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Deaza Analogues of Adenosine as Inhibitors of Blood Platelet Aggregation

IPPOLITO ANTONINI *, GLORIA CRISTALLI *, PALMARISA FRANCHETTI *, MARIO GRIFANTINI *x, SANTE MARTELLI *, and FRANCO PETRELLI ‡

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Abstract □ A number of deaza analogues of adenosine were prepared and tested as inhibitors of platelet aggregation induced by ADP and collagen to investigate the structure–activity relationships in this class of nucleoside analogues. The results showed that the presence of a 6-amino group and nitrogen atoms at positions 3 and 7 of the purine moiety are required for inhibitory activity.

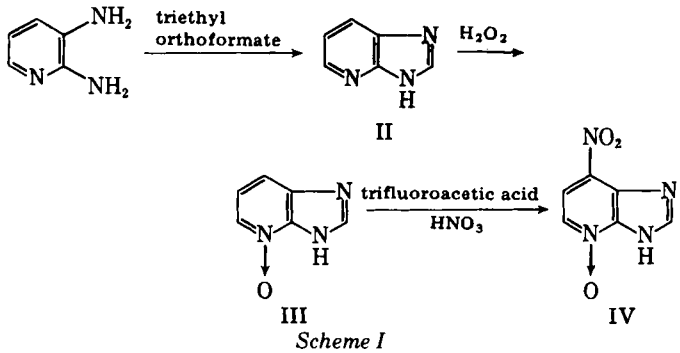
Keyphrases □ Adenosine—deaza analogues, inhibition of ADP- and collagen-induced platelet aggregation □ Platelet aggregation—induction by ADP and collagen, inhibition by deaza analogues of adenosine □ Nucleoside analogues—deazaadenosines, inhibition of ADP- and collagen-induced platelet aggregation

Blood platelets seem to play a dominant role in both the physiological and pathological events of hemostasis (1). In fact, platelet aggregation leads to the formation of either hemostatic plugs or thrombi. Several tissue constituents can give rise to platelet aggregation (2, 3). Among these substances, adenosine diphosphate (ADP) has been shown to be important as it can initiate aggregation (4) and can also mediate aggregation induced by other agents (3, 5).

Adenosine, a structural analogue of ADP, is a powerful inhibitor of platelet aggregation induced by ADP (6). A number of adenosine derivatives and analogues have been synthesized and tested in an attempt to clarify the structure–activity relationships in this class of platelet-aggregation inhibitors (6–9). The present paper investigates the influence of the purine nitrogen atoms on adenosine activity. Some deaza analogues of adenosine and purine riboside have been prepared and tested *in vitro* as inhibitors of platelet aggregation induced by ADP and collagen.

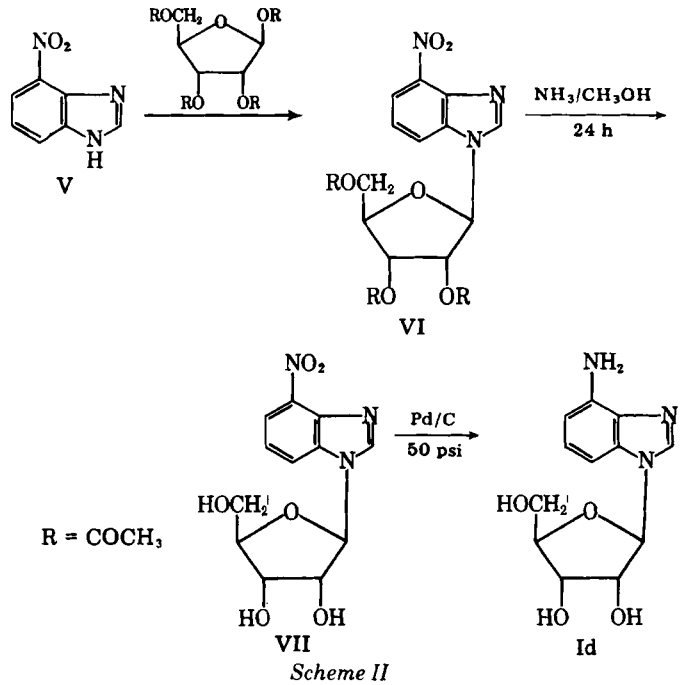
DISCUSSION AND RESULTS

Adenosine[1] (Ia), 7-deazaadenosine[2] (Ie), and purine riboside[2] (Ig) were purchased from commercial sources. 3-Deazaadenosine (Ic) was synthesized using a method previously described (10). 1-Deazaadenosine (Ib) was synthesized using a modification of previous methods (11–14) (Scheme I). Imidazo[4,5-*b*]pyridine (II) (11) was prepared in a 73% yield by the condensation of 2,3-diaminopyridine with triethyl orthoformate.



Scheme I

Nitration of *N*-oxide III, employing a nitric acid–trifluoroacetic acid mixture instead of a nitric acid–acetic acid mixture (12), gave 7-nitroimidazo[4,5-*b*]pyridine-4-oxide (IV) in good yield. The other steps involved following known procedures (13, 14). 1,3-Dideazaadenosine (Id) was synthesized as shown in Scheme II instead of the method reported



Scheme II

[1] Fluka.
[2] Sigma Chemical Co.

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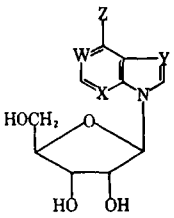
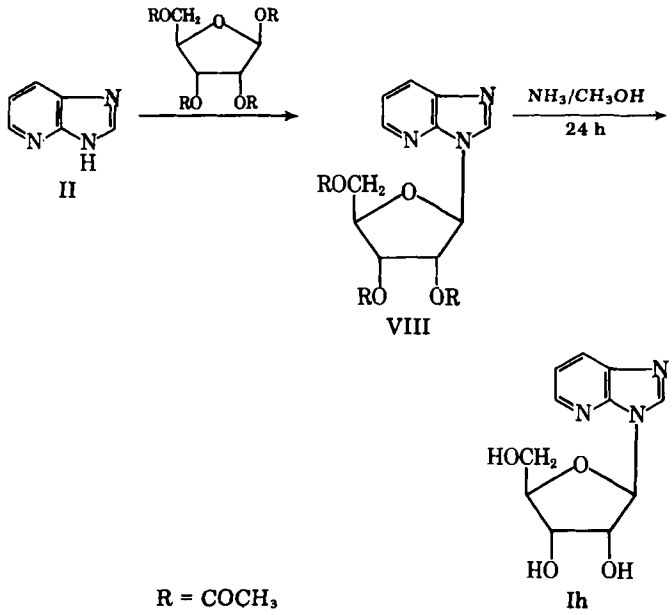
Table I—Effect of Deaza Analogues of Adenosine on Human Platelet Aggregation



	Compound	W	X	Y	Z	Inhibition of Aggregation[a] ADP Induced %	ADP Induced RAD[b]	Collagen Induced %	Collagen Induced RAD[b]
Ia	Adenosine	N	N	N	NH_2	93	1	97	1
Ib	1-Deazaadenosine	CH	N	N	NH_2	82	0.88	87	0.89
Ic	3-Deazaadenosine	N	CH	N	NH_2	<10	<0.1	<10	<0.1
Id	1,3-Dideazaadenosine	CH	CH	N	NH_2	<10	<0.1	<10	<0.1
Ie	7-Deazaadenosine (tubercidin)	N	N	CH	NH_2	<10[c]	<0.1	<10	<0.1
If	1,7-Dideazaadenosine	CH	N	CH	NH_2	<10	<0.1	<10	<0.1
Ig	Purine riboside	N	N	N	H	<10[c]	<0.1	<10	<0.1
Ih	1-Deazapurine riboside	CH	N	N	H	<10	<0.1	<10	<0.1

[a] Compounds were tested at 1.10^{-4} M final concentration. [b] RAD is the relative potency of the compounds to adenosine tested at the same conditions. [c] Values obtained for Ie and Ig are in agreement with literature data (6, 8).

by Mizuno *et al.* (15). Fusion of 4(7)-nitrobenzimidazole (V) (16) with tetra-*O*-acetyl-β-ribofuranose followed by deacetylation with methanolic ammonia at room temperature gave VII in good yield. Ribonucleoside Id was then obtained by catalytic hydrogenation of VII with palladium-on-carbon (17). The same synthetic procedure was followed to prepare 1-deazapurine riboside (Ih) as shown in Scheme III, instead of the syntheses previously described (18, 19). The synthesis 1,7-dideazaadenosine (If) has been described elsewhere (20).



Scheme III

The compounds in Table I were tested as inhibitors of ADP- and collagen-induced human platelet aggregation *in vitro* according to the method of Born and Cross (6). The inhibitory activity of each compound was estimated by the extent of the decrease in optical density after the addition of ADP and collagen and compared with adenosine activity (RAD). 7-Deazaadenosine (Ie) and purine riboside (Ig) were tested previously and found to be ~1% as active as adenosine (6, 8). The results of the aggregation tests are shown in Table I.

1-Deazaadenosine (Ib) proved to be the only deaza analogue of adenosine showing significant inhibitory activity at 1×10^{-4} M concentration. When 1-deazaadenosine was tested at different concentrations between 5×10^{-6} M and 1×10^{-4} M, its effect on platelet aggregation induced by ADP and collagen was dose dependent, as shown in Fig. 1. The activity, also affected by the incubation period, was maximal after 7.5 min of preincubation (Fig. 2).

When adenosine and 1-deazaadenosine were coadministered, the cumulative effect was almost equal to the sum of the effects elicited by the substances separately. The results of aggregation tests clearly indicated that the nitrogen atoms at positions 3 and 7 of the purine ring are required for inhibitory activity. In fact 3-deazaadenosine (Ic), 7-deazaadenosine (Ie), 1,3-dideazaadenosine (Id), and 1,7-dideazaadenosine (If) did not show any significant inhibitory activity at a concentration of 1×10^{-4} M.

Elimination of the 6-amino group both in adenosine and 1-deazaadenosine resulted in loss of activity, as shown by the fact that purine riboside (Ig) and 1-deazapurine riboside (Ih) did not inhibit platelet aggregation induced by ADP and collagen (Table I). On the other hand, the nitrogen atom at position 1 of the purine ring appeared to be less critical than the other nitrogen atoms suggesting that some substituent changes can be made without loss of the inhibitory activity against platelet aggregation.

EXPERIMENTAL[3]

Imidazo[4,5-*b*]pyridine (II)—A mixture of 20 g (0.183 mol) of 2,3-diaminopyridine[1] and 400 mL of triethyl orthoformate was heated at reflux for 3 h. The solution was evaporated to dryness *in vacuo*, and then the residue was heated at reflux with 200 mL of concentrated hydrochloric acid for 1 h. The mixture was allowed to cool, neutralized with solid Na_2CO_3, and extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4), and the solvent was removed at reduced pressure. The residue was dissolved in absolute ethanol, treated with charcoal, filtered, and then the solvent was evaporated to give 16 g of II as colorless needles (73% yield), mp 152–153°C [lit. (11) mp 153–154°C]; ^{1}H-NMR (DMSO-d_6): δ 7.3 (m, 1, 6-H), 8.11 (d, 1, J = 8 Hz, 7-H), 8.45 (d, 1, J = 5 Hz, 5-H), and 8.54 ppm (s, 1, 2-H).

7-Nitroimidazo[4,5-*b*]pyridine-4-oxide (IV)—To a cold (0°C) solution of 13.6 g (0.1 mol) of imidazo[4,5-*b*]pyridine-*N*-oxide (III) (12) in 100 mL of trifluoroacetic acid was added, in a dropwise manner, 65 mL of 90% fuming nitric acid. The mixture was heated at 90°C for 3 h, cooled, and then poured into crushed ice. The mixture was neutralized with concentrated ammonium hydroxide while maintaining the temperature below 30°C. The resulting solid was filtered, washed with ice water, and dried to give 13.5 g of IV as light-yellow needles (75% yield). The structure of IV was confirmed by comparison of the data obtained with that in the literature (12).

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-nitrobenzimidazole (VI)—A mixture of 4(7)-nitrobenzimidazole (1g, 6.13 mmol) (17), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.9 g, 12.25 mmol) and *p*-toluenesulfonic acid (50 mg) was heated at 160°C with stirring *in vacuo* (25 mm) for 20 min. The mixture was cooled, and the resulting solid was neutralized with a saturated Na_2CO_3 solution and extracted with chloroform. The organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was chromatographed on silica gel (ethyl acetate) to give 1.6 g (60%) of VI as light-yellow needles, mp 75–77°C; ^{1}H-NMR ($CDCl_3$): δ 2.08–2.15 (m, 9, 3 CH_3), 4.4–4.63 (m, 3, 4′-H and 5′-CH_2), 5.36–5.63 (m, 2, 2′-H and 3′-H), 6.16 (d, 1, J = 5 Hz, 1′-H), 7.3 (t, 1, 6-H),

[3] The melting points were determined with a Büchi apparatus and are uncorrected. The NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer, using tetramethylsilane as the internal standard. For column chromatography silica gel 60 Merck was used.

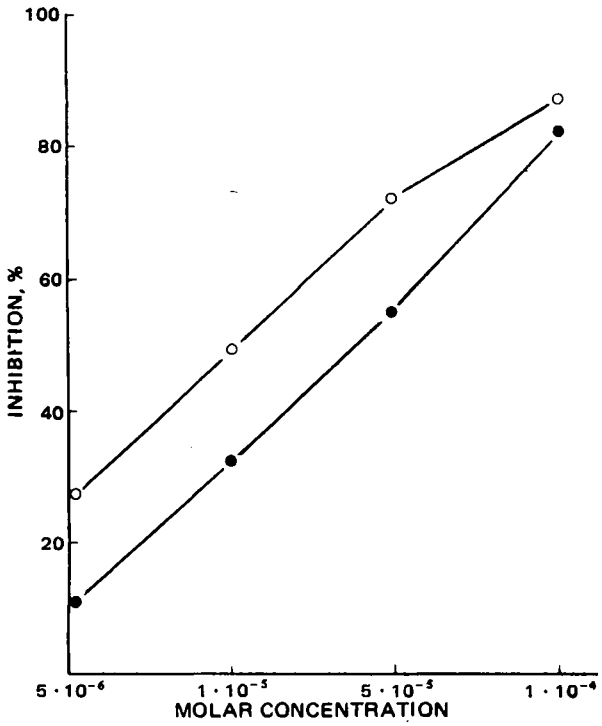


Figure 1—*Inhibitory effects of 1-deazaadenosine (Ib) at different concentrations on human platelet aggregation induced by ADP (●) and collagen (○). Ib was incubated in platelet-rich plasma at 37°C for 5 min before the addition of ADP and collagen.*

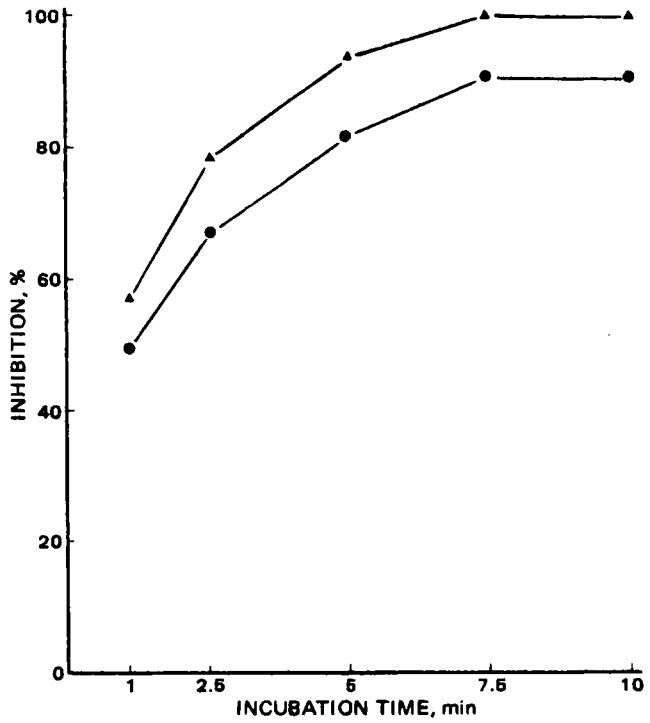


Figure 2—*Effect of 1-deazaadenosine (Ib) (●) and adenosine (Ia) (▲) on ADP-induced human platelet aggregation. The compounds were added to platelet-rich plasma at 1 × 10⁻⁴ M concentration and incubated at 37°C for increasing intervals before the addition of ADP.*

7.98 (d, 1, J = 8 Hz, 7-H), 8.17 (d, 1, J = 5 Hz, 6-H), and 8.41 ppm (s, 1, 2-H).

Anal.—Calc. for $C_{18}H_{19}N_3O_9$: C, 51.31; H, 4.55; N, 9.97. Found: C, 51.18; H, 4.59; N, 10.06.

3 - (2',3',5'-Tri -*O*- acetyl-β-D-ribofuranosyl)-3*H*-imidazo-[4,5-*b*]pyridine (VIII)—An intimate mixture of II (200 mg, 1.66 mmol), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1 g, 3.3 mmol), and *p*-toluenesulfonic acid (20 mg) was heated at 160°C with stirring *in vacuo* (25 mm) for 20 min. The solid was neutralized with a saturated Na_2CO_3 solution and extracted several times with ethyl acetate. The combined extracts were concentrated *in vacuo* to dryness, and the residue was chromatographed on silica gel (ethyl acetate–methanol, 9:1) to give 320 mg (50%) of VIII as a vitreous solid.

Anal.—Calc. for $C_{17}H_{19}N_3O_7$: C, 54.11; H, 5.08; N, 17.28. Found: C, 53.98; H, 5.11; N, 17.42.

3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (Ih)—A solution of VIII (280 mg, 0.74 mmol) in methanol saturated with ammonia (15 mL) was set aside at room temperature for 24 h. The solvent was removed *in vacuo*, and the residue was crystallized from water to yield 160 mg (85%) of Ih, mp 220–221°C [lit. (18) mp 220–222°C]. ^{1}H-NMR (DMSO-d_6): δ 3.65 (m, 2, 5'-CH_2), 4.02 (m, 1, 4'-H), 4.22 (t, 1, 3'-H), 4.7 (t, 1, 2'-H), 6.1 (d, 1, J = 5.5 Hz, 1'-H), 7.36 (m, 1, 6-H), 8.16 (d, 1, J = 8 Hz, 7-H), 8.4 (d, 1, J = 4 Hz, 5-H), and 8.72 ppm (s, 1, 2-H).

Inhibition of Platelet Aggregation—Platelet aggregation was measured by the method of Born and Cross (5), using a platelet aggregation meter[4]. The aggregative agents ADP and collagen were purchased[5]; blood was obtained by venipuncture in the forearms of apparently healthy humans and collected in polyethylene tubes containing a 1:9 volume of 3.8% sodium citrate. Platelet-rich plasma was obtained by centrifugation at 1200 rpm for 10 min, while platelet-poor plasma was obtained by centrifugation at 4500 rpm for 20 min. The platelet-rich plasma was adjusted to ~250,000 platelets/mL by adding platelet-poor plasma.

A 50-μL aliquot of the test sample dissolved in Michaelis buffer was added to a cuvette containing 0.5 mL of platelet-rich plasma, and a 50-μL aliquot of Michaelis buffer was added to the test control. The cuvette was placed in the aggregation meter and allowed to incubate at 37°C for 5 min, after which 50 μL of ADP or collagen was added to the platelet-rich plasma. The final concentration of each sample is reported in Table I and in Figs. 1 and 2. The percent inhibition of aggregation by a test compound was calculated by dividing the maximal deflection in the optical density curve in the presence of the compound by that observed in the control, then multiplying by 100. As inhibition percentage differed from preparation to preparation of platelet-rich plasma, relative potency (RAD) to a reference standard (adenosine) in the same concentration was a direct measure of the potency of inhibition.

[4] 1060 S Elvi 840 platelet aggregation meter.
[5] Centropa A6P test; Mascia Brunelli.

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Degradation and Epimerization Kinetics of Moxalactam in Aqueous Solution

NAOFUMI HASHIMOTO ˣ, TAKENOBU TASAKI, and HIROYOSHI TANAKA

Received September 7, 1982, from the *Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.* Accepted for publication January 26, 1983.

Abstract □ The kinetics of epimerization and degradation of moxalactam in aqueous solution was investigated by HPLC. The pH–rate profiles of the degradation and epimerization were determined separately over the pH range of 1.0–11.5 at 37°C and constant ionic strength 0.5. The degradation and simultaneous epimerization were followed by measuring both of the residual *R*- and *S*-epimers of moxalactam and were found to follow pseudo-first-order kinetics. The degradation was subjected to hydrogen ion and hydroxide ion catalyses and influenced by the dissociation of the side chain phenolic group. The epimerization rates were influenced significantly in the acidic region by the dissociation of the side chain carboxylic acid group and in the basic region by hydroxide ion catalysis. The pH–degradation rate profile of moxalactam showed a minimum degradation rate constant between pH 4.0 and 6.0. The pH–epimerization rate profiles of moxalactam showed minimum epimerization rate constants at pH 7.0. The epimerization rate constants of the *R*- and *S*-epimers were not very different.

Keyphrases □ Moxalactam—degradation and epimerization in aqueous solutions, kinetics, HPLC □ Degradation—moxalactam in aqueous solutions, kinetics, epimerization □ Epimerization—moxalactam in aqueous solutions, kinetics, degradation □ Kinetics—moxalactam degradation and epimerization in aqueous solutions, HPLC

Stereoisomers, occurring widely in nature, display different biological and pharmacological effects than their racemic mixtures. Many β-lactam antibiotics have stereoisomers with different antibacterial activities (1). Few reports (2, 3) describe the kinetics of the epimerization of β-lactam antibiotics, a process which should offer valuable information for predicting and improving antibacterial activity.

Moxalactam (6059-S)[1] is a new semisynthetic (4), broad-spectrum (5) 1-oxacephalosporin which exists as the *R*- and *S*-epimers, epimeric at C-7. The *in vitro* activity of the *R*-epimer is twice that of the *S*-epimer (1). In the present study, the epimerization and degradation of moxalactam were investigated kinetically.

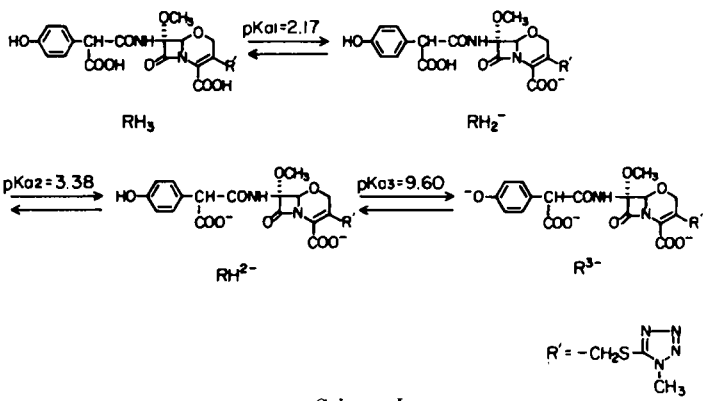
EXPERIMENTAL

Materials—*R*- and *S*-epimers of moxalactam disodium and decarboxy-moxalactam monosodium (decarboxylated in the 7-side chain)[1] were used as obtained. All other chemicals were of reagent grade. Water was purified with an ion-exchange column and distilled before being used.

pH—The pH of the solution was controlled throughout the reaction by a pH-stat[2]. The titrated volume of diluted hydrochloric acid or sodium hydroxide solution was at most 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 pH meter[3] was standardized with the combination of standard buffer solutions of pH 4 and 7 or pH 7 and 9 at the temperature of the kinetic experiments.

Analytical Procedures—HPLC[4] was used to determine the concentrations of *R*- and *S*-epimers of moxalactam and decarboxy-moxalactam. Quantification was based on integration of peak areas using an integrator[5]. The elution was carried out on a 4.0 × 250-mm stainless steel column packed with octadecylsilane chemically bonded on silica gel[6] at room temperature. The mobile phase employed to resolve *R*- and *S*-epimers in the HPLC operation consisted of 0.05 M ammonium acetate–methanol (50:3). The mobile phase employed to resolve the decarboxy-moxalactam consisted of a solution containing 5.2 g of tetra-*n*-butylammonium hydroxide (10% in water), 6.1 g of tetra-*n*-propylammonium hydroxide (10% in water), 1.0 g of dibasic sodium phosphate, and 1.0 g of monobasic sodium phosphate, adjusted to pH 6.0 by acetic acid, and methanol (7:3). The mobile phases were prepared by micropore filtration[7] and deaerated. No difference in absorbance between *R*- and *S*-epimers was observed at any wavelength of the UV spectrum (6). Accordingly, the peak areas of *R*- and *S*-epimers represent their intact concentrations. The calibration curves of the peak area against the concentration of moxalactam was satisfactorily linear.

Kinetic Procedure—All kinetic experiments were carried out at 37



Scheme I

[1] Latamoxef; Shionogi & Co., Ltd., Osaka, Japan.

[2] pH-Stat titrator assembly consisting of TTT80 titrator, ABS80 autoburet, PHM84 research pH meter, REC80 servo graph, TTT81 digital titrator, and TIK801 titration keyboard; Radiometer, Copenhagen, Denmark.

[3] Radiometer PHM84 research pH meter.

[4] Waters ALC/GPC 204 series with U6K universal injector, Model 440 absorbance detector (254 nm), and Model M6000A pump, Waters Associates, Milford, Mass.

[5] Chromatopac C-E1B; Shimadzu, Kyoto, Japan.

[6] Nucleosil $7C_{18}$, particle size 5 μm; Macherey-Nagel Co., Düren, West Germany.

[7] 0.45 μm HAWP; Millipore Corp., Bedford, Mass.

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