Photoisomerization Kinetics of Cefuroxime Axetil and Related Compounds

H. FABRE*x, H. IBORK*, AND D. A. LERNER‡

Received March 30, 1993, from the *Laboratoire de Chimie Analytique, Faculté de Pharmacie, 34060 Montpellier Cedex 01, France, and ‡Laboratoire de Chimie Physique et Informatique, Ecole Nationale Supérieure de Chimie, 34053 Montpellier Cedex 1, France. Accepted for publication October 18, 1993®.

Abstract □ The photoisomerization kinetics of aqueous solutions of cefuroxime axetil under irradiation at 254 nm was investigated by HPLC. The overall degradation is the result of a competition between the isomerization of the alkoxyimino group and the photolysis of the β-lactam ring. Cefuroxime axetil exists as a mixture of two diastereomers which are shown to react at different rates. This is true not only for the photoisomerization step but also for ground-state hydrolysis in alkaline conditions. Photoisomerization of the alkoxyimino group is also observed for the *anti* isomer of cefuroxime axetil and for some of its degradation products. The quantum yields for all these photoisomerizations are always lower than 1%, which explains the relative importance of the photolysis step. A stationary *syn* to *anti* ratio of 1 is measured for cefuroxime axetil and of 2.1 for cefuroxime. From this and previous studies, it appears that cefuroxime axetil is the most sensitive under irradiation at 254 nm when compared to other antibiotics bearing the alkoxyimino group. Aztreonam is the most stable followed by cefotaxime, cefuroxime, and cefuroxime axetil.

Cefuroxime axetil, an ester prodrug of cefuroxime, was the first oral cephalosporin of the second generation to be commercially available as tablets in 1989. It exists as a mixture of diastereomers A and B due to the esterification of cefuroxime by the racemic 1-acetoxyethyl bromide. Its stability *in vitro* has been monitored by HPLC in buffer solutions[1] and in common beverages.[2,3] The hydrolysis kinetics follow a first-order reaction in a pH range 1–9.[1] The pH–rate profile for the total isomeric mixture shows a maximum stability in the pH range 3.5–5.5 and different hydrolysis rate constants for the two isomers. Isomer A is always more reactive than isomer B with a maximum difference in reactivity (27%) being observed at pH 1. Acetate or phosphate buffer catalyzes the degradation, but ionic strength does not have a significant effect on the kinetics. The hydrolysis proceeds through different routes, yielding the Δ-2-isomer, cefuroxime, and small quantities of sulfoxides (Chart 1). The present study is a continuation of previous works[4,5] related to the photochemical degradation of antibiotics possessing an alkoxyimino linkage on the side chain of the β-lactam ring. We had previously shown that, under UV light, aqueous solutions of cefotaxime[4] and aztreonam[5] aside from photolysis undergo a conversion to their *anti* isomer.

We have investigated the stability of the diastereomers of cefuroxime axetil in methanol–water solutions under UV light (254 nm).

Experimental Section

Materials—Cefuroxime axetil (I): CAS (64544-07-6) (registry numbers provided by author), batch number AWS76 B. *Anti* I isomer (II): CAS (127181-39-9 and 127181-37-7 for isomer A and B, respectively) batch number STD 87/101. Δ-2-Cefuroxime axetil (III): CAS (127304-68-8 and 127304-64-7 for isomer A and B, respectively) batch number STD 87/100. Cefuroxime sodium (IV): CAS (55268-75-2) and batch number AWS72A. All these compounds were kindly provided by Glaxo Laboratories (Greenford, Middlesex, England) and were used as received. The other chemicals used throughout the study were of analytical or HPLC grade.

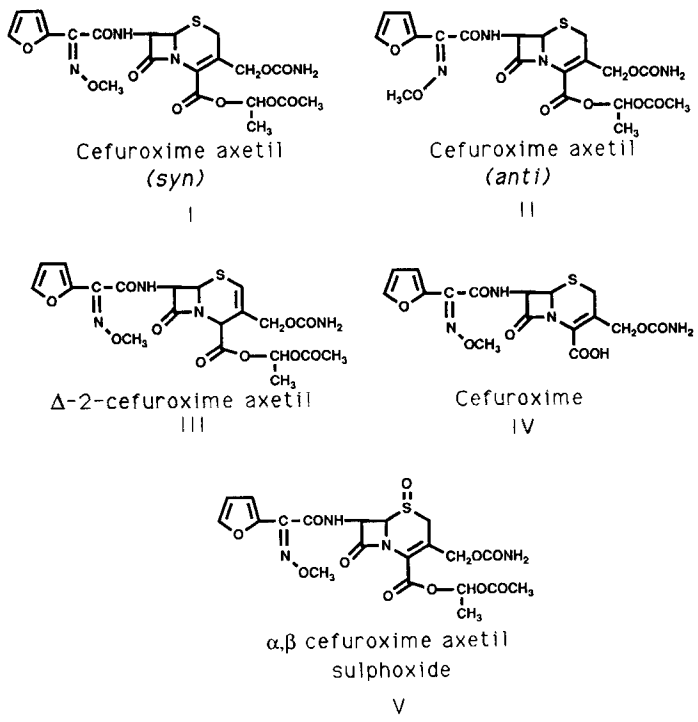

Chart 1—Structure of cefuroxime axetil and its major potential decomposition products

Photodegradation—*Solutions*—Since cefuroxime axetil and its potential decomposition products are poorly soluble in water, kinetics were carried out (unless stated otherwise) in a water–methanol mixture (50:50, v/v) adjusted at an apparent pH of 5 using a diluted hydrochloric solution. Solutions of I, II, III, and IV were 2×10^{-3} M. For chromatographic analysis, the solution were diluted 1:10 (v/v) in water.

Photoreactor—Standard Teflon-stoppered quartz cells for fluorescence were used to hold the samples. The photoreactor and the conditions used for photodegradation (λ = 254 nm; 25 °C) were the same as those previously used for aztreonam.[5]

Actinometry—The actinometry was carried out with monochloroacetic acid as previously described.[4,5] The absorptivity of the solutions to be irradiated was measured at 254 nm and calculated to be about 12 200 for I, 9350 for II, 7200 for III, and 11 300 for IV in the same methanol–water mixture. All the incident light could be considered as absorbed in the first 2 mm of the irradiated solution. The intensity absorbed by the solution was about 4.4×10^{17} photons/s.

Chromatography—*Apparatus*—The HPLC system consisted of an isocratic pump (Waters 510, Milford, MA), a diode array detector (Waters 991), fitted with a Rheodyne loop (20 μL) and an integrator (Waters 5200). An isocratic pump (HP 1050, Hewlett-Packard, Waldbronn, Germany) fitted with a Rheodyne loop (20 μL) and an integrator (Shimadzu C-R4A) were alternatively used.

The separation was carried out on a Lichrocart cartridge 250 mm × 4-mm i.d., packed with Lichrosorb RP 18, 7 μm; a guard column Merck 4 × 4 mm packed with Lichrosorb 100 RP 18 was fitted prior to the analytical column.

Chromatographic Conditions—The mobile phase was 0.2 M sodium phosphate buffer pH 4.5–CH_3OH (62:38, v/v) filtered on a 0.45-μm Millipore filter (Milford, MA). The flow rate was 1 mL·min^{-1} and the pressure around 200 bar. The compounds were detected at λ = 282 m

® Abstract published in *Advance ACS Abstracts*, January 15, 1994.

0022-3549/94/1200-553$04.50/0

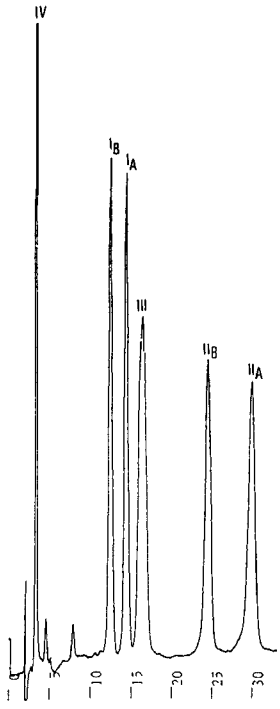

Figure 1—HPLC chromatogram of a mixture solution of I, II, III, and IV (2×10^{-4} M) in H_2O–MeOH (50:50, v/v).

(band width = 5 nm) which corresponds to the maximum absorbance for each compound. The band width for peak identity and homogeneity assessment was set at 1.3 nm. Peak heights were used for quantitation.

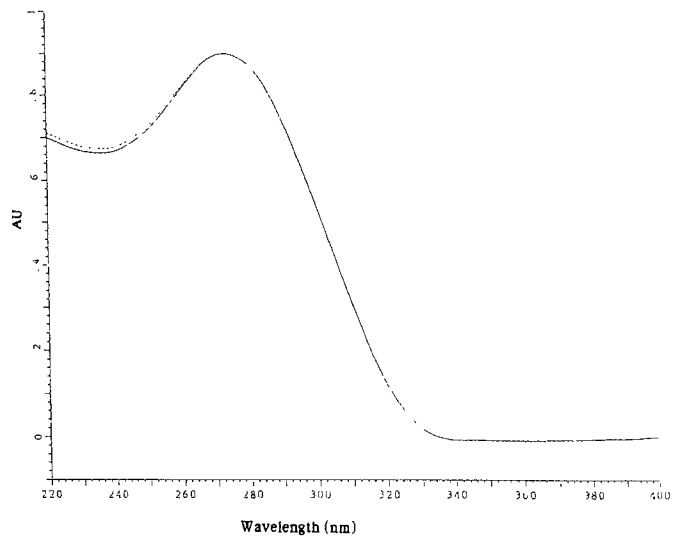

Figure 3—Spectral overlay of II and the major compound formed by irradiation of I.

Results and Discussion

Validation of HPLC Technique for Cefuroxime Axetil—The mobile phase we used[6] was the one indicated for the separation of cefuroxime axetil and its related compounds on a MOS stationary phase. However, the present separation was carried out on a C_{18} stationary phase. The technique was validated for a simultaneous determination of cefuroxime and its related compounds (Chart 1). A chromatogram of a mixed standard solution of the compounds is given in Figure 1. The

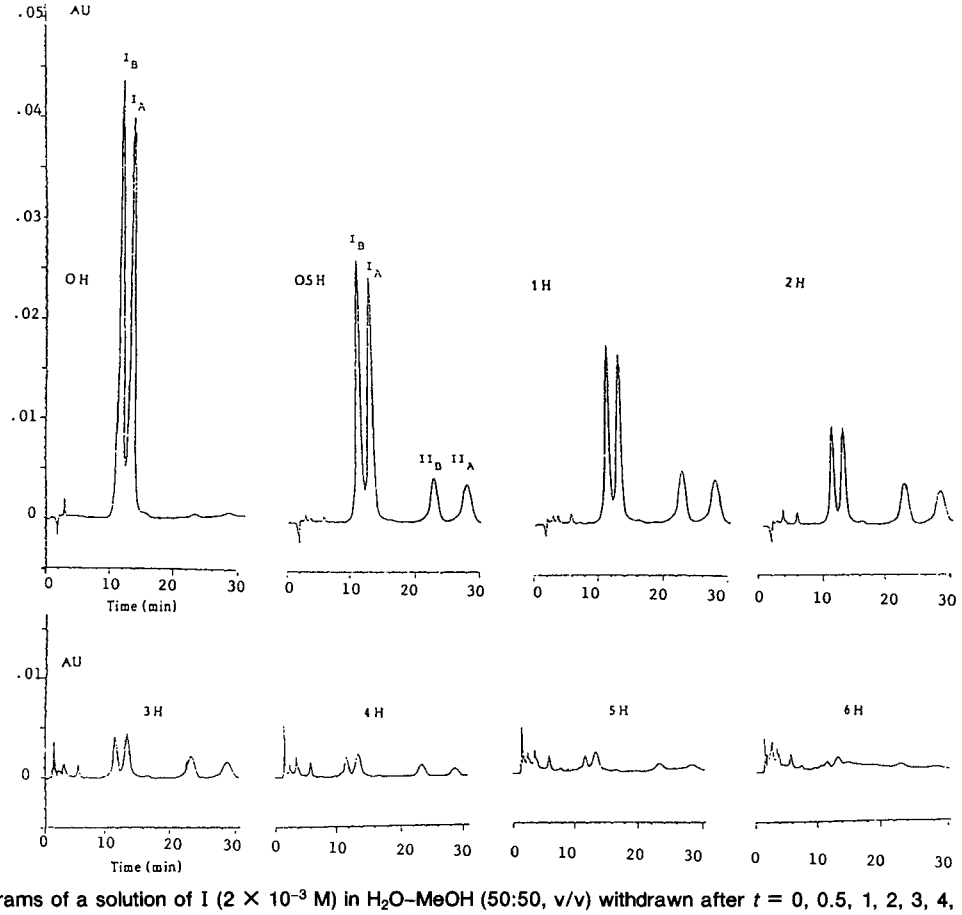

Figure 2—Chromatograms of a solution of I (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) withdrawn after t = 0, 0.5, 1, 2, 3, 4, 5, and 6 h of irradiation at 254 nm (25 °C).

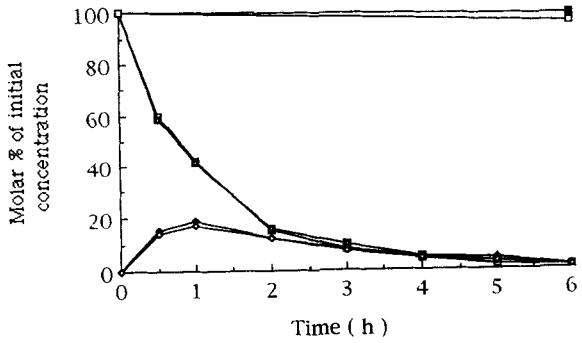

Figure 4—Photoisomerization kinetics for I at 254 nm (25 °C). Key: (⊡) isomer B *syn*; (□) isomer A *syn*; (◆) isomer B *anti*; (◇) isomer A *anti*; (■) isomer B *syn* kept in the dark; (□) isomer A *syn* kept in the dark.

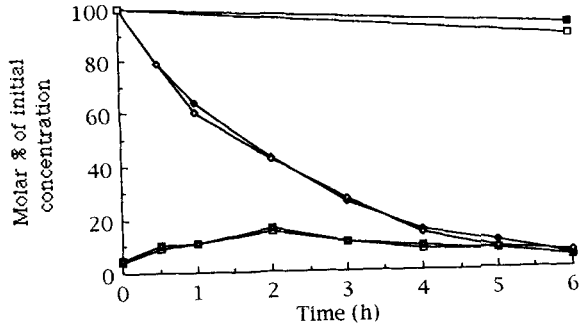

Figure 6—Photoisomerization kinetics for II at 254 nm (25 °C). Key: (◆) isomer B *anti*; (◇) isomer A *anti*; (⊡) isomer B *syn*; (□) isomer A *syn*; (■) isomer B *anti* kept in the dark; (□) isomer A *anti* kept in the dark.

respective couples of diastereomers for I and II were separated. Typical capacity factors were 6.1 (I_A), 5.1 (I_B), 13.1 (II_A), 10.6 (II_B), 6.9 (III_A and III_B), and 0.9 (IV).

The linearity of the calibration graphs (height–concentration) was assessed by injecting six mixed standard solutions ranging between 2×10^{-4} and 2×10^{-5} M for each compound. The graph was linear ($r > 0.999$) and went through the origin ($p = 0.05$) in all cases.

The repeatability of the chromatographic system was assessed at two different concentrations (2×10^{-5} and 2×10^{-4} M). The RSD using peak height concentration measurement was about 1.1% (seven injections) in all cases.

The detection limits (S/N = 2) were about 34 ng for I, 94 ng for II, 85 ng for III, and 8.5 ng for IV.

The limits of quantitation were evaluated to be 5 times the detection limits.

Photodegradation Kinetics at 254 nm—*Irradiation of Cefuroxime Axetil (I) Solutions*—The chromatograms (Figure 2) from solutions withdrawn at different time intervals during the kinetics display the growth of two major peaks under UV irradiation. The major peaks were identified as the *anti* isomers (A and B) of I by comparison of their RT and spectra to those of II in standard solutions (Figures 1 and 3). It can consistently be seen from the relative peaks heights (I_B/I_A) measured at 2, 3, and 4 h that I_B undergoes a slightly faster degradation than I_A.

Figure 4 represents the degradation course of I and the formation of II under UV light. The degradation kinetics of an identical solution kept in the dark are given on the same graph

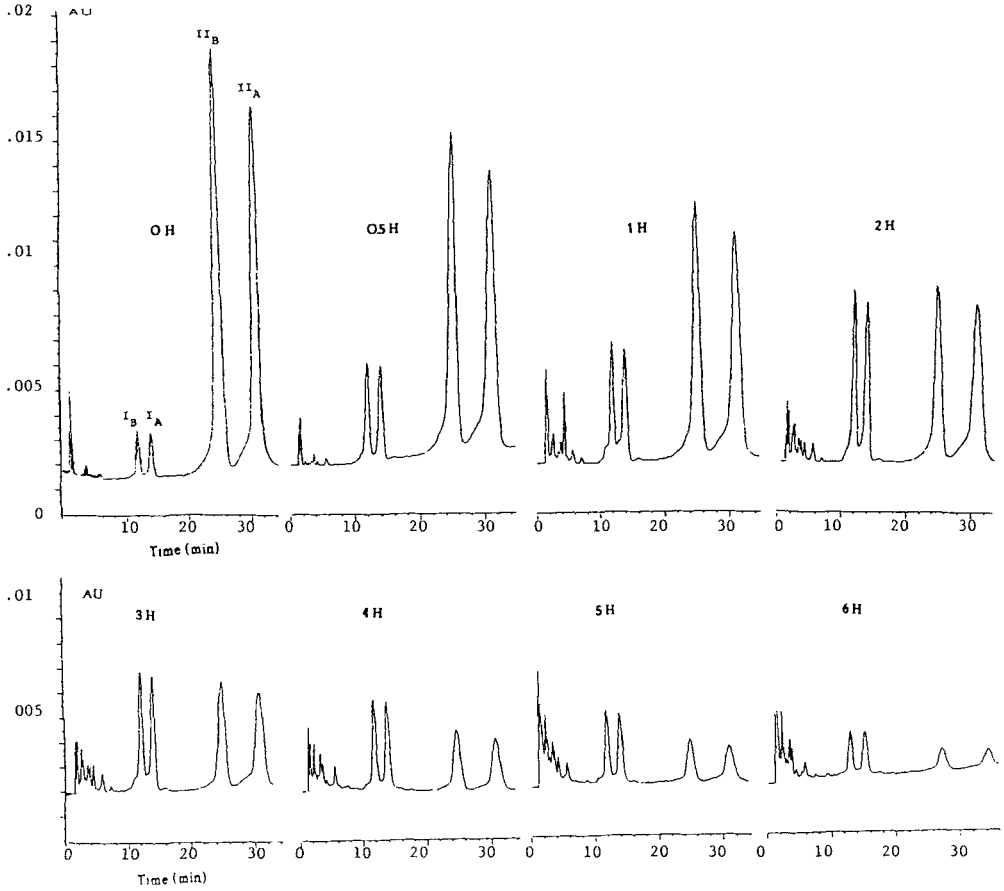

Figure 5—Chromatograms of a solution of II (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) withdrawn after t = 0, 1, 2, 3, 4, 5, and 6 h of irradiation at 254 nm (25 °C).

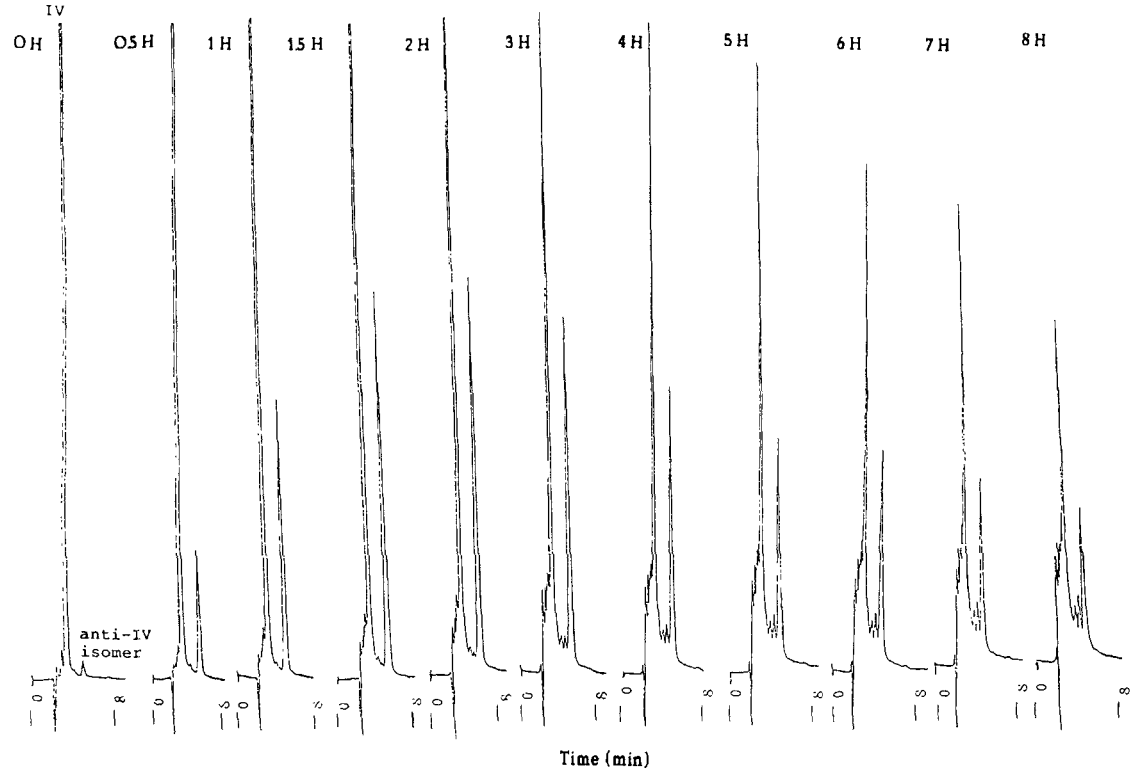

Figure 7—Chromatograms of a solution of IV (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) withdrawn after t = 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h at 254 nm (25 °C).

for comparison. They reflect the sensitivity of I to UV irradiation. The quantum yield for the *syn–anti* isomerization (calculated from the amount of II formed after a 30-min irradiation), is about 0.08% for the two diastereomers. A *syn* to *anti* ratio of 1 is estimated for the photostationary step. In addition to photoisomerization, a rapid destructive photolysis is taking place: after 1 h of irradiation, the sum (I + II) is approximately 60% of the initial concentration of I. It is assumed that the β-lactam ring is photolyzed and that low absorption products are formed. This is consistent with the molecular structure of I in which the side chains including the furan ring have a negligible absorption in the region of interest,[7] in contrast to cefotaxime or aztreonam. The chromatograms from the kinetics of I examined at different wavelengths did not show any significant peak within the 220–300-nm range.

Irradiation of anti-Cefuroxime Axetil (II) Solutions—The chromatograms recorded throughout the kinetics, and the degradation kinetics of II are given in Figures 5 and 6, respectively. It can be noted that compound II contained I as an impurity amounting approximately to 7% of II. From the retention times and the spectra, it can be concluded that the irradiation of II yields, as expected, the formation of I, with a quantum yield of isomerization about 0.03% for each isomer. In addition to isomerization, a photolysis has taken place, which in contrast to the photolysis of I, leads to more detectable polar decomposition products (t_R < 8 min). There is presently no explanation for this observation yet. The total degradation rate is slower than for I, but is still fast.

The photoisomerization of II in acetonitrile is one of the synthesis route for I.[8] In this process, irradiation is carried out in acetonitrile through Pyrex with a high-pressure mercury lamp. This means that the useful radiations fall in the range 300–400 nm. Conversion yields of II to I are claimed to be 85%. We carried out experiments in acetonitrile at 254 nm to see if this high yield could be related to the choice of the solvent, but did not observe a significant difference with water–methanol mixture in which the conversion yield is about 15%. Since it was shown

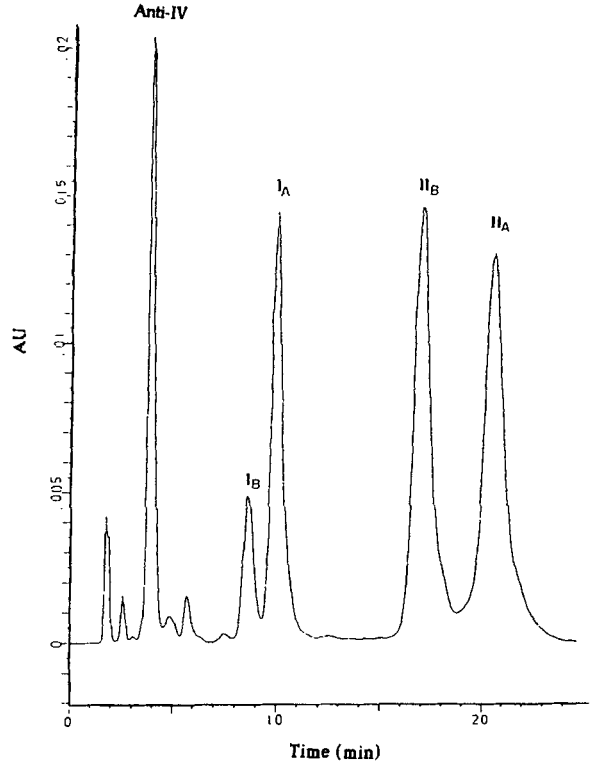

Figure 8—Chromatogram of a solution of II (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) degraded at pH 9 for 1 h at 35 °C in the dark.

before that 360-nm radiation was effective in promoting the isomerization of aztreonam to its *anti* isomer,[5] it may be assumed that the photolysis path associated to irradiation at 254 nm explains the observed difference. It is assumed that an irradiation

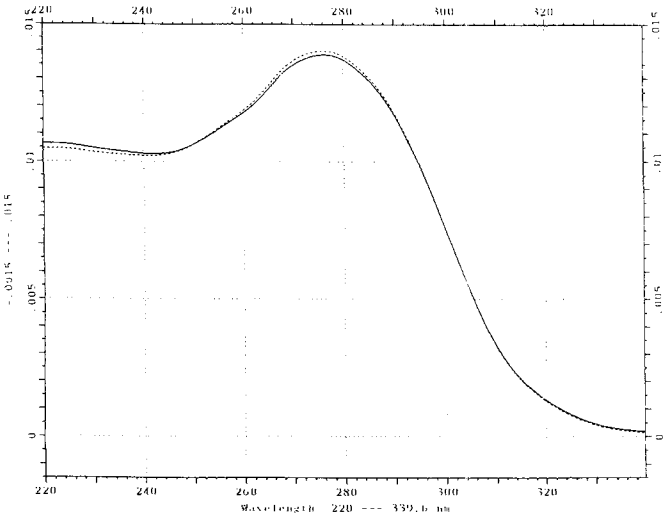

Figure 9—Spectral overlay of *anti* IV and the compound formed by alkaline hydrolysis of II.

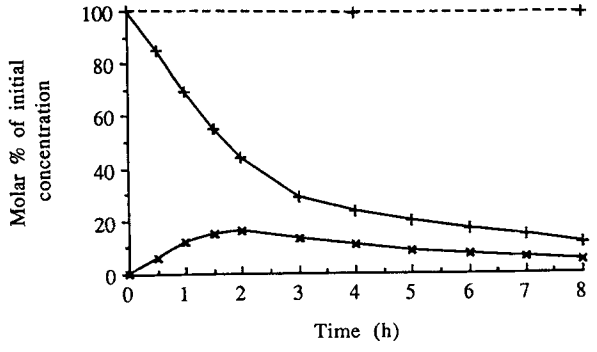

Figure 10—Photoisomerization kinetics for IV (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) at 254 nm (25 °C). Key: (+) cefuroxime *syn*; (×) cefuroxime *anti*; (– + –) cefuroxime kept in the dark.

at 254 nm favors the photolysis route at the expense of isomerization.

Irradiation of Cefuroxime Sodium (IV) Solutions—The irradiation of a solution of IV was carried out to investigate the possible involvement of IV as an intermediate in the photolysis path of I. The irradiation of IV (Figure 7) yields the formation of its *anti* isomer; the latter was identified by comparison of its RT (Figure 8) and spectrum (Figure 9) to that obtained from the *anti* IV formed by alkaline hydrolysis of II in the dark (at 35 °C and pH 9 to match the conditions used in ref 1). The formation of *anti* IV isomer was evaluated by assuming that its absorptivity is similar to that of IV at 282 nm. The quantum yield was about 0.03%. The comparison of the degradation course of IV (Figure 10) with that of I (Figure 4) shows that the degradation of IV is slower than that of I, which makes it highly improbable that the former is an intermediate.

The photostationary state for IV is reached after about 8 h with a *syn–anti* ratio of 2.1. Ioro et al.[9] have reached a stationary equilibrium (*syn–anti* 1.3:1 and 1:4) after a 24-h irradiation of cefuroxime sodium solutions in water–acetone (1:4, v/v) and acetone–ethanol (4:1, v/v), respectively. They have also stated that cefuroxime sodium is isomerized more slowly in aqueous solutions, but the stationary equilibrium was not given. The light source in their experiment consisted of four Sylvania Black light blue lamps (15 W) emitting mostly around 360 nm. The flask flushed by nitrogen was placed 2 cm from the light source. Here also the difference in our experiments mostly relates to the choice of the irradiation wavelength as well as to the fact that full kinetics were undertaken in our work.

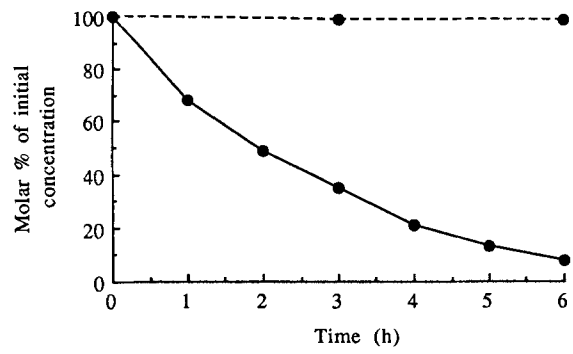

Figure 11—Photodegradation kinetics for III (2×10^{-3} M) in H_2O–MeOH (50:50, v/v) at 254 nm (25 °C). Key: (●) III; (– ● –) III kept in the dark.

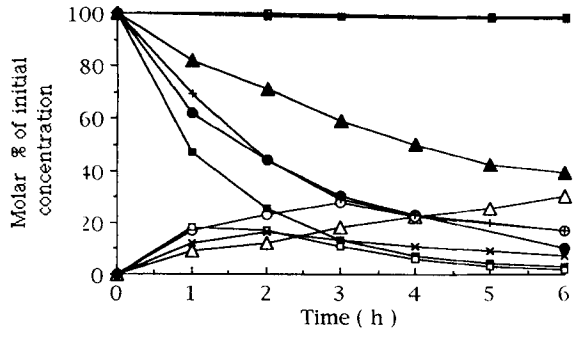

Figure 12—Comparison of the photodegradation kinetics of cefotaxime (●), aztreonam (▲), cefuroxime (+), and cefuroxime axetil (■) under the experimental conditions used in this study. The symbols (○), (△), (×), and (□) refer to their respective *anti* isomers and the symbols (□), (□), (×), and (□) refer to the solutions of isomer *syn* kept in the dark.

It is also interesting to note that the alkaline hydrolysis of II in the dark (Figure 8) yields a nonnegligible amount of I with a dramatic difference in the proportions of I_A and I_B ($I_a \gg I_B$). The alkaline hydrolysis of I in the dark was then carried out to explain this disporportion. B and A remain in roughly equal amount, and the conversion of I to II does not take place. It is therefore assumed that the difference noted in the alkaline hydrolysis of II is due to a higher conversion of II_A under the operating conditions. The alkaline hydrolysis of IV does not give the *anti* IV isomer. Therefore, the *anti* IV isomer is directly issued from the hydrolysis of II.

Irradiation of Δ-2 Isomer (III) Solution—The irradiation of III yielded the formation of numerous peaks. On the basis of the observation that the *anti* isomers of similar formula are always eluted at t_R's higher than their *syn* homologues (this statement is true for many cephalosporins and monobactam, see, e.g., refs 4, 5, and 10), the poorly resolved peaks at a t_R of 27 min were assumed to be the Δ-2 *anti* isomers A and B. The degradation kinetics of III are given in Figure 11.

Conclusions

The *syn* to *anti* isomerization observed for cefuroxime axetil and its main degradation products, under UV excitation at 254 nm, results from the presence of an alkoxyimino group on the side chain. Another characteristic feature of cefuroxime axetil is that it exists as a mixture of two diastereomers A and B which react at slightly different rates and with different quantum yields. This is true not only for the photoisomerization but also for the ground-state isomerization resulting from the treatment with a strong base. For instance, treating II with sodium hydroxide leads to I in which the amount of I_A is larger than that of I_B. However, compound I treated in the same condition leads to a mixture with more I_A than I_B, confirming previous observations.[1]

So the inversion in the ratio of the two diastereomers of II occurs during the isomerization step.

Enough data is now available on the antibiotics bearing an alkoxyimino group, such as aztreonam, cefuroxime, and cefotaxime, to compare their relative photosensitivities. For all these compounds, the quantum yields of the *syn* to *anti* isomerization under the same experimental conditions are lower than 1%. The simultaneous formation of degradation products, induced by the energy of photons at 254 nm, makes it impossible to obtain a pure photostationary state for the *anti* and *syn* couples. A closer examination of these molecules reveals variations in their half-lives under irradiation in solution at 254 nm. The most stable is aztreonam followed by cefotaxime and cefuroxime which come very close together. Cefuroxime axetil comes last (Figure 12): its increased reactivity with respect to cefuroxime is due to the ester group which makes it a prodrug. Nevertheless, this ester group is not hydrolyzed during irradiation, a fact which points to the sensitivity of the photoisomerization to conformational effects.

The results of these studies suggest the necessity of proper shielding of the above-mentioned antibiotics against photodegradation during their storage and handling in order to preserve their biological activity.

References and Notes

1. Nguyen, N. T. *Pharm. Res.* **1991**, *8*, 893–898.
2. St. Claire, R. L.; Wilbourne, D. K.; Caudill, W. L. *Pediatr. Infect. Dis. J.* **1988**, *7*, 744.
3. St. Claire, R. L.; Caudill, W. L. *Am. J. Hosp. Pharm.* **1989**, *46*, 256.
4. Lerner, D. A.; Bonnefond, G.; Fabre, H.; Mandrou, B.; Simeon de Buochberg, M. *J. Pharm. Sci.* **1988**, *77*, 699–703.
5. Fabre, H.; Ibork, H.; Lerner, D. A. *J. Pharm. Biomed. Anal.* **1992**, *10*, 645–650.
6. Bassler, B. J.; Hartwick, R. A. *J. Chromatogr. Sci.* **1989**, *27*, 162–165.
7. *Absorption Spectra in the Ultraviolet and Visible Region*; Lang, L., Ed.; Akademia Kiado: Budapest, 1967; pp 297–298.
8. Grey, M. G. U.K. Pat. Appl. GB 2218095A, 1989; *Chem. Abstr.* **1989**, *106*, 72773j.
9. Ioro, M. A.; Nicoletti, M. *Il Farmaco-Ed. Sc.* **1986**, *41*, 801–807.
10. Coomber, P. A.; Jefferies, J. P.; Woodford, J. D. *Analyst* **1982**, *107*, 1451–1456.