Stability of the Penems SCH 29482 and FCE 22101 in Aqueous Solution

Rosa MÉNDEZ, Teresa ALEMANY and Javier MARTÍN-VILLACORTA*

Departmento de Bioquímica y Biología Molecular, Facultades de Biología y Veterinaria, Universidad de León, 24071 León, Spain.
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The catalytic effect of various buffer systems (citrates, acetates, phosphates, borates and carbonates) on the degradation of SCH 29482 and FCE 22101 in aqueous solution was studied at 35 °C and a constant ionic strength of 0.5 mol·dm^{-3} over a pH range of 3.50 to 10.0. The observed degradation rates, obtained by measuring the remaining intact antibiotic, were shown to follow pseudo-first-order kinetics with regard to antibiotic concentrations and to be significantly influenced by general acid and general base catalysis. The changes in the concentration of intact SCH 29482 in the solutions were established by spectrophotometric determination of the reaction product with imidazole, while those of intact FCE 22101 were determined by reverse-phase high performance liquid chromatography with ultraviolet-detection. Of the buffer systems employed in the kinetic studies the borate one had the greatest catalytic effect. The pH-rate profiles for SCH 29482 and FCE 22101 showed a degradation minimum at pH 5.86 and 5.22, respectively. The SCH 29482 is more reactive with hydrogen ion than the FCE 22101 and less reactive with hydroxide ion. The Arrhenius activation energies were determined for SCH 29482 and FCE 22101 at pHs 4.23, 6.59 and 8.60.

Keywords β-lactam antibiotic; penem; stability; aqueous solution; degradation kinetics; catalytic effect; buffer system; pH-rate profile

Introduction

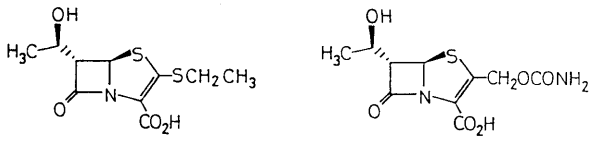
In 1975, a new β-lactam skeleton, previously unknown in nature, was synthesized.[1,2] This type of compound, called a penem, can be regarded as a hybrid between penicillins and cephalosporins. Penems are β-lactams with a number of interesting properties including a broad antibacterial spectrum and low susceptibility to hydrolysis by β-lactamases.[3-8] Chemical structures of these penems are shown in Fig. 1.

In general, the drugs of the β-lactam antibiotic group are characterized by a pronounced susceptibility to attack on the β-lactam ring by acid-base reagents, metal ions, β-lactamases, organic catalytic agents, and even water molecules and their ions.[9-20]

The purpose of this work was to investigate the stability of these penems in various solutions as a function of catalytic components, and was begun with the hope that a systematic quantitative approach to the stabilization would help to produce much better β-lactam antibiotics in future. In accordance with this it is interesting to note that the evidence available is suggestive of a correlation between biological activity on the one hand and chemical reactivity of the β-lactam system on the other, and, although no simple quantitative relationship of these two features is obvious today, the search for structures with reactive β-lactam rings has proved useful in designing new biologically active derivatives.

Experimental

Materials SCH 29482 was supplied by Schering Corporation (Bloomfield, U.S.A.) and FCE 22101 was supplied by Farmitalia Carlo Erba (Milán, Italy).



SCH 29482 FCE 22101

Fig. 1. Chemical Structures of the Penems SCH 29482 and FCE 22101

Buffer salts used and other chemicals were commercial products of analytical grade. All the water used was purified by a Milli-Q-Reagent Water System (Millipore Bedford, MA, U.S.A.).

Buffer Solutions The buffer systems used in the kinetic studies were: citrate buffer (pH 3.50—4.75), acetate (pH 3.96—5.65), phosphate (6.30—7.95), borate (8.25—8.85) and carbonate (pH 9.60 and 10.50). A constant ionic strength of 0.5 mol·dm^{-3} was maintained for each buffer by adding an appropriate amount of sodium perchlorate monohydrate and a 1×10^{-4} mol·dm^{-3} concentration of ethylenediaminetetraacetic acid (EDTA) was used in order to avoid the catalytic effect induced by small amounts of metal ions possibly present as impurities. The buffers were prepared by dissolving the citric acid, acetic acid, sodium phosphate, boric acid and sodium bicarbonate together with sodium perchlorate monohydrate and EDTA in distilled water and adjusting the pH with concentrated sodium hydroxide. The solutions were freshly prepared and the pH's measured at 35°C using a pH meter equipped with a combination electrode.

Analytical Procedures 1) Liquid Chromatography: A reverse-phase high-performance liquid chromatographic (RP-HPLC) method was used to follow the kinetics of the degradation of FCE 22101. The HPLC system consisted of a Konik KNK-500-A liquid chromatograph, a Rheodyne 7125 loop injector (volume 20 μl), a Waters 441 UV detector and a Varian 4290 computing integrator. The detector was set at 305 nm. The separation was carried out using a Spherisorb ODS-2 RP-18 column (10 μm; 25 cm × 0.4 cm i.d.) with a phosphate (0.1 mol·dm^{-3}, pH 7.0): methanol (90 : 10) mobile phase. A pre-column (3 cm × 0.4 cm i.d.) packed with μ-Bondapak C18 (30 μm) was used to guard the main column. The flow-rate was 1.0 ml·min^{-1} and the capacity factor 1.37. All chromatographic operations were carried out under ambient conditions.

Analyses of the experimental reproducibility and of the various time plots indicate the relative uncertainty of the observed rate constant, k_{obs}, to be 10% at the 95% confidence level.

2) Spectrophotometric Method: A spectrophotometric method was used to follow the kinetics of the degradation of SCH 29482. This is based on the spectrophotometric measurement at 386 nm of the reaction product with imidazole formed at 35 °C in a 1.5 mol·dm^{-3} imidazole/ 2×10^{-3} mol·dm^{-3} mercuric chloride solution at pH 7.50 for 15 min.[21]

Kinetic Procedure Weighed amounts of the antibiotic were dissolved in the buffer solutions preheated to a desired temperature to produce a final antibiotic concentration of 2×10^{-4} mol·dm^{-3}. Aliquots (10 ml) of the solutions were each sealed in a glass vial. To avoid possible changes in the pH of the kinetic solution during the reaction, it was maintained with a pH-stat. All reactions were conducted in a constant-temperature water bath at 35.0 ± 0.1 °C with the total buffer concentration greatly exceeding the reacting substrate concentration to maintain pseudo-first-order kinetics. Aliquots of the solution were withdrawn at appropriate time intervals and assayed immediately. The pH values of the reaction solutions were measured at the experimental temperature initially and at the end of each experiment on a pH meter. No significant

changes in pH were observed.

pK_a Determination The apparent pK_a values of the penems studied for the carboxyl group were determined potentiometrically[22] at an ionic strength of 0.5 mol·dm^{-3} at 35 °C. The initial concentration of penems was 0.01 M and the titration was a process of proton association (proton gain) with acid (HCl).

Results and Discussion

Order of Reaction and Observed Rate Constants The degradation kinetics of SCH 29482 and FCE 22101 were investigated at various pH values and buffer concentrations at a constant temperature (35 °C) and ionic strength (μ=0.5 mol·dm^{-3}). In the presence of excess buffer at constant pH, the degradation of the penems SCH 29482 and FCE 22101 at concentrations of up to at least 5×10^{-3} mol·dm^{-3} followed pseudo-first-order kinetics and gave rate constants k_{obs} for their β-lactam ring cleavage estimated by means of the least-squares method from the slopes of time *versus* log(residual%) plots (Fig. 2).

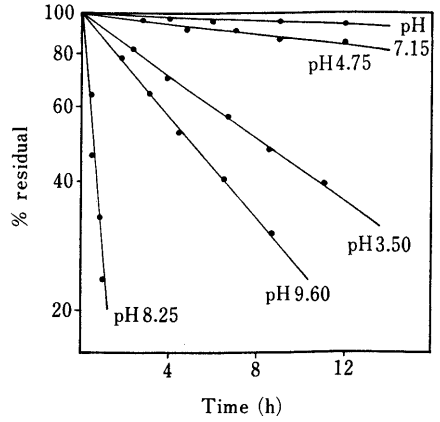


Fig. 2. Plots of the Observed Pseudo-First-Order Kinetic Degradation of SCH 29482 in Aqueous Solution at Different pH's at 35 °C (μ=0.5 mol·dm^{-3})

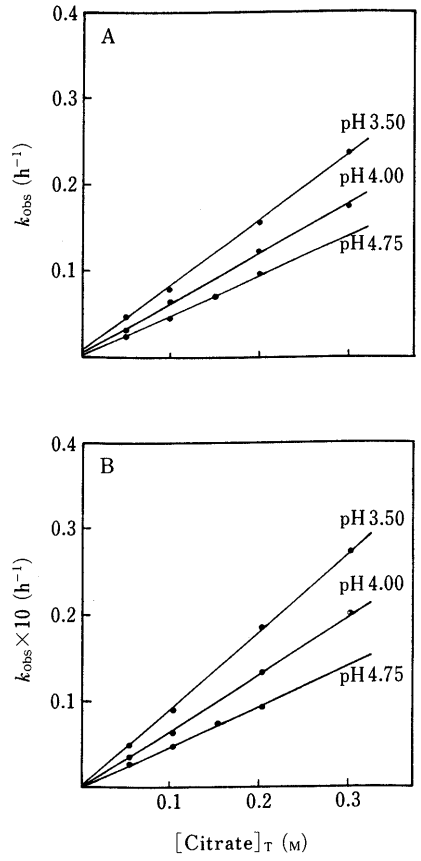


Fig. 3. Plots of the Pseudo-First-Order Rate Constants *versus* the Total Citrate Buffer Concentration at Several pH's at 35 °C and μ=0.5 mol·dm^{-3} for the Degradation of SCH 29482 (A) and FCE 22101 (B)

Catalytic Effect of Buffer Systems The catalytic effect of the buffer systems used in the kinetic studies was determined by experiments at constant pH, temperature, ionic strength and antibiotic concentration, the only variation being in the buffer concentration at a given pH. This was repeated at several pH values within the effective range of the buffer employed.

Figure 3 shows the catalytic effect of citrate buffers between pH 3.50 and 4.75 for SCH 29482 and FCE 22101, which increased linearly with the buffer concentration. Therefore, the rates of degradation of these penems in the citrate buffer at constant pH and 35 °C were shown to be significantly affected by general acid catalysis. In addition, the degradation rate was greater at more acidic conditions for both antibiotics. These results indicate that the catalytic effect of the citrate species is in the order of $[H_3A]>[H_2A^-]>[HA^{2-}]>[A^{3-}]$, which are, respectively, the nondissociated, monoanionic, dianionic, and trianionic forms of citric acid.

Since the A^{3-} species exhibits the weakest catalytic effect and, in addition, approximately only 3% of total citrate exists as this species, the observed rate constant may be expressed by the following equation:

$$k_{obs}=k_{pH}+k_{H_3A}[H_3A]+k_{H_2A^-}[H_2A^-]+k_{HA^{2-}}[HA^{2-}] \quad (1)$$

where k_{pH} is the rate constant at zero buffer concentration; the other k's are the catalytic rate constants due to the citrate buffer species.

The total citrate concentration, c_T, is

$$c_T\simeq[H_3A]+[H_2A^-]+[HA^{2-}] \quad (2)$$

From the dissociation constants, $pK_1=2.85$ and $pK_2=4.40$

$$K_1=\frac{[H^+][H_2A^-]}{[H_3A]} \quad (3)$$

$$K_2=\frac{[H^+][HA^{2-}]}{[H_2A^-]} \quad (4)$$

and from Eqs. 1 and 2 the following overall rate expression, k_{obs}, was obtained.

$$k_{obs}=k_{pH}+c_T\frac{k_{H_3A}[H^+]^3+k_{H_2A^-}[H^+]^2K_1+k_{HA^{2-}}[H^+]K_1K_2}{[H^+]^3+[H^+]^2K_1+[H^+]K_1K_2} \quad (5)$$

A plot of k_{obs} *versus* the total citrate concentration will yield a straight line with an intercept of k_{pH} and a slope of

$$\text{slope}=\frac{k_{H_3A}[H^+]^3+k_{H_2A^-}[H^+]^2K_1+k_{HA^{2-}}[H^+]K_1K_2}{[H^+]^3+[H^+]^2K_1+[H^+]K_1K_2} \quad (6)$$

From the slopes we calculated the citrate buffer catalytic rate constants on degradation of each antibiotic at 35 °C (Table I).

A similar treatment, described by us in a previous publication,[20] was used to study the catalytic effects of the other buffers used, including acetate ones in the

TABLE I. Catalytic Rate Constants of Citrate, Acetate, Phosphate, Borate and Carbonate Buffer Ions on Degradation of SCH 29482, FCE 22101 and Imipenem at 35 °C and $\mu = 0.5\,\mathrm{mol \cdot dm^{-3}}$

Buffer solution	Catalytic rate constants ($\mathrm{mol^{-1} \cdot dm^3 \cdot h^{-1}}$)	Antibiotic		
		SCH 29482	FCE 22101	Imipenem[a]
Citrate	k_{H_3A}	1.96	0.194	397
	$k_{H_2A^-}$	0.527	6.11×10^{-2}	23.6
	$k_{HA^{2-}}$	0.402	2.73×10^{-2}	6.17
Acetate	k_{AcH}	0.279	1.10×10^{-2}	4.93
	k_{Ac^-}	1.91×10^{-2}	1.34×10^{-3}	0.149
Phosphate	$k_{H_2PO_4^-}$	2.32×10^{-2}	2.25×10^{-2}	1.23
	$k_{HPO_4^{2-}}$	2.24×10^{-2}	2.27×10^{-2}	0.099
Borate	$k_{H_3BO_3}$	8.13	4.94	67.7
	$k_{H_2BO_3^-}$	39.4	93	333
Carbonate	$k_{HCO_3^-}$	8.31×10^{-2}	0.230	0.554
	$k_{CO_3^{2-}}$	1.91	1.18	9.65

a) From reference 20.

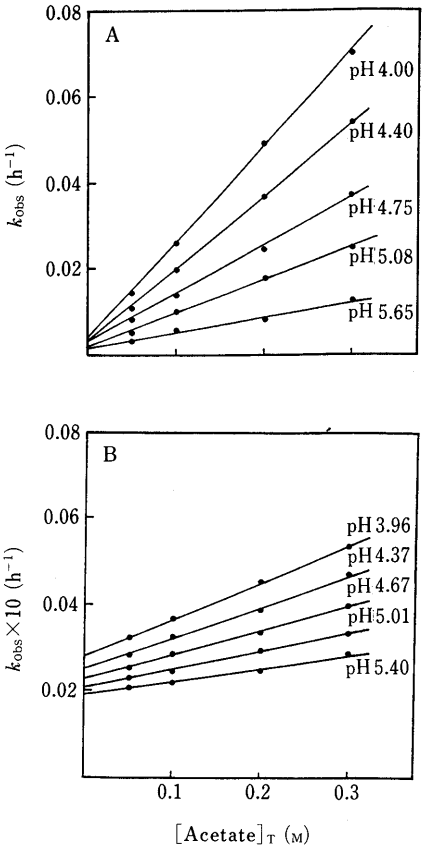


Fig. 4. Plots of the Pseudo-First-Order Rate Constants *versus* the Total Acetate Buffer Concentration at Several pH's at 35 °C and $\mu = 0.5$ $\mathrm{mol \cdot dm^{-3}}$ for the Degradation of SCH 29482 (A) and FCE 22101 (B)

pH range 3.96—5.65, the rate constant observed being expressed by the following equation.

$$k_{obs} = k_{pH} + c_T \frac{k_{AcH}[H^+] + k_{Ac^-}K_a}{[H^+] + K_a} \quad (7)$$

where k_{pH} is the rate constant at zero buffer concentration, c_T is the total acetate concentration, k_{AcH} and k_{Ac^-} are the catalytic rate constants due to the undissociated acetic acid and the acetate ion, respectively, and $pK_a = 4.55$. Figure 4 shows the catalytic effect of acetate buffer on the

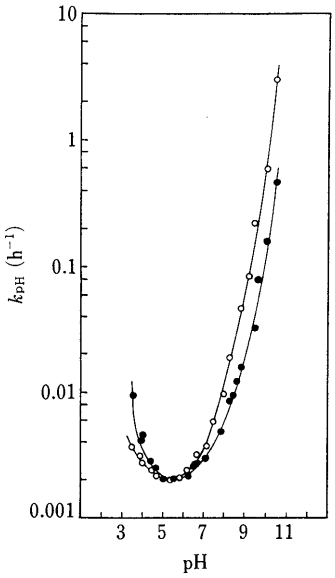


Fig. 5. log k_{pH}–pH Profiles for the Degradation of SCH 29482 (●) and FCE 22101 (○) in Aqueous Solution at 35 °C and $\mu = 0.5\,\mathrm{mol \cdot dm^{-3}}$

degradation of SCH 29482 and FCE 22101. The plots allow us to obtain the k_{pH} values and the catalytic rate constants. These constants are shown in Table I.

We also studied the catalytic effects of phosphate buffer of pH 6.30 to 7.95, borate buffer of pH 8.25 to 8.85 and carbonate buffer of pH 9.60 and 10.0. The observed rate constants may be expressed by the following equations:

$$k_{obs} = k_{pH} + c_T \frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^{2-}}K_2}{[H^+] + K_2} \quad (8)$$

$$k_{obs} = k_{pH} + c_T \frac{k_{H_3BO_3}[H^+] + k_{H_2BO_3^-}K_a}{[H^+] + K_a} \quad (9)$$

$$k_{obs} = k_{pH} + c_T \frac{k_{HCO_3^-}[H^+] + k_{CO_3^{2-}}K_2}{[H^+] + K_2} \quad (10)$$

The plots of Eqs. 8, 9 and 10 allow us to obtain the k_{pH} values and the catalytic rate constants due to the phosphate, borate and carbonate buffer species for the degradation of SCH 29482 and FCE 22101. These constants are shown in Table I. If we compare the catalytic effects, we can see that phosphate, borate and carbonate buffers exert a comparable effect on the degradation of both penems. Stress should be laid on the great catalytic effect presented by the acid and basic species on borates, which may be up to 2000 times greater than the catalytic effect of phosphates.

The catalytic effects of citrate and acetate buffers on the degradation of SCH 29482 are greater than on that of FCE 22101 (*ca.* 10—25 fold) (Table I).

pH-Rate Profile Figure 5 shows $\log k_{pH}$ *versus* pH for the degradation of SCH 29482 and FCE 22101 at 35 °C and $\mu = 0.5\,\mathrm{mol \cdot dm^{-3}}$. These pH-rate profiles exhibit a U-shape with three important pH regions: one where a hydrogen-ion-catalyzed reaction took place, a pH-independent region where the predominating reaction is the attack by water molecules and a region where the reaction was hydroxide-ion catalyzed.

The degradation rates of the penems studied were not influenced by the dissociation of the 3-carboxylic acid

Table II. Catalytic Rate Constants of the Water Species in β-Lactam Antibiotic Hydrolysis at 35 °C and $\mu = 0.5\,mol \cdot dm^{-3}$

β-Lactam antibiotic	k_H ($mol^{-1} \cdot dm^3 \cdot h^{-1}$)	k_{H_2O} ($h^{-1} \times 10^3$)	k_{OH} ($mol^{-1} \cdot dm^3 \cdot h^{-1} \times 10^{-2}$)	Ref.
Penems				
SCH 29482	16.2±2.06	2.17±0.13	14.97±1.06	—
FCE 22101	4.24±0.66	2.25±0.15	72.80±8.95	—
Carbapenem				
Imipenem	1207±220	6.61±1.63	89.16±17.31	20
Penicillins				
Penicillin G	601	0.90	11.9	12
Carbenicillin	52.2	2.04	12.1	12
Cloxacillin	35.6	0.94	13.4	12
Propicillin	30.7	0.89	17.3	12
Cyclacillin	4.61	2.49	11.0	12
Ampicillin	1.82	0.75	25.7	12
Cephalosporins				
Cephalotin	0.172	10.9	10.6	12
Cephaloridine	0.134	4.40	38.8	12
Cephaloglycin	0.148	5.00	13.1	12

group of FCE 22101 ($pK_a = 3.10$) and SCH 29482 ($pK_a = 2.98$). These observations imply that the degradation rates of both undissociated and dissociated penems are of almost the same magnitude. Therefore, the apparent first-order rate constant at a given pH obeys the general rate law:

$$k_{pH} = k_{H_2O} + k_H[H^+] + k_{OH}[OH^-] \quad (11)$$

where k_H and k_{OH} represent second-order rate constants of proton- and hydroxide ion-catalyzed degradation, respectively; k_{H_2O} is the rate constant of spontaneous or water-catalyzed degradation; and $[H^+]$ and $[OH^-]$ are the hydrogen ion concentration and the hydroxide ion concentration, respectively. The values of these constants, computed by means of a non-linear least-squares method, are given in Table II. The value for the autoprotolysis constant of water, K_w, at 35 °C is 2.09×10^{-14}.[23]

The theorical profiles generated using these constants adequately describe the behavior of each antibiotic and can be interpreted kinetically as follows: The hydrogen-ion-catalyzed reaction is important at pH 5.0 for both antibiotics and the hydroxide-ion-catalyzed reaction occurs at pH 6.0 for FCE 22101 and at pH 6.5 for SCH 29482. The horizontal portion between pH 5.0 and 6.0, for FCE 22101, and between pH 5.0 and 6.5 for SCH 29482, is pH-independent.

The theoretical pH minimum for the degradation of each antibiotic, obtained by taking the derivative of Eq. 11 and equating it to zero was pH 5.86 and 5.22 for SCH 29482 and FCE 22101, respectively.

If we compare the catalytic effects of water species in the degradation of the penems studied in acidic and basic solutions (Table II) we find that SCH 29482 is more reactive with hydrogen ion than the FCE 22101 and less reactive with hydroxide ion.

Effect of Temperature The temperature dependence of the hydrolytic reactions of FCE 22101 and SCH 29482 in solution was determined at three different pH values in the acidic, neutral and alkaline regions and a constant ionic strength of $0.5\,mol \cdot dm^{-3}$. These determinations were made at pH 4.23 (acetate buffer), pH 6.59 (phosphate buffer) and pH 8.60 (borate buffer). The observed rate constants (k_{obs}) at 20, 30 and 40°C, activation energies (E_a) and frequency factor ($\log A$) are given in Table III, and the corresponding Arrhenius plots are shown in Fig. 6. As the Table shows, the values of activation energies for the two antibiotics are very similar, although their frequency factors are quite different, especially in an acid medium, where FCE 22101 has a significantly lower one than SCH 29482. In this medium, the amino group of FCE 22101 will be protonated, which, in hydrolysis, will bring on an effect of repulsion toward the hydrogen ions attacking it, which would result in a lower frequency factor for this penem. In a base medium, a higher frequency factor for FCE 22101 might increase the reactivity.

Table III. Rate Constants and Arrhenius Activation Parameters for Degradation of SCH 29482 and FCE 22101 at $\mu = 0.5\,mol \cdot dm^{-3}$

Antibiotic	pH	k_{obs} ($h^{-1} \times 10^2$) 40 °C	k_{obs} ($h^{-1} \times 10^2$) 30 °C	k_{obs} ($h^{-1} \times 10^2$) 20 °C	E_a (kcal/mol)	$\log A$ (h^{-1})
SCH 29482	4.23	6.76	3.10	1.66	12.70	7.66
	6.59	1.03	0.47	0.24	13.56	7.46
	8.60	260	102	48.2	15.25	11.01
FCE 22101	4.23	0.642	0.352	0.165	12.29	6.37
	6.59	1.29	0.582	0.280	13.82	7.73
	8.60	501	215	85.4	16.01	11.84

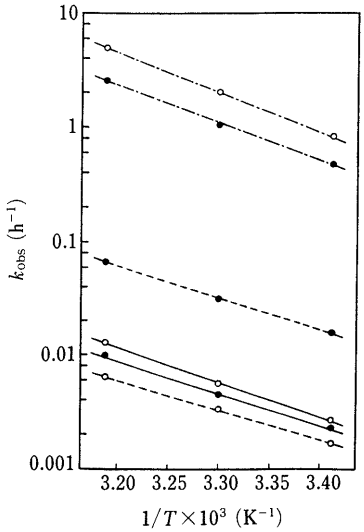


Fig. 6. Arrhenius Plots of the Degradation of SCH 29482 (●) and FCE 22101 (○) at pH 4.23 (– – – Acetate Buffer), pH 6.59 (—— Phosphate Buffer) and pH 8.60 (—·— Borate Buffer)

We observed that, in general, the E_a and frequency factor values of the penem studied are lower than those of certain bicyclic β-lactamic antibiotics such as ampicillin,[10] cefotaxime,[24] imipenem[20] and clavulanic acid.[16]

Stability Comparison of Penems Studied and Other β-Lactam Antibiotics The high reactivity of the β-lactam system in penicillins has been explained by a reduction in amide resonance coupled with a fusion of the four- and five-membered rings giving rise to a pyramidal geometry of the β-lactam nitrogen[25-27] and a consequent distortion of the orbitals. On the other hand, the high reactivity of cephalosporins would appear to be related to the presence and specific location of the double bond in the annelated six-membered ring which facilitates the cleaving of the

amide C–N bond, as a conjugative interaction of the unshared electron pair on nitrogen with the double bond is competitive with the usual stabilization of the amide C–N bond.[27] Penems combine both these structural elements.

If we compare the catalytic effects of buffer solutions of phosphates on the degradation of the penems studied with those found in the literature for some penicillins and cephalosporins, we find that the catalytic constants of penems are similar to those of penicillins and cephalosporins.[13,28] On the other hand, the reactivities of the Imipenem with all buffer species are significantly greater than those of the penems studied (Table I).[20]

The reactivities of penems studied, penicillins, cephalosporins and other β-lactam compounds with hydrogen ions, water, and hydroxide ions are listed in Table II. A comparison of the catalytic constants in penem hydrolysis with those obtained from several penicillins and cephalosporins shows that FCE 22101 is more reactive with hydroxide ions (k_{OH}) than the penicillins and cephalosporins, while SCH 29482 exhibits a reactivity comparable to that of these β-lactam antibiotics. The reactivities of the penems studied with water (k_{H_2O}) are about the same as those of penicillins and cephalosporins. These penems are less reactive with hydrogen ions (k_H) than are the penicillins (with the exception of ampicillin), but they exhibit reactivities greater than those of cephalosporins.

As the only difference in the β-lactam skeletons of the two penems is the side chain at C-2 (Fig. 1), we conclude that the nature of this side chain has a significant effect on the reactivity of FCE 22101 and SCH 29482 in relation to the hydrolysis catalyzed by buffer solution species and water species.

It would be interesting, from the point of view of obtaining clinical antibiotics, to ascertain the influence of the side chains and of the structure of β-lactam on the chemical reactivity of β-lactam antibiotics, and also to establish the relationship between all these factors and biological activity. To do so it would be necessary to strike a balance between, on the one hand, the reactivity of a β-lactam system and, on the other, a reasonable stability in its conditions of application, and thus obtain a high degree of biological activity. The activity of β-lactam antibiotics has been reported to be related to the lability of their β-lactam ring structure,[29] and correlations have also been established between the stability of this ring and antibiotic activity. Therefore, the more labile a β-lactam drug is, then generally the greater will be the antibacterial activity observed.[30]

As the penem family is still very young and the evaluation of its biological potential still incomplete, we cannot draw any far-reaching conclusions about structure–activity relationships. At the moment we can only make a few generalizations. Alterations in the positions of C-2 and C-6 have, however, been proposed as structural changes which would allow us to obtain acceptably stable and biologically active compounds.[31–33] The results of such studies will probably soon enable us to establish clear relationships between structure and activity.

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