipes, G. Keller, J. Woog, T. Vickroy, R. Deering, and A. Keith, *Photochem. Photobiol.*, 1979, **29**, 785.

[195] T. Ito and K. Kobayashi, *Photochem. Photobiol.*, 1977, **26**, 581.

[196] T. Ito, *Photochem. Photobiol.*, 1977, **25**, 47.

[197] L. I. Grossweiner and A. G. Kepka, *Photochem Photobiol.*, 1972, **16**, 305.

[198] R. Nilsson and D. R. Kearns, *Photochem. Photobiol.*, 1973, **17**, 65.

[199] A. G. Kepka and L. I. Grossweiner, *Photochem. Photobiol.*, 1973, **18**, 49.

[200] G. Jori, G. Galiazzo, and O. Buso, *Arch. Biochem. Biophys.*, 1973, **158**, 116.

[201] G. Jori, M. Folin, G. Gennari, G. Galiazzo, and O. Buso, *Photochem. Photobiol.*, 1974, **19**, 419.

was published recently.[202] The microbicidal action of polymorphonuclear leukocytes depends on (i) recognition of the invading organism, (ii) enclosure into a membrane-bound vesicle (phagosome), and (iii) fusion with enzyme-containing granules. During the enclosure and fusion steps $O_2^{\cdot -}$ and H_2O_2 are released and it has been speculated that $O_2(^1\Delta_g)$ is also involved. This latter was specifically examined[203] by incubating human polymorphonuclear leukocytes with two strains of the coccus *Sarcina leutea*, one containing carotenoid and the other a mutant without carotenoid. The wild strain showed no significant killing, whereas the mutant was rapidly killed under the experimental conditions. Parallel experiments with Toluidine Blue as photosensitizer showed the same type of effect, *i.e.* protection by carotenoid. It was inferred that protection against bacteriocidal action parallels that against photodynamic killing, which is consistent with the proposal that singlet oxygen may be a microbicidal agent in leukocytes.

Suggestions that oxidase–substrate systems generate $O_2(^1\Delta_g)$ stem from observations of chemiluminescence during the enzyme–substrate reaction.[46] Further experimental work during the past decade has failed[47,204–206] to show conclusively that singlet oxygen is formed. The most that can be stated is that $O_2(^1\Delta_g)$ production does not occupy a major role in oxidase activity. Many aspects of oxidase and other enzyme biochemistry are presented in a 1979 review.[202]

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[202] N. I. Krinsky, in Ref. 14, Chapter 12.

[203] N. I. Krinsky, *Science*, 1974, **186**, 363.

[204] T. C. Pederson and S. D. Aust, *Biochem. Biophys. Res. Commun.*, 1973, **52**, 1071.

[205] E. W. Kellog and I. Fridovich, *J. Biol. Chem.*, 1975, **250**, 8812.

[206] K.-L. Fong, P. B. McCoy, J. L. Poyer, H. P. Misra, and B. B. Keele, *Chem. Biol. Interact.*, 1976, **15**, 77.