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The photoreactions of trimethoprim in solution

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Abstract

The photochemical behaviour of Trimethoprim, a trimethoxybenzylpyrimidine used as bactericide, in solution has been investigated. Direct irradiation causes a slow reaction (e.g. Φ in methanol 3×10^{-4}). The molecule is quite sensitive to benzylic hydrogen abstraction by $n\pi^*$ ketones yielding dimers in the absence of oxygen or oxidised products (the corresponding benzoylpyrimidine and trimethoxybenzaldehyde) in its presence. The drug is also sensitive to singlet oxygen, likewise undergoing oxidative cleavage, though inefficiently. The reported photolability of drug preparations is probably due to sensitised oxidation by impurities or other components.

Keywords: Trimethoprim; Drugs photoreactivity; Drugs photolability; Type I and II photooxidation

1. Introduction

Increasing attention has recently been given to the problem of the photolability of drugs [1–3]. Although it is generally possible to protect commercial drug preparation by appropriate packing, occasional exposure before or after commercialisation may result in some photoreaction and as a consequence in a significant activity loss or even in the formation of toxic by-products. Furthermore, the knowledge of the photochemistry of drug substances often helps in predicting or rationalising phototoxic effects. This make it desirable that the photochemical behaviour of commonly used drug substances is known in detail, and that the study is extended to different conditions (solid state or solution, additives used in pharmaceutical preparations etc.). This is also advisable in view of the recently implemented ICH Guidelines on the Drug Photostability [4]. At present, the available information is often limited to a generic indication of photolability.

As a part of our program on drug photochemistry we presently report a study on the photoreactivity of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (**1**), a largely used bactericide (Trimethoprim) in solution.

2. Experimental

2.1. General

Trimethoprim was a commercial sample (Sigma). Spectrophotometric grade solvents were used for photochemical reactions. The course of the reaction was monitored by GLC (HP-1 capillary column, 25 m) and by HPLC (Hypersil ODS-2 column, 5 μ, 200 × 4.6 mm, eluting with a MeOH-aqueouos acetic (pH 5) 6–4 mixture).

2.2. Explorative irradiations

2.5×10^{-4} M solutions of compound **1** in quartz or Pyrex tubes were flushed with argon or oxygen as appropriate and irradiated in a multi-lamp apparatus fitted with six lamps (15 W) emitting at the chosen wavelength (254 nm for direct irradiation, centred at 360 nm for benzophenone sensitisation, at 410 nm for tetraphenylporphine and at 490 for Rosa Bengal, respectively). The substrate consumption was monitored by HPLC, and was limited to 30%. For the 254 nm experiment, the light flux was monitored by ferrioxalate actinometry.

2.3. Preparation irradiations

2.3.1. Direct irradiation in acetonitrile

230 mg of compound **1** in 320 ml acetonitrile were subdivided in three quartz tubes and irradiated by means of six

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external 15 W low pressure mercury arcs for 8 h. GLC examination showed the presence of 3,4,5-trimethoxybenzaldehyde (**2**, 11.5%). Evaporation of the solvent gave a solid. Washing with cyclohexane gave aldehyde **2**, identical to an authentic sample (Fluka). Chromatography of the residue on silica gel eluting with chloroform-methanol 9–1 mixture gave 13 mg of ketone **3**. This showed: Mp 190°C. Anal. Found: C, 55.3; H. 5.3; N, 18.4. Calc. for $C_{14}H_{16}N_4O_4$, C, 55.25; H, 5.23; N, 18.42. ^{1}H NMR [$(CD_3)_2SO$)]δ 3.75 (s, 3H), 3.8 (s, 6H), 6.8 (s, 2H, H-2′,6′), 7.0 (br s, 2H), 7.4 (br s, 1H), 8.15 (s, 1H, H-6), 8.3 (br s, 1H). IR (KBr) 3390, 3070, 1675, 1580 cm^{-1}. These properties corresponded well with those reported previously for this compound [5].

2.3.2. Benzophenone-sensitised irradiation

290 mg of compound **1** and 728 mg of benzophenone in 100 ml acetonitrile were argon flushed and irradiated by means of a Pyrex filtered medium pressure arc for 3 h. A yellowish precipitate (30 mg) formed and was filtered (product **4**). The solution was evaporated and chromatographed on silica gel yielding benzopinacol (20 mg) and a mixture of compounds **4** and **5** (25 mg). The last products were recognised on the basis of the analytic and spectroscopic properties reported below as isomeric dimers, though we were unable to assign the stereochemistry of each compound.

Compound **4** showed: Mp > 300°C. Anal. Found: C, 58.4; H. 5.7; N, 19.2. Calc. for $C_{28}H_{34}N_8O_6$, C, 58.12; H, 5.92; N, 19.37. Mass spectrum (CI with ammonia): 579 ($M^+ + H^+$), 596 ($M^+ + NH_4^+$). ^{1}H NMR [$(CD_3)_2SO$]δ 4.82 (s, 2H, benzylic H), 3.58 (s, 6H), 3.78 (s, 12H), 5.5 (br, s, 4H), 6.45 (br s, 4H), 6.78 (s, 4H, H-2′, 6′), 8.18 (s, 2H, H-6).

Compound **5** was present in a fraction also containing some of isomeric **4**. The spectrum was in part superimposed, and by a number of diagnostic signals was well distinguished, allowing to recognise this product as an isomer of **4**: ^{1}H NMR [$(CD_3)_2SO$] δ 4.63 (s, 2H, benzylic H); 6.62 (s, 4H, H-2′, 6′); 7.65 (s, 2H, H-6).

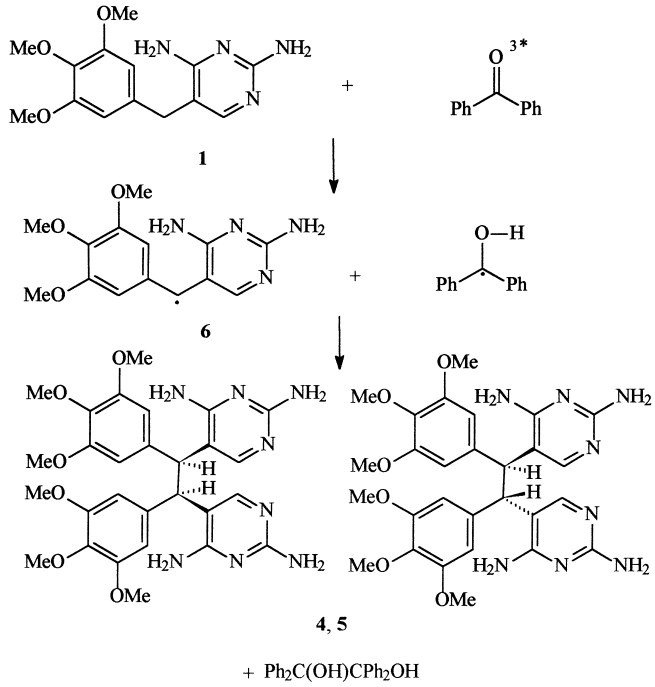
2.3.3. Dye-sensitised irradiation

100 mg of compound **1** and 3 mg of Rosa Bengal in 69 ml acetonitrile were continuously flushed with oxygen and irradiated by means of four external phosphor coated lamps (centre of emission 410 nm) for 3 h. The solution was evaporated. Washing with cyclohexane gave trimethoxybenzaldehyde (**2**, 13 mg, 20%).

3. Results

3.1. Direct irradiation

The absorption spectrum of compound **1** (Scheme 1) has a maximum centered at 285 nm ($\varepsilon 7 \times 10^3$). It shows neither fluorescence nor phosphorescence in fluid solution or in a glassy matrix.



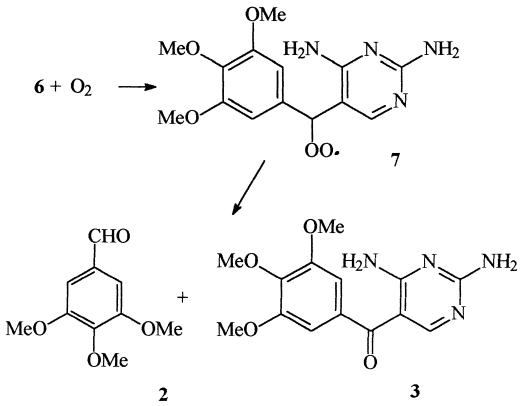
Scheme 1.

Direct irradiation (low pressure mercury arc) of this compound in argon flushed solutions in methanol, ethanol, acetonitrile and water causes very little change after several hours. In dichloromethane and choroform acidity was evolved, and after some hours caused the precipitation of **1** as the hydrochloride.

The decomposition quantum yield in MeOH was measured as 3×10^{-4} and in the other solvents the rate of decomposition was similar. This value remained the same (within 20%) when an oxygen or air equilibrated solution was used. In the presence of oxygen, some 3,4,5-trimethoxybenzaldehyde (**2**) was always formed in organic solution, as determined by gas-chromatography. Besides aldehyde **2**, a preparative irradiation of a MeCN solution led to the isolation of a small amount of trimethoxybenzoylpyrimidine **3** (see Scheme 2), which could be obtained by column chromatography. In water the photodecomposition was somewhat faster, but no tractable product (in particular none of **2**) was formed.

3.2. Benzophenone-sensitised irradiation

Irradiation (360 nm) in the presence of benzophenone in argon flushed acetonitrile (Pyrex filtered medium pressure arc) caused a reaction of **1**. A precipitate separated out during the irradiation. This was identified from its analytical and spectroscopic properties as the tetraryletane **4**. Column chromatography of the residue afforded some benzopinacol and a fraction containing compound **4** admixed with its diastereoisomer **5** (see Scheme 1).



Scheme 2.

When an identical air-equilibrated acetonitrile solution was irradiated, trimethoxybenzaldehyde (**2**) was the main product (yield 12%).

3.3. Dye-sensitised irradiation

Irradiation (visible light) of a chloroform solution in the presence of tetraphenylporphine as well as in acetonitrile and in methanol in the presence of Rosa Bengal under oxygen flushing led to a slow consumption of **1** with formation of **2** as the main product. The initial rate of reaction was in the order $CHCl_3 > MeCN > MeOH$ (see Fig. 1).

3.4. Supporting voltammetric data

A voltammetric analysis on a solution of **1** in acetonitrile showed a first anodic peak (non reversible) at 1.2 V SCE followed by a reversible peak at 1.4 V (see Fig. 2).

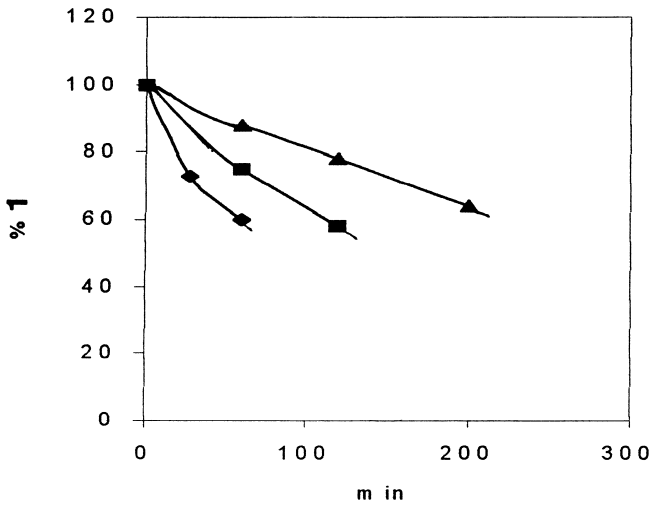


Fig. 1. Decomposition of Trimethoprim (**1**) upon dye-sensitised photo-oxidation in chloroform (◆), acetonitrile (■) and methanol (▲).

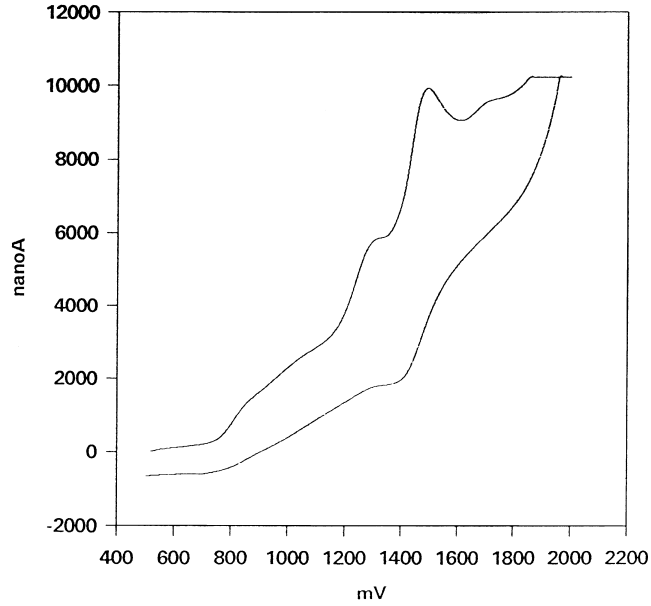


Fig. 2. Cyclic voltammogramme of Trimethoprim (**1**) in acetonitrile, scan speed 50 mV/s.

4. Discussion

Trimethoprim is largely used as an antibacterial [6]. The photosensitivity of this substance has been noted, and the various Pharmacopeas [7,8] caution that it has to be adequately protected from light. However, the type of reaction involved in the photodegradation has not been clarified. A study by Bergh et al. [5] shows that some ketone **3** was formed in solutions or suspensions of this drug in various aqueous buffers (pH 4.5–8) when exposed to sunlight [5]. The nature of the lowest singlet state poses some problem since compound **1** contains two separate chromophores, and both of them absorb at about the same wavelength [9]. The fact that neither fluorescence nor phosphorescence is observed with **1** suggested, however, that both the lowest singlet and the lowest triplet are centred on the pyrimidinediamine chromophore, since aromatic ethers usually are both fluorescent and phosphorescent.

The present investigation shows that direct irradiation in solution causes only a slow reaction. This may be expected, since aromatic amines mostly show little photochemical reactivity and their excited states decay mainly by emission (which is not the case here) or by internal conversion or inter system-crossing.

The formation of acidity observed in dichloromethane may be reasonably attributed to electron transfer to excited **1** from the solvent, and decomposition of the radical anion of the latter (e.g. Eq. (1)).

$$1^* + CH_2Cl_2 \rightarrow 1^{+\bullet} + CH_2Cl_2\text{-}\bullet \rightarrow CH_2Cl^{\bullet} + Cl^- \quad (1)$$

Compound **1** is a good electron donor, as shown by the voltammetric analysis in Fig. 2. The first anodic peak is attributed to the diaminopyridine moiety, and the latter

(reversible) one to the trimethoxybenzyl moiety by analogy with the literature [10]. Electron excitation (3.4 eV for the singlet) makes SET e.g. from dichloromethane (E_{red}−2.3 V) to **1** (E_{0X}1.2 V, see above) viable. There is precedent for fragmentation induced by electron transfer from halogenated alkanes to excited amines [11,12].

As for the triplet state, no information is available at present about the extent of intersystem crossing. If formed at all, the triplet is as unreactive as the singlet.

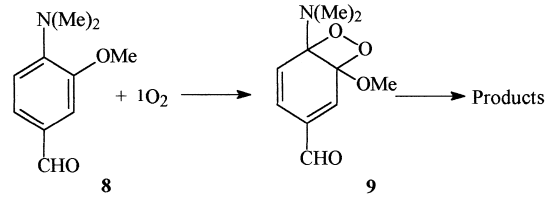
These results, coupled with the fact that the absorption by Trimethoprim in the UV-A region of the spectrum is weak, suggest that photochemical reactions directly from the excited states have scarcely a role in the observed photolability of this drug.

On the other hand, compound **1** is sensitive under two sets of photosensitised conditions. Thus, the reaction in the presence of benzophenone is clearly due to homolytic hydrogen abstraction by the $n\pi^*$ triplet state of the latter from the activated benzylic position. The thus formed benzyl radicals (**6**, see Scheme 1) are highly stabilised, and couple to yield the diastereoisomeric tetraarylethanes **4** and **5** as long with benzopinacol.

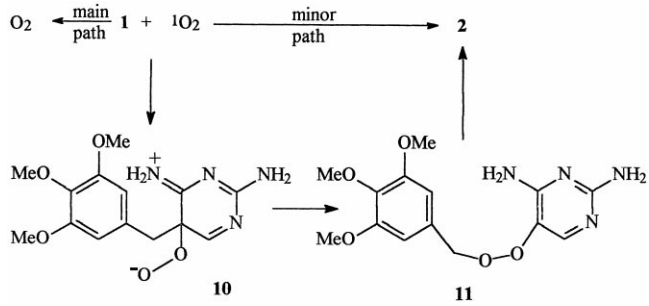
It is reasonable that in the presence of oxygen the benzyl radical is trapped to yield a peroxy radical (**7**, see Scheme 2) and that the observed aldehyde **2** and ketone **3** arise from the decomposition of the latter intermediate. The formation of the same compounds by direct irradiation in oxygen equilibrated solution is likely to be due to the sensitization by some ketone present as an impurity in the drug or formed during the photolysis.

Trimethoprim is slowly decomposed also by dye-sensitized oxidation. Under this condition singlet oxygen is formed. That this is the active species is supported by the fact that the rate of decomposition follows the order $CHCl_3$ > MeCN > MeOH (see Fig. 1), i.e., the same as that of the singlet oxygen lifetime in those solvents. This reaction leads again to the aldehyde **2** as the main product. Singlet oxygen is not involved in the above discussed ketone-sensitised oxidation of **1** to **2**, however. In a non hydrogen donating solvent such as acetonitrile, where the only paths for benzophenone triplet are quenching by oxygen and hydrogen abstraction from **1**, the ketone-sensitised oxidation is about twice as fast as the dye-sensitised reaction with a light flux of similar intensity. Thus, two different mechanisms are involved in the oxidation, Type I with ketones (Scheme 1) and Type II with dyes.

That the latter path is inefficient is not surprising, in view of the fact that arylamines are physical rather than chemical quenchers of singlet oxygen, and at any rate benzylic oxidation is not an expected reaction with this species. In fact, we irradiated under the same conditions 3,4,5-trimethoxytoluene and found that no reaction occurred. However, some chemical reactions of aromatic amines with singlet oxygen have been reported. As an example, the photo-oxidation of amine **8** has been rationalised as proceeding via the dioxetane **9** [13] (see Scheme 3; a singly



Scheme 3.



Scheme 4.

bonded zwitterion may equally be well envisaged). Analogy with this finding suggests that with **1** zwitterion **10** is formed, and a 1,3 benzyl shift occurs at this stage leading to peroxide **11** and finally to the observed product as shown in Scheme 4.

In conclusion, reactions from the excited states of **1** are inefficient. More likely paths inducing photodegradation of **1** involve: a. homolytic abstraction from the benzylic position by a triplet ketone or species of similar reactivity, e.g., alkoxy radicals, which are easily formed from additives used in drug preparations and b. reaction with singlet oxygen, which likewise leads to attack at the benzylic position. The usual therapeutic association of Trimethoprim with sulfamido drugs, known to form radicals upon irradiation [14], may make the photodegradation more important in practice.

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