The photosensitising properties and photostability of stilbene fluorescent whitening agents

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Several stilbenes have been shown to sensitise the formation of singlet oxygen, which is capable of oxidising indole residues, e.g. tryptophan. Some stilbenes were found to quench singlet oxygen, e.g. disodium 4,4′-diaminostilbene-2,2′-disulphonate. Such materials have been shown to be capable of sensitising the photoyellowing of wool and proved to be no more stable than conventional stilbene fluorescent whitening agents.

INTRODUCTION

There is no doubt that a most popular type of fluorescent whitening agent (FWA) for wool contains the stilbene chromophore [1]. Nevertheless such FWAs photodegrade on exposure to sunlight and also sensitise the degradation of wool [2,3]. Degradation on the surface of wool appears to involve reaction of the stilbene with wool [4] and this may not be a photo-oxidative process. There is also good evidence that stilbenes, on the surface of wool, can sensitise the formation of singlet oxygen, which then reacts with tryptophyl residues located some distance from the stilbene molecule [5]. It is undoubtedly true that the amino acids most susceptible to photodegradation are those which are the most susceptible to attack by singlet oxygen [6]. A further twist to the story is that stilbenes themselves may not only act as sensitisers of singlet oxygen production, but also as quenchers (physical and chemical) [7]. Thus, like pyrazolines [8,9] stilbenes have the facility to self destruct in photochemical processes. In this context it is interesting to note that time-resolved fluorescence studies have shown that triplet stilbene (in benzene solution) produces singlet oxygen with an efficiency of 18±5%[10]. In view of this result and the fact that most stilbene FWAs have high quantum yields of fluorescence and short fluorescence lifetimes, it is likely that such compounds are inefficient sensitisers of singlet oxygen.

We now present experimental evidence that some stilbenes sensitise singlet oxygen formation and that others quench singlet oxygen. Furthermore FWAs that are inefficient sensitisers of singlet oxygen, when applied to wool, photoyellow the wool and are photodegraded at similar rates to conventional sensitisers.

EXPERIMENTAL

Materials

The following chemicals were used as supplied: *trans*-stilbene, thioanisole, 4,4′-diaminostilbene dihydrochloride, anhydrous sodium acetate, 3-methylindole and ethylenediamine tetraacetic acid (disodium salt, dihydrate) (all Aldrich), indole (Fisons), methylene blue, *N*-acetyl-*dl*-tryptophan and *dl*-tryptophan (all Sigma), Rose Bengal (Eastman Kodak Co.), potassium chromate (BDH), 4,4′-diaminostilbene-2,2′-disulphonic acid (Vickers Laboratories Ltd), Photine-HV (HWL) (Figure 1), Lissapol N (ICI), hydrogen peroxide (100 vol., about 300 g/l peroxide, BDH), formic acid (95–97%, Aldrich), glacial acetic acid and pyridine (both Fisons SLR grade), methanol (Fisons AnalaR and HPLC grades), methanol-d_1 and methanol-d_4 (Aldrich Gold Label grade). Spectroscopic solvents for fluorescence quenching experiments (acetonitrile and methanol) were used as supplied (Aldrich Gold Label spectrophotometric grade).

The wool fabric employed was a botany serge (2/2 twill of weight 200 g/m^2) supplied by Salts of Saltaire.

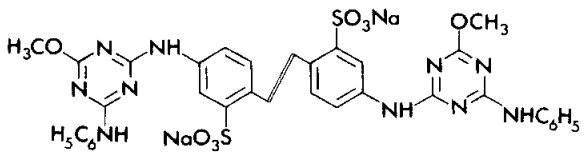

Figure 1 – Photine-HV

Disodium 4,4′-diaminostilbene-2,2′-disulphonate

This was prepared from 4,4′-diaminostilbene-2,2′-disulphonic acid according to the procedure described by Zahradnik [11].

4,4′-Diacetamidostilbene-2,2′-disulphonic acid

This was prepared by the method described by Needles and Seiber [11] and its structure was confirmed by n.m.r. and i.r. spectroscopy.

Phenylmethylsulphone

This was synthesised from thioanisole using the method described by Bell and Bennett for the oxidation of 1,4-dithian by hydrogen peroxide [12]. The product was identified by its melting point (m.p. 81°C, lit. m.p. 88°C [13]) and elemental analysis (found: C 53.83, H 5.10; $C_7H_8O_2S$ requires C 53.82, H 5.16%).

Methods

U.v. spectra were recorded using a Perkin-Elmer 402 u.v./vis. spectrophotometer. Solutions of specific absorbance values were made up using a Cecil CE 272 linear read-out u.v. spectrophotometer. Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. G.l.c. analyses were carried out

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using a Perkin-Elmer Sigma 3 gas chromatograph fitted with a 1.5% OV225 (80–100 mesh; length 2 m; outer dimension 0.125 in, inner dimension 2 mm; support material Chromosorb W acid washed dimethyldichlorosilane) column. Peak areas were calculated using a Pye Unicam DP88 computing integrator, and compared with those of the internal standard. H.p.l.c. analyses were carried out using an ODS Hypersil (5 μm) column (150×4 mm) and degassed (2 h in an ultrasonic bath at room temperature), eluent (70% water, 20% methanol and 10% glacial acetic acid). U.v. spectroscopy (Cecil CE 2012 reference channel variable wavelength u.v. monitor) was used for detection of the peaks.

Fluorescence quenching

Fluorescence spectra were recorded on a Perkin-Elmer MPF-4 spectrofluorimeter and were uncorrected. Solutions of *trans*-stilbene were made up to an absorbance value of 0.1 at the excitation wavelength (320 nm in methanol and 326 nm in acetonitrile). Fluorescence spectra of the degassed (flushed with dry argon for 5 min) solutions were obtained in the presence and absence of indole (10^{-2} mol/l), under similar conditions.

Photo-oxidation of indoles

Mixtures of indoles (10^{-2} mol/l) and stilbenes (10^{-2} mol/l) in pyrex photolysis tubes were irradiated within a circular array of 16×8 W (F8T5/BLB) Sylvania black light lamps giving a maximum output at 350 nm, which is absorbed only by the stilbenes. Also included in the above was a photostable internal standard phenylmethylsulphone, 1 mg being employed for subsequent post-irradiation g.l.c. analyses and 72 mg for other analyses; total volume of the irradiated solution solvent was 20 ml (water or methanol). Dry oxygen was continuously bubbled through the reaction mixture. Changes in the kinetic stability of a particular indole were followed by monitoring the decrease in its concentration with time. This was achieved by measuring the area of its g.l.c. peak or the height of its h.p.l.c. absorption peak (at 270 nm) relative to those of the internal standard.

Measurement of kinetic solvent isotope effects

Solutions containing indole (10^{-3} or 10^{-4} mol/l) *trans*-stilbene (10^{-2} mol/l) and phenylmethylsulphone (2.4 mmol/l) were made up in methanol CH_3OD (methanol-d_1) and CD_3OD (methanol-d_4). The solutions were photolysed using the above procedure with the following modifications: the solutions were flushed with dry oxygen for 30 min and stoppered prior to being irradiated in a reactor fitted with a carousel apparatus. The photochemical reactions of the 10^{-3} mol/l indole solutions were monitored by g.l.c. analysis whilst h.p.l.c. was used for the 10^{-4} mol/l indole solutions.

Stern–Volmer rate constant measurements

Solutions of indole (10^{-3} mol/l) containing phenylmethylsulphone (641 μmol/l) and either methylene blue (sufficient to give an absorbance value of 1.0 at 661 nm) and disodium 4,4′-diacetamidostilbene-2,2′-disulphonate (10^{-4}, 5×10^{-4} or 10^{-3} mol/l) or Rose Bengal (sufficient to give an absorbance value of 1.0 at 548 nm) and disodium 4,4′-diaminostilbene-2,2′-disulphonate (10^{-4}, 5×10^{-4} or 10^{-3} mol/l) were made up in water. The dye-sensitised photo-oxidation of the indole in these solutions was carried out in a similar manner to the indolic oxidations using daylight lamps (8×20 W Chryselco) filtered through a 2% aqueous potassium chromate light filter solution. The reactions were followed by g.l.c. analysis.

Wool bleaching

Wool serge fabric was bleached overnight by a pad–batch (room temperature) method, padding on (100% wet pick-up) 80 g/l hydrogen peroxide (100 vol.), 5 g/l formic acid (95–97%) and 10 g/l Lissapol N [14]. This was followed by rinsing in cold water and drying in air at room temperature.

Application of 4,4′-diacetamidostilbene-2,2′-disulphonic acid and Photine-HV to bleached wool and subsequent photoyellowing

Pre-bleached wool samples were treated in baths set at pH 4 (4% o.w.f. sodium acetate plus required acetic acid), at a liquor ratio of 30:1 in the presence of ethylenediamine

TABLE 1

Photo-oxidation[a] of indoles sensitised by stilbenes

Stilbene sensitiser	Indole	Solvent	Amounts of indole oxidised (%)
trans-Stilbene	Indole	Methanol	74[b]
	3-Methylindole	Methanol	96[b]
	N-Acetyl-*dl*-tryptophan	Methanol	74[c]
4,4′-Diaminostilbene dihydrochloride	Indole	Methanol	88[b]
	N-Acetyl-*dl*-tryptophan	Methanol	100[c]
Disodium-4,4′-diaminostilbene-2,2′-disulphonate	Indole	Water	40[b]
	dl-Tryptophan	Water	11[c]
	Indole	Methanol	0[b]
	N-Acetyl-*dl*-tryptophan	Methanol	10[c]
4,4′-Diacetamidostilbene-2,2′-disulphonic acid	Indole	Water	45[b]
	dl-Tryptophan	Water	10[c]

(a) Irradiation time 24 h
(b) Determined by g.l.c.
(c) Determined by h.p.l.c.

tetraacetic acid (disodium salt, dihydrate), 1% o.w.f., and Lissapol N, 1% o.w.f., with no FWA, or with either 4,4′-diacetamidostilbene-2,2′-disulphonic acid or Photine-HV at 0.5% o.w.f. The temperature was raised to 80°C at 1 degC/min and maintained for 1 h, followed by cold water rinsing and drying at room temperature. Both FWAs gave exhaustion values above 80% by this application method.

The whitened samples were photoyellowed for 24 h as described previously [3].

Extractions

Strips of equivalent weight from each of the whitened and photoyellowed fabrics (see above) were wetted with aqueous Lissapol N solution and rinsed in cold water. Extraction of the brighteners and photodegradation products was achieved by boiling (5 min) each strip in 25% aqueous pyridine (50 ml) five times [15]. The amount of brightener extracted from each sample was determined by u.v. spectroscopy.

Yellowness index measurements

The yellowness index (YI) formula employed is shown in Eqn 1:

$$(YI) = \frac{100\ (1.316X - 1.164Z)}{Y} \qquad (1)$$

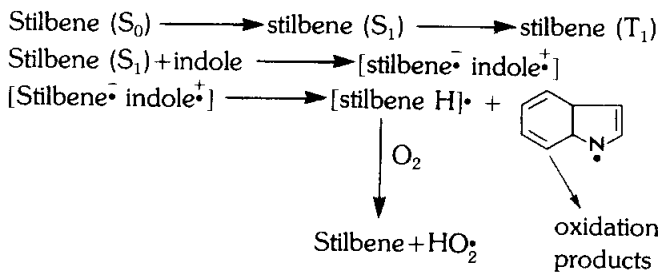
where X, Y and Z are the CIE tristimulus values obtained from the Macbeth Micromatch reflectance spectrophotometer. The lower the yellowness index value, the whiter is the wool colour.

RESULTS AND DISCUSSION

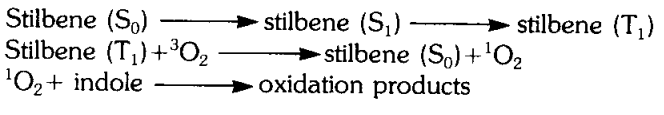
One of the amino acids that is most prone to photodegradation in proteins such as wool is tryptophan [2]. Studies were therefore made of the degradation of this amino acid using stilbenes as photosensitisers. In addition some closely related indoles were also examined. From Table 1 it can be seen that stilbene and 4,4′-diaminostilbene dihydrochloride successfully photosensitise the degradation of indolic compounds. The other two stilbenes proved to be very inefficient sensitisers.

There are two possible mechanisms for the oxidation reactions, mechanisms A and B, shown below.

Mechanism A



Mechanism B



where S_0 = ground state
S_1 = excited singlet state
T_1 = excited triplet state

In mechanism A the excited state of the stilbene interacts primarily with the indole to give radicals that subsequently react with oxygen. To prove that this mechanism is operating, the effect of indole upon the fluorescence was studied. With indole concentrations as high as 10^{-2} mol/l no quenching was observed. Similarly the short lifetime of triplet stilbene [16] makes it unlikely that it will undergo a bimolecular reaction with indole at a concentration of 10^{-2} mol/l. Thus, although it is known that amines can react with stilbenes via a charge-transfer process [17,18], this process does not appear to be operating under the reaction conditions employed. In order to establish that the oxidation involves singlet oxygen, the reactions were examined to see if they exhibit a solvent isotope effect [19]. This technique is far more reliable than using quenchers of singlet oxygen [20], but even so it is not without its pitfalls [21]. A comparison of the rates of oxidation of indole (10^{-3} mol/l) in methanol, monodeuterated methanol (CH_3OD) and fully deuterated methanol (CD_3OD) sensitised by stilbene (10^{-2} mol/l) yielded the following isotope effects:

$$\frac{K_{indole}\ (CH_3OD)}{K_{indole}\ (CH_3OH)} = 1.4$$

$$\frac{K_{indole}\ (CD_3OD)}{K_{indole}\ (CD_3OH)} = 2.1$$

The values found for the isotope effects are remarkably low. Without any complicating factors the expected isotope effect for the CD_3OD/CH_3OH system is 22 [22]. A possible reason for the low value found is the high concentration of indole employed [21]. When the concentration was reduced to 10^{-4} mol/l the isotope effect for the system CD_3OD/CH_3OH rose to a value of 5.2, which is still lower than expected; this may be because the stilbene itself acts as a quencher of singlet oxygen. Recently many well known sensitisers have been shown to act as quenchers of singlet oxygen [23] and this leads to the observed kinetic solvent isotope effects being lower than expected. Nevertheless the observation of an isotope effect in this work demonstrates that singlet oxygen is involved.

Attempts were also made to see if the diaminostilbene and diacetamidostilbene are sensitisers for singlet oxygen production using time-resolved fluorescence spectroscopy. The stilbenes were excited with a neodymium YAG laser at 355 nm. Attempts were made to detect the singlet oxygen produced by looking for its fluorescence at 1.27 μ using a germanium detector [10]. No emission could be detected, suggesting that the yield of singlet oxygen is probably less than 10%.

From Table 1 the two stilbenes disodium 4,4′-diaminostilbene-2,2′-disulphonate and 4,4′-diacetamidostilbene-2,2′-disulphonic acid can be seen to be inefficient sensitisers for indole oxidation. It would appear that these compounds are quenchers of singlet oxygen. To test this hypothesis the methylene blue and Rose Bengal (dyes which are capable of sensitising singlet oxygen formation) sensitised oxidation of indole in the presence and absence of 4,4′-diacetamidostilbene-2,2′-disulphonic acid (as disodium salt) and disodium 4,4′-diaminostilbene-2,2′-disulphonate respectively were studied. By measuring the rates of reaction in the presence of varying known amounts of the stilbenes Stern–Volmer

plots were constructed (Figure 2). The slope of each line is given by Eqn 2:

$$\text{Slope } (K_{sv}) = \frac{k_q}{k_d + k_r \text{ (indole)}} \qquad (2)$$

where k_q = bimolecular quenching rate constant for quenching singlet oxygen

k_d = unimolecular decay constant for singlet oxygen in solvent employed

k_r = bimolecular rate constant for reaction of singlet oxygen with indole (7.7×10^5 l/(mol s) [24]).

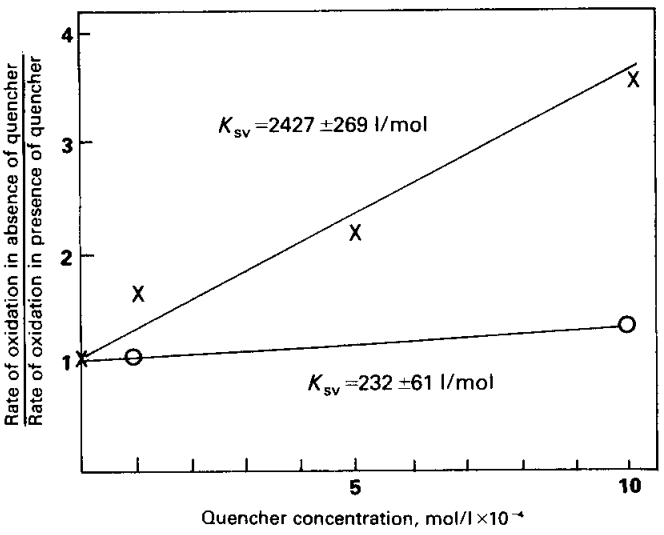


Figure 2 – Stern–Volmer plots of the oxidation of indole using 4,4'-diacetamidostilbene-2,2'-disulphonic acid and 4,4'-diaminostilbene-2,2'-disulphonic acid (disodium salt) as quenchers of singlet oxygen

Using the data in Figure 2 and the value shown above for k_r, quenching rate constants of $(1.8\pm0.5)\times10^5$ and $(1.9\pm0.2)\times10^6$ l/(mol s) are obtained for 4,4'-diacetamidostilbene-2,2'-disulphonic acid (as disodium salt) and disodium 4,4'-diaminostilbene-2,2'-disulphonate quenchers respectively. Clearly the stilbenes are not particularly efficient quenchers of singlet oxygen.

Since the 4,4'-diacetamidostilbene-2,2'-disulphonic acid is a quencher of singlet oxygen, albeit of moderate efficiency, it might be expected to be a stable FWA when applied to wool. The stilbene was applied to wool and the treated wool exposed in a damp state to light. Both photoyellowing of the wool and degradation of the stilbene was observed. The rate of degradation of a commercial stilbene FWA (Photine-HV) applied to wool was also assessed and it was found that the diacetamido derivative was no more stable than Photine-HV (Table 2).

TABLE 2

Photoyellowing of stilbenes applied to wool

Stilbene	(YI)[a]	(YI)[b]	Δ(YI)
4,4'-diacetamidostilbene-2,2'-disulphonic acid	23.55	42.44	18.89
Photine-HV	15.34	37.09	21.75
Control (i.e. no FWA)	23.71	34.39	10.68

(a) Before irradiation
(b) After irradiation

The amounts of stilbenes degraded during yellowing were determined by extracting undegraded FWAs with aqueous pyridine [12], and it was found that 37% of Photine-HV and almost 100% of 4,4'-diacetamidostilbene-2,2'-disulphonic acid were degraded. These results confirm earlier work [3] which showed that the photodegradation of FWAs on wool does not solely involve singlet oxygen. It is remarkable that 4,4'-diacetamidostilbene-2,2'-disulphonic acid, which can act as a physical quencher of singlet oxygen, possesses so little photostability when applied to wool.

We conclude from these studies that stilbene FWAs will photosensitise the yellowing of wool and will degrade upon irradiation when applied to wool, irrespective of whether they are efficient sensitisers of singlet oxygen production or whether they are efficient quenchers of singlet oxygen.

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