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Photochemical decomposition of sulfamethoxazole

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Abstract

Sulfamethoxazole (4-amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide) is extremely photolabile in acidic aqueous solution, giving rise to at least five primary photoproducts. The major product has been identified as 4-amino-*N*-(5-methyl-2-oxazolyl)benzenesulfonamide resulting from photoisomerization of the isoxazole ring. This product was found to exist predominantly in its imido tautomeric form. Other products include sulfanilic acid, aniline, 3-amino-5-methylisoxazole and a hydrated product. The pathways leading to the formation of the products are postulated.

Key words: Sulfamethoxazole; NMR; Photodegradation; Photoisomerization; Photoproduct; Tautomerism

1. Introduction

Sulfamethoxazole (Fig. 1), an intermediate-acting antibacterial sulfonamide, is an essential component of co-trimoxazole, a fixed combination product containing sulfamethoxazole and trimethoprim in a 5 : 1 ratio. The formulations are regularly applied in the treatment of respiratory tract infections such as bronchitis (Hughes and Russell, 1982) and *Pneumocystis carinii* pneumonia with AIDS (Amyes et al., 1986; Fischl et al., 1988), severe urinary tract infections (Lude, 1987) and enteric infections (Yunus et al., 1982). However, co-trimoxazole has been implicated in adverse phototoxic and photoallergic skin reactions in patients exposed to sunlight (Australian Drug Evaluation Committee, 1987) and sulfamethoxazole is believed to be the prime suspect in causing such adverse effects (Martindale, 1989) although the photosensitization mechanism remains unknown. The adverse photosensitivity effects may arise from sensitization by either of the drugs or from some of the photoproducts formed following irradiation. The photoproducts can be toxic, or unstable and react further to give toxic derivatives (Greenhill and McLelland, 1990). To date there has been one report relating the degradation of trimethoprim in which one photoproduct

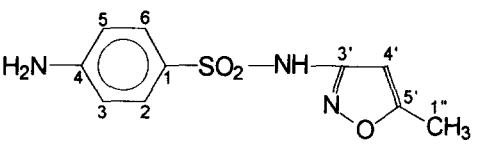

Fig. 1. Structure of sulfamethoxazole (4-amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide).

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was identified (Bergh et al., 1989). Studies on sulfamethoxazole have not been specifically reported, but sulfanilamide and sulfacetamide have been investigated in terms of photodecomposition (Reisch and Niemeyer, 1972; Pawlaczyk and Turowska, 1976) and free radical formation on photolysis (Chignell et al., 1980, 1981; Motten and Chignell, 1983). We have been studying the photochemical reactions of sulfamethoxazole alone and in combination with trimethoprim to gain information clarifying the photosensitization mechanism. Here we report the isolation and identification of the products from the photolysis of sulfamethoxazole in aqueous solution.

2. Materials and methods

2.1. Chemicals

Sulfamethoxazole was obtained from Sigma Chemical Co. (St. Louis, U.S.A.) and used without further purification after being proven pure by HPLC. Dimethyl-d_6 sulfoxide (DMSO-d_6) and the other deuterated solvents were purchased from Cambridge Isotope Laboratories (Woburn, MA, U.S.A.). All other reagents were of analytical reagent or higher grade. Organic solvents used were purified by fractional distillation. The water was double distilled in an all-glass still.

2.2. Photodegradation of sulfamethoxazole

1 l of aqueous solution of sulfamethoxazole (1.0×10^{-4} M, pH 3.4, acetate buffer) was irradiated at $\lambda > 290$ nm for 4–6 h under magnetic stirring in a Hanovia photochemical reactor with a 125 W medium pressure mercury lamp shielded by a Pyrex sleeve as previously described (Moore, 1987). Nitrogen was bubbled through the solution for 15 min prior to switching on the UV lamp and continued throughout the irradiation period. The composition of the solution was monitored by withdrawing samples for HPLC analysis to determine the time of irradiation giving optimal yield of the primary products. The preparative irradiation was carried out at pH 3.4 because of a higher rate of degradation obtained, compared to irradiation at pH 7.0 which led to identical products but at a much slower rate.

2.3. HPLC assay

High-performance liquid chromatography was carried out on an isocratic system consisting of an Altex 110A pump equipped with a Spectra-Physics model 100 UV/Vis detector or a Hewlett-Packard HP 1040A diode array spectrophotometric detector and a Rheodyne 7125 syringe loading sample injection value. The column used was a Brownlee RP-18 (Spheri-5, 5 μm, 100 mm $\times$ 4.6 mm). The mobile phase consisted of 0.05 M phosphate buffer (pH 6.0) and acetonitrile in the proportion 95:5. The UV spectra of the photoproducts formed, and the spectral homogeneity of the chromatographic peaks, were assessed using the diode array detector with 2 nm bandwidth.

2.4. Preparative chromatography

The irradiated aqueous solution was reduced in volume to 5 ml under reduced pressure at room temperature. The small amount of brown amorphous precipitate was removed by centrifugation and the supernatant was loaded onto a short reverse-phase preparative column for initial fractionation. The packing material for the flash chromatography was Merck 10 μm Lichrosorb RP-8. The crude fractions were then purified by preparative HPLC using a Spectra-Physics Iso Chrom LC pump and an Alltech Econosil C8 column (10 μm, 250 mm $\times$ 10 mm). The sample was eluted using a mixture of ethanol and water (20:80) as mobile phase flowing at 2 ml/min. The irradiated samples and collected fractions were stored at 4°C and protected from light.

2.5. Spectrophotometry

UV absorption spectra were obtained on a Perkin-Elmer Lambda 5 UV/Vis spectrophotometer or the Hewlett Packard HP 1040A diode array HPLC detector. IR spectra were recorded using a KBr disc with a Bio-Rad FTS 20/80 spectrometer.

2.6. Mass spectrometry

Chemical ionization mass spectra (CIMS) using CH_4 as reagent gas were obtained by desorption probe on a Finnigan Mat TSQ46 GC/MS/MS with an Incos data system. High resolution electron impact mass spectrometry (EIMS) was performed at 70 eV on an AEI MS-902 (with Kratos MS 50 upgrade) mass spectrometer.

2.7. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H- and ^{13}C-NMR spectra were obtained in DMSO-d_6 using a Varian Gemini 300 MHz NMR spectrometer. Chemical shifts are quoted on the δ scale relative to DMSO (δ 2.62 ppm) unless stated otherwise. Proton chemical shift assignments were achieved through selective spin-spin decoupling experiments and two-dimensional ^{1}H-^{1}H homonuclear correlation spectroscopy (COSY). ^{13}C resonances were assigned by two-dimensional ^{1}H-^{13}C heteronuclear correlation spectroscopy (HETCOR) and distortionless enhancement by polarization transfer (DEPT).

3. Results and discussion

3.1. Structure elucidation

Although sulfamethoxazole is relatively thermostable (Rudy and Senkowski, 1973), it decomposes rapidly in aqueous solutions under the influence of UV light. The photodegradation time course for irradiation of 1×10^{-4} M sulfamethoxazole at pH 3.4 and pH 7.4 is shown in Fig. 2. The degradation is strongly dependent on the state of ionization (pK_a 5.6; Rudy and Senkowski, 1973) with the sulfamethoxazole anion being the more stable form. The quantum yields for sulfamethoxazole photodegradation were determined by use of monochromatic light and ferroxalate chemical actinometry (Moore, 1987), the values of 0.47 (pH 3.4) and 0.084 (pH 9.0) being obtained. Separated by reverse-phase chromatography (Fig. 3), the primary photoproducts elute

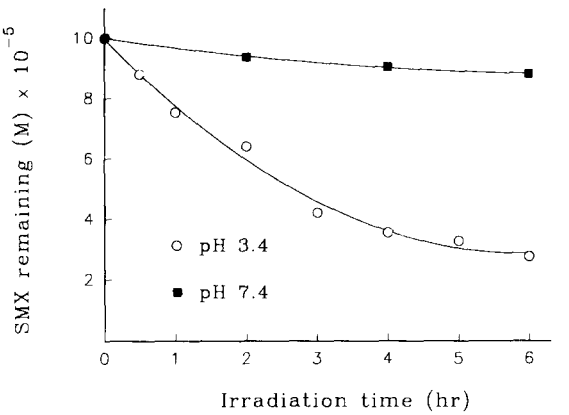

Fig. 2. The photodegradation time course of sulfamethoxazole in aqueous solution (1×10^{-4} M).

before sulfamethoxazole and are therefore more polar than the parent. Less polar products were formed in trace amount when a more concentrated solution was irradiated for a lengthy period. These are believed to be secondary products. Irradiation of an air-saturated solution produced the same product profile. However, a nitrogen atmosphere was maintained to enhance the stability of the photoproducts.

A homogeneous peak eluting at 10.9 min in the HPLC, designated I, represents the major photoproduct from photolysis of sulfamethoxazole in aqueous media. The quantum yield of formation of product I is estimated to be about

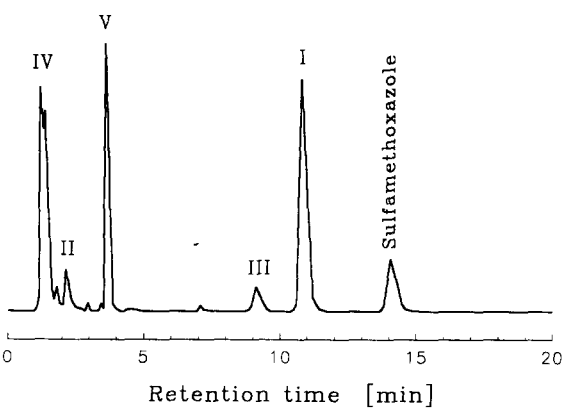

Fig. 3. Reverse-phase HPLC profile of sulfamethoxazole aqueous solution at pH 3.4 irradiated for 4 h.

0.15 in acidic solution, while the quantum yield of the photodegradation of sulfamethoxazole under this condition was determined to be 0.47, i.e., product I represents about 30% of the total products formed.

The CIMS spectrum of product I showed a quasimolecular ion at m/z 254 with adduct ions at m/z 282 ($[M + C_2H_5]^+$) and 294 ($[M + C_3H_5]^+$), respectively. The empirical formula $C_{10}H_{11}N_3O_3S$, which is the same as that of sulfamethoxazole, was established by high resolution mass spectrometry. The observed value, 253.0510, is in good agreement with the calculated value of 253.0521. These findings lead to the conclusion that the major product is an isomer of sulfamethoxazole formed through a photorearragement pathway. The EIMS spectrum of product I displayed the typical fragments from the sulfonamide moiety, such as 92 ($H_2NC_6H_4$), 140 ($H_2NC_6H_4SO$), 156 ($H_2NC_6H_4SO_2$) and 189 (M-SO_2).

^{1}H- and ^{13}C-NMR data of product I and sulfamethoxazole are presented in Table 1. The resonances of I were assigned according to several criteria, beginning with a comparison with those of sulfamethoxazole. In the ^{1}H-NMR spectrum, signals at δ 2.08 ppm (3H), δ 5.79 ppm (2H), δ 6.53 ppm (2H) and δ 7.45 ppm (2H) were similar to those of sulfamethoxazole, suggesting that the H_2N-C_6H_4-SO_2- group and the methyl group were intact. The most downfield signal, appearing at δ 11.3 ppm as a very broad peak which disappeared after addition of a drop of D_2O, can be assigned to the exchangeable proton of the -NH- group (Turczan and Medwick, 1972). The peak broadening might be attributable to quadrupole relaxation associated with the nitrogen (Rahman, 1986; Kemp, 1987).

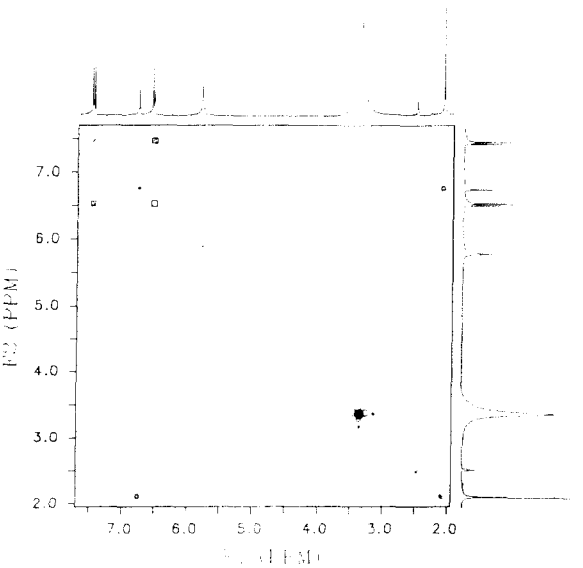

Fig. 4. Two-dimensional COSY spectrum of photoproduct I.

A homonuclear correlation (COSY) spectrum is shown in Fig. 4. Apart from those caused by the aromatic protons, the cross-peaks suggested a coupling between $H_{4'}$ and the methyl protons. The coupling was also observed by one-dimen-

Table 1
^{1}H- and ^{13}C-NMR data of product I and sulfamethoxazole in DMSO-d_6

Number of H or C	δ ^{1}H (ppm) (multiplicity)		$J_{H,H}$ (Hz)		δ ^{13}C (ppm)	
	Product I	SMX	Product I	SMX	Product I	SMX
1					130.9	124.0
2, 6	7.45 (d)	7.46 (d)	$J_{2,3}$, $J_{6,5} = 8.7$	$J_{2,3}$, $J_{6,5} = 8.8$	129.2	128.7
3, 5	6.53 (d)	6.57 (d)	$J_{3,2}$, $J_{5,6} = 8.7$	$J_{3,2}$, $J_{5,6} = 8.8$	114.2	112.5
4					153.7	153.1
2′ or 3′					143.4	169.7
4′	6.76 (d)	6.09 (d)	$J_{4',1''} = 1.2$	$J_{4',1''} = 0.82$	112.3	95.26
5′					158.8	157.8
1″	2.08 (d)	2.28 (d)	$J_{1'',4'} = 1.2$	$J_{1'',4'} = 0.82$	10.96	12.18
NH_2	5.79 (s, b)	6.06 (s, b)				
NH,	11.3 (s, b)	10.9 (s, b)				

s, singlet; d, doublet; q, quartet; b, broad.

sional homonuclear decoupling experiments and the coupling constant was determined to be 1.2 Hz, while $J_{4',1''}$ in sulfamethoxazole was 0.82 Hz.

The ^{13}C-NMR data of product I showed that the 10 carbon atoms in the molecule exhibited eight distinct resonances, all of which were similar to those of sulfamethoxazole except those related to the isoxazole ring. Assignment of all the carbon resonances was achieved with the aid of DEPT and HETCOR experiments and also by comparison with those of sulfamethoxazole and other sulfonamides. The DEPT spectrum revealed the existence of three CH resonances and one methyl resonance. The resonances at δ 114.2 and 129.2 ppm represent the two pairs of protonated aromatic carbons, verified by the HETCOR experiment, which further proved that $H_{4'}$ (δ 6.76 ppm) is directly attached on $C_{4'}$ (δ 112.3 ppm) as expected from ^{1}H-NMR.

From the MS and NMR information, it was concluded that the structure of product I is 4-amino-*N*-(5-methyl-2-oxazolyl)benzenesulfonamide which is depicted in Fig. 5. The UV spectrum of product I exhibits two absorption bands with maximum wavelengths at 240 and 264 nm, respectively, which is significantly different in shape from that of sulfamethoxazole (Fig. 6). An explanation is that the compound exists predominantly in its imido tautomeric form in aqueous solution (Fig. 7), in contrast to sulfamethoxazole for which the amido form dominates (Bult, 1983). The same UV feature was observed for sulfamoxol, a sulfonamide containing the oxazole ring, which also exists predominantly in imido form (Bult and Klasen, 1978). Furthermore, it has been shown that the SO_2 symmetric stretching band, one of the strongest absorptions in the IR spectra of sulfonamides, could be used as a criterion of the tautomeric forms, i.e., sulfonamides in the amido form absorb in the region 1170–1145 cm^{-1}, while sulfonamides in the imido form absorb in the region 1145–1130 cm^{-1} (Uno, 1963). The position of this band of product I was observed at 1132 cm^{-1} in the IR spectrum, confirming the imido structure.

Product II eluted at 2.4 min on the reverse-phase HPLC. Its UV spectrum resembled that of sulfamethoxazole with λ_{max} at 256 nm. From the proton NMR data (Table 2), it was evident that the sulfonamide moiety was present. In the CIMS spectrum the quasimolecular ion at m/z 272 with appropriate adduct ions at m/z 300 ([M + C_2H_5]$^+$) and 312 ([M + C_3H_5]$^+$) suggested a formula of $C_{10}H_{13}N_3O_4S$. The resonance in the ^{1}H-NMR spectrum at about δ 6.5 ppm was determined to be an exchangeable proton assigned to

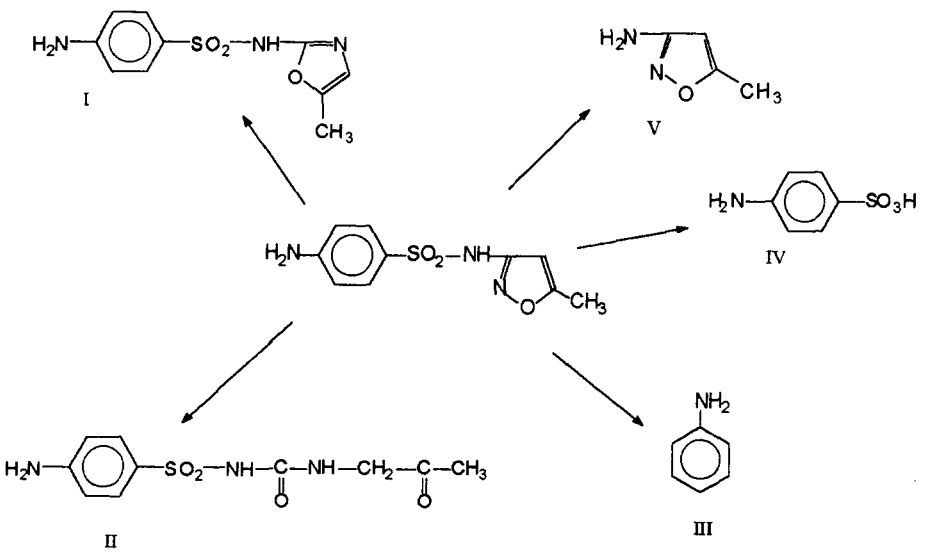

Fig. 5. Photochemical decomposition products from sulfamethoxazole.

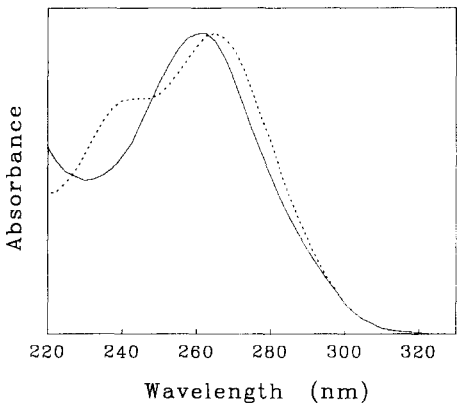

Fig. 6. Normalized UV spectrum of sulfamethoxazole (continuous line) and photoproduct I (broken line) in aqueous solution at pH 6 obtained using the diode array HPLC detector.

an -NH- group. The methylene protons are magnetically nonequivalent, appearing as a split signal at δ 3.8 ppm. A test for the carbonyl functional group using 2,4-dinitrophenylhydrazine (Shriner et al., 1980) indicated the presence of a carbonyl (C = O) group. On the basis of the spectral data, product II was deduced to be a hydrated product (Fig. 5) of 2H-azirine, a metastable product formed as discussed below.

Product III eluted at 9.2 min on the reverse-phase HPLC. The compound possessed identical chromatographic features and UV and mass spectra to those of an authentic sample of aniline.

Product IV, one of the most polar photoproducts, was formed in a relatively high yield. It coeluted on the HPLC at very short retention time (1.7 min) with some other components as shown in Fig. 3. After further purification by preparative HPLC, the fraction afforded a single peak on the analytical HPLC using a more polar mobile phase (1% acetonitrile in 0.05 M acetate buffer, pH 3.0; flow rate, 0.25 ml/min). The UV spectrum was uniform throughout the peak and was identical to that of sulfanilic acid. The CIMS and EIMS data were also identical to those of a sulfanilic acid standard.

Table 2
^{1}H-NMR data of product II in DMSO-d_6

H	δ (ppm) (multiplicity)	$J_{H,H}$ (Hz)
H_2, H_6	7.46 (d)	$J_{2,3}$, $J_{6,5} = 8.5$
H_3, H_5	6.53 (d)	$J_{3,2}$, $J_{5,6} = 8.5$
$H_{2'}$	3.79 (d)	
$H_{4'}$	2.00 (s)	
NH_2	5.82 (s, b)	
SO_2NH	10.6 (s, b)	
NH	6.57 (s, b)	

s, singlet; d, doublet; b, broad.

Product V, eluted at 3.8 min on the reverse-phase HPLC, was observed more strongly when the detection wavelength was shifted to 230 nm. The UV spectrum of V exhibited a maximum absorption at 210 nm, suggesting a lack of aromatic characteristics. The CIMS spectrum displayed a quasimolecular ion at m/z 99 ($[M + H]^+$) with adduct ions at m/z 127 ($[M + C_2H_5]^+$) and m/z 139 ($[M + C_3H_5]^+$). The EIMS spectrum of V gave a highest mass of 98. The empirical formula $C_4H_6N_2O$ was then established by high resolution mass spectrometry. The NMR data of product V are presented in Table 3. In the ^{1}H-NMR spectrum, three proton resonances were observed at δ 2.30 ppm (3H), δ 3.53 ppm (2H) and δ 5.56 ppm (1H), respectively. The signal at δ 3.53 ppm was broad and exchangeable, and was assigned as an amino group. The resonance at δ 2.30 ppm represents a methyl group which was found to couple with H_4 ($J_{1'4} =$ 0.72 Hz). The ^{13}C-NMR spectrum and DEPT

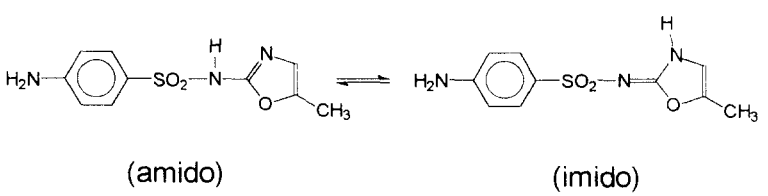

Fig. 7. Tautomerism of photoproduct I.

Table 3
^{1}H- and ^{13}C-NMR data of product V in $CDCl_3$

Number of H, C	δ ^{1}H (ppm) (multiplicity)	$J_{H,H}$ (Hz)	δ ^{13}C (ppm)
3			169.4
4	5.56 (s)		94.24
5			163.4
1′	2.30 (d)	$J_{1',4} = 0.72$	12.55
NH_2	3.53 (s, b)		

s, singlet; d, doublet; b, broad.

experiment revealed the existence of two quaternary carbons, one CH carbon and a methyl carbon. The evidence allowed the assignment of product V as 3-amino-5-methylisoxazole.

3.2. Photodecomposition pathways

Formation of the identified photoproducts indicates that sulfamethoxazole photodegrades in aqueous solution by several pathways, the most important of which is a rearrangement of the isoxazole ring resulting from rupture of the nitrogen-oxygen bond. A postulated pathway for the photoisomerization is summarized in Fig. 8. The initial step is the UV-induced cleavage of the labile N-O bond of the five-membered ring. The reactive diradical so formed then undergoes recyclization to produce an intermediate believed to be the 2H-azirine (X) which has not been successfully isolated. The intermediate quickly rearranges to form the oxazole ring via the carbonyl-stablized nitrile ylide as suggested previously for the general isoxazole ring (Singh and Ullman, 1967; Sato and Saito, 1974). Product II can be formed by hydration of intermediate X. Aniline and sulfanilic acid have been identified as photoproducts from photolysis of other sulfonamides (Pawlaczyk et al., 1974; Pawlaczyk and Turowska, 1976). These two products were believed to be formed from free radicals following homolytic γ-fission and δ-fission of sulfonamides (Chignell et al., 1981). Formation of 3-amino-5-methylisoxazole could be via free radical reaction initiated either by δ-fission or by hydrolysis of sulfamethoxazole under UV irradiation.

4. Conclusions

The main photoproducts from sulfamethoxazole are closely related to known sulfonamides,

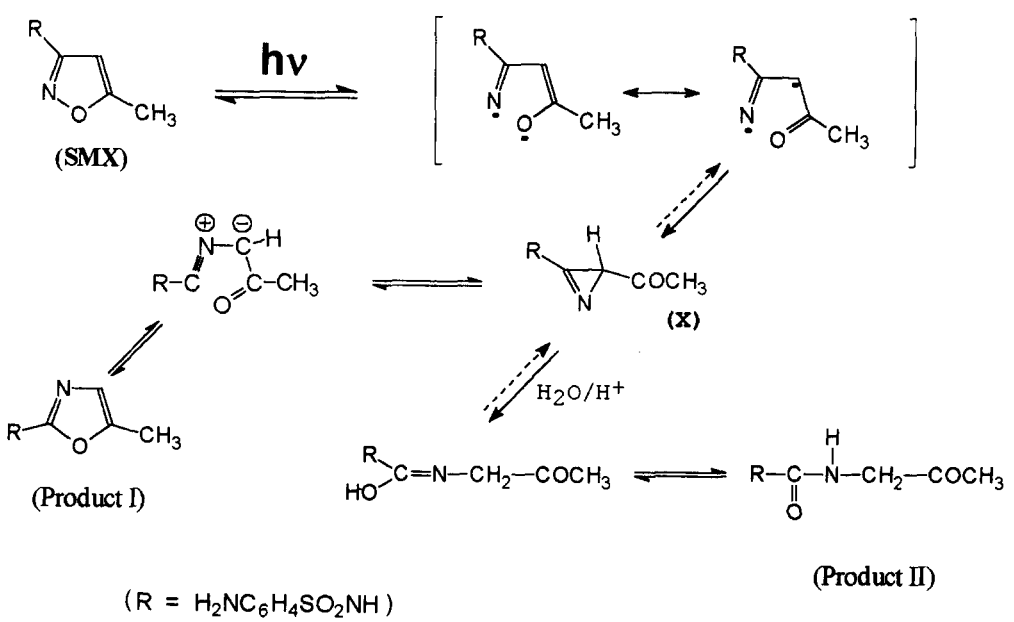

Fig. 8. Postulated pathways of formation of photoproducts I and II.

but that does not exclude them from suspicion of involvement in adverse photosensitivity. Chlordiazepoxide is one instance where a closely related metabolite is responsible for a phototoxic effect (Cornelissen et al., 1979). Alternatively, some metastable intermediates and/or free radicals may be responsible for the adverse photoinduced effect. Irradiation of sulphanilamide caused covalent binding to nucleic acids and proteins, nicking of closed circular DNA as well as crosslinking of calf thymus DNA and human serum albumin (Sinha et al., 1982). These effects have been attributed to the free radical intermediates, among which $SO_3^{\cdot-}$ is believed to be highly toxic (Motten and Chignell, 1983). Undoubtedly, the photoallergic response of sulphanilamide results from the covalent modification of biological macromolecules by metastable photoproducts to produce an antigen (Chignell et al., 1980). Although there is some resemblance between sulfamethoxazole and sulphanilamide in their photodecomposition pathways, it may be premature to conclude that their adverse photosensitization mechanisms are similar. Further experimental investigations are being undertaken in which biologically relevant macromolecules are present during the irradiation of sulfamethoxazole.

Acknowledgments

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