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# Photodegradation kinetics under UV light of aztreonam solutions

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**Abstract**: A photodegradation study of aztreonam solutions exposed to UV irradiation showed that the major product of degradation was the *anti*-isomer together with some unidentified products. This result was similar to that obtained with cefotaxime and it is postulated that this would be generally true of compounds containing an alkoxyimino group.

**Keywords**: *Aztreonam; cefotaxime; photodegradation;* syn–anti *isomerism.*

## Introduction

Aztreonam is the first antibiotic from the monobactam family to have been therapeutically approved. It was introduced in 1987 in the form of injections in the USA and is now used in most European countries in the treatment of infections against Gram-negative bacteria.

In a review article recently published [1] on this compound, the degradation profile of the antibiotic as a function of pH was extensively described, but its stability under UV light was not of major concern; only the yellowing of the powder after light exposure was mentioned.

However, the structural analogy (Fig. 1) between aztreonam and cefotaxime, a third generation cephalosporin (both having a β-lactam structure and a side-chain with an alkoxyimino group in the *syn*-configuration), raises some doubt as to its photochemical stability.

The study presented here was undertaken to complete previous work [2] concerning the sensitivity of some antibiotics from the β-lactam family to UV irradiation. The degradation kinetics of aqueous solutions of aztreonam irradiated at 254 nm, has been monitored through HPLC and UV absorption with special attention to the *syn–anti* isomerization. The results have been compared to those obtained for cefotaxime under the same experimental conditions.

## Experimental

### *Materials*

Aztreonam (I) CAS [78110-38-0], batch number 12783, Aztreonam *anti* (or E-isomer) (II), batch number NN004 and open ring aztreonam (IV), CAS [87500-74-1] batch number NN006 were gifts from Squibb Laboratories (Paris, France).

Cefotaxime sodium salt (VII), CAS [60846-21-1], batch number 4 and cefotaxime *anti*-isomer (or E-isomer), (VII) batch number 2, were gifts from Roussel UCLAF Laboratories (Romainville, France). See Fig. 1 for details.

### *Photodegradation*

*Solutions.* Aqueous solutions used for photodegradation were $2 \times 10^{-3}$ M in distilled water for (I), (II) and (VII), respectively. Their pH was adjusted to 5 with diluted sodium hydroxide (I, II) or hydrochloric acid (VII).

*Photoreactor.* The radiation source was a UV lamp emitting at 254 nm (TUV, 6 W Philips Eindhoven). The lamp (25 cm long and 4 cm in diameter) was set horizontally under a metallic reflector. The photoreactor itself was a $1 \times 1 \times 4.5$ cm quartz cell fitted with a conical Teflon stopper, containing 3 ml of the solution under study. During irradiation, the cell was laid flat on one side and immersed (flushed with water to its upper surface) in a 1 cm depth of circulating water. The solution

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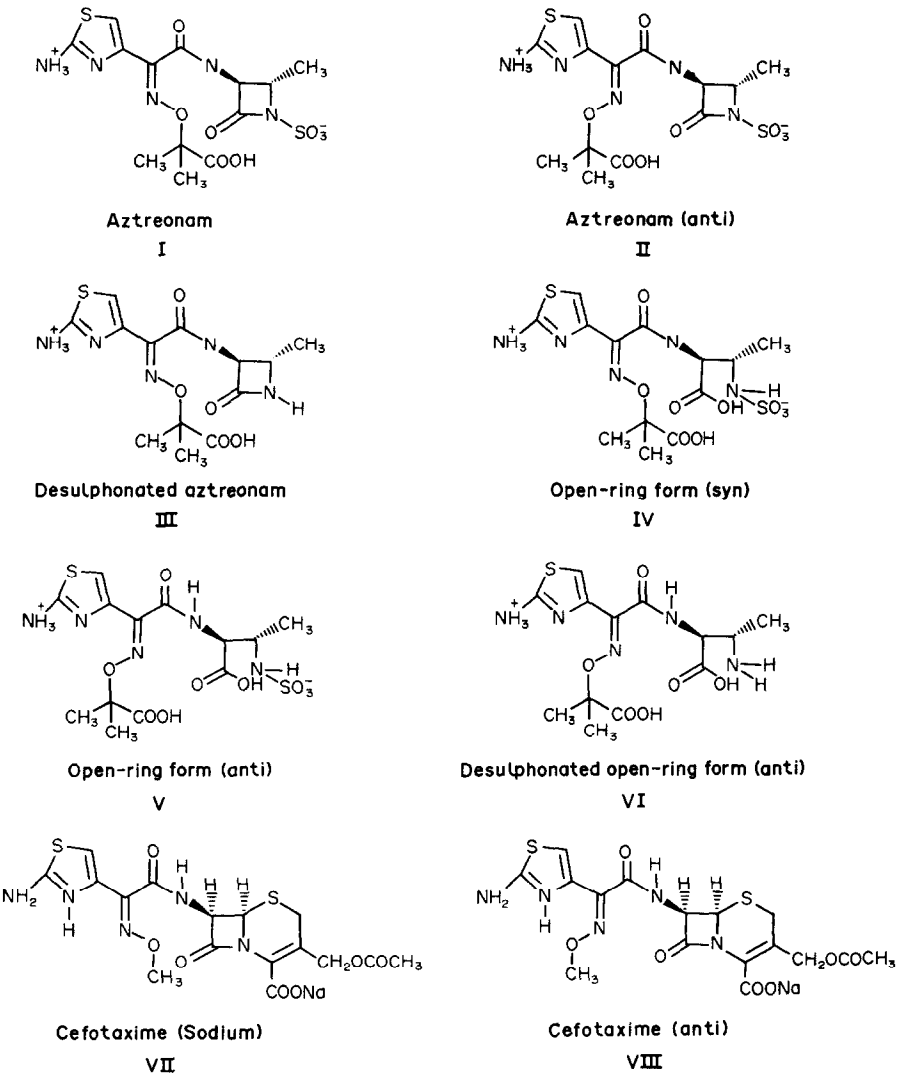


**Figure 1**
Chemical structures for aztreonam, its major potential decomposition products and cefotaxime and its *anti*-isomer.

inside the cell was stirred by means of a small bar magnet coated with glass. The water circulating around the cell was pumped from a thermostated bath, keeping the cell at a temperature of 25 ± 0.5°C. The vessel containing the water and cell was flat at the bottom and open at the top. The cell was laid on a marked area, with its long side parallel to the axis of the lamp, at a fixed distance from the latter. The UV light fell on the horizontal upper face of the quartz cell.

*Actinometry*. Chemical actinometry based on monochloroacetic acid hydrolysis was used [2–4]. The plot of the number of photolysed molecules versus time was linear (correlation coefficient 0.9998) and passed through the origin.

The measured molecular absorptivities at 254 nm for the solutions of aztreonam, its *anti*-isomer and cefotaxime were 9330, 11200 and 18000, respectively. Therefore, the incident UV light can be considered to be totally absorbed by the upper 2 mm of the solutions. Under these conditions, the intensity of the light flux absorbed by the sample and calculated from the slope of the graph was $4.4 \times 10^{17}$ photons $s^{-1}$.

*Analytical techniques*

*Materials and reagents*. $KH_2PO_4$, $H_3PO_4$ and $CH_3OH$ were of analytical grade. Water was bidistilled in a glass apparatus.

*Apparatus.*

*Spectrophotometry*. UV measurements were

carried out with a UV–vis spectrophotometer (Shimadzu UV 2100, Kyoto, Japan) using a quartz cell having a 10 mm pathlength.

*Liquid chromatography*. The liquid chromatograph used for monitoring the kinetics consisted of an isocratic pump (HP 1050, Hewlett–Packard, Waldbronn, Germany) fitted with an injection loop of 10 µl. The compounds were separated on a Lichrocart$^R$ cartridge of 250 mm × 4 mm i.d. (Merck, Darmstadt, Germany) packed with Lichrosorb$^R$ RP 18, 7 µm particles. A guard column, Merck Lichrocart$^R$ 4 × 4 mm packed with Lichrosorb$^R$ 100 RP 18 was fitted prior to the analytical column. The mobile phase was 0.05 M phosphate buffer pH 3–methanol (80:20, v/v). Standard solutions for quantification were mixed aqueous solutions of I and II (or VII and VIII), each $2 \times 10^{-4}$ M. Test solutions withdrawn from the kinetics were diluted 1 to 10 in water before injection.

For the detection, a UV programmable multi-wavelength detector (Hewlett–Packard 1050) was used. In order to detect the isomers at their maximum absorbance wavelength, the detector was programmed at $\lambda = 262$ nm (from 0 to 10 min), $\lambda = 239.5$ nm (from 10 min) after injection for the degradation kinetics of I and II, and $\lambda = 237.5$ nm (from 0 to 10 min), $\lambda = 262$ nm (from 10 min on) for the degradation kinetics of VII and VIII. The bandwidth was set to 4 nm. A reference wavelength of 450 nm together with a bandwidth of 80 nm was used to minimize the background noise.

The identity of the compounds formed and the homogeneity of the chromatographic peaks were assessed using a diode array detector (Waters 991, Milford, MA, USA) with a 2 nm bandwidth.

## Results and Discussion

*Validation of the analytical method*

As the method proposed in the monograph sterile aztreonam [5] is given only for the determination of (I), the technique was validated for a simultaneous determination of I and II during the photodegradation. The capacity factors were $k'_I = 3.5$ and $k'_{II} = 7.5$ and the resolution 13.2. The symmetry factors were $As_I = 1.25$ and $As_{II} = 1.0$. The linearity of the calibration graphs (peak area vs concentration) was assessed for I and II between $2 \times 10^{-5}$ and $20 \times 10^{-5}$ M ($n = 6$ solutions). The graphs were linear and went through the origin. The repeatability was assessed at two different concentrations ($2 \times 10^{-5}$ and $20 \times 10^{-5}$ M). The relative standard deviation using peak area measurements was about 1.1% ($n = 7$ injections) in all cases. The detection limits evaluated for a signal-to-noise ratio of 2 were about 3.25 ng (I) and 5.4 ng (II). The limit of determination was estimated to be about 16 ng (I) and 27 ng (II).

*Degradation kinetics at* 254 nm

*Irradiation of aztreonam solutions*. A specimen chromatogram recorded during the degradation kinetics of I is given in Fig. 2. The peak, the retention time of which was similar to that of II, had a spectrum which was superimposable after normalization to that of II in the standard solution. This confirms that the major compound formed after irradiation at 254 nm was the *anti*-isomer, which is microbiologically inactive [6].

The plot of the percentage of I remaining and II formed (with respect to the initial concentration of I), as a function of the time of irradiation, is given in Fig. 3 (the average of three different kinetic studies). The plot of a solution of I kept in the dark is given for comparison and showed no change. Therefore, the degradation and isomerization observed after irradiation were due to the effect of UV light. The degradation proceeds through an isomerization of the alkoxyimino group yielding the formation of II on the one hand, and

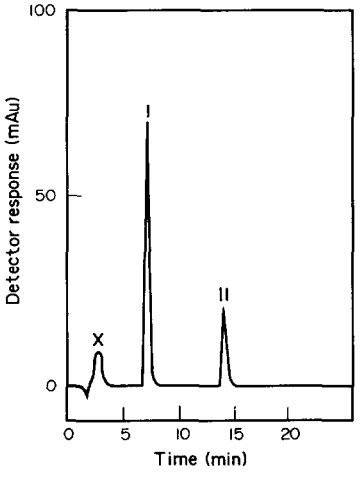


**Figure 2**
Chromatogram from a $2 \times 10^{-3}$ M aztreonam solution, pH = 5.0, after 3 h irradiation.

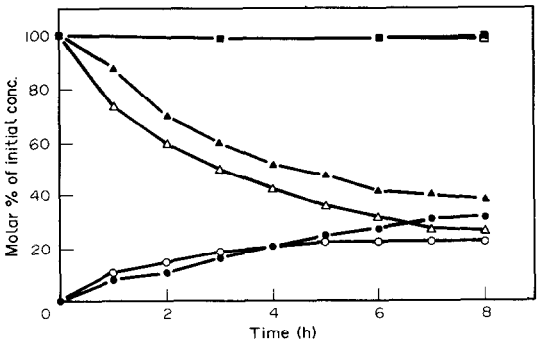


**Figure 3**
Data for I (▲, △) and its corresponding isomer (●, ○) formed during the photodegradation kinetics of an aqueous solution of I ($2 \times 10^{-3}$ M) pH = 5.0, and data for I (■, □) stored in the dark. Filled and open symbols refer to the kinetics without and with arginine, respectively.

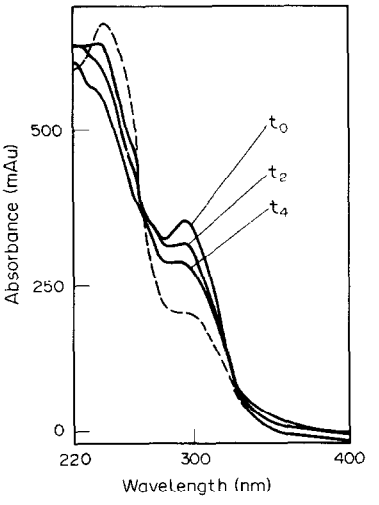


**Figure 4**
Spectral changes during the photodegradation of a $2 \times 10^{-3}$ M aqueous solution of (I); pH = 5.0 after 0, 2 and 4 h irradiation; the broken line features the spectrum of a standard solution of II, for comparison.

the formation of photodegradation products on the other, as shown on the chromatogram (Fig. 2). These photoproducts (*X*) have a retention time which differ from that of IV, which is a potential decomposition product of I (Fig. 1). Under the same chromatographic conditions, IV was eluted at 240 s.

The total percentage of I and II after 3 h irradiation, was about 80%. If we assume that the absorptivity of the photoproducts is similar to that of I, the formation of the photoproducts (retention time approx. 180 s) corresponded to about 20% (with respect to the initial I concentration). The conversion is slow and the photostationary equilibrium is reached only after 8 h. From this moment on, the ratio of concentration I:II is about 1.2. The kinetics and the composition of the photostationary solution depends of course on the power of the lamp. The absolute quantum yield of the reaction, which depends only on the irradiation wavelength, was about 0.03%.

The degradation kinetics of I was simultaneously monitored by UV absorption spectrophotometry. The evolution of the spectra recorded during the kinetics are shown in Fig. 4. A gradual decrease in the absorbance at 262 nm can be noted. At this wavelength, which corresponds to the maximum absorbance of aztreonam at pH = 5, II has a lower absorptivity. The formation of II during the kinetics may be partly responsible for the spectral evolution observed. An isosbestic point is observed at 265 nm. An increase in the absorbance in the 350–400 nm wavelength range can also be observed, corresponding to the yellowing of the solutions, which was visually observed after 5 h irradiation. An important change in the pH values of the solution (from 5.0 to 7.5) was also noted during the degradation kinetics. This increase could be due to the formation of III and VI (Fig. 1), potential degradation products cited in ref. 7, and/or their analogue *anti*-isomers.

*Irradiation of a mixed solution of aztreonam and L-arginine.* Aztreonam is marketed as a powder containing 41% (w/w) of L-arginine, which yields a pH of 5 after reconstitution in aqueous solution.

The kinetic protocol previously described was conducted on a mixed aqueous solution of aztreonam ($2 \times 10^{-3}$ M) and L-arginine in a proportion corresponding to the formulated product. The degradation kinetics of a solution of aztreonam alone, at the same pH value was conducted in parallel for comparison.

The degradation curve (Fig. 3) shows that the rate of aztreonam loss is slightly increased by the presence of arginine. The interaction noted between aztreonam and L-arginine [8, 9], which leads to the formation of an amide, may be responsible for the difference in the degradation rate observed. The photostationary mixture in the presence of L-arginine showed a lower ratio of I to II of approximately 10%.

*Irradiation of a solution of E-isomer at* 254 nm. The photochemical interconversion

between *syn*- and *anti*-isomers according to the following scheme

$$anti \leftrightarrow \text{syn} \rightarrow \text{stationary equilibrium}$$

is observed for many organic compounds possessing an alkoxyimino group [10]. Therefore the kinetics of the photodegradation of II was conducted under the same conditions as for I.

The irradiation of a solution of II at 254 nm showed (Fig. 5) a compound the retention time and absorption spectrum of which were similar to that of I. Figure 6 shows the plot of the degradation kinetics of II, both under UV irradiation and in the dark, for comparison.

The comparison of these two kinetics allows the degradation and isomerization, observed under irradiation, to be attributed to UV light.

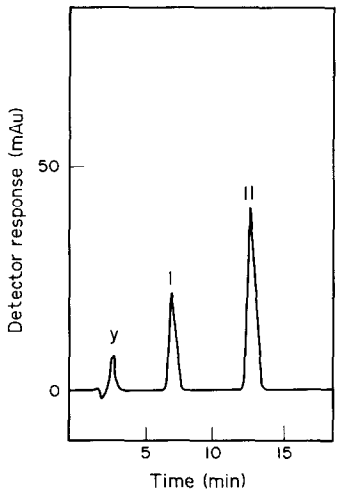


**Figure 5**
Chromatogram from a $2 \times 10^{-3}$ M aztreonam *anti*-isomer solution, pH = 5.0, after 3 h irradiation.

The concentration of the *anti*-isomer decreased regularly as a function of time, whilst aztreonam concentration increased. A plateau was reached after about 5 h. The stationary equilibrium was not yet reached after 8 h irradiation. The photostationary states evaluated at infinite time for the kinetics of I and II were similar within 5% (Figs 3 and 6). The total percentage of I + II after 3 h irradiation was about 85%. If we consider that the absorptivity of the photoproducts is similar to that of II, the peak y, the retention time of which was about 180 s (Fig. 5), represented about 15% with respect to the initial concentration of II.

In order to assess whether y was V (Fig. 1) this latter (obtained from alkaline hydrolysis of II [11]) was injected under the same chromatographic conditions; its RT (465 s) was different from that of *Y* (180 s).

During the photodegradation of I and II, a yellowing of the irradiated solutions was also visually observed after 6 h irradiation.

*Photodegradation of (I) at pH* 3.5. The photodegradation of (I) at 254 nm was also conducted at pH 3.5 (pH of aztreonam aqueous solutions without any adjustment of pH). At pH 3.5, the disappearance of (I) followed an overall similar course (Fig. 7) but II formed in greater amount and no other product was detected. The larger amount of II is consistent with the superimposition of a thermal isomerization [1], in addition to the chemical process. It was also observed that in contrast to pH 5.0, the pH remained constant during the degradation and furthermore, no yellowing of the solution took place. So, at pH

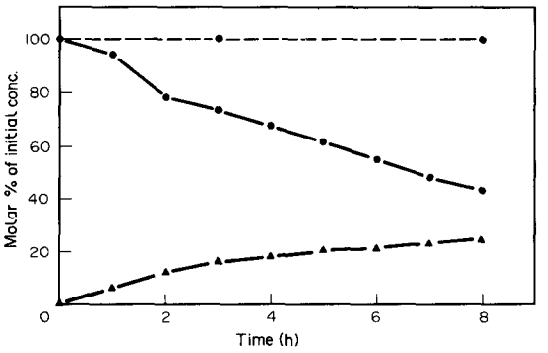


**Figure 6**
Data for II (●) and its *syn*-isomer I (▲) during the photodegradation kinetics of an aqueous solution of II ($2 \times 10^{-3}$ M) pH = 5.0 and data for II ($2 \times 10^{-3}$ M) stored in the dark (–●–).

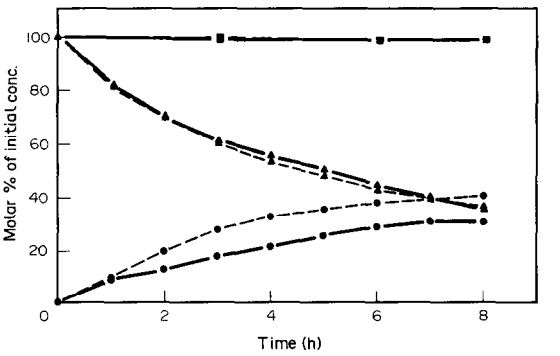


**Figure 7**
Comparative data of the photodegradation kinetics of I, conducted at pH = 3.5 and pH = 5.0, I (▲), II (●). Kinetics conducted in the dark, I (■). Broken and solid lines refer to the kinetics at pH 3.5 and 5.0, respectively.

5.0, the observed yellowing and pH change could be correlated to the formation of other products than II (x or y, Figs 2 and 5). It must be noted that the irradiation of thiazoximic acid [2] and azitidinone ring, two main substructures of (I), did not lead to any yellowing.

## Conclusion

The degradation of aztreonam under 254 nm UV light leads mainly to its *anti*-isomer. Very similar results were previously obtained with cefotaxime [2] and it is expected that all antibiotics with an alkoxyimino group will behave in an analogous way. We have also observed (unpublished results), that for cefotaxime and aztreonam, such an isomerization also takes place under irradiation at 360 nm, although a quantitative study was made difficult because of the very low absorbances of these molecules at this wavelength. This should be true also for other cephalosporins bearing an oxyimino group.

In fact, irradiating at 254 nm would be expected to convert the molecule into its second or third excited state whereas irradiating at 360 nm must be close to exciting the 0–0 band of the first excited state as is frequently the case for organic molecules; relaxation from an upper excited state (second or third) will lead to the first excited state responsible for the observed isomerization.

This study shows also that if the same general scheme holds for the degradation under UV light of all antibiotics containing an alkoxyimino group, there will be nevertheless differences due to the various substituents present. For instance, we carried out the photolysis of cefotaxime under the same experimental conditions as for aztreonam (Fig. 8). The photoisomerization yield of aztreonam (0.03%) points to a better stability compared to cefotaxime (0.2%). The half-lives of these compounds differ by a factor of 3, the faster degradation of cefotaxime having certainly to do with the concurrent photolysis of the cephem ring or of the ester chain [2]. As a concluding remark, it must be noted that, since the isomerization of these antibiotics result in the formation of an inactive *anti*-isomer in all cases, the above results constitute a strong indication to avoid handling solutions of the above molecules in the vicinity of UV sources such as the sun, neon tubes or germicidal lamps.

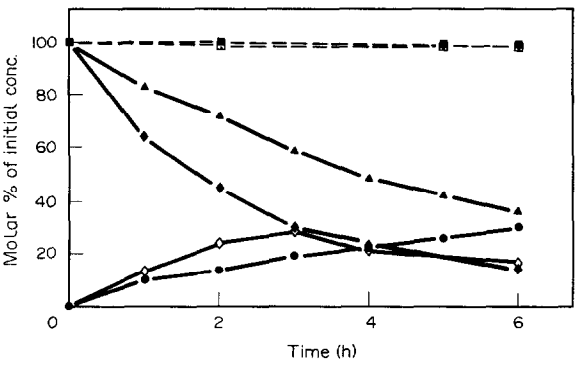


**Figure 8**
Comparison of the photodegradation kinetics of cefotaxime (◆) and aztreonam (▲) in aqueous solutions, under the experimental conditions used in this study. The symbols ◇ and ● refer to the corresponding *anti*-isomer. The broken line refers to both kinetics in the dark.

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