199. HPLC and ^{1}H-NMR Studies of Alkaline Hydrolysis of Some 7-(Oxyiminoacyl)cephalosporins

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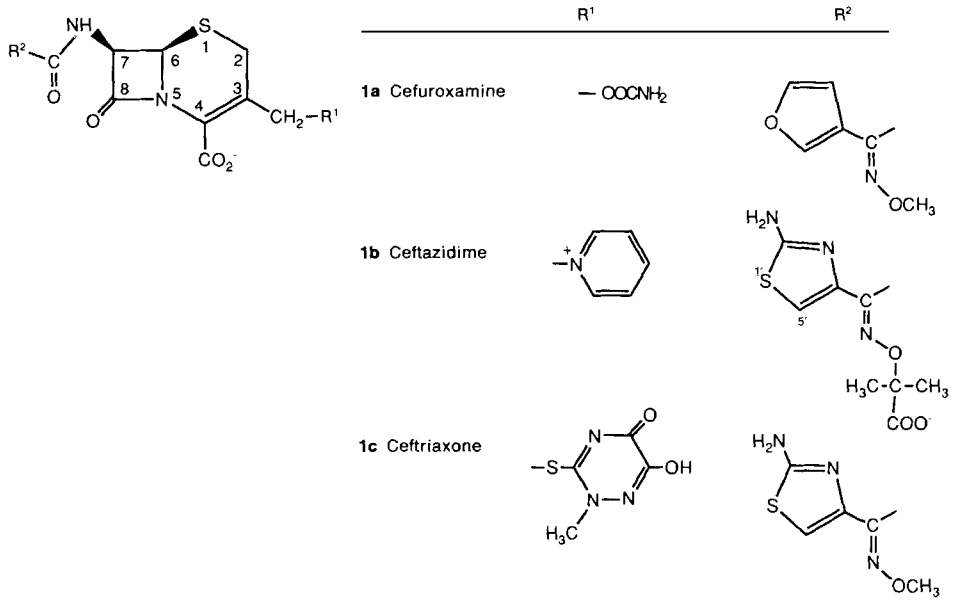
(21.VII.93)

Alkaline hydrolysis (pH 10.5) of the three 7-(oxyiminoacyl)cephalosporins **1a–c** (cefuroxime, ceftazidime, and ceftriaxone) was studied at 37° using HPLC and ^{1}H-NMR techniques. The 7-epicephalosporin **2**, the 3-methylidene compound **3**, and the 6-epimer **4** of the 3-methylidene compound **3** were identified for each cephalosporin as the major degradation products under the conditions used; ceftazidime (**1b**) yielded also the Δ^2-isomer **5b** (*Scheme 1*). A kinetic scheme was developed to account for the production of these compounds, and the different kinetic constants involved in the process were calculated. The experimental results show that the presence of a pyridinio group at position *C*–C(3) favours the appearance of the Δ^2-isomer, which was detected mainly in cephalosporins bearing an ester function at C(4). The presence of an oxyimino group at *C*–CONH–C(7) facilitates epimerization at C(7) (→ **2**), whereas that of an electron-withdrawing group at *C*–C(3) results in a increased formation constant for the 3-methylidene compound **3**. The 3-methylidene compounds **3a–c** produced by the three cephalosporins on cleavage of the β-lactam ring all underwent epimerization at C(6) to yield the corresponding 6-epimer **4**.

1. Introduction. – The first cephalosporin, cephalosporin C, was isolated over 40 years ago by *Abraham* and *Newton* from a *Cephalosporium acremonium* culture from Sardinia [1]. By introducing appropriate substituents at position 7 of the original compound, a number of substances – the first of which was cephalothin – were obtained that improved on the antibacterial potency of the parent compound. However, the new cephalosporins were scarcely resistant to β-lactamases which are enzymes produced by bacteria that hydrolyse the β-lactam ring. This problem was solved in two ways, *viz.* by isolating cephamycins (cephalosporins with an α-oriented MeO group at C(7)) and by synthesizing 7-(oxyiminoacyl)cephalosporins. Both groups of substances are highly resistant to most β-lactamases [2].

The alkaline hydrolysis of cephalosporins was found to resemble the enzymatic acylation involved in transpeptidation at the bacterial cell wall [3]. Such a similarity has fostered studies on the reactions of the β-lactam ring in basic media aimed at determining a potential relationship between the ring cleavage and antibacterial activity [4–13]. Many of such studies involved UV spectroscopy, although HPLC and ^{1}H-NMR spectroscopy have proved to be more effective, since the former provides information on the number of hydrolysis products and the latter supplies valuable evidence of their structures.

Recent studies performed in our laboratory on cephaloridine [14] [15] and cefotaxime [16] revealed the occurrence of two poorly known reactions in the alkaline hydrolysis of



cephalosporins, *viz.* epimerization at C(7) in both substances and isomerization of the double bond in the dihydrothiazine ring of cephaloridine.

To determine the potential influence of the substituents at C(3) and C(7) on the hydrolysis of cephalosporins, in this work, we carried out a kinetic study of the alkaline hydrolysis of cefuroxime (**1a**), ceftazidime (**1b**), and ceftriaxone (**1c**), three cephalosporins of clinical interest that bear an oxyiminoacyl group at C(7) and various substituents at *C*–C(3). The study was performed by HPLC and ^{1}H-NMR spectroscopy. Whenever possible, the reaction products were isolated by lyophilization to determine their structure unequivocally.

2. Experimental. – *Materials.* Sodium ceftriaxone (Na salt of **1c**) was purchased from *Sigma*, while cefuroxime (**1a**) and ceftazidime (**1b**) were complimentary supplied by *Glaxo España*. The three antibiotics were used without further purification. Deuterium oxide (99.8% D) was obtained from *Sigma*.

NMR Spectroscopy. Bruker AMX-300 spectrometer, sample-tube diameter 5 mm; initial cephalosporin concentration $1 \cdot 10^{-2}$ M in D_2O; solns. thermostated at 37° and stabilised at pD 10.5 (pD = $-\log[D^+]$ using a carbonate buffer; ionic strength 0.5M; chemical shifts δ in ppm rel. to 3-(trimethylsilyl)propane-1-sulfonic acid (DSS) as internal reference; assignments based on reported data [14–20].

High-Performance Liquid Chromatography (HPLC). Shimadzu-LC-9A chromatograph with a *Rheodyne-7125* universal injector and a *Shimadzu-SPD-M6A* UV/VIS photodiode array detector for products from **1a** and **1b**; *Waters-510* instrument equipped with a *Waters-M-712* autoinjector and a *Waters-M-490* programmable multi-wavelength detector for products from **1c**. The products were isolated by using a *Spherisorb ODS-2* column (25 × 0.46 cm × 10 μm for products from **1a** and **1b**, 25 × 0.46 cm × 5 μm for those from **1c**); eluent 0.1M NH_4OAc/MeCN 95:5 (**1a**), 97:3 (**1b**), and 99.5:0.5 (**1c**); V_i 50 (**1a** and **1b**) and 20 μl (**1c**); flow 1.7 (**1a**), 1.2 ml (**1b**), and programmed (**1c**); detection at 255 (**1a**), 256 (**1b**) and 245 nm (**1c**). The solns. of **1** were kept at 37° and stabilised at pH 10.5 by using a carbonate buffer; ionic strength 0.1M, and cephalosporin concentration *ca.* $(2–7) \cdot 10^{-3}$ M.

3. Results. – 3.1. *Degradation Products.* The HPLC's (*Figs. 1–3*) of the alkaline-degradation mixtures obtained from cefuroxime (**1a**), ceftazidime (**1b**), and ceftriaxone (**1c**)

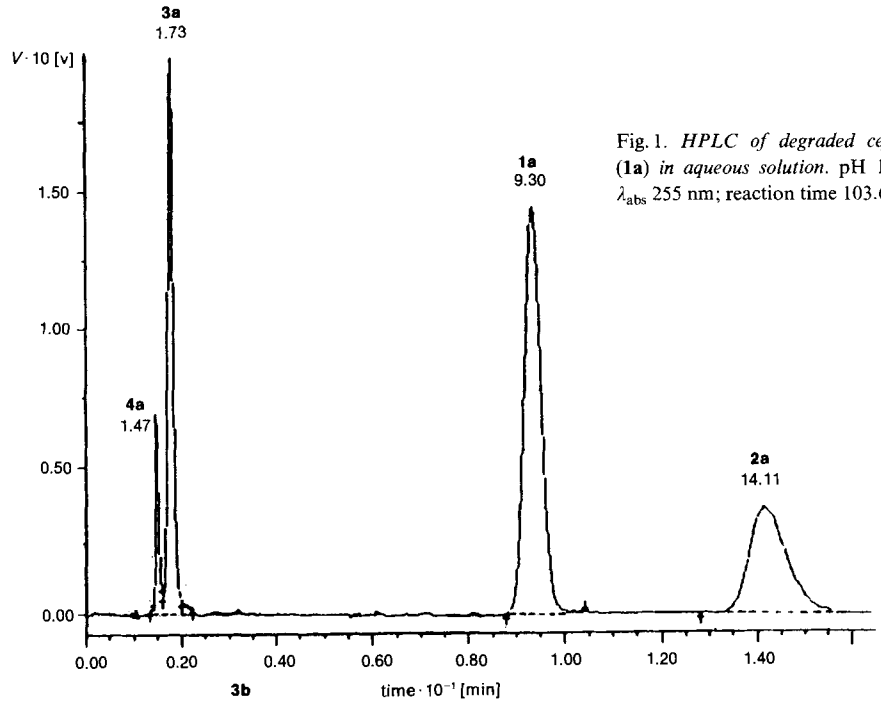


Fig. 1. *HPLC of degraded cefuroxime* (**1a**) *in aqueous solution.* pH 10.5, 37°, λ_{abs} 255 nm; reaction time 103.6 min.

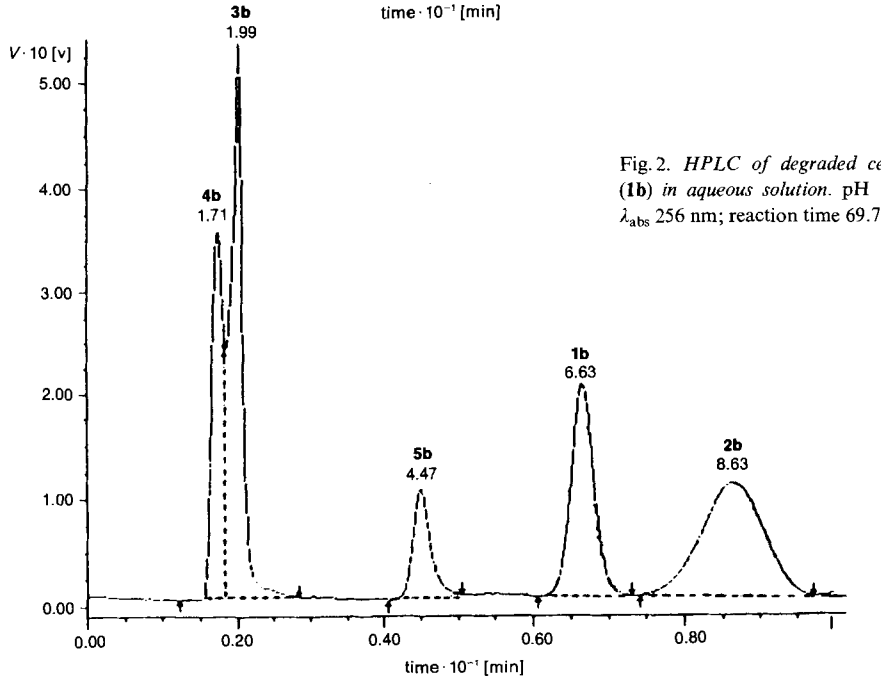


Fig. 2. *HPLC of degraded ceftazidime* (**1b**) *in aqueous solution.* pH 10.5, 37°, λ_{abs} 256 nm; reaction time 69.7 min.

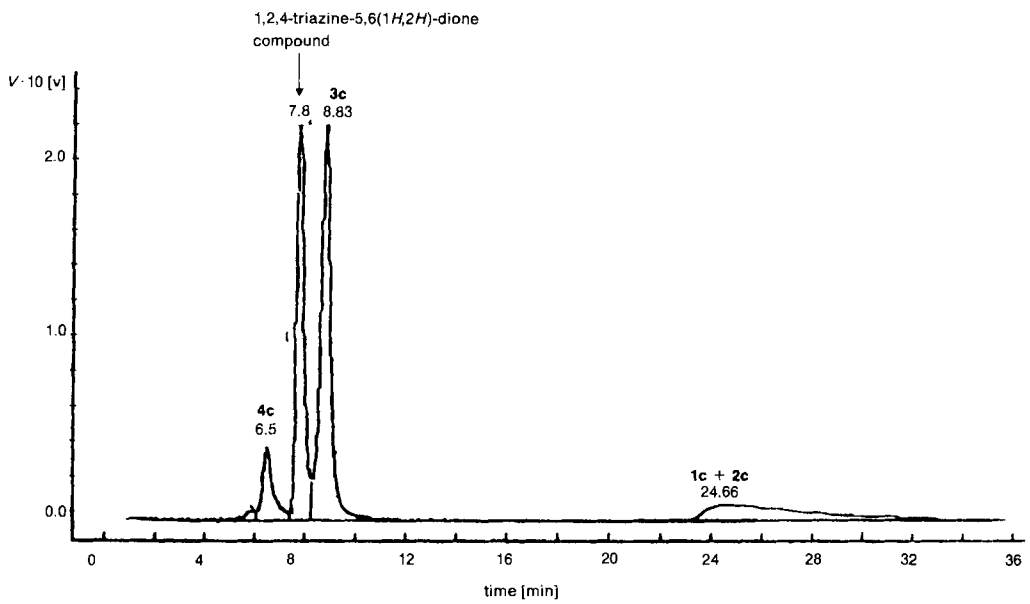


Fig. 3. *HPLC of degraded ceftriaxone* (**1c**) *in aqueous solution.* pH 10.5, 37°, λ_{abs} 245 nm; reaction time 269 min.

showed well resolved product peaks, which allowed for isolation and identification. Thus, all cyclosporins **1a–c** yielded the corresponding 7-epimer **2**, the 3-methylidene compound **3**, and the 6-epimer **4** of the latter; in the case of **1b**, also the Δ^2-isomer **5b** was isolated. Some of these products were already detected in the alkaline hydrolysis of other cephalosporins [10] [14] [16].

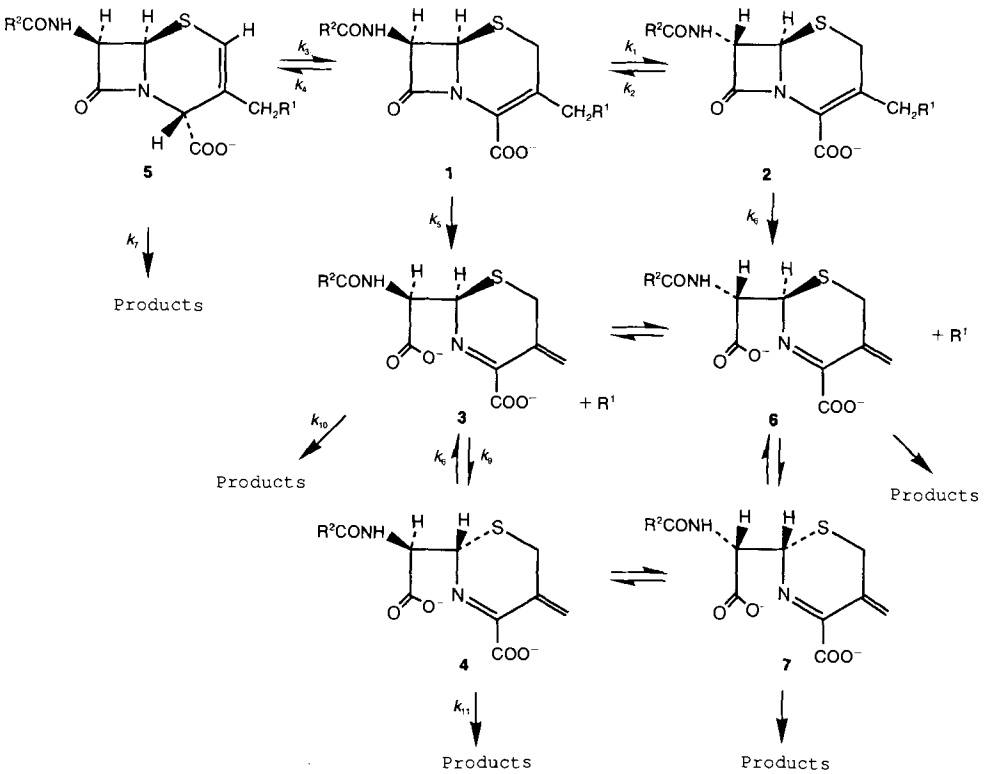
7-Epimers **2a–c**. The HPLC obtained from the hydrolysis products of cefuroxime (**1a**) (*Fig. 1*) showed a peak at t_R 14.11 min, which had the same UV spectrum as **1a**, thus suggesting that the corresponding product **2a** had preserved the closed β-lactam ring [14] [16] [19]. The ^{1}H-NMR spectrum (*Table 1*) of isolated **2a** exhibited an upfield shift of the *d*'s of H–C(6) and H–C(7) with respect to **1a** (from 5.23 and 5.82 to 4.99 and 5.11 ppm, resp.) as well as a smaller J(6, 7) (4.8 *vs.* 2.2 Hz). According to *Holden* [21], this indicates that **2a** is the 7-epimer of **1a**.

A similar product **2b** obtained from ceftazidime (**1b**) appeared at t_R 8.6 min in the corresponding HPLC (*Fig. 2*) and had the same UV spectrum as **1b**. However, **2b** was not isolated. The kinetic study performed by the ^{1}H-NMR technique revealed the presence of the *s* at 5.0 ppm, characteristic of H–C(6) in the 7-epimer, which became a *d* on hydrolysis in H_2O since the exchange of H by D during the epimerization in D_2O cancelled the signal corresponding to H–C(7) and prevented coupling of the two nuclei.

In the HPLC (*Fig. 3*) of the hydrolysis products from ceftriaxone (**1c**), the peak of **2c** overlapped with that of **1c** as its high polarity hindered separation; however, the ^{1}H-NMR technique confirmed the presence of **2c** by the *s* at 4.9 ppm (H–C(6)) and that at 6.97 ppm (H–C(5′) of the thiazolyl group).

3-Methylidene Compounds **3a–c**. The 3-methylidene compounds **3**; present in the hydrolysis mixtures appeared at the following t_R: 1.73 (**3a**), 1.99 (**3b**), and 8.86 min (**3c**). The considerably diminished band at 260 nm in their UV spectra [14] [16] [19] revealed that opening of the β-lactam ring had occurred. Only **3a** was isolated (lyophilized solid). The ^{1}H-NMR spectrum of **3a** featured two characteristic *s*'s for the methylidene protons at 5.59 and 5.68 ppm and 2 *d*'s at 4.82 and 5.49 ppm (J = 3 Hz) for H–C(6) and H–C(7) of the open ring. All this suggests that the product in question is the excocyclic-olefin compound which appears in the alkaline hydrolysis of cephalosporins with a good leaving group at *C*–C(3) [14–16] [19]. The ^{1}H-NMR study clearly confirmed the presence of **3b** and **3c** in the corresponding hydrolysis mixture by 2 *s*'s at *ca.* 5.6 and 5.7 ppm (CH_2–C(3)) and 2 *d*'s at *ca.* 4.8 (H–C(6)) and 5.5 ppm (H–C(7)).

Scheme 1



6-Epimers **4a–c** *of the 3-Methylidene Compounds.* The 6-epimer **4a** of the 3-methylidene compound **3a** appeared at t_R 1.47 min in the HPLC of the hydrolysis mixture (*Fig. 1*) obtained from **1a**. The UV spectrum was similar to that of **3a**, thereby suggesting that it must bear the same chromophores. The ^{1}H-NMR spectrum of the isolated (lyophilization) **4a** showed 2 *s*'s at 5.65 and 5.69 ppm and 2 *d*'s at 4.99 and 5.37 ppm, the positions of the 2 *s*'s being slightly different from those of **3a** and the *d* of H–C(7) being shifted upfield and that of H–C(6) downfield. On the other hand, the kinetic study performed in D_2O showed the *d* at 4.99 ppm to disappear and that at 5.35 ppm to become a *s*. All this suggests that **4a** is the 6-epimer of **3a**. It is also worth noting the slight shift in the signals of CH_2(2) (from 3.51 and 3.70 to 3.50 and 3.66 ppm, resp.), resulting from the conformational change in the dihydrothiazine ring. The 6-epimers **4b** and **4c** appeared at t_R 1.71 and 6.5 min, respectively in the HPLC of the corresponding hydrolysis mixture (*Figs. 2* and *3*). Neither of them was isolated, but the ^{1}H-NMR kinetic study showed for each a *s* at *ca.* 5.35 ppm and 2 others at *ca.* 5.65 and 5.70 ppm.

Δ^2-Cephem **5b**. Only the alkaline hydrolysis of ceftazidime (**1b**) yielded a Δ^2-cephem, *i.e.* **5b**, with t_R 4.47 min. Its UV spectrum showed a slightly decreased band at 260 nm. Compound **5b** was isolated by liquid chromatography. A comparison of its ^{1}H-NMR spectrum with that of **1b** revealed a shift in the signals at 5.28 (H–C(6)) and 5.87 ppm (H–C(7)) to 5.43 and 5.55 ppm, respectively. In addition, the usual CH_2(2) signals (3.17 and 3.64 ppm) were absent, and two new signals appeared at 4.60 (H–C(4)) and 6.87 ppm (H–C(2)), thereby indicating that the double bond in the dihydrothiazine ring had undergone isomerization [21]. Compound **5b** was also formerly detected in cephaloridine, a cephalosporin with a pyridine ring at *C*–C(3) [14] [15] and cefpirome [10] which has a similar substituent at that position.

Pyridine. This compound should have appeared in the HPLC of the hydrolysis mixture from **1b** (*Fig. 2*), since it was formed simultaneously with the 3-methylidene compound **3b**. However, under the HPLC conditions used, pyridine was retained by the column. In any case, it was detected in the ^{1}H-NMR study.

Table 1. *^{1}H-NMR Data* (D_2O, 25°) *of the Antibiotics* **1** *and their Degradation Products*

	CH_2(2)	CH_2–C(3) or CH_2=C(3)	H–C(6)	H–C(7)	Other signals
1a[a])	3.42 (*d*, *J* = 18.0), 3.69 (*d*, *J* = 18.0)	4.66 (*d*, *J* = 12.3), 4.86 (*d*, *J* = 12.3)	5.23 (*d*, *J* = 4.8)	5.82 (*d*, *J* = 4.8)	4.00 (*s*, MeO); 7.69 (*s*), 6.89 (*d*, *J* = 3.5), and 6.63 (*m*, furan)
2a[a])	3.38 (*d*, *J* = 18.0), 3.67 (*d*, *J* = 18.0)	4.63 (*d*, *J* = 12.1), 4.86 (*d*, *J* = 12.1)	4.99 (*d*, *J* = 2.2)	5.11 (*d*, *J* = 2.2)	3.99 (*s*, MeO); 7.68 (*s*), 6.85 (*d*, *J* = 3.5), and 6.63 (*m*, furan)
3a[a])	3.51 (*d*, *J* = 14.7), 3.70 (*d*, *J* = 14.7)	5.59 (*s*), 5.68 (*s*)	4.82 (*d*, *J* = 3.0)	5.49 (*d*, *J* = 3.0)	3.96 (*s*, MeO); 7.67 (*s*), 7.02 (*d*, *J* = 3.5), and 6.62 (*m*, furan)
4a[a])	3.50 (*d*, *J* = 14.7), 3.66 (*d*, *J* = 14.7)	5.65 (*s*), 5.69 (*s*)	4.99 (*d*, *J* = 5.0)	5.37 (*d*, *J* = 5.0)	3.96 (*s*, MeO); 7.65 (*s*), 6.92 (*d*, *J* = 3.5), and 6.60 (*m*, furan)
1b[a])	3.22 (*d*, *J* = 18.0), 3.64 (*d*, *J* = 18.0)	5.35 (*d*, *J* = 14.5), 5.57 (*d*, *J* = 14.5)	5.28 (*d*, *J* = 5.0)	5.87 (*d*, *J* = 5.0)	7.02 (*s*, H–C(5′)); 1.46 (*s*, Me_2C); 8.97 (*d*), 8.57 (*m*), and 8.09 (*m*, Py)
2b	3.17 (*d*, *J* = 18.0), 3.64 (*d*, *J* = 18.0)	5.35 (*d*, *J* = 14.6), 5.57 (*d*, *J* = 14.6)	5.00 (*d*, *J* = 2.4)	5.10 (*d*, *J* = 2.4)	6.87 (*s*, H–C(5′)); 1.46 (*s*, Me_2C); 8.97 (*d*), 8.57 (*m*), and 8.09 (*m*, Py)
3b	3.52 (*d*, *J* = 14.6), 3.73 (*d*, *J* = 14.6)	5.59 (*s*), 5.67 (*s*)	4.78 (*d*, *J* = 3.6)	5.47 (*d*, *J* = 3.6)	7.22 (*s*, H–C(5′)); 1.43 (*s*, Me_2C)
4b	3.52 (*d*, *J* = 14.8), 3.70 (*d*, *J* = 14.8)	5.65 (*s*), 5.68 (*s*)	4.99 (*d*, *J* = 5.0)	5.33 (*d*, *J* = 5.0)	7.10 (*s*, H–C(5′)); 1.43 (*s*, Me_2C)
5b[a])	6.87 (*s*)[b])	5.37 (*d*, *J* = 14.6), 5.53 (*d*, *J* = 14.6)	5.43 (*d*, *J* = 3.9)	5.55 (*d*, *J* = 3.9)	7.02 (*s*, H–C(5′)); 1.46 (*s*, Me_2C); 8.87 (*d*), 8.58 (*m*), and 8.08 (*m*, Py)
1c[a])	3.47 (*d*, *J* = 19.0), 3.75 (*d*, *J* = 19.0)	4.04 (*d*, *J* = 13.4), 4.37 (*d*, *J* = 13.4)	5.19 (*d*, *J* = 4.8)	5.78 (*d*, *J* = 4.8)	7.01 (*s*, H–C(5′)); 3.98 (*s*, MeO); 3.63 (*s*, MeN)
2c	3.41 (*d*, *J* = 17.8), 3.75 (*d*, *J* = 17.8)	4.00 (*d*, *J* = 13.6), 4.29 (*d*, *J* = 13.6)	4.99 (*d*, *J* = 3.6)	5.06 (*d*, *J* = 3.6)	6.97 (*s*, H–C(5′)); 3.98 (*s*, MeO); 3.63 (*s*, MeN)
3c	3.51 (*d*, *J* = 15.0), –	5.61 (*s*), 5.71 (*s*)	4.82 (*d*, *J* = 3.3)	5.48 (*d*, *J* = 3.3)	7.27 (*s*, H–C(5′)); 3.95 (*s*, MeO)
4c	3.51 (*d*, *J* = 15.0), –	5.65 (*s*), 5.69 (*s*)	5.02 (*d*, *J* = 4.5)	5.37 (*d*, *J* = 4.5)	7.13 (*s*, H–C(5′)); 3.95 (*s*, MeO)

[a]) ^{1}H-NMR chemical shifts of these compounds were obtained from the isolated compounds. All others signals were estimated from the spectra of mixtures.

[b]) H–C(2) instead of CH_2(2).

1,2,4-Triazine-5,6(1H,2H)-dione Compound. This compound appeared in the alkaline-hydrolysis mixture from ceftriaxone (**1c**; *Fig. 3*) at t_R 7.8 min. It was isolated, and its ^{1}H-NMR spectrum only showed a *s* at 3.71 ppm for the Me group at N(2) of the triazine.

3.2. *Determination of Rate Constants. HPLC Study.* At a given reaction time, the initial concentration of antibiotic **1** can be given by *Eqn. 1*,

$$[\mathbf{1}]_0 = \sum^{n} c_i = \sum_{i=1}^{n} a_i A_i \qquad (1)$$

where c_i and A_i are the concentration and peak area in the HPLC of species *i*, and a_i is a constant of proportionality between both. From A_i values measured at different reaction times, *Eqn. 1* was solved using a multi-variable non-linear regression programme based on the *Marquardt* algorithm [22], which provided the a_i values and the concentrations of the different reaction products. From such concentrations, the kinetic constants in *Scheme 1* were determined with the aid of the programme GIT [23] [24], which allows numerical integration of the differential equations corresponding to a given kinetic sequence.

On the basis of the degradation products involved in the HPLC's (*Fig. 1–3*), we developed the kinetic sequence shown in *Scheme 1*, which is a modification of the scheme formerly proposed for the alkaline hydrolysis of the cephaloridine [14–16]. *Scheme 1* assumes that, in a basic medium, cephalosporin **1** can react simultaneously *via* a reversible epimerization at C(7) to 7-epimer **2**, by nucleophilic attack of the OH^- ion on the carbonyl group of the β-lactam ring to the 3-methylidene compound **3**, and, in ceftazidime (**1b**) only, *via* base-catalysed isomerization of the double bond at C(3) to Δ^2-isomer **5b**. The isomerization **1** → **5** creates a new asymmetric centre at C(4); however, it was reported that only a single isomer is produced, *viz.* that with the COOH group lying below the dihydrothiazine ring [25].

The nucleophilic attack on the β-lactam carbonyl group of 7-epimer **2** yields a 3-methylidene compound **6** that differs from **3** in its configuration at C(7). However, **6** was not detected due to its low concentration. Therefore, we did not calculate the kinetic rate constants of reversible epimerization at C(7) for **3** or **6**. Chemically, one should expect the formation of the Δ^2-isomer of the 7-epimer by epimerization at C(7) in **5** or by isomerization of the double bond in **2**. However, inasmuch as **2** and **5** were obtained at fairly low concentrations, this Δ^2-compound must be produced in very small amounts, so it was excluded from the kinetic scheme.

Compound **5** may undergo a nucleophilic attack on the carbonyl group of the β-lactam ring to yield products containing an open ring. The 3-methylidene compounds **3** and **6** can undergo reversible epimerization at C(6) to yield the corresponding 6-epimer **4** and 6,7-diepimer **7** of the 3-methylidene compound. It should be noted that compound **7** is obtained in low concentration, so the constant of epimerization of **6** at C(6) to yield **7** and *vice versa* was not calculated. Finally, compounds **3** (**6**) and **4** (**7**) may undergo subsequent degradation reactions.

Fig. 4 shows fittings of the experimental data to theoretical equations obtained from *Scheme 1* and using the constant values listed in *Table 2*.

^{1}H-NMR Study. The alkaline hydrolysis of **1a–c** was studied by ^{1}H-NMR at 37°, pD 10.5, and $I = 0.5$M. It should be borne in mind that the utilization of D_2O results in

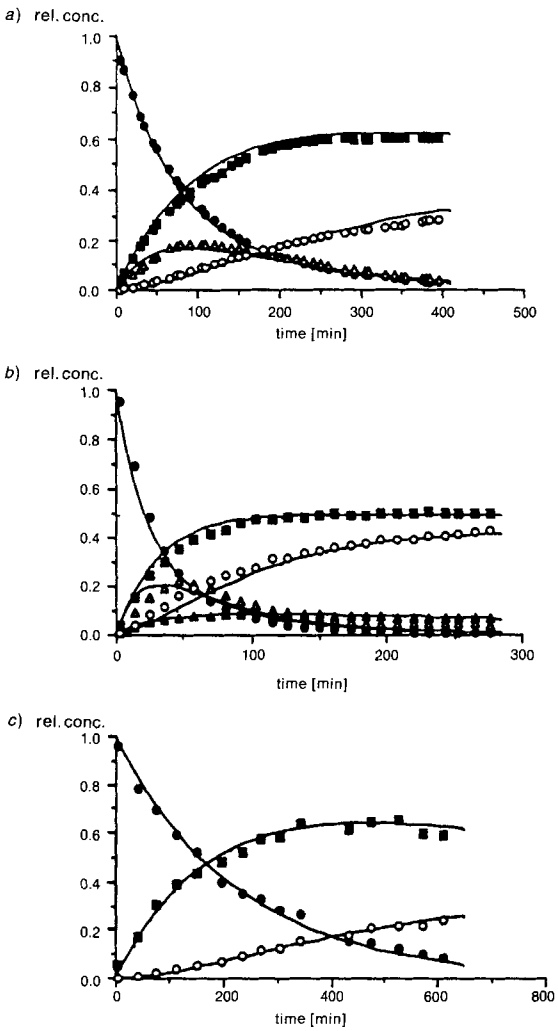


Fig. 4. *Time course of the alkaline degradation of* a) *cefuroxime* (**1a**), b) *ceftazidime* (**1b**), *and* c) *ceftriaxone* (**1c**) *in carbonate buffer solution.* The continuous lines between the HPLC experimental values are the best theoretical fitting; ●: cephalosporin **1**, △: 7-epimer **2**, ■: 3-methylidene compound **3**, ○: 6-epimer **4** of **3**, ▲: Δ^2-cephem **5b**.

Table 2. *Rate Constants* $\cdot 10^2$ [h^{-1}]

		k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9
1a	HPLC	31	24			43	41		17	25
	^{1}H-RMN	33	29			33	30		15	18
1b	HPLC	108	96	19	12	95	90	3	65	70
	^{1}H-RMN	60	57	10	5	61	55	1	32	33
1c	HPLC	–	–			26	26		8	8
	^{1}H-RMN	10	10			10	10		6	6

slightly smaller kinetic constants relative to those obtained in H_2O owing to the isotopic effect of the D-atom.

Figs. 5–7 show the ^{1}H-NMR spectra of cefuroxime (**1a**), ceftazidime (**1b**), and ceftriaxone (**1c**) as well as of their degradation products. In the region of 3.1–3.8 ppm, the initial spectra exhibit an *AB* system for the $CH_2(2)$ group of **1** that decreases in favour of two new signals for $CH_2(2)$ of 7-epimer **2**. In addition, the spectra show the signals of the 3-methylidene compound **3** and those of its 6-epimer **4**.

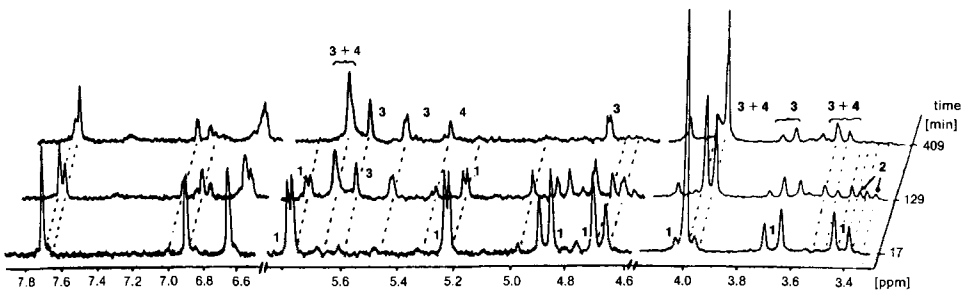


Fig. 5. *Change in intensities of ^{1}H-NMR signals during degradation of cefuroxime* (**1a**) *in deuterated carbonate buffer solution.* pD 10.5, 37°.

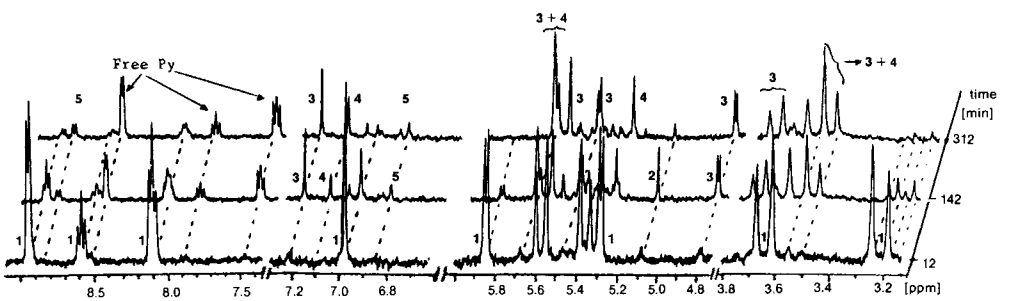


Fig. 6. *Change in intensities of ^{1}H-NMR signals during degradation of ceftazidime* (**1b**) *in deuterated carbonate buffer solution.* pD 10.5, 37°.

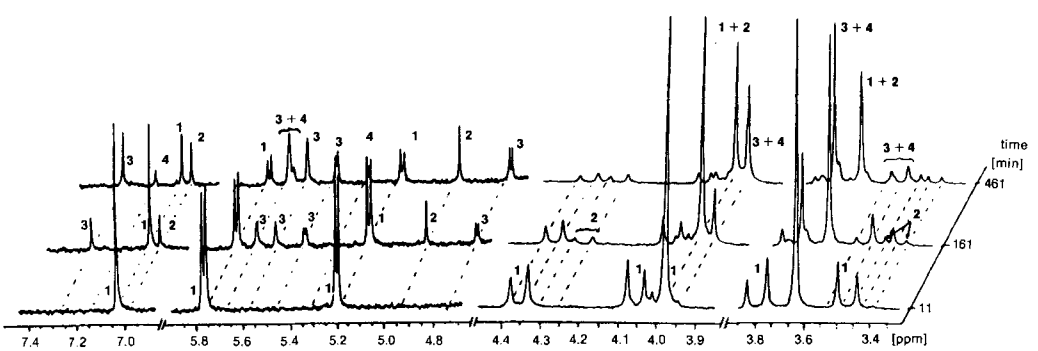


Fig. 7. *Change in intensities of ^{1}H-NMR signals during degradation of ceftriaxone* (**1c**) *in deuterated carbonate buffer solution.* pD 10.5, 37°.

The 4.6–6.0-ppm regions initially include 2*d*'s corresponding to H–C(6) and H–C(7), the areas of which decrease through epimerization at C(7) (→ **2**), cleavage of the β-lactam ring (→ **3**) and, **1b**, isomerization of the double bound (→ **5b**). As the reaction develops, the *s* for **2** appears at 5.0–5.1 ppm (H–C(7)) and 4.8 ppm (H–C(6)) plus 2 *s*'s at 5.6–5.7 ppm (CH_2=C(3)) for **3**, and a *s* at 5.3 (H–C(7)) for **4**, which appears as a *s*, because the epimerization in D_2O results in the replacement of H–C(6) by a D-atom.

Finally, the regions from 6.6–9 ppm include the signals corresponding to the protons of the furan ring at the C(7) side chain in the cefuroxime (**1a**) and to H–C(5′) of the thiazolyl group in ceftazidime (**1b**) and ceftriazone (**1c**). In *Fig. 6*, this region includes also the signals of the pyridine group. Initially, they occur as 2 *t*'s (8.60 and 7.87 ppm) and a *d* (8.94 ppm) corresponding to pyridine bonded to the dihydrothiazine ring. As the reaction develops, the signals corresponding to free pyridine appear at 8.58 (*d*), 7.87 (*m*), and 7.46 (*m*). Also, a *d* corresponding to the Δ^2-isomer **5b** appears at 8.86 ppm.

The ^{1}H-NMR signals used to calculate the kinetic constants are shown in *Table 3*. Once the concentration of each compound was determined, the kinetic constants were calculated as in the HPLC study by applying the programme GIT to the above kinetic

Table 3. *^{1}H-NMR Signals Used to Calculate the Kinetic Constants*

	Signal	δ [ppm]	Compound
Cefuroxime hydrolysis	*d*	5.22	**1**
	s	4.99	**2**
	s	5.59	**3**
	s	5.37	**4**
Ceftazidime hydrolysis	*d*	5.87	**1**
	s	5.05	**2**
	d	4.78	**3**
	s	5.37	**4**
	d	8.87	**5**
Ceftriaxone hydrolysis	*d*	5.19	**1**
	s	4.99	**2**
	s	5.61	**3**
	s	5.37	**4**

scheme. It should be emphasized that the Δ^2-isomer was only obtained as a degradation product from ceftazidime (**1b**), but not from the other two antibiotics. The kinetic constants obtained are listed in *Table 2*. *Fig. 8* shows the fitting of the experimental data to *Scheme 1* using the constant values obtained for each cephalosporin.

Discussion. – The kinetic constants of hydrolysis at pH 10.5 of the three antibiotics **1a–c** obtained by HPLC were larger than those determined by ^{1}H-NMR spectroscopy, owing to the H/D-isotopic effect (*Table 2*). Due to the determining process of peak areas in the ^{1}H-NMR, the constants obtained by this method are subjected to larger errors than those provided by HPLC. In addition, the fitting of the ^{1}H-NMR experimental results (*Fig. 8*) was poorer than that of the HPLC results (*Fig. 4*).

Isomerization of the 3-double bond in the dihydrothiazine ring to give a mixture of Δ^3- and Δ^2-cephalosporins were described for compounds whose carboxyl group at C(4) is esterified [26–28]. The proposed mechanism [28] [29] for the isomerization, which is a reversible base-catalyzed process, involves the abstraction by the base of a H–C(2). The resulting ambident carbanion can be protonated in the C(4) position, giving the Δ^2-isomer. In previous papers [14] [15], we described the formation of Δ^2-isomer of

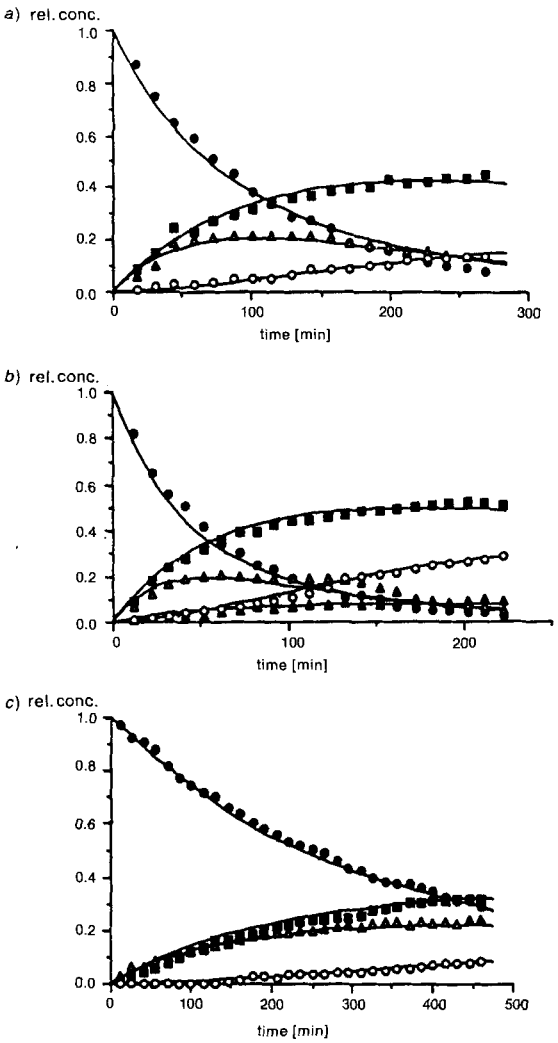


Fig. 8. *Time course of the alkaline degradation of* a) *cefuroxime* (**1a**), b) *ceftazidime* (**1b**), *and* c) *ceftriaxone* (**1c**) *in deuterated carbonate buffer solution.* The continuous lines between the ^{1}H-NMR experimental values are the best theoretical fitting; ●: cephalosporin **1**, △: 7-epimer **2**, ■: 3-methylidene compound **3**, ○: 6-epimer **4** of **3**, ▲: Δ^2-cephem **5b**.

cephaloridine in its free-acid form. The kinetic constant for this process, *ca.* $20 \cdot 10^{-2}\ h^{-1}$, is almost identical to the corresponding value given in this paper for ceftazidime (**1b**), $19 \cdot 10^{-2}\ h^{-1}$. Both cephalosporins bear the same group at *C*–C(3′), the pyridinio group, which has a very strong electron-withdrawing effect. Presence of the Δ^2-isomer was not detected for any of the other antibiotics studied, likely because their substituent at *C*–C(3) is not an as good electron-withdrawing group as the pyridinio ion. *Morin et al.* [25] suggested that the equilibrium between Δ^3- and Δ^2-isomer is shifted to Δ^2, if there are bulky groups at *C*–C(3), in order to minimize steric repulsions. Ceftriaxone (**1c**) has a very bulky group at *C*–C(3), but no Δ^2-isomer was formed on hydrolysis, because the electron-withdrawing effect of such a group is not sufficient to promote the abstraction of

a H–C(2). Ceftazidime (**1b**) presents a slightly higher kinetic constant for the reaction towards Δ^2-isomer than towards Δ^3-isomer which would confirm *Morin*'s hypothesis.

Since the three antibiotics studied bear similar substituents at C(7) and these have little influence on their chemical reactivity, differences between their kinetic constants must chiefly arise from the substituents at C–C(3) which modulates the electrophilicity of the β-lactam carbonyl group [5] [30].

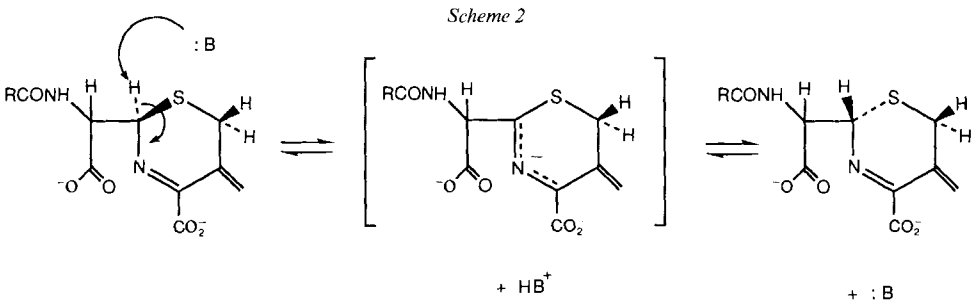
k_1 and k_2 refer to the epimerization at C(7), *via* an intermediate carbanion formed by the abstraction of the acidic proton by a base [11]. Because the acidity of H–C(7) is the result of the resonance effect of the neighbouring carbonyl group, it is indirectly influenced by the substituent at C–C(3) [16]. Values of 7-epimerization constants shown in *Table 2* are in agreement with this hypothesis. Ceftriaxone (**1c**), ceftoaxime, and deacetylcefotaxime are 7-(oxyiminoacyl)cephalosporins with the same substituent at C(7). Among them, cefotaxime which bears an Ac group at C–C(3) shows the higher epimerization constant, *ca.* $30 \cdot 10^{-2}$ h^{-1}. Deacetylcefotaxime and **1c** show smaller constants, $k_1 = 16 \cdot 10^{-2}$ and $10 \cdot 10^{-2}$ h^{-1}, respectively [16]. Moreover, k_1 also appears to be influenced by the substituent at C(7). *E.g.* cefaloridine has a k_1 of *ca.* $66 \cdot 10^{-2}$ h^{-1} (by HPLC) [14], which is smaller than that presented here for ceftazidime (**1b**), in spite of having the same group at C–C(3).

Nishikawa et al. [17], who studied the alkaline hydrolysis (pD 10.4) of various oxacephem derivatives by ^{1}H-NMR spectroscopy, proposed that the decomposition of an oxacephem with the same substituent as cefuroxime at C–C(3) takes place *via* two parallel reactions. The first one involves opening of the β-lactam ring by the OD^- ion, yielding the 3-methylidene compound, NH_3 and CO_2. The second reaction involves the aminolysis of the antibiotic by *in situ* produced NH_3. They obtained a value of 6840 $l \cdot mol^{-1}$ h^{-1} for the second-order aminolysis constant and 0.228 h^{-1} for the pseudo-first-order hydrolysis constant. This latter value is equivalent to a second-order constant of 907 $l \cdot mol^{-1}$ h^{-1}, which lies within the range of values reported by *Page* [31] for various cephalosporins (*viz.* 97.2–1800 $l \cdot mol^{-1}$ h^{-1}). Based on these results, one can conclude that NH_3 is more nucleophilic than OH^- ion, which is rather surprising. We could detect no aminolysis reaction, so none is included in *Scheme 1*.

Constants k_5 and k_6 correspond to the cleavage of the β-lactam ring. Both were somewhat larger for **1b** than they were for **1a** and **1c**. Consequently, these constants depend essentially on the electron-withdrawing character of the group at C–C(3).

Finally, it is worth emphasizing the occurrence of the 6-epimer **4** of the 3-methylidene compound **3**. Such a product must be formed according to the mechanism depicted in

Scheme 2



Scheme 2. A planar carbanion is formed followed by the uptake of a proton by the base catalyst. In HPLC experiments, peaks corresponding to the 3-methylidene compound **3** and its 6-epimer **4** are very closer together, and sometimes their resolution is very poor. Thus constants refering to this last epimerization are rather approximative, and no conclusion can be drawn from their variation from one to another cephalosporin.

In summary, alkaline hydrolysis of cephalosporins **1** with an oxyimino group at *C*–CONH–C(7) also involves epimerization at C(7) in (→ **2**). In addition, presence of a pyridinio group at *C*–C(3) greatly favours appearance of the Δ^2-isomer (→ **5**). The presence of a good electron-withdrawing group at *C*–C(3) results in increased constants of formation of the 7-epimer **2** and cleavage of the β-lactam ring (→ **3**). Data from HPLC and ^{1}H-NMR experiments strikingly show production of the 6-epimer **4** of the 3-methylidene compound **3**, previously undetected in the alkaline hydrolysis of cephalosporins.

Finally, it should be noted that the present study was conducted under alkaline conditions, and obviously most of the degradation products described will not be formed in neutral medium or in the body. Nevertheless, the knowledge of these reactions is very useful for the design and the synthesis of new potential antibiotics. *E.g.*, it is known that a method to enhance the oral bioavailability of cephalosporins is to form prodrugs through reversible esterification of the carboxyl group of these antibiotics [27]. It was observed that these compounds tend to undergo reversible isomerization of the double bond of the dihydrothiazine moiety in both aqueous buffer systems and human plasma yielding the microbiologically inactive Δ^2-isomer [27]. In this work, we demonstrated that the presence of a good electron-withdrawing group at *C*–C(3) increases the rate of this reaction and so, must decrease the antibacterial properties of esters of cephalosporins, due to the increase of formation of the inactive Δ^2-isomer.

This work was possible thanks to a grant from the Spanish Government (DGICYT, PB90-0358 and PB90-1024). One of us (*B.V.*) wishes to acknowledge a fellowship from the Spanish Government (*F.P.U.I.*).

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