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PHOTOCHEMICAL ASPECTS OF THIOPHENE
AND POLYACETYLENE PHOTOTOXICITY

by

CHRISTOPHER EVANS, B.Sc.

A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfillment of the requirements
for the degree of
Master of Science

Department of Chemistry

Carleton University

Ottawa, Ontario

September 2, 1995

The undersigned recommend to the Faculty of Graduate Studies
and Research acceptance of the thesis

PHOTOCHEMICAL ASPECTS OF THIOPHENE
AND POLYACETYLENE PHOTOTOXICITY

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September 30, 1985

Abstract.

An examination of excited state properties of the thiophene derivative α -terthienyl (α -T) and the polyacetylene phenylheptatriyne (PHT), both phototoxins, was undertaken to gain insight into their mechanisms of action *in vivo* (photooxidative vs. non-oxidative). Several derivatives of each parent compound were also investigated. Laser excitation (337.1nm or 308nm) yielded intense triplet signals with modest quantum yields in each case. Triplet lifetimes were between 3 and 30ns in methanol. Energy transfer quenchers induced triplet decay rates between 10^8 and $10^{10} \text{ M}^{-1}\text{s}^{-1}$, giving approximate triplet energies of 42-47 kcal/mol for the α -T series and 58-63 kcal/mol for PHT and the thiophene DTR. Triplet oxygen quenching rates were on the order of 1/9 the diffusion controlled rate suggesting that this deactivation process occurs via energy transfer leading to singlet oxygen formation. This is supported by the observed bleaching of 1,3-diphenylisobenzofuran (DPBF) in each case.

The lowest excited singlet states of the thiophenes were found to be too short-lived to contribute to the singlet oxygen quantum yields of these compounds.

Electron transfer from the triplets to methyl viologen occurs at a rate sufficient to compete with oxygen quenching. The reverse process, transfer of an electron from methyl viologen back to the semi-oxidized sensitizer, was quantitative for the thiophenes but reached only 50% completion in acetylenes. This

suggests that acetylene radical cations decay by modes other than recombination with the methyl viologen radical cation. None of the triplet states examined were good electron acceptors.

Laser excitation of the iodinated thiophene, DIT, resulted in cleavage of at least one of the C-I bonds. This is the first report of such behaviour for thiophenic systems.

The magnetic circular dichroism (MCD) spectrum of α -T indicates that the strong absorption band at 350nm can be assigned as a singlet-singlet transition. The absorption band of PHT centered at 310nm exhibits a vibronic sequence of MCD A terms suggesting that this transition has singlet-triplet character. This result is unusual because spin-orbit coupling in saturated carbon compounds is small. In delocalized unsaturated systems this coupling can be larger and significantly enhance singlet-triplet intensities.

The results of this study suggest that α -T is a purely photodynamic (type II, or singlet oxygen, mechanism) photosensitizer while PHT may function as either a type II or a type I (non-oxidative, or radical, mechanism) sensitizer depending on reaction conditions. Related thiophenes and acetylenes seem to exhibit behaviour similar to that of these model compounds. These conclusions agree with the behaviour of these compounds reported in vivo and with the known behaviour of their excited states.

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1. Introduction.

1. Introduction to the problem.

1.1 General.

One of the major processes in photochemistry is that known as photosensitization [1]. In a broad sense this may be defined as the process by which light energy absorbed by one component of a system causes another component of the system to undergo chemical reaction [2].

Typical photosensitizing molecules are organic compounds such as aromatic hydrocarbons [1]. They are characterized by a capacity for allowing the initial excitation energy to be transferred from the excited singlet state (usually S_1) to the (generally) lowest triplet state, T_1 . The following scheme shows a typical photosensitization occurring via the triplet state [3].

where S is the sensitizer, A the substrate (the "other component" of the system), X is a third system component, SA is some

chemical species formed by direct interaction of the sensitizer and substrate, P and Q are the final product(s) formed and * represents an electronic excited state.

Once populated the vast majority of organic triplets are quite long-lived (100-100000ns) [4] because of the forbidden nature of the $T_1 \rightarrow S_0$ transition. As a result, these highly energetic states play an important role in a wide variety of photochemical [1] and photobiological [5] reactions.

Biologically relevant photosensitizers fall into two categories: photodynamic and non-photodynamic [6]. The former behaviour is characterized by requiring the presence of molecular oxygen. Non-photodynamic, or non-photooxidative, sensitizers do not require oxygen to exhibit photoreactivity in biological systems.

One important subfield of photosensitization, as it relates to biology, is that of photosensitized oxidations (or photooxidations), also known as photodynamic action. The usually detrimental effects of these reactions have been implicated in many living systems [5], particularly in animal and human medicine [7]. They are also relevant to chemiluminescent organic reactions [8].

Photooxidations are fundamentally dependent on the excited state properties of the sensitizing molecule as well as on the nature of the substrate. It is the nature of the excited states involved in the photooxidative behaviour of certain phototoxic plant metabolites, known as thiophenes and polyacetylenes (see

Table 1.), that is the main subject of this thesis.

Since the characteristics of the molecular triplet states are of the utmost importance in such a study, two powerful techniques have been employed to examine them. These are magnetic circular dichroism (MCD) and laser flash photolysis (LFP). The former is especially useful in assigning transitions [9] while the latter is best suited for studying the dynamic properties of triplets directly [10]. In addition, standard absorption and emission techniques may elucidate the role, if any, that the excited singlet states play in the phototoxicity of these compounds.

1.2 Thiophenes and polyacetylenes.

1.2.1 Nature and distribution.

Polyacetylenes are a group of over 500 highly unsaturated compounds derived (in vascular plants) from oleic acid via a series of dehydrogenations to form triple bonds, and β -oxidations leading to chain shortening [11]. The polyacetylenes are C12, C13, C17 and C18 structures and may be aliphatic or being stabilized by phenyl, furan, or spiroketalenol groups. Thiophenes are derived biosynthetically from polyacetylenes by addition of sulphur across a diacetylene group and may incorporate various terminal groups including alcohol, aldehyde, methyl and halogen. Mixed thiophene-acetylenes are also possible. The thiophene and

Table 1. Structures and abbreviations of the sensitizers.

Structure	Abbreviation	Compound No.
DT	I
MIT	II
DIT	III
DTB	IV
PHT	V
YEP	VI

(L) = (X-terphenyl)
 MIT = monoiodo- (L-terphenyl)
 DIT = diiodo- (L-terphenyl)
 DTB = 1,4-dithienylbenzene
 PHT = phenylthienylbenzene
 YEP = "yne-ene-furan"
 = 5-(b-ene-8,10,12-tri-phenyl)furan.

polyacetylene analogue structures examined in this study are illustrated in Table 1. Of these α -T, PHT and YFF are naturally occurring substances, while MIT, DIT and DTB are synthetic analogues of α -T; the most active polyacetylene derivative yet investigated.

About twenty families of vascular plants synthesize polyacetylenes and thiophenes [11] but they reach their greatest diversity in the Asteraceae. Higher plants may also contain monoacetylenic compounds but these are often derived from different biosynthetic pathways [12]. All parts of a plant may contain polyacetylenes and thiophenes [13] but the roots and flowers contain the widest variety and the greatest concentration [11]. They are somewhat less common in plant leaves but can be at high concentrations [11,14]; up to 700ug/g fresh weight.

1.2.2 Toxicity of the thiophenes and acetylenes.

Both polyacetylenes and thiophenes exhibit toxicity under dark conditions [11,15,16,17] but in many cases their toxicity is enhanced significantly in the presence of light. This phototoxicity was first observed by Gommers [18,19] and by Chan et al. [20] in the mid-1970's. Light enhances the toxicity of these compounds to nematodes [18,19,21] and fungi [14,22] as well as to a variety of yeasts and bacteria [14,20,22,23] and marine and fresh water algae [24]. α -terthienyl inhibits photosynthesis at a site near photosystem II [25]. Human skin [26] and fibroblast cells are also susceptible [22].

The fact that such compounds photosensitize haemolysis of erythrocytes [27,28] and photoinactivation of membrane bound (but not membraneless) viruses [29,30] indicates that they act primarily on membranes. Since these substances were not found to photobind DNA [14], do not induce cytogenetic damage [30] and are equally toxic to wild-type and DNA repair deficient *E.coli* [31], the site of action is not DNA. This behaviour is in contrast to that of the non-photodynamic sensitizers the furocumarins. The photoactivity of these compounds results from photobinding of DNA [5,6].

Thiophanes and polyacetylenes are also toxic towards insects. In fact several are highly toxic to mosquito larvae [32,33]. The larvicidal activity of α -T exceeds that of DDT while that of YEF is comparable to DDT's [33]. Others have been shown to be photoovicidal [34,35]. α -T, PHT and matricaria ester show antifeedant activity to herbivorous insects, the suggestion being that the role these materials play is to protect plants from insect predation [36,37,38].

1.3 Photooxidations.

1.3.1 General.

The involvement of what are now known as photosensitized oxidations in biological systems was first noted by Raab in 1900 [39]. It was observed that low concentrations of certain dyes led to rapid killing of paramecia in the presence of oxygen and light but not in the dark. Such processes are called photodynamic effects by biologists [5]. Since that time hundreds of photooxidation reactions using a variety of sensitizers have been discovered in living systems [40]. For example, rose bengal can cause gene conversion in microorganisms [41], methylene blue can lead to hemolysis in human blood [42] as can protoporphyrin [43] and acridine orange can bring about mutation in the Salmonella bacterium [44].

An early proposal by Kautsky [45] that a metastable excited state of oxygen might be important in photooxidation mechanisms was ignored until revived by Foote and Wexler [46] and Corey and Taylor [47] in 1964. These authors concluded that the excited species proposed by Kautsky was that which has come to be known as singlet oxygen [48]; that is the $^1\Delta_g$ state of O_2 . Since that time the involvement of singlet oxygen in photooxidations has been amply verified [7,49,50] and in fact has become a major driving force in research dealing with the photodynamic effect [40]. Radical oxygen species are also relevant to photodynamic behaviour.

1.3.2 Mechanisms of photocoridation.

Most photosensitized phenomena in vivo appear to be mediated by sensitizer triplet states, mainly because of their long lifetimes [6,7]. The best sensitizers are compounds with long lived triplets which are formed in moderate to high quantum yield. Typical sensitizers of biological interest are dyes such as rose bengal and methylene blue, pigments such as chlorophyll and certain aromatic hydrocarbons [7,43].

Two important pathways leading to photosensitized oxidation have been recognized [7,50]. The first is known as the type I mechanism [7]. In these systems the triplet sensitizer interacts directly with a substrate resulting in either hydrogen abstraction or electron transfer. The resulting radicals may be involved in a wide variety of reactions including electron transfer to oxygen, reaction with other substrates, initiation of free radical chain autooxidation or back reaction [5,7]. These processes are illustrated below [7]

Reaction with
other reagents

Reaction with
oxygen

The reaction between the partly reduced sensitizer and oxygen yielding the superoxide radical anion ($O_2^{\cdot -}$) or the

conjugate acid ($\text{H}_2\text{O}^\bullet$) [7] is of particular biological significance [51].

$\longrightarrow$ Reactions

The role of $\text{O}_2^\bullet-$ in oxygen toxicity is well established [51]. Such species have been implicated in such phenomena as inactivation of DNA [52], inflammation of joints [53] and the aging of cells and animals [54].

The non-oxidative type I mechanism is relatively rare in biological systems. The best known sensitizers of this sort are the furocumarins (vide supra).

The second route to photooxidation is the singlet oxygen or type II mechanism [7,49,50]. In this case it is the excited singlet oxygen that is the reactive species. The process of singlet oxygen generation via energy transfer is illustrated here

where O_2 is the ground state of molecular oxygen ($^3\Sigma_g^-$) and O_2^* is the lowest excited singlet state ($^1\Delta_g$).

The involvement of this latter species in phenomena of consequence to biology and chemistry has been demonstrated. For example, singlet oxygen plays a critical role in the photo-

degradation of polymers [48] and in the formation of 1,2-dioxetenes which subsequently decompose with chemiluminescence [49]. Singlet oxygen interacts with alkyl-substituted olefins to give allylic hydroperoxides with a shifted double bond (the "ene" reaction) and it may react with a variety of heterocyclic compounds [49]. From a biological viewpoint singlet oxygen has been implicated in photooxidation of proteins [55], photooxidation of lipids and tocopherols [7] and plays a role in animal and human porphyrias [7]. Singlet oxygen is also the active species in one of the few known beneficial photodynamic effects, namely the radiative treatment of neonatal jaundice using bilirubin as the sensitizer [56].

1.4 Mechanisms of phototoxicity.

The observation of the phototoxicity of thiophenes and polyacetylenes has generated considerable interest in these species over the past few years [19,27,31,35,57]. In particular the photosensitized toxicity of α -T and related compounds to mosquito larvae and their potential application as a larvicide makes their mechanism of action a topic of considerable importance [57,58].

The involvement of superoxide anion as the primary source of toxicity has been ruled out in each case because superoxide dismutase does not significantly inhibit the phototoxicity of thiophenes or polyacetylenes [32,59]. On the

other hand 2,1- and terthienyls have now been shown to be potent singlet oxygen sensitizers [21,32,39,50,51] (in fact the singlet oxygen generating capacity of α -T is greater than that of methylene blue [38]). In vivo evidence suggests that the mechanism of thiophene phototoxicity in biological systems involves only type II photooxidations. For example, photosensitization of *E. coli* in the presence of α -T was enhanced under aerobic conditions while the singlet oxygen quencher sodium azide (NaN_3) protected yeast from the photodynamic action of α -T [32]. In contrast the ring stabilized polyacetylene PHT, while having a similar quantum yield of singlet oxygen as α -T, shows little change in phototoxicity in the presence or absence of oxygen [59]. In addition NaN_3 does not protect *E. coli* or *S. cerevisiae* from PHT photosensitization [59]. The lifetime of singlet oxygen is significantly longer in D_2O than H_2O [62]. Therefore if a singlet oxygen mechanism were operating the killing of the test organism would be expected to increase in the deuterated solvent. No D_2O effect on the microbicidal activity of PHT was observed [59]. Further, it is well documented that irradiation of acetylenic chromophores often leads to radical formation [63,64] and it has been recently demonstrated [59] that PHT and related acetylenes are rapidly and extensively photodegraded. The same study indicated that α -T and other thiophenes are very resistant to photodegradation under similar irradiation conditions. This has been taken as evidence that the phototoxic behaviour of PHT may involve competing type I and type

II mechanisms. This is controversial, however, and Kagan [65,66] has proposed that only a type II mechanism occurs with PHT. Even in the case of A-T the question as to whether its phototoxicity is due exclusively to a singlet oxygen mechanism is not fully resolved [67,68].

2. Theory.

2.1 Excited state properties and measurement.

This section outlines primary photoprocesses that may occur upon electronic excitation of a molecule and techniques which may be used to study them, with respect to thiophene and acetylene phototoxicity.

2.1.1 Primary photoprocesses.

Primary photoprocesses are, as the name implies, those reactions that occur as a direct result of absorption of a photon leading to transitions to electronic excited states [69]. Several physical events can take place in a molecule upon absorption of a photon as shown in Figure 1 [70]. Table 2 gives approximate rate constants for these processes [71]. Since vibrational relaxation is so rapid, photochemically relevant states (S_1 or T_1) are usually in their lowest vibrational level (Kasha's rule) [72]. Emission will also occur from the $v=0$ level. Once the excitation energy has been directed to one or both of these states a variety

Figure 1. photophysical processes connecting the low-lying states of a "typical" organic molecule (20).

Table 2. Approximate rates of the various photoprocesses illustrated in Figure 1.

Process	Rate s ⁻¹
Absorption	10 ¹⁵
Fluorescence	10 ⁷ - 10 ¹¹
Phosphorescence	10 ⁻¹ - 10 ³
Internal Conversion	
S _n → S ₁	10 ¹¹ - 10 ¹⁴
S ₁ → S ₀	10 ⁷ - 10 ⁹
T _n → T ₁	10 ¹¹ - 10 ¹⁴
Intersystem Crossing	
S ₁ → T _n	10 ⁸ - 10 ¹⁴
T ₁ → S ₀	10 ⁻¹ - 10 ³

of photochemical changes may occur [59] including radical formation, intramolecular rearrangement, isomerisation, hydrogen abstraction, external electron transfer and energy transfer.

2.1.2 Absorption and emission phenomena.

The usefulness of electronic absorption and emission spectra for understanding the structure, energetics, and dynamics of electronic excited states is well documented [1]. The relevant processes are

Absorption involves interaction between the electronic vector of light and the electrons of a molecule leading to excitation of an electron from an initial state, described by a wavefunction Ψ_2 , to a final state described by Ψ_1 [73]. Emission is a related process involving emission of photons from electronically excited states [74].

Since molecular electronic states may only have certain discrete energies, absorption and emission occur only at certain frequencies given by the expression

$$(6) \quad \Delta E = h\nu$$

where ΔE is the energy difference between the final and initial states, ν is the frequency of the absorbed or emitted photon and h is Planck's constant. Experimentally the intensity of absorption, I_a , is given by the Beer-Lambert law

$$(7) \quad I_a = (I_0) \exp[-\epsilon c l]$$

where I_0 is the intensity of the incident radiation, c is the molar concentration of the absorbing species, l the irradiated path length and ϵ a constant of proportionality called the molar extinction coefficient.

The oscillator strength concept is widely used to describe transition intensities [73]. In a classical sense the oscillator strength, f , is the effective number of electrons that give rise to a particular absorption band. The main interest in this theoretically derived quantity is its relation to the experimental observable ϵ

$$(8) \quad f \propto \int \epsilon d\nu$$

where $\int \epsilon d\nu$ is the integrated absorption band for the transition from ψ_1 to ψ_2 . Thus ϵ is a measure of the intensity of a transition.

The inherent or radiative lifetime of an excited state (that is, its lifetime in the absence of all non-radiative reactivation processes) is inversely related to f and therefore to ϵ

$$(9) \quad \tau_0 \propto 1 / \int \epsilon d\nu$$

Thus for emission processes τ_{00} is an estimate of the allowedness of the transition. In addition, emission lifetimes give information regarding the involvement of a particular state in a given photoreaction. For example, a singlet state that radiates in ≤ 1 ns will not be involved in a reaction that proceeds at a rate of 10^8 M $^{-1}$ s $^{-1}$. Fluorescence lifetimes are readily measured by the single photon counting technique [10]. A description of this technique is given below.

The intensities of electronic transitions may be related to the square of the transition moment, R_{12} , connecting the initial and final states [3].

$$(10) \quad R_{12} = \langle \Psi_1 | \hat{\mu} | \Psi_2 \rangle$$

where $\hat{\mu}$ is the dipole moment operator. For a transition to be allowed R_{12} must be non-zero.

If we make the Born-Oppenheimer approximation, assume that the total electronic wavefunctions can be written as the product of one-electron wavefunctions (ϕ_1 and ϕ_2) and that only one electron is involved in a given transition, expression (10) becomes

$$(11) \quad R_{12} = \int \Theta_1 \Theta_2 d\tau_v / \int \phi_1 \hat{\mu} \phi_2 d\tau_e$$

where Θ_1 and Θ_2 are vibrational wavefunctions. $\hat{\mu}$ operates only on the electronic wavefunctions. Further, if it is assumed that the ϕ_i may be separated into a spin orbital (S_i) and a space orbital (ψ_i) one may write

$$(12) \quad P_{12} = \int \psi_1 \psi_2 d\tau_v / \int \psi_1 \psi_2 d\tau_s / \int \psi_1 \psi_2 d\tau_e$$

This expression allows one to identify factors that influence transition intensities. These apply to both emission and absorption.

The first important point concerning transition intensities is the symmetry of ψ_1 and ψ_2 . Generally, if the product of these with the dipole moment operator is an odd function, the electronic integral in expression (12) will be zero and the transition forbidden. In addition if the extent of overlap between ψ_1 and ψ_2 is small, the transition will have low intensity. The same will be true if the overlap between the vibrational functions is small.

Spin restrictions may also limit the intensity of a given transition. In a transition between states of the same multiplicity ($S \rightarrow S$ or $T \rightarrow T$) no change in the spin vector direction takes place. The spin overlap integral in expression (12) is then equal to 1. If the spin direction does change upon excitation ($S \rightarrow T$), the spin wavefunctions are orthogonal and the transition is forbidden.

In general the spin restriction is quite strict [73] but may be relaxed if a perturbation "mixes" states such as S_1 and T_1 . The most common such perturbation is spin-orbit (SO) coupling. Electronic orbital motion generates a magnetic field over the electron. The spin of the electron is influenced by this field and its vectorial direction may be changed by it if the interaction is strong enough. The extent of SO coupling is an

inverse function of the energy gap between the two states involved and a direct function of the potential field in which the electron is moving. Thus, the SO interaction is greatest for two states of relatively close energy in the presence of a heavy nucleus.

The intensities of the vibrational structure of electronic transitions are rationalized by the Franck-Condon principle [73]. This states that nuclear motion is slow relative to the rate of an electronic transition and therefore molecular nuclei have the same relative positions before and after the transition. The most probable transitions will therefore be those that require the least change in the position and momentum of nuclei. This is illustrated in Figure 2 [73]. In the case of absorption the vertical transition is from $S_0(v=0)$ to $S_1(v=2)$. The $0 \rightarrow 2$ vibrational band is therefore the most intense in the resulting absorption spectrum. The situation is similar for emission spectra. In fact there is often an (approximate) mirror image relationship between the absorption and emission spectrum of a molecule [74].

2.1.3 Radiationless transitions.

Along with optical transitions, two types of radiationless processes, internal conversion (IC) and intersystem crossing (ISC) are illustrated in Figure 1. Information regarding these processes can also be obtained from absorption and emission data.

IC occurs between states of the same multiplicity and is

Figure 2. Potential energy curves for a diatomic molecule illustrating (a) the Franck-Condon principle and (b) radiationless transitions [27].

very rapid (10^{11} to 10^{13}s^{-1}). This accounts for the observation that emission is nearly always observed from S_1 . ISC occurs between states of different multiplicity and similar energy. If there were no spin restriction this would be as rapid as IC. In fact, ISC proceeds at about 10^7 to 10^{11}s^{-1} , similar to the rate of fluorescence deactivation of S_1 [73].

Figure 2 [73] can give some insight into how these transitions come about. The figure shows typical potential energy curves for a diatomic molecule. Initial absorption leads to population of an upper singlet state followed by a rapid cascade of energy until the vibrational level of S_1 corresponding to point A is reached. Vibrational relaxation to $S_1(v=0)$ follows. This is isoenergetic with $T_1(v=3)$. In the presence of SO coupling the molecule is in equilibrium between $S_1(v=0)$ and $T_1(v=3)$. Since $T_1(v=3) \rightarrow T_1(v=0)$ is very rapid the molecule will be confined to the T_1 state if the energy gap between $S_1(v=0)$ and $T_1(v=0)$ is large enough.

Point B is a vibrational turning point for both S_1 and T_1 . This means the nuclear arrangement, the potential and kinetic energies of S_1 and T_1 are the same at this point. Among upper electronic states of the singlet (or triplet) manifold such turning points are more common. This facilitates the rapid cascading of energy to S_1 (or T_1) from the upper states of the same multiplicity, especially since the spin restriction does not apply.

2.1.4 The triplet state.

As has been implied there are two commonly observed excited states in organic molecules. One of these, in which the spins of the excited and unexcited electrons are paired, is known as the singlet state because it remains unsplit in the presence of an external magnetic field. The other type of state shows splitting into three quantized states when a magnetic field is applied and is known as the triplet state. In the triplet state the spins of the two electrons involved are unpaired [1]. Because of the spin restriction $S \rightarrow T$ absorptions are rare and generally weak. On the other hand population of the triplet (at least to some extent) by ISC is thought to be completely general [74].

The key difference between singlet and triplet states is their different spin arrangement. This accounts for most of the observed triplet properties. A spinning electron possesses magnetic angular momentum which may be represented by the spin vector S [1]. This vector precesses about the electronic "axis" as the electron spins. The spin vector is allowed two measureable orientations along an arbitrary axis, namely "up" and "down" which correspond to $\alpha = +\pi/2$ and $\beta = -\pi/2$, respectively. It is the existence of these two spin directions that accounts for the derivation of S and T states from a single electronic configuration. For example, if an electron is excited from a ground state orbital ψ_1 to an excited orbital ψ_2 the possible orientations of the two spins are as shown in Figure 3 [1]. The

Figure 3. Allowed electron spin orientations [17].

only stable configurations of the spin vectors are those in which $\hat{S}_1$ and $\hat{S}_2$ are in phase or completely out of phase (separated by 180°). The former situation leads to three possible orientations (the triplet) while the latter leads to only one (the singlet).

Since the triplet state has two spin parallel electrons it must be paramagnetic. This is indeed the case and was one of the earlier tests used to prove the existence of the triplet state [75]. Further, the triplet state is that associated with phosphorescence. The long lived nature of this type of emission is immediately rationalized when one realizes it involves a spin change.

2.1.5 Quenching and the Stern-Volmer analysis.

The following processes commonly occur upon excitation leading to an excited singlet or triplet state, S^* [9].

This scheme implies that no reactions such as $S^* + S^*$ or $S^* + S$ occur.

Excited states are simultaneously oxidizing and reducing

agents. The electron in the unstable orbital is readily transferred to any available acceptor if distance and overlap conditions are met. Similarly, the hole remaining in the stable orbital may accept an electron from any source when distance and overlap conditions permit. This leads to a variety of chemical alternatives to radiationless decay of sensitizer excited states.

The last three steps shown here are quenching reactions. Quenching of electronic excitation is important in many areas including synthetic photochemistry [1], assignment of excited states [71], photosynthesis [76], and stabilization of polymers [48].

Steps 4 and 6 proceed via some form of energy transfer while step 5 is an electron transfer. All three require close approach of the molecules involved and are therefore diffusion controlled [1]. The rate of diffusion of, say, S^* and A in the chosen solvent places an upper limit on the rate of quenching. The diffusion controlled rate constant, k_d , is usually 10^9 to $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ [1].

Quenching experiments are typically analyzed by the Stern-Volmer method [77]. This is based on competition between the inherent unimolecular decay of S^* (steps 2 and 3) and bimolecular quenching by A, (steps 4 to 6), i.e.

If no other processes occur the observed lifetimes of S^* in the

absence (T_1) and presence (T_2) of quencher are given by

$$(15) \quad k_1 = 1/T_1$$

$$k_2 = 1/T_2 = 1/T_1 + k_2[A].$$

A plot of the observed lifetime, k_2 , versus concentration of A should be linear with a slope equal to k_2 , the bimolecular quenching rate constant.

2.1.6 Magnetic circular dichroism.

Magnetic circular dichroism, or "CD", is the preferential absorption of left and right circularly polarized light by a sample placed in a magnetic field [9] and is a property of all matter. The technique is based on the Faraday effect, i.e., a substance placed in a magnetic field rotates the plane of polarized light [29]. Experimentally the Faraday effect follows the relation [29].

$$(16) \quad \alpha = d l H \sin \theta$$

where α is the magnetic rotation, l the path length the light travels through the sample, H the magnetic field strength and θ the angle between the magnetic lines of force and the optical beam. d is a constant of proportionality.

Theoretical investigations [2,9] have concluded that three terms are important for predicting the Faraday effect for a given molecule: the A term which is related to the Zeeman splitting of the spectral line of a given transition into left and right

circularly polarized components; the B term generated by mixing of the ground and excited states by the magnetic field; the C term associated with electronic population changes in a split ground state.

The contribution of an isolated transition $a \rightarrow j$ to the MCD of a molecule is given by [79].

$$(17) \quad [\Theta]_M = -21.3458[f_1A + f_2(B + C/kT)]$$

where $[\Theta]_M$ is the molar ellipticity per unit magnetic field and f_1 and f_2 are functions which describe the shapes of the MCD A term and B and C terms, respectively. The $[\Theta]_M$ value is also proportional to the difference in extinction coefficients for the absorption of the right and left circularly polarized light ($\epsilon_l - \epsilon_r$) [80].

Equation (18) is the expression for the MCD A term for a transition between states a and j [79].

$$(18) \quad A(a \rightarrow j) = 1/2d_a \sum [\langle j | \hat{\mu} | j \rangle - \langle a | \hat{\mu} | a \rangle] \cdot \text{Im}[\langle a | \hat{m} | j \rangle \times \langle j | \hat{m} | a \rangle]$$

where d_a is the degeneracy of a and $\hat{\mu}$ and $\hat{m}$ are the magnetic and electric dipole operators, respectively. From this it follows that the A term will be observed if either a or j has a non-zero magnetic moment [9]. A triplet state would fulfil this requirement. Figure 4 illustrates how the A term arises for a transition from a non-degenerate ground state to a degenerate excited state [79] when a magnetic field of strength H is

Figure 4. Illustration of the "A" and "B" terms (731).

applied. A terms are generally observed in such cases and are indeed diagnostic for them [9].

C terms arise upon transition from a degenerate ground state to a non-degenerate excited state. Such situations are very rare, especially among organic molecules, and will not be considered further.

It is the B term that is responsible for the observation of the Faraday effect in all matter. This term arises from the mixing of the ground and two or more excited states by the magnetic field [79]. The expression is

$$(19) \quad B(a \rightarrow j) = 1/\Delta a \sum_{k \neq a} \frac{\langle k | \hat{\mu} | a \rangle \langle a | \hat{m} | j \rangle \langle j | \hat{m} | k \rangle}{\Delta E_{ka}} + \sum_{k \neq j} \frac{\langle j | \hat{\mu} | k \rangle \langle a | \hat{m} | j \rangle \langle k | \hat{m} | a \rangle}{\Delta E_{ka}}$$

and the related processes are illustrated in Figure 4 for a situation where only the ground state, a , and two excited states j and k are involved. We can simplify the situation by assuming the contribution of the second summation to B is greater than that of the first. If no magnetic field is present there will be no preferential absorption of polarized light by either j or k and the normal absorption spectrum is observed. When there is a field present, mixing of j and k will occur [79] and each will acquire some character of the other. The mixed states exhibit differential absorption of the polarized light resulting in a B type MCD band. B terms are observed for allowed transitions [79].

2.1.7 Laser flash photolysis.

2.1.7a Basic principles.

Flash photolysis (FP) was originally proposed by Norrish and Porter in 1949 [81]. The laser manifestation of the technique evolved in the late 1960's due to the work of Lindqvist [82] and others. Laser flash photolysis (LFP) is a powerful technique for examining early photoprocesses, fast chemical kinetics, and identifying reactive intermediates such as triplet states, free radicals and ions. Since laser pulses in the 5-20ns time domain are typically used, the LFP technique has time-resolution that is at least three orders of magnitude greater than that of the conventional technique. In fact, with the advent of ps lasers, vibrational lifetimes can now be observed. Two types of photochemistry become resolved, that straight out of the singlet following a selected vibration and that out of triplets following randomized reaction coordinates [83].

As with regular FP, the principle behind LFP is fairly simple, and is essentially based on the Beer-Lambert law (vide supra). A solution containing a substrate (eg. an organic molecule) is irradiated with a short-lived (ps-ns), high-intensity burst of light. This leads to a relatively high population of excited states and/or other intermediates (eg. radicals) which are called transient species [69].

Figure 5 shows a schematic diagram of the LFP system used in this study. Upon excitation the monitoring beam is attenuated by

Figure 5. Schematic diagram of the laser flash photolysis system.

the transient (at its absorbing wavelengths) according to the Beer-Lambert law and this attenuation is measured by the photomultiplier tube (PMT). Thus the data from a LFP experiment consists of the monitoring beam intensity, I_0 , and a set of a few hundred voltage signal points acquired as a function of time.

These are converted to change in optical density by the relation [34]

$$(20) \quad \Delta O.D. = -\log(1 - \text{Signal}/I_0).$$

This equation illustrates an important difference between transient absorption and conventional ground to excited state absorption. In the latter the concentration and extinction coefficient of the initially absorbing species are known. In the former instance these parameters are generally not known. Thus only the difference between the optical density of the starting materials (substrate) and the transient may be measured. A negative signal is possible and this "bleaching" occurs when the transient signal is less intense than that of the starting materials [34].

Figure 6 shows a typical transient decay trace obtained during the present work. As indicated in equation (20), the $\Delta O.D.$ (or voltage signal) is a function of time. Transient absorption spectra can be readily built-up by acquiring a transient decay (or growth) at several different wavelengths and plotting the maximum $\Delta O.D.$ (top OD) for each as a function of wavelength. The measurement can be made at different delay times after

excitation which allows for time-resolution of several transients if more than one is generated by a given substrate. Since $\Delta O.D.$ is being measured, transient spectra are difference spectra—showing the difference between the spectrum of the transient and that of the substrate. Thus transient spectra are generally precursor dependent in the spectral region where substrate absorbs [71,84].

The ideal transient for a LFP study would be one which is generated in the duration of the laser pulse, decays with first- or pseudo first-order kinetics and the absorbance of which returns to the pre-excitation level [84]. In such a case the rate law is of the form

$$(21) \quad \ln(O D_0 / O D_t) = k t$$

where k is the (pseudo) first-order rate constant for transient decay and $O D_0$ and $O D_t$ are the transient optical densities immediately following excitation ("end of pulse") and at time t after excitation, respectively. This expression implicitly assumes that there is only one absorbing species present at the monitoring wavelength. This is often not the case and at long times a residual absorption may be observed due to product formation from the transient or solvent. In such cases it can be shown [85] that

$$(22) \quad \ln(O D_0 - O D_{\infty} / O D_t - O D_{\infty}) = k t.$$

A plot of $\ln(O D_t - O D_{\infty})$ vs. time provides an appropriate treatment

of the data in such cases.

The measurement can be made as a function of wavelength, temperature, quencher concentration, laser dose, substrate concentration and other exogenous factors. In addition, LFP may be used to obtain extinction coefficients and quantum yields, of transient formation; usually by comparison to a standard absorption [84].

FP generally has a number of advantages and disadvantages inherent in it [69]. The technique may be applied to gases, liquids and solids over a wide range of temperatures, wavelengths, and substrate concentrations as well as in the presence of quenchers. On the other hand the sample must be optically clear for monitoring or scattering effects will distort the signal. Although the technique is quite sensitive there are many transients with low extinction coefficients that are not detectable. Transient identification can be facilitated by the transient absorption spectrum but such assignments may be ambiguous at best if the spectrum is not characteristic or well resolved from those of other transients present.

In addition to these points there are several problems specific to LFP [84]. Laser irradiation can lead to energy deposition in a very thin layer of sample if the ground state OD of the substrate is too high. The heat that results can lead to acoustic shock waves which may seriously distort the signal. Laser irradiation may lead to different photo products than conventional flash lamp irradiation. This is observed in systems

where products may form from reactions which are second-order in transient concentration (eg. radical-radical processes, triplet-triplet annihilation). Since the reactive intermediate concentration can easily exceed that in steady-state photolysis by three orders of magnitude such processes can be expected except at low substrate levels and low laser dose. In addition, extensive sample decomposition may occur, especially with more high power lasers.

2.1.7b The Lasers.

Lasers or related devices have been available since the mid-1950's [86]. All such devices operate on the principle of light amplification by stimulated emission of irradiation. This process is illustrated in Figure 7. There are three competing processes [87]: stimulated absorption (Einstein coefficient B_{12}); spontaneous emission (Einstein coefficient A_{21}); stimulated emission (Einstein coefficient B_{21}). Absorption occurs at a rate of

$$(23) \quad I(B_{12})(N_1)$$

where I is the intensity of absorption and N_1 is the number of molecules in the ground state. Spontaneous emission occurs when the excited molecule returns to the ground state with a rate of

$$(24) \quad (A_{21})(N_2).$$

Stimulated emission occurs when an additional photon having the

Figure 2. Physical processes relevant to laser action (top) and schematic diagram of a laser cavity (bottom) [22].

same energy as the absorbed photon strikes the molecule and it is excited state. The incident photon is joined by a second photon from the excited state i.e., there is an amplification of photons. The second photon wave is precisely in phase with that of the incident photon giving a perfectly coherent beam of radiation emitted at a rate of

$$(25) \quad I(\nu_2)(\nu_2) = I(\nu_1)(\nu_1)$$

From expressions (23), (24) and (25) it is evident that the rate of population of state 2 will be equal to its rate of depopulation if

$$(26) \quad I(\nu_2)(\nu_2) = I(\nu_1)(\nu_1) = I(\nu_1)(\nu_1)$$

The three Einstein coefficients are related to each other by

$$(27) \quad A_{21} = c^2 B_{21} \\ g_2(\nu_2) = g_1(\nu_1)$$

where c is a constant for a given wavelength and g_1 and g_2 are the statistical weights of states 1 and 2, respectively. If 1 and 2 are non-degenerate

$$(28) \quad B_{21} = B_{12}$$

For laser action to occur emission must dominate absorption. From expressions (26), (27) and (28) this may only occur when

$$(29) \quad \nu_2 > \nu_1$$

i.e., when population inversion takes place. This is the fundamental requirement for laser action [87].

Lasers have three essential components: the active material (a gas, liquid or solid); two energy levels radiatively connected which can be pumped to achieve population inversion; a resonant cavity. These are illustrated in Figures 7 and 8 [87]. Photons are trapped in the resonant cavity, amplifying the photon flux continually. Some of the photons escape the cavity through the semi-transparent mirror to form the laser beam. The resultant beam has high pulse peak power, low divergence, monochromaticity, spatial coherence and short-lived pulses (ps-ns) [87].

Many lasers operate on a four-level system for optical pumping [87] as illustrated in Figure 8. This is more efficient than a three-level system since the lower laser level is kept empty by rapid relaxation to the ground state.

The lasers used in this study were an N_2 gas laser and a XeCl excimer laser. The former uses a four-level electronic energy level system to lase. The upper laser level has a lifetime of about 5ns while that of the lower level is several μ s. The laser action is therefore self-terminating and the laser can only operate in the pulsed mode. Pumping of the desired excited state is achieved by capacitor discharge [69].

Excimer lasers are based on excimer (or exciplex) formation between a ground state atom and one in an electronic excited state [88]

Figure 2. A four level laser system.

The excimer decays radiatively to the ground state and then dissociates

A population inversion can be easily developed between the excimer (A_2^*) level and the dissociative (lifetime $\leq 10^{-13}$ s) ground state (A_2). The XeCl laser emits at 308nm while the F_2 laser emits at 337.1nm.

2.2 The nature of singlet oxygen.

The potential energy curves and orbital occupancy corresponding to the three lowest lying electronic states of molecular oxygen are shown in Figure 9. The $^1\Delta_g$ state, that which is now known as singlet oxygen, was first observed experimentally in 1934 by Herzberg [39] though its existence had had been predicted by Mulliken in 1928 [90]. In the absence of collisions the lifetime of singlet oxygen is on the order of 45min. to 1h [48]. Even in fluid solution it decays on a μ s time-scale. This long lifetime is reasonable as the only decay route open to $^1\Delta_g$ is spin forbidden. The higher energy state $^1\Sigma_g^+$ has a much shorter lifetime because its decay to $^1\Delta_g$ is spin allowed. Thus the former state is thought to be unimportant

Figure 2. Potential energy curves and orbital occupancy of the three lowest electronic states of N_2 .

in reactions of excited state oxygen (2,3).

Singlet oxygen may be generated by several ways and among microwave discharge and chemical reaction (2,4), the most common means is via photosensitization according to oxygen quenching of organic molecular triplet states. Two principal principles govern the energy transfer process (2,5). The first is conservation of energy. That is, the total energy of the organic triplet state encounter complex initially formed must be maintained in the final products. The final state must therefore be either excited oxygen and ground state sensitizer or vibrationally excited ground states.

The second relevant point is that conversion of electronic energy into vibrational energy is relatively inefficient compared to direct transfer of electronic energy (2,3,6,7). This means that generation of singlet oxygen is favoured when oxygen quenching of organic triplet states leads to energy transfer.

Oxygen quenching in solution is a diffusion controlled process. Spin statistical factors indicate that only 1/3 of diffusional encounters will lead to energy transfer as shown in the following scheme.

where k_d is the diffusion controlled rate constant. If oxygen quenching proceeds at rates that approach $k_d/2$ and if the quantum yield of singlet oxygen is 1, the quenching is solely by energy transfer.

3. Objectives and approach.

The compounds examined in this study are illustrated in Table 1. α -T, PHT and YEF are naturally occurring toxins. The naphtho- and benzo- thiophene derivatives were prepared especially for phototoxicity testing. They have been included for comparison. At the time this study was initiated there was little information concerning the excited states of these molecules in the literature. For example, apart from the absorption spectrum and a possible assignment of these transitions [93], the only other study relating to excited state α -T was one examining the polarization of its fluorescence spectrum [94]. Even less information was available on PHT.

The primary goal of this study is to gather chemical evidence that either supports or contradicts the views that (i) the phototoxicity of α -T and related species is mediated solely by singlet oxygen while (ii) that of PHT and analogues involves competing type I (non-oxidative or radical) and type II (singlet oxygen) mechanisms. Since excited state properties of the sensitizers are of critical importance to this type of behaviour an examination of these properties has been undertaken using both

conventional (absorption/emission spectroscopy) and unconventional (MO/LFP) techniques. where possible an attempt has been made to characterize the states in terms of their energy, degeneracy, intensity, lifetimes, energy transfer and electron transfer properties, quantum yield, and oxygen generation properties.

II. Experimental

1. Instruments.

1.1 Absorption spectrophotometer.

Ground state absorption spectra were recorded on a Varian Cary-219 double beam spectrometer. The light sources are an air-cooled tungsten-halogen lamp for the visible range and an air-cooled deuterium lamp for the ultra-violet. The accessible wavelength range is 200-800nm.

1.2 Fluorimeter.

Luminescence spectra were recorded on a Perkin-Elmer LS-5 Luminescence Spectrophotometer equipped with a Perkin-Elmer 3600 Data Station. Figure 10 [95] shows an outline of the optical system.

The spectrometer can scan an active range of 230 to 720 nm in the case of excitation and 250 to 300 nm in the case of emission. The excitation source is a pulsed Xe flash lamp. Two reflection grating monochromators (1200 lines/mm for excitation, 960 lines/mm for emission) are used in the optical system and in sample and reference photomultipliers. The monochromator slit widths are independently adjustable to four discrete values. Neutral density and cut-off filters were used when appropriate.

MCD / ABSORBANCE FLOWCHART OF DATA ACQUISITION

Figure 10. Block diagram of the MCD/Absorbance data acquisition system. The system is a computer-based system for the acquisition of MCD data.

1.3.1 Magnetic circular dichroism spectrometer.

The MCD data were obtained with a custom-built, combination absorption / circular dichroism / magnetic circular dichroism instrument [96]. The operation is fully computer controlled over the range of 200 to 800 nm. The signal-to-noise ratio is controlled by the standard deviation of the signal, and the scan rate between digitized points is controlled by the mean slope, to produce the best spectrum available in a single scan. In the current experiment spectra were run from 25000 cm^{-1} to 13333 cm^{-1} , merged with an ethanol background scan and plotted by computer. Figure 10 shows a schematic diagram of the instrument.

1.4 Single photon counting instrument.

The system used consisted of components from Photochemical Research Associates (PRA). Figure 11 shows a schematic diagram of the instrument [69].

The excitation source (PRA 5103) is a low pressure (~ 0.5 atm) or "triggered" hydrogen lamp. Lamp triggering is provided by supplying high voltage (typically 6kV) to one tungsten electrode via a charging resistor and grounding the other electrode through a HY-2 gating thyatron whose grid is pulsed at 31 kHz. The electrode gap is between 2 and 3 mm and the lamp profile decays in about 2 ns.

The lamp pulse is converted to electrical signal by the lamp photomultiplier tube (PMT) which is built into the lamp housing.

As a consequence of this arrangement, no beam splitter is

Figure 11. Block diagram of the single channel counting instrument [19].

required.

The lamp signal is directed to the input of a PRA 1719, 100 MHz discriminator which transmits a "start" pulse to the PRA 1701 time-to-peak height converter (TPHC) which initiates its constant ramp time sweep.

The remainder of the excitation radiation is directed through the excitation monochromator (PRA 1280) and onto the sample. Sample emission is collected at 90° relative to excitation. The spectra were analysed using suitable band pass or cut-off filters.

The emitted light is focussed on the second PMT which is housed in, and cooled by, the PRA 1551 thermoelectric refrigeration unit. This cooling system improves the signal-to-noise by reducing the "dark" counts at the PMT.

The PMT signal is next transmitted to the TPHC. The first incoming photon registered acts as a "stop" prompt terminating the sweep of the TPHC. The resulting output voltage is fed to the multi-channel analyzer (TN-7200) where it is displayed as photon counts as a function of time.

The data is finally dumped to the PDP11/73 computer for storage and data processing.

1.5 Laser flash photolysis system.

Figure 5 shows an outline of the laser flash photolysis (LFP) system used in this study. This system has been described in detail elsewhere [97].

1.5.1 The lasers.

The samples (at 300 K) were excited with the pulses (338 nm, ~5ns, up to 80 mJ/pulse) from a Lumonics TE860-2 excimer laser operated with Xe-HCl mixtures, or (337.1nm, ~8ns, up to 10mJ/pulse) from a Molectron UV-24 nitrogen laser. The pulses were concentrated on the samples by a combination of spherical and cylindrical lenses as indicated in the Figure 5 [84]. The lasers are pulsed at 1Hz and the shutters are included to allow regular pulsing of the laser without irradiating the sample with each shot. This regular pulsing leads to very reproducible pulse energies.

1.5.2 The monitoring system.

The monitoring system consists of three important components; a pulsed 200-w Xenon lamp with vertical electrodes, a high intensity PRA-9204 monochromator, and a RCA-4840 photomultiplier tube (PMT) at the detection end which operates on six dynodes.

The monitoring system includes cut-off filters which eliminate second order effects in the monochromator and a shutter which limits the irradiation of the sample by the monitoring beam.

The angle between the nitrogen laser beam and the monitoring beam is small. This decreases the volume irradiated giving a higher signal/pulse energy ratio [88] and so is better suited for use with a lower energy excitation source. Further,

by having the nitrogen laser at right angles to the excimer, one can change the excitation wavelength without altering the optical set-up.

1.5.3 Data capture, processing, storage, and experimental control.

In this system the output of the PMT is captured by a R7912-Tektronix transient digitizer containing 4K local memory. These data are then transferred to a PDP11/23 computer.

The data entering the computer consist of the intensity of the monitoring beam (I_0) and a set of a few hundred voltage signals that are a function of time. The computer transforms these signals into change in optical density (relative to starting materials i.e., baseline) by the equation

$$(34) \quad \Delta O.D. = -\log(I - \text{signal} / I_0)$$

One of two corrections were applied in order to improve the quality of the data. These were:

1) a "baseline" correction to take care of noise originating in the laser and Xenon lamp as well as baseline drift due to changes in I_0 . This is generally applied at longer time scales ($> 2 \mu s$).

2) a "fluorescence" correction to compensate for sample fluorescence (at time scales $< 2 \mu s$) and any photon source other than the monitoring beam, such as scattered light.

These corrections were introduced by a ternating signal and

correction laser shots.

Transient decay traces were typically obtained using 10 (occasionally 20) laser shots per decay. Only five shots per decay were used to generate spectra. The decays were initially stored in the 4K floating memory mentioned above and then transferred to "floppy" discs from which the data could be readily recalled. This form of data acquisition gives better signal-to-noise because of the averaging effect.

In addition to its role in data acquisition, processing, and storage the computer is important in experimental control. The computer signals the opening and closing of the shutters, thus allowing for simple correction; it activates the lamp pulses; it controls the movements of the cut-off filters as well as the neutral density filters (used to attenuate laser dose). The computer also drives the monochromator stepping motor and sets the high voltage so that a suitable current will be drawn from the PMT. It bases this on the intensity of the monitoring beam at a given wavelength and the signal from the previous pulse. Consequently, if the signal from the first pulse is weak the computer will increase the PMT voltage to compensate; this is very useful for automatic recording of transient spectra.

1.5.4 Time sequence of events.

Figure 12 shows a plot of output from the photomultiplier versus time for the period from one second before to one second after a particular pulse. The first event is the opening of the

shutter in front of the monochromator which allows some light in. Next the monitoring lamp is pulsed, increasing the intensity of light reaching the monochromator by about 50 times. The A/D converter reads the voltage (this corresponds to I_0), then detection starts. Shortly after this, the laser fires and the important signal is seen represented in the diagram by the small square. This corresponds to a decay trace of the sort shown in Figure 6. Finally, an electric switch dumps the charges on the capacitor producing the spike shown, then the shutter closes ready for the next pulse.

2. Procedures.

2.1 Reagent additions and degassing.

All reagent additions were performed using Hamilton microlitre syringes in the 10 to 250 μ l range. To deliver volumes greater than 250 μ l Hamilton tuberculin syringes of 1 or 2 ml capacity or glass pipettes were used.

In many experiments it was necessary to eliminate oxygen from the system. This was readily accomplished by bubbling oxygen-free nitrogen through the solution of interest in a septum-sealed cell for 10 to 15 minutes. A similar method was used for adding gas mixtures to solution (vide infra).

The septa used were soaked in benzene for two days to remove any surface materials that might act as quenchers of transient species.

2.2 Ground state absorption spectra.

UV-VIS ground state absorption spectra and molar extinction coefficient data were obtained on a Cary-219 spectrophotometer. Samples were contained in either $1 \times 1 \text{ cm}^2$ standard quartz cells or in $7 \times 7 \text{ mm}^2$ cells made of Suprasil tubing. Data was always obtained relative to a baseline reference of air or, when necessary, solvent.

2.3 Luminescence studies.

Room temperature fluorescence spectra in cyclohexane were obtained from deaerated samples contained in $1 \times 1 \text{ cm}^2$ standard quartz cells using a Perkin-Elmer LS-5 luminescence spectrophotometer. Typical parameters were: excitation slit = 10 nm, emission slit = 5 nm for emission spectra, excitation slit = 5 nm, emission slit = 10 nm for excitation spectra. The scan speed was always 1 nm per second. G type cut-off filters (Schott) and neutral density filters were used when appropriate.

Fluorescence spectra at 77K in deaerated methylcyclohexane solution were obtained on the same instrument. Rather than $1 \times 1 \text{ cm}^2$ cells, quartz tubes of about 5 mm internal diameter were used.

Singlet lifetimes were estimated using a Photochemical Research Associates time correlated single photon counting instrument operated by the PDP 11/73 computer. Again deaerated cyclohexane samples were contained in $1 \times 1 \text{ cm}^2$ cells. The lamp pulse profile was easily obtained by suspending silicalite in

PLC grade H_2 and collected a decay trace in a quartz cell. Singlet decay traces were then collected using the same excitation wave length. These were derived from the first pulse by the PDP 11/73 which also calculated the lifetime. Since PHT proved to be non-fluorescent this experiment was carried out only for the triophenes.

2.4. MCP experiments.

Magnetic Circular Dichroism (MCD) spectra were obtained for A-T and PHT as model compounds. These sensitizers were diluted to an absorbance of 1.0 in ethanol at the absorption maximum between 300 and 400 nm, the normal range for photosensitization of these substances. The MCD spectra were determined in 10-cm quartz fluorescence cells, merged with an ethanol flash lamp and calibrated against aqueous $CoSO_4$ standards (1987).

2.5. Laser flash photolysis.

2.5.1. General.

For all six sensitizers, transient signals can be generated in degassed solutions upon excitation with 337 nm and 277 nm laser light. Unless noted otherwise, the transient signals have been assigned to the triplet states of these molecules, and monitored at the λ_{max} for their triplet-triplet absorptions. This does not apply to triplet-triplet spectra for these compounds. The monochromator of the LFP system was stepped in general 10 nm intervals.

LFP experiments were carried out in 1×10^{-2} , 1×10^{-2} cell, rarely 1×10^{-2} cells of Suprasil, quartz. For most experiments sensitizer concentrations were on the order of $2 \times 10^{-5}M$. This led to suitable ground state absorbance (<1.5) typically about 0.5 at the laser wavelength. The use of static cells, usually containing 2ml of solution, only led to minor substrate depletion during the experiments. UV-VIS spectra recorded before and after the experiments showed that photoconversion was $<10\%$ in the case of the thiophenes and $<20\%$ for PAT. This suggests that irreversible photoprocesses occur with very low quantum yields.

In certain experiments (vide infra) it was necessary to use sensitizer concentrations as high as $10^{-3}M$. For such experiments the ground state absorption optical density (O.D.) at the laser wavelength was kept below 1.5 by using the 303nm laser (all the compounds have relatively low extinction coefficients at this wavelength) and short path lengths. It is important to ensure that the O.D. at the exciting wavelength is not too great so the laser's energy will be largely deposited in a very thin layer of sample. The resulting heat generation can lead to acoustic shock waves which may lead to misinterpretation of LFP data (847).

1.5.2 Quenching experiments.

A series of quenching experiments were performed on the sensitizers using a variety of triplet quenchers including oxygen, B-carotene, methyl viologen, 1,3-octadiene, 1,3-cyclohexadiene, triethylamine, anthracene. The solvent was

benzene were recorded. The excimer laser was used for excitation in each case. The spectra and decays were obtained on the same time base, with the same delay after excitation and the same laser dose.

2.5.5 Furan bleaching.

This procedure followed that of Merkel and Kearns [62,49]. Sample cells containing air-saturated methanol solutions of $10^{-3}M$ sensitizer plus $4.4 \times 10^{-5}M$ 1,3-diphenylisobenzofuran (DPBF) were irradiated with the excimer laser, the dose being attenuated to 36% of the full dose. Irradiation led to bleaching of the furan as monitored at 440nm. Since the solutions were air-saturated the triplets decayed very rapidly and so did not interfere on the time scale of this experiment (5 μ s).

The samples were prepared so that their ground state absorbances at 308nm were equal. This ensured that the same population of excited sensitizer singlet was achieved in each case.

A blank run was performed for each experiment to correct for direct photolysis of the furan which was small in each case.

2.5.6 Determination of $ET \times \Phi_{ISC}$.

An approximate value for the product of the triplet extinction coefficient and the intersystem crossing quantum yield, $ET \times \Phi_{ISC}$, for an unknown triplet may be obtained relative to a standard - benzophenone ($\Phi_{ISC} = 1.0$, $ET = 7600$ at 525nm).

[102] in this case.

In this experiment a solution of $2 \times 10^{-5} M$ sensitizer (D.O. = 0.3 at 308nm) in methanol was optically matched to a solution containing benzophenone in benzene. The samples were irradiated with the excimer laser and the top 0.2% of the triplet decays monitored at 526nm for benzophenone, 450nm for the thiophenes and 420nm for PHT. A short (200ns) time scale was used for these measurements. In addition the triplet decay rate and triplet-triplet absorption spectrum of the ketone were recorded and these agreed with the literature [103].

2.5.7 Determination of E^+ .

An independent determination of E^+ for the sensitizers was carried out following the method of Bensasson and Land [104,105]. The method is subject to a variety of errors and assumptions which limit its accuracy to about 20%. However, this is the most widely used means of estimating E^+ .

Deaerated solutions of the compounds in cyclohexane were prepared such that the D.O. at 308nm was between 0.4 and 0.5. This corresponds to concentrations in the $10^{-4} M$ range.

The triplet decay rates in the absence of quenchers were determined on a long time scale (10 μ s). The top 0.2% of the unquenched triplet was determined on a μ s time scale. Very $1.3 \times 10^{-5} M$ 8-carotene was added. At this concentration the D.O. at 308nm did not exceed 1.3% that of the sensitizers. The top 0.2% of the triplets was again determined together with the

the rate of decay of sensitizer triplet in the presence of quencher. The monochromator was then re-set to the λ_{max} of triplet β -carotene (515nm) and the top O.D. of this transient observed together with the growth rate, k_G . These values were used to calculate ET for each system.

3. Chemicals.

3.1 Sensitizers.

The thiophene type sensitizers were prepared by a nickel-phosphine complex-catalyzed Grignard coupling of thiophene bromides following the method of Tamao et.al. [104]. Phenylheptatriyne was extracted from greenhouse grown Bidens pilosa L. following the procedure of Wat et.al. [33]. YEF was a natural product extracted from Chrysanthemum leucanthemum L. by the method of Wat et.al [33]. The purity of all six compounds was determined by capillary GC and HPLC (impurities <1%). The sensitizers were kept refrigerated and protected from light.

3.2 Solvents and bases.

The following solvents were used in this study: methanol, cyclohexane, methylcyclohexane, benzene, ethyl ether, acetone, ethanol. All were from Aldrich, Fisher or BDH and were of the highest quality commercially available. They were used as received.

The bases used, 4% and 0.1% were Linde products of very high purity (99.9+).

3.3 Reagents.

All-trans- β -carotene (Aldrich) was purified [105] by recrystallization from ether-acetone. The purified material was stored under nitrogen in a freezer.

Benzophenone (Aldrich) was purified [105] by recrystallization from methanol followed by sublimation. The purified material was protected from light and air.

1,3-diphenylisobenzofuran (Aldrich) was purified [62] by recrystallization from methanol-water. The purified furan was stored under nitrogen in a freezer.

Methyl viologen, or paraquat (Aldrich), was purified by double recrystallization from ethanol.

1,3-octadiene (Wiley Organics) was of high purity (99%+) and used as received.

1,2-cyclohexadiene (Aldrich) was fractionally distilled. The fractions distilling between 78° and 79°C contained no polymer material as judged by a methanol spot test [105].

Triethylamine (Aldrich) was purified [105] by shaking with NaOH followed by fractional distillation. The collected fractions distilled at 89°C.

Anthracene (Eastman) was purified by column chromatography [105] followed by vacuum drying.

III. Results.

1. Ground state absorption spectra.

Ground state absorption spectra of the thiophenes in their typical photosensitization region are shown in Figure 13. These spectra (recorded with an air blank) have wavelength resolution of $\pm 2\text{nm}$.

The spectra of α -T and its analogues are all related, showing one transition in this wavelength range (there are two more transitions at higher energy but they will not be considered here). This low energy band is apparently a π - π^* transition [55, 23, 206] to the lowest excited singlet state, S_1 . MO spectra and the fluorescence lifetime obtained in the present study (vide infra) support this view. Splitting of the α -T moiety by a benzene ring (OTB) alters the spectrum markedly; only one band is observed and at much higher energy. Presumably this is also a π - π^* transition based on its large extinction coefficient and the short fluorescence lifetime [10].

Figure 14 shows spectra for the two polyacetylenes examined. Again they were recorded against air and the resolution is $\pm 2\text{nm}$.

The spectrum of PBT shows two separate band systems, both very intense. The lower energy system has well resolved vibrations separated by about 2230cm^{-1} , i.e., the carbon-carbon triple bond stretching frequency [207]. The higher energy system

The spectrometer used in the present work was a photoelectric
spectrometer. In the original experiments the filter used was
a thin film of aluminum. The use of the present type of filter
has made it possible to use the spectrometer in the present work.

Acetic acid extinction coefficients for a series of sensitizers have been determined and their values are tabulated in Table 3 along with the λ_{max} of these compounds. These extinction coefficient values are the averages of two determinations based on Beer's Law. They are precise to about $\pm 7\%$. Figure 15 shows a typical Beer's Law plot for α -T. All systems followed Beer's Law in the zero to $100\mu M$ concentration range. In the cases of α -T and P4T, where values are available [38, 39], the present data agree very well with the literature.

2. Emission spectra.

Figure 16 shows room temperature fluorescence emission and excitation spectra for α -T in cyclohexane while Figure 17 shows the corresponding spectra for DTB. The wavelength resolution is 2nm. Only the spectra of α -T have been reported previously [55,94] and the present data agrees very well with the literature. The emission spectra of MIT and DIT were very similar

Table 3. Absorption and fluorescence characteristic of the sensitizers in methanol at 300K.

Sensitizer	λ_{max}^a nm	ϵ^b $\text{M}^{-1}\text{cm}^{-1}$	λ_{em}^a nm	ν_{OO}^c cm^{-1}
αT	352	22300	426	25100
MIT	362	27900	426	24900
DIT	368	32600	430	24900
DTB	284	27000	359	30600
PHT	310	19310		
YEF	362	34770		

± 2 nm

$\pm 3\%$

$\pm 0.6\%$

Figure 15. Beer's law plot for α -T in benzene.

Figure 16. Room temperature fluorescence (---) and excitation (—) spectra of α -T in cyclohexane. α -T = 5.

Figure 17. Room temperature fluorescence emission and excitation spectra of A-1 in cyclohexane. $[A-1] = 5 \mu M$.

α -T. and its analogues had short fluorescence lifetimes; in

Figure 18. Single photon counting data for ^{125}I PTE in cyclohexane. The solid curve is the fit of the calculator to the experimental emission decay profile. The short lived signal is the lamp profile.

The appearance of A terms in an EPR spectrum indicates transitions from a non-degenerate ground state to a degenerate excited state (22). It has been suggested (23) that the appearance of similar A terms in the MCD of several related polyacetylenes indicates that the corresponding, relatively weak, near-UV absorption bands represent S $\rightarrow$ T transitions. In the case of PHT the presence of A and B terms indicates that both S $\rightarrow$ S and S $\rightarrow$ T transitions may occur between 300 and 400 m μ . These

Table 1. Fluorescence and triplet lifetimes at 300K.

Sensitizer	τ_F^a	$\tau_T^{b,c}$
	ns	ns
CI	30	±3
MIT		11.5±0.5
DMC		9.4±0.5
DTB	24.5	3.1±0.5
PHT		30
YEF		2.8±0.5

^aIn cyclohexane.

^bIn methanol.

^cErrors as ±2σ.

assignments and their implications for the photoreactivity of these compounds. The discussion follows.

5. Transient decays.

Laser excitation (339 or 337 nm) of 10⁻⁵M solutions of each of the test compounds led to the formation of very strong transient absorption signals which have been assigned to the molecular triplet states in each case (see Table 1). Figure 3 shows a typical decay trace for α -I. Those for the other transients were very similar. In each case the decay is predominantly first-order, but at higher laser doses a significant second-order contribution is observed. This may be attributed to triplet-triplet annihilation (11).

6. Transient spectroscopy.

The decay traces observed upon laser irradiation of the sensitizers were identical at all wavelengths examined in this work, that each spectrum (Figures 20 and 21) corresponds to a single transient species. The spectra were essentially the same in all the solvents tested (methanol, cyclohexane) except that the 430 nm band of α -I and the 400 nm band of PHT were slightly broader in the alcohol. The inverted peaks at shorter wavelength should be interpreted as points of maximum difference between the ground state extinction coefficient and that of the transient. The

Figure 1. Absorbance spectra of the solutions of the monomers of triphenyl-
 30 methanol.

Figure 21. Triplet-triplet absorption spectra of polyacetylenes in methanol.

Table 5. Triplet-triplet absorption characteristics of the sensitizers in cyclohexane at 300K.

Sensitizer	λ_{max}^a nm	Isobestic ^a nm	$\epsilon_T^b \phi_{\text{ISC}}^b$ $\text{M}^{-1}\text{cm}^{-1}$	ϕ_{ISC}^c	ϵ_T^b $\text{M}^{-1}\text{cm}^{-1}$
AT	450(550) ^d	338	26550	0.26	102100
MIT	470(550)	402	13900	0.14	99200
-DIT	490(550)	408	10400	0.20	51750
DIB	390(340)	310	2760	0.04	68900
	610				
PHT	400	312	12940	0.12	107800
YEF	410	372			

^a ± 2 nm

^b $\pm 20-30\%$ at λ_{max} of the TT absorption

^c $\pm 40-60\%$ at λ_{max} of the TT absorption

^d () are shoulders.

Figure 22. Effect of laser dose (top O.D.) on the decay rate of thionine photochromic dye in ethanol.

$T(\blacksquare)$, $TTT(O)$, $TTT(\square)$, $TTT(\bullet)$.

It has also been noted that the "zero dose" lifetime is a function of substrate concentration. As in an earlier report [30], we attribute this to self-quenching, e.g.

This is illustrated in Figure 23 for MIT and gives the values for k_{ST} included in Table 6.

3. Quenching studies.

3.1 Oxygen quenching.

All quenching studies were carried out at the λ_{max} of the triplet-triplet absorption unless noted otherwise.

The pseudo first-order rate constants for the decay of all six triplets were determined for solutions containing oxygen at concentrations between zero and 0.003M in methanol or cyclohexane. In the samples containing oxygen the decays followed simple mono-exponential behaviour, even at the higher laser doses. For example, in a methanol solution of $\alpha\text{-T}$ ($2.0 \times 10^{-5}\text{M}$) containing $5.0 \times 10^{-4}\text{M}$ oxygen the lifetime was 400ns and the correlation coefficient for the fit 0.9999.

The rate constants for oxygen quenching (k_q) were obtained by plotting the rate constants for triplet decay, k_{OBS} , as a function of oxygen concentration according to the Stern-Volmer relation [10]

22
OF / DE

Figure 23. Self-quenching of MIT in methanol at 30°C.

Table 6. Self-quenching rate constants for the thiophenes in methanol at 300K.

Sensitizer	k_{SQ}^a $\times 10^{-8} \text{ M}^{-1} \text{ s}^{-1}$
dT	5 ± 1
MIT	3.6 ± 0.6
DIT	15.7 ± 0.2
DTB	$.8 \pm 1$

^aErrors at $\pm 2\sigma$.

$$(36) \quad k_{obs} = k_0 + k_1 I_0^2$$

where k_0 is the rate of decay ($k_0 = 1/\tau_T$) in a degassed solution. Figure 24 shows representative quenching plots for several of the substrates; the corresponding rate constants are given in Table 2.

3.2 Methylviologen quenching.

The reduced form, MV^{+} , of the well-known electron acceptor methylviologen (111), MV^{2+} , has a strong and readily detectable spectrum [100] showing two bands with maxima at 300 and 603nm.

Photoexcitation (333.1nm) of methanol samples of various sensitizers containing MV^{2+} leads to the efficient formation of MV^{+} , which was characterized from its absorption spectrum and monitored at 603nm. The kinetic analysis is similar to that used for oxygen quenching except that k_{obs} is now based on the growth of MV^{+} (rather than on the decay of the triplet). The corresponding plots are shown in Figure 25; the insert shows a typical growth trace for MV^{+} obtained for a sample containing $6.0 \times 10^{-5} M$ MV^{2+} in the presence of I_2 recorded at 603nm. The molecular rate constants obtained are displayed in Table 3 and

Figure 24. Oxygen quenching of thiophene triallates in methanol at 20°C. α -T (○), β -T (●), DTB (□).

Table 7. Energy transfer quenching rate constants for the sensitizers at 300K.

Sensitizer	Quencher Solvent	kQ^a $\times 10^{-9} \text{ M}^{-1} \text{ s}^{-1}$			
		MeOH	O_2 Cy.Hex.	β -carotene Cy.Hex.	Anthracene Cy.Hex.
αT		3.3 ± 0.2	3.7 ± 0.4	17 ± 4	1.7 ± 0.1
MIT		3.4 ± 0.2	2.2 ± 0.2	24 ± 2	1.0 ± 0.1
DIT		3.0 ± 0.1	2.1 ± 0.2	20 ± 4	0.3 ± 0.1
DTB		1.5 ± 0.2	1.2 ± 0.3	12 ± 1	
PHT		3.0 ± 0.4		84 ± 3	
YEF		7 ± 1		8.0 ± 0.2	

^aErrors as $\pm 2\sigma$

Figure 25. Methyl viologen quenching of phosphene triplets in methanol at 300K. Insert shows growth of 1O_2 monitored at 403nm.
 α -T (●), NO_2^- (□), NO_3^- (○).

Table 8. Electron transfer characteristics of the sensitizers in methanol of 300K.

Quencher	MV ²⁺	TEA	
Sensitizer	kG ^a ×10 ⁻⁹ M ⁻¹ s ⁻¹	kQ ^b ×10 ⁻⁴ M ⁻¹ s ⁻¹	
	% Back Electron transfer		
αT	6.8±0.3	80-90	5±1
MIT	9 ±2	90-100	~1
DIT	9 ±2	90-100	3±1
DIB			8±2
PHT	1.4±0.1	~50-60	10±0
YEF	1.5±0.2	30-40	

^aErrors as ±2σ

^bActual errors may be significantly greater than indicated here.

See text.

correspond to the electron transfer illustrated in equation (37) for α -T.

8.3 Back electron transfer.

Several measurements were carried out in order to determine the efficiency of back electron transfer, illustrated here for α -T

It is known that the reduction of MV^{2+} is a reversible process [111]. Thus the extent of back reaction was estimated from the fraction of $\text{MV}^{+\bullet}$ which decayed following their formation. Figure 26 illustrates this process for α -T and PHT. We find that thiophenes generally undergo close to 100% recovery, while acetylenes clearly do not (see Table 8).

No direct evidence for the formation of $\alpha\text{-T}^{+\bullet}$ or $\text{PHT}^{+\bullet}$ was observed. This may be due to spectral screening by the strongly absorbing $\text{MV}^{+\bullet}$ ($\epsilon_{510} = 13000 \text{ M}^{-1} \text{ cm}^{-1}$) [112].

8.4 Energy transfer quenching.

Conjugated dienes are well known triplet quenchers [113, 114]. The quenching rate constants generally approach diffusion controlled when the process is exothermic by at least a few kcal/mol [115]. Quenching studies of the triplet sensitizers (excluding YEF) with 1,3-octadiene (triplet energy, E_T , about 58

Figure 26. Efficiency of back electron transfer to V^{+} from PHT (upper trace) and α -T (lower trace) in methanol. $[V^{2+}] = 3.77 \times 10^{-5} M$, $[PHT] = 2.0 \times 10^{-4} M$.

to 60 kcal/mol) [4] were carried out in a manner similar to that outlined above. α -T, MIT and DIT were not quenched even at rather high concentrations (up to 0.34M). On the other hand DTA and PHT were readily quenched with rate constants of $(3.1 \pm 0.2) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ and $(1.6 \pm 0.1) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, respectively (see Figure 27).

A second series of quenching experiments were carried out using 1,3-cyclohexadiene (ET= 52kcal/mol) [4] as quencher. Again no quenching was observed for the α -T type thiophenes.

A third series of such experiments was performed with all-trans- β -carotene which is also an excellent triplet quencher with very low triplet energy (ET= 23kcal/mol) [116,117,118]. The quenching process can be monitored at or below 450nm where triplet β -carotene is relatively transparent [117] and the sensitizer triplets absorb strongly. Table 7 summarizes the quenching rate constants in cyclohexane at 25°C; the corresponding quenching plots are shown in Figure 28. The insert in this diagram illustrates the spectrum of triplet β -carotene which in this experiment was readily detected following the decay of the donor triplet state. Since $\phi_{isc}=0$ for β -carotene [71] its triplet can be populated by energy transfer only.

Since the α -T type triplets seemed to be rather low-lying, quenching experiments were attempted with anthracene. The triplet of this quencher (ET= 42kcal/mol) [4] lies between those of β -carotene and 1,3-cyclohexadiene.

Anthracene was chosen since it can be readily dissolved in

Figure 27. 1,3-octadiene quenching of $\text{CH}_3\text{CH}=\text{CH}\cdot$ (●) and $\text{CH}_2=\text{CH}\cdot$ (○) triplets in methanol at 70°C.

Figure 26. β -carotene quenching of tripletene triplets in cyclohexane at 300K. Inset shows spectrum of triplet β -carotene. α -T (●), DIT (□), and DIT (○).

cyclohexane at mM concentrations and because of its triplet energy. Unfortunately its triplet can be populated directly by the 308nm laser. To limit this problem the ground state optical density of anthracene at 308nm was kept to 1/10 of that of the thiophenes. Quenching was observed in each case and the rate constants are presented in Table 7. Figure 29 shows the corresponding quenching plots.

To verify that the observed quenching was physical rather than chemical a TT spectrum of α -T containing 80um anthracene was recorded. All observed peaks were due to either anthracene or α -T triplets. At longer times after excitation the triplet α -T signal decayed completely leaving only the anthracene triplet signal (T_T anthracene = 160us) [102]. Furthermore, when the anthracene triplet was monitored at 420nm in the presence of α -T it showed a time resolved growth while the same signal, recorded for anthracene alone did not show this time resolved behaviour (see Figure 30). This indicates that energy transfer does take place between α -T and anthracene. Since the quenching rate decreases as the triplet energy decreases, i.e. on going from α -T to PIT [4], it seems reasonable to conclude that these triplets do not lie much above that of anthracene. Table 9 summarizes the estimated triplet energies for the compounds studied here. The value for PIT agrees with those reported [109] for similar compounds.

Figure 2. Anthracene quenching of thiophene triplets in cyclohexane at 300K. α -T (●), IT (○), PIT (□).

Figure 30. Growth of anthracene triplet in the presence (upper trace) and absence (lower trace) of α -T.

Table 9. Approximate triplet energies of the sensitizers.

Sensitizer	E_T kcal/mol	E_{T_2} ^a kcal/mol
α T	42-47	106-111
MIT	42-47	103-108
DIT	42-47	100-105
DTB	58-63	105-110(131-136) ^b
PHT	58-63	130-135

^aDerived from λ_{max} of the corresponding TT absorption spectrum.

^b() calculated from the higher energy band.

8. Triethylamine quenching.

Excited states are both better electron donors and acceptors than the precursor ground state. To test the electron acceptor properties of the sensitizer triplets (excluding AET), quenching experiments were performed with triethylamine (TEA), a good electron donor.

In each case the measurement was quite difficult because of the slow quenching rate. Table 1 gives the rate constants obtained. It should be noted that reactions which are as slow as these are difficult to measure with laser photolysis techniques and the values may be subject to substantial error. On the other hand the triplets which are the worst electron acceptors based on this experiment are the best electron donors based on the methylviologen experiments (vide supra).

9. Formation of the benzene-iodine complex.

There have been several reports [119, 120, 121, 122] of photo-aromatic compounds losing an iodine atom upon laser excitation. If the experiment is performed in the presence of benzene formation of an iodine-benzene complex is expected [120, 121, 122]. In spite of the apparent photo-stability of ^1IT and ^3IT an attempt was made to detect formation of this complex for the DIT-benzene system.

Air saturated samples of 10^{-5}M ^1IT and ^3IT in benzene were

irradiated with the 308 laser. Figure 31a shows the transient spectrum recorded after decay of most of the DIT triplet signal. Figure 31b shows the transient recorded under identical conditions when $I_2(s)$ was dissolved in benzene. The spectra are essentially the same and agree with the literature spectrum for this same species [121,122]. No corresponding spectrum was observed when a $10^{-5}M$ benzene solution of α -T was irradiated. It seems reasonable to conclude that the long-lived transient observed when DIT is irradiated in benzene is the benzene-iodine complex and is formed due to loss of at least one iodine atom from the DIT moiety.

Figure 32 shows two traces corresponding to decay of DIT triplet and benzene-iodine complex in air saturated (solid line) and oxygen saturated (broken line) benzene. It is evident that the amount of complex formed is independent of the triplet concentration.

10. Singlet oxygen generation.

Since all the test species had oxygen quenching rates on the order of $k_d/9$, where k_d is the diffusion controlled rate constant, it was of interest to determine if singlet oxygen was being generated as well. The method used was that of Merkel and Kearns [62].

Essentially, the method can be described by the following reaction scheme [123]:

Figure 71. The benzene-iodine complex. (a) and (b) in air saturated benzene. Time delay, 7 ns.

Figure 32. The effect of PIT triplet concentration on formation of the benzene-iodine complex; air saturated (solid curve) vs. nitrogen saturated (broken curve) benzene.

where S is the sensitizer; $3O_2$ and $1O_2^*$ are ground state and singlet oxygen, respectively; A is the acceptor; $1O_2$ are the products of the chemical reaction between $1O_2^*$ and A; τ_{SO} is the lifetime of $1O_2^*$ in the chosen solvent; and the k values are the rate constants for the various steps.

Ideally the acceptor should be a compound whose reaction with $1O_2^*$ is well characterized, fast and for which physical quenching is negligible. One of the most commonly used acceptors, one which fulfils these criteria [43], is 1,3-diphenylisobenzofuran (DPBF).

The reaction of $1O_2^*$ with DPBF leads to a peroxide product [48]

The rate constant of this reaction in methanol is fast (about 10^8 s^{-1}) [49] and there is no measurable physical quenching.

[403].

With this acceptor and using pulsed (< 2 ns) excitation the two main pathways for decay of 1O_2 are steps 3 and 4 above. If [DPPF] does not change by more than about 10^{-10} during this type of experiment it can be shown that [133]

$$(42) \quad [A]_t - [A]_{\infty} = (k_4 I_2 + 7k_5 [A]_{av}) \exp(-t/\tau_{SO})$$

where $[A]$ on the right hand side is assumed to have a constant value $[A]_{av}$; $k_4 = k_3 + k_4[A]_{av}$, $k_5 = k_4 + k_5$ and I_2 is the concentration of singlet oxygen at time equal to zero. From this it follows that

$$(43) \quad -\ln([A]_t - [A]_{\infty}) = (1/\tau_{SO} + k_5[A]_{av})t + \text{Constant}$$

or

$$(44) \quad -\ln(O_2^t - O_2^{\infty}) = (1/\tau_{SO} + k_5[A]_{av})t + \text{Constant}$$

where O_2^t is the optical density of the acceptor measured at some convenient wavelength (440 nm in this case) as a function of time, t . Thus, plots of $-\ln(O_2^t - O_2^{\infty})$ vs t should be linear, based on first-order kinetics, and, when determined at two different $[A]_0$, should give values for $1/\tau_{SO}$ and k_5 . The method can therefore give qualitative information regarding singlet oxygen generation.

Figure 33 shows the bleaching of 440 m DPPF in the presence of nM concentrations of α -T and PHT in air saturated methanol. The initial rapid decay represents relaxation of the triplet sensitizer. Calculation of the kinetics for the bleaching

Figure 33.. Bleaching of 44 μ M DPBF in the presence of mM α -T (top frame) and PHT (bottom frame) in air saturated methanol at 300K.

portion of the curve gives a very good first-order fit in both cases. Good first-order bleaching was observed for the other four sensitizers as well. Furthermore if one uses the data shown in the first two columns of Table 10 to calculate k_1 and T_{50} (by solving the resulting simultaneous equations based on relation (3)), one finds these to be in agreement with literature data.

None of this is conclusive evidence that $1O_2$ is being generated by these systems. However, in view of the large body of data showing that α -T, β -T, γ -T, δ -T and chlorophyll *a* act as $1O_2$ sensitizers it seems safe to conclude that this is also true of α -T, β -T, γ -T, and δ -T.

The bleaching traces of Figure 10 were obtained using critically matched solutions. One may therefore take 0.000 as a rough measure of the amount of peroxide product formed, i.e. the amount of $1O_2$ formed. All compounds tested show roughly equal values. From this one may conclude that the $1O_2$ generating capacity of all six sensitizers is approximately the same. This agrees with data published previously for α -T and β -T (10). It must be kept in mind that this conclusion is based on purely qualitative data and is therefore open to criticism.

11. Determination of E^* vs. λ .

There are a variety of methods available for estimating extinction coefficients of various species (11). One of the better ones is to use a secondary standard. In this case:

Table 10. Lifetime of singlet oxygen and rate of it's reaction with DPBF in methanol at 300K calculated from bleaching rate of DPBF in the presence of α T and PHT.

Sensitizer	[DPBF] M	$k_B \times 10^{-5}$ $M^{-1}s^{-1}$	τ_{SO} μs	$k_A \times 10^{-8}$ $M^{-1}s^{-1}$
α T	44	2.91	10	21
	88	2.84		
PHT	44	2.06	5	1.4
	88	2.25		

^a k_B =rate constant for bleaching of DPBF in the presence of a sensitizer.

molecule leading to a transient of known extinction coefficient is used as a reference and is compared to an optically matched solution containing the precursor of the unknown transient. For samples of equal and low optical density for which ground state depletion is minimal it can be shown [124,125] that

$$(45) \quad E_T \phi_{ISC} = (\Delta OD_u / \Delta OD_r) E_T \phi_{ISC}$$

where u and r refer to unknown and reference standards respectively.

In this study the reference substrate used was benzophenone in benzene, for which we used a value of $E_T = 7640 M^{-1} cm^{-1}$ and $\phi_{ISC} = 1.0$ at 525nm [102].

It was not convenient to use 525nm as the monitoring wavelength for these experiments so a TT spectrum of benzophenone in benzene was obtained in order to determine values for E_T of benzophenone at other wavelengths. In particular $E_{T450} = 2983 M^{-1} cm^{-1}$ and $E_{T420} = 2204 M^{-1} cm^{-1}$. ϕ_{ISC} was assumed to be independent of wavelength.

The values of $E_T \phi_{ISC}$ for five of the sensitizers are tabulated in Table 5. Given the relatively large spread of literature values for benzophenone triplet's E_T [102] the true errors in $E_T \phi_{ISC}$ are probably around 20-30%.

12. Determination of ϵ_T .

The most common method for obtaining separate values for ϵ_T was originally proposed by Land [103] and is based on an energy transfer.

In a typical system a donor substrate (D) is excited by the laser. The sample also contains an acceptor molecule (A). The following scheme may be used to describe the system

where k_0 is the rate constant for donor decay in the absence of acceptor, k_1 is the rate of donor decay in the presence of acceptor ($k_1 = k_0 + k_0[A]$) and k_2 is the decay rate of acceptor triplet.

By selecting a suitable reference substrate to act as either donor or acceptor it is possible to quench essentially all the donor triplet with a small amount of quencher. Further, if the acceptor quenches sufficient donor triplet so that $k_2 \ll k_1$ on the time scale of the experiment one may write [71]

$$(47) \quad \epsilon_{Td} / \epsilon_{Ta} = \Delta OD_{Td} / \Delta OD_{Ta}$$

where

$$(48) \quad \Delta OD_Ta = \Delta OD_Ta(max) [k_1/k_1 + k_0]$$

ϵ_{Td} and ϵ_{Ta} are the extinction coefficients for the donor and acceptor triplets, respectively; ΔOD_{Td} is the maximum optical density of the donor triplet (measured at its own λ_{max}) in the absence of acceptor; $\Delta OD_{Ta}(max)$ is the maximum observed optical density of the acceptor triplet (at its λ_{max}) in the presence of donor. It is immaterial which triplet is used as the standard - acceptor or donor. In this study all trans β -carotene was used as acceptor (and reference) and the sensitizers were used as donors.

The validity of this technique involves a series of assumptions which are outlined here [103]. 1) The donor triplets should not react chemically with either the solvent or the acceptor. No evidence was found to suggest any of the five sensitizers tested reacts with cyclohexane or with β -carotene. 2) All acceptor triplet states are formed via energy transfer from the donor. Since β -carotene has $\phi_{ISC}=0$ [71] its triplet cannot be populated by irradiation. In every case the β -carotene triplet signal was observed to show time-resolved growth which indicates that quenching is via energy transfer. 3) Each donor triplet gives rise to one acceptor triplet. This is generally held to be true [71,103] but is not readily testable. This assumption is the weak point in this method. If it is incorrect, i.e. if production of acceptor triplets from donor triplets is less than 100% efficient, the values of ϵ_T obtained by this method would be low. 4) The donor singlet states formed are too short-lived to

interfere. In this study the lifetimes of the singlet donors are $<10\text{ns}$ in each case so they cannot interfere with the determination of ET.

All-trans- β -carotene was a good choice for acceptor because of its low triplet energy and negligible ISC. The triplet β -carotene absorption is fairly narrow [117] allowing for the separation of the donor and acceptor signals based on judicious choice of monitoring wavelength. Its extinction coefficient is quite large (130000 to $170000\text{ M}^{-1}\text{cm}^{-1}$) [102] so its triplet absorption can be readily distinguished from that of the donor in regions of overlap. Furthermore, preliminary tests showed that a small amount (10^{-5}M) of β -carotene could quench most of the triplet generated by 10^{-4}M donor. Therefore, even though β -carotene does absorb the laser it was possible to keep its ground state OD at 308nm to about $1/10$ of that for the donor and still obtain significant quenching. Finally, the lifetime of triplet β -carotene ($12.5\mu\text{s}$) [71] is long enough that its rate of decay may be ignored on the time scales used to determine k_1 for the donors.

Table 5 gives IT extinction coefficients for the various sensitizers at the wavelengths indicated. Again, because of the range of ET values available for β -carotene, these values cannot be accurate to much more than 30%.

Table 5 also gives the values of ϕ_{ISC} obtained, at the wavelengths indicated, by dividing the values of ET/ISC by the extinction coefficients given here. The estimated error in these

values is about 60% which is rather large. On the other hand the ϕ ISC values are all small so that a 60% error in the case of α -T corresponds to a range of ϕ ISC values between 0.11 and 0.42. i.e. values of ϕ ISC are still low. Therefore any conclusions based on the values of ϕ ISC being small are still valid.

The values of E_T and ϕ ISC for α -T agree with those reported for α -T in methanol ($E_T = 85000 M^{-1} cm^{-1}$, ϕ ISC = $0.20 \pm 20\%$) [65]. The value of E_T for PHT agrees with an earlier report from this laboratory [126] in which its extinction coefficient in heptane was estimated to be $70000 M^{-1} cm^{-1}$ by the singlet depletion method [71].

10. Discussion.

10.1 Absorption and emission spectroscopy.

The lowest energy absorption band of α -T (ant. 277 nm, ϵ 1.5 species) has been taken to be a π - π^* transition [see 27, 274]. This has been supported by the large extinction coefficient, the small red-shifting (change from polar to nonpolar solvents), and the lowest fluorescence quantum yield. In addition, the lowest energy transition in thiophene monomer is known to be π - π^* [107]. The very short fluorescence lifetime measured in the present study supports this assignment.

It seems likely that this low energy band is the $S_0 \rightarrow S_1$ transition of α -T. This is supported by the observed energy, the simple MO spectrum (R term only) and the observation that the fluorescence emission is in approximate mirror image of the low energy band of the absorption spectrum [74].

The present study is the first to report excited state properties of "IT", "BIT" and "OTB". An earlier report gives data on mono- and diiodo- α -bithienyl [27] which agree with those of the present study. That is, upon increasing iodination the intensity of the lowest absorption band of the α -T system increases while the band maximum shifts to lower energies. As in the case of α -T we propose that the lowest energy transitions of the iodinated analogues are π - π^* absorptions from S_0 to S_1 based on similar arguments.

Breaking the α -T moiety, as in the case of DTA, leads to significant changes in the absorption spectrum. Only one band is observed in the accessible ultra-violet and this is at much higher energy than the lowest energy band of the α -T systems. However, the short fluorescence lifetime, high extinction coefficient and the mirror image relationship between the fluorescence emission and absorption spectra suggest that this is again a $S_0 \rightarrow S_1$ transition of the $\pi-\pi^*$ type.

Laser excitation of all substrates leads to population of their lowest lying triplet state. It is clear that the TT spectra of α -T, MIT and DIT are all related indicating that the chromophore is the same in each case. The very high extinction coefficients for these transitions show that they are strongly allowed.

If the assignment of the S_1 state of these molecules as $\pi-\pi^*$ is correct, a corresponding assignment for the triplet states might be expected since the T_1 level of organic molecules is generally derived from the same electronic orbital configuration as the S_1 level [1]. Furthermore the estimated S_1-T_1 energy splitting for these molecules is about 30 kcal/mol for α -T, MIT and DIT. Such splittings correspond to those commonly observed for $\pi-\pi^*$ states [1]. Therefore the T_1 states of the α -T series triplets are assigned to be $\pi-\pi^*$ in first order, ignoring interelectron repulsion and spin orbit coupling. Self-quenching results also support this conclusion (vide infra).

Iodination broadens the TT band of α -T. However, the decrease in intensity and red shift observed may be attributed to corresponding behaviour in the ground state. That is, these changes in the triplet signal are due to increased bleaching by the ground state.

The triplet spectrum of PIT is quite different from those of the other thiophenes with λ_{max} at 375 nm (as compared to 250 nm for α -T). In addition a second, weaker band is observed at 410 nm. Both of these bands exhibit the same decay kinetics indicating that they are due to the same transient. The two bands probably represent two different TT absorptions from T₁ or T₂. Quite clearly "breaking" the thiophene ring drastically alters the characteristics of the chromophore.

As in the α -T series, the T₁ state of PIT is also assigned as $\pi-\pi^*$. The S₁-T₁ splitting is about 70 kcal/mol in this case.

The TT spectra of PIT and VET are also closely related. The absorption band of the latter molecule is slightly broader but occurs at essentially the same energy as that of the former. This again implies that the same chromophore (the acetylene group) is involved in both cases.

If we take 250 nm as an approximate value for the S₀ $\rightarrow$ S₁ energy of PIT (vide infra) the results of energy transfer quenching experiments give an S-T splitting on the order of 50 kcal/mol. The lowest triplet of PIT is therefore also probably

$\pi-\pi^*$.

7. Excited state lifetimes.

For the thiophene series the measured fluorescence lifetimes are quite short indicating that the process

is strongly allowed [1,7,73].

Although it is spin forbidden, quenching of excited singlet states by ground state molecular oxygen is well documented [1,73]. This process amounts to oxygen enhanced intersystem crossing and may lead to singlet oxygen if the S_1-T_1 energy gap is greater than the singlet oxygen excitation energy (22 kcal/mol) [49]. For all thiophenes little oxygen-quenching of S_1 may take place because of the short lifetimes. This may be illustrated by a solution of NTB ($\tau_{S_1} = 6$ psec) in air saturated cyclohexane. This system is about 2.3×10^{-4} M in oxygen [4] and the diffusion controlled rate constant is $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ [47]. Therefore S_1 of NTB will undergo 0.15 collisions with oxygen within one radiation half-life (this calculation assumes that it is the oxygen molecule that is diffusing). The same argument applies to the α -T series and implies that generation of singlet oxygen from thiophene S_1 states need not be considered in discussions of their role as type II phototoxins.

All the species examined here have relatively long triplet lifetimes in fluid solution. This is consistent with the known singlet oxygen generating capacity of α -T₂ thiophenes as a class

and PHT [21,32,39,60,61].

In the course of this study it was observed that the triplet lifetime of PHT decreased as solutions of the substrate aged. The value of this triplet lifetime given in Table 4 was determined with a fresh solution. YEF was available in alcoholic solution only because of instability of the solid. Therefore the triplet lifetime obtained in this study is probably too short.

A variety of factors were found to affect the triplet lifetime of the thiophenes. As expected, iodination decreased the lifetime in the α -T series. This may be attributed to enhanced $S_1 \rightarrow T_1$ intersystem crossing as a result of greater spin-orbit coupling [1,3,73]. In addition to this heavy atom effect, the thiophene systems also exhibit triplet-triplet annihilation and self-quenching.

At low triplet concentrations, bimolecular interactions between these excited states are improbable. However, if T_1 is long-lived and is formed at fairly high concentrations, such interactions may occur resulting in a rate law for triplet decay of the form [1]

$$(5F) \quad \text{Rate} = k_D[T1] + k_{TT}[T1]^2.$$

Therefore at higher triplet concentrations a second order contribution to the decay should be observed. Such is the case for the thiophenes and PHT. Second order contributions were evident at higher laser dose for a given substrate concentration. This indicates a process of the type

occurs [11]. Decay of S_1 , when generated by TT annihilation, should appear as delayed fluorescence. No attempt to detect S_1 in emission was made in this study.

Self-quenching (the variation of "zero" laser dose triplet lifetime as a function of substrate concentration) was first reported for α -T by Seyfranz et al. [55]. This has been confirmed by our work and extended to other thiophene derivatives. It is worth noting that $n-\pi^*$ states are less prone to self-quenching than $\pi-\pi^*$ states [12]. The self-quenching rate constants obtained here are quite high indicating that this is an efficient process. This is further evidence to support the assignment of thiophene T_1 states as $\pi-\pi^*$.

3. Energy transfer quenching; state energy diagrams.

Estimates of the energies of the T_1 states of the thiophenes and PBT were derived from comparative energy transfer quenching studies. When 1,3-octadiene and anthracene were used as quenchers the rate of quenching, while rapid, was not diffusion controlled. This is usually taken to mean that the triplet level of the energy donor is not more than about 5 kcal/mol above that of the quencher [113, 114, 115]. These rates may be compared to those obtained using the much lower energy p-carotene triplet as quencher. In the latter case quenching was always diffusion controlled.

Using the values of Table 2 along with the estimates

$S_0(v=0) \rightarrow S^1(v=0)$ energies of α -T and DPB, and taking 250nm (114 kcal/mol) as the value of this energy for PHT (vide infra), we may generate an approximate state energy diagram for each of these molecules. These are shown in Figure 34, and give energies, multiplicities and tentative state types. These may be compared to the potential energy curve diagram of molecular oxygen presented in Figure 9. It is immediately evident that any of these molecules can populate the singlet states of oxygen by energy transfer.

Oxygen quenching of all sensitizer triplet states is rapid but significantly less than the diffusion controlled rate. Except for YEF, all oxygen rate constants are on the order of $k_d/9$ i.e., oxygen quenching via energy transfer. This is supported by the observation of DPBF bleaching in each case. We conclude, therefore, that all systems examined may act as singlet oxygen sensitizers.

4. Electron transfer reactions.

Excited states are expected to be both better electron donors and electron acceptors than the corresponding ground states. The rate constants for reaction with the electron acceptor methyl viologen show that thiophene and acetylene triplets are excellent electron donors.

An interesting difference is observed between the back electron transfer reaction efficiency of the α -T series and that of the acetylene systems. In the latter case the back transfer is

Figure 34. State energy diagrams for α -T (I), 24T (IV) and 24T (V).

only 50% efficient suggesting that PHT^+ (or YFF^+) is a rather unstable species which finds alternate modes of decay in addition to reaction with MV^+ . The thiophene radical cations on the other hand, undergo extensive recombination with MV^+ to give the ground state starting materials.

All four thiophene triplets and that of PHT are relatively poor electron acceptors as indicated by the low rates for reaction with triethylamine.

5. Carbon-iodine bond cleavage.

The spectra shown in Figure 31 are very similar and indicate that photolysis of DIT in the presence of benzene leads to cleavage of at least one of the C-I bonds followed by formation of a complex with benzene [117,120,121,122]. The present study is the first to report such behaviour for terthiophene systems. The earlier work concentrated primarily on halogenated aromatic hydrocarbons.

In the case of DIT the present results indicate that dissociation does not proceed via T1. This is based on the observation that oxygen quenching of T1 does not decrease the amount of complex formed (see Figure 32). This is consistent with earlier observations since the energy of T1 is 42 kcal/mol while the bond dissociation energy of the C-I bond in iodobenzene is 64 kcal/mol [121]. Even if the thiophenic C-I bond were 20% weaker than this, the triplet energy of DIT would be insufficient to break it. The dissociation of the C-I bond in DIT is apparently

due to channeling of the S^1 excitation energy into some dissociative $\pi\pi^*$ singlet state localized in the C-I bond. This is in contrast to the proposed mechanism in the corresponding bond cleavage of iodo-aromatic hydrocarbons. In these molecules the triplet energies are on the order of the C-I bond strength and a dissociative $\pi\pi^*$ triplet is thought to be involved [117,121]. This concept is used to explain the very short room temperature lifetimes (10-100ns) reported for these triplets [117,129]. The comparatively longer triplet lifetime of PIT is further evidence that this state does not mediate the breaking of the C-I bond.

b. Mechanism of the type I behaviour of polyacetylenes.

The MCD of A-T in the photosensitizing region is a typical B term indicating that this transition is purely $S \rightarrow S$ in character [9,79,80]. On the other hand the appearance of strong A terms superimposed on a weak B term in the MCD of PHT is an unexpected and unusual result. Such A terms indicate transitions from a non-degenerate ground state to degenerate excited states ($S \rightarrow T$ for example) [9,79,80]. A recent article [110] reports the appearance of similar A terms in the MCD of other polyacetylenes without ring-stabilization. An explanation has been proposed which should apply to PHT as well.

The absorption spectra of unsubstituted polyacetylenes ($n \leq 5$ triple bonds) have been examined in the gas phase and dilute solution. Two intense band systems have been observed and

assigned as $S \rightarrow S$ transitions [130] resulting in a bent (trans) excited state [131]. A bent excited state means that any orbital degeneracies for singlet spin states which might be expected to arise based on the high symmetry of the ground state would be broken and could not give rise to A terms.

A recent theoretical examination of di and triacetylene has shown that, in addition to the two singlet states, at least two triplets are expected at lower energy [132]. Such transitions are normally forbidden because of the spin selection rules but may gain intensity from enhanced spin-orbit coupling through conjugation of multiple C-C bonds [133]. In the case of PHT the band centered at about 310nm is quite intense ($\epsilon = 19300$ M⁻¹cm⁻¹) but is relatively weak compared to the transition at 250nm ($\epsilon = 101000$ M⁻¹cm⁻¹). This suggests that the former is forbidden to some extent.

Herzberg has defined several criteria to distinguish between different types of forbiddenness in transitions [134]. Axis switching [135] (corresponding to a change of symmetry) or $S \rightarrow T$ transitions are the most important sources of forbiddenness. Both are expected to obey unusual selection rules. That the long wavelength absorptions of PHT do so is suggested by the fact that both of the acetylenic $S \rightarrow S$ transitions may be accounted for at shorter wavelengths.

Axis switching and $S \rightarrow T$ transitions may be readily distinguished as the latter is expected to show a strong Zeeman effect while the former is not. Therefore appearance of A terms

in the MCD of PHT, combined with the relatively low intensity of the corresponding absorption bands, suggests that the band system centered around 310nm has $S \rightarrow T$ character. The fact that the A terms are superimposed on a weak B term suggests that these transitions also have some $S \rightarrow S$ character. Therefore the ring-stabilized polyacetylene PHT is spectroscopically intermediate between α -T (MCD B term only) and polyacetylenes without ring stabilization (MCD A terms only).

If we draw the conclusion that the "fingerlets" of PHT are due to the $S_0 \rightarrow T_1$ absorption we must also conclude that this transition is strongly allowed based on its extinction coefficient. However we know from the LFR data that the lifetime of this triplet, even in fluid solution, is quite long (300ns) which indicates that the process $T_1 \rightarrow S_0$ is not favourable. We can explain this discrepancy by suggesting that the initial excitation is $S_0 \rightarrow T_n$ rather than to T_1 . This T_n state rapidly relaxes to T_1 from which relaxation to S_0 is inefficient. This would also account for the difference in the value of the $S_0 \rightarrow T_1$ energy obtained from quenching studies and the energy of the longest wavelength absorption of PHT. That is, if these bands do represent the $S_0 \rightarrow T_1$ transition, the vibrational structure is well defined enough to take 330nm (87 kcal/mol) as the 00 energy. The quenching studies give a value of only 60 kcal/mol. The difference would be explained if the "fingerlets" are due to a $S_0 \rightarrow T_n$ transition.

If this assignment of the PHT "fingerlets" is correct the

absorption band at 255nm would be the $\pi \rightarrow \pi^*$ transition (vide supra).

Both PHT and YEF transfer an electron efficiently at a rate comparable to that at which they are quenched by O_2 . However, back transfer is inefficient, leading to a radical species which decays by routes other than recombination with O_2 . This suggests that radical acetylene species are important to the phototoxicity of these systems. This is supported by earlier work on the flash photolysis of diacetylene [64], the lack of an oxygen requirement for PHT phototoxicity [59] and the extensive photodegradation of polyacetylenes [30].

Triplet states are diradical like in nature since they involve two unpaired electrons. The $\pi \rightarrow \pi^*$ band system of PHT involves a transition within the acetylene group as the vibrational spacing (2030 cm^{-1}) corresponds closely to that of the carbon-carbon triple bond stretch [17]. The promotion of an electron directly to one of the vibronically coupled triplet states (which are primarily π^*) would therefore weaken a triple bond. This directly populated vibronic triplet state (T_0), or a dissociative state derived from it, appears to initiate PHT radical formation by leading to breaking of a triple bond. There is some experimental evidence to support this scheme. Flash photolysis of diacetylene vapour resulted in formation of C3 but not C2 fragments [64].

While the weakness of the carbon-carbon single bond, relative to the corresponding triple bond, may be important in

determining the thermal reactivity of acetylenes, the π is not observed for photochemical reactivity. In the photoreactions studied here the π anti-bonding nature of the excited state is dominant, leading to triple bond cleavage. Thus proposed reactions for type I behaviour have the general form of a homolysis of the triple bond system:

The free radical fragments may then proceed to secondary chain reactions in the surrounding medium.

The semi-oxidized P^{+}T^{-} radical is likely to be the potent agent involved in non-oxidative sensitizations observed with P^{+}T^{-} (33,532). Photoreactions involving radical S_2 but not necessarily formation of O_2 radical S_2 are the typical species in several other phototoxic reactions. For example, the antibacterial activity of anthraquinone methanols (1753). In addition radical processes have been proposed to explain photopolymerization of vinyl monomers containing synthetic diacetylenes (1754).

Mechanisms of type II behaviour of A^+ and diacetylenes.

Triplet A^+ is formed with great quantum yield and the acquired energy is sufficient to generate singlet oxygen via energy transfer. This conclusion is supported by the observed oxygen quenching rate constant k_q and the reactions of

22. In addition it has been recently shown that the quantum yield of singlet oxygen from α -T triplets is close to unity (53).

The "00 indicates that the absorption band of α -T used for photosensitization leads to excitation of a singlet state of α -T and therefore conclude that the T_1 state of α -T is populated primarily by intersystem crossing from S_1 .

Although α -T triplet is an efficient electron donor the reaction is highly reversible. This state is also a very poor electron acceptor. These results suggest that direct electron transfer reactions are important in the phototoxicity of α -T. In addition, the recent article by Refnans et al (55) has shown that triplet α -T does not abstract hydrogen from cysteine, a poor hydrogen donor.

These results indicate that α -T functions as a photosensitizer in which the singlet excitation energy transfers to O_2 by intersystem crossing. The T_1 state can then interact with ground state molecular oxygen to give singlet oxygen.

This conclusion agrees with earlier in vitro and in vivo results (21, 22, 38, 40, 41) and explains the oxygen requirement for the near UV mediated phototoxicity of this compound (52).

Generation of singlet oxygen via energy transfer is the simplest apparent explanation for the observed oxygen requirement for thiophene phototoxicity. However, recent spectroscopic results

... a more detailed analysis of the excited state ...
usually identified as ...
Thiophene ... is an aromatic system with a resonance
stabilization close to that of benzene ...
the p orbitals of sulfur contribute to the π electron
delocalization ...
suggests that while sulfur orbitals have little effect on the
total energy of the molecule ...
electron density of the sulfur atom at the expense of ...
... This indicates that there is
substantial interaction between the ... and the π system.

It has recently been shown ... that the lowest excited
state of thiophene is ...
This ... resonance
stabilization in the excited state since the overlap of the
atomic orbitals with the ... π orbital orbitals is ...
... expect an interaction which ...
the π electron density to be ...

Ground state molecular orbital has ... π ...
orbitals and may therefore ... as a π ...
... is a ...
reasonable then that ... between ... and the ...
... of excited state thiophene ...
... of π electron density from the thiophene ...
... into the ... of ... This process would be ...
by the ... orbitals as indicated in Figure 3A. This

Figure 75. The thioether-epoxide π complex: (a) orbital interaction between thioether and epoxide; (b) hypothetical intermediate.

electron donation would decrease the ionization character of the excited state, permitting the thiophene to retain planar geometry and the resonance stabilization associated with π overlap of carbon $p\pi$ and sulphur d orbitals.

Since the LUMO of α_2 is π^* , an interaction of this sort would weaken the oxygen-oxygen bond resulting in an intermediate complex like that shown in Figure 35b. Such a species would be subject to electrophillic attack at the terminal oxygen atom in biological media leading to radical and/or ionic species. Such reactive species would certainly be relevant to the phototoxicity of thiophenes. Although this analysis is tentative it provides a detailed, rational alternate mechanism to energy transfer. This model may be tested more fully in further studies.

In general, the points just noted may be extended to the other thiophene systems. However, the C-I bond cleavage results obtained with PHT suggest that radical mechanisms may be relevant when halogenated thiophenes exhibit phototoxicity.

The situation is somewhat different for the polyacetylene systems. First of all it is evident that such molecules may first generate singlet oxygen based on the triplet energy, oxygen quenching rates, triplet quantum yields and furan bleaching observed with PHT. This agrees with the results of earlier work based on the phototoxic activity of PHT towards microorganisms [32]. This type II behaviour of polyacetylenes can now also be rationalized as a successive step to the initial type I photolysis of the carbon-carbon triple bond. The radical fragments are both

strong Lewis bases, capable of donating electron density to any acceptor species, including oxygen molecules. As with the thiophene-oxygen complex, the O_2 molecule can act as a π acid in an end-on complex with a polyacetylene fragment, accepting the unpaired electron into its π^* orbital:

The electron migration again makes the terminal oxygen atom subject to electrophilic attack.

In summary, then, the evidence collected in the present study supports the view that A-T is a type II sensitizer while PHT may act as both a type I and a type II sensitizer. Related thiophenes and acetylenes show similar behaviour. We therefore expect A-T phototoxicity to be mediated only by singlet (or complexed) oxygen while that of PHT may involve singlet (or complexed) oxygen or radical species depending on the reaction conditions of the reaction system. These conclusions agree with those of several workers in the field but are in contrast to those of Kacan et al. (67, 68) who suggest that PHT is a type I sensitizer only. Radical formation due to carbon-halogen bond cleavage may also play an important role in any observed phototoxicity of halo-thiophenes.

8. New directions.

Several areas for future research are suggested by the thiophene results. One of the most useful would be an attempt to establish a relationship between structure and phototoxicity. In the present study too few variations of structures have been examined. A continuation of this work including structures with electron donating and withdrawing substituents as well as mixed thiophene-acetylenes would be valuable. Halogen substituted systems are of particular interest in order to extend the preliminary results obtained here relating to the C-I bond cleavage.

Although it is now established that PHT^+ exists and does not recombine quantitatively with MV^+ , its final fate is still unknown. Further investigation of the PHT type radicals is indicated. An electron spin resonance study would be the most obvious approach. Another possibility would be to vary the electron accepting capacity of species used to quench triplet PHT. This would give an approximate value for the oxidation potential of PHT. Knowing this would give more detailed insight into the type of conditions that favour PHT radical formation.

Nothing is known of the ability of excited state polyacetylenes to abstract hydrogen. This is an important type I process and should also be examined.

The observation of high intensity $S \rightarrow T$ absorptions in the acetylene systems is a very unusual result and clearly requires more extensive study. Some obvious questions are: if this

transition is from S_0 to T_0 what vibronic triplet is initially populated? how long-lived is it? does the rate of population growth of T_1 match the rate of decay of the population of T_0 ? Such questions could best be examined using LFP with ps resolution by which the behaviour of the various triplet states might be time-resolved.

A final consideration is the magnitude of the spin orbit coupling mechanism which lends such high intensity to the $S \rightarrow T$ transitions of these polyacetylenes. It must be unusually large when one considers that the extinction coefficients of $S \rightarrow T$ transitions of aromatic hydrocarbons do not generally exceed 10^4 l cm^{-1} , even in the presence of heavy atoms [74]. At any rate it is evident that the spectroscopy of polyacetylenes is not as straightforward as their simple structures might suggest.

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