# Epimerization Kinetics of Moxalactam, Its Derivatives, and Carbenicillin in Aqueous Solution

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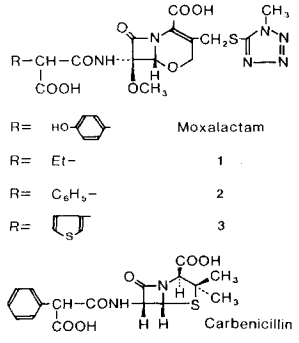
**Abstract** □ The mechanism of the epimerization of moxalactam was studied by measuring the rate of epimerization after deuteration of the C-7 side-chain chiral carbon, introduction of different substituents on the side chain, and variation of the ring system. Deuteration slowed the epimerization rate considerably. The rate was also influenced by the choice of the ring system and the substituent on the C-7 side-chain chiral carbon. When the penicillin ring system with the 2-carboxy-2-phenylacetamide was studied, the epimerization rate decreased indicating that the same ring system needed to be used throughout the epimerization studies. Thus, experiments were conducted with different substituents replacing the phenolic group at the C-7 side-chain chiral carbon of moxalactam. The epimerization rate decreased in the substituent order thienyl, phenyl, 4-hydroxyphenyl, the ionized form of 4-hydroxyphenyl, and ethyl. These results showed that dehydrogenation of the chiral carbon seems to be the rate-determining step and that the stronger the electron-donating effect of the substituent, the slower the epimerization rate becomes.

The epimers of several β-lactam antibiotics often show different in vitro activity.[1,2] Moxalactam (*N*-[(6*R*, 7*R*)-2-carboxy-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-en-7-yl]-2-(*p*-hydroxyphenyl)malonamic acid) (6059-S, Latamoxef), a new semisynthetic antibiotic with a 1-oxacephem nucleus, exists as *R*- and *S*-epimers, epimeric at C-7. It is active against a broad range of Gram-negative microorganisms, including those resistant to cephalosporins. The *R*-epimer of moxalactam is two to three times as active in vitro as the *S*-epimer.[3,4]

In a previous report[5] the epimerization and the degradation of moxalactam were examined in aqueous solution, and the stability of the *R*- and *S*-epimers was determined. In the present study we investigated the mechanism of this epimerization by studying the influence of the ring system and substituents in the side chain of the β-lactam antibiotics moxalactam, moxalactam derivatives, and carbenicillin.




## Experimental Section

**Materials**—The following materials were used as obtained from Shionogi & Co., Ltd., Osaka. The *R*-epimer of moxalactam disodium salt deuterated at the chiral carbon in the C-7 side-chain (deuterated moxalactam),[6] *R*- and *S*-epimers of moxalactam,[7] an *R*- and *S*-mixture of the ethylmalonylamino derivative (**1**),[8] an *R*- and *S*-mixture of the phenylmalonylamino derivative (**2**),[7,9] and the *R*-epimer and an *R*- and *S*-mixture of the 3-thienylmalonylamino derivative (**3**)[7,9] were synthesized at Shionogi Research Laboratory. Carbenicillin disodium salt was supplied by Taito Pfizer Co., Tokyo. All other chemicals were reagent grade. Water was purified with an ion-exchange column and distilled before being used to prepare all solutions.

**pH**—The pH of the solution was controlled throughout the reaction by a pH-stat titrator assembly consisting of a 632 pH meter, 614 Impulsomat, 625/3 Dosigraph, 655 Multi-Dosimat, and 649 Swing-Out Magnetic Stirrer, Metrohm, Ltd., Herisan, Switzerland. The titrated volume of diluted hydrochloric acid or sodium hydroxide solution was ≤2% of the reaction volume. The ionic strength was adjusted to 0.5 by the addition of potassium chloride. No significant pH change was observed throughout the reaction. The Metrohm 632 pH meter was standardized with a combination of standard buffer solutions of pH 4 and 7, or of pH 7 and 9, at the temperature of the kinetic experiments.

**Preparative Fractionation**—The *R*- and *S*-epimers were prepared from *R*- and *S*-mixtures of moxalactam derivatives and carbenicillin. Compounds **1**, **2**, and **3** were fractionated by HPLC (Waters ALC/GPC 204 series with U6K Universal Injector, Model 440 Absorbance Detector (254 nm) and Model 600A pump) using a stainless steel column (10 × 300 mm) packed with Nucleosil 10-$C_8$, particle size 10 μm, Macherey-Nagel Co., Düren, FRG. The eluants employed to separate the *R*- and *S*-epimers were 0.05 M potassium chloride for **1** and 0.05 M potassium chloride:methanol (20:1) for **2** and **3**. The eluants were prepared by micropore filtration (0.45 μm HAWP, Millipore) and deaerated. The *R*- and *S*-epimers of moxalactam and **3** could be identified using the pure epimers, but those of **1** and **2** were not distinguishable since the pure epimers were not available. The peaks with the shorter retention times for both **1** and **2** were assigned to the *R*-epimer and those with the longer retention times to the *S*-epimer, based on the elution patterns of moxalactam and **3** where the *R*-epimer preceded the *S*-epimer under the quantitative HPLC conditions described in the next section. The elution patterns of the *R*- and *S*-epimers of **1** and **2** for fractionation also coincided with those used for quantitative HPLC.

The *R*- and *S*-epimers of carbenicillin appeared as one peak but could be separated by taking fractions of the peak using a 3- × 86-cm glass column packed with porous polystyrene (Diaion, HP-20, Mitsubishikaseikogyo Co., Ltd., Tokyo) and using water as the eluant. Others have also used HPLC to separate the diastereoisomers of carbenicillin,[10-13] but no stereochemical assignments were made. For the reasons explained above, we considered the front edge of the peak to be the carbenicillin *R*-epimer and that appearing toward the tailing edge to be the carbenicillin *S*-epimer.

The fractions of the *R*- and *S*-epimers were immediately



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frozen in a dry ice–acetone bath and lyophilized in a Labconco freeze-dryer consisting of a Vac-stop tray dryer and freeze-dryer 5, Kansas City, MO.

**Analytical Procedures**—Quantitation of the *R*- and *S*-epimers by HPLC was based on integration of peak area using a Model 5000A System Instruments Intelligent Integrator (Tokyo). Moxalactam and deuterated moxalactam were eluted on a stainless steel column (4.6 × 150 mm) packed with Nucleosil 5-$C_8$, particle size 5 μm, Macherey-Nagel Co., at a flow rate of 2.0 mL/min. The eluant employed to separate *R*- and *S*-epimers was 0.1 M potassium chloride. Compounds **1**, **2**, and **3** were eluted on a Waters Radial Pak-A and RCM 100 radial separation–compression system at a flow rate of 2.0 mL/min. The eluants used to separate the *R*- and *S*-epimers were 0.1 M ammonium acetate:methanol (25:2) for **1** and 0.1 M ammonium acetate:methanol (6.45:1) for **2** and **3**. Carbenicillin was eluted on a stainless steel column (4.6 × 150 mm) packed with Nucleosil 5-$C_{18}$, particle size 5 μm, Macherey Nagel Co. The eluant used to separate the *R*- and *S*-epimers was 0.05 M ammonium acetate:methanol (17:1).

**Kinetic Procedures**—All kinetic experiments were carried out at 37 ± 0.1°C and an ionic strength of 0.5. An accurately weighed sample (*R*- or *S*-epimer of moxalactam, *R*-epimer of deuterated moxalactam, *R*- or *S*-epimer of carbenicillin, or *R*-epimer of **3**) was dissolved in 1 mL of 0.5 M potassium chloride solution. It was immediately diluted with 7 mL of 0.5 M potassium chloride solution adjusted to the appropriate pH by the pH-stat titrator and preheated to 37°C by a Taiyo Thermo Unit C-550, Taiyo Kagaku Kogyo Co., Ltd., Tokyo, thermoregulator with ±0.1°C precision. The final sample concentration was made to ~$1 \times 10^{-4}$ M–$1 \times 10^{-3}$ M.

The freeze-dried samples of *R*- and *S*-epimers of **1**, **2**, and **3**, which were obtained by fractionation, consisted mostly of potassium chloride and were treated as only potassium chloride when weighed. The freeze-dried sample was weighed to obtain an ionic strength of 0.5 and dissolved in water adjusted to the appropriate pH by the pH-stat titrator and preheated to 37 ± 0.1°C. The final sample concentration was made to ~$1 \times 10^{-4}$ M–$1 \times 10^{-3}$ M. Portions were removed from the solution at appropriate intervals and quickly frozen in a dry ice–acetone bath. These were thawed just before HPLC analysis and the remaining substances were determined.

## Results and Discussion

**Primary Kinetic Deuterium Effect**—The carbon–deuterium bond (C—D) cleaves at a slower rate than the corresponding carbon–hydrogen bond (C—H).[14] If the cleavage of the bond between the chiral carbon and the hydrogen in the C-7 sidechain is involved in the rate-determining step of the epimerization reaction, the epimerization rate of deuterated moxalactam should be slower than that of moxalactam.

The epimerization reaction of moxalactam proceeds according to the following reaction mechanism: $k_1$ is the epimerization rate constant from the *R*-epimer to the *S*-epimer, $k_2$ is the reverse, and $k_3$ is the degradation rate constant of moxalactam (*R*- and *S*-epimers).[5]

Figures 1 and 2 show the residual percent plots of the *R*- and *S*-epimers and the semilogarithmic plots of moxalactam and deuterated moxalactam versus time at pH 1.47 and 10.46 in aqueous solution. The observed *R*-epimer of deuterated moxalactam in residual percent plots shows the total of the residual *R*-epimer of deuterated moxalactam and the amount of *R*-epimer of moxalactam formed because the peaks of moxalactam could not be separated from the peaks of deuterated moxalactam by HPLC. On the other hand, the observed peak for the *S*-epimer includes almost all the *S*-epimer of moxalactam formed. In the semilogarithmic plot, the observed residual percent of deuterated moxalactam shows the total of the residual deuterated moxalactam and the moxalactam formed. The epimerization reaction starting with the *R*-epimer of deuterated

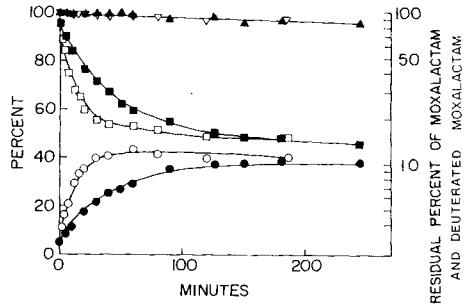


**Figure 1**—*Time courses for the* R*-epimer, the* S*-epimer, and the total of* R- *and* S*-epimers of moxalactam and deuterated moxalactam at pH 1.47 (disappearance of* R*-epimer and appearance of* S*-epimer). The total of the* R- *and* S*-epimers is plotted semilogarithmically versus time. Key: (□)* R*-epimer of moxalactam; (○)* S*-epimer of moxalactam; (△) the total of the* R- *and* S*-epimers of moxalactam; (■)* R*-epimer of deuterated moxalactam; (●)* S*-epimer of deuterated moxalactam; (▼) the total of* R- *and* S*-epimers of deuterated moxalactam.*

moxalactam was slower than that observed for moxalactam (Figs. 1 and 2). Wolfe et al.[15] and Nayler and co-workers[16] suggested that dehydrogenation is the rate-determining step of the epimerization reaction at the 6-position on the penicillin nucleus and that the carbanion is an intermediate in the epimerization reaction. Accordingly, retardation of the rate of epimerization of deuterated moxalactam could be attributed to the fact that C—D bond cleavage is slower than C—H bond cleavage, and the process of C—D bond cleavage or C—H bond cleavage is the rate-determining step of the epimerization reaction.[17]

The semilogarithmic plots of the residual percent deuterated moxalactam and the residual percent moxalactam versus time were reasonably linear and showed almost the same slope. Thus, deuterated moxalactam and moxalactam seem to decompose at almost the same degradation rate. If the degradation rate of deuterated moxalactam differed from that of moxalactam, the semilogarithmic plots of the residual percent deuterated moxalactam would have curved early in the decomposition.

**Epimerization Reaction of Moxalactam in the Basic Region**—The epimerization rate was previously reported[5] to increase by positive unity with increasing pH in the basic region. However, the present study, with strict and fine pH intervals, revealed that the epimerization rate is influenced slightly by dissociation of the phenolic group at the C-7 sidechain and follows eq. 1 in the basic region:

$$k_{obs} = k_{OH}\left(\frac{a_H}{a_H + Ka_3}\right)\left(\frac{Kw}{a_H}\right) + k_O\left(\frac{Ka_3}{a_H + Ka_3}\right)\left(\frac{Kw}{a_H}\right) \quad (1)$$

where $k_{obs}$ is the observed epimerization rate, $k_{OH}$ and $k_O$ are the second-order rate constants for the hydroxide-ion-catalyzed

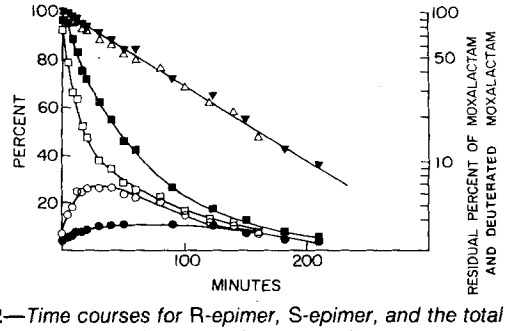


**Figure 2**—*Time courses for* R*-epimer,* S*-epimer, and the total of the* R- *and* S*-epimers of moxalactam and deuterated moxalactam at pH 10.46 (disappearance of* R*-epimer and appearance of* S*-epimer). The total of the* R- *and* S*-epimers is plotted semilogarithmically versus time. Key: (□)* R*-epimer of moxalactam; (○)* S*-epimer of moxalactam; (△) the total of the* R- *and* S*-epimers of moxalactam; (■)* R*-epimer of deuterated moxalactam; (●)* S*-epimer of deuterated moxalactam; (▽) the total of the* R- *and* S*-epimers of deuterated moxalactam.*

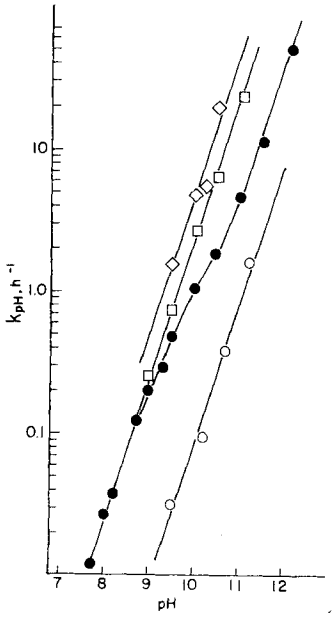


**Figure 3**—*Log $k_{pH}$–pH profiles for moxalactam, 1, 2, and 3 in aqueous solution at 37°C and an ionic strength of 0.5. The points are experimental values and the solid lines are the theoretical curves calculated from the constants in Table I. Key:* (●) $k_1$ *of moxalactam;* (○) $k_1$ *of 1;* (□), $k_1$ *of 2;* (◇) $k_1$ *of 3.*

epimerization of the undissociated and the dissociated forms of the C-7 side-chain phenolic group, and $a_H$ and $Ka_3$ are the activity of the hydrogen ion measured by the glass electrode and the dissociation constant of the C-7 side-chain phenolic group ($2.51 \times 10^{-10}$ at 37°C).[5] The value for the autoprotolysis constant of water, $Kw$, at 37°C is $2.38 \times 10^{-14}$.[18]

The log $k_{pK}$–pH profiles for the epimerization of moxalactam are shown in Figs. 3 and 4. The solid lines represent the theoretical curves calculated by NONLIN.[19] The rate constants in eq. 1 giving the best fits to the log $k_{pH}$–pH profiles are listed in Table I. These results suggested that the sigmoid dependency of the epimerization rates on pH values <11 comes from the

**Table I—Hydroxide-Ion-Catalyzed Epimerization Rate Constants of Moxalactam and Its Derivatives, and $pK_a$ of Carboxylic Acid Derivatives**

| Compounds | Log $k_1^{+a}$ | Log $k_2^{+a}$ | $pK_a^b$ |
|---|---|---|---|
| **1** | 2.63 | 2.84 | 4.87 |
| Moxalactam[c] | 3.18 | 3.21 | — |
| Moxalactam[d] | 4.03 | 4.05 | 4.58 |
| **2** | 3.98 | 4.12 | 4.21 |
| **3** | 4.30 | 4.46 | 4.10 |

[a] $k_1^+$ and $k_2^+$, are the hydroxide-ion-catalyzed epimerization rate constants of $k_1$ and $k_2$, respectively, expressed in $M^{-1} \cdot h^{-1}$. [b] Dissociation constants of carboxylic acid derivatives R—COOH with each substituent in water at 25°C from ref. 20. [c] Moxalactam dissociated at the phenolic group. [d] Moxalactam undissociated at the phenolic group.

specific hydroxide-ion-catalyzed epimerization reaction of undissociated and dissociated moxalactam of the C-7 side-chain phenolic group in the basic region.

**Influence of the Ring System on the Epimerization Rate Constants**—Carbenicillin and **2** are β-lactam antibiotics which have the same side chain (at the 6-position of carbenicillin and at the 7-position of **2**) but different ring systems. We investigated how their epimerization rate constants in the basic region are influenced by the ring systems of carbenicillin and **2**. Both the semilogarithmic plots of carbenicillin and **2** were reasonably linear and indicated that their degradation followed pseudo-first-order kinetics at constant pH and temperature. All of the rate constants were obtained by nonlinear regression analysis (NONLIN),[5,19] and the experimental data fitted well to the theoretical curves.

The log $k_{pH}$–pH profiles for the rate constants ($k_1$, $k_2$, and $k_3$) are shown in Fig. 5. The degradation and epimerization rates increased by one with increasing pH, showing hydroxide-ion-catalyzed degradation and epimerization. The hydroxide-ion-catalyzed degradation and epimerization rate constants of carbenicillin and **2** are listed in Table II. The $k_1$ and $k_2$ of **2** were three times as large as those of carbenicillin; this was attributed to the difference between the ring systems.

**Influence of the Substituents on the Epimerization Rate Constants**—The substituent effect on the epimerization rate constants was investigated using moxalactam derivatives with the same fundamental skeleton but different substituents

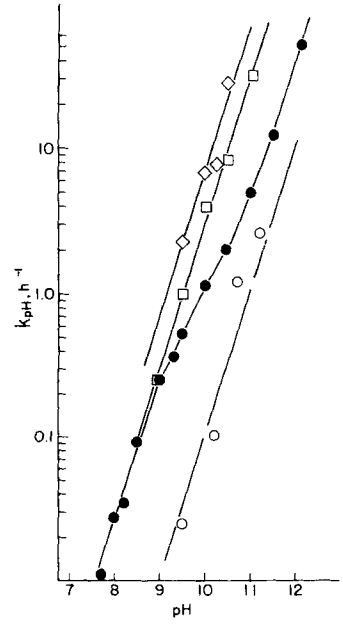


**Figure 4**—*Log $k_{pH}$–pH profiles for moxalactam, 1, 2, and 3 in aqueous solution at 37°C and an ionic strength of 0.5. The points are experimental values and the solid lines are the theoretical curves calculated from the constants in Table I. Key:* (●) $k_2$ *of moxalactam;* (○) $k_2$ *of 1;* (□) $k_2$ *of 2;* (◇) $k_2$ *of 3.*

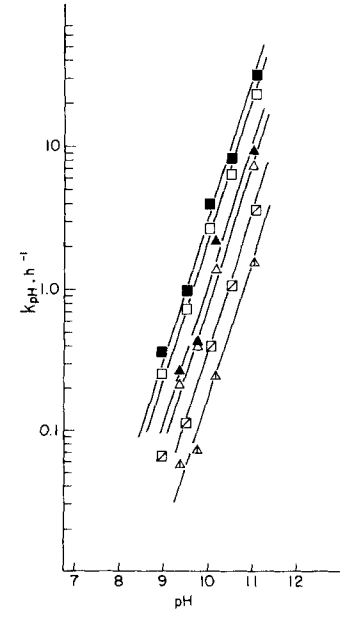


**Figure 5**—*Log $k_{pH}$–pH profiles for 2 and carbenicillin in aqueous solution at 37°C and an ionic strength of 0.5. The points are experimental values and the solid lines are the theoretical lines calculated from the constants in Table II. Key:* (□) $k_1$ *of 2;* (■) $k_2$ *of 2;* (▨) $k_3$ *of 2;* (△) $k_1$ *of carbenicillin;* (▲) $k_2$ *of carbenicillin;* (◬) $k_3$ *of carbenicillin.*

**Table II—Hydroxide-Ion-Catalyzed Rate Constants for Epimerization and Degradation of 2 and Carbenicillin at 37°C and an Ionic Strength of 0.5**

| | $k_1^{+a}$ | $k_2^{+a}$ | $k_3^{+a}$ |
|---|---|---|---|
| **2** | 9592 ± 955 | 13110 ± 1806 | 1481 ± 84.2 |
| Carbenicillin | 3383 ± 465 | 4740 ± 1316 | 756 ± 207 |

[a] $k_1^+$, $k_2^+$, and $k_3^+$, are the hydroxide-ion-catalyzed rate constants of $k_1$, $k_2$, and $k_3$, respectively, expressed in $M^{-1} \cdot h^{-1}$.

at the C-7 side-chain chiral carbon. Both the semilogarithmic plots of **1** and **3** were reasonably linear and indicated that their degradation followed pseudo-first-order kinetics. The $k_1$, $k_2$, and $k_3$ of each derivative was obtained by NONLIN and the experimental data fitted the theoretical curves well.

The log $k_{pH}$–pH profiles for $k_1$, $k_2$, and $k_3$ of **1**, **2**, and **3** are shown in Figs. 3, 4, and 6. The degradation and epimerization rates increased by one with increasing pH, showing hydroxide-ion-catalyzed degradation and epimerization. As shown in Fig. 6, the hydroxide-ion-catalyzed degradation rate constants of the derivatives were found to be almost the same from the log $k_{pH}$–pH profiles for the degradation of the derivatives.

The log values of the hydroxide-ion-catalyzed epimerization

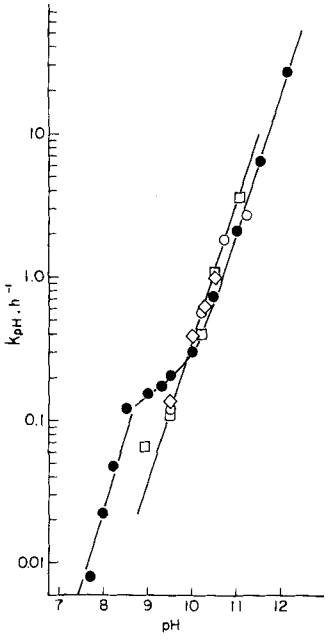


**Figure 6**—*Log $k_{pH}$–pH profiles for moxalactam, 1, 2, and 3 in aqueous solution at 37°C and ionic strength 0.5. The points are experimental values and the solid lines are the theoretical curves calculated from constants in Table I. Key:* (●) $k_3$ *of moxalactam;* (○) $k_3$ *of 1;* (□) $k_3$ *of 2;* (◇) $k_3$ *of 3.*

rate constants of moxalactam and the derivatives, and the $pK_a$ values of the carboxylic acid with the same substituent,[20] are listed in Table I. The smaller the dissociation constant of the carboxylic acid derivative, the larger the epimerization rate constant. This implied that the substituent with a larger electron-withdrawing effect decreased the electron density of the chiral carbon, made the C—H bond easier to cleave, and increased the epimerization rate. On the other hand, the substituent with a larger electron donating effect, i.e., the ethyl group in Table I, increased the electron density of the chiral carbon and decreased the epimerization rate. These inductive effects, in spite of the narrow $pK_a$ range, were also confirmed when the dissociation of the phenolic group of moxalactam decreased the epimerization rate.

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