

STUDIES OF THE TRAPPING OF
SINGLET OXYGEN IN AQUEOUS SOLUTION

A thesis submitted for the Degree of
Doctor of Philosophy in the Faculty
of Science of the University of London.

by

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ABSTRACT

A series of anthracene-derived water-soluble compounds has been synthesized and their suitability for use as traps for singlet oxygen in aqueous media investigated. One of these compounds, anthracen-9,10-yl-1'-bis-ethylsulphonic acid, was selected as offering the optimum combination of high chemical reactivity and negligible physical quenching towards singlet oxygen, adequate water solubility, stability and ease of quantification and analysis.

Ni(II)-N,N'-ethylene-bis-2-hydroxy-5-sulphonic acid-phenylaldehyde has been synthesized and shown to possess adequate water solubility and an extremely high efficiency of physical quenching of singlet oxygen.

The water-soluble traps and quencher of singlet oxygen prepared have been employed in the investigation of singlet oxygen involvement in a number of aqueous systems, with the following results:

- i) During the uncatalysed decomposition of peroxy acids in aqueous solution singlet oxygen is probably generated only from one of the pathways of decomposition, that involving initial interaction between the peroxidic oxygens of two peroxy acid moieties. Evidence has been found of charge-transfer complex formation between one of the anthracene derivatives used and intermediates of the peroxy acid decomposition reaction. The implications of this for the mechanism of singlet oxygen quenching by anthracene derivatives are discussed.
- ii) Singlet oxygen has been shown to be formed during the lipxygenase-catalysed oxidation of linoleic acid. Its possible relationship to the enzyme's action is discussed.
- iii) The decomposition of H_2O_2 , uncatalysed or catalysed by a series of catalysts of complexity ranging from Fe^{2+} ions to catalase, appears not to give rise to any singlet oxygen. The significance of this vis-a-vis the mechanisms of H_2O_2 decomposition is discussed.

CHAPTER 1

General Introduction

1. Historical review of singlet oxygen (1, 2)

Oxygen was discovered and isolated by Scheele and Priestley independently in about 1772, although Priestley believed in the phlogiston theory as late as 1800.

Avogadro in 1811 showed that oxygen is a diatomic molecule, while Faraday in 1848 demonstrated that it is unique among diatomic molecules with an even number of electrons in being paramagnetic in its ground state.

The paramagnetism of the triplet ground state ($^3\Sigma_g^-$) of oxygen together with the prediction for two low lying singlet states ($^1\Delta_g$, $^1\Sigma_g^+$) were accounted for in terms of the Molecular Orbital theory by Mulliken in 1928 (3). The existence of the $^1\Sigma_g^+$ state was verified spectroscopically by Childe and Mecke in 1931 (4) while that of the $^1\Delta_g$ state by Herzberg

in 1934 (5), lying 37.5 kcal/mole and 22.5 kcal/mole above the ground state respectively. It is the $^1\Delta_g$ state of oxygen which has come to be called singlet oxygen.

The role of singlet oxygen in a variety of dye-sensitized photooxygenation reactions has been demonstrated by many workers since 1964. However the initial proposition, made in 1931, that singlet oxygen is the active intermediate in dye-sensitized photooxygenations came from Kautsky (6, 7, 8). He demonstrated that excitation of sensitizer molecules, adsorbed on silica gel, caused the oxygenation of acceptor molecules adsorbed on a different set of silica gel particles physically separated from the dye-coated ones. He concluded that oxygenation must have involved the formation of some metastable species able to migrate in the gas phase from the sensitizer to the acceptor and as such he suggested singlet oxygen.

A recent version of Kautsky's experiment has been carried out using a system where the sensitizer and the acceptor were applied to opposite sides of a stearate film at a distance of 500 Å. Significant photosensitized oxygenation was observed owing to diffusion of singlet oxygen through the film (9, 10):

Kautsky's proposal about singlet oxygen was rejected by the chemists working in the field and a moloxide mechanism

(Sens....O₂) for photooxygenation proposed in 1935 by Schönberg (11) and reintroduced in the 1940's by Terenin and Schenck (12a, 12b) remained dominant until 1964.

So until 1964 singlet oxygen was considered as of no importance in the oxygenation of organic compounds. The situation changed dramatically in 1963-1964 as a result of the work of Khan and Kasha (13a, 13b, 13c), Arnold, Ogryzlo and Witzke (14), Foote and Wexler (15), and Corey and Taylor (16).

Through spectroscopic investigation of the red chemiluminescence accompanying the reaction of hydrogen peroxide with sodium hypochlorite, it was shown by the first two groups of workers that the emission was caused by the transition $[2O_2 \ ^1\Delta_g] \longrightarrow [2O_2 \ ^3\Sigma_g^-]$ and consequently that singlet oxygen was generated during the decomposition of hydrogen peroxide.

The connection between these spectroscopic observations and the entire field of photosensitized oxygenation was provided by Foote and Wexler who showed that the hydrogen peroxide-sodium hypochlorite mixture is an effective reagent for oxygenating a number of organic acceptors and that the products and product distribution were the same as those obtained by sensitized photooxygenation of these compounds.

Corey and Taylor at the same time demonstrated that excited oxygen molecules, generated by radiofrequency discharge,

reacted with various substituted anthracenes and olefins to give oxygenation products that were identical with those formed during their photosensitized oxygenation.

Mainly on the basis of these comparisons it was concluded that singlet oxygen was the reactive intermediate in photosensitized oxygenation reactions as Kautsky had suggested many years before.

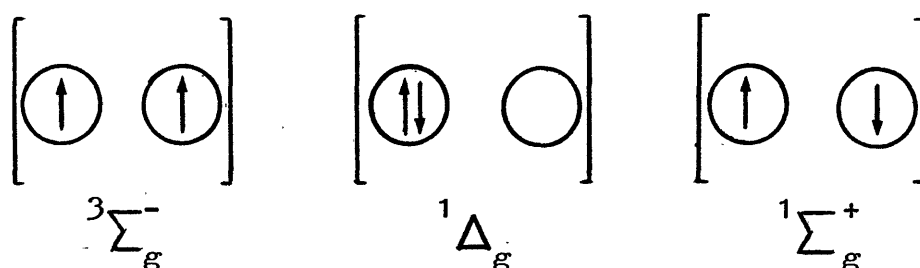
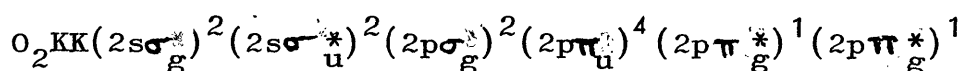
The range of phenomena, reactions and systems in which singlet oxygen is now believed to be directly or indirectly involved is indeed remarkable and will be discussed in more detail in subsequent sections. Generally singlet oxygen is or may be involved in dye-sensitized photooxygenation, photodynamic action, photosynthesis, chemiluminescence phenomena, enzyme systems, ozonolysis reactions and even in metal-catalyzed oxygenation reactions (17, 18, 19).

2. The electronic structure of the states of molecular oxygen (1, 18, 20, 21)

Molecular oxygen is somewhat unique amongst molecules with an even number of electrons (16 electrons) in that the highest-energy occupied orbitals are a pair of doubly degenerate antibonding orbitals holding only two electrons. Taking into account the electron spin wave function, this arrangement

gives rise to three resolved electronic states, designated $^3\Sigma_g^-$, $^1\Delta_g$ and $^1\Sigma_g^+$, of different energies.

So we have the following molecular orbital description of molecular oxygen and representation of the three states:



According to Hund's law the state of highest multiplicity is the one with the lowest energy, therefore the ground state of oxygen is the triplet, $^3\Sigma_g^-$, with the two electrons in two separate orbitals having their angular momentum opposed but with parallel spins. Consequently ground-state oxygen can act as a diradical in one-electron reactions.

The first excited state, $^1\Delta_g$, has both electrons in the same orbital with the same angular momentum. Since the two electrons have opposed spins, the state is a singlet. It has an energy of 22.5 kcal/mole above the ground state and is expected to undergo two-electron reactions. It resembles ethylene electronically, but should be more electrophilic.

The second excited state, $^1\Sigma_g^+$, has the two electrons in separate orbitals with their angular momentum opposed. The spins are also opposed, so it too is a singlet. It has an energy of 37.5 kcal/mole above the ground state and might be expected to undergo one-electron, free radical reactions.

With regard to their magnetic properties, ground-state oxygen is paramagnetic, $^1\Delta_g$ oxygen is ~~para~~paramagnetic and $^1\Sigma_g^+$ oxygen is diamagnetic.

The electronic transitions of the oxygen species are given below:

Single - molecule transition	Energy cm^{-1} (nm)
$^1\Delta \longleftarrow ^3\Sigma$	7,882 (1269)
$^1\Sigma \longleftarrow ^3\Sigma$	13,120 (762)
Simultaneous transitions	
$2(^1\Delta) \longleftarrow 2(^3\Sigma)$	15,765 (634)
$^1\Sigma + ^1\Delta \longleftarrow 2(^3\Sigma)$	21,003 (476)
$2(^1\Sigma) \longleftarrow 2(^3\Sigma)$	26,462 (381)

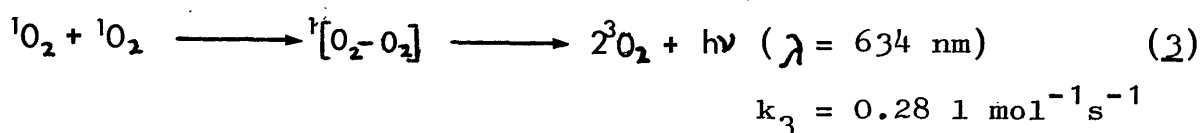
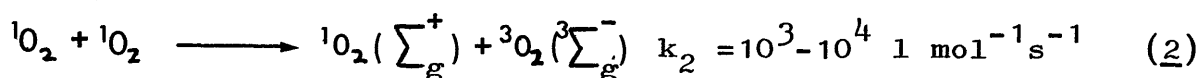
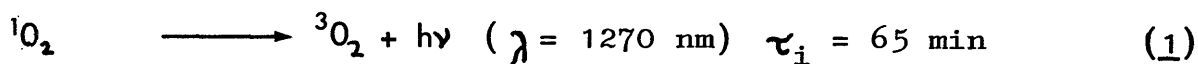
The lifetime of the $^1\Sigma_g^+$ state in the absence of collisions is 12 sec (22) but it is reduced dramatically by collisions so that in the condensed phases it is 10^{-9} - 10^{-11} sec. The $^1\Sigma_g^+$ state decays to $^1\Delta_g$ state by a spin allowed process, before appreciable chemistry can occur.

Both excited states have been unequivocally identified spectroscopically as products of microwave discharge and photosensitized excitation of oxygen in the gas phase, and of the chemiluminescent reaction of sodium hypochlorite with hydrogen peroxide. No unequivocal evidence for the photosensitized formation or reactions of $^1\Sigma_g^+$ oxygen in solution exists, although a theoretical treatment suggests it may be the primarily formed singlet state with some sensitizers (1).

The lower excited state, $^1\Delta_g$, is much longer-lived, with a lifetime of 64 min (23, 24), in the absence of collisions, because the transition to the ground state is spin forbidden. Its lifetime in the condensed phase is highly solvent-dependent and of the order of 10^{-6} - 10^{-3} s. It is now believed to be the only singlet oxygen species which reacts in solution, so the term "singlet oxygen" will subsequently be used to refer to the $^1\Delta_g$ state.

3. Singlet oxygen lifetimes

In the gas phase singlet oxygen has an intrinsic lifetime of about 64 min (23, 24); the gas phase lifetime may be shortened considerably by energy pooling (rn.(2)), by dimole formation (rn.(3)), by collision with inert molecules Q (quenchers) (rn.(4)) and by reaction with suitable substrates A (rn.(5)), as follows:



The rates of the quenching of $^1\text{O}_2$ by gas molecules are found to be mainly of the order of 10^3 (47); for $\text{Q} = \text{O}_2$, $k_4 = 1.4 \times 10^3 \text{ l mol}^{-1} \text{ s}^{-1}$; for $\text{Q} = \text{CO}_2$, $k_4 = 2.4 \times 10^3 \text{ l mol}^{-1} \text{ s}^{-1}$; for $\text{Q} = \text{H}_2\text{O}$, $k_4 = 8.4 \times 10^3 \text{ l mol}^{-1} \text{ s}^{-1}$ and for $\text{Q} = \text{N}_2$, $k_4 = 4 \times 10^1 \text{ l mol}^{-1} \text{ s}^{-1}$. The decay of singlet oxygen in air at atmospheric pressure is largely due to the quenching by O_2 and its lifetime is reduced to $\ll 100 \text{ ms}$ (55).

The lifetime of singlet oxygen in the liquid phase is reduced to $2 \times 10^{-6} - 10^{-3} \text{ s}$ and is highly dependent upon solvent. The accepted mechanism of quenching by solvent is that of transfer of electronic energy to vibrational levels of the solvent and especially those of O-H moiety. On this basis Kearns and coworkers (56, 57, 58) have developed an interesting theory to explain the three orders of magnitude difference in solvent

quenching rate constants (k_d). They found that these rate constants were correlated with the optical densities of the solvents according to the following empirical expression:

$$k_d \times 10^{-6} \propto 0.5[\text{O.D.}_{7780 \text{ cm}^{-1}}] + 0.05[\text{O.D.}_{6280 \text{ cm}^{-1}}]$$

where the optical densities of the solvent (1 cm cell) at 7880 and 6280 cm^{-1} correspond to the $0 \longrightarrow 0$ and $0 \longrightarrow 1$ components of $\text{O}_2(^1\Delta_g) \longrightarrow \text{O}_2(^3\Sigma_g^+)$ transition.

This theory explains also the 10-fold increase in lifetime observed when D_2O (with lower optical densities at 7880 and 6280 cm^{-1}) replaces H_2O as the solvent (57). The heavy-water effect on the lifetime of singlet oxygen became a powerful diagnostic tool for investigating the involvement of $^1\text{O}_2$ in various chemical and physical processes, especially in biological systems.

Some representative lifetimes of singlet oxygen in various solvents including the lower and the higher ones, are given below (56, 57, 58, 59).

Table 1: Lifetimes of singlet oxygen in solution at room temperature

Solvent	τ (μ s)	$k_d \times 10^{-4} \text{ s}^{-1}$	Solvent	τ (μ s)	$k_d \times 10^{-4} \text{ s}^{-1}$
H ₂ O	2	50	CHCl ₃	60	1.7
D ₂ O	20	5	CS ₂	200	0.5
CH ₃ OH	7	14.3	CDCl ₃	300	0.33
CH ₃ CH ₂ OH	12	8.3	C ₆ F ₆	600	0.17
Benzene	24	4.2	CCl ₄	700	0.14
Acetone	26	3.8	CF ₃ Cl	1000	0.1
			(Freon-11)		
			Freon-113	2200	0.0455

4. Sources and occurrence of singlet oxygen

Some of the reactions and processes which lead to singlet oxygen are given briefly below:

4.1. Physical sources

Photosensitization (20, 25)



Microwave discharge (16, 26)

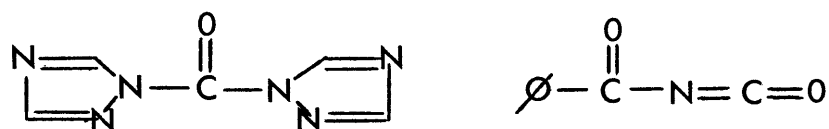


4.2. Chemical sources

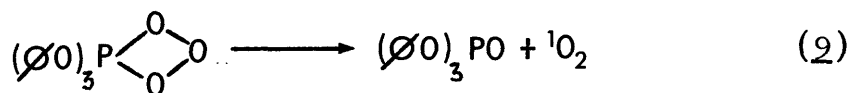
Reaction of hydrogen peroxide with sodium hypochlorite, chlorine or bromine (13a, 13c, 27a, 27b, 27c).



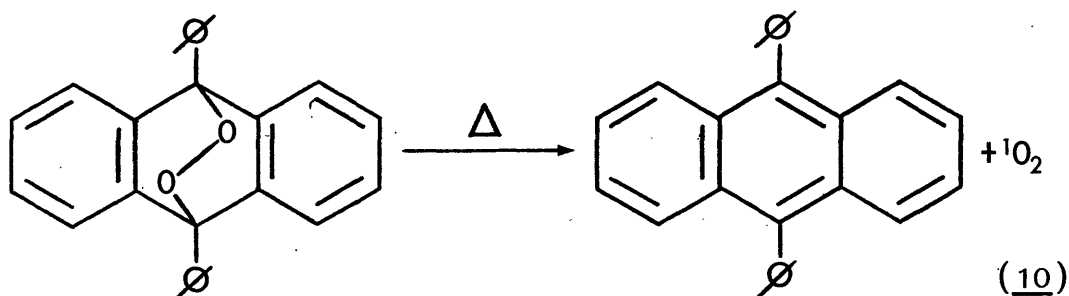
Hydrogen peroxide reaction with dehydrating agents (28)
such as:



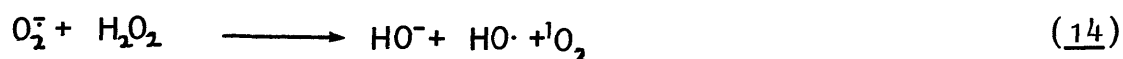
Decomposition of triphenyl phosphine or phosphite ozonides (29, 30), or 1-phospha,2,8,9-trioxa-adamantane ozonide (31).



Decomposition of endoperoxides of aromatic hydrocarbons
(32).



Superoxide anion reactions (33-35).



4.3. Atmospheric generation

Singlet oxygen is postulated to be a reactant in polluted air (36, 37, 38), and a number of studies have provided evidence that it is involved in photochemical air pollution (39, 40). It is produced in the photochemical smog at a rate of 200 pphm/h (37) and the main reactions responsible for this production are as follows:

- (1) Photosensitized generation probably caused by aldehydes, ketones, olefins or aromatic hydrocarbons acting as sensitizers (37, 18).
- (2) Excitation of ground-state oxygen by excited NO_2 (41, 42, 43).
- (3) Hydrolysis of peroxyacetylnitrate, an air pollutant (44).
- (4) Ozone photolysis (253.7 nm) under certain conditions (45-49, 1).
- (5) Direct excitation of oxygen, although inefficient at normal pressures (50, 51).

4.4. Singlet oxygen in biological systems (18,19)

The best known and unequivocally established action of singlet oxygen in biological systems is photodynamic action, which is the photosensitized oxygenation leading to cell damage. Photodynamic action is the result of the effects of three components (light, dye and oxygen) on cell constituents such as amino acids (e.g. methionine, histidine, tryptophan, tyrosine, cysteine), nucleic acid bases (e.g. guanine), lipids and others. These chemical effects lead to enzyme inactivation, mutagenesis, damage of the cell membranes and they may sometimes be lethal.

A number of photosensitized diseases found in higher animals and in humans are caused by dietary photosensitizers or drug photosensitizers. Some porphyrias resulting in the deposition of sensitizing blood porphyrins in the skin and causing severe skin damage on brief light exposure also belong to this category. Evidence of the involvement of singlet oxygen in these cases is the fact that they can be treated effectively with large dosages of carotenes which are powerful singlet oxygen quenchers. A disease involving photosensitized oxygenation in its treatment is neonatal jaundice caused by the accumulation of the lipid-soluble bilirubin in the skin and the brain of infants. It can be treated by irradiation which results in photosensitized (by bilirubin) production and reaction of singlet oxygen with bilirubin and its consequent conversion into water-soluble products. A disease where singlet oxygen involvement must be encountered with considerable scepticism is photocarcinogenicity and related phenomena, where singlet oxygen is considered as activator of carcinogenic substances (54).

Singlet oxygen has been invoked in several enzyme systems most of which are still highly speculative. Several enzymes mediate oxidation reactions which resemble the singlet oxygen ones. Such examples are dioxygenases, tryptophan pyrrolase and lipoxygenase. It is possible that an enzyme-oxygen complex of some type might mimic singlet oxygen but there is no convincing evidence for this.

An extensive search is under way for singlet oxygen generation in sites where probable precursors of it (as $O_2^{\cdot-}$, H_2O_2 , $HO\cdot$) are generated, but without any definite results so far. In particular the reaction of H_2O_2 with $O_2^{\cdot-}$ has proved to be too slow to be of any significance (52).

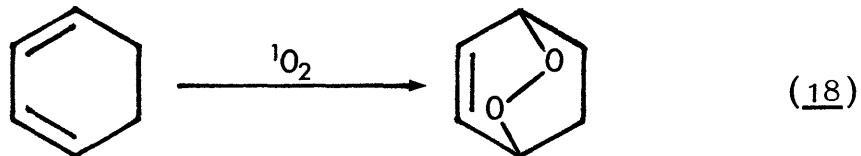
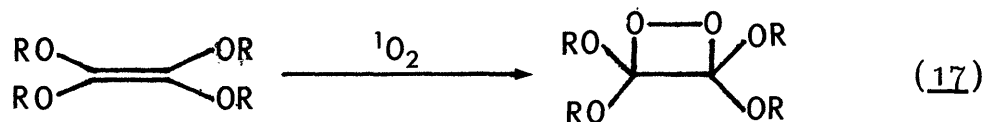
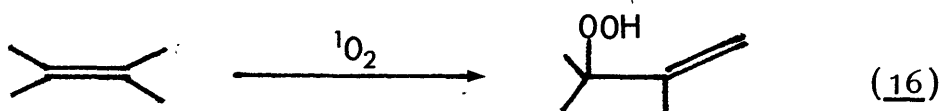
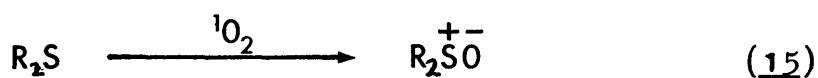
More successful has been the search establishing the role of lipid peroxides in singlet oxygen involvement during the lipid peroxidation (35d). Krinsky (53) has produced evidence that singlet oxygen is involved in phagocytosis. He has shown that human polymorphonuclear leucocytes kill colorless mutant strains of *Sarcina lutea* more effectively than they kill the carotenoid-containing wild type.

Singlet oxygen may also be involved in chemi- and bioluminescence though the mechanism is unknown. A possible one is through the production and breakdown of 1,2 dioxetanes.

5. Reactions of singlet oxygen (1, 60, 61)

The four principal pathways of singlet oxygen reactions are:

i) oxidation of heteroatom-bearing compounds, ii) allylic hydroperoxide formation with olefins, iii) 1,2 cycloaddition and iv) 1,4 cycloaddition.

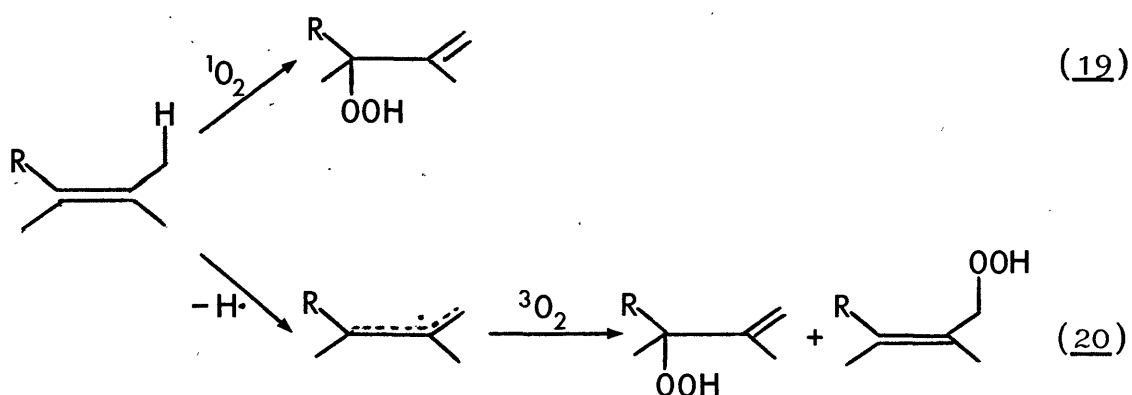


5.1. Oxidation of heteroatom-bearing compounds

Singlet oxygen oxidises sulphides to sulfoxides and sulphones by electrophilic attack, possible via a charge transfer complex (60). A similar mechanism may operate in the oxidative dealkylation of substituted amines and in the oxidation of some alcohols and α -keto-carboxylic acids. The rate constants for these reactions generally lie in the range $10^4 - 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$.

5.2. Allylic hydroperoxide formation

This reaction is characterized by a stereospecific shift of the double bond (20) which distinguishes it from one involving addition of ground-state oxygen to a free radical intermediate.



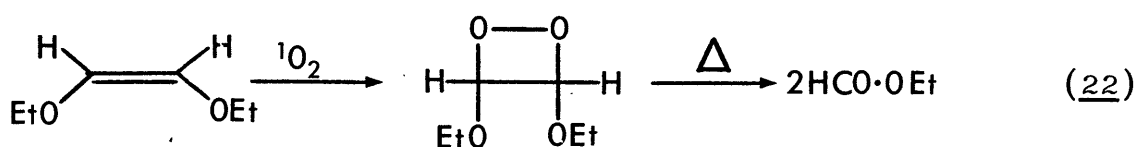
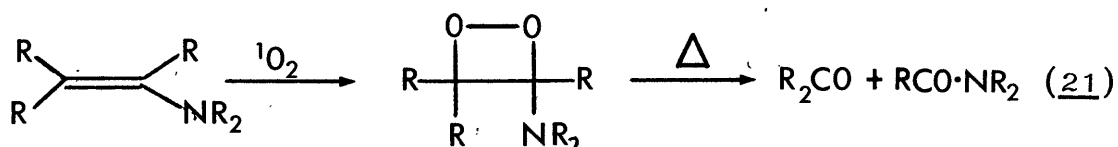
It appears likely that the mechanism involves concerted addition analogous to the classical "ene" reaction (20, 60a, 60b, 62-65). Where an olefin possesses more than one allylic hydrogen, a number of product hydroperoxides may be formed. The proportion of each will depend on the precise electronic and steric environment around each of the allylic hydrogens. The finding of a characteristic pattern of products from an olefin has been proposed as a powerful diagnostic tool for the detection of singlet

oxygen (66). Among the olefins used for this purpose are cholesterol (67) and limonene (68).

Rate constants for hydroperoxide formation are usually in the range $10^5 - 5 \times 10^6 \text{ l mol}^{-1} \text{ s}^{-1}$ (60).

5.3. 1,2-Cycloaddition

This reaction occurs primarily with olefins bearing strongly electron-donating substituents, particularly heteroatoms, e.g. enamines (69) and vinyl ethers (70, 71). The product dioxetanes are usually unstable at room temperature undergoing C-C cleavage.



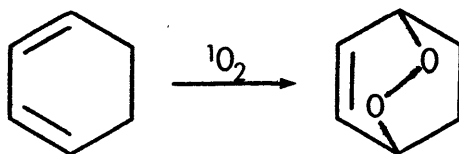
The mechanism of this reaction has been the subject of some controversy, with ion pairs, charge-transfer complexes and perepoxides being invoked as intermediates (60, 63, 70-75), and no clear picture has yet emerged.

Some of the rate constants for this type of reaction are reported to be in the range $8 \times 10^3 - 3 \times 10^6 \text{ l mol}^{-1} \text{ s}^{-1}$ (60, 63).

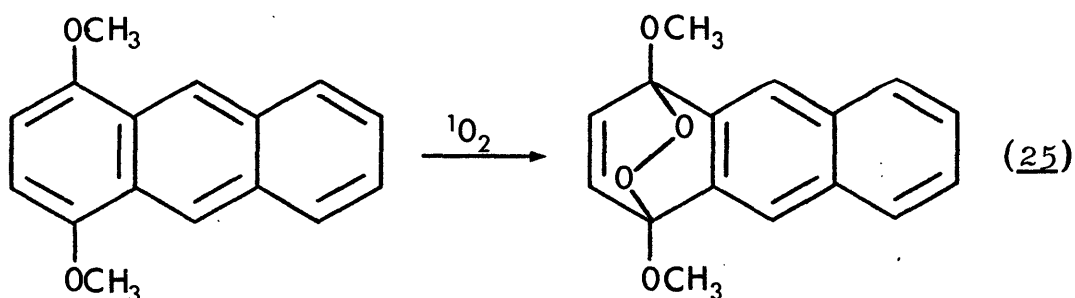
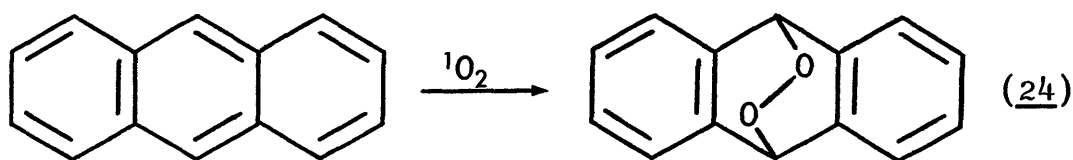
5.4. 1,4-Cycloaddition (76)

Among the compounds which commonly give this kind of reaction are simple cyclic dienes, anthracenes, and heterocyclic systems, particularly furans (60). Table 2 gives some representative results of such studies illustrating some general trends.

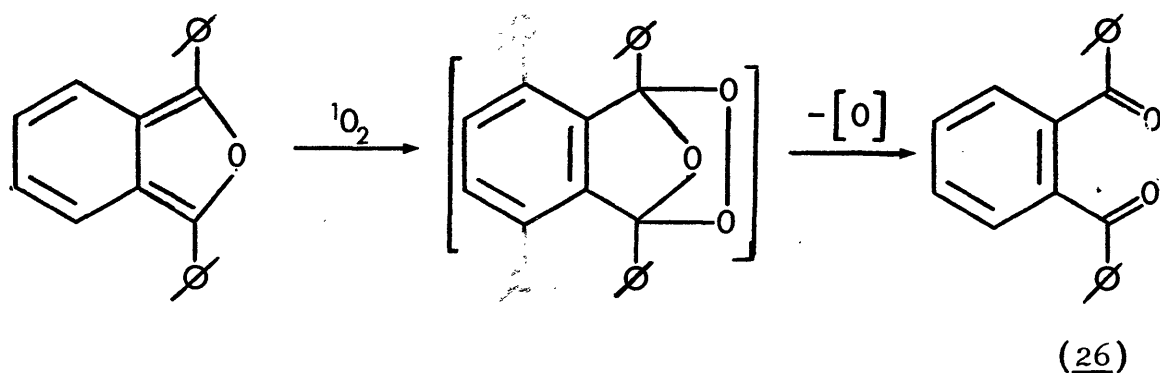
The products of 1,4-cycloaddition are endoperoxides which may occasionally undergo further reaction. With simple cyclic dienes and homonuclear aromatic hydrocarbons, the transannular endoperoxide product is normally stable and can be isolated. 1,3-cyclohexadiene gives 2-cyclohexene-1,4-endoperoxide (rn. (23)), while with anthracene derivatives the 9,10-endoperoxide is normally the product (rn. (24)). However addition of oxygen to a position other than 9,10- may be achieved by suitable choice of substituents. Thus 1,4-dimethoxyanthracene is oxidised to the 1,4-endoperoxide (rn. (25)).



(23)

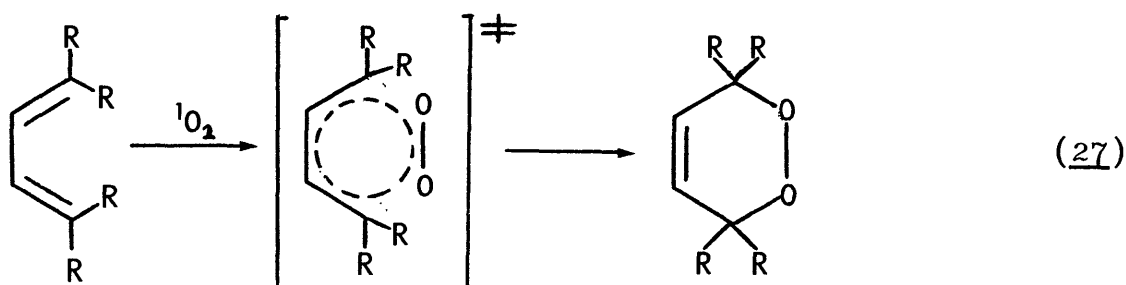


With heterocyclic 1,3-dienes, the endoperoxide initially formed is frequently unstable, readily undergoing ring-opening reactions. Thus with 1,3-diphenylisobenzofuran, one of the most widely used singlet oxygen substrates, the isolated product is not the endoperoxide but *o*-dibenzoylbenzene, formed by the reaction of a second molecule of the substrate with the endoperoxide initially produced (rn. (26)) (77). An analogous product is obtained from the corresponding oxidation of 3,5-dimethylfuran (20, 78).



Turning now to the rates of these reactions, as Table 2 (p.23) shows most of the rate constants are in the range $10^6 - 5 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$. The reaction rate constants reported in the literature frequently do not distinguish between k_r , the rate constant for the chemical reaction, and $(k_r + k_q)$, the rate constant for the total interaction, chemical and physical (quenching), of the substrate with singlet oxygen. This is a serious drawback of some of the earlier kinetic studies and the relative magnitudes of k_r and k_q in such cases can be estimated only indirectly.

The introduction of electron-donating substituents in the substrates increases the reaction rates, in agreement with the well established electrophilic nature of singlet oxygen. There is general agreement that 1,4-cycloaddition is a concerted reaction analogous to the Diels-Alder reaction (rn.(27)), (60, 76).



The finding that similar substituents successively introduced into anthracene alter the reaction rates by similar amounts, has been used as evidence of the concerted nature of the reaction. Further evidence for this is found in the small effect which the polarity of the solvent exerts on the reaction rate (60, 79). It should be noted that the effect of the solvent is mainly due to the variation of the lifetime of singlet oxygen rather than the rate constant itself.

Table 2: Substrates of 1,4-cycloaddition of $^1\text{O}_2$

Substrate	Solvent	Rate constant ($\text{l mol}^{-1} \text{s}^{-1}$)	Ref
1,3-cyclopentadiene	MeOH/BuOH 1:1	5.6×10^7	80
1,3-cyclohexadiene	MeOH/BuOH 1:1	3.8×10^6	80
2,5-diphenylfuran	MeOH	4.6×10^7	81
2,5-dimethylfuran	MeOH	4.0×10^8	57
1,3-diphenylisobenzofuran	MeOH	8.0×10^8	57
Anthracene	Benzene	1.5×10^5	82
9-phenylanthracene	Benzene	4.2×10^5	82
9,10-diphenylanthracene	Benzene	1.2×10^6	82
9-methylanthracene	Benzene	3.2×10^6	82
9,10-dimethylanthracene	Benzene	2.1×10^7	82
9-methoxyanthracene	Benzene	2.5×10^6	82
9,10-dimethoxyanthracene	Benzene	1.4×10^7	82
rubrene	Benzene	2.1×10^9	82
bilirubin	Chloroform	4.3×10^8	82

CHAPTER 2

The Development Of A Water-Soluble Singlet Oxygen Trap

1. Introduction

1.1. The kinetics of trapping

The trapping of singlet oxygen can be used to demonstrate qualitatively its presence in a system or to provide quantitative reactivity data. In both cases a knowledge of the kinetic processes underlying trapping is necessary for the correct assessment of the results.

A number of kinetic methods have been used. These fall into two broad categories: (a) studies under "equilibrium" conditions and (b) studies prior to establishment of equilibrium.

In studies under "equilibrium" conditions singlet oxygen is usually produced by a rate-limiting process (chemical

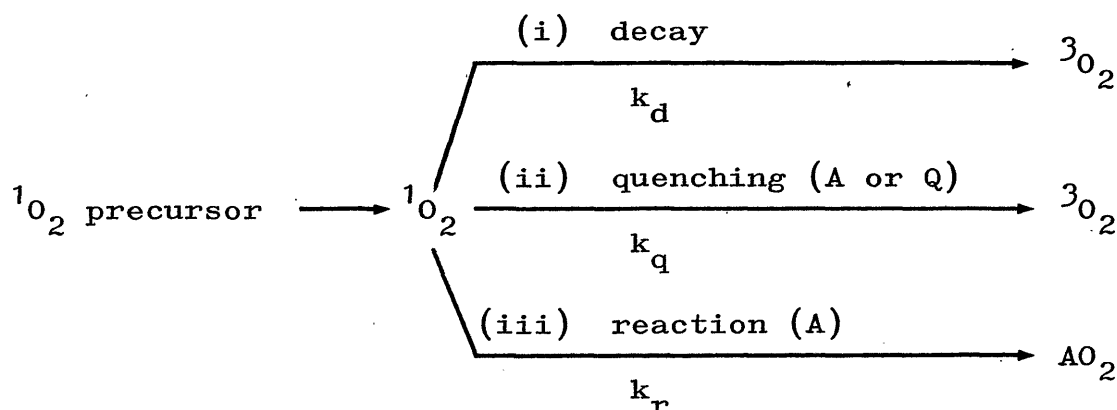
or photochemical) and it never accumulates. When measurements of substrate disappearance or product formation are taken, all, or practically all, the singlet oxygen produced up to that point has either reacted or decayed (83-86).

This method may be used either in the presence of just one substrate, whose reaction is being followed, or in the presence of that and another compound which may be a second substrate (competitive experiment) or a quencher of singlet oxygen (20, 83, 86, 87). As this type of method has been used in some of our studies, its basic kinetic equations will be described later in this section.

In studies under conditions of non-equilibrium, singlet oxygen is produced very rapidly (usually by a short pulse of laser radiation) so that a significant pool of it accumulates. This subsequently decays by reaction with the substrate or by quenching, and this reaction of the substrate (which typically has a lifetime of the order of milliseconds) is monitored by means of suitable rapid-reactions apparatus (81, 88-90). This method too can be used with just one substrate or in the presence of a second substrate or a quencher. It has proved a very valuable method because it provided the first direct estimates of absolute rate constants for singlet oxygen reactions.

Once singlet oxygen is produced it may follow one of three principle pathways (Scheme 1) : (i) it may return to

the ground state spontaneously or through quenching by the solvent, (ii) it may be physically quenched back to the ground state by added molecules, which may be those of a substrate (A) or a quencher (Q), and (iii) it may react chemically with a substrate (A).



Scheme 1

Once all the singlet oxygen produced up to a certain moment has reached equilibrium with its environment (by decaying, being quenched or reacting), the fraction which has reacted with A will be given by equation (1), provided that no significant change in the concentration of A has occurred, i.e. $\Delta[A] \ll [A]_0$.

$$E = \frac{[AO_2]}{[^1O_2]} = \frac{-\Delta[A]}{[^1O_2]} = \frac{k_r[A]_0}{k_d + (k_r + k_q^A)[A]_0} \quad (1)$$

$$\frac{1}{E} = \frac{k_r + k_q^A}{k_r} + \frac{k_d}{k_r} \cdot \frac{1}{[A]_0} \quad (2)$$

In this equation it is assumed that quenching occurs only through the solvent (k_d) and A (k_q^A) and that no other quencher is present. The ratios $[AO_2]/[{}^1O_2]$ and $-\Delta[A]/[{}^1O_2]$ represent the efficiency E with which the singlet oxygen produced is trapped by A. If E is measured at different initial concentrations of A, a plot of $1/E$ against $1/[A]$ (a type of Stern-Volmer plot) would be linear, with slope (k_d/k_r) and vertical intercept ($1 + k_q/k_r$). The first of these is a frequently measured parameter, often referred to as the β -value or Foote constant (20). It gives the ratio of the rate constants for solvent quenching and chemical reaction by A. In the absence of physical quenching by A (i.e. if $k_q \ll k_r$), β is numerically equal to the concentration of A at which 50% of the singlet oxygen is chemically trapped by A. It must be emphasized that this relation does not hold when a substrate brings about significant physical quenching, a fact that was ignored in most of the earlier reported kinetic studies (20, 87). The relative importance of physical quenching by a substrate is provided by the ratio k_q/k_r obtained from the vertical intercept of the plot.

In the presence of an added quencher Q, equations (1) and (2) have to be modified into (3) and (4):

$$E = \frac{k_r[A]_0}{k_d + (k_r + k_q^A)[A]_0 + k_q^Q[Q]} \quad (3)$$

$$\frac{1}{E} = \frac{k_d + (k_r + k_q^A)[A]_0}{k_r[A]_0} + \frac{k_q^Q}{k_r[A]} \cdot [Q] \quad (4)$$

If now the efficiency is measured at a fixed $[A]_0$ but with different concentrations of Q , a plot of $1/E$ against $[Q]$ would provide a value for k_q^Q/k_r . It can be seen that the technique described provides values for the ratios of rate constants and not for individual ones. Such values could be calculated if one, e.g. k_d , were obtained by an independent method, such as a non-equilibrium method.

As stated in Ch.1, Sect.5, the increase in the reactivity of singlet oxygen substrates in going from water to heavy-water, resulting from its increased lifetime in the latter solvent, has been widely used as a diagnostic tool for the involvement of singlet oxygen in a reaction. The corresponding isotope effect on the observed efficiency E , can be calculated as follows:

In heavy-water the lifetime of singlet oxygen is tenfold longer than that in water, i.e.

$$k_d^{D_2O} = 0.1 k_d^{H_2O} \quad (5)$$

From (2), and writing E_H for the efficiency in water and E_D for the efficiency in heavy water, we have:

$$\frac{1}{E_H} = \frac{k_d^{H_2O}}{k_r[A]_0} + \frac{k_r + k_q^A}{k_r} \quad (6)$$

$$\frac{k_d^{H_2O}}{k_r[A]_0} = \frac{1}{E_H} - \frac{k_d + k_q^A}{k_r} \quad (7)$$

On the other hand, in heavy-water

$$\frac{1}{E_D} = \frac{k_d^{D_2O}}{k_r[A]_0} + \frac{k_r + k_q^A}{k_r} \quad (8)$$

$$\frac{1}{E_D} = \frac{0.1 k_d^{H_2O}}{k_r[A]_0} + \frac{k_r + k_q^A}{k_r} \quad (9)$$

And substituting (7) into (9)

$$\frac{1}{E_D} = \frac{0.1}{E_H} + 0.9 \left(1 + \frac{k_q^A}{k_r} \right) \quad (10)$$

$$E_D = \frac{E_H}{0.1 + 0.9 E_H \left(1 + \frac{k_q^A}{k_r} \right)} \quad (11)$$

Equation (11) in the case of $k_q^A = 0$, gives

$$E_D = \frac{E_H}{0.1 + 0.9 E_H} \quad (12)$$

Clearly the solvent isotope effect will approach 10 at low substrate concentrations (and efficiencies) while it will decrease toward unity as the latter are increased. Consequently for optimum effect the lowest practicable concentration of the substrate must be chosen.

1.2. Traps for singlet oxygen

A fairly wide range of compounds has been used for the specific trapping of singlet oxygen for the purpose of qualitative detection or kinetic studies. It includes furans and other heteroatom-bearing conjugated diene systems, anthracenes and other polycyclic aromatics, simple olefins and inorganic compounds. All the organic substrates used with the exception of the olefins react with singlet oxygen by a 1,4-cycloaddition pathway while the olefins that have been used give allylic hydroperoxides. Table 3 gives some relevant data on the most important of these compounds with the exception of the anthracenes which are discussed in the next Section.

Table 3: Traps of singlet oxygen

Trap	k_r ($l \text{ mol}^{-1} \text{ s}^{-1}$)	$\beta_{\text{calc.}} = \frac{k_d^{\text{H}_2\text{O}}}{k_r}$ (M)	Ref
Diphenylisobenzofuran	8×10^8 (MeOH)	6×10^{-4}	57
Dimethylfuran	4×10^8 (MeOH)	1.2×10^{-3}	57
Histidine	3.2×10^7 (H_2O)	1.6×10^{-2}	60
Imidazole/NDMA*	—	—	96,97
Cholesterol	3.4×10^4 (pyridine)	15	67
Limonene	—	—	68

* NDMA: Nitrosodimethylaniline

Of the two furans of Table 3, diphenylisobenzofuran is one of the most widely used singlet oxygen traps (51, 83, 87, 90, 91) and the most reactive of the common ones, having a calculated β value in water of $6 \times 10^{-4} \text{ M}$. Its high reactivity, coupled with a high extinction coefficient in its fluorescent spectrum, explains its wide use. On the other hand, the use of this compound is limited by its low solubility in water and by the fact that its reaction with singlet oxygen is not stoichiometric and the starting material is slowly regenerated from the reaction products (92). Dimethylfuran, although less reactive, has

also been extensively used, partly because of its higher water-solubility. However it suffers from problems associated with a non-stoichiometric reaction, volatility and relatively difficult monitoring of its reaction. Furthermore, diphenylisobenzofuran and dimethylfuran are known to undergo reaction with some chemical precursors of singlet oxygen, such as for example with sodium hypochlorite (92, 24).

With both of the compounds just described, substrate disappearance is the method of monitoring the reaction, and the same is true of histidine (95) and of imidazole/p-dimethylaniline (96, 97), two other water-soluble singlet oxygen-detecting systems. Histidine has been shown (93, 94) to react with hypochlorite and to quench singlet oxygen, and it is likely that the same is true of imidazole. The imidazole/p-dimethylaniline system is an interesting one in that it exhibits a rather unique kinetic pattern with singlet oxygen which can be used to help distinguish between the latter and other oxidizing intermediates.

All the traps so far described, relying as they do on the monitoring of substrate disappearance, are liable to give false positive results in the presence of reactive species other than singlet oxygen (e.g. hydroxyl radicals). This drawback is avoided in the case of tetramethylethylene,

cholesterol and limonene, where the observation of the products or their deliberately modified derivatives is carried out. Limonene gives a characteristic pattern of six oxidation products with singlet oxygen, two of which are obtained exclusively from the singlet oxygen reaction (68). An analogous situation holds with cholesterol, whose hydroperoxide products are analyzed by TLC without further modification (67). The existence of this "fingerprint" pattern of products makes these compounds very useful for the specific and relatively unambiguous detection of singlet oxygen (98). However, these compounds too suffer from lack of adequate water solubility.

In conclusion, the main disadvantages of the water-soluble traps so far used include the lack of easy monitoring of their reactions and non-stoichiometry, things which limit their use, particularly in the search for singlet oxygen involvement in biological systems. The search for and synthesis of a soluble trap of singlet oxygen were conducted bearing in mind that a good singlet oxygen trap should satisfy as far as possible the following criteria:

- (i) it should be stable under the conditions of its use, and particularly it should be inert towards the chemical precursors of singlet oxygen,
- (ii) it should react specifically with singlet oxygen to give characteristic, stable products,

- (iii) the extent of reaction should be easily quantifiable, preferably in terms of both substrate consumption and, independently, product formation, and
- (iv) it should not physically quench singlet oxygen to any significant extent.

1.3. Anthracene derivatives as traps of singlet oxygen

The photooxidation of anthracene derivatives to give transannular endoperoxides was first carried out by Duffraisse and Etienne in 1935 (99). Since that time a large number of anthracene derivatives have been investigated for this reaction (60, 66, 100), and the first clear demonstrations of singlet reactions in solution involved the formation of endoperoxides with substituted anthracenes (15, 16).

The mechanism of the 1,4-cycloaddition of singlet oxygen to anthracenes has been investigated by Stevens and his group (79, 82). Addition always takes place across the nucleophilically active 9 and 10 positions, unless stronger electron-donating substituents are present elsewhere in the molecule (Ch.1, Sect. 5). Table 2 gives a series of rate constants which illustrates the high reactivity of substituted anthracenes towards singlet oxygen. The reaction appears to be quite specific in that only one product is found and in that this product is generally formed only

with singlet oxygen. However, some caution should be exercised because anthracenes are known to act as photosensitizers and it is therefore possible that under some circumstances they might sensitize their own photooxygenation (66).

Anthracene derivatives have also been used by McKeown and Waters (101) as probes for singlet oxygen formation during peroxyacid decomposition. The results of these studies are discussed in some detail in a subsequent section. It is noteworthy that anthracene derivatives do not undergo epoxidation with peroxy acids (28, 102); in particular 9,10-diphenylanthracene does not react with *m*-chloroperoxybenzoic acid, in contrast to diphenylisobenzofuran which is initially epoxidized (102).

Evans and Tucker (85) have carried out a detailed kinetic study of the interaction between 9,10-dimethylanthracene and singlet oxygen. They have shown that this compound physically quenches singlet oxygen as well as chemically reacting with it, with $k_q/k_r = 0.6$. This is the only study in which the degree of physical quenching of singlet oxygen by an anthracene derivative has been reported.

The endoperoxides of polycyclic aromatic hydrocarbons are generally stable compounds undergoing few reactions under mild conditions (100, 103). One unusual property of some

of them is the regeneration to the parent hydrocarbon with the simultaneous release of singlet molecular oxygen on heating (32, 100). The degree of reversibility is increased by aryl substituents at the positions of oxygen attachment. Table 4 (taken from ref. 100) demonstrates the effect of substituents on the extent of disassociation after heating in vacuo up to 250°C.

Table 4: Thermal decomposition of anthracene endoperoxides

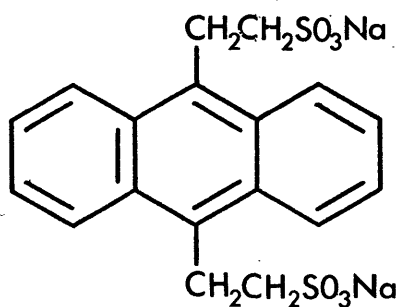
Parent compound	Percent decomposition
9-methylanthracene	0
9,10-dimethylanthracene	0
9-phenylanthracene	12
9-phenyl-10-methyl	20
9,10-diphenylanthracene	96

2. Results and discussion

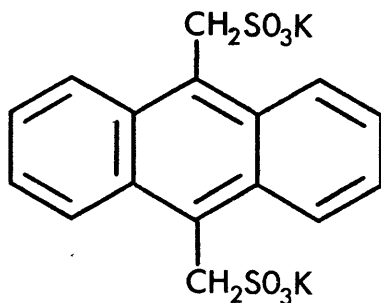
2.1. Anthracene derivatives as traps of singlet oxygen in aqueous solutions

One of the purposes of this work was the preparation of a

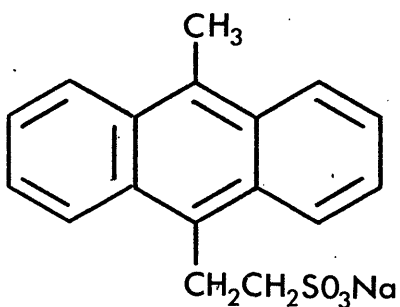
Table 5: Anthracene-derived traps of singlet oxygen examined in the present work



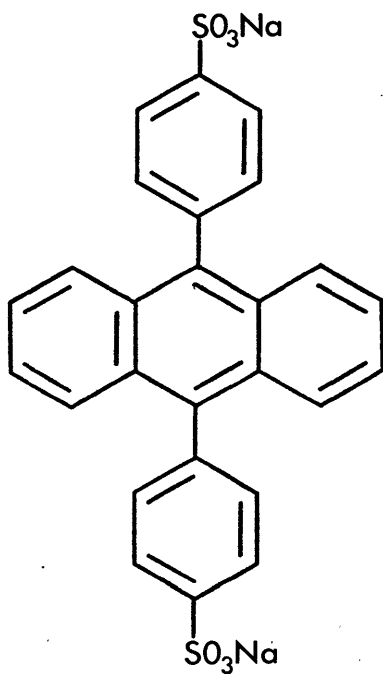
Anthracen-9,10-yl-1'-bis-ethylsulphonic acid, disodium salt (AES).



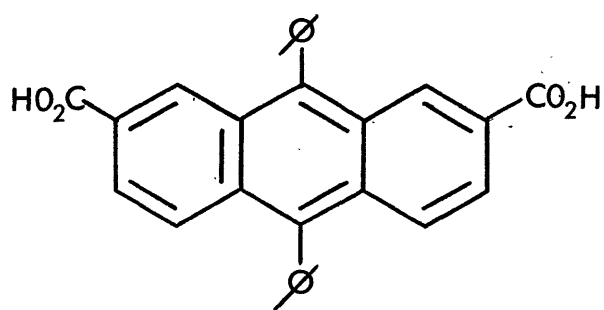
Anthracen-9,10-yl-bis-methylsulphonic acid, dipotassium salt (AMS).



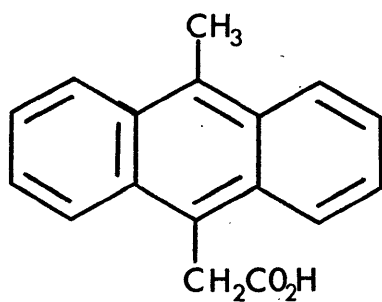
9-Methylanthracen-10-yl-1'-ethylsulphonic acid, sodium salt (AS).



Anthracen-9,10-yl-bis-benzene-4'-sulphonic acid, disodium salt (DPADS).



2,7-Dicarboxyl-9,10-diphenylanthracene (DPADC).

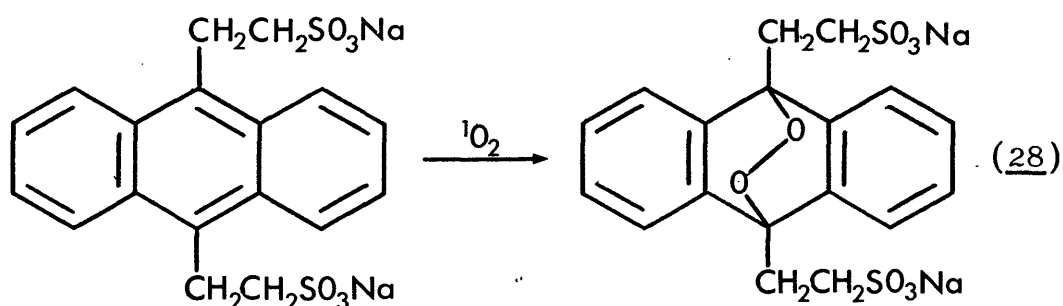


9-Methylantracen-10-yl-acetic acid (AC).

water-soluble singlet oxygen trap with the desirable characteristics (Section 1.2.). The search was based on the anthracene family of substrates known to give only one product on singlet oxygen addition. Consequently, a series of anthracene derivatives (some of them new) containing hydrophilic groups ($-\text{COOH}$ or $-\text{SO}_3^-$) were prepared (Table 5). Of these some were rejected for reasons of solubility and reactivity, two, AES and AMS, were selected for more detailed study and one, AES, was proposed as the chosen trap for singlet oxygen.

First is described in detail the work done on the selected two compounds AES and AMS, and then are given briefly some of the properties of the remaining compounds.

2.2. AES as a trap of singlet oxygen



2.2.1. Preparation and characteristics

The preparation of AES together with its characteristics are given in detail in the experimental part (Section 3.3.1.). This compound was stable over a wide range of pH's (1.5-13) and adequately soluble in aqueous buffers up to a concentration of 0.08 M at least.

2.2.2. The AES product from photosensitized oxygenation

AES was used as a trap in photosensitized oxygenation with Rose Bengal as the triplet sensitizer. The characteristics of the product obtained (TLC, polarography, UV-vis. and NMR spectroscopy) were consistent with the expected 9,10-endoperoxide. TLC examination of the reaction mixture in a number of solvent systems showed that only one product was formed. This product failed to oxidize KI but it did oxidize Fe^{2+} to Fe^{3+} which subsequently gave the characteristic red colour with SCN^- , in accordance with the known properties of dialkyl peroxides (104).

The UV-vis. and NMR spectra are also consistent with the product being the 9,10-endoperoxide of AES. In particular the UV-vis. spectrum lacked the characteristic absorbance of AES in the region 400-340 nm, making it possible to follow the progress of the reaction by monitoring the decrease in AES absorbance at 399 nm.

This way of monitoring the product formation was permitted by the fact that only one product was found and its validity was confirmed by the internal agreement of subsequent results (Section 2.2.5.).

The NMR spectrum of the AES-9,10-endoperoxide in water showed the singlet at 3.33 δ and a singlet at 7.44 δ . Clearly the chemical shifts of the two pairs of $-\text{CH}_2$ -groups were by coincidence practically identical and thus gave rise to no resolved spin-spin splitting, and the same applied to the aromatic protons.

Further evidence for the nature of the product was obtained by polarography using the dropping mercury electrode. No polarographic studies of anthracene endoperoxides have been published, so the polarographic examination is discussed in detail in Section 2.2.4.

The product 9,10-endoperoxide from the photosensitized oxygenation was subsequently used as a standard for confirming products of other reactions.

2.2.3. The AES product from reaction with $\text{H}_2\text{O}_2/\text{NaOCl}$

AES was used as a trap for singlet oxygen in the $\text{H}_2\text{O}_2/\text{NaOCl}$ system. The stability of AES towards H_2O_2 and NaOCl

separately was found to be very satisfactory after incubation times greatly exceeding those used during the reaction in question. The product obtained was shown to be the only product of the reaction (TLC), exhibiting the same characteristics as the standard 9,10-endoperoxide. Incubation of the reaction mixture after completion of the reaction, in the absence or presence of excess of H_2O_2 or NaOCl did not produce any changes in the product, indicating that the latter was stable under the conditions of its formation.

2.2.4. The polarography of AES-9,10-endoperoxide

Polarographic investigation were carried out on the AES-9,10-endoperoxide obtained by photosensitized oxygenation by $\text{H}_2\text{O}_2/\text{NaOCl}$ reaction (Section 2.2.2.).

The polarographic curves obtained (Fig. 1) are of the irreversible reduction type and show a major wave with half-wave potential at -1.02 ± 0.04 volts. Neither the position nor the size of this wave were affected by sodium sulphite, a reagent known to reduce hydroperoxides. The half-wave potential observed was within the range of the corresponding parameters for dialkyl peroxides (104). Polarographic examination of AES failed to give any wave in the region 0-(-1.9) volts. The only transannular endoperoxide for which polarographic studies have been reported is ascaridole (1-methyl-4-i-propylcyclohex-2-ene-

1,4-endoperoxide), which has been studied in organic solvents (105, 106). Under these conditions its half-wave potential varied in the range -0.89 to -1.22 volts, close to the value found for AES-9,10-endoperoxide.

The size of the polarographic wave was linearly related to the concentration of the endoperoxide up to a concentration of 1×10^{-3} M (Fig. 2). This linear relationship allowed the construction of a calibration curve enabling the measurement of the endoperoxide concentration in unknown solutions (Chapter 4, Section 2.2.).

At concentrations above 1×10^{-3} M the polarographic wave began to shift to less negative voltages until at 2×10^{-3} M it stabilized at -0.75 volts with a greatly increased magnitude and the appearance of a maximum. This effect although not investigated properly, was probably due to adsorption of the endoperoxide onto the mercury at these concentrations. Because of this effect all measurements with unknown solutions were conducted at dilutions where the endoperoxide was at a concentration of 1×10^{-3} M or less. The remaining components of the system (in particular buffer ions) were also found to affect the range over which the quantitative measurements at -1.02 volts could be made (probably by affecting the adsorption process). For this reason such measurements were carried out only where all these parameters could be controlled (0.1 M lithium sulphate and carbonate buffer not greater than 0.04 M).

Fig. 1: Polarographic curve for AES-9,10-endoperoxide

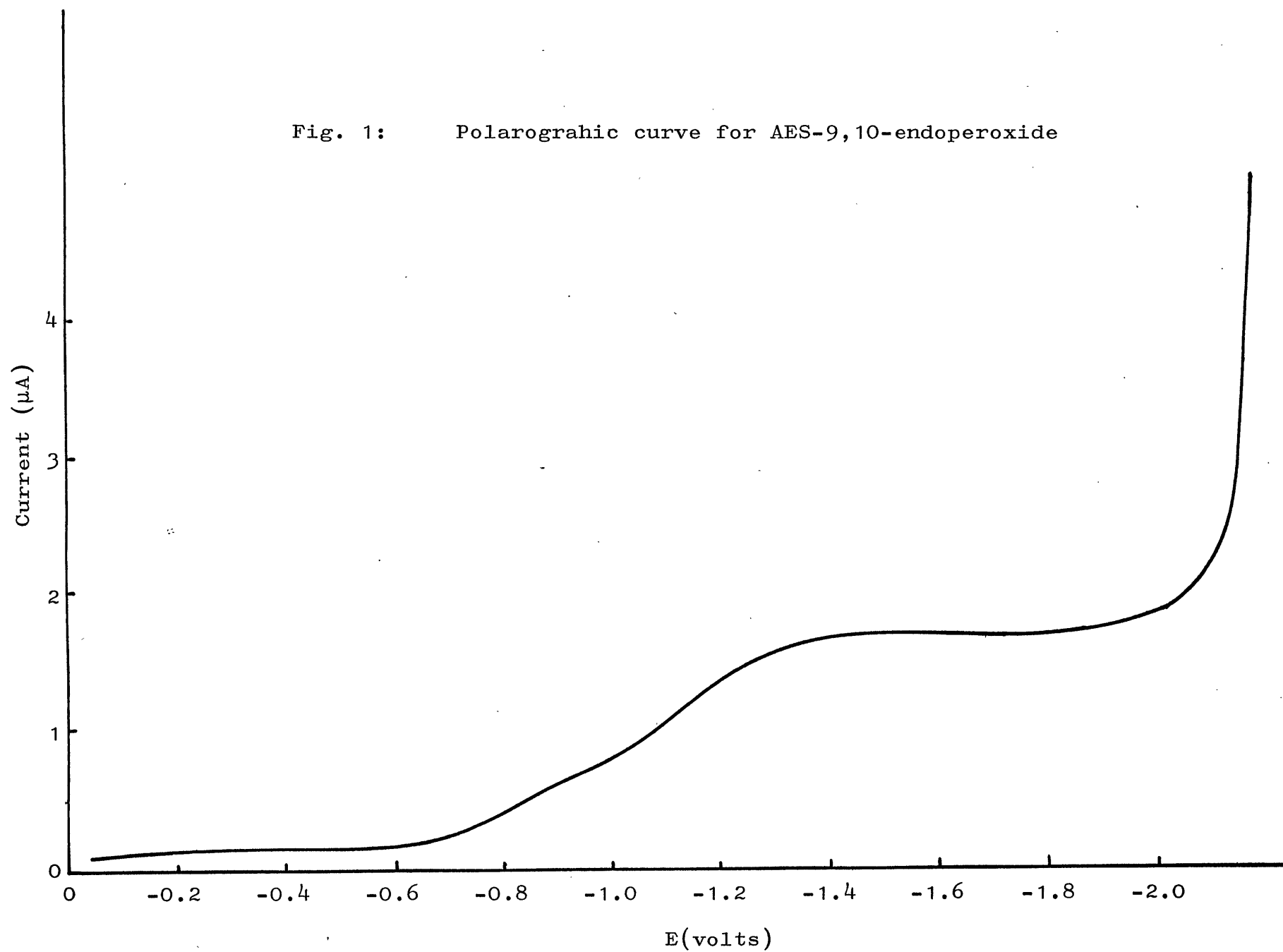
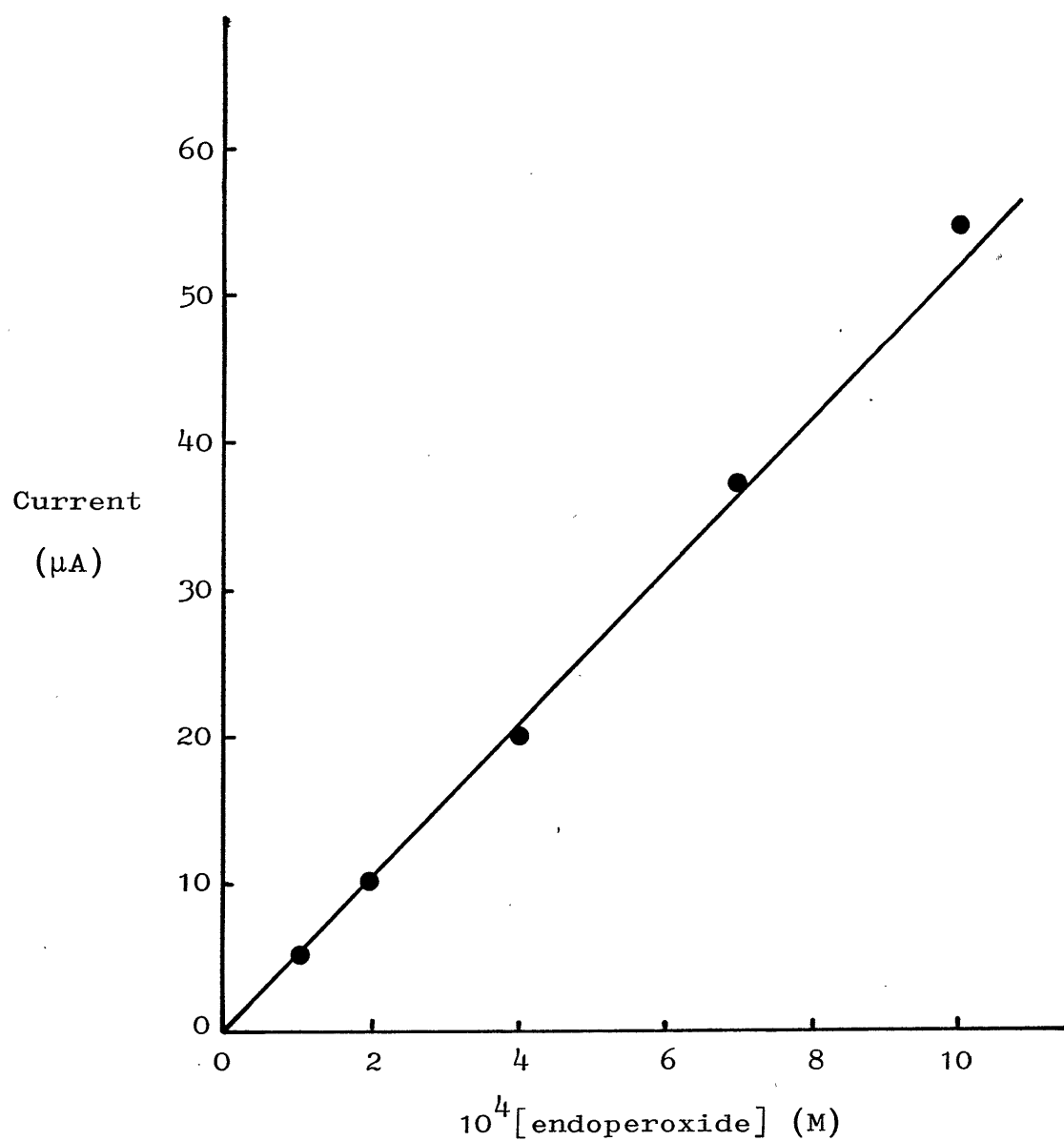


Fig. 2: The measurement of AES-9,10-endoperoxide
by polarography



2.2.5. The reactivity of AES as a trap

With the system $\text{H}_2\text{O}_2/\text{NaOCl}$ as a source, and AES as a trap for singlet oxygen, a Stern-Volmer plot of $1/E$ versus $1/[A]$ (where $[A]$ = average concentration of AES before and after reaction) was constructed (Table 6 and Fig. 3).

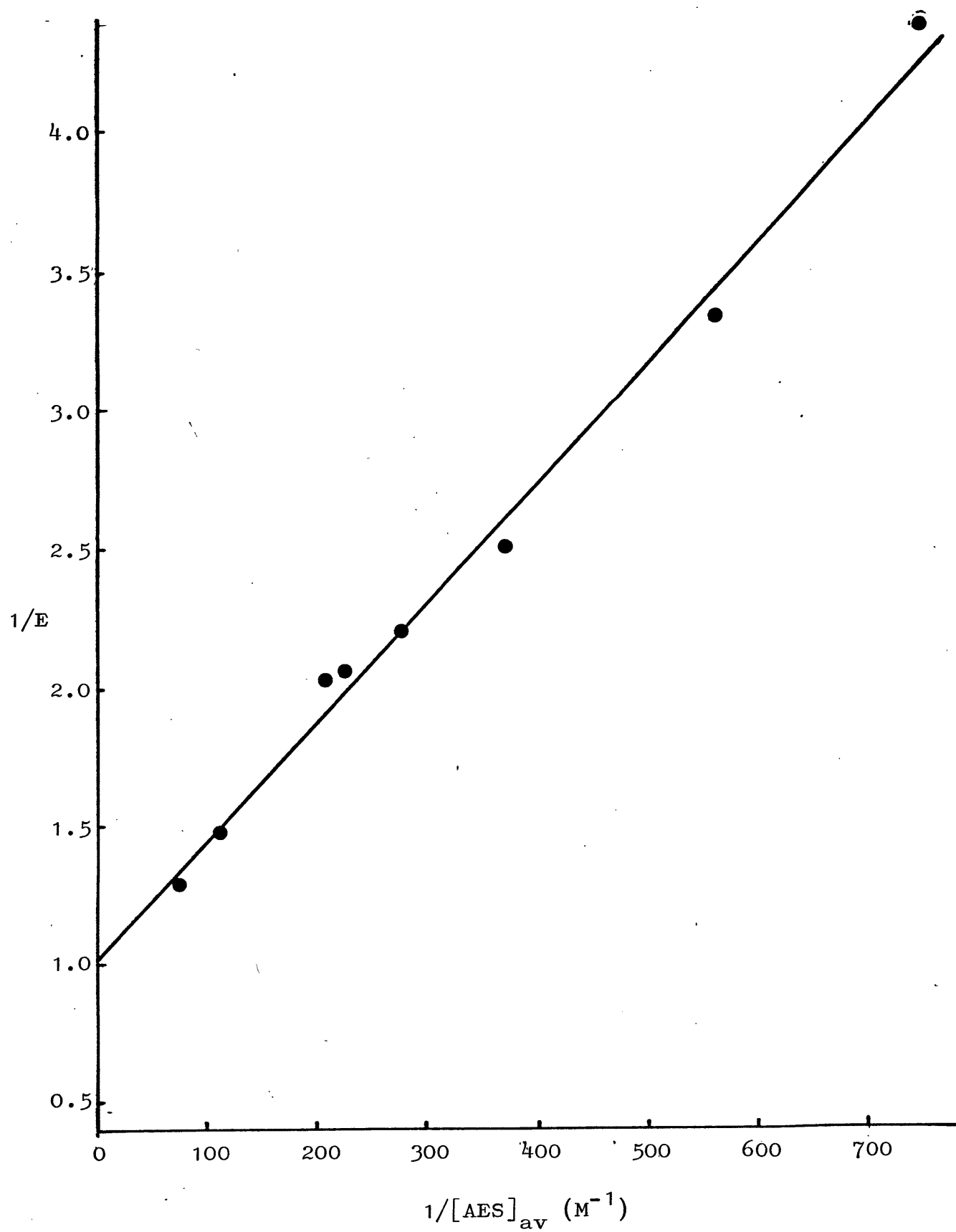
The plot is linear (correlation coefficient = 0.9959) with a slope of $(4.34 \pm 0.17) \times 10^{-3}$ and an intercept of 1.02 ± 0.06 .

Table 6: Kinetic data for AES reactivity

$[\text{AES}]_{\text{av}}$ (M)	$1/[\text{AES}]_{\text{av}}$	$100E$	$1/E$
1.34×10^{-3}	746.2	22.9	4.36
1.79×10^{-3}	558.6	30.1	3.32
2.69×10^{-3}	371.7	39.8	2.51
3.64×10^{-3}	274.7	45.5	2.20
4.43×10^{-3}	225.4	48.3	2.07
4.81×10^{-3}	207.6	49.0	2.04
9.10×10^{-3}	109.8	67.6	1.48
13.7×10^{-3}	73.0	76.9	1.30

In all cases AES consumption was kept between 5% and 13%

Fig. 3: The kinetics of singlet oxygen trapping by AES



The linearity of the plot confirms the participation of an active intermediate (singlet oxygen) in the reaction. The near-unity intercept indicates strongly that no significant quenching of singlet oxygen is caused by AES. An upper limit for any quenching caused is 12.7% (at the 95% confidence level) of the total interaction between AES and singlet oxygen, with $k_q^A/k_r = 0.02 \pm 0.13$. In addition the intercept obtained indicates that the yield of singlet oxygen from the $H_2O_2/NaOCl$ reaction is very close to 100%, confirming the findings of Held et al. (107), and Foote et al. (108).

The slope of the curve allows the calculation of k_r as $(1.15 \pm 0.05) \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$. The slope, being numerically equal to the Foote constant β , indicates that, since no significant quenching is occurring, half of the total singlet oxygen available can be trapped at an $[AES] = 4.34 \times 10^{-3} \text{ M}$, and that for the trapping of 95% of the singlet oxygen available one needs $[AES] = 8.2 \times 10^{-2} \text{ M}$.

A simple calculation, taking into account the molar absorptivity of AES ($\epsilon_{399} = 1.260 \times 10^4$) and the method of monitoring the reaction, shows that the smallest amount of singlet oxygen accurately measurable by AES is of the order of 10^{-7} moles/ml of reaction mixture.

2.2.6. The AES/H₂O₂/NaOCl reaction in D₂O

The effect of D₂O on the efficiency of AES in the H₂O₂/NaOCl system was examined at two AES concentrations (Table 7). The increase in the efficiency, expected from a ten-fold increase in the singlet oxygen lifetime in D₂O, can be calculated according to eq. (12) (Section 1.1.). As Table 7 shows the observed efficiencies are in good agreement with the calculated ones.

Table 7: Heavy-water effect on the efficiency of AES

[AES] (M)	100E _H ^{obs.}	100E _D ^{obs.}	100E _D ^{calc.}
1.46x10 ⁻³	24.9	69.1	75.9
4.3x10 ⁻³	49.5	87.2	89.5

2.2.7. Reaction of AES with HO• radicals

As expected, AES was found to react with HO• radicals generated by the Fenton system as described in Chapter 5, Section 2.1. However the products of this reaction do not react with KI or the Fe²⁺/SCN⁻ system and have different

R_f values from the 9,10-endoperoxide, so they can easily be distinguished from it. Nevertheless, in cases where $HO\cdot$ radicals are expected, it is advisable for specific scavengers to be added (e.g. mannitol).

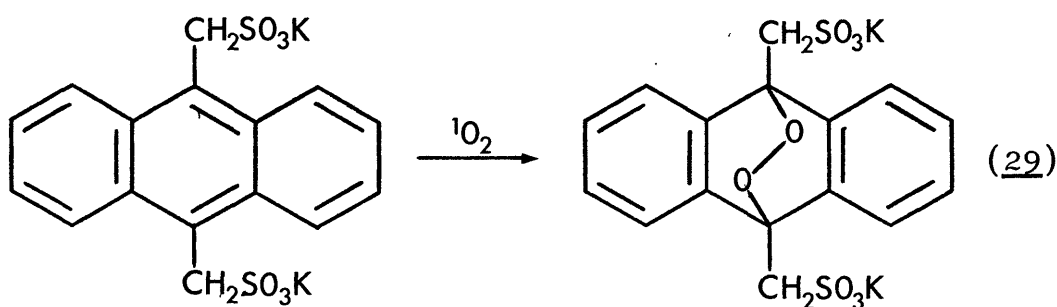
2.2.8. General conclusions regarding the utility of AES

AES is a stable, water-soluble compound which can be used over a wide range of pH. It has a high molar absorptivity in the visible ($\epsilon_{399} = 1.260 \times 10^4$), which allows the straightforward determination of changes in its concentration. It reacts with singlet oxygen rapidly ($k_r = (1.15 \pm 0.05) \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$) and without causing any significant physical quenching, to give one characteristic, stable product. If desired, this product can be easily characterized and quantified by TLC and polarography, while the extent of its formation could be routinely estimated through the decrease in the AES absorbance at 399 nm.

The rate of reaction of AES with singlet oxygen is of the same order as, or greater than, those of other traps commonly used in aqueous systems, such as 2,5-dimethylfuran and histidine (Table 3, p. 31), and its β value lies between those of the other two. However, it has the advantages of easy monitoring and increased stability towards singlet oxygen precursors together with giving a clean,

stoichiometric reaction with singlet oxygen, with a single, stable product.

2.3. AMS as a trap of singlet oxygen



AMS was prepared as described in Section 3.3.2. It was then used as a singlet oxygen substrate in a way exactly analogous to that used for AES, described in the preceding section.

AMS was stable in solution over the pH range 1.5-13 and readily soluble in aqueous buffer up to a concentration of 0.08 M at least.

On photooxidation it gave only one product (TLC), the 9, 10-endoperoxide, which was subjected to the same tests and

characterisations as the corresponding product of AES.

The polarographic examination of the product showed a wave with a $E_{\frac{1}{2}} = -1.06$ volts. A similar product was obtained from the $H_2O_2/NaOCl$ system. This product was shown to be stable under the conditions of its production.

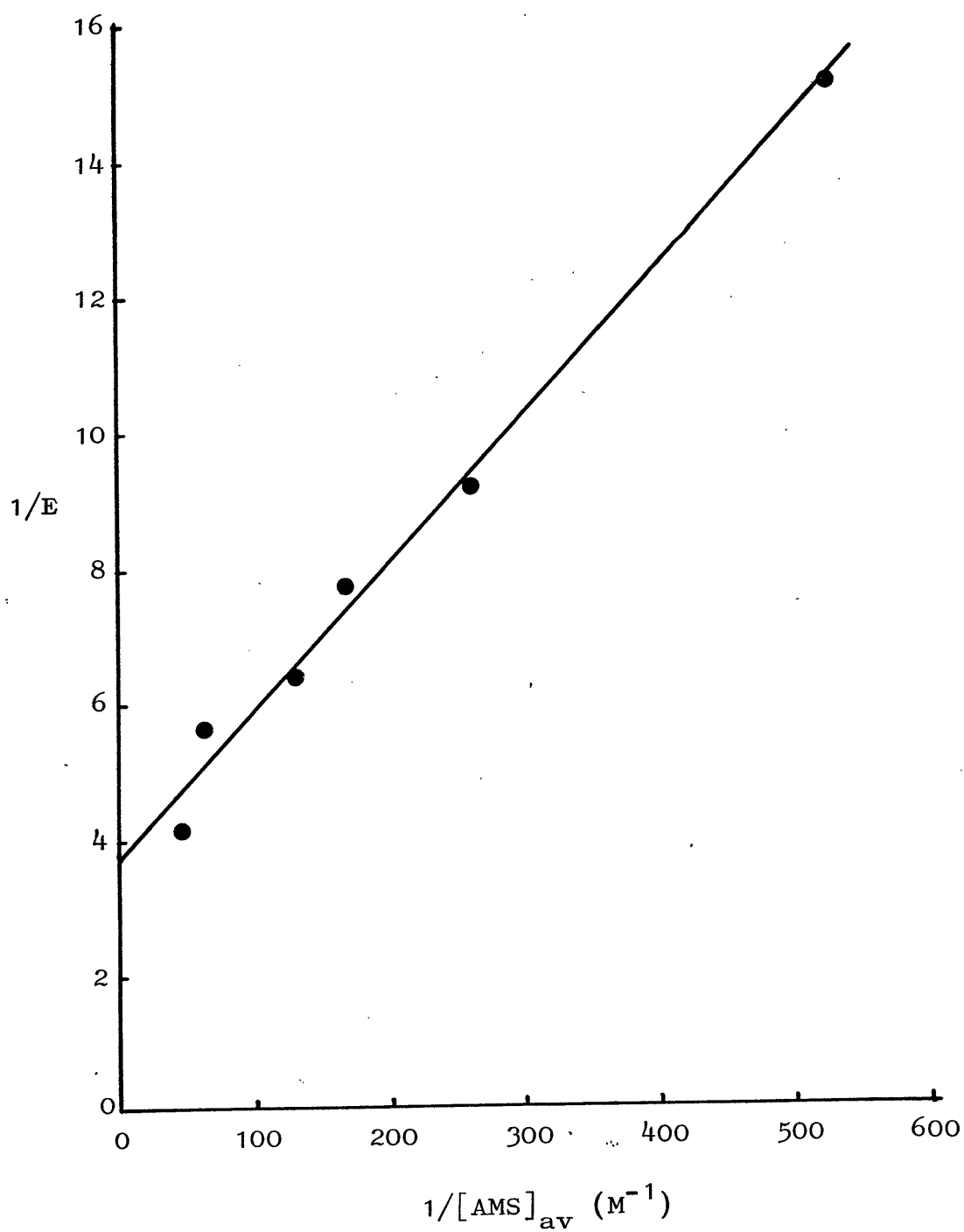
In kinetic experiments using the $H_2O_2/NaOCl$ system, the efficiency of AMS was measured at different initial AMS concentrations. A Stern-Volmer plot was constructed (Table 8, Fig. 4). This plot is linear (correlation coefficient 0.9945), with a slope of $(2.17 \pm 0.11) \times 10^{-2}$ and intercept of 3.76 ± 0.29 .

Table 8: Kinetic data for AMS reactivity

$[AMS]_{av}$ (M)	$1/[AMS]_{av}$	$100E$	$1/E$
1.91×10^{-3}	523.5	6.6	15.10
3.86×10^{-3}	259.0	10.9	9.20
6.02×10^{-3}	166.0	13.0	7.70
7.78×10^{-3}	128.5	15.7	6.37
1.68×10^{-2}	59.6	17.8	5.61
2.27×10^{-2}	44.1	24.0	4.16

The consumption of AMS never exceeded 10%

Fig. 4: The kinetics of singlet oxygen trapping by AMS



The intercept shows that AMS causes significant physical quenching of singlet oxygen. From the value of the intercept and equation(2)(Section 1.1.) it can be calculated that $k_q^A/k_r = 2.76 \pm 0.29$, i.e. quenching constitutes $(73.4 \pm 6.1)\%$ of the total interaction between AMS and singlet oxygen.

The value of the slope of the plot gives $k_r = (2.30 \pm 0.12) \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$. Consequently the rate constant for the quenching is calculated as $k_q = (6.35 \pm 0.74) \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$.

The significant quenching caused by AMS means that the maximal efficiency with which it can intercept singlet oxygen is well below 100%. In fact a simple calculation shows that:

$$E_{\text{max}}^{\text{AMS}} = \frac{1}{\text{intercept}} = 26.6 \pm 2.1\%$$

The value of the Foote constant β , given by the slope of the curve $(2.17 \pm 0.11) \times 10^{-2}$, does not in this case give the AMS concentration which traps one half of the singlet oxygen available (this much can never be trapped by AMS) or show half-maximal efficiency.

Table 9 shows the various kinetic parameters measured for AMS and AES.

Table 9: Kinetic parameters of AES and AMS

Substrate	$10^{-8}k_r$ (l mol ⁻¹ s ⁻¹)	$10^{-7}k_q$ (l mol ⁻¹ s ⁻¹)	$10^3\beta$ (M)	$E^{\max}$ (%)
AES	1.15 ± 0.05	—	4.34 ± 0.17	98.3 ± 3.7
AMS	0.230 ± 0.012	6.35 ± 0.74	21.7 ± 1.1	26.6 ± 2.1

Further evidence for the correctness of the figures just presented for AMS was obtained from measurements of the effect of D₂O on AMS efficiency (Table 10). Taking into account the degree of quenching caused by AMS (eq. (11), Section 1.1.) one can calculate the expected efficiencies in close agreement (calculated with k_q taken from the Stern-Volmer plot) with the ones actually observed.

Table 10: Heavy-water effect on the efficiency of AMS

[AMS]	$100E_H^{\text{obs.}}$	$100E_D^{\text{obs.}}$	$100E_D^{\text{calc.}}$
4.10×10^{-3}	11.5	23.9	23.5
7.94×10^{-3}	14.9	26.5	24.7
2.29×10^{-2}	20.5	27.9	25.8

AMS is clearly less reactive than AES and, more importantly, it brings about significant quenching of singlet oxygen a fact which limits its usefulness. It is not possible to explain the difference between AMS and AES in their ability to quench singlet oxygen on the basis of previous results. A hint for a possible explanation was found in the studies of peroxyacid decomposition (Chapter 4).

2.4. Other singlet oxygen traps

The preparation and characteristics of the remaining substrates synthesized (DPADCA, AC, AS and DPADS) are given in the experimental part.

All of them were stable in aqueous solution, but they were significantly less soluble than AES and AMS. AS had a solubility limit at ca. 3×10^{-3} M, while DPADCA and AC were soluble only above pH 9.

All substances were tested as singlet oxygen substrates in the photosensitized oxygenation, $H_2O_2/NaOCl$ reaction and peroxymonophthalic acid uncatalyzed decomposition. This last reaction was carried out as described in Chapter 4 with the exception that for DPADCA and AC the pH of the reaction was 9. All three reactions gave a single product (TLC) which as usual was identified as the 9,10-endoperoxide.

On heating to 180°C in vacuo, the DPADCA 9,10-endoperoxide formed by photosensitized oxygenation, gave back the original hydrocarbon with a yield of 91%, very close to the 96% reported for the degree of thermal decomposition of the similar substrate diphenylanthracene-9,10-endoperoxide (100).

Because of the solubility problem no full kinetic examination of these compounds with singlet oxygen was carried out. Table 11 shows their efficiencies, (together with those of AES and AMS) in the $\text{H}_2\text{O}_2/\text{NaOCl}$ system at a fixed concentration of 4×10^{-3} (apart from $[\text{AS}] = 2 \times 10^{-3}$ M for solubility reasons).

Some of the substrates of Table 11 showed efficiencies higher than those of AES and AMS at similar concentrations. However, because of solubility and pH limitations they were not considered useful singlet oxygen traps.

3. Experimental

3.1. Reagents

Sodium hypochlorite was prepared by passing chlorine gas through an ice-cold 2N NaOH solution until the pH reached the value of 11.0. The resulting hypochlorite solution was

Table 11: The efficiencies of various singlet
oxygen substrates

Substrate	Conc. (M)	100 E
DPADC	4×10^{-3}	16.5
AC	4×10^{-3}	50.6
AS	2×10^{-3}	51.3
DPADS	4×10^{-3}	17.2
AES	4×10^{-3}	47.6
AMS	4×10^{-3}	10.9

analysed by $\text{Na}_2\text{S}_2\text{O}_3/\text{KI}$ titration and was found to be 0.92 M. Stock solutions were kept in dark bottles in a refrigerator and were always analysed volumetrically prior to use. They were generally found to be stable.

Hydrogen peroxide was 30% w/v BDH Aristar. It was kept in a refrigerator and was always analysed volumetrically prior to use.

All other reagents (apart from those whose preparation is described herein) were commercial products of the highest purity available.

All aqueous solutions were prepared using deionised water.

3.2. Instruments

U.V. spectra were ran on a Perkin Elmer 402 or a Pye Unicam SP8000 UV spectrophotometer, IR spectra on a Perkin Elmer 257 IR spectrometer and NMR spectra on a Perkin Elmer R32 NMR spectrometer. Polarography was conducted on a Radiometer Polariter polarograph with a timer, using a dropping mercury electrode (DME).

All reactions and measurements were carried out at 20°C unless otherwise indicated in the "Results and Discussion" Sections.

3.3. The preparation of water-soluble anthracene derivatives for use as singlet oxygen substrates

Of the various anthracene derivatives used, one (AS) was kindly provided by Dr D.F. Evans.

UV (H_2O) λ_{max} 401, 380 ($\log \epsilon = 3.940$), 360, 343 nm.

The remaining anthracene derivatives used were of two types. One type carried carboxy groups to increase their water-solubility, while the other type carried sulphonic acid groups.

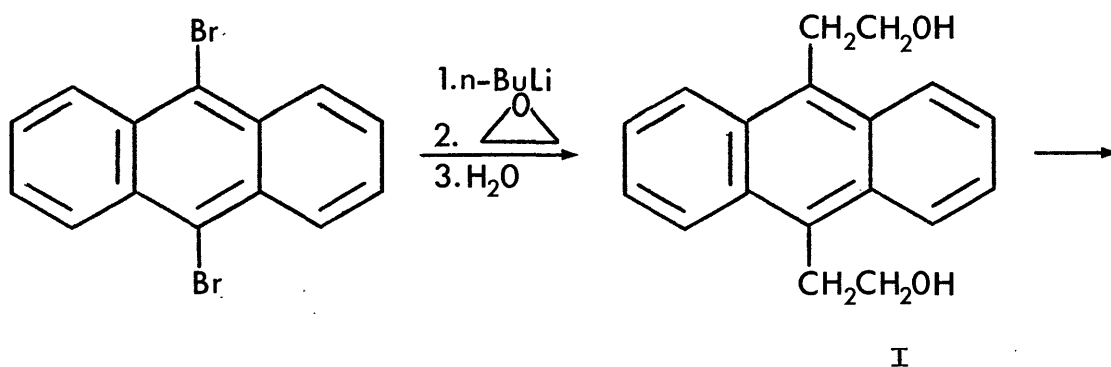
Alkyl or aralkylsulphonates are normally prepared by the reaction of the corresponding bromide or iodide with excess sodium sulphite(109). The reaction can be carried out in 50% acetone-water mixtures to allow dissolution of both substrates. This method however suffers from one drawback: Sodium sulphite is not very soluble in the solvent mixture, and it is difficult to isolate the pure sodium salt of the sulphonic acid in the presence of the other sodium salts.

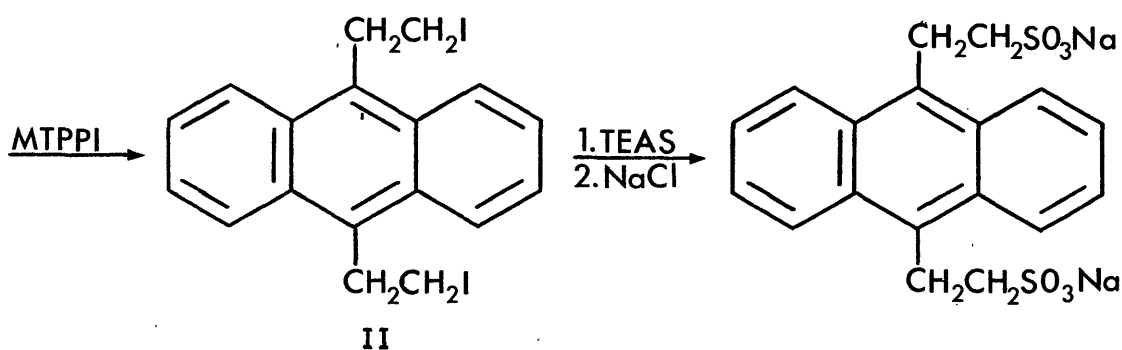
The use of tetraethylammonium sulphite in place of sodium sulphite has been found to help overcome this problem. A homogenous solution is easily obtained, and the tetra-

ethylammonium salts of sulphonic acid are extremely soluble in water. Any unreacted organic material can thus be easily removed. The sodium or potassium salt can then be obtained by precipitation from aqueous solution with NaCl or potassium acetate. Furthermore, since the sodium salts are very soluble in water but not in ethanol, a successful method of removing the excess NaCl is to wash with 50% NaClO₄ and then with ethanol in which NaClO₄ is quite soluble.

3.3.1. Preparation of AES

AES (Na salt) was prepared as outlined in Scheme 2.





Scheme 2

The dialcohol (I) was initially prepared by a modification of a literature method (110). 9,10-Dibromoanthracene (49 g, 0.146 moles) was added to anhydrous diethyl ether (350 ml) and the mixture was stirred with a magnetic bar stirrer under argon while being cooled to 5°C. A solution of *n*-butyllithium (1.6 M in hexane) (200 ml) was added to the mixture over a period of 10 min and stirring was continued for a further 20 min. An excess of ethylene oxide dissolved in cold diethyl ether (70 ml) was added over a period of 10 min, and the mixture was then poured over 100 g of crushed ice. After filtration, the precipitated dialcohol (I) was washed with a little cold ether. The yield of crude product was 33.4 g (86.2%) while that of the product obtained after recrystallisation from ethanol was 14.7 g, 38.3% m.p. 221-225°C (lit. 223.5-224.5°C).

Anal. calcd. for $C_{18}H_{18}O_2$: C, 81.2; H, 6.82.

Found: C, 80.81; H, 6.64.

The dialcohol (14.7 g, 0.055 moles) was dissolved in anhydrous DMF (dried over 3A molecular sieves), methyltriphenoxyphosphonium iodide (MTPPI) (99.8 g, 0.221 moles) (prepared according to Veheyden and Moffatt (111)) was added and the mixture was stirred for 1 hour at 15°C. Methanol (150 ml) and water (150 ml) were then added slowly and the mixture was cooled to 0°C. The crystals of the diiodide (II) which formed were filtered, washed with a little methanol, dried in air and recrystallised from benzene. Yield: 17.0 g, 63.9% m.p. 180° (dec.).

Anal. calcd. for $C_{18}H_{16}I_2$: C, 44.41; H, 3.32.

Found: C, 44.99; H, 3.18.

The diiodide (4.3 g, 0.0088 moles) was dissolved in a mixture of aqueous 0.75 M tetraethylammonium sulphite (TEAS) (32 ml, 0.24 moles) and dioxan (32 ml) and refluxed while being stirred for 6 hours. (Dioxan was used in place of acetone to allow the use of higher temperatures. The TEAS had been prepared by passing SO_2 gas through a solution of tetraethyl ammonium hydroxide). The mixture was evaporated to dryness on a rotatory evaporator and the residue redissolved in water (30 ml). After removing any undissolved material by filtration, the filtrate was heated to boiling and NaCl (8.5 g) was dissolved in it. After cooling to 0°C, the precipitated sodium salt of AES was collected by filtration, washed successively with 20% NaCl, 50% aqueous

NaClO_4 and extensively with ethanol, and dried in air. Yield 3.2 g, 83.0%. Overall yield (relative to 9,10-dibromoanthracene): 20.3%.

Anal. calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_2\text{S}_2\text{Na}_2$: C, 49.33; H, 3.68; S, 14.64.

Found: C, 48.51; H, 3.53; S, 13.45.

UV (H_2O) λ_{max} 399 ($\log \epsilon = 4.093$), 378, 361, 342 nm (Fig.5).

NMR (D_2O) - 2.96 δ (q, 4H), 3.54 δ (q, 4H), 7.55 δ (q, 4H), 8.04 δ (q, 4H).

In order to check further the purity of the compound, the less hygroscopic tetraphenylphosphonium salt was prepared by extraction into CHCl_3 . After drying, this compound was subjected to elemental analysis.

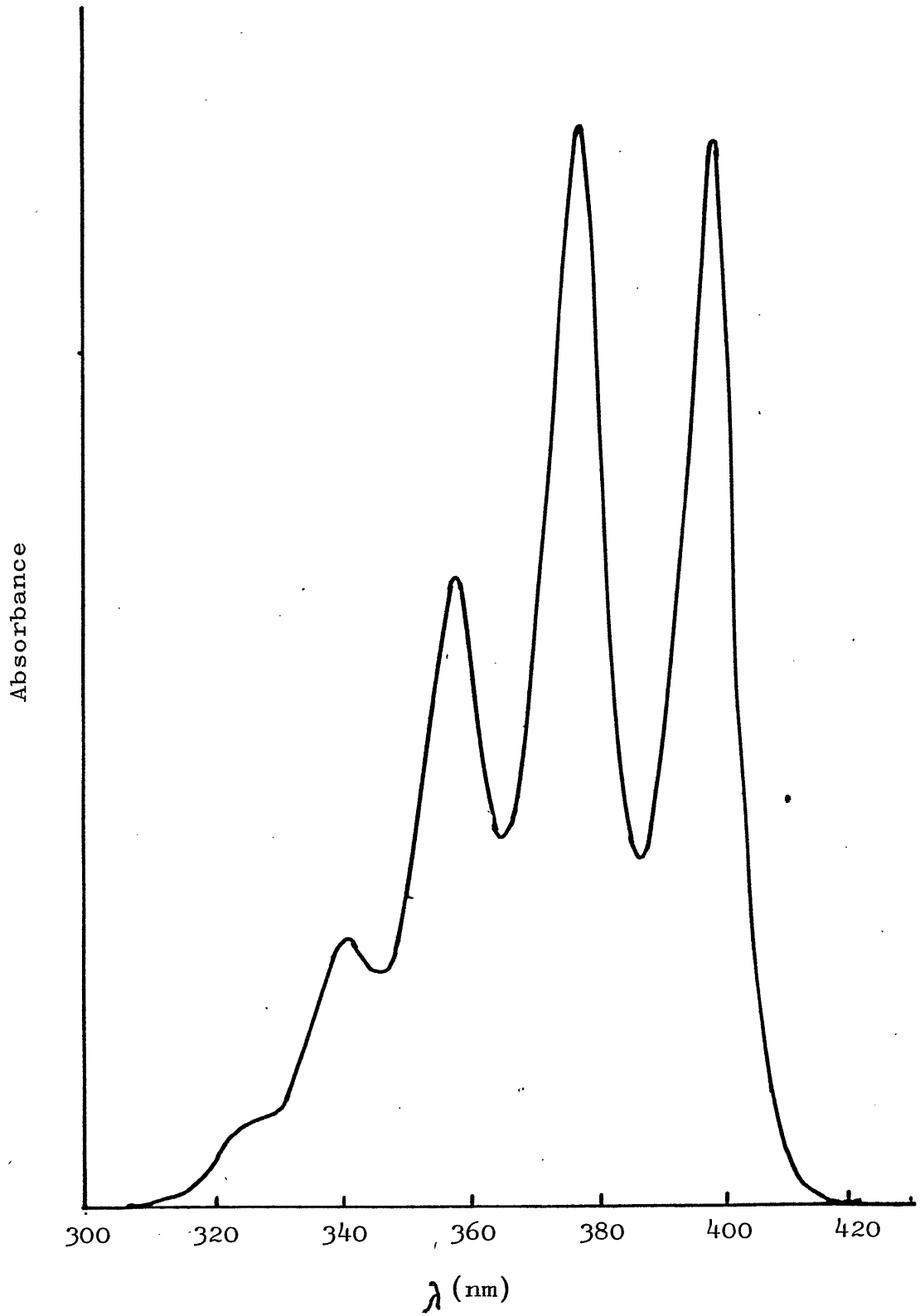
Anal. calcd. for $\text{C}_{66}\text{H}_{56}\text{O}_6\text{S}_2\text{P}_2$: C, 73.99; H, 5.27.

Found: C, 73.74; H, 5.28.

Finally TLC analysis of the product (Section 3.8.) showed only one spot.

Apart from the sodium salt of AES, the potassium and caesium salts were also prepared as part of the general attempt to obtain as pure a compound as possible. The potassium salt was prepared by adding KCl instead of NaCl to

Fig. 5: The UV spectrum of AES



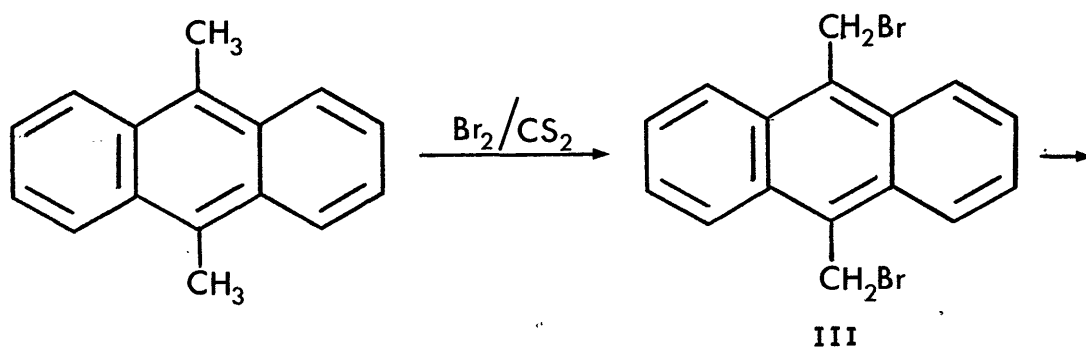
the aqueous mixture obtained from reaction of the diiodide with TEAS. The precipitate obtained on cooling was filtered and washed successively with potassium acetate solution and ethanol.

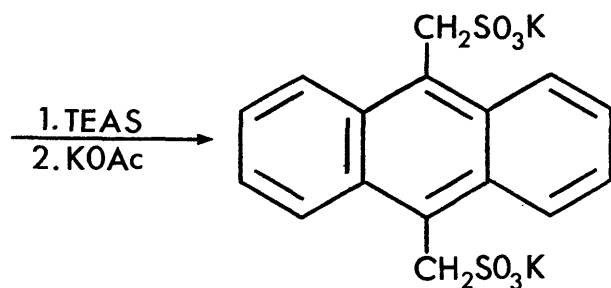
The caesium salt was obtained in a similar way and washed with strong CsCl solution and only a small volume of ethanol as this salt is soluble in the latter.

The elemental analysis of these salts did not indicate that they offered any significant advantage over the sodium salt.

3.3.2. Preparation of AMS

AMS (K salt) was prepared as outlined in Scheme 3.



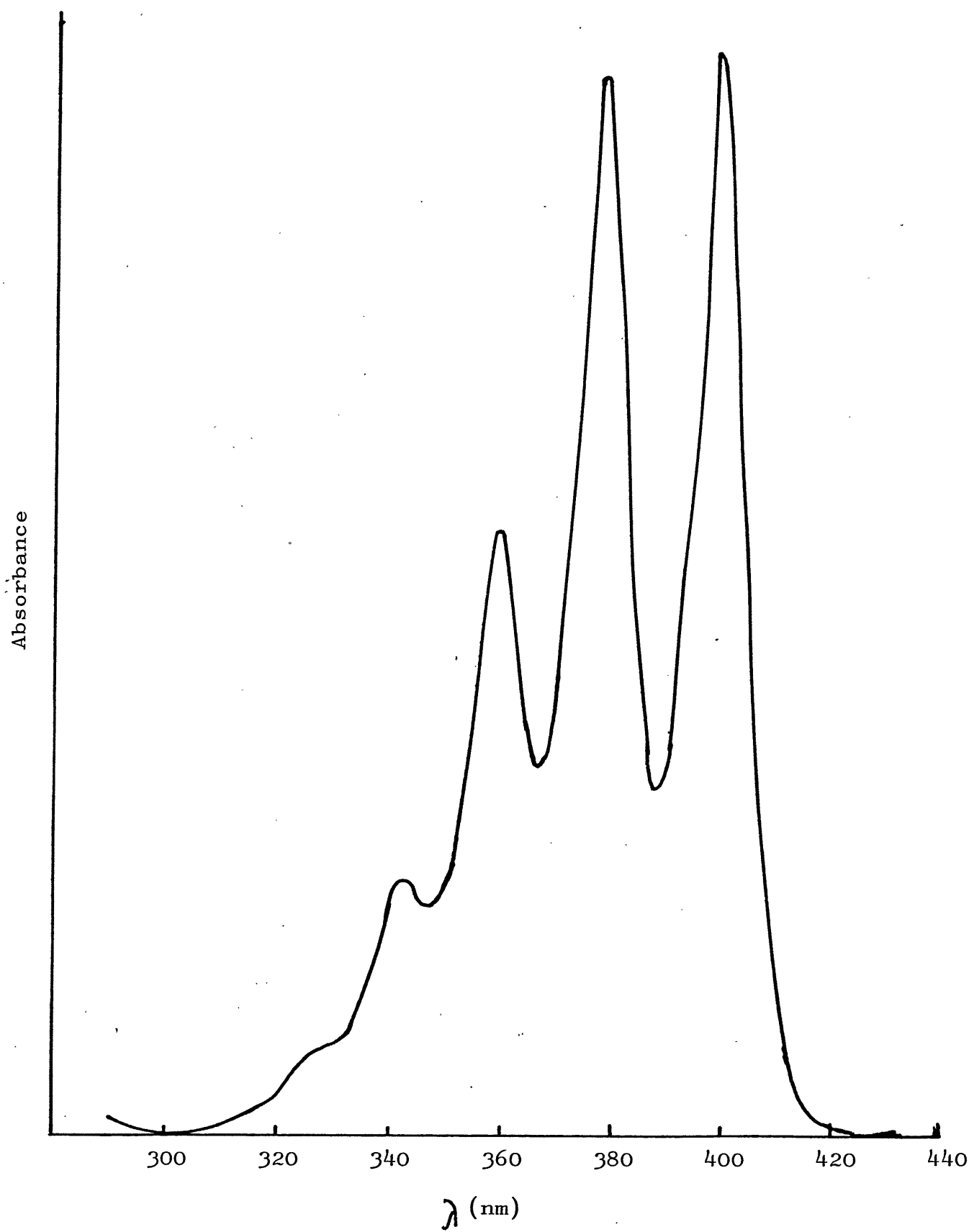


Scheme 3

9,10-dimethylantracene was brominated by the method of de Barnett and Mathews (112). Thus 9,10-dimethylantracene (4.1 g, 0.02 moles) was dissolved in ice-cold CS_2 (100 ml) and a solution of Br_2 (2.1 ml, 0.079 moles) in CS_2 (20 ml) was added slowly with stirring. The precipitated dibromide (III) was filtered, washed with a little benzene and dried in vacuo. Yield: 6.4 g, 88.1%. m.p. 310–315°C (decomp.) (lit. 315°C).

The dibromide (6.4 g, 0.0176 moles) was dissolved by stirring and refluxing for about 2 hours in a mixture of 0.7 M aqueous TEAS (60 ml) and acetone (60 ml) to give an almost clear solution. Water (60 ml) was slowly added to the mixture which was then concentrated to about 100 ml by boiling. After cooling and filtering potassium acetate

Fig. 6: The UV spectrum of AMS



(45 g) added to the filtrate and the precipitate collected by filtration, washed with strong aqueous potassium acetate and ethanol. The product was purified by shaking with water (75 ml), filtering and incubating the precipitate with ethanol in the cold. After 2 hours the mixture was filtered and the precipitate washed with cold ethanol and air dried. Yield: 3.0 g, 38.6%. Overall yield relative to 9,10-dimethylantracene: 34.0%.

Anal. calcd. for $C_{16}H_{12}O_6S_2K_2$: C, 43.43; H, 2.73.

Found: C, 43.57; H, 2.87.

UV (H_2O)- λ_{max} 400.5 ($\log \epsilon = 4.124$), 379, 359.5, 343 nm (Fig. 6)

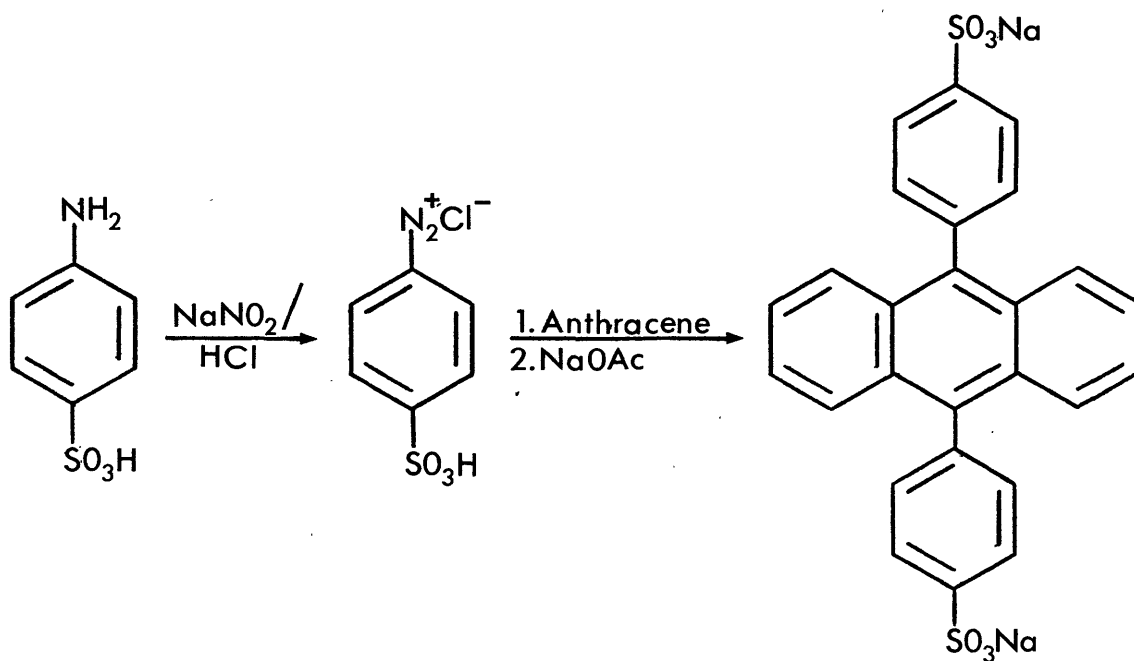
NMR (D_2O) - 4.68 δ (s, 4H), 7.57 δ (q, 4H), 8.08 δ (q, 4H).

The sodium salt of AMS was also prepared in a similar manner to the sodium salt of AES.

TLC analysis indicated the absence of any impurities.

3.3.3. Preparation of DPADS

DPADS was prepared as outlined in Scheme 4.



Scheme 4

~~Sulph~~anilic acid was diazotised as described in (113). Briefly, ~~sulph~~anilic acid was added to an aqueous solution of NaNO_2 (1 equivalent). The mixture was cooled to 0°C and one equivalent of HCl was added. The diazonium chloride precipitate was filtered and recrystallised from aqueous ethanol. One sample of this material was dried in a vacuum dessicator but the dried product on being touched with a spatula exploded with some violence. (It has been

reported(113)as being explosive). Accordingly, a second sample was washed with a little ethanol, followed by ether and merely sucked dry on a filter pump.

The diazonium salt (11 g, 0.05 moles), together with anthracene (4.45 g, 0.025 moles) was dissolved in a mixture of glacial acetic acid (60 ml) and acetic anhydride (40 ml) containing sodium acetate (25 g). The mixture was stirred mechanically for 12 hours at 80°C under an atmosphere of argon. After this time, water (150 ml) was added and the mixture was filtered. Solid sodium acetate (30 g) was added to the filtrate and agitated until dissolved. The mixture was then allowed to stand for 2 hours at 0°C. The precipitate which formed was filtered, washed with aqueous 50% NaClO₄ and cold ethanol and air dried. Yield: 3.1 g, 11.6%.

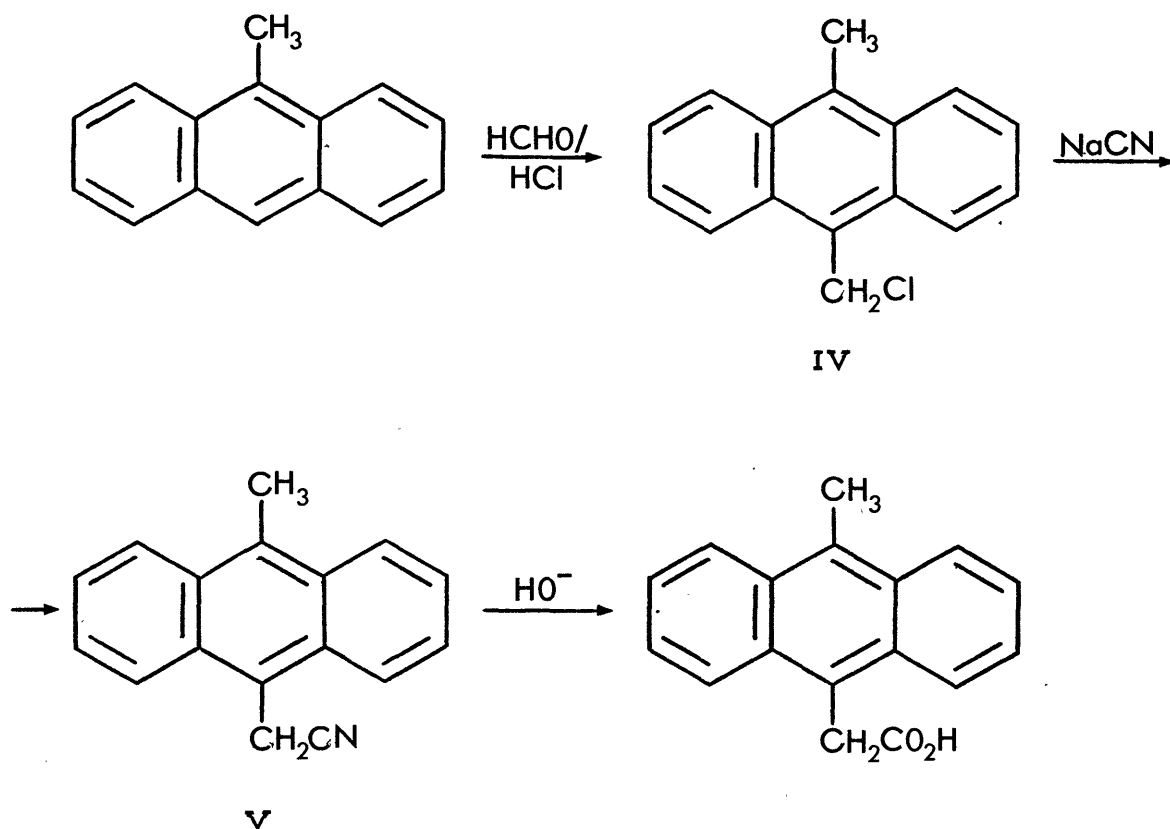
Anal. calcd. for C₂₆H₁₆O₆S₂Na₂: C, 58.44; H, 3.02.

Found: C, 59.68; H, 3.10.

UV (H₂O) -λ_{max} 395 (log ε = 4.130), 357, 342 nm.

3.3.4. Preparation of AC

AC was prepared as outlined in Scheme 5.



Scheme 5

The chloromethylation of 9-methylanthracene was carried out by a modification of the method of Adelfang and Daub (114) Paraformaldehyde (2 g) was suspended in glacial acetic acid (20 ml), the mixture was cooled in ice and hydrogen chloride gas was bubbled through for a few minutes to give a saturated solution. This was added to 9-methylanthracene (5 g, 0.024 moles) dissolved in glacial acetic acid (30 ml) and the mixture was kept at 45°C for two hours. It was then cooled to room temperature (30 min) and crushed ice (200 g) was added. The pale yellow

product (IV) was filtered, washed with water and dried in a vacuum dessicator. Yield: 1.66 g, 30.1%, m.p. 183-187°C, decomp. (lit. 180-190°C decomp.). This intermediate was converted to the nitrile (V) by adding it as pulverised powder (1.66 g, 0.0072 moles) to a suspension of NaCN (1.4 g, 0.029 moles) in dry DMSO. After stirring for 2 hours at 40°C, ice-water (300 ml) was added to the mixture and the precipitated nitrile was filtered, washed with water and dried. It was recrystallised from toluene. Yield: 1.24 g, 73.6%. m.p. 230 -235°C (decomp.).

Anal. calcd. for $C_{17}H_{13}N$: C, 88.27; H, 5.62.

Found: C, 86.70; H, 5.45.

Finally the nitrile (1.24 g, 0.0053 moles) was hydrolysed to the carboxylic acid by refluxing for 8 hours in a mixture prepared by mixing an aqueous solution (2.5 ml) of KOH (5 g) with ethyleneglycol monoethylether (25 ml). The mixture was then cooled, diluted with water (220 ml), filtered and the precipitate washed with water (50 ml). The washings were mixed with the filtrate which was then neutralised with conc. HCl. The precipitated carboxylic acid was filtered, washed with water and dried in air. It was recrystallised from glacial acetic acid. Yield: 1.03 g, 77.3%. m.p. 240 -245°C (decomp.).

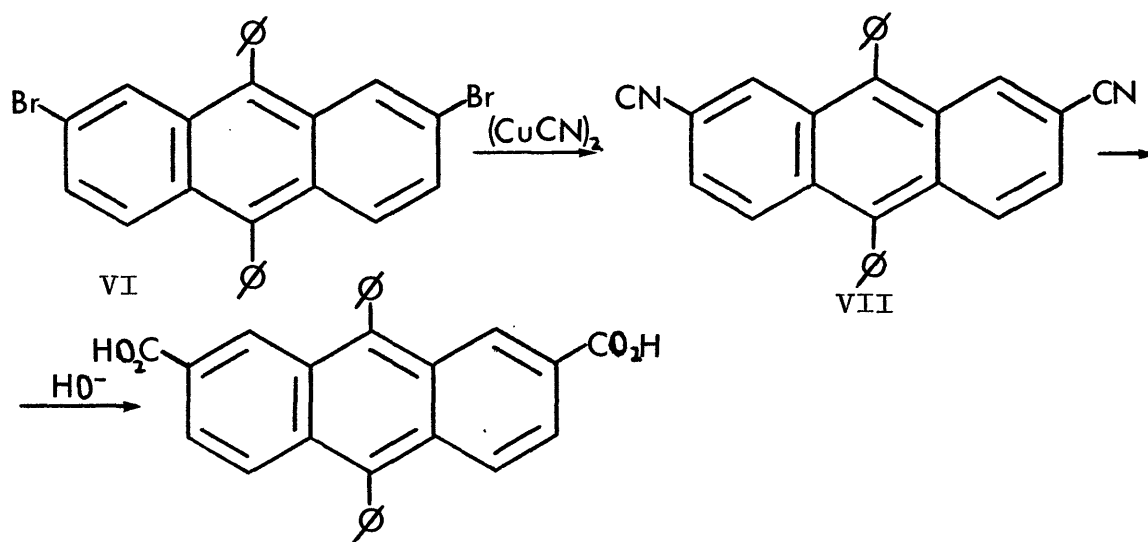
Anal. calcd. for $C_{17}H_{14}O_2$: C, 81.56; H, 5.67.

Found: C, 83.33; H, 5.40.

UV (H_2O) - λ_{max} 401, 380 ($\log \epsilon = 3.929$), 360, 343 nm.

3.3.5. Preparation of DPADC

DPADC was prepared as outlined in Scheme 6.



Scheme 6

2,7-Dibromo-9,10-diphenylanthracene (VI) (kindly provided by Dr D.F. Evans) was converted into the nitrile (VII) by the method of Newman (115). Compound (VI) (9.8 g, 0.02 moles) was dissolved in 10 ml dry pyridine. Powdered dry cuprous cyanide (4.4 g, 0.024 moles dimer) was added and, once a homogeneous solution was obtained, it was heated to 180°C under reflux (with the exclusion of moisture) for 14 hours. After cooling to 80°C , the mixture was poured into conc. NH_4OH (50 ml diluted with 50 ml water), and benzene (50 ml) was added and the mixture shaken. After cooling at room temperature, ether

(35 ml) was added and the precipitate which formed was filtered. The organic layer of the filtrate was separated and extracted repeatedly with dilute NH_4OH , then twice with 6N HCl , water and concentrated aqueous NaCl . After drying over Na_2SO_4 , the organic solvents were removed on a rotary evaporator.

The nitrile thus obtained was hydrolysed to the carboxylic acid by a method exactly analogous to that described in Section 3.3.4. Thus to the crude residue obtained from the last stage, ethyleneglycol monoethylether (120 ml) was added followed by a concentrated aqueous solution of KOH (20 g in 10 ml water). After a homogeneous solution was obtained, the mixture was refluxed for 10 hours. It was then cooled, filtered, and the carboxylic acid was precipitated from the filtrate with conc. HCl . The precipitate was collected by filtration, washed with water and dried in air. It was purified by recrystallisation from glacial acetic acid. Yield: 4.18 g, 50.0%. m.p. 210-215°C (decomp.).

Anal. calcd. for $\text{C}_{28}\text{H}_{18}\text{O}_4$: C, 80.36; H, 4.34.

Found: C, 82.11; H, 4.47.

UV (H_2O)- λ_{max} 413, 393 ($\log \epsilon = 3.989$), 373.

3.4. Measurement of molar absorptivity

Molar absorptivities were measured by two methods. In one method, the substrate was dried at 125°C in vacuo to constant weight and was then carefully weighed and used to prepare a standard solution whose absorbance was subsequently measured at the appropriate wavelength. In the second method, the tetraphenylphosphonium salt of the substrate was isolated by extraction with CHCl_3 , removal of the solvent on a rotatory evaporator at 28°C and further drying at 125°C in vacuo. This salt was used to prepare a standard solution as above. The two values of the molar absorptivity obtained where both methods were used (as for AES and AMS) agreed to within 2%.

3.5. The $\text{H}_2\text{O}_2/\text{NaOCl}$ reaction

The stability of all the substrates towards H_2O_2 and NaOCl separately was tested by incubating appropriate mixtures and monitoring the absorbance of the substrate. All substrates tested were unaffected by NaOCl over 24 hours, and by H_2O_2 over at least 2 hours.

The reaction itself was carried out by weighing an amount of solid substrate in a volumetric flask and then adding water (or bicarbonate buffer pH 9.5-10) followed by the

appropriate volume (a few microlitres) of NaOCl solution. The pH was adjusted to 9.5-10.0 with a few drops of NaOH solution and finally the volume was taken up to the mark with water. A small measured aliquot (usually 100 μ l) of the mixture was withdrawn and its absorbance at the appropriate wavelength measured after dilution. This measurement was used for the calculation of the initial substrate concentration. A small magnetic bar stirrer was then introduced into the mixture and finally appropriately diluted H_2O_2 (usually 100 μ l) was added slowly (over ca. 5 min) from a microsyringe, with vigorous stirring.

The stability of H_2O_2 during its addition was confirmed by carrying out the addition from a glass syringe with a metallic plunger and needle or from an all-plastic micropipette, with identical results.

The amount of H_2O_2 added was always approximately 10% less than that of the NaOCl present. After 15 min the absorbance of the mixture was again read as before, making allowance for the dilution caused by the addition of H_2O_2 . The final absorbance was stable for at least 60 min after addition of the last portion of H_2O_2 .

The wavelengths used to monitor the reactions are shown in Table 12. The characteristic anthracene absorption bands in this region were completely absent in the spectrum of

of the product endoperoxides which had molar absorptivity coefficients of zero at wavelengths longer than 320 nm.

Table 12: Wavelengths used in monitoring anthracene-derivative reactions

Substrate	λ (nm)	ϵ ($M^{-1} cm^{-1}$)
AES	399	1.24×10^4
AMS	400.5	1.33×10^4
AS	380	8.70×10^3
DPADS	375	1.35×10^4
AC	380	8.50×10^3
DPADCA	393	9.75×10^3

The extent, efficiency and products of the reaction were unaffected when the order of addition of NaOCl and H_2O_2 was reversed as well as when bicarbonate buffer or unbuffered water were used in the initial solution. Duplicate experiments gave identical absorbance readings and generally the reproducibility and consistency of this method were excellent.

In kinetic experiments the concentrations of the reagents were chosen such that the consumption of substrate was never greater than 15%, and usually under 10%. Kinetic

experiments were conducted at least three times and the measurements reported are averages of values which differed by not more than 3% from their mean.

3.6. Experiments in D₂O

In experiments with D₂O care was taken for the H₂O-content of solutions never to exceed 1%. The value of pD was calculated according to the formula $pD = pH \text{ (measured)} + 0.4$ (116).

3.7. Photosensitized oxygenation

Such experiments were carried out with Rose Bengal as sensitizer, using an 12V,100 W tungsten halogen arc projector lamp with a dichroic reflector (A231) and a +K2 yellow heat filter.

Reactions were carried out in unbuffered water or in aqueous solutions brought to pH 8.2, 9.0 or 10.5 with bicarbonate buffer or NaOH. Oxygen gas was passed through the solutions during the irradiation, which was maintained until all the substrate had been consumed (30-60 min).

The stability of all the substrates towards light was tested by incubating as above in the absence of added

sensitizer. No consumption or alteration of substrate was ever detected under those conditions.

3.8. Thin layer chromatography (TLC)

All the work published so far on the endoperoxides of anthracene derivatives involved compounds which were soluble in organic solvents (28). Consequently, it was necessary to develop a system for the TLC separation of water-soluble substrates and products.

A large number of systems was examined including silica, alumina and cellulose-coated plates, and benzene, toluene, chloroform, acetone, ethyl acetate, ethanol, methanol, DMF, dioxan and water as solvents.

In order to have the possibility of detecting minor products it was desired to apply concentrated reaction mixtures to the plates. This was achieved in a number of ways.

In the case of two of the substrates (DPADC and AC) which had particularly low solubility in water, acidification of the reaction mixtures precipitated the anthracene derivatives as the free acids. These were filtered, redissolved in a small volume of solvent and applied to the TLC plates.

For the remaining substrates (the sulphonic acid derivatives) two techniques were used initially: In the first technique successive aliquots of the reaction mixture were applied directly to the plate until a fairly large amount accumulated (but not so large as to cause streaking of the spots). In the alternative technique, the reaction mixtures were extracted three times with one volume of chloroform each time, after addition to the aqueous mixture of an amount of tetraphenylphosphonium chloride calculated to be in a 10% excess over the substrate. This procedure was shown to result in at least 90% extraction of the anthracene derivatives into the organic layer. After removal of the bulk of the chloroform on a rotatory evaporator at 28°C, a small quantity of the remaining solution was placed on a plate.

After it was confirmed that the above two methods gave identical results (in terms of the number of products detected) only the second one was used routinely.

With the two carboxylic derivatives (DPADC and AC) the solvents used normally for development were (i) chloroform-acetone 7:3, (ii) chloroform-acetone 1:1 and (iii) chloroform-methanol 7:3.

With the sulphonic acid derivatives the solvents used for elution included (iv) water, (v) toluene-formamide-water

60:38:2 and (vi) chloroform-dioxan-methanol 60:35:5. An additional solvent devised and used with great success in these cases was a 3% aqueous solution of $\text{Ba}(\text{NO}_3)_2$ (vii). This was used because it was found that the Ba salts of the endoperoxides were water-soluble while those of the parent compounds were insoluble.

The plates were cellulose-coated ones. The chromatography tanks were wrapped in aluminium foil to minimise exposure of the plates to light. The spots were located by observation under UV light and by using as locating reagent the $\text{Fe}^{2+}/\text{SCN}^-$ system which caused the development of a red colour on the endoperoxide spot. Sometimes KMnO_4 solution was also used as a general locating reagent.

Table 13 shows a series of representative R_f values for AC and AES, and their endoperoxides obtained from photooxygenation or the $\text{H}_2\text{O}_2/\text{NaOCl}$ system.

It can be seen that a fairly broad range of mobilities is covered with the solvents used, allowing the unambiguous identification of spots. In all cases the photooxygenation product was used as the authentic material for identification of endoperoxide spots. All reaction mixtures examined were analysed in all of the systems described.

Table 13: R_f values for AC and AES and their endoperoxides*

Substrate	Solvent	R_f (Substrate)	R_f (Endoperoxide)
AC	(i)	0.41	0.20
AC	(ii)	0.52	0.31
AC	(iii)	0.82	0.70
AES	(iv)	0.60	0.81
AES	(v)	0.10	0.19
AES	(vi)	0.81	0.29
AES	(vii)	0.20	0.78

* On cellulose plates, 0.25 mm

3.9. Polarography

Measurements were carried out using a dropping mercury electrode (DME) with a saturated calomel one (SCE) as reference.

The drop-time was 2s and the height of the mercury pool was 50 cm. The value of m was approximately 2.1 mg s^{-1} . The applied voltage was increased at the rate of 0.4 volts per minute.

Lithium sulphate (0.1 M) was the supporting electrolyte. All solutions were carefully deoxygenated by passing oxygen-free N_2 through them for 30 min. A stream of oxygen-free N_2 was passing over the surface of the solutions during the measurements.

Because H_2O_2 is known to give a polarographic wave in the region of -1 volt, this compound was removed from the reaction mixtures by freeze-drying the solutions. This method was devised in place of the addition of La^{3+} (117, 118) as the latter was found to precipitate the products. The effectiveness of freeze-drying was confirmed by examining solutions of H_2O_2 and H_2O_2 /AES-endoperoxide before and after freeze-drying. No H_2O_2 wave was found after the freeze-drying.

3.10. The stability of endoperoxides

The endoperoxides of AES and AMS were stable in aqueous solution at 4°C for at least one month as judged by TLC and polarographic examination. They were also unchanged after incubation in a mixture containing H_2O_2 and NaOCl at such initial concentrations as to have produced an excess of singlet oxygen. Consequently, it can be concluded that the endoperoxides were stable under the conditions of their production.

CHAPTER 3

A New, Water-Soluble Singlet Oxygen Quencher

1. Introduction (119, 120)

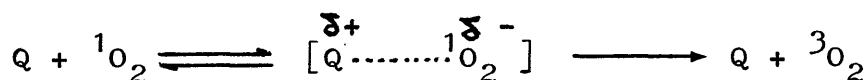
A singlet oxygen quencher is any compound which accelerates the deactivation of singlet oxygen. Deactivation can take the form of chemical reaction or return to ground state. The term quenching is reserved for the latter situation which, strictly speaking, should be referred to as physical quenching. Since it is possible (and indeed frequently the case) for a compound to react chemically with, as well as physically quench, singlet oxygen, only those compounds for which the rate of the latter interaction predominates (i.e. $k_q \gg k_r$) are classified as quenchers. The kinetics of quenching have already been described in outline (Chapter 2, Section 1.1.).

In practice, in order for a compound to bring about significant quenching at reasonable concentrations (10^{-2} - 10^{-3} M), it must have a $k_q \gg 5 \times 10^7$ - 10^8 l mol s^{-1} (remembering that

the decay rate constant in water is $k_d = 5 \times 10^5 \text{ s}^{-1}$). Many important naturally occurring and synthetic quenchers have, in fact, k_q approaching diffusion-controlled values (10^9 - $10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$).

Two major mechanisms of singlet oxygen quenching are recognised, known respectively as charge-transfer and energy-transfer quenching.

Charge-transfer quenching involves the diffusion-controlled formation of a partial charge-transfer complex between the quencher and singlet oxygen, which subsequently breaks down with the release of ground-state oxygen.



Typical of this mechanism is the mode of action of amines (121, 122) sulphides (122), phenols (123), azomethine dyes (124) and bilirubin (83, 125). In the case of amines the operation of this mechanism is indicated by the observed inverse correlation of the quenching rate constant with the ionisation potential of the amines (121). Accordingly the rates of quenching by different amines varies in the order primary < secondary < tertiary (122).

Some rate constants for the charge-transfer quenching of singlet oxygen are shown in Table 14. Some of the compounds shown

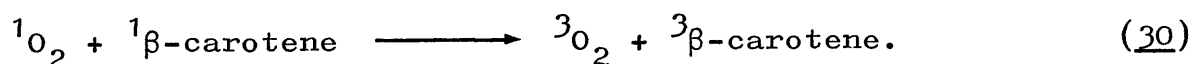
react chemically with singlet oxygen in addition to quenching.

Table 14: The quenching of singlet oxygen by the charge-transfer mechanism

Quencher	$k_q (1 \text{ mol}^{-1} \text{ s}^{-1})$	Ref
Triethylamine	2.8×10^6	126
Nicotine	4.3×10^6	128
DABCO*	2.7×10^7	120
Di-n-butylsulfide	5×10^5	126
Bilirubin	2.1×10^9	83
α -Tocopherol	6.2×10^8	38
Histidine	5×10^7	129
Azide	3.5×10^8	130

* 1,4-Diazabicyclo[2.2.2.]octane

Turning now to energy-transfer quenching, a number of compounds, the most important of which is β -carotene, have been shown to interact with singlet oxygen, converting it to ground-state oxygen while they themselves are excited to a triplet state (rn. (30)), (57, 127, 131).



This process takes place extremely rapidly ($k_q = 1.5 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$) and it is energetically allowed because at 17-25 kcal mol^{-1} the triplet state of β -carotene is nearly degenerate with or energetically below that of singlet oxygen (127). Other substances which are believed to quench by this mechanism are shown in Table 15.

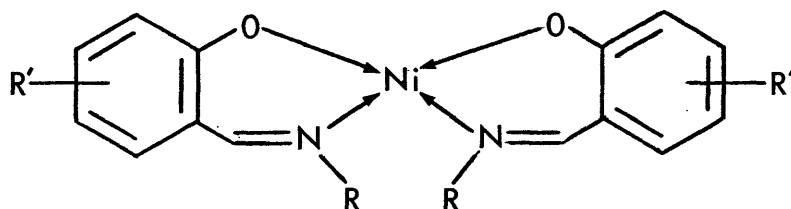
Table 15: The quenching of singlet oxygen by the energy-transfer mechanism

Quencher	$k_q (\text{l mol}^{-1} \text{s}^{-1})$	Ref
β -carotene	1.5×10^{10}	127
Polymethane pyrulium dye	3.0×10^{10}	131
Ni(II) bis-isooctane (di-n-butyl-dithio-carbonate)	1.7×10^{10}	57

Amongst the various quenchers examined, metal chelates have provoked particular interest because of their occurrence in nature as well as their commercial potential. Such quenchers include complexes of Zn(II), Cu(II), Pd(II), Mn(II), Co(II) and Ni(II), (120, 132-135). Within a particular type of chelates (the hydrated chlorides) they quench with efficiencies varying in the order $\text{Mn(II)} \ll \text{Co(II)} < \text{Ni(II)}$. Energy-transfer quenching is thought to operate.

The high quenching efficiency of Ni(II) complexes has resulted in a large number of them being studied, and some have been found to be extremely powerful quenchers with quenching constants k_q of the order of 10^9 - 10^{10} $\text{l mol}^{-1} \text{s}^{-1}$.

One such family of complexes is the Ni(II) bis-2-hydroxyphenylaldimine chelates, studied by Zweig and Henderson (132).



These have k_q values in the range 1.7 - 12×10^9 $\text{l mol}^{-1} \text{s}^{-1}$ (Table 16). One of these was chosen (with $R' = \text{H}$ and $R-R =$ ethylene) and modified in order to make it water soluble by introducing sulphonic groups at position 5 of the aromatic rings.

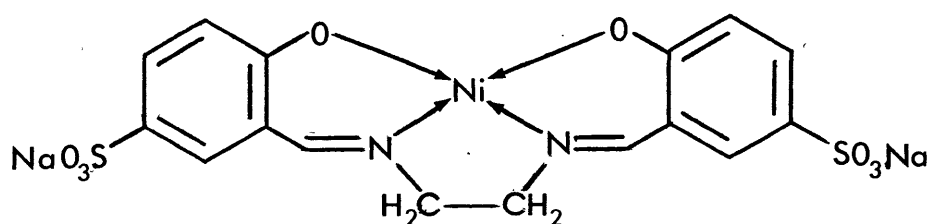
Amongst the singlet oxygen quenchers used up to now most have low water solubility. The ones most frequently used in water are DABCO, histidine and azide. Apart from having relatively low quenching efficiencies, all three suffer from the disadvantage of reacting chemically with singlet oxygen (120). By contrast Ni(II) complexes are reported to have high resistance to singlet oxygen (134, 135). This, together with the extremely high quenching efficiency of these compounds, encouraged the present search for a water-soluble quencher of this type.

Table 16: k_q for Ni(II)-bis-2-hydroxyphenylaldimine chelates*

R' = H	R = Phenyl	7.8×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$
	R = NH_2	7.5×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$
	R = i-propyl	5.9×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$
	R = sec-butyl	4.0×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$
	R-R = ethylene	5.3×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$
	R-R = 1,8-naphthylene	12×10^9	$1 \text{ mol}^{-1} \text{ s}^{-1}$

* From ref. 132.

2. Results and Discussion



Ni(II)-N,N'-ethylene-bis-2-hydroxy-5-sulphonic acid-phenylaldimine (Trivial name: Niphalds)

2.1. Preliminary search for a Ni(II)-quencher

Because Ni(II) complexes are known to be efficient quenchers of singlet oxygen, an initial attempt was made to use a simple complex of Ni(II) with cyclohexane-1,2-diamino tetraacetic acid (CDTA). A mixture containing 4×10^{-4} M $\text{Ni}(\text{NO}_3)_2$ and 6×10^{-4} M CDTA was checked for the presence of free nickel ions by the dimethylglyoxime test and found to be free of them, indicating complete complexing of the metal. An aliquot of this mixture diluted ten times had no effect at all on the efficiency of trapping of singlet oxygen by 4×10^{-3} M AES.

2.2. Niphalds, a water-soluble quencher

This compound was prepared as described in the experimental part (Section 3.), the rationale being to modify, with the addition of hydrophilic sulphonic acid groups, a Ni(II)-bis-2-hydroxyphenylaldimine chelate known to be an efficient singlet oxygen quencher (Section 1.). The characteristics of this compound are also given in the experimental part.

The solubility of Niphalds in water was examined in the pH range 1.5-13 and was found to be approximately 5×10^{-3} M and constant throughout this range.

The stability of Niphalds was checked by incubation with H_2O_2 and NaOCl separately or together, or with peroxymonophthalic acid (PPA). The absorbance of the quencher at 545 nm was monitored as well as the concentration of the other species. The results shown in Table 17, indicate that the quencher was stable towards H_2O_2 and singlet oxygen but underwent slow reaction with NaOCl. For this reason in experiments involving NaOCl the latter was added last and in small portions.

PPA underwent rapid decomposition with much frothing in the presence of Niphalds, presumably owing to the known extreme sensitivity of peroxy acids to metal ions (104).

Niphalds was also stable in dilute alkali, acid, CDTA and dimethylglyoxime, but was decomposed (presumably to $\text{Ni}(\text{CN})_4^{2-}$) by KCN.

Table 17: The stability of Niphalds

[S] (M)	[Q] (M)	t	ΔQ (%)	ΔS (%)
H_2O_2 , 1.5×10^{-2}	1×10^{-3}	5 h	0	0
NaOCl , 2×10^{-3}	1×10^{-3}	5 min	10%	-
NaOCl , 2×10^{-3}	1×10^{-3}	10 min	20%	100
NaOCl , 2×10^{-3}	1×10^{-3}	30 min	20%	100
$\text{H}_2\text{O}_2/\text{NaOCl}$, $8 \times 10^{-3}/8 \times 10^{-3}$	1.5×10^{-3}	10-30 min	0	100
PPA, 8×10^{-3}	1.5×10^{-3}	24 h	0.1%	100

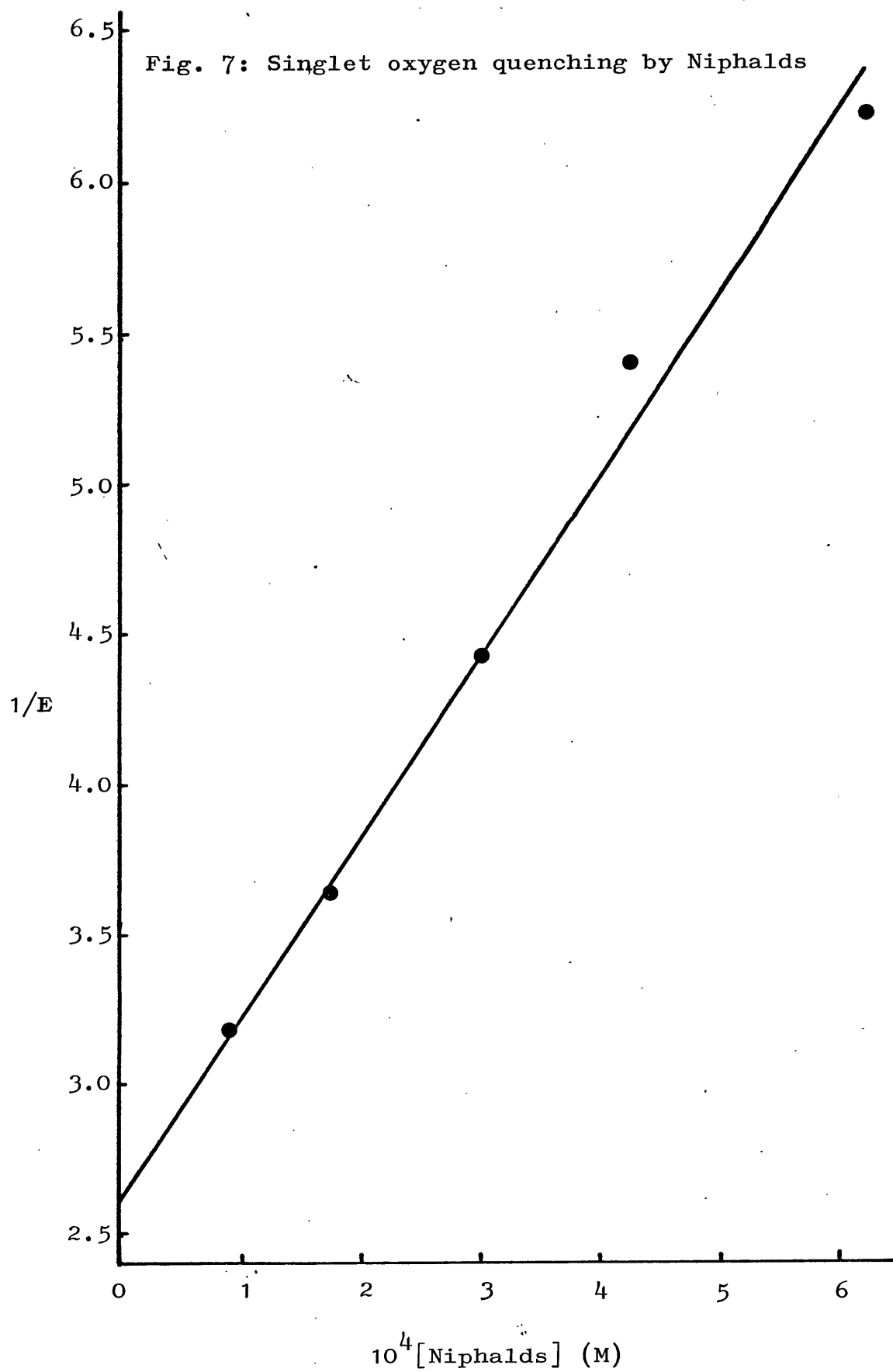
A kinetic experiment was subsequently carried out in which different concentrations of Niphalds were included in a system of $\text{H}_2\text{O}_2/\text{NaOCl}/\text{AES}$. The effect of the quencher concentration on AES efficiency is shown in Table 18. The results of this Table can be plotted in the form of a Stern-Volmer plot. As explained in Chapter 2, Section 1.1., a plot of $1/E$ versus $[Q]$ should be linear, with slope equal to $k_q^Q/k_r^{\text{AES}}[\text{AES}]$. Such a plot is shown in Fig. 7 which is indeed linear (correlation coefficient = 0.9952)

with slope $(6.03 \pm 0.22) \times 10^3$. Consequently, the rate constant for the quenching can be calculated as $k_q^Q = (1.87 \pm 0.07) \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$. This value can be compared to the corresponding value for a compound closely related to the one used here, but without the sulphonic acid groups, found to be $5.3 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$ in toluene (Table 16, Section 1.).

The value k_q^Q obtained for Niphalds, although somewhat lower than this, is still very high, indicating that the compound prepared is an extremely efficient quencher of singlet oxygen. A concentration of $3.7 \times 10^{-3} \text{ M}$ would quench most (95%) of the singlet oxygen present in a system.

Table 18: Kinetic data for singlet oxygen quenching by Niphalds

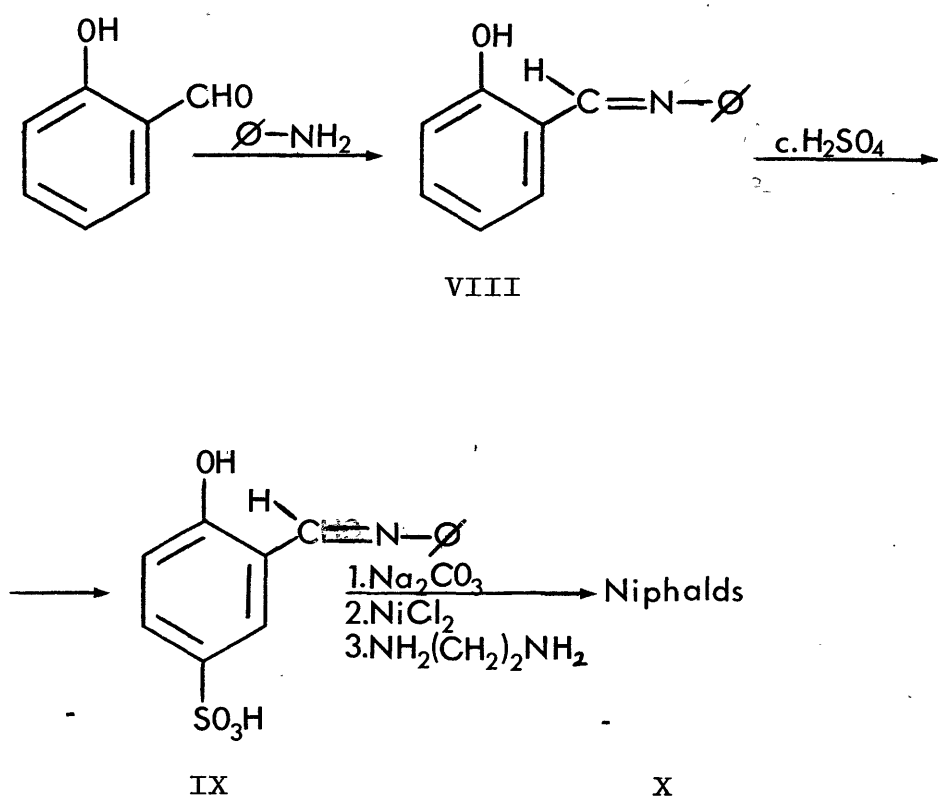
$[^1\text{O}_2]_{\text{tot.}} = 1.10 \times 10^{-3}$ $[\text{AES}]_0 = 2.70 \times 10^{-3} \text{ M}$		
$10^4 [\text{Q}] \text{ (M)}$	100E	1/E
0	39.1	2.56
0.91	31.4	3.18
1.76	27.5	3.64
3.00	22.6	4.42
4.25	18.5	5.41
6.20	16.1	6.21



3. Experimental

3.1. Preparation of Ni(II)-N,N¹-ethylene-bis-2-hydroxy-5-sulphonic acid-phenylaldimine.

The synthetic procedure used for the preparation of the title compound is shown in outline in Scheme 7.



Scheme 7

The initial preparation of salicylaldehyde-5-sulphonic acid was based on the method of Blau (136). Freshly distilled aniline (10.7 ml, 0.095 moles) and salicylaldehyde (10.1 ml, 0.095 moles) were warmed together to 60°C for 15 min.

Methanol (40 ml) was added to the mixture which was then cooled to -10°C . The crystalline salicylaldehyde anil (VIII) was filtered, washed with ice-cold methanol and air dried. Yield: 12.0 g, 64.1%.

This material was carefully dissolved in conc. H_2SO_4 (33 ml) and the solution heated to 80°C until a small aliquot of it gave no oily drops in water (20 min). The mixture was then slowly poured without external cooling into water (110 ml) and allowed to cool in the refrigerator. The crystalline sulphonic acid (IX) was filtered off, washed with a little ice-cold water and recrystallised from water. Yield: 10.5 g, 54.7% relative to the aniline.

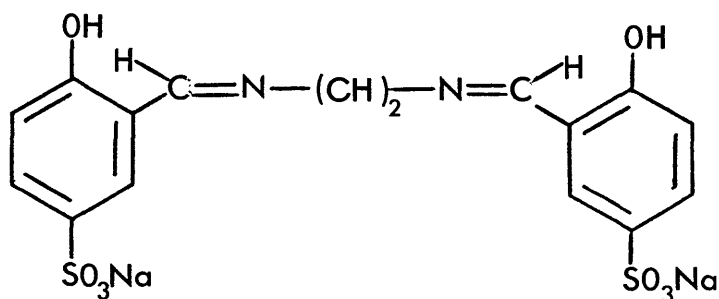
This product was converted to the sodium salt by dissolving it in an aqueous solution of Na_2CO_3 (5.5 g in 50 ml water). The mixture was concentrated by boiling to a final volume of 40 ml over a period of $1\frac{1}{2}$ hours. The mixture was then cooled to room temperature, the pH adjusted to 5.5 with a few drops of conc. HCl , and the mixture placed in the refrigerator. The precipitated sodium salt was filtered, washed successively with 50% NaClO_4 and ethanol and dried in vacuo at 125°C . Yield: 4.4 g, 37.7% relative to the free acid.

The sodium salt was converted into the final complex by two methods.

In the first method, the salt (2.1 g, 0.010 moles) was mixed with $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (1.19 g, 0.005 moles) and sodium acetate (1.65 g) and the mixture was dissolved in water (50 ml). After warming the mixture to 60°C for ca. 10 min and cooling to room temperature, ethylenediamine monohydrate (0.40 ml, 0.005 moles) was added with vigorous stirring. The mixture was then heated to 80°C for 10 min and allowed to cool in a refrigerator. The orange crystalline precipitate (X) was filtered, washed with 20% sodium perchlorate and ethanol and dried in vacuo at room temperature. Yield: 2.5 g, 94.5%.

This product was sparingly soluble in water, although it had an identical visible spectrum to that of a more soluble material whose preparation is described next.

In a second method, the sodium salt of IX (2.1 g, 0.010 moles) was suspended in 15 ml of boiling ethanol and water was added slowly until all the solid dissolved. To this hot solution, ethylenediamine monohydrate (0.46 ml, 0.0057 moles) was added with vigorous stirring. After 10 min the mixture was cooled to room temperature and the precipitated adduct XI filtered. It was washed with 60% ethanol and recrystallised from water. Yield: 1.14 g, 48.0%.



XI

This product (0.94 g, 0.002 moles) was dissolved in water (25 ml) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.47 g, 0.002 moles) with sodium acetate (0.8 g) dissolved in a small volume of water were added with stirring. The precipitated orange product was filtered, washed with 10% NaClO_4 and ethanol and dried in vacuo at room temperature. Yield: 0.66 g, 63.0%.

All attempts to prepare the complex from hot water or to recrystallise it from ethanol-water led to a sparingly soluble product similar to that obtained by the first method. This was probably related to the "soap"-like nature of the complex, as evidenced by its slow rate of dissolution into water.

Anal. calcd. for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{NiO}_8\text{S}_2$: C, 36.32; H, 2.29.

Found: C, 36.20; H, 2.25.

3.2. Spectral characteristics

The UV spectrum was characterised by major absorbance maxima at 315 and 390.2 nm ($\log \epsilon = 3.681$) and shoulders at 340, 423 and 545 nm (Figs. 8 and 9).

Addition of excess KCN destroyed the characteristic absorption and gave a maximum at 380 nm.

3.3. Kinetic arrangements

In kinetic experiments a weighed amount of the quencher was placed in a volumetric flask together with a weighed amount of singlet oxygen substrate. Buffer and the other substrates were added, and the remaining part of the procedure was exactly as described in Chapter 2, Section 3.

Fig. 8: The UV-vis spectrum of Niphalds

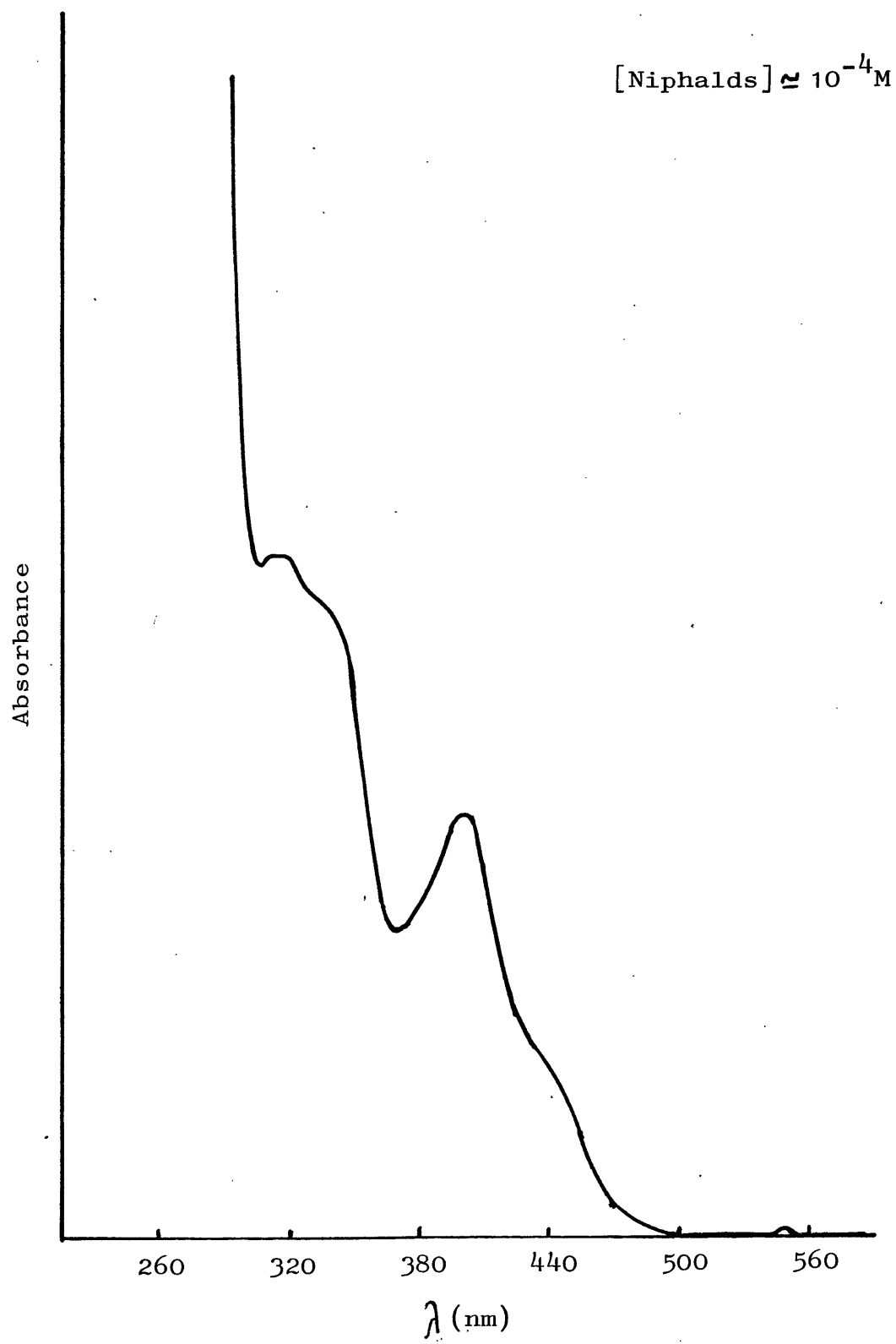
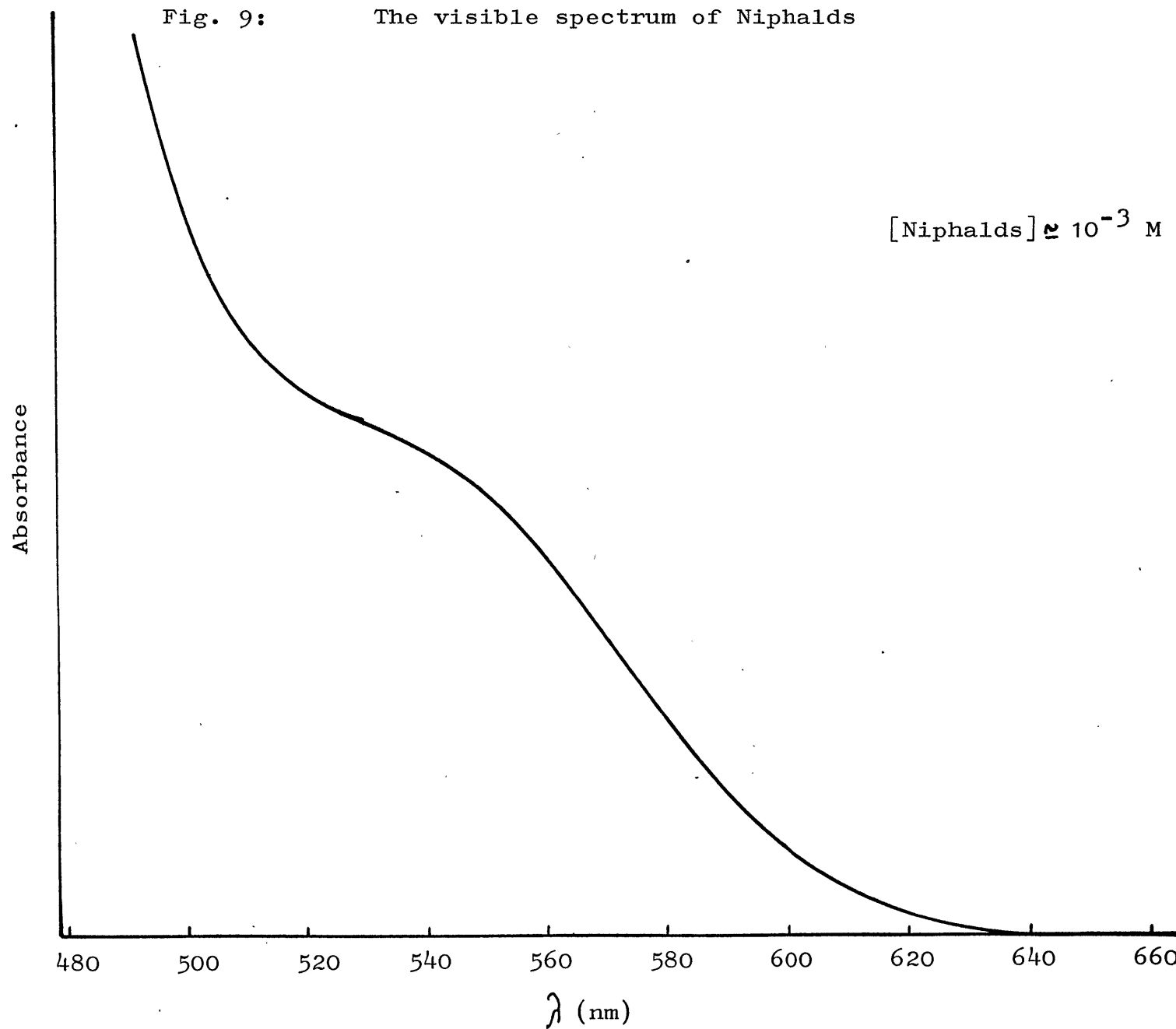


Fig. 9: The visible spectrum of Niphalds



CHAPTER 4

The Uncatalysed Decomposition of Peroxy Acids

1. Introduction

1.1. The mechanism of the uncatalysed decomposition of peroxy acids

Peroxy acids can undergo uncatalysed decomposition to form the corresponding carboxylic acid and molecular oxygen.



Relatively few studies of the uncatalysed peroxy acid decomposition in water have been published (138-143).

One of the problems that makes the study of this reaction difficult is the great facility with which it is catalysed by transition metal ions (104). Concentrations of Co(II) ions as low as 10^{-8} M are sufficient to cause significant diversion of the decomposition to catalysed pathways.

Because of this problem the studies of the spontaneous pathway have been carried out using highly purified materials in specially cleaned vessels, often in the presence of added EDTA to sequester any metal ions present (139, 143).

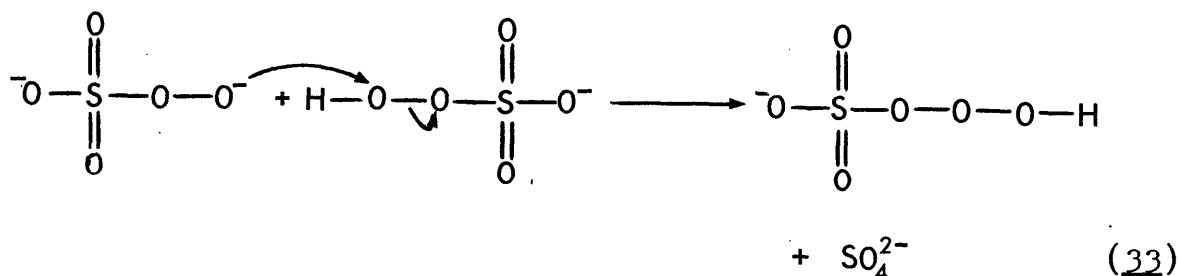
The use of EDTA has been shown to reduce the rate of peroxy acid decomposition in aqueous media to a residual one believed to represent only the spontaneous (uncatalysed) reaction. Even so the rates of decomposition obtained by different groups of workers have not always been in agreement.

The first kinetic study of the spontaneous decomposition of a peroxy acid to be published dealt with the decomposition of Caro's acid (peroxysulfuric acid) in aqueous alkaline buffers.

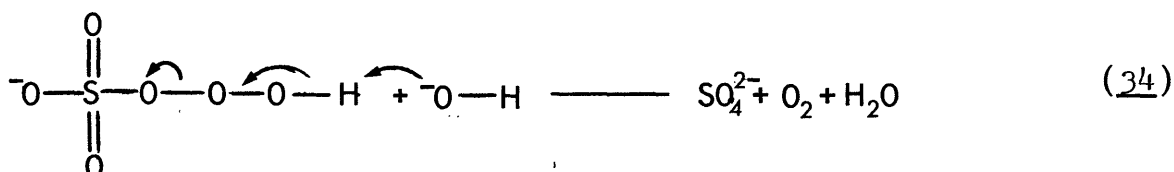


Ball and Edwards, (137) found that the reaction was second order with respect to peroxy acid, and the second order rate constant had a maximum value at a pH numerically equal to the pK_a of the acid, i.e. when the acid was 50% dissociated. This led them to suggest that the reaction mechanism involved

initial nucleophilic attack by the negatively charged peroxidic oxygen of the peroxy acid on the peroxidic oxygen of the undissociated peroxy acid molecule.

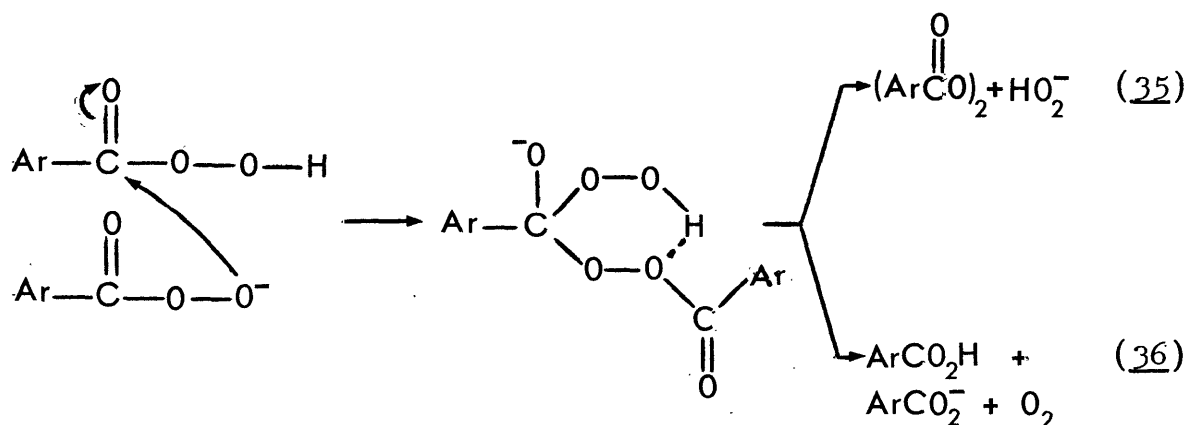


The intermediate HSO_6^- formed hydrolyses rapidly.



In 1962 Goodman, Robson and Wilson published a similar kinetic study of the spontaneous decomposition of a series of peroxybenzoic acids (139). Their results were exactly analogous to those of Ball and Edwards, but, basing themselves on the observed substituent effects, these workers suggested that the attack of the peroxidic oxygen anion is directed at the carbonyl carbon of the undissociated molecule, to form an internally hydrogen-bonded cyclic intermediate. This intermediate then breaks down to form the carboxylic acid

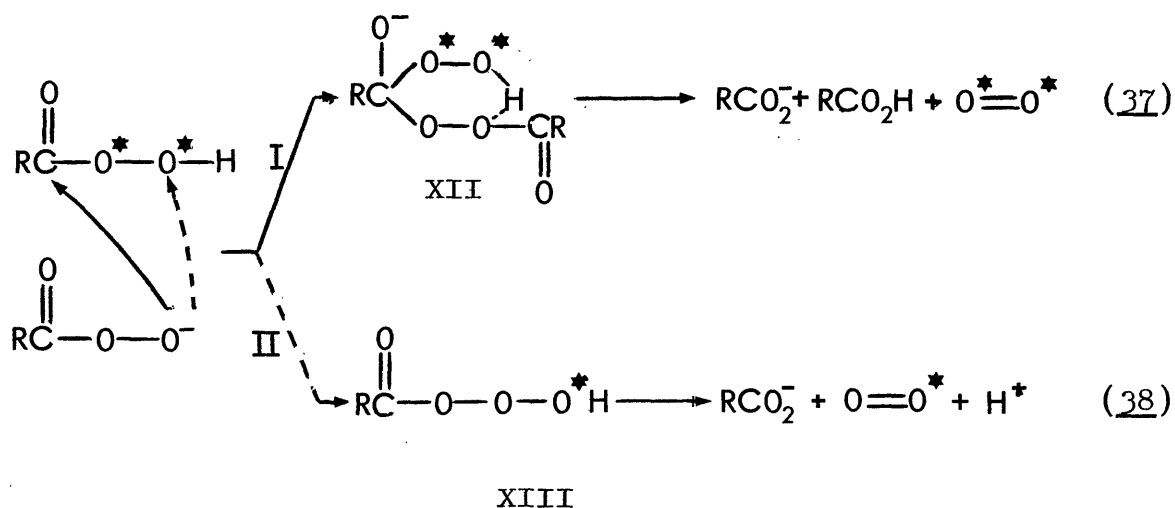
and oxygen or, alternatively, to give diaryl peroxide, a minor product detected in less than 5% yield.



The operation of a free radical pathway was considered and excluded experimentally. It was further suggested that an analogous mechanism might operate in the decomposition of Caro's acid, with an initial nucleophilic attack on the sulfur atom.

Two mechanisms for peroxy acid decomposition thus emerged, which were kinetically indistinguishable. One way of distinguishing between them makes use of the fact that nucleophilic attack on oxygen would result in molecular oxygen carrying one atom from each of the reacting peroxy acid species, whereas attack on the carbon (or sulfur) would result in

molecular oxygen with both of its atoms originating from only one of the reacting molecules (Scheme 8). Thus isotopic distribution studies, utilising ^{18}O -labelled peroxy acids offer a way of distinguishing between the two mechanisms.



Scheme 8

This technique was first used by Edwards and his group (139) who were thus able to show that peroxyacetic acid decomposes primarily (to an extent of 83%) via pathway I, involving nucleophilic attack on the carbonyl carbon. The same technique was subsequently used to study the mechanism of decomposition of a number of peroxy acids (142, 143). The results obtained, summarised in Table 19, suggest that both pathways operate side by side, pathway I being possibly the favoured one in the absence of adverse steric effects (i.e. bulky

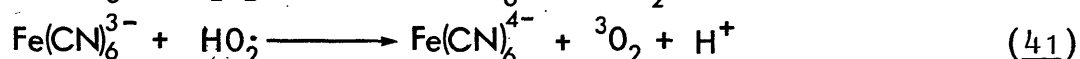
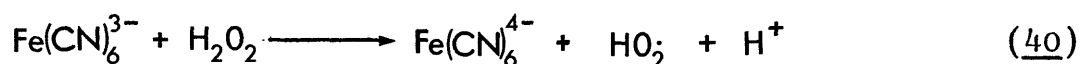
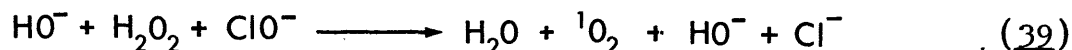
groups in place of R).

Table 19: The decomposition of peroxy acids

Peroxy acid	Pathway I(%)	Pathway II(%)	Ref
peroxyacetic	83	17	138
Caro's	9	91	142
peroxymonophthalic	26	74	143
peroxypivalic	24	76	149

1.2. Singlet oxygen formation during peroxy acid decomposition

The suggestion that singlet oxygen may be produced during the decomposition of peroxy acids was first made by McKewon and Waters in 1966 (101). These workers speculated that the heterolytic decomposition of peroxy compounds may result in the production of singlet-state oxygen, while their homolytic decomposition may result in ground-state oxygen. They noted, for example, that the oxidation of hydrogen peroxide by hypochlorite in alkaline media results in singlet oxygen production and is accompanied by chemiluminescence, while one-electron oxidising systems oxidise hydrogen peroxide to ground-state oxygen without chemiluminescence.



They therefore postulated that singlet oxygen might be formed during the decomposition of peroxy acids since this reaction is thought to involve heterolytic bond fission. In support of this suggestion these workers claimed to have detected singlet oxygen by isolating the 9,10-endoperoxide of dimethylantracene during the decomposition of di-isoperoxyphthalic acid in the presence of dimethylantracene. On the other hand, no such product could be obtained with peroxyacetic acid.

Clearly the situation is more complex than that envisaged by the initial suggestion of McKewon and Waters. Not only were quantitative measurements of singlet oxygen formation not made in their experiments (which were mainly qualitative), but also control experiments to test the possibility of endoperoxide formation by direct reaction with the peroxy acid were not carried out.

In fact, Boyer et al. (102) have subsequently shown that ~~diphenylisobenzofuran~~ diphenylisobenzofuran can undergo epoxidation (followed

by further, rapid oxidation to the endoperoxide) with *m*-chloroperoxybenzoic acid.

Consequently, there is a need for a careful quantitative study of the interaction between singlet oxygen substrates and peroxy acids with the aim of ascertaining whether singlet oxygen is formed during peroxy acid decomposition.

2. Results and Discussion

2.1. TLC analyses

Peroxyacetic and chloroperoxyacetic acids with AES gave rise to two product-spots on TLC plates. The major one had an R_f of 0.41 with toluene-formamide-water (60:38:2) and gave no colour with $\text{Fe}^{2+}/\text{SCN}^-$. Furthermore, it gave no reaction when sprayed with a solution of KI and starch, indicating that it was not a readily reducible substance such as a hydroperoxide. The second spot, present only in trace amounts, had an R_f corresponding closely to that of the endoperoxide and gave a positive test with $\text{Fe}^{2+}/\text{SCN}^-$.

With *m*-chloroperoxybenzoic acid and AES, the same two products were obtained, but this time the endoperoxide was the major one by far.

All the other peroxy acids examined (peroxy-pivalic, -sulfuric and -monophthalic) gave with AES only one product which on TLC corresponded closely to the endoperoxide.

2.2. Polarography.

Polarography was conducted on the same solutions as those used for the TLC analyses.

Peroxyacetic and chloroperoxyacetic acids gave a polarographic signal with a half-wave potential of -1.35 volts. This was the only wave observed and it differed significantly from the one expected for endoperoxide.

All the remaining acids gave polarographic traces which were quite similar to those observed with authentic AES-endoperoxide. Table 20, gives the half-wave potentials observed in each case.

Clearly, with the exception of the first two acids, the polarographic traces obtained correspond to those of endoperoxide, indicating that the latter was present.

In the case of peroxy-monophthalic acid some quantitative polarographic measurements were also made. By constructing

Table 20: Polarography of the products of peroxy acids with AES*

Acid	$E_{\frac{1}{2}}$ (volts)
Peroxyacetic	-1.35
Chloroperoxyacetic	-1.35
Chloroperoxybenzoic	-1.10
Peroxypivalic	-1.05
Peroxysulfuric	-0.95
Peroxymonophthalic	-1.02

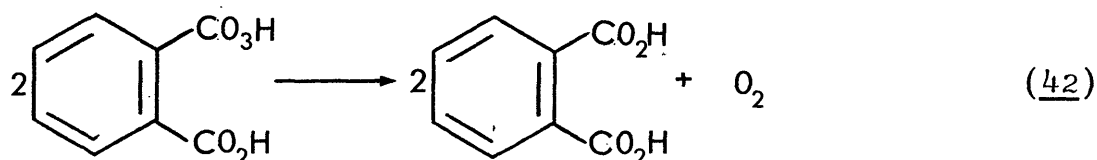
* Addition of Na_2SO_3 did not alter the polarographic waves.

a calibration curve with authentic endoperoxide (Fig.2,p.45) it was possible to quantify the endoperoxide present in the reaction mixture .

With initial concentrations of 2×10^{-3} M and 4×10^{-3} M peroxy acid and AES, respectively, the polarographic wave obtained after complete decomposition of the peroxy acid had a height of 15 μA , which, by reference to Fig. 2, can be seen to correspond to 2.8×10^{-4} M endoperoxide. The corresponding value obtained spectrophotometrically was 3.2×10^{-4} M in fair agreement.

In conclusion, both TLC and polarography indicate that very little endoperoxide is produced from AES and peroxyacetic or chloroperoxyacetic acids, while the endoperoxide is the major product with chloroperoxybenzoic and the only product with peroxypivalic, peroxysulfuric and peroxymonophthalic acids. Further discussion of the possible origin of this product is postponed until after consideration of the kinetic studies.

2.3. Kinetic studies of peroxymonophthalic acid (PPA) decomposition



2.3.1. Preliminary studies

In all kinetic studies of peroxy acid decomposition, a concentration of cyclohexane-1,2-diaminetetraacetic acid (CDTA) (1×10^{-3} M) was included in order to block any metal ion-catalysed decomposition, according to Edwards and others (137, 142, 143).

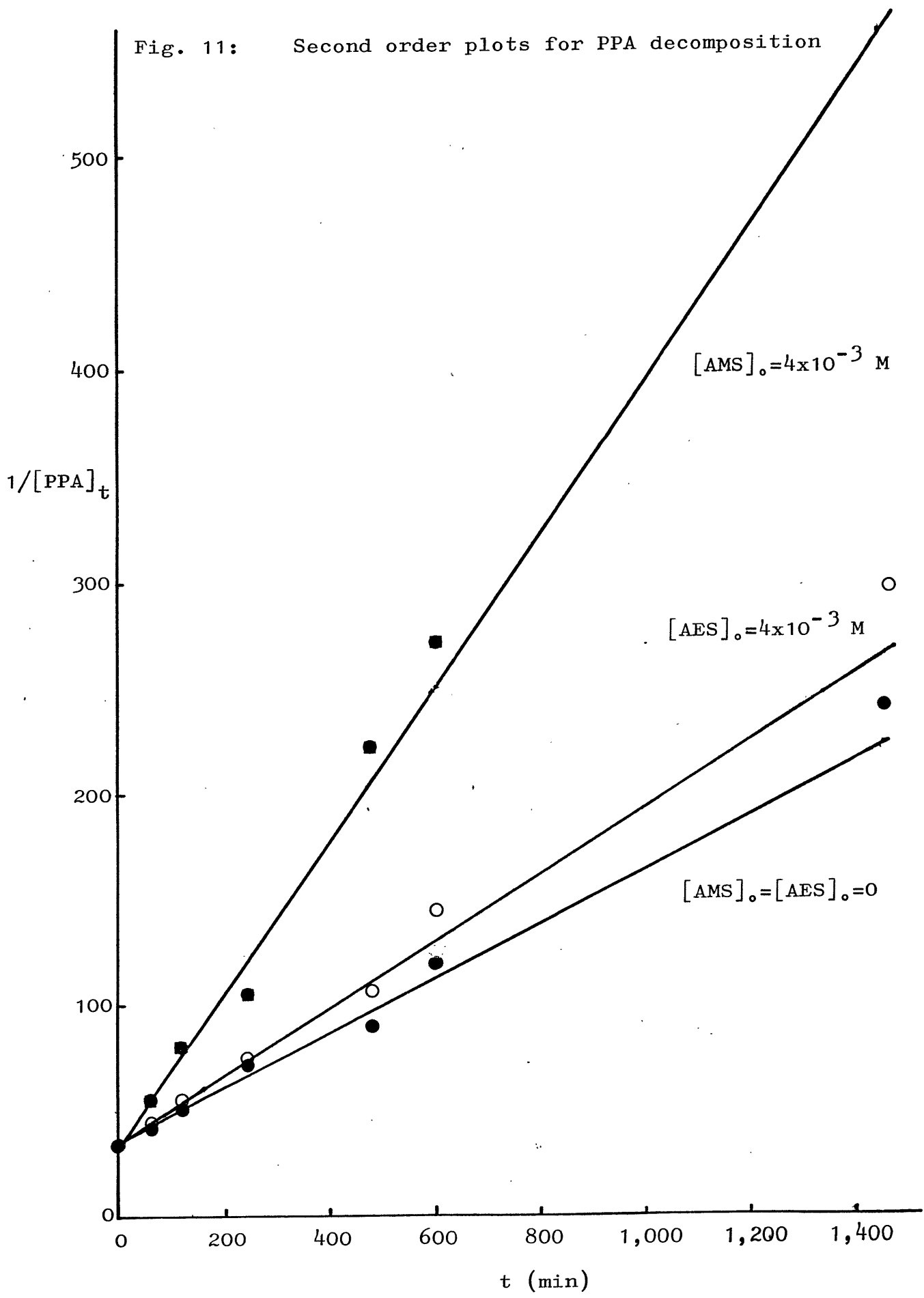
Independent experiments with the systems $\text{H}_2\text{O}_2/\text{NaOCl}/\text{AES}/\text{CDTA}$ and $\text{H}_2\text{O}_2/\text{NaOCl}/\text{AMS}/\text{CDTA}$ established that CDTA does not quench singlet oxygen at that concentration.

The progress of PPA decomposition at pH 8.2 was followed over an extended period of time by sampling and analysing for peroxy acid remaining by iodometric titration. As Fig. 11 shows, second order plots were linear both in the absence and in the presence of added AES or AMS. The measured second order constant in the absence of AES or AMS was $k_{\text{obs}} = 2.18 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$ at 20°C , in reasonable agreement with the value ($5.6 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$) obtained by Edwards et al. (143) at 25°C . The corresponding rate constants in the presence of $4 \times 10^{-3} \text{ M}$ AES or AMS were $2.61 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$ and $6.08 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$ respectively.

The conclusions that can be drawn from these results are that (i) the uncatalysed decomposition was being observed in the absence of AES or AMS, and (ii) the decomposition was second order with respect to peroxy acid in all three cases.

In all subsequent experiments the extent of reaction was measured after seven minutes, when the extents of PPA decomposition and AES or AMS consumption were within the desirable limits.

Fig. 11: Second order plots for PPA decomposition



The extent of PPA decomposition was determined by iodometric titration while AES or AMS consumption was measured by spectrophotometry. Because it had already been established that 9,10-endoperoxide was the only product arising from AES or AMS, a figure for the apparent percent efficiency of singlet oxygen trapping was calculated on the basis of one oxygen molecule being formed from two molecules of PPA as follows:

$$E = \frac{2\Delta[\text{AES}]}{\Delta[\text{PPA}]} \cdot 100$$

This figure gives the proportion of PPA which reacts with a trap in a "singlet oxygen"-fashion.

3.1.2. PPA decomposition in the presence of AES

The results obtained from the study of PPA decomposition in the presence of different amounts of AES are summarized in Table 21 and Figs. 12 and 13.

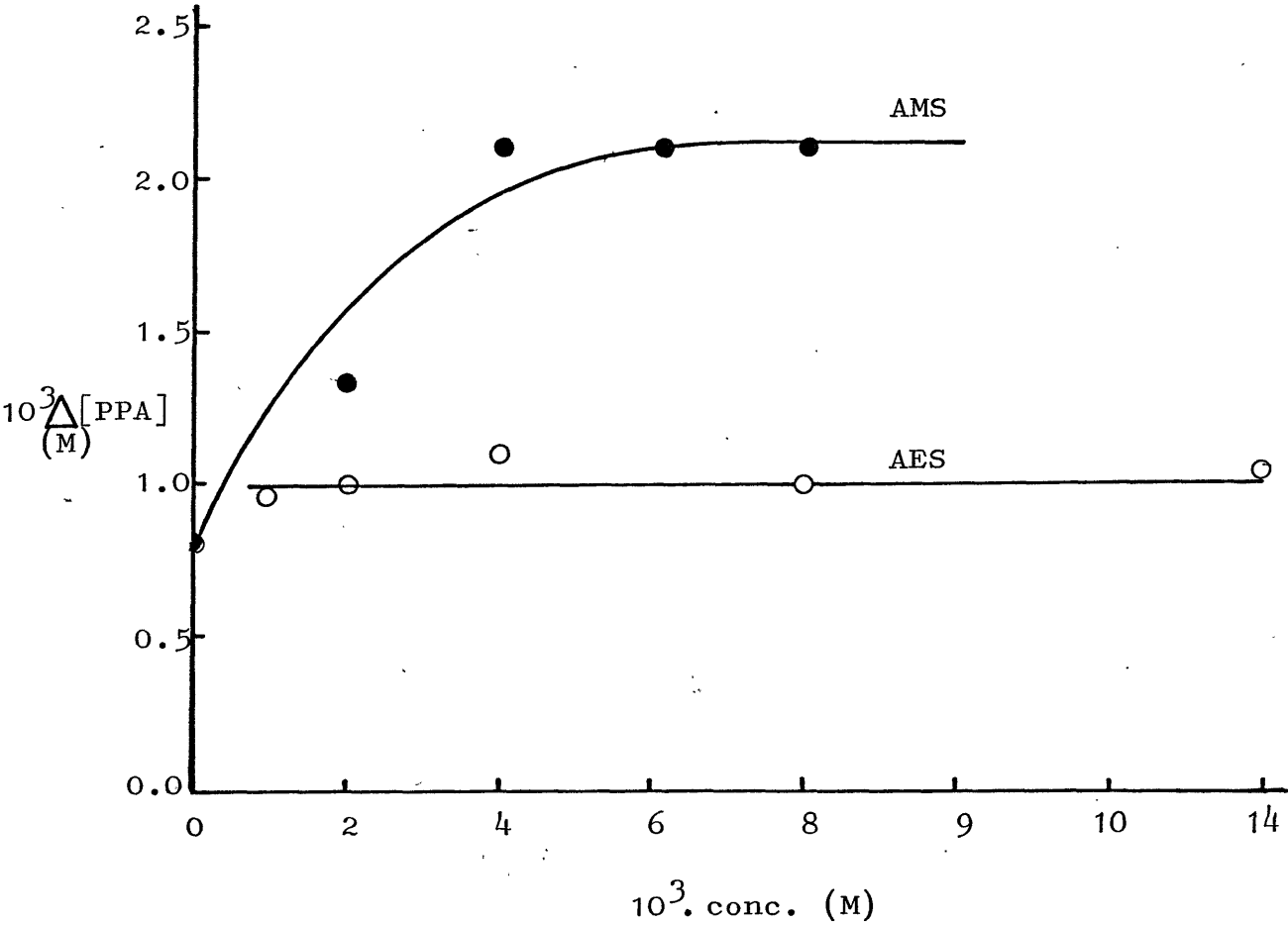
The following conclusions can be drawn from these data:

- (i) AES does not bring about an increase in the rate of PPA disappearance (Fig. 12). This means that the observed

Table 21: The interaction between PPA and AES

$[PPA]_0 = 3.0 \times 10^{-2} \text{ M}; \quad [CDTA] = 10^{-3} \text{ M}; \quad t_{inc} = 7 \text{ min}; \quad 0.15 \text{ M bicarbonate buffer, pH } 8.2; \quad T = 20^\circ\text{C}$				
$[AES]_0$ (M)	$\Delta[AES]$ (M)	$\Delta[PPA]$ (M)	E (%)	E_{10^2} (%)
—	—	0.80×10^{-3}	—	—
1×10^{-3}	0.67×10^{-4}	0.96×10^{-3}	14.0	18.8
2×10^{-3}	1.33×10^{-4}	1.00×10^{-3}	26.6	31.5
4×10^{-3}	1.81×10^{-4}	1.10×10^{-3}	32.9	47.7
8×10^{-3}	2.26×10^{-4}	1.00×10^{-3}	45.2	64.2
14×10^{-3}	2.79×10^{-4}	1.05×10^{-3}	53.1	75.8

Fig. 12: The effects of AES and AMS on PPA decomposition



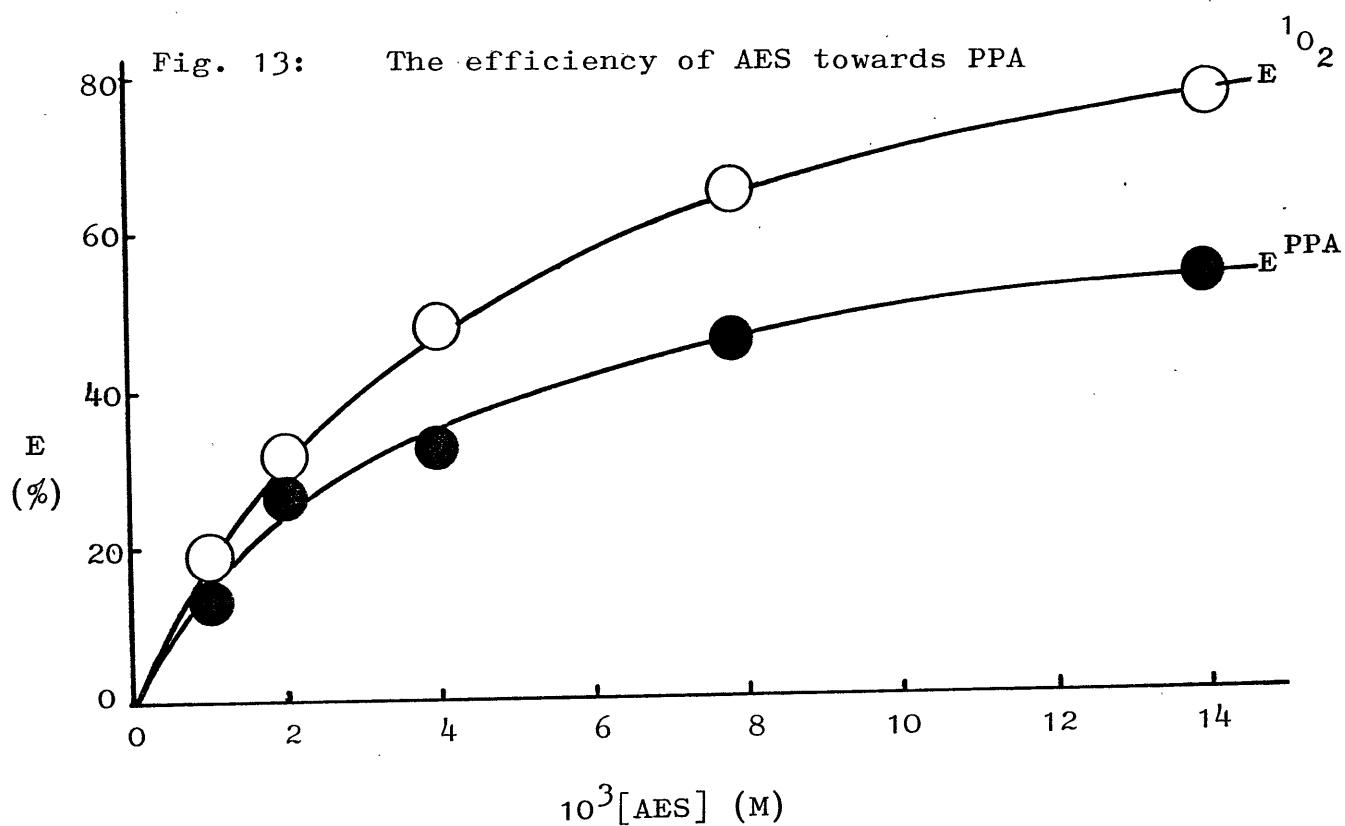
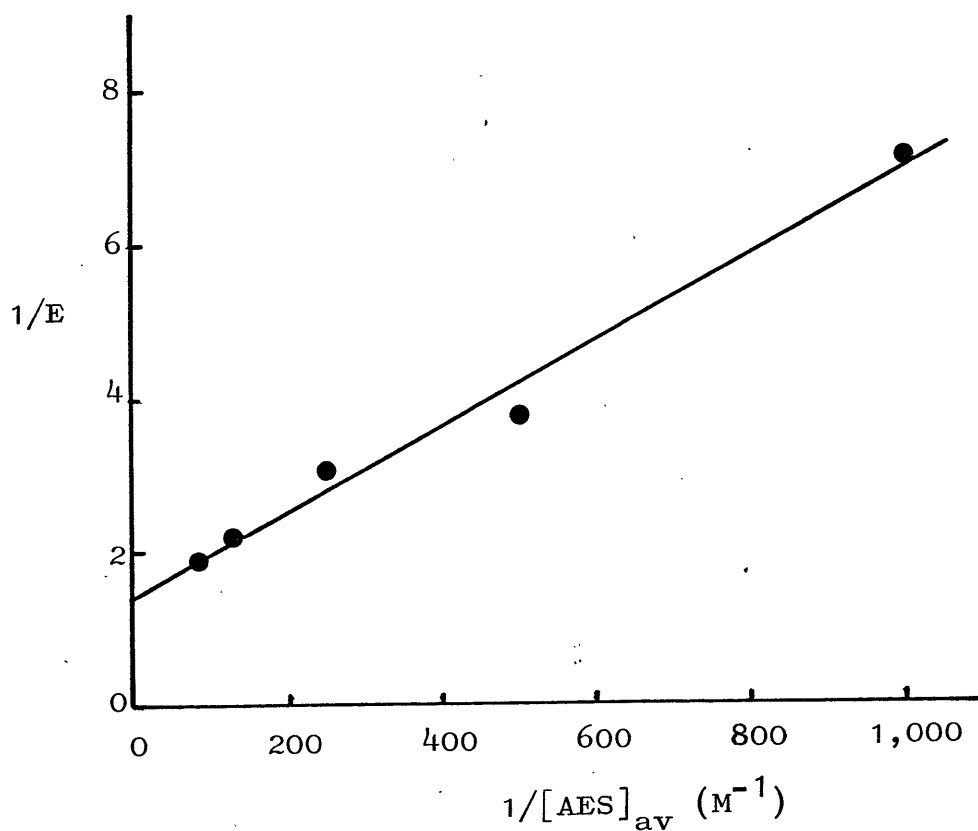


Fig. 14: The efficiency of AES towards PPA (double inverse plot)



endoperoxide formation is not due to a direct reaction between AES and PPA but it must be the result of a reaction of AES with an intermediate on the PPA decomposition pathway.

(ii) The efficiency of AES towards PPA is lower than its efficiency towards free singlet oxygen, and varies in parallel with it (Fig. 13). A plot of $1/E$ versus $1/[AES]$ is linear and allows from its intercept the calculation of the maximum achievable efficiency of AES towards PPA (Fig. 14). This maximal efficiency, which has a value of $(68.5 \pm 3.5)\%$, may be due to one of two things:

- (a) singlet oxygen may be generated during PPA decomposition and suffer quenching by AES to the extent of 31.5%, in contrast to the normal mode of interaction between AES and singlet oxygen which does not involve quenching,
- (b) singlet oxygen or a similarly-acting species may be produced during PPA decomposition with a yield of 68.5% and trapped by AES with its normal maximal efficiency of 100%.

In the first case, it is difficult to see how AES could acquire quenching properties towards singlet oxygen. Furthermore, it is striking that, the ratios of the slope and intercept of Fig. 3 (p. 47), to the corresponding parameters of Fig. 14 (p. 118) are very close to each other

(0.77 and 0.68 respectively). The implications of this can be examined by a simple mathematical analysis. Case (a) predicts the following equations:

$$E = E_{\text{AES}}^{1\text{O}_2} = \frac{k_r[\text{AES}]}{k_r[\text{AES}] + k_d} \quad (13)$$

$$E' = E_{\text{AES}}^{\text{PPA}} = \frac{k_r'[\text{AES}]}{(k_r' + k_q)[\text{AES}] + k_d} \quad (14)$$

From the above equations it can be derived that:

$$\frac{\text{slope}}{\text{slope}'} = \frac{k_r'}{k_r}$$

$$\frac{\text{intercept}}{\text{intercept}'} = \frac{k_r'}{k_r' + k_q}$$

If these ratios are approximately equal, $k_r \simeq k_r' + k_q$. This is a necessary condition for case (a) to describe the observed behaviour of the system and it requires a rather unique quantitative relationship between the various independent parameters.

On the other hand, case (b) predicts the following equation:

$$E' = E_{\text{AES}}^{\text{PPA}} = \frac{k_r[\text{AES}]}{k_r[\text{AES}] + k_d} \times Y \quad (15)$$

where $Y = \%$ yield of "singlet oxygen".

From eqs. (13) and (15)

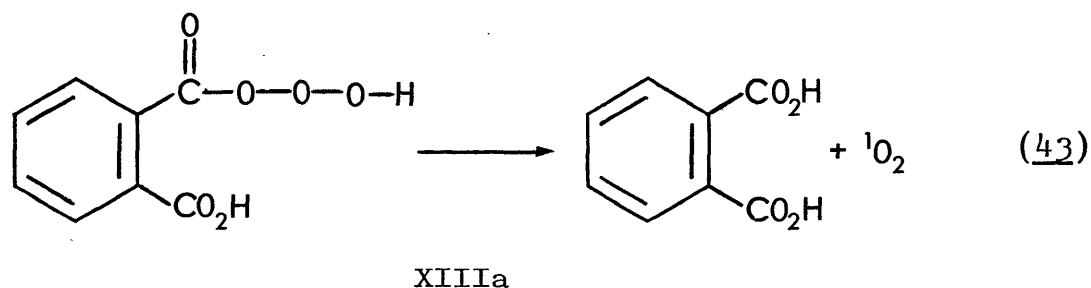
$$\frac{\text{slope}}{\text{slope}'} = \frac{\text{intercept}}{\text{intercept}'} = Y$$

Clearly the model of case (b) satisfies the observed kinetics in a more reasonable way than that of case (a). It can be tentatively concluded that singlet oxygen or a similarly-acting species is generated during PPA decomposition with a yield of $(68.5 \pm 3.5)\%$ and is trapped by AES in a similar manner to singlet oxygen. It is therefore reasonable to suggest that this species is in fact free singlet oxygen.

The figure of $(68.5 \pm 3.5)\%$ is strongly reminiscent of the figure $(74 \pm 4)\%$ obtained by Edwards and his group (143) for the proportion of PPA decomposition proceeding via oxygen-oxygen attack and intermediate XIII (Section 1, Scheme 8).

This correlation leads to the tentative proposition that singlet oxygen is produced during PPA decomposition only

from the breakdown of intermediate XIIIa



Further evidence for this model and a deeper insight into the mechanism of PPA decomposition was obtained from the study of the PPA/AMS system described in the next section.

2.3.3. PPA decomposition in the presence of AMS

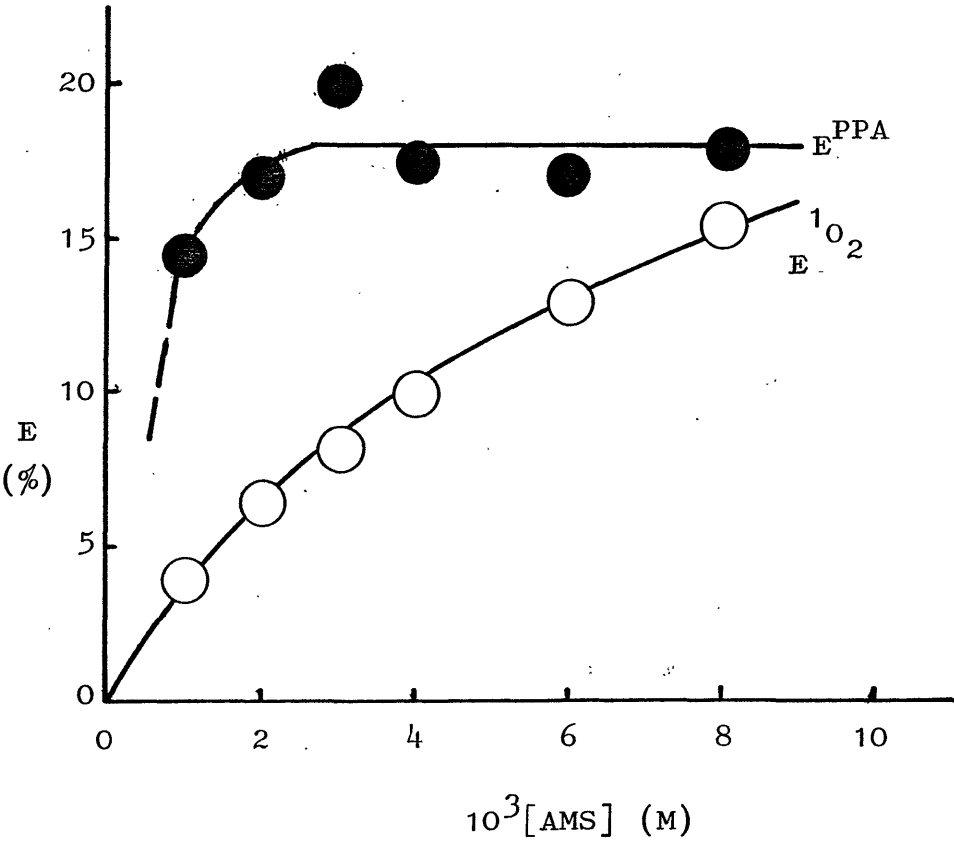
The results obtained from the study of PPA decomposition in the presence of different amounts of AMS are summarized in Table 22 and Figs. 12 and 15. The following conclusion can be drawn from these data:

- (i) AMS brings about a significant increase in the rate of decomposition of PPA (Fig. 12). The maximal increase in the rate of PPA decomposition is 1.3×10^{-3} M in seven minutes, while the maximum amount of AMS consumption in the same period of time is ca. 1.9×10^{-4} M. This shows that

Table 22: The interaction between PPA and AMS

Conditions as in Table 21.				
$[AMS]_0$ (M)	$\Delta[AMS]$ (M)	$\Delta[PPA]$ (M)	E (%)	E^{1O_2} (%)
—	—	0.80×10^{-3}	—	—
1×10^{-3}	0.83×10^{-4}	1.14×10^{-3}	14.6	3.9
2×10^{-3}	1.13×10^{-4}	1.33×10^{-3}	17.0	6.6
3×10^{-3}	1.82×10^{-4}	1.81×10^{-3}	20.0	8.3
4×10^{-3}	1.84×10^{-4}	2.10×10^{-3}	17.5	10.0
6×10^{-3}	1.78×10^{-4}	2.10×10^{-3}	17.0	13.0
8×10^{-3}	1.89×10^{-4}	2.10×10^{-3}	18.0	15.4

Fig. 15: The efficiency of AMS towards PPA



the increase in PPA consumption represents catalysis of the decomposition and not, primarily, reaction with AMS.

(ii) The maximal efficiency of AMS towards PPA is 18% in contrast to the corresponding efficiency towards free singlet oxygen of 26.6% (Fig. 4, p. 53).

(iii) The above maximal efficiency of 18%, is reached significantly more rapidly than the maximal efficiency towards free singlet oxygen. Thus half-maximal efficiency towards PPA is reached at ca. 6×10^{-4} M [AMS], while with free singlet oxygen 6×10^{-3} M [AMS] is required.

If singlet oxygen is produced during PPA decomposition and its yield is 68.5% as indicated in the preceding section, it would be expected to be trapped by AMS with a real efficiency of 26.6% leading to an apparent one of 18.2%, in excellent agreement with the observed value. This finding reinforces the proposition about the mechanism of singlet oxygen production during PPA decomposition made in the preceding section.

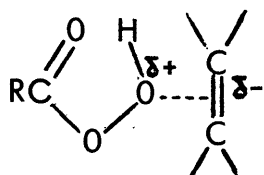
Although the singlet oxygen produced appears to interact with AMS in a similar manner to free singlet oxygen, maximal efficiency in the former case is reached at lower concentrations of AMS than in the latter. This indicates

that this species is quenched by the solvent to a smaller degree than free singlet oxygen. The most reasonable way of accounting for this is by suggesting that singlet oxygen is generated during PPA decomposition in close proximity with AMS, thus interacting with it faster than being quenched by the solvent. This situation would be expected to arise if PPA decomposition occurred via a complex of the peroxy acid (or an intermediate derived from it) with AMS. Indeed, the formation of such a complex is strongly indicated by the observed catalysis by AMS of PPA decomposition. This catalysis has a parallel in the effects of olefins on peroxy acid decomposition, a subject which will be briefly considered in the following section.

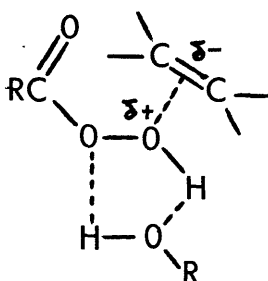
2.3.4. The interactions of peroxy acids with olefins

While in non-basic solvents peroxy acids exist mainly in an intramolecularly H-bonded form and epoxidise via charge-transfer complexes of type XIV, in basic protic solvents (e.g. trifluoroethanol or acetic acid-DMF mixtures) intermolecularly H-bonded species (XV and XVI) have been detected by kinetic analyses of the decomposition and epoxidation reactions (104, 144).

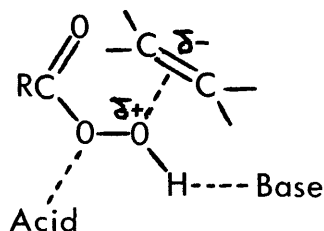
Detailed studies in acetic acid-DMF mixtures have shown that bases catalyse peroxy acid decomposition while



XIV

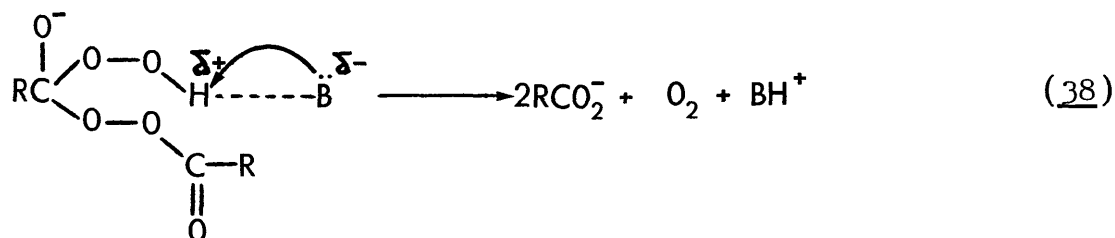
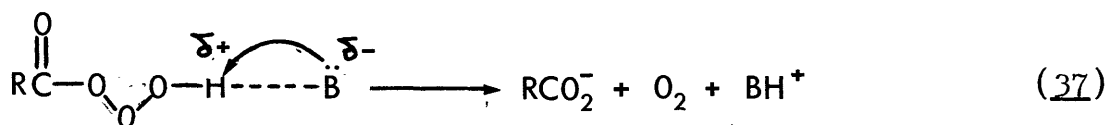


XV



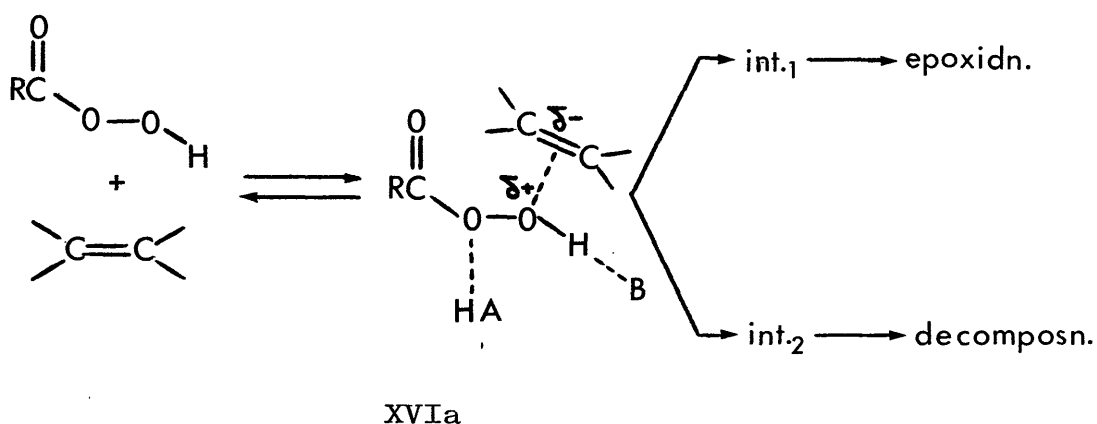
XVI

inhibiting olefin epoxidation. The first of these effects has been explained primarily in terms of acceleration of the breakdown of intermediates formed following reaction between two peroxy acid molecules (Section 1) (144).



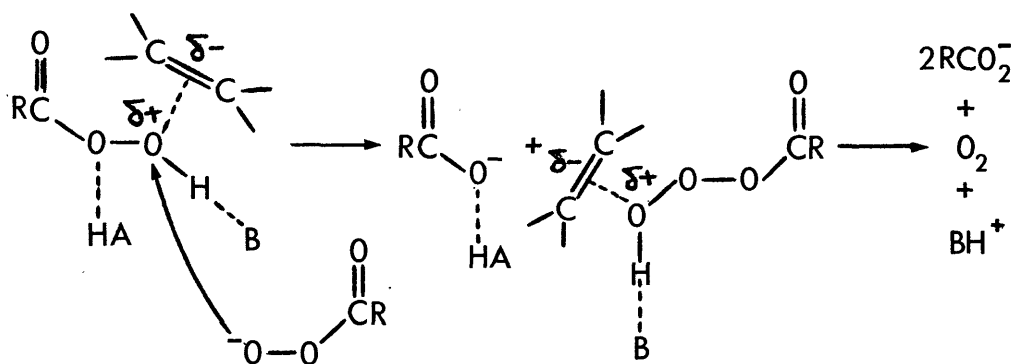
Olefin epoxidation is inhibited by base because of the decreased electrophilicity induced in the peroxidic oxygen by H-bonding with the base, making nucleophilic attack by the olefin on it more difficult.

Olefins inhibit the decomposition of some peroxy acids (e.g. allyl bromide with peroxyacetic acid) while catalyzing that of others (e.g. allyl bromide with PPA). Dryuk has suggested (144) that an olefin-base-peroxy acid charge-transfer complex similar to XVI is initially formed which is then transformed into intermediates leading to epoxidation or decomposition (Scheme 9).



Scheme 9

Olefin inhibition of the decomposition is explained by the antagonistic roles of the olefin and base in XIVa. On the other hand, the reason for catalysis (where this is observed) is more obscure. It is suggested that catalysis may be related to specific solvation effects playing a role in facilitating attack by a peroxy acid anion on V (Scheme 10),



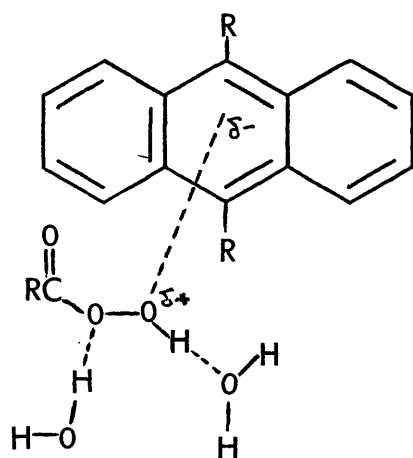
Scheme 10

That the balance between catalysis and inhibition by olefins of the decomposition is a fine one is illustrated by the fact that in some cases either effect may be observed, depending on the precise solvent composition (ref. 144 and references therein).

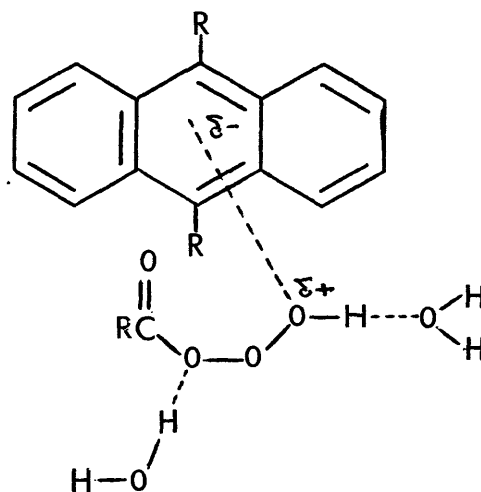
2.3.5. The interaction of PPA with AMS

It was seen in the preceding section that charge-transfer intermediates dominate the pathways of peroxy acid interactions with olefins in basic protic solvents. It is not unreasonable to expect that what holds in acetic acid-DMF and trifluoroethanol solvents may also hold in water, also a basic protic solvent.

Returning now to the PPA/AMS system, it may be reasonably postulated that a complex involving charge-transfer between the electron-rich central ring of AMS and the electron-deficient peroxidic oxygen of solvated PPA (or a similar intermediate on the decomposition reaction pathway) is reversibly formed (XVII or XVIII).



XVII



XVIII

Such complexes might have led to AMS epoxidation, by analogy to the corresponding olefin complexes. However, Boyer et al. (102) have shown that 9,10-diphenylanthracene

does not react with m-chloroperoxybenzoic acid (a strong epoxidising agent) even after refluxing in CH_2Cl_2 . Wolf (28) has also shown that benzene, toluene and other aromatic hydrocarbons show a similar lack of reactivity.

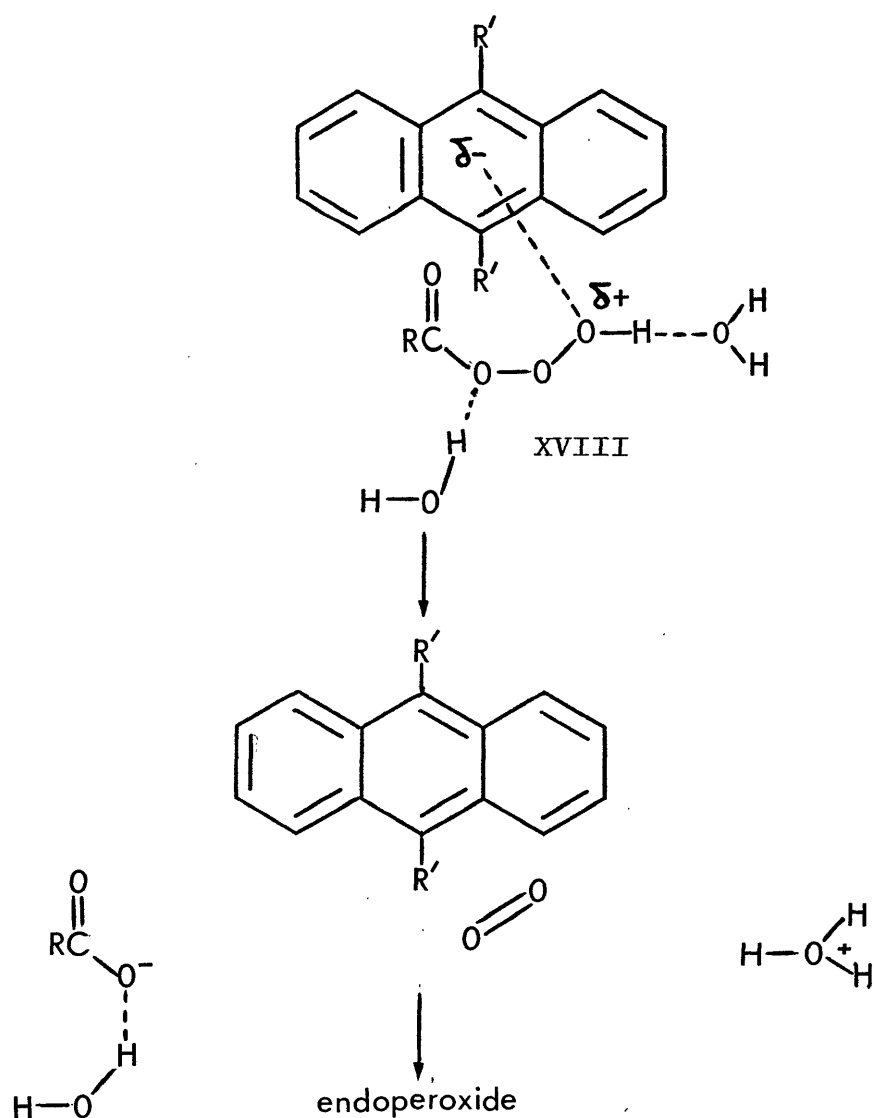
Furthermore, PPA decomposition was second order in peroxy acid even in the presence of AMS (Fig. 11), indicating that no rate-determining reaction involving one molecule of PPA was occurring. Finally no evidence of any product other than the 9,10-endoperoxide was found by TLC or polarography. It is consequently concluded that epoxidation of AMS did not take place.

On the other hand, still by analogy with the corresponding olefin complexes, XVII and XVIII might be expected to undergo further reaction along the pathway of PPA decomposition.

In such a case, XVII (if formed) would undergo attack by a PPA anion to form XVIII which would then decompose to form free singlet oxygen in close proximity with AMS (Scheme 11).

Scheme 11 can account admirably for all the observations:

(i) Because singlet oxygen is generated in close proximity with AMS, interaction between the two is more likely than if singlet oxygen were generated independently to AMS in the bulk of the solution. Consequently, the degree of



Scheme 11

quenching by the solvent is reduced and maximal efficiency is reached at lower AMS concentrations than in the case of singlet oxygen generated by other means (e.g. the $\text{H}_2\text{O}_2/\text{NaOCl}$ system).

(ii) Because the species generated is molecular singlet

oxygen and not a similarly-reacting, "singlet-oxygen-like" species, interaction with AMS and AES is as observed with singlet oxygen generated by the standard methods. That is, chemical reaction with and physical quenching by AMS and AES in the PPA system occur in the same proportions as they do in the case of singlet oxygen generated by the standard methods. Evidently, the effect of proximity is to increase the rates of both to an equal extent by raising the number of collisions.

(iii) The catalysis of PPA decomposition by AMS is compatible with the formation of a complex between AMS and PPA or an intermediate along the decomposition pathway. The fact that AMS, while increasing the overall rate of PPA decomposition by ca. 2.5 times, does not alter the proportion of PPA decomposing via oxygen-oxygen attack (and singlet oxygen) may be explained in one of the two ways: Either AMS in combination with PPA (complex XVII) facilitates to an equal extent both decomposition pathways (oxygen-carbon and oxygen-oxygen attack), or AMS complexes not with PPA but with an intermediate (or intermediates) formed after the initial reaction between two PPA molecules. In order to distinguish between these possibilities it is necessary to examine the kinetic behaviour of the system, considered in a subsequent section.

2.3.6. The interaction of PPA with AES

The finding that AES has a true efficiency towards PPA-generated singlet oxygen similar to that which it exhibits towards singlet oxygen generated by standard methods and the absence of any significant effect of AES on PPA decomposition, both argue against the formation of an active complex in this system.

Alternatively, one might argue that a complex is formed in this system also, but any singlet oxygen finally arising from it escapes into the bulk of the solution before being locally trapped by AES. However, given the higher reactivity of AES compared to that of AMS (Table 9, p. 55) this is not a plausible suggestion.

It is somewhat surprising that AES, which is clearly more reactive than AMS towards singlet oxygen (presumably because of the greater electron density at positions 9 and 10), should form less easily charge-transfer complexes of the types suggested. It is possible that the greater bulkiness of the substituents prevents sufficiently close approach of the other molecule to the central ring of the anthracene nucleus. Inspection of molecular models indicates that the substituents on AES can prevent access to the central ring significantly more successfully than the substituents on AMS.

It is consequently concluded that AES does not interact

with PPA directly, but it reacts normally with the singlet oxygen formed during PPA decomposition and released into the bulk of the solution.

The behaviour of AES and AMS towards PPA (in terms of the formation of charge-transfer complexes) parallels their behaviour as physical quenchers of singlet oxygen (Table 9, p. 55). Thus it is striking that AES, which causes no significant quenching of singlet oxygen, appears not to form any charge-transfer complex in the PPA system. On the other hand, AMS quenches significantly singlet oxygen and also forms a charge-transfer complex in the PPA system. Although the mechanism of singlet oxygen quenching by aromatic hydrocarbons is at present unknown, it is tempting to suggest on the basis of this correlation that charge-transfer may be playing a role, somewhat analogously to the mechanism of quenching by amines (121). In this context it is interesting to note that charge-transfer complexes have been detected spectrophotometrically in solutions of oxygen in aromatic hydrocarbons (145, 146).

2.3.7. Solvent-isotope effects in the PPA/AES and PPA/AMS systems

The argument presented in the preceding sections suggests that PPA-generated singlet oxygen in the presence of AMS

is less liable to quenching by the solvent than that formed in the presence of AES. This argument leads to the prediction that D_2O , through its reduced rate of quenching of singlet oxygen relative to H_2O , should increase the efficiency of AES towards PPA but not (or to a much smaller degree) that of AMS.

This prediction was tested by measuring the respective efficiencies in H_2O and D_2O . The results, shown in Table 23, show clearly that a significant increase in efficiency in D_2O is observed only with AES and not with AMS. This result constitutes strong evidence for the fundamental correctness of the proposed mechanism.

Table 23: Heavy-water effects in the PPA-AES and PPA-AMS systems.

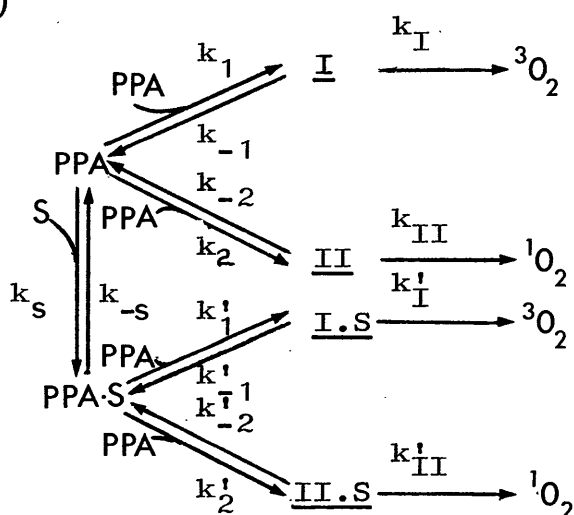
Substrate	$[PPA]_0$ (M)	Solvent	$\Delta[\text{Substrate}]$ (M)	$\Delta[PPA]$ (M)	E (%)
AES, 2×10^{-3} M	3×10^{-2}	H_2O	1.33×10^{-4}	1.00×10^{-3}	26.6
AES, 2×10^{-3} M	3×10^{-2}	D_2O	3.23×10^{-4}	0.95×10^{-3}	68.0
AMS, 4×10^{-3} M	3×10^{-2}	H_2O	1.84×10^{-4}	2.10×10^{-3}	17.5
AMS, 4×10^{-3} M	3×10^{-2}	D_2O	1.97×10^{-4}	2.00×10^{-3}	19.7

Analogous results are predicted by the mechanism for the effects of an added quencher. Unfortunately the already mentioned instability of PPA in the presence of Niphalds precluded such experiments.

2.3.8. Kinetic analysis

In Section 2.3.5. it was pointed out that two possible models are compatible with the observations as presented so far. One model involves a complex of AMS with PPA itself while the other involves complexes of AMS with the intermediates formed following initial reaction between two PPA molecules. In what follows, the kinetic behaviour predicted by each of these models is deduced and compared to the observed behaviour of the system.

(i)



Scheme 12*

* Intermediates I and II are not related to I and II of Chapter 2.

Scheme 12 shows in outline a general form of the first model. AMS combines reversibly with PPA to give a complex (PPA.S) which reacts by two processes exactly analogous to, but faster than, those of uncomplexed PPA. One pair of these processes, corresponding to k_1 and k_1' , results in ground-state oxygen formation and involves oxygen-carbon attack between two PPA molecules. The other process, corresponding to k_2 and k_2' , results in singlet oxygen formation and involves oxygen-oxygen attack. Processes following molecular oxygen formation have been left out for simplicity.

In order to account for the observed constant proportion of singlet oxygen formation in the absence and presence of AMS, it is necessary that:

$$\frac{k_1}{k_2} = \frac{k_1'}{k_2'} \quad \text{and} \quad \frac{k_{-1}}{k_{-2}} = \frac{k_{-1}'}{k_{-2}'}$$

As Fig. 12 shows, the maximal rate of PPA decomposition with $[PPA]_0 = 3 \times 10^{-2}$ M is reached with $[AMS] 4 \times 10^{-3}$ M. This indicates clearly that maximal rate is not due to all PPA being converted to the PPA.S complex, i.e. equilibrium conditions do not prevail for PPA.S.

The total rate of PPA decomposition, as measured by iodometric titration, and assuming that all the intermediate species shown in Scheme 12 are equally reactive in the iodometric titration, is given by equation (15).

$$-\frac{d[\text{PPA}]}{dt} = 2(k_I[\underline{\text{I}}] + k_{II}[\underline{\text{II}}] + k_I'[\underline{\text{I.S}}] + k_{II}'[\underline{\text{II.S}}]) \quad (15)$$

Applying the steady-state treatment to complexes I, II, I.S and II.S, the corresponding steady-state concentrations can be calculated.

$$[\underline{\text{I}}] = \frac{k_1[\text{PPA}]^2}{k_{-1} + k_I} \quad (16)$$

$$[\underline{\text{II}}] = \frac{k_2[\text{PPA}]^2}{k_{-2} + k_{II}} \quad (17)$$

$$[\underline{\text{I.S}}] = \frac{k_1'[\text{PPA}][\text{PPA.S}]}{k_{-1}' + k_I'} \quad (18)$$

$$[\underline{\text{II.S}}] = \frac{k_2'[\text{PPA}][\text{PPA.S}]}{k_{-2}' + k_{II}'} \quad (19)$$

Applying the same treatment to PPA.S,

$$[\text{PPA.S}] = \frac{k_S[\text{PPA}][\text{S}] + k_{-1}'[\text{I.S}] + k_{-2}'[\text{II.S}]}{k_{-S} + [\text{PPA}](k_1' + k_2')} \quad (20)$$

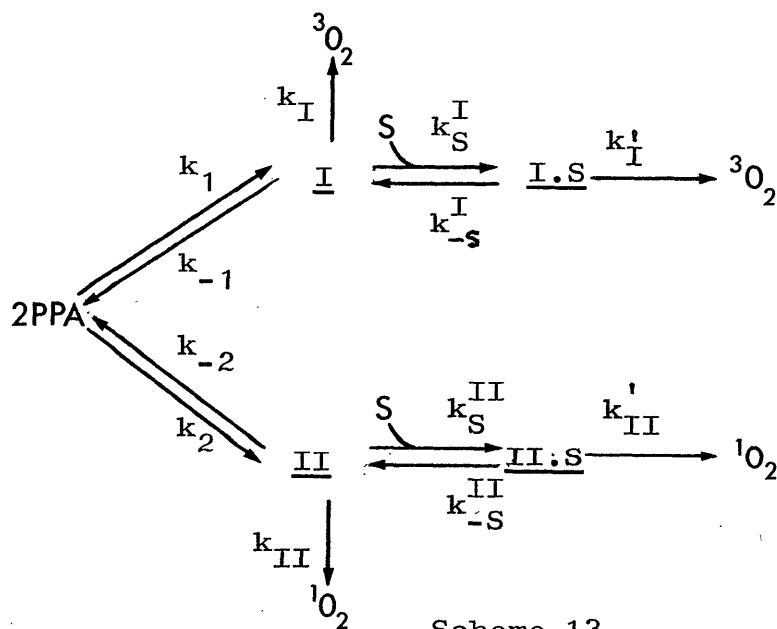
If the way is examined in which the concentrations of the various intermediates just calculated would vary with $[\text{S}]$, it can be seen that $[\text{I}]$ and $[\text{II}]$ would be independent of $[\text{S}]$, while $[\text{I.S}]$ and $[\text{II.S}]$ would show the same dependence as $[\text{PPA.S}]$. The latter can be obtained by substituting (18) and (19) into (20). When this is done, the resulting rather complex expression is of the form

$$[\text{PPA.S}] = \frac{k_A[\text{PPA}][\text{S}]}{k_B + k_C[\text{PPA}]} \quad (21)$$

where k_A , k_B and k_C are combinations of rate constants. Equation (21) shows that $[\text{PPA.S}]$ would be proportional to $[\text{S}]$ at all values of $[\text{S}]$. This means that the rate of PPA decomposition would also show a linear dependence on $[\text{AMS}]$, in clear contradiction with what is observed. Consequently, this model can be rejected.

(ii)

Scheme 13 shows the alternative model, in which two PPA molecules combine reversibly to form two types of complex, I and II. Each complex may then break down to form molecular



Scheme 13

oxygen (with rate constant k_I or k_{II}) or it may associate with AMS to form a new complex (I.S or II.S) which finally also breaks down to form molecular oxygen. It is not necessary for both I and II to form a complex with AMS, but this possibility is included here for generality.

The possibility that intermediates I, I.S, II and II.S are in equilibrium with each other and with PPA can be rejected for the same reason that equilibrium conditions were rejected in the previously considered model. Consequently, steady-state conditions operate.

Applying the steady-state treatment to the various intermediates, their steady-state concentrations can be calculated as follows:

$$[\underline{\text{I}}] = \frac{k_1(k_{-S}^{\text{I}} + k_{\text{I}}')[\text{PPA}]^2}{(k_{-1} + k_{\text{I}})(k_{-S}^{\text{I}} + k_{\text{I}}') + k_{\text{S}}^{\text{I}}k_{\text{I}}'[S]} \quad (22)$$

$$[\underline{\text{II}}] = \frac{k_2(k_{-S}^{\text{II}} + k_{\text{II}}')[\text{PPA}]^2}{(k_{-S} + k_{\text{II}})(k_{-S}^{\text{II}} + k_{\text{II}}') + k_{\text{S}}^{\text{II}}k_{\text{II}}'[S]} \quad (23)$$

$$[\underline{\text{I.S}}] = \frac{k_{\text{S}}^{\text{I}}k_1[S][\text{PPA}]^2}{(k_{-2} + k_{\text{I}})(k_{-S}^{\text{I}} + k_{\text{I}}') + k_{\text{S}}^{\text{I}}k_{\text{I}}'[S]} \quad (24)$$

$$[\underline{\text{II.S}}] = \frac{k_{\text{S}}^{\text{II}}k_2[S][\text{PPA}]^2}{(k_{-2} + k_{\text{II}})(k_{-S}^{\text{II}} + k_{\text{II}}') + k_{\text{S}}^{\text{II}}k_{\text{II}}'[S]} \quad (25)$$

Remembering that

$$-\frac{d[\text{PPA}]}{dt} = 2(k_{\text{I}}[\underline{\text{I}}] + k_{\text{II}}[\underline{\text{II}}] + k_{\text{I}}'[\underline{\text{I.S}}] + k_{\text{II}}'[\underline{\text{II.S}}])$$

and substituting for the various concentrations

$$-\frac{d[\text{PPA}]}{dt} = 2 \frac{k_1[\text{PPA}]^2(k_{\text{I}}(k_{-S}^{\text{I}} + k_{\text{I}}') + k_{\text{S}}^{\text{I}}k_{\text{I}}'[S])}{(k_{-1} + k_{\text{I}})(k_{-S}^{\text{I}} + k_{\text{I}}') + k_{\text{S}}^{\text{I}}k_{\text{I}}'[S]} +$$

$$\begin{aligned}
& k_2[\text{PPA}]^2(k_{\text{II}}(k_{-\text{S}}^{\text{II}} + k_{\text{II}}') + k_{\text{S}}^{\text{II}}k_{\text{II}}'[\text{S}]) \\
& + \frac{(k_{-\text{S}} + k_{\text{II}})(k_{-\text{S}}^{\text{II}} + k_{\text{II}}') + k_{\text{S}}^{\text{II}}k_{\text{II}}'[\text{S}]}{(k_{-\text{S}} + k_{\text{II}})(k_{-\text{S}}^{\text{II}} + k_{\text{II}}') + k_{\text{S}}^{\text{II}}k_{\text{II}}'[\text{S}]} \quad (26)
\end{aligned}$$

Equation (26) clearly shows that with increasing $[\text{S}]$ the rate of decomposition would increase to reach a maximal value, as indeed observed.

Equation (26) also predicts that PPA decomposition in the presence of AMS should be second order in PPA, again as observed (Fig. 11, p. 115).

It can therefore be concluded that the kinetic behaviour of the PPA/AMS system is compatible with a model in which AMS combines with one (II) or both of the intermediates formed after reaction between two PPA molecules.

In the absence of any information on the production of ground-state oxygen, during the reaction, it is not possible to use eq. (26) for the quantitative analysis of the available data. However, it is possible to make some general comments regarding the variation with $[\text{AMS}]$ of its efficiency towards PPA.

The maximal efficiency of AMS towards PPA is reached when all singlet oxygen is formed via II.S, i.e. when all II is converted into II.S. The concentration of AMS required to

achieve this is related to the steady-state concentration of II. The latter is a complex function of the various rate constants (eq. (23)) and is probably only a small fraction of the total PPA concentration present. Consequently, maximal AMS efficiency can be reached at concentrations well below that of PPA, as observed.

At any concentration of AMS, the observed efficiency is made up of contributions from the efficiencies of trapping of singlet oxygen arising via II or via II.S, and it lies between these. The efficiency of trapping of free singlet oxygen (such as arises via II) is known to reach a half-maximal value when $[AMS] = 5.8 \times 10^{-3}$ M (Fig. 4, p. 53). The concentration at half-maximal efficiency is numerically equal to the ratio of the rate constant for solvent quenching (k_d) to that of the total interaction with a substrate ($k_r + k_q$), (Chapter 2, Section 1). Consequently, for AMS and free singlet oxygen,

$$\frac{k_d}{k_r + k_q} = 5.8 \times 10^{-3}$$

On the other hand, for AMS and singlet oxygen produced via II.S, for which half-maximal efficiency is ca. 5×10^{-4} M (Fig. 15),

$$\frac{k_d'}{k_r' + k_q'} = 5 \times 10^{-4}$$

This means that the singlet oxygen formed by decomposition of II.S shows approximately a 12-fold greater preference for interaction with AMS than with the solvent than does free singlet oxygen. This is a clear reflection of the increased probability of collision with AMS inside the solvent cage immediately following breakdown of II.S.

2.3.9. General conclusions regarding the PPA/AMS and PPA/AES systems

All the chemical and kinetic evidence presented in the preceding sections is compatible with a model whose main characteristics are summarised below:

- (i) Singlet oxygen is formed during the decomposition of PPA only via the pathway of oxygen-oxygen attack.
- (ii) AMS forms a charge-transfer complex with the intermediate formed following oxygen-oxygen reaction between two PPA molecules.
- (iii) Breakdown of the above complex results in the formation of molecular singlet oxygen which preferentially

interacts with the AMS molecule of the complex rather than with the solvent.

(iv) AES is unable to form an analogous complex, possibly because of sterically reduced accessibility to the PPA intermediate.

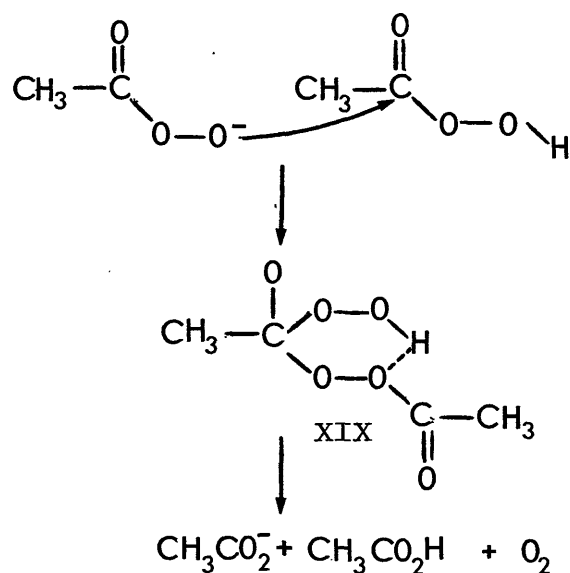
2.4. Kinetic studies of peroxyacetic acid decomposition

In view of the TLC and polarography indications that AES-endoperoxide was only a minor fraction of the products formed with peroxyacetic acid, kinetic experiments with this (and all the remaining acids) were limited.

The rate constant for peroxy acid consumption was measured in the presence and absence of AES. The results (Table 24) show that at pH 8.1 the observed rate constant in the absence of AES was $k_o = 2.4 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$, which corresponds to a molecular rate constant $\underline{k} = 4.8 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$, in fair agreement with the figure of Koubek et al. ($5.7 \times 10^{-3} \text{ l mol}^{-1} \text{ s}^{-1}$) (138). It can consequently be concluded that the process observed was indeed the uncatalysed decomposition.

In the presence of AES the rate of decomposition is not altered, indicating absence of catalysis. However, the

apparent efficiency (assuming that decomposition was generating only singlet oxygen) is over 100%. This, together with the TLC and polarography findings, clearly suggests that the main pathway of peroxyacetic acid reaction with AES does not involve singlet oxygen or any similar species. This observation is in agreement with that of McKewon and Waters (101) (discussed in Section 1.2.). It also accords with, and reinforces, the model proposed in the preceding sections. Peroxyacetic acid decomposes primarily (to the extent of 83%) by oxygen-carbon attack, generating a cyclic intermediate (XIX).



Scheme 14

According to the proposed model, the molecular oxygen formed from this kind of intermediate is not in the singlet state. This would be expected if breakdown of the

intermediate occurred homolytically. The finding of a major product other than endoperoxide trapped with an observed efficiency of more than 100% (i.e. with the reacting ratio of peroxy acid to AES less than 2:1) may in fact represent the interception by AES of an intermediate with significant free radical character. Identification of the unknown product might provide further evidence for such a process.

2.5. Other peroxy acids

Table 24 shows the apparent efficiencies observed in single experiments with the remaining peroxyacids examined. These results, in combination with the TLC and polarography ones, lead to the following conclusions:

i) Chloroperoxyacetic acid behaves similarly to peroxyacetic, reacting extensively with AES but forming little endoperoxide.

ii) With all the remaining peroxy acids, where the endoperoxide is the major or only product, the AES efficiencies are in the region of 40-45% with $[AES]_0 = 4 \times 10^{-3}$ M. At this concentration the efficiency with free singlet oxygen would be 48%. Therefore, if singlet oxygen is being formed its yield is probably 80-90% and it is being trapped

Table 24: The interaction of the remaining peroxy acids with AES

[AES] = 4×10^{-3} M; [CDTA] = 10^{-3} M; 0.15 M bicarbonate buffers, pH = pK_a of peroxy acid; T = 31°C.

[Peroxy acid] (M)	Δ [AES] (%)	E (%)	$k_{\text{decomp.}}$ 1 mol ⁻¹ s ⁻¹
Peroxyacetic,* 3×10^{-3}	—	—	2.4×10^{-3}
Peroxyacetic, 3×10^{-3}	41.6	111	2.5×10^{-3}
Chloroperoxyacetic, 8×10^{-3}	100	100	—
Chloroperoxybenzoic, 2×10^{-3}	10.6	42.5	—
Peroxypivalic, 2×10^{-3}	11.2	44.8	—
Peroxysulphuric, 2×10^{-3}	10.0	40.0	—

* In the absence of AES.

by AES as free singlet oxygen in the bulk of the solution.

Both conclusions are in perfect accordance with the proposed model. Peroxyacetic acid (and probably its chloro-substituted derivative) decomposes by oxygen-carbon attack to give an intermediate which probably has free radical character and breaks down to give ground-state oxygen. The remaining acids, known or expected to react primarily via oxygen-oxygen attack, give singlet oxygen. No active complex is formed with AES and therefore, the latter intercepts singlet oxygen in the bulk of the solution with an efficiency corresponding to free singlet oxygen.

3. Experimental

3.1. Preparation and purity of peroxy acids

3.1.1. Peroxyacetic acid

This compound was obtained commercially. Its purity was estimated iodometrically(104) to be 46% (w/v), with a H_2O_2 content of 11.4%. It was used with no further purification.

3.1.2. Chloroperoxyacetic acid

This compound was prepared by the method of Panizzans (147) as modified by Koubek and Edwards ((148) 86% H_2O_2 was used, and the resulting peroxy acid had a purity of 89% (w/v), with a 1.7% H_2O_2 content.

3.1.3. m-Chloroperoxybenzoic acid

Commerical peroxybenzoic acid (85% pure) was washed with phosphate buffer (pH 7.5), then with a little cold water and dried in vacuo. The final purity was 98% (w/w) with no evidence of H_2O_2 presence.

3.1.4. Peroxypivalic acid

The method of Koubek and Welsh (149) was used for this preparation. To an ice-cold mixture containing conc. H_2SO_4 (11.6 ml), pivalic acid (5.6 ml) and water (3.4 ml) was added dropwise 50% H_2O_2 (5.1 ml, 0.07 moles). The mixture was allowed to stand at room temperature for 1 hour and was then extracted twice with 50 ml CH_2Cl_2 . After removal of the solvent at reduced pressure the oily residue was dissolved in water (25 ml), re-extracted with CH_2Cl_2 and the solvent removed as before. The final product was H_2O_2 -free and had a purity of 92% (w/v).

3.1.5. Peroxysulphuric acid (150)

Potassium peroxydisulphate $K_2S_2O_8$ (45 g, 0.18 moles) was slowly added to conc. H_2SO_4 (45 ml) at $0^\circ C$. The mixture was stirred for 1 hour at $0^\circ C$, after which time 200 g of finely crushed ice was slowly added with vigorous stirring. The unreacted salt was filtered and the filtrate allowed to stand at room temperature for 24 hours. After being filtered again it was stored in a refrigerator.

Iodometric and volumetric analyses showed the final mixture to be 0.45 N in H_2SO_5 and 3.8 N in H_2SO_4 with no detectable H_2O_2 . This corresponds to a final purity of 28% (w/w).

3.1.6. Peroxymonophthalic acid

This compound was prepared as described by Swern (104). Briefly, phthalic anhydride was incubated for 2 hours at $30^\circ C$ with a four-fold excess of 50% H_2O_2 .

The product peroxy acid had a purity of 62%, the remaining material being phthalic acid. No H_2O_2 impurity was present.

Table 25 summarizes the purities of the various peroxy acids used.

Table 25: The purity of the peroxy acids

	% Peroxy acid	% H ₂ O ₂
Peroxyacetic	46 (w/v)	11.4
Chloroperoxyacetic	89 (w/v)	1.7
Peroxypivalic	92 (w/v)	—
m-Chloroperoxybenzoic	98 (w/w)	—
Peroxysulphuric	28 (w/v)	—
Peroxymonophthalic	62 (w/w)	—

3.2. Kinetic studies of the decomposition of peroxy acids

All peroxy acid decompositions were carried out in aqueous buffers (phosphate or bicarbonate) of pH numerically equal to the pK_a of the acid. All aqueous solutions were prepared with distilled water which had been passed through deionising resin. Particular care was taken to use carefully cleaned vessels because of the ease with which metal ions catalyse peroxy acid decomposition. The use of metallic spatulas was avoided, and small volumes were measured or handled by means of all-plastic micropipettes. Furthermore, in all experiments CDTA (10^{-3} M) was included to sequester any residual metal ions (137).

Experiments were carried out in vessels wrapped with aluminium foil, to minimise and effects of light. Sufficiently large volumes of reaction mixtures were prepared to allow spectrophotometric and iodometric measurements on a number of aliquots to be carried out.

In a typical experiment, a weighed quantity of AES was placed in a 50 ml volumetric flask and buffer was added up to the mark. After dissolution of the solid AES the flask was placed in a thermostatted water bath for 10 min, and a known amount of peroxy acid added with vigorous shaking. After replacing the flask in the water bath, aliquots were withdrawn at different times for analysis.

3.3. TLC and polarography with peroxy acid mixtures

TLC and polarographic analyses were carried out on reaction mixtures which had been allowed sufficient time for all of the peroxy acid to have decomposed. The analytical methods used are as described before (Chapter 2, Section 3).

CHAPTER 5

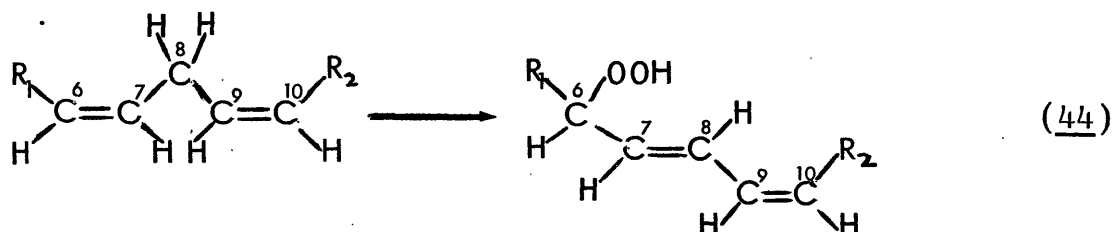
Singlet Oxygen Formation During Lipoxygenase Action

1. Introduction

1.1. The mechanism of action of lipoxygenase

Soybean lipoxygenase is an enzyme which catalyses the aerobic oxidation of unsaturated fatty acids to hydroperoxides (151). The enzyme shows a high degree of substrate stereospecificity, requiring that the substrate contain the cis,cis-1,4-pentadiene moiety between positions ω -6 and ω -10. The conjugated cis,trans-6-hydroperoxide is usually the major product (151, 152). The formation of an allylic hydroperoxide is reminiscent of the corresponding singlet oxygen reaction and has led to speculation about the possible involvement of singlet oxygen in the mechanism of action of lipoxygenase (rn. (44)).

The natural substrate of lipoxygenase is linoleic acid (9,12-octadecanoic acid) (or its esters) which is converted

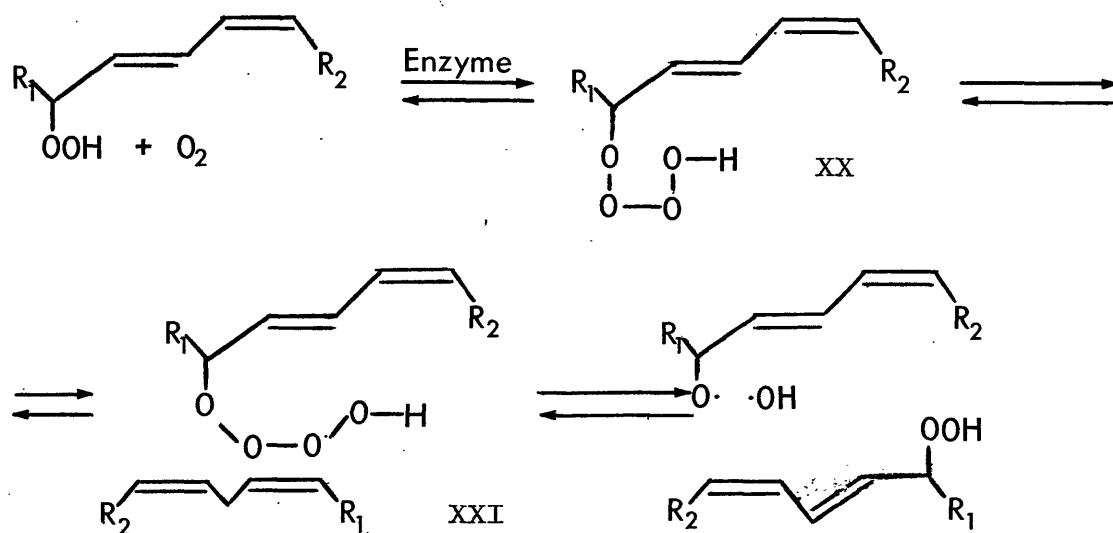


primarily to 13L-hydroperoxy-9(cis),11(trans)-octadecadienoic acid(153). Although the hydroperoxy-group is primarily introduced at the ω -6 position of substrates, varying amounts of products bearing the hydroperoxy function at the ω -10 position have been found, depending on the substrate and the precise origin of the enzyme.

The reactions of lipoxygenase are characterised by an initial lag period (increased by high initial concentrations of substrate and decreased or abolished by the addition of product hydroperoxide) and by inactivation of the enzyme during the reaction (154, 155).

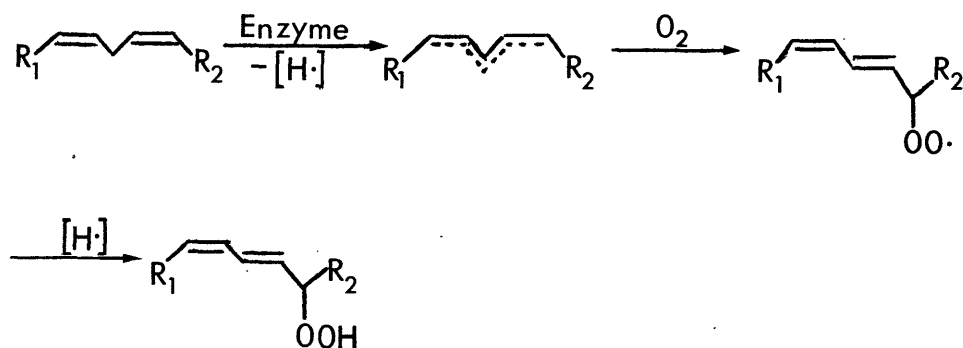
Smith and Lands(155) have suggested that a complex consisting of one molecule each of product, substrate and oxygen specifically bound to the enzyme is formed. This complex can then rearrange to give the products or an inactivated form of the enzyme. They propose that the bound product and oxygen molecules initially combine to form a non-planar

tetroxide intermediate XX which, under the influence of an enzyme-bound substrate molecule, takes up a strained configuration in which the four oxygen atoms lie in a plane XXI. This species, which may represent an intermediate or only a transition state, may be in equilibrium with $^1\text{O}_2$ resulting from the liberation of the two central oxygen atoms as excited molecular oxygen. Alternatively, species XXI may react with the substrate to form a perepoxide intermediate which rearranges to the final product. In either case, $\text{HO}\cdot$ radicals are formed which may lead to the deactivation of the enzyme (Scheme 15).



An alternative model (151, 156) proposes that a hydrogen atom is removed in a rate-determining step from the methylene group at the w-8 position of the substrate. The free radical thus formed adds stereospecifically a molecule of ground-state oxygen adsorbed at the enzyme active site to

give initially a hydroperoxy radical and then a hydroperoxide (Scheme 16).



Scheme 16

Nakano et al. (157) have demonstrated that self-reactions of linoleic acid peroxy radicals generate singlet oxygen, implying that any such species detected during lipoxygenase action is only a byproduct and is not involved in the enzyme's catalytic action.

1.2. The search for singlet oxygen in lipoxygenase-catalysed reactions

In view of the similarity of the reactions catalysed by lipoxygenase to those brought about by singlet oxygen, a number of investigations have searched for evidence that the latter species may be formed during the action of lipoxygenase.

Chan has incubated ethyl linoleate and soybean lipooxygenase in the presence of tetraphenylcyclopentadiene or (separately) diphenylisobenzofuran (158). From the reaction mixtures he was able to isolate the endoperoxide of the first compound or 1,2-dibenzoylbenzene, the final product of the second compound's reaction with singlet oxygen. He concluded from these results that singlet oxygen, or a closely related analog is generated, although he was unable to suggest whether such an intermediate may play any direct role in the overall reaction.

Faria-Oliveira et al. (159) have examined the effect of D_2O on the rate of bleaching of cytochrome c in the linoleate-lipoxygenase system. They found the initial rate in D_2O 4-5 times faster than in H_2O , although the rates of hydroperoxide formation are the same. They concluded that the species responsible for the bleaching is singlet oxygen, although this could not be the cause of the enzyme's inactivation.

Since it seems fairly well established that singlet oxygen, or a similarly-reacting species, is produced during lipooxygenase action, it was decided to test the activity of AES in this system. Previously, AES has been used in simple chemical systems producing large amounts of singlet oxygen (photosensitized oxygenation and the $H_2O_2/NaOCl$ system). It seemed desirable to use it also under the

conditions of a biochemical system.

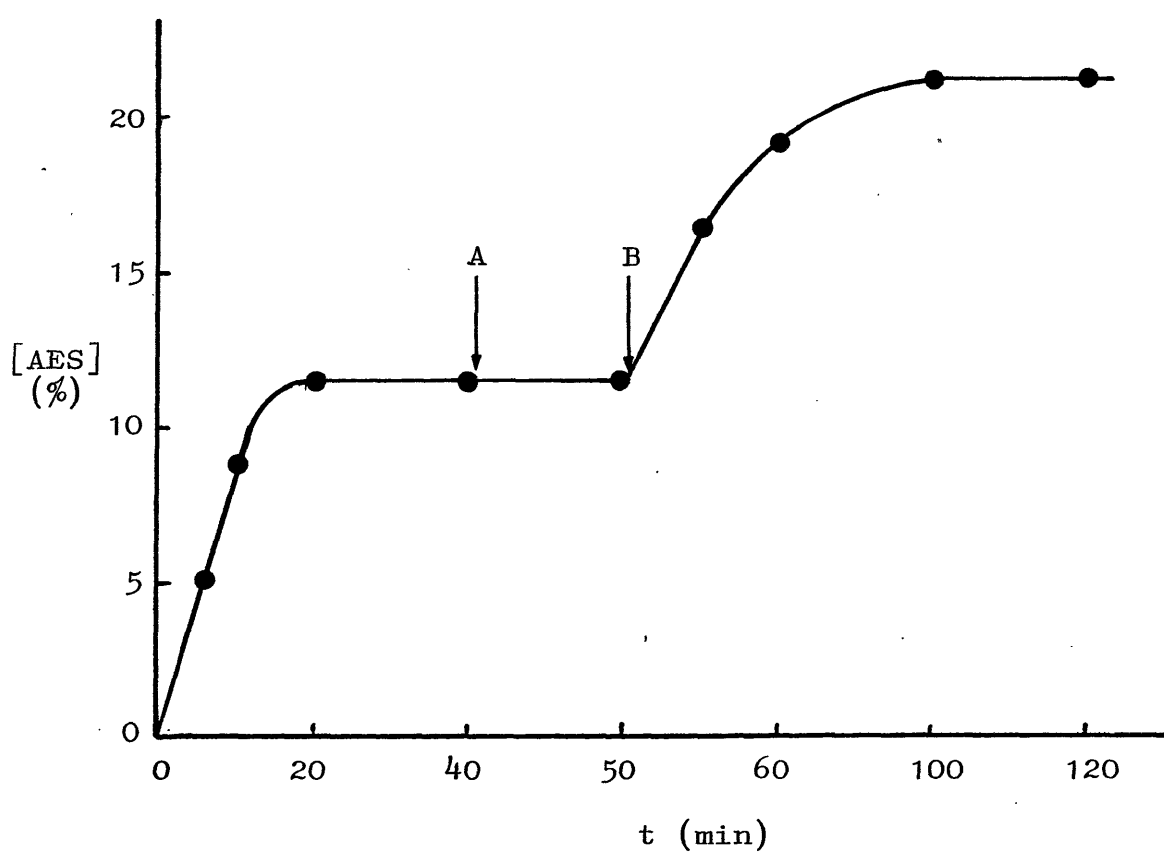
2. Results and Discussion

Lipoxygenase is known to show a lag period in its reaction with linoleic acid, which is completely abolished in the presence of product hydroperoxide(155). However, the lag period is shortened (in the absence of product) if the enzyme concentration is increased, so that with $21 \mu\text{g ml}^{-1}$ the lag time is 0.16 min.

In the experiments whose results are shown in Fig. 16, the initial enzyme concentration was $100 \mu\text{g ml}^{-1}$ and therefore, as expected, no lag period was observed, and maximal reaction rate was reached practically from the beginning of the reaction. This indicates that sufficient product formed rapidly to completely abolish the lag period.

TLC analysis of a linoleic acid/lipoxygenase/AES reaction mixture showed the presence of two products arising from AES (control TLC analyses were carried out with mixtures obtained in the absence of AES). One of these products, subjectively estimated as 5-10% of the total, corresponded to the 9,10-endoperoxide as judged by the usual TLC tests (Chapter 2, Section 3). The major product, which with aq. BaCl_2 as the TLC solvent had a smaller R_f than the endoperoxide, was

Fig. 16: The consumption of AES during lipoxygenase oxidation of linoleic acid



$[AES]_0 = 4 \times 10^{-3} \text{ M}$; $[\text{linoleic acid}]_0 = 1.3 \times 10^{-3} \text{ M}$;
 $[\text{Enzyme}]_0 = 100 \mu\text{g ml}^{-1}$; 0.1 M Tris-HCl buffer,
 pH 9.0; $T = 30^\circ\text{C}$

A: Addition of fresh linoleate ($8.7 \times 10^{-4} \text{ M}$)

B: Addition of fresh enzyme ($100 \mu\text{g ml}^{-1}$)

clearly not of peroxidic nature as judged by the $\text{Fe}^{2+}/\text{SCN}^-$ system.

Fig.16 also shows that the enzyme undergoes inactivation during the reaction, causing the reaction to stop while unconsumed substrate is still available. The inactivation which accompanies lipxygenase action has been shown by Smith and Lands(146) to be such that the total amount of substrate consumed by the time the enzyme is inactivated is independent of the initial substrate concentration. Table 26 shows the results obtained when different initial concentrations of linoleic acid are incubated with fixed concentrations of enzyme and AES. It can be seen that increasing the linoleic acid concentrations decreases the extent of reaction of AES. Since the overall amount of reaction (linoleic acid oxidation) is constant*, it appears that linoleic acid may be inhibiting AES reaction by competing with it for a reactive intermediate. It is not possible on the basis of these results to gauge the reactive intermediate(s) involved.

The effect of D_2O on the extent of AES consumption was examined in an attempt to elucidate the question of the quantitative importance of the singlet oxygen reaction. A similar

* It was not possible to measure spectrophotometrically the extent of linoleic oxidation in the presence of AES.

Table 26: Linoleic acid oxidation in the presence of AES

$[\text{AES}]_0 = 4 \times 10^{-3} \text{ M}$; $[\text{Enzyme}]_0 = 100 \mu\text{g ml}^{-1}$; $t = 60 \text{ min}$;
 0.1 M Tris-HCl buffer, pH 9.0; $T = 30^\circ\text{C}$.

[Linoleate] (M)	[AES] (%)
0.008	17.0
0.016	12.7

attempt was made using the quencher Niphalds. The results, shown in Table 27, indicate that the effects of both, D_2O and Niphalds, on the extent of reaction of AES are smaller than would be expected if all AES consumption were due to free singlet oxygen. Estimates of the proportion of AES consumption which may be due to free singlet oxygen are 21% (from the D_2O results) and 61% (from the Niphalds results). The variation between these results is evidently quite large and consequently they must be considered only as indicative.

On the basis of the results presented above it can be concluded that singlet oxygen is generated during lipoxygenase action, together with other reactive intermediates (probably radicals), and it probably causes 20-60% of the total consumption of AES observed. Both AES and linoleic acid appear able to react with one or both of the above species. This

Table 27: D₂O and Niphalds effects on AES consumption

[AES]₀ = 2×10^{-3} M; [Linoleate]₀ = 1.6×10^{-3} M; [Enzyme]₀ = $2 \mu\text{g ml}^{-1}$; t = 90 min; 0.1 M Tris-HCl buffer, pH 9.0; T = 30°C.

Medium	[Niphalds] (M)	$\Delta[\text{AES}]$ (%)	$\Delta[\text{AES}]_{\text{calc}}^*$ (%)
H ₂ O	—	14.7	—
D ₂ O	—	19.4	37.5
H ₂ O	8×10^{-4}	9.0	5.1

* Calculated on the assumption that all AES consumption is due to reaction with free singlet oxygen.

leaves open the possibility that even if singlet oxygen is not on the main pathway of the enzyme's action, part of the hydroperoxide formation observed may still be due to its reaction with linoleic acid.

3. Experimental

Soybean lipoxygenase, Type I, was obtained from Sigma as a lyophilised powder of activity 152,000 units per mg protein. It was stored at -20°C and, when needed, it was dissolved in

buffer just before use.

Linoleic acid (99% pure) was also obtained from Sigma as the sodium salt. It too was stored at -20°C until used.

All experiments were carried out in 0.1 M Tris-HCl buffer, pH 9.0 at 30°C . Fresh enzyme and substrate solutions were prepared daily and kept on ice while not in use. Reactions were initiated by the addition of enzyme to appropriate mixtures and the latter were exposed to air during the reaction.

Spectrophotometric measurements and TLC analyses were carried out by the standard methods (Chapter 2, Section 3.).

CHAPTER 6

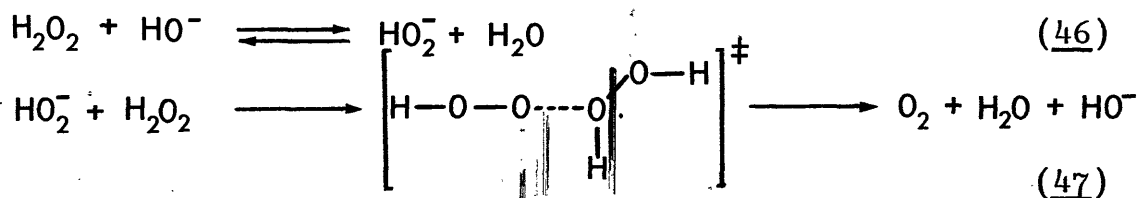
Singlet Oxygen Generation From H_2O_2

1. Introduction

That singlet oxygen may be produced during some reaction of H_2O_2 has been known for many years (15, 160). Many reactions of H_2O_2 in which molecular oxygen is produced are accompanied by a chemiluminescence which has been attributed to the decay of $^1\text{O}_2$ produced during the reaction (160–162). The first conclusive demonstration of the formation of singlet oxygen during a chemical reaction involved the reaction of hydrogen peroxide with alkaline hypochlorite (15). Since then a number of systems involving hydrogen peroxide have been shown (more or less conclusively) to produce singlet oxygen, while there is speculation about its possible formation in others. Some of the latter systems are discussed briefly in the next sections.

1.1. The disproportionation of H_2O_2

The base-catalysed disproportionation of H_2O_2 (rn. (45)) has been shown (163) to follow kinetics analogous to those of the decomposition of peroxy acids and is believed to involve a nucleophilic attack of an ionised molecule of hydrogen peroxide on a non-ionised molecule.



The electronic state in which the molecular oxygen is generated is not known with any certainty. Evidence that it is in the singlet state has been furnished by Smith and Kulig (164) who examined the oxidation of cholesterol by H_2O_2 in aqueous solutions (pH 8.5-9.5) at 25-60°C. Among the products which they isolated were some which, they suggest could only be accounted for by the intermediacy of singlet oxygen and they estimated that the ratio

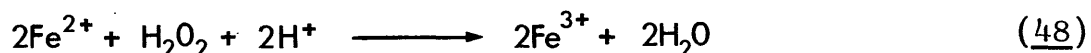
of singlet to triplet oxygen formed during the decomposition of hydrogen peroxide is not less than 1:3.

1.2. The $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ system

The ability of Fe^{2+} to catalytically decompose H_2O_2 has been known for a long time (165-168). The pathway of the decomposition depends on the conditions of the reaction: If Fe^{2+} is added to a solution of H_2O_2 so that the latter is always in excess, the H_2O_2 is catalytically decomposed to molecular oxygen and water.



If, on the other hand, the Fe^{2+} is in excess, the net reaction is the oxidation of Fe^{2+} to Fe^{3+} and the reduction of the peroxide to water.



In practice both reactions occur to different extents

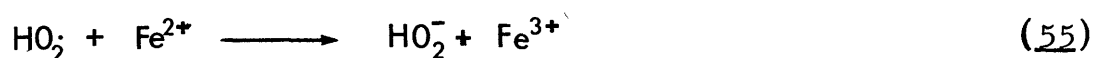
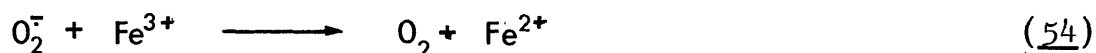
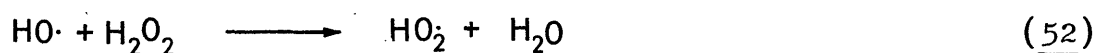
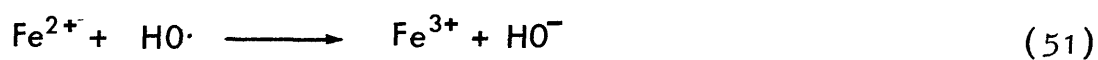
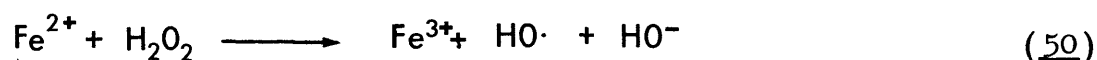
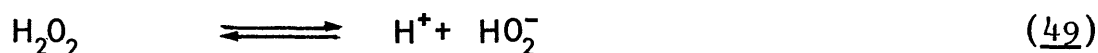
under most conditions.

Mixtures of Fe^{2+} and H_2O_2 (particularly with the former in excess) have the ability to readily oxidise many organic compounds. This property was first noted by Fenton in 1894, when he discovered (165) that such mixtures oxidised effectively organic hydroxylic compounds. The Fenton reagent, as the mixture has come to be called, has since been used widely for oxidising compounds to products that resemble those of the corresponding biological oxidations (169-170).

The mechanism of action of the Fenton reagent has been extensively investigated (170-174). Most studies point strongly to the formation of hydroxyl radicals as reactive intermediates. The detailed mechanism, to the extent to which it has been established, is as follows:

The reaction is a chain reaction, with the reduction of the anion of H_2O_2 as the initiating step (rn. (49)).

The hydroxyl radicals produced propagate the reaction by attacking a second molecule of H_2O_2 , resulting in the production of molecular oxygen (rns. (53) and (54)). Reaction (55) is a terminating step. In the presence of excess Fe^{2+} (as in the Fenton system), the $\text{HO}\cdot$ radicals may react with Fe^{2+} rather than H_2O_2 (rn. (51)), effectively terminating the sequence there.

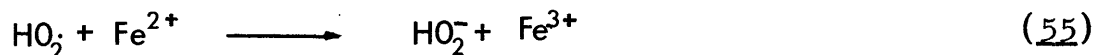
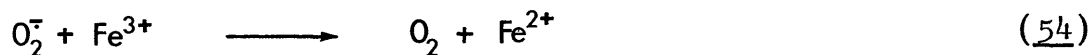
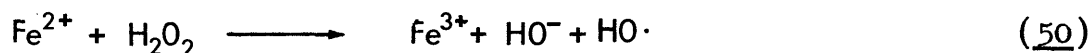
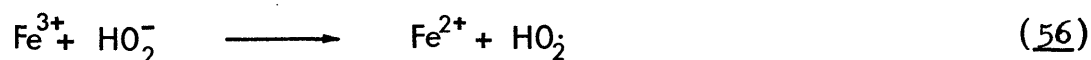
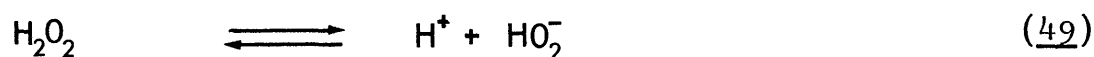


The hydroxyl radicals produced may be intercepted by organic species present, bringing about the latter's oxidation.

1.3. The $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system

Fe^{3+} decomposes H_2O_2 (more slowly than Fe^{2+}) to molecular oxygen and water (168, 173). Furthermore, mixtures of Fe^{3+} and H_2O_2 oxidise organic compounds and bring about the polymerisation of substituted ethylenes, albeit more slowly than the Fenton reagent. These observations,

together with kinetic evidence, indicate that the same intermediates are involved in the $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system as in the Fenton reagent. The main steps of its mechanism appear to be the following:



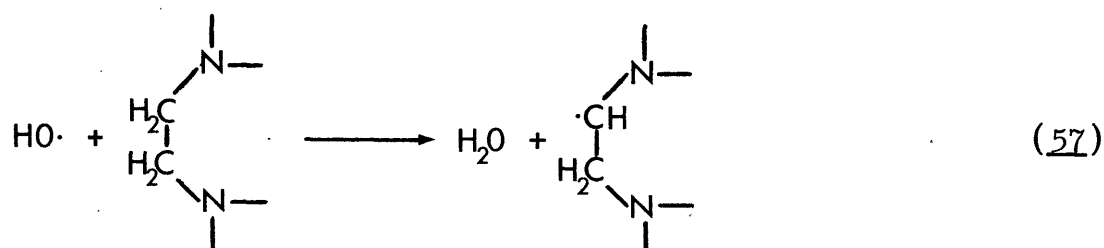
It can be seen that following the initial oxidation of H_2O_2 by Fe^{3+} (rns. (49) and (56)), with the formation of Fe^{2+} , all the subsequent steps are the same as those found in the Fenton system. However, as Fe^{2+} is present

as an intermediate (hence in low transient concentrations relative to H_2O_2), the hydroxyl radicals react with H_2O_2 rather than Fe^{2+} , i.e. reaction (52) will predominate over reaction (51). The result of this is that the net reaction is the decomposition of H_2O_2 to water and molecular oxygen, as observed with the $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ system under conditions of low $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ratio. This decomposition is inhibited by oxidisable compounds which intercept the hydroxyl radicals. Thus, as in the case of the Fenton system, chemical and kinetic evidence points to a mechanism in which hydroxyl radicals are formed as reactive intermediates.

1.4. The $\text{Fe}^{3+}/\text{EDTA}/\text{H}_2\text{O}_2$ system

This system has been studied by Walling et al. (175, 176) who found that it behaves in a similar manner to the $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system, showing catalytic decomposition of H_2O_2 to molecular oxygen and oxidation of added organic molecules. One added feature of this system arises from the fact that the hydroxyl radicals produced can be intercepted by EDTA, free or complexed (as well as by H_2O_2 and Fe^{2+}), i.e. reactions (51) and (52) may become of secondary importance relative to (57).

The EDTA-derived radical intermediate may be oxidised by

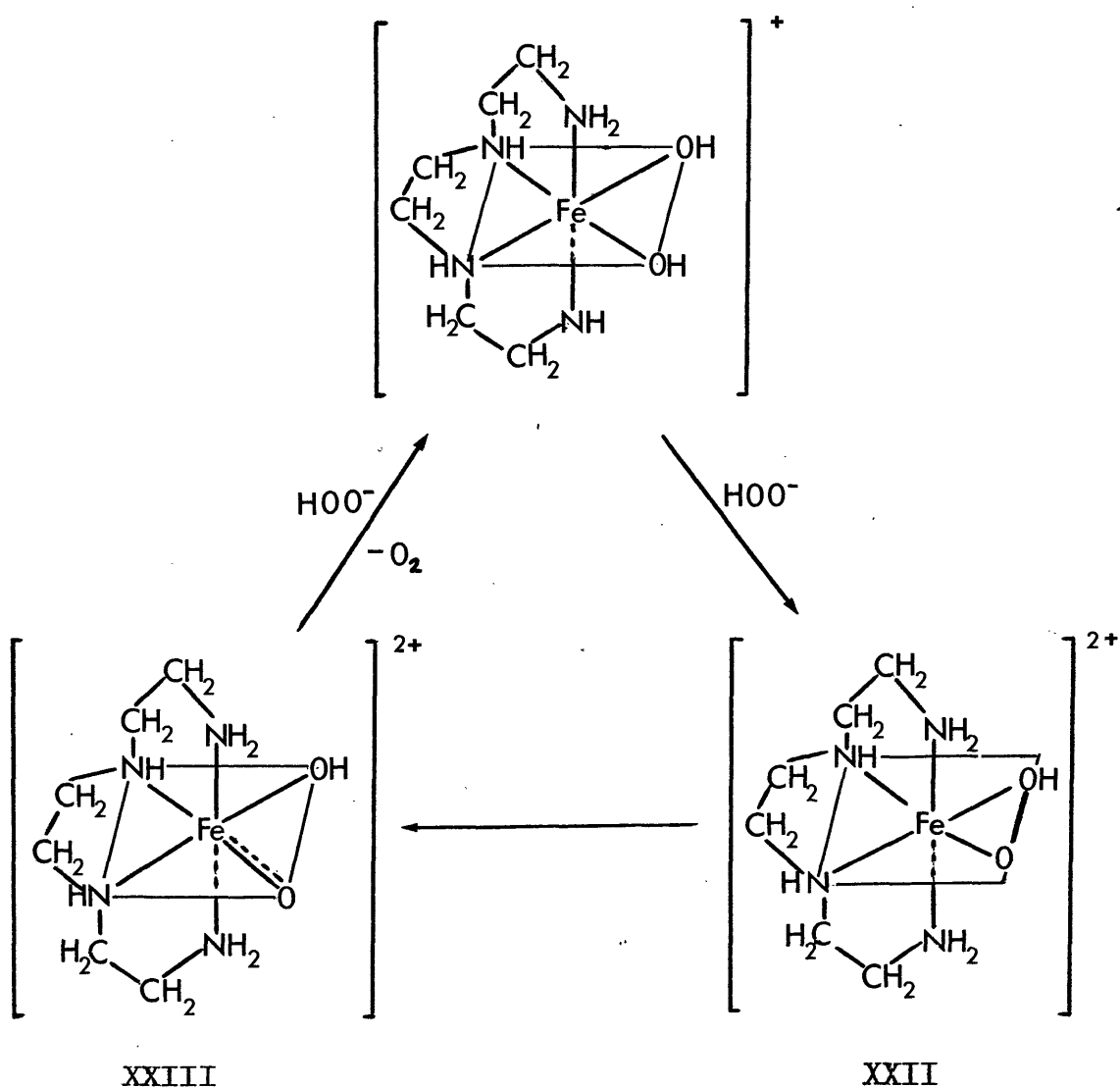


molecular oxygen (resulting in chain termination) or by Fe^{3+} (resulting in chain propagation through the formation of Fe^{2+}). While reaction (57) predominates, steps (51) and (52) are suppressed, with the result that oxygen evolution is inhibited.

1.5. The $\text{H}_2\text{O}_2/\text{Fe}^{3+}/\text{TETA}$ system

Other Fe^{3+} chelates have been studied for their ability to catalyse H_2O_2 decomposition, and they appear to act by the same mechanism as the system just described (177). However, there is an additional mechanism which predominates in the case of the complex of Fe^{3+} with triethylene tetramine (TETA), $\text{H}_2\text{NCH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2$. The complex of TETA with Fe^{3+} in aqueous solution, $(\text{TETA})\text{Fe}(\text{OH})_2^+$, has been shown by Wang (169) to be an extremely efficient catalyst of the decomposition of H_2O_2 , and it has been proposed as a model for the enzymatic action of catalase.

Detailed kinetic and other studies have shown that the mechanism of action of this complex is distinctly different from that of the $\text{Fe}^{3+}/\text{EDTA}$ complex, in that no free radical intermediates are involved. The mechanism is shown diagrammatically in Scheme 17 (taken from ref. 178) which illustrates the importance of steric factors in the activity of the complex.



Scheme 17

After one of the hydroxyl ligands in the complex is replaced by a hydroperoxy anion, the second hydroxyl is expelled by the second oxygen of the hydroperoxy ligand in an intramolecular displacement step. Because of the precise steric arrangement of the Fe^{3+} octahedral orbitals, the O-O bond in XXII is stretched and partially broken while at the same time the overlap of the electrons of the two oxygens increased. This increased interaction partially compensates for the activation energy for the splitting of the O-O bond with the result that intermediate XXIII is formed rapidly. This complex, bearing an electron-deficient oxygen atom, rapidly reacts with a second hydroperoxide ion to oxidise it to molecular oxygen.

Other chelating ligands, bearing a greater or a smaller number of basic groups, are practically ineffective in bringing about catalysis by this mechanism, because of the changed degree of occupancy of Fe^{3+} orbitals and/or the reduced stability of the corresponding complexes.

1.6. The catalase/ H_2O_2 system

Catalase ($\text{H}_2\text{O}_2:\text{H}_2\text{O}_2$ oxidoreductase, EC 1.11.1.6) is a metalloenzyme which specifically and with great efficiency catalyses the decomposition of H_2O_2 to water and molecular oxygen (179). There have been some suggestions that singlet

oxygen may be formed during the action of catalase, based mainly on the finding by Anbar (180) that N_2O can be produced if the reaction is carried out in the presence of N_2 . Singlet oxygen has also been implicated in the weak chemiluminescence detected during the catalase reaction.

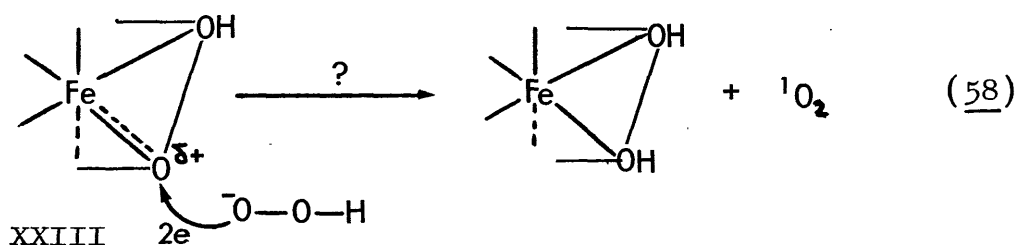
These suggestions have been disputed by Porter and Ingraham who were unable to trap singlet oxygen using 2,5-diphenylisobenzofuran during the catalase-catalysed decomposition of H_2O_2 (181) they concluded that singlet oxygen cannot constitute more than 0.5% of the oxygen released into the solution during catalase action and suggested that if more is formed, it must be rapidly quenched by the enzyme.

1.7. The possibility of singlet oxygen production from H_2O_2 systems

Mixtures of Fe^{3+} or Fe^{2+} and H_2O_2 (e.g. the Fenton system) are widely used as sources of hydroxyl radicals. Because, however, molecular oxygen is, or can be, produced in all of them, the question arises whether this is ever produced in the excited singlet state.

In all the systems discussed in the previous paragraphs, with the exception of the $H_2O_2/Fe^{3+}/TETA$ system, molecular

oxygen ultimately arises as a result of the one-electron oxidation of the superoxide radical anion by Fe^{3+} . As pointed out by McKeown and Waters (102), one-electron oxidations of hydrogen peroxide appear to result in triplet molecular oxygen, and one might expect the same thing to occur here. On the other hand, the catalysis of H_2O_2 decomposition by $(\text{TETA})\text{Fe}(\text{OH})_2^+$ is postulated to be mediated by a two-electron oxidation of the hydroperoxide anion (rn. (58)), a reaction which has been said to result in the production of singlet molecular oxygen. Although the details of the oxidation of the hydroperoxide ion by intermediate (XIII) (Scheme 17) are not known, the possibility of a direct two-electron oxidation with accompanying singlet oxygen formation cannot be excluded.



Finally, the mechanism of action of catalase may involve either one- or two-electron oxidation. In the latter case singlet oxygen may well be initially produced (at the active site), and some of it may escape any quenching mechanisms of the enzyme and diffuse into the solution.

Because of the importance of catalase in biological systems, the possibility of the release of such a potentially damaging species is one that needs to be examined carefully.

It has been shown in the preceding Chapters that AES is a water-soluble singlet oxygen substrate that does not suffer from many of the disadvantages of other substrates used in such cases. For this reason it was decided to employ it in a series of brief experiments to search for any tentative, qualitative evidence of singlet oxygen formation during H_2O_2 decomposition. The systems examined were of increasing complexity, starting from H_2O_2 disproportionation, via decomposition catalysed by Fe^{2+} , Fe^{3+} , $\text{Fe}^{3+}/\text{EDTA}$ and $\text{Fe}^{3+}/\text{TETA}$, finally reaching the enzymatic action of catalase.

2. Results and Discussion

In what follows a presentation of the results obtained with each system is initially given, followed by a general discussion and assessment of their implications.

2.1. The disproportion of H_2O_2

The disproportion of H_2O_2 was briefly examined by incubating H_2O_2 (6×10^{-3} M) with AES (4×10^{-3} M) in aqueous bicarbonate

buffer (0.15 M, pH 9.7) at 37°C for 48 h.

Under these conditions 53% of the H_2O_2 and 10% of the AES were consumed. TLC analysis showed the presence of two products, none of which corresponded to the 9,10-endoperoxide of AES. One of the products (the minor one) had a strong yellow colour, while the other one was similar to one of the products subsequently obtained during the decomposition of H_2O_2 in the presence of Fe^{2+} or Fe^{3+} (described in subsequent sections). This implies that some metal-catalysed decomposition may have occurred.

2.2. The $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ system

In this system, H_2O_2 was slowly added in a small volume to a stirred solution of Fe^{2+} and AES in 0.1 N H_2SO_4 .

The concentrations of the reactants were as follows:

$$[\text{Fe}^{2+}] = 0.04 \text{ M}; \quad [\text{H}_2\text{O}_2] = 0.04 \text{ M}; \quad [\text{AES}] = 0.004 \text{ M}.$$

Effervescence was observed during the addition of H_2O_2 , and most of the latter had been consumed by the end of the addition. AES consumption was 54%.

TLC analysis showed the presence of two products, none of which corresponded to the endoperoxide. One of these products was very similar to the main product obtained from the uncatalysed decomposition of H_2O_2 (Section 2.1.).

2.3. The $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system

In this system the H_2O_2 needs to be presented in an excess of 30 or more over the Fe^{3+} ions. Therefore, an Fe^{3+} solution was added to an aqueous mixture of H_2O_2 and AES so that the nominal initial concentrations were as follows: $[\text{Fe}^{3+}] = 1.3 \times 10^{-4} \text{ M}$; $[\text{H}_2\text{O}_2] = 4 \times 10^{-3} \text{ M}$; $[\text{AES}] = 4 \times 10^{-3} \text{ M}$.

After 22 hours all the H_2O_2 had been consumed while the consumption of AES was 66%. When the initial concentration of H_2O_2 was doubled (while keeping the $[\text{H}_2\text{O}_2]/\text{Fe}^{3+}$ ratio constant), all the AES was consumed, as could have been expected on the basis of the first result. When the initial concentration of AES was also doubled (to $8 \times 10^{-3} \text{ M}$), its final consumption was still 100%. These consumption figures (corresponding to efficiencies of 100% or more in terms of singlet oxygen production and trapping) indicate that a reactive species other than singlet oxygen was being trapped.

TLC analysis showed the formation of two products exactly analogous to those found with the $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ system.

2.4. The $\text{Fe}^{3+}/\text{EDTA}/\text{H}_2\text{O}_2$ system

In this system, H_2O_2 was added to a solution containing

Fe^{3+} , EDTA and AES in 0.15 M bicarbonate buffer, pH 9.0.

Two concentration ranges were examined. In the first, the following initial concentrations were used:

$[\text{Fe}^{3+}] = 4.4 \times 10^{-4} \text{ M}$; $[\text{EDTA}] = 8.8 \times 10^{-4} \text{ M}$; $[\text{H}_2\text{O}_2] = 8.8 \times 10^{-3} \text{ M}$.

The initial yellow colour of the solution changed to purple on addition of the H_2O_2 owing to the formation of an $\text{Fe}^{3+}/\text{EDTA}/\text{H}_2\text{O}_2$ complex (176). After 17 hours, when all the AES and H_2O_2 had been consumed, TLC analysis of the reaction mixture again showed two products analogous to those found with Fe^{2+} and Fe^{3+} described in the preceding two subsections.

In a second experiment the concentration of H_2O_2 was tenfold greater than before, i.e. $[\text{H}_2\text{O}_2]_0 = 8.8 \times 10^{-3} \text{ M}$. Again, after 17 hours complete H_2O_2 consumption was observed and the same products were detected by TLC.

2.5. The $\text{Fe}^{3+}/\text{TETA}/\text{H}_2\text{O}_2$ system

In this system the proportions of Fe^{3+} and TETA were as used by Wang who discovered the complex's catalytic activity (178). The concentrations used were as follows:

$[\text{Fe}^{3+}] = 2.0 \times 10^{-6} \text{ M}$; $[\text{TETA}] = 6.0 \times 10^{-3} \text{ M}$; $[\text{H}_2\text{O}_2] =$

8.0×10^{-2} M; [AES] = 4×10^{-3} M. The medium was bicarbonate buffer, 0.15 M and pH 10.0

Some effervescence was observed and after 60 min, when all the H_2O_2 had decomposed, there had still been no decrease in the absorbance of AES, hence no reaction. It can be concluded therefore that no singlet oxygen was generated.

2.6. The catalase/ H_2O_2 system

In the study of this system, H_2O_2 (6×10^{-2} M) was mixed with AES (4×10^{-3} M) in 0.1 M phosphate buffer, and 200 units of enzyme were added. Effervescence was observed and after 1 h all the H_2O_2 had decomposed, while no decrease in the AES concentration could be detected spectrophotometrically.

2.7. General discussion

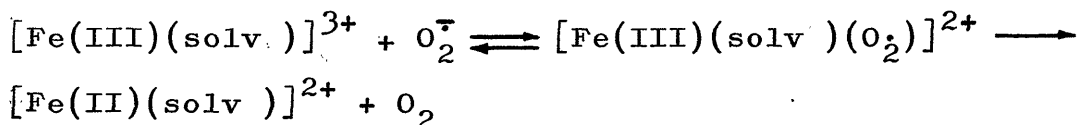
The uncatalysed decomposition of H_2O_2 has been kinetically described by Duke and Haas (163) and has also been claimed to have been detected by Smith and Kulig (164). However, Edwards and his group (138) have presented evidence to suggest that the truly uncatalysed (by metals) reaction is so extremely slow that it has probably never been observed. The findings described above do indeed suggest that some metal catalysis may still take place even in the presence of EDTA and with all the precautions taken to avoid the presence of uncomplexed metals.

The question of singlet oxygen production from the decomposition of H_2O_2 or from reactions of species related to it (e.g. the superoxide anion) has been the subject of a lively debate particularly amongst people interested in the mechanisms of biochemical oxidations (164, 182 - 185). Theoretical considerations shows that singlet or triplet oxygen could be generated from the oxidation of superoxide depending on whether an electron is removed from its half-filled or its filled π_g^*2p orbitals. Apart from questions relating to orbital symmetries, thermodynamic and kinetic factors determine which course the reaction may take.

One of the problems encountered during attempts to detect singlet oxygen in such systems by trapping is that the trap itself may react with superoxide or a related species to give the characteristic "singlet oxygen product". The trap used in the present study (AES) has the advantage that, like other anthracene derivatives, it is probably stable towards such species (186).

The oxidation of a superoxide radical anion $\text{O}_2^{\cdot-}$, although apparently very slow with H_2O_2 , is expected (185) and indeed has been shown to be faster with Fe^{3+} (183). Both Fe (aq)^{3+} and Fe (EDTA)^{3+} have been shown to bring

about this reaction under physiological conditions (184). The superoxide anion is formed, and its oxidation by Fe^{3+} species to molecular oxygen takes place, in three of the systems examined: the $\text{Fe}^{2+}/\text{H}_2\text{O}_2$, the $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ and the $\text{Fe}^{3+}/\text{EDTA}/\text{H}_2\text{O}_2$ (Section 1). That no singlet oxygen was detected in any of them may indicate that electron transfer from O_2^- to Fe(III) takes place from the filled π_g^{*2p} orbital, perhaps via an intermediate complex involving bonding with the metal via the two electrons of the filled orbital.



Thus molecular oxygen is released with two half-filled orbitals, i.e. in the triplet state.

In the case of the $\text{Fe}^{3+}/\text{TETA}$ complex, it was speculated in Section 1 that a final two-electron transfer may be occurring (Scheme 17, Section 1) which might have given rise to singlet oxygen. However, the failure to detect the latter species argues against this suggestion. An alternative mechanism might be one involving step-wise one-electron reduction of XXIII. In this case triplet oxygen formation would be expected.

The above arguments may have some bearing on the mechanism of action of catalase, since the $\text{Fe}^{3+}/\text{TETA}$ system has been suggested as a model for catalase. Porter and

Ingraham (181) have suggested that the absence of singlet oxygen may indicate quenching by the enzyme or it may be due to mechanistic factors. Since simple complexes of Fe(III) are not known to be quenchers of singlet oxygen (121), it is unlikely that Fe(III)(TETA)(H₂O)₂ is one, hence it is likely that here (and in the case of catalase) electronic and mechanistic factors operate to produce triplet oxygen.

3. Experimental

H₂O₂ (Aristar, BDH) was distilled in order to get rid of the added stabiliser. It was stored in a plastic bottle in the refrigerator.

Metal salts were added as the sulphate for Fe²⁺ and the nitrate for Fe³⁺.

Catalase (from bovine liver) was obtained from BDH as an aqueous solution and had an activity of 2×10^5 units ml⁻¹. All reactions were carried out in plastic containers at 25°C unless otherwise indicated. The precautions outlined in Chapter 2 regarding the handling of H₂O₂ were also observed in the series of experiments described in this Chapter.

Generally reaction mixtures were prepared containing all the reactants (including AES) but without H_2O_2 which was added last to initiate the reaction. Aliquots of the reaction mixtures were withdrawn at appropriate intervals and analysed spectrophotometrically (for AES) or iodometrically (for H_2O_2).

TLC was carried out as described in Chapter 2, Section 3.

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