Kinetic and Energetic Aspects of the Photodimerisation of Some Systemic Pyrimidine Fungicides

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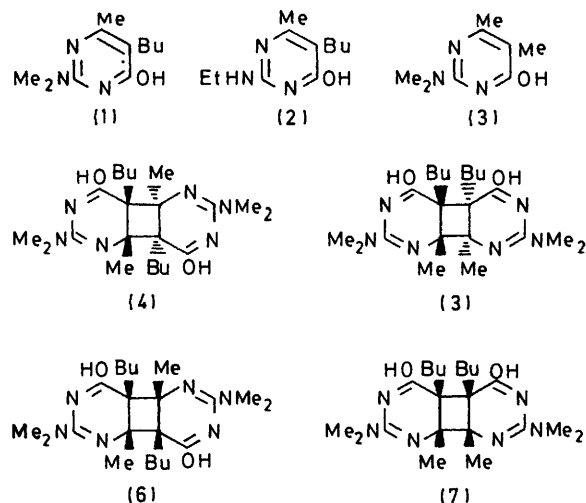
The photodimerisation of 2-dimethylamino-4-hydroxy-6-methyl-5-n-butyl- (1), 2-ethylamino-4-hydroxy-6-methyl-5-n-butyl- (2), and 4-hydroxy-5,6-dimethyl-2-dimethylamino-pyrimidine (3) has been investigated in acetonitrile. Quenching experiments have shown that the formation of photodimers involves a triplet state of (1)—(3), the energies of which have been estimated. The quantum yields for intersystem crossing from the singlet to triplet manifolds of (1)—(3) fall in the range 0.09—0.11, and the quantum yields for photodimerisation fall in the range 0.07—0.08. Rate constants for radiationless decay of the triplet state pyrimidines have been determined (2.1—3.0 × 10^5 s^{-1}), and also the rate constants for reaction of the triplet states of (1)—(3) with the corresponding ground state pyrimidine (1.5—2.0 × 10^8 l mol^{-1} s^{-1}). The efficiency of the triplet state dimerisation reactions of (1)—(3) is high compared with that of the analogous triplet state reactions of the biologically important pyrimidines, thymine and uracil.

EXPOSURE of agricultural chemicals to u.v.–visible radiation may reduce their active lifetime and may give rise to a variety of products. Consequently it is important to have an understanding of the mechanism whereby such chemicals undergo photochemical reaction so that, if necessary, the reactions can be prevented or modified.

2-Dimethylamino-4-hydroxy-6-methyl-5-n-butyl- (1) and 2-ethylamino-4-hydroxy-6-methyl-5-n-butyl-pyr-

imidine (2) are used as systemic fungicides for the control of powdery mildew on cucurbits and cereals re-pectively,[1,2] and we report herein on the mechanism of the photoreactions of (1) and (2) in acetonitrile, and of

(1)   (2)   (3)

(4)   (3)

(6)   (7)

the structurally related compound 4-hydroxy-5,6-dimethyl-2-dimethylaminopyrimidine (3), in the same solvent. The products from each of these photoreactions have been characterised previously [3] as stereoisomers formed by dimerisation across the 5,6-bond of the parent pyrimidine; the structures of the products from (1) being (4)—(7).
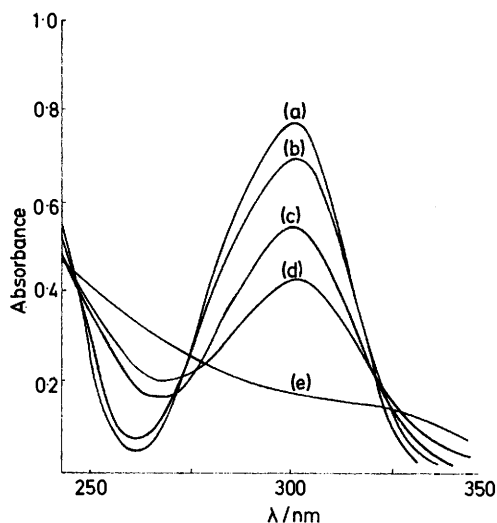

FIGURE 1 Absorption spectrum of a 4.0 × 10⁻⁴M degassed solution of (1) in acetonitrile after photolysis for (a) 0, (b) 6, (c) 15, (d) 20, and (e) 30 h

RESULTS AND DISCUSSION

Photolysis of (1)—(3) in degassed acetonitrile solutions with Hg radiation in the range 290—330 nm brings about photochemical reaction as indicated by the disappear-

ance with time of the longest wavelength absorption band of the substrate. A typical set of absorption spectra recorded after different photolysis times are shown in Figure 1. Monitoring the disappearance of the substrates with time gave plots which showed that the reactions followed first-order kinetics. A plot for (1) is shown in Figure 2(a); similar plots were obtained for (2) and (3).

The effect of added cis-penta-1,3-diene on the photo-reaction of (1) is also shown in Figure 2, where it can be seen that the added diene virtually completely quenches the reaction. The same effect was observed for the photolysis of (2) and (3) in the presence of cis-penta-1,3-diene. G.l.c. analysis of the reaction mixtures revealed that trans-penta-1,3-diene had been formed as a result of the photolysis. Since cis-penta-1,3-diene does not absorb
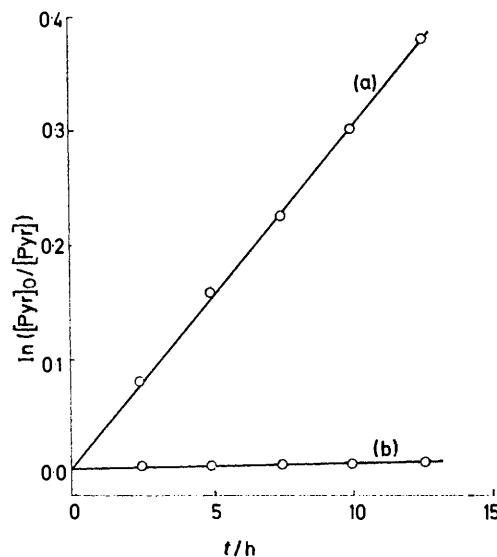

FIGURE 2 First-order kinetic plots for photolysis of a 5.0 × 10⁻⁴M degassed solution of (1) in acetonitrile in (a) the absence of cis-penta-1,3-diene and (b) the presence of cis-penta-1,3 diene (1.0 × 10⁻²M)

at the wavelengths used for irradiation it is apparent that energy transfer from the pyrimidines had brought about the cis–trans isomerisation of penta-1,3-diene. As the energy of the lowest excited singlet state of cis-penta-1,3-diene is 544 kJ mol⁻¹, considerably higher than that of the pyrimidines under study (ca. 370 kJ mol⁻¹), singlet–singlet energy transfer is an unlikely mechanism for quenching of the photodimerisation of (1)—(3). Since the cis–trans isomerisation of penta-1,3-diene can proceed via a triplet state diene species,[4] it can be concluded that the quenching of photodimerisation occurs by triplet energy transfer to the diene.

Prolonged irradiation of (1)—(3) in the presence of either cis- or trans-penta-1,3-diene leads to the establishment of a photostationary state, as shown for (1) in Figure 3. The trans : cis ratio at the photostationary

[1] R. M. Bebbington, D. H. Brooks, M. J. Geoghegan, and B. K. Snell, Chem. and Ind., 1969, 1512.

[2] R. S. Elias, M. C. Sheppard, B. K. Snell, and J. Stubbs, Nature, 1968, 219, 1160.

[3] B. D. Cavell, S. J. Pollard, and C. H. J. Wells, Chem. and Ind., 1976, 566

[4] G. S. Hammond, J. Saltick, A. A. Lamola, N. J. Turro, J. S. Bradshaw, D. O. Cowan, R. C. Counsell, V. Vogt, and C. Dalton, J. Amer. Chem. Soc., 1964, 86, 3197.

state being close to 2.1 : 1 in each case enables the energy of the lowest triplet state of (1)—(3) to be estimated from the results of Hammond *et al.*[4] as either *ca.* 230 or 270 kJ mol^{-1}. The latter value may be compared with values in the range 291—305 kJ mol^{-1} obtained from the phosphorescence spectra of (1)—(3).[5] Since the method of Hammond *et al.* for deriving triplet state energies invariably gives low values for unsaturated compounds, the value of 270 kJ mol^{-1} is as expected in relation to that obtained from phosphorescence measurements.

As the energies of the lowest triplet state of *cis-* (238) and *trans*-penta-1,3-diene (246 kJ mol^{-1}) are lower than that of the lowest triplet state of (1)—(3), the most probable mechanism for quenching of the photodimerisation
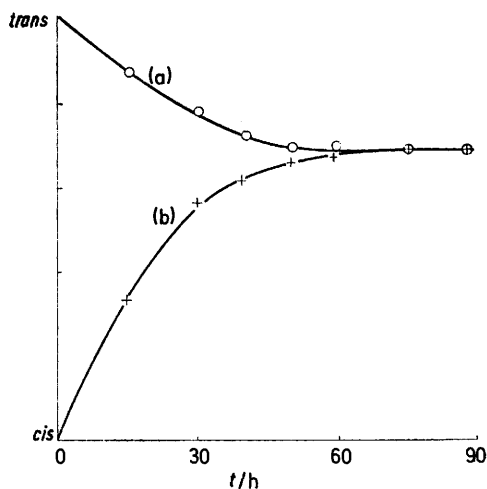

FIGURE 3 Photolytic *cis–trans* isomerisation of penta-1,3-diene in a 4.8 × 10^{-4}M solution of (1) in degassed acetonitrile containing initially (a) 100% *trans*-penta-1,3-diene (1.0 × 10^{-3}M) and (b) 100% *cis*-penta-1,3-diene (1.0 × 10^{-3}M). Composition at photostationary state is 67.7% *trans*- and 32.3% *cis*-penta-1,3-diene

of (1)—(3) is by direct triplet–triplet energy transfer. Furthermore, since the quenching was, to all extents and purposes, 100% efficient it is apparent that, at the concentrations used, the photodimerisation of (1)—(3) proceeds virtually entirely by way of triplet state pyrimidine. It is to be noted that for such a situation, the kinetics of addition of triplet state to ground state pyrimidine to form a dimeric species would be first order with respect to the pyrimidine.

The quantum yield for formation of triplet state pyrimidine is equal to the quantum yield, Φ_{isc}, for intersystem crossing from the singlet to triplet state manifold, and was determined for each pyrimidine by using the photosensitised *cis–trans* isomerisation of penta-1,3-diene as a means of counting the triplet states formed on irradiation.[6] The values so determined for Φ_{isc} are given in the table. In a similar fashion, irradiation of solutions of the pyrimidines both in the absence and presence of added *cis*-penta-1,3-diene, followed by product analysis, allowed measurement of the ratio of the quantum yield of

dimerisation, Φ_{dim}, to that for intersystem crossing Φ_{isc}. The values of Φ_{dim} derived from this ratio are listed in the Table.

Quantum yield and kinetic data for photodimerisation

Pyrimidine	Φ_{isc}	Φ_{dim}	$10^5 k_{\mathrm{d}}/\mathrm{s}^{-1}$	$10^8 k_{\mathrm{r}}/$ $\mathrm{l\ mol^{-1}\ s^{-1}}$
(1)	0.09 ± 0.01	0.07 ± 0.01	2.09 ± 0.07	1.72 ± 0.13
(2)	0.09 ± 0.01	0.06 ± 0.01	2.87 ± 0.07	1.46 ± 0.15
(3)	0.11 ± 0.01	0.08 ± 0.01	2.99 ± 0.06	2.04 ± 0.20

The quantum yield data given in the Table show that, at the pyrimidine concentrations employed, between 66 and 77% of the triplet state molecules formed react to give photodimers. This is in marked contrast to the photodimerisation of thymine (2,4-dihydroxy-5-methyl-pyrimidine) and uracil (2,4-dihydroxypyrimidine), two biologically important pyrimidines which play a direct role in the photodenaturation of DNA and RNA respectively.[7,8] Thus, photoexcitation of thymine and uracil under similar experimental conditions to those used in the present work gives rise to triplet states of which only 1.4% in the case of thymine and 4.9% in the case of uracil react to give photodimers.[9] The low conversion to photodimers in the case of thymine and uracil is a consequence of the formation of a metastable dimeric species (by addition of a triplet state molecule to a ground state molecule) which either stabilises to form products or reverts to ground state molecules, with the latter route predominating. If such a metastable dimeric species is formed during the reaction of the triplet state of (1)—(3) with ground state pyrimidine, then obviously this species has a higher efficiency of conversion into products and a smaller efficiency of splitting to ground state pyrimidine than is the case for thymine and uracil.

Applying the steady-state approximation method to a simple reaction mechanism in which the pyrimidine substrate is consumed *via* reaction of triplet with ground state pyrimidine gives expressions (1) and (2) where Φ_0

$$\Phi_0/\Phi = 1 + k_{\mathrm{q}}\tau[\mathrm{Q}] \tag{1}$$

$$1/\tau = k_{\mathrm{d}} + k_{\mathrm{r}}[\mathrm{Pyr}] \tag{2}$$

and Φ represent the respective quantum yields for consumption of pyrimidine in the absence and presence of quencher [Q], τ is the lifetime of the triplet state, [Pyr] is the concentration of ground-state pyrimidine, and k_{q}, k_{d}, and k_{r} are the respective rate constants for quenching of the triplet state, unimolecular radiationless decay of the triplet state, and reaction of triplet state with ground state pyrimidine. A Stern–Volmer plot according to equation (1) is shown for compound (1) in Figure 4; similar plots were obtained for (2) and (3). The plots were linear up to high values of Φ_0/Φ reinforcing the conclusion that, at the concentrations of pyrimidine used, the photodimerisation occurs *via* the triplet state.

In view of the difference between the triplet state energies of the pyrimidines and of the quenching agents

[5] D. Sen and C. H. J. Wells, unpublished results.

[6] A. A. Lamola and G. S. Hammond, *J. Chem. Phys.*, 1965, **43**, 2129.

[7] J. G. Burr, *Adv. Photochem.*, 1968, **6**, 193 and references cited therein.

[8] R. B. Setlow, *Photochem. and Photobiol.*, 1968, **7**, 643.

[9] P. J. Wagner and D. J. Bucheck, *J. Amer. Chem. Soc.*, 1970, **92**, 181.

used in these studies, it may be assumed that quenching is diffusion controlled and that the rate constant, k_q, will
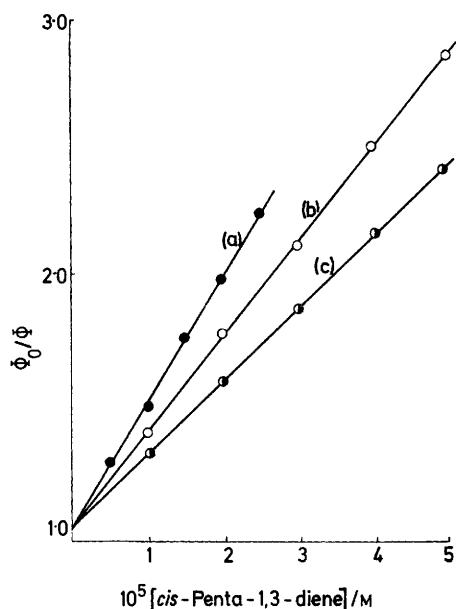

FIGURE 4 Representative Stern–Volmer plots for photolysis of degassed acetonitrile solutions of (1): (a) 1.0×10^{-4}, (b) 5.0×10^{-4}, and (c) 10.0×10^{-4}M

have the diffusion controlled value of $1.1 \times 10^{10}\,\text{l mol}^{-1}\text{s}^{-1}$ in acetonitrile.[9] Using this value, the slopes of the Stern–Volmer plots yielded values for the triplet state lifetimes, τ, which when plotted against pyrimidine concentration according to equation (2) gave the straight line plots shown in Figure 5. The intercepts and slopes of these plots yielded the values for k_d and k_r listed in the Table.

The rate constant values for (1) were checked by monitoring the rate of formation of photodimers from 2-^{14}C labelled (1) using t.l.c. for product separation followed by liquid scintillation analysis. The build-up of photo-
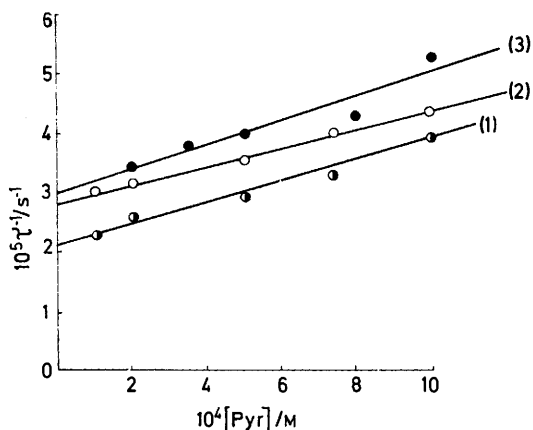

FIGURE 5 Plot of reciprocal of triplet state lifetime versus pyrimidine concentration

dimers with time is plotted in Figure 6 where the total dimer yield is expressed as a percentage of the initial

activity. For a mechanism in which photodimers are formed by reaction of triplet state with ground state pyrimidine, the initial rate, R, of dimer formation is given by expression (3) [10] where a is a constant specific to

$$\frac{1}{R} = \frac{1}{a} + \frac{k_d}{ak_r} \cdot \frac{1}{[\text{Pyr}]} \qquad (3)$$

each pyrimidine. Since a dimer represents two pyrimidine molecules, the initial rate of dimer formation is given by one-half the initial slope of the graph of dimer
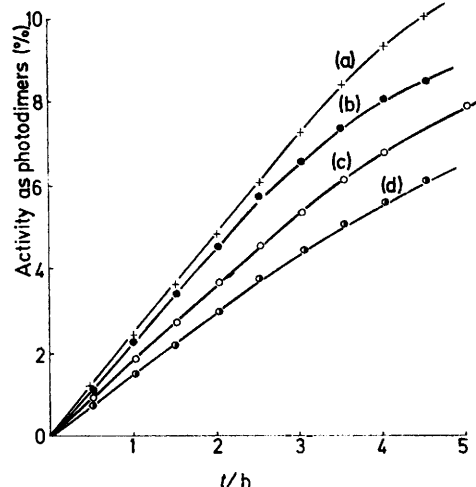

FIGURE 6 Yield of dimers from photolysis of degassed acetonitrile solutions of 2-^{14}C labelled (1): (a) 4.0×10^{-4}, (b) 5.0×10^{-4}, (c) 7.0×10^{-4}, and (d) 10.0×10^{-4}M
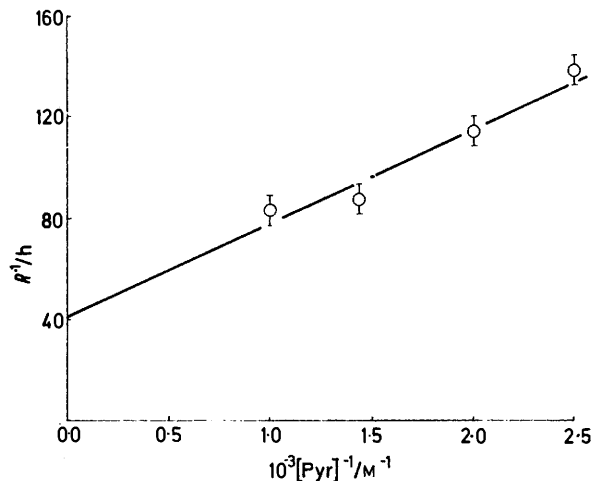

FIGURE 7 Plot of reciprocal of initial rate of dimer formation versus reciprical of concentration of (1)

yield versus time (Figure 6). A plot according to equation (3) is shown in Figure 7. The ratio k_d/k_r derived from the slope and intercept of the straight line shown in the plot is $0.9 \times 10^{-3}\,\text{mol l}^{-1}$ which may be compared with the value of $1.2 \times 10^{-3}\,\text{mol l}^{-1}$ derived from the values given in the Table.

The rate constants for addition of triplet state to ground state pyrimidine for (1)—(3) fall in the range

[10] I. H. Brown and H. E. Johns, Photochem. and Photobiol. 1968, 8, 273.

1.5—2.0 × 10^8 l mol^{-1} s^{-1} which is markedly lower than the values determined [9] for the corresponding rate constants for thymine (7.0 × 10^8 l mol^{-1} s^{-1}) and uracil (20.0 × 10^8 l mol^{-1} s^{-1}). The similarity in the rate constants for (1)—(3) is not unexpected in view of the similarity in substituents, and hence electron density, at the C(5)–C(6) bond across which dimerisation occurs. Photodimerisation in thymine and uracil also occurs across the C(5)–C(6) bond, and the change in rate constant values in the order (1) ∼ (2) ∼ (3) < thymine < uracil most probably reflects the decreasing steric hindrance at the C(5)–C(6) bond is going from (1)—(3) to uracil.

EXPERIMENTAL

Acetonitrile was purified by the method of O'Donnell et al.[11] and distilled through a 30 plate spinning band column so that there was no absorption above 260 nm. The distilled acetonitrile was stored in the dark under nitrogen and used within three weeks. The purity of cis- (K and K) and trans-penta-1,3-diene (K and K) was checked by g.l.c., only material of >99% purity being used. Compounds (1) and (2) were supplied by Plant Protection Division, I.C.I. Limited, and were recrystallised twice from methanol prior to use. Compound (3) was prepared by an adaptation of the method of Hull et al.[12] 1,1-Dimethylguanidine sulphate (27.2 g) was added to ethanolic sodium ethoxide, prepared by adding clean sodium (4.6 g) to redistilled absolute ethanol (150 cm^3). The mixture was shaken for 15 min, then ethyl 2-methylacetoacetate (28.8 g) was added, and the solution allowed to stand for 18 h, after which time it was refluxed for 2 h, cooled, filtered, and the filtrate evaporated to low volume whence (3) crystallised as needles. Recrystallisation from methanol yielded product (8.4 g), m.p. 169—173°.

G.l.c. analysis of cis- and trans-penta-1,3-diene were performed on an Aerograph 660 chromatograph using an 8.5 m × 3 mm column packed with 25% 1,2,3-tris-(β-cyanoethoxy)-propane on 40—60 mesh Chromosorb W. Absorption spectra were recorded on a Unicam SP 8000 spectrophotometer and quantitative analysis of (1)—(3) was carried out at 302 nm on a Unicam SP 500 spectrophotometer. T.l.c. was performed on Merck silica gel F_{254} plates (0.2 mm × 20 cm × 20 cm) using chloroform–methanol (95 : 5) as eluant. Autoradiograms were taken using Ilford X-ray film. Liquid scintillation was carried out on an Ekco Electronics N664A instrument connected to a Nuclear Enterprises ST3 digital counter.

Samples were prepared for photolysis by pipetting sample solution (4 cm^3) into 10 mm i.d. quartz tubes, connected by graded seal to a Pyrex socket, and degassed by a repeated freeze–thaw cycle until a vacuum of at least 0.3 N m^{-2} was achieved, and then sealed in vacuo. Each sample was prepared in duplicate or triplicate. Up to 28 sample solutions were simultaneously photolysed using a 500 W Hanovia medium-pressure Hg arc lamp located in a quartz well at the centre of a ' merry-go-round ' apparatus [13] which itself was located in a constant temperature bath maintained at 25°. For photolysis using radiation in the range 290—330 nm, the lamp radiation was filtered by means of a Chance OX7 glass filter (2 mm) in combination with a filter solution of potassium chromate (1.05 g in 2 dm^3 of 1% aqueous sodium carbonate) pumped through the annulus in the quartz well

[11] J. F. O'Donnell, J. T. Ayres, and C. K. Mann, Analyt. Chem., 1965, 37, 1161.

and through a heat exchanger. This filter combination had a transmission maximum at 313 nm and cut-offs at 290 and 330 nm.

Kinetic plots, such as shown in Figure 1, were derived from data obtained by photolysing solutions of the pyrimidines (3.5—5.0 × 10^{-4}M) in acetonitrile with solutions of the same concentration of pyrimidine but containing cis-penta-1,3-diene (5.0 × 10^{-3}M). Samples were withdrawn at various times and, after appropriate dilution, the absorbance of the parent pyrimidine in the solutions measured at 302 nm. The ratio of the absorbance of the solutions before photolysis to that at intervals during photolysis gave the ratio [Pyr]$_0$/[Pyr]. Values for the ratio Φ_0/Φ used in plots of the type shown in Figure 4 were derived from data obtained by photolysing for a specified time degassed solutions of the pyrimidines of fixed concentration (2—10 × 10^{-4}M) containing various concentrations of cis-penta-1,3-diene (1—5 × 10^{-5}M) alongside degassed solutions containing the same concentration of pyrimidine but without added cis-penta-1,3-diene. The ratio of the drop in absorbance at 302 nm for the unquenched solutions on photolysis to the drop in absorbance for each quenched solution gave the ratio Φ_0/Φ.

The procedure for the determination of the quantum yield, Φ_{isc}, of intersystem crossing from the singlet to triplet state manifold was as follows. Two sets of degassed acetonitrile solutions of composition (a) 5.0—7.0 × 10^{-4}M in pyrimidine and 5 × 10^{-4}M in cis-penta-1,3-diene, and (b) 2.0 × 10^{-2}M in benzophenone and 5.0 × 10^{-2}M in cis-penta-1,3-diene were simultaneously photolysed and after a suitable time the percentage conversion of cis- to trans-penta-1,3-diene in each solution was determined by g.l.c. The photolysis time was chosen such that the photostationary state at which the ratio cis : trans is constant was not reached, typical cis–trans conversions for the pyrimidine solutions were in the range 15—20% for a photolysis time of 10 h. The same amount of radiation was absorbed by solutions (a) and (b) at the concentrations used, and all triplet states formed would be quenched by energy transfer to the penta-1,3-diene. The ratio of the quantum yield of intersystem crossing in each of the pyrimidines to that in benzophenone was determined using the expressions of Lamola and Hammond,[6] and the Φ_{isc} values for the pyrimidines were calculated from these ratios on the basis that Φ_{isc} for benzophenone is unity.[6]

The ratio of the quantum yield, Φ_{dim}, for formation of dimers to the quantum yield for intersystem crossing in the pyrimidines was determined by simultaneous photolysis of degassed acetonitrile solutions of the pyrimidines (5.0—7.0 × 10^{-4}M) in the absence and in the presence of the quencher, cis-penta-1,3-diene (5.0 × 10^{-2}M). The percentage drop in the absorbance of the pyrimidines at 302 nm in the unquenched solutions gave a measure of the number of pyrimidine triplets reacting to give dimers. Also, the percentage conversion from cis- to trans-penta-1,3-diene in the totally quenched systems when divided by the factor of 0.55 to take into account the reverse conversion trans to cis,[6] gave a measure of the total number of pyrimidine triplets formed. The two sets of percentage values gave the ratio Φ_{dim} : Φ_{isc}.

Liquid scintillation data was obtained from four sets of degassed acetonitrile solutions of 2-^{14}C labelled (1) (4.0—

[12] R. Hull, B. J. Lovell, H. T. Openshaw, L. C. Payman, and A. R. Todd, J. Chem. Soc., 1946, 357.

[13] F. G. Moses, R. S. H. Liu, and B. M. Monroe, Mol. Photochem., 1969, 1, 245.

10.0 × 10⁻⁴M) which were simultaneously photolysed in the reactor. Samples from each of the four sets were withdrawn at regular intervals, evaporated down, the residue dissolved in a small volume of methanol, and the products separated by t.l.c. The regions on the plates shown by autoradiography to contain the four photodimers were combined, and the activity residing in the combined photodimers in each sample was determined by liquid scintillation analysis.