Stability of *N*-Formimidoylthienamycin in Aqueous Solution

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Abstract □ The stability of *N*-formimidoylthienamycin in aqueous solution at 25 and 40° was determined. In pH 5–8 nonnucleophilic inert buffers, pseudo-first-order ring opening was observed with dilute solutions (1 or 2 mg/ml initially). At higher initial concentrations, a second-order reaction also was evident. The rate data were compared with literature data for various cephalosporins and penicillins.

Keyphrases □ *N*-Formimidoylthienamycin—stability in aqueous solution at 25 and 40° □ Stability—*N*-formimidoylthienamycin in aqueous solution, reaction kinetics, compared to other β-lactam antibiotics □ Kinetics—stability of *N*-formimidoylthienamycin in aqueous solution □ β-Lactam antibiotics—stability of *N*-formimidoylthienamycin in aqueous solution

Thienamycin (I) is a new β-lactam antibiotic, discovered in culture broths of *Streptomyces* MA4297 (1). Although a detailed kinetic study of thienamycin has not been made, it is known to decompose in dilute aqueous solution by apparent first-order reaction and has its greatest stability at neutral pH. However, the decomposition accelerates as the antibiotic concentration is increased (1). This behavior is believed to be due to aminolysis of the β-lactam by the primary amine of a second thienamycin molecule. Analogous reactions of 6-aminopenicillanic acid (2) and ampicillin (3) have been reported. *N*-Formimidoylthienamycin (II) was designed to mitigate this bimolecular reaction (4).

The present study determined the stability of II in aqueous solution; the results are related to data for other β-lactam antibiotics.

EXPERIMENTAL

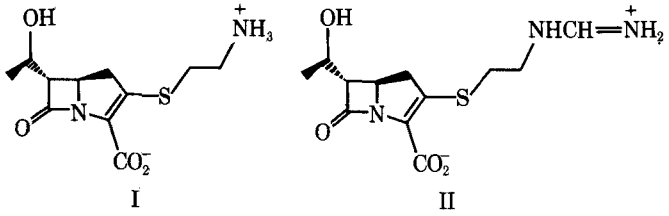
Materials—2-(*N*-Morpholino)ethanesulfonic acid[1], 3-(*N*-morpholino)propanesulfonic acid[1], monobasic potassium phosphate[2], and 50% sodium hydroxide solution[3] were used for buffers.

The ^{13}C-NMR, PMR, and IR spectra were consistent with the structure of II monohydrate (4).

Anal.—Calc. for $C_{12}H_{17}N_3O_4S{\cdot}H_2O$: C, 45.41; H, 6.04; N, 13.25; S, 10.08. Found: C, 45.11; H, 6.14; N, 13.09; S, 10.07.

TLC—Aged aqueous solutions of II were examined by TLC as follows. Solution aliquots containing ~50 μg of solute were loaded onto 250-μm silica gel GF[4] plates. The plates were developed in a saturated chamber with toluene–acetone–*n*-butanol–acetic acid–water (1:1:1:1:1) and were viewed under short- and long-wavelength UV lights.

Kinetic Runs—Aqueous buffer solutions of monobasic potassium phosphate, 2-(*N*-morpholino)ethanesulfonic acid (pKa 6.15), and 3-(*N*-morpholino)propanesulfonic acid (pKa 7.2) were adjusted to the desired pH with concentrated aqueous sodium hydroxide. These buffers, to be used as solvents for kinetic runs, were prepared at the reaction temperatures.



I

II

Compound II was weighed into 3-ml glass reaction vials equipped with open-top screw caps and silicone septa. Each reaction vial contained 1–30 mg of II and 1 ml of buffer. Nitrogen containing <10 ppm of oxygen was used to protect the reaction solutions from air.

Each kinetic run was initiated by adding nitrogen-purged buffer to the screw-capped vial. The vial was closed immediately and purged with a subsurface nitrogen stream injected through the septum with a 25-gauge syringe needle. A second needle through the septum served as the vent. The vials were stored in constant-temperature water baths.

Aliquots (100 μl) of the reaction solutions were removed periodically with a microsyringe equipped with a 25-gauge needle. The reaction vials were purged with nitrogen while aliquots were removed. The aliquots were diluted quantitatively to UV concentration, 0.2 μmole of II/ml initially, with 0.1 *M*, pH 7, 3-(*N*-morpholino)propanesulfonic acid, which had been purged with nitrogen; the UV spectra then were recorded[5]. Then 0.1 ml of 0.2 *M* aqueous hydroxylamine hydrochloride[2] was mixed with 3 ml of each diluted aliquot (hydroxylamine concentration of 6.5 μmoles/ml). The UV spectra were recorded again after the hydroxylamine mixtures had aged for 20 min at room temperature. (Hydroxylamine hydrochloride was used for all kinetic runs except the preliminary runs with phosphate buffer.)

RESULTS AND DISCUSSION

Reaction of II and Phosphate—In preliminary runs with pH 7.2 phosphate buffer, a complex reaction was evident by direct UV assay of the solutions exposed to air. After a weakening of the II spectrum initially (absorbance maximum at 297 nm), a consecutive reaction was apparent with the appearance of absorbance peaks at 308 and 270 nm, and the reaction solutions turned brown. The consecutive reaction was due to oxygen. With air excluded, the solutions remained nearly colorless, and pseudo-first-order behavior was observed. The UV spectra of II and its product with oxygen excluded are shown in Fig. 1 as curves A and B, respectively.

Curve B was observable only if the reaction mixture had been purged with nitrogen initially and during aliquot removal and only if the buffer used for dilution had been purged with nitrogen. Then the entire absorbance curve could be recorded twice (about 10 min) before the peaks at 308 and 270 nm appeared as the result of exposing the cell to room air.

The results of four runs with pH 7.2 phosphate buffer at 23° are shown in Fig. 2. The percent of the initial II unreacted, measured at 300 nm, is plotted on the logarithmic scale *versus* reaction time. The reaction was first order with respect to II, as shown by the linear log plots for the runs under nitrogen. Furthermore, the half-life was unchanged when the initial II concentration was halved from 6 to 3 m*M*. When the phosphate concentration was reduced from 1 to 0.5 *M*, the half-life doubled. This result means that II hydrolysis was catalyzed by phosphate and that essentially all of the reaction observed in these runs was due to phosphate. The apparent initial rate for the run unprotected from air was about the same as that for the runs under nitrogen. However, the final spectrum used to calculate the rate could be obtained only when air was excluded.

Analytical Methods—After the runs with phosphate buffer, kinetic data for unreacted II were obtained by hydroxylamine UV assay. When diluted aliquots of hydrolysis mixtures were treated with hydroxylamine, ~90% of the II absorbance was extinguished, but the absorbances of the hydrolysis products obtained in the presence or absence of oxygen were unaffected. The loss of absorbance at 300 nm, measured on treatment

[1] Calbiochem.
[2] Merck.
[3] Baker.
[4] Analtech.
[5] Cary model 14, Varian Associates.

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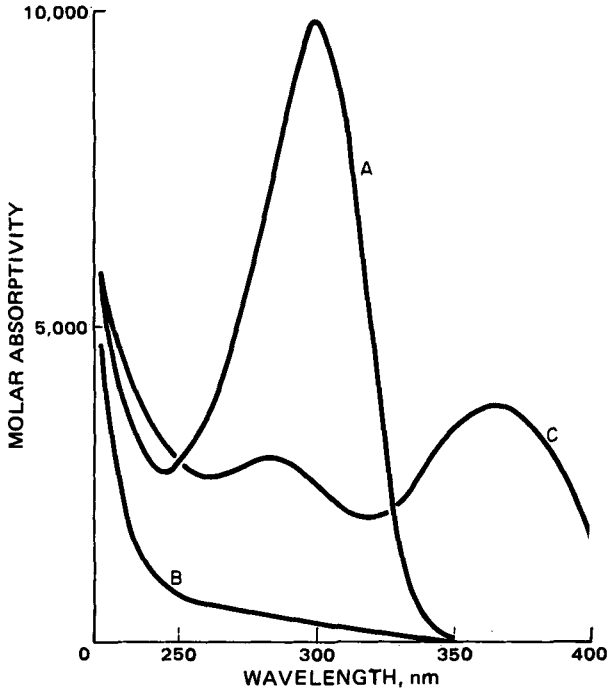


Figure 1—*UV spectra measured with 0.1* M, *pH 7, 3-(*N*-morpholino)-propanesulfonic acid buffer as the UV solvent. Key: A, II; B, decomposition product formed in pH 7.2 phosphate buffer protected from air; and C, decomposition product formed in pH 5 or 6 2-(*N*-morpholino)-ethanesulfonic acid buffer.*

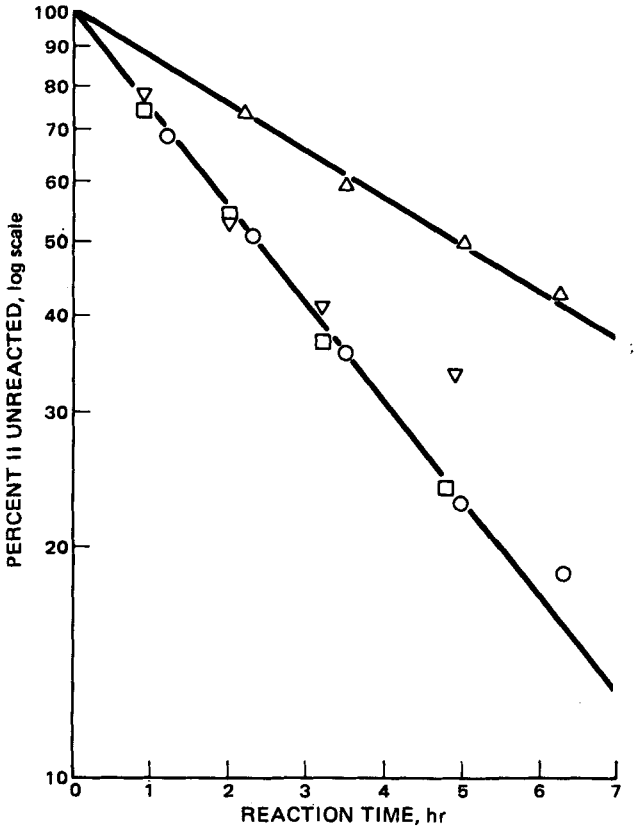


Figure 2—*Stability of II in pH 7.2 phosphate buffer at 23°. Key:* ○, *6 mM substrate in 1.00* M *buffer under nitrogen;* △, *6 mM substrate in 0.50* M *buffer under nitrogen;* □, *3 mM substrate in 1.00* M *buffer under nitrogen; and* ▽, *6 mM substrate in 1.00* M *buffer unprotected from air.*

with hydroxylamine, was proportional to the concentration of unreacted β-lactam.

Although the hydroxylamine UV assay for II was adequate and did not require oxygen exclusion, all kinetic runs were performed with oxygen excluded so that the direct UV assay data could be used. In every run, the transition from the initial spectrum of II to that of the product appeared as a simple one-step reaction, although the product UV spectra, always measured at pH 7, varied depending on the reaction pH (discussion to follow). Although not completely stable by UV assay, the products were more stable than the starting material, and the reaction rates were calculated readily from the crude UV data. Good agreement of the rate data obtained by the two UV methods was observed.

Reaction Products—The UV assays provided measurements of the concentration of II during reaction but did not characterize the products. Brief studies by Fourier transform IR spectroscopy (FTIR) and TLC revealed that hydrolysis of II led to complex product mixtures.

Opening of the β-lactam ring was observed by FTIR of unbuffered aqueous II, 5 mg/ml. The disappearance of the β-lactam band at 1774 cm^{-1} was not accompanied by appearance of any distinct features in the spectrum.

Decomposition of an aqueous solution of II (5 mg/ml), adjusted to pH 4 with trifluoroacetic acid, was monitored by TLC. This procedure was done at pH 4 because the consecutive reaction with oxygen at higher pH, evident by UV assay, did not occur at pH 4. Even so, several degradates were apparent in short-wavelength UV light by fluorescence quenching and in long-wavelength UV light by fluorescence. Therefore, the slow, rate-determining opening of the β-lactam ring in the absence of oxygen, for which the kinetics are described later, preceded the relatively rapid formation of many unidentified degradates.

Table I—Apparent First-Order Rates in Nonnucleophilic Buffers

Buffer pH	k_{pH}, hr^{-1}, at 25°		k_{pH}, hr^{-1}, at 40°	
	Observed	Calculated	Observed	Calculated
5.0[a]	0.0315	0.0318	0.111	0.113
5.5[a]	0.0139	0.0128	0.0533	0.0465
6.0[a]	0.0069	0.0069	0.0257	0.0257
6.5[a]	0.0048	0.0050	0.0169	0.0197
6.5[b]	0.0048	0.0050	0.0169	0.0197
7.0[b]	0.0040	0.0047	0.0169	0.0197
7.5[b]	0.0055	0.0054	0.0262	0.0255
8.0[b]	0.0083	0.0082	0.0462	0.0461

[a] 2-(*N*-Morpholino)ethanesulfonic acid. [b] 3-(*N*-Morpholino)propanesulfonic acid.

Rate–pH Profiles for II at Low Concentration—Kinetic runs with nonnucleophilic 2-(*N*-morpholino)ethanesulfonic acid at pH 5.0, 5.5, 6.0, and 6.5 and 3-(*N*-morpholino)propanesulfonic acid at pH 6.5, 7.0, 7.5, and 8.0 were performed at 25 and 40°. Each buffer was used at 0.5 and 1.0 *M* with an initial II concentration of 3 or 6 m*M*, *i.e.*, 1 or 2 mg/ml. Kinetic data for these runs were accumulated over 1 week since the reaction mixtures had half-lives of several days. Then the reaction mixtures were stored at 60° to hasten completion of the reactions, and the final UV spectra were recorded at pH 7. (The products were quite stable by UV assay.)

The final UV spectra for runs at 40° were about the same as those for runs at 25° at the corresponding pH values. The final UV spectrum with the pH 8 buffer was the same as that observed with pH 7.2 phosphate buffer (Fig. 1, curve B). The product formed at pH 8, like that formed in pH 7.2 phosphate, gave absorbance peaks at 308 and 270 nm upon exposure to air. The final UV spectra for runs with buffers at pH 5–6 denoted a different product (Fig. 1, curve C). An isosbestic point at 328 nm was observed. By UV assay, the product formed in pH 5–6 buffers, unlike the pH 8 product, was stable to air at pH 5 or 7, with or without added phosphate. The final spectra for reactions at pH 6–8 were intermediate between curves B and C of Fig. 1.

At low initial II concentration, pseudo-first-order decomposition was observed. Plots of log (percent of II unreacted) *versus* time were linear, similar to the runs under nitrogen shown in Fig. 2; at each pH, the apparent rate constant was measurably the same with initial II concentrations of 1 and 2 mg/ml. The reaction rates were independent of the nonnucleophilic buffer concentrations; *i.e.*, at each pH, the reaction rate was about the same in 0.5 or 1.0 *M* buffer. Thus, the rate effect of ionic strength was insignificant. Moreover, the rates with 2-(*N*-morpholino)-ethanesulfonic acid and 3-(*N*-morpholino)propanesulfonic acid at pH 6.5 were about the same.

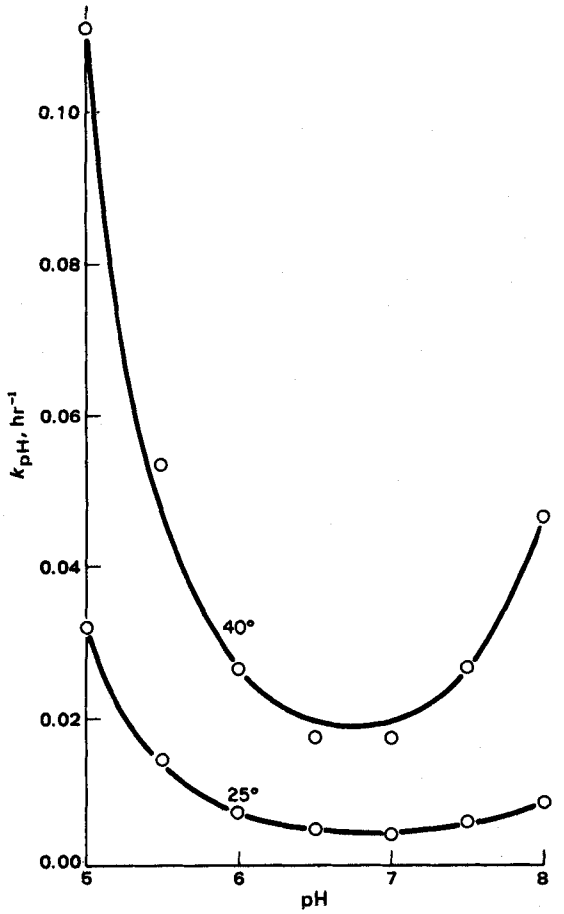


Figure 3—*The pH profiles of pseudo-first-order rate constants: data points (○) and least-squares curves calculated according to Eq. 1 (—).*

The apparent pseudo-first-order rate constants observed at 25 and 40° are listed in Table I. At each temperature, II was most stable at neutral pH, *i.e.*, pH ~ 7.0 at 25° and pH ~ 6.75 at 40° [$K_W = 10^{-14.00}$ at 25° and $K_W = 10^{-13.53}$ at 40° (5)]. The rate–pH profiles were consistent with:

$$k_{pH} = k_H a_H + k_0 + k_{OH}(K_W/a_H) \qquad \text{(Eq. 1)}$$

where k_{pH} is the apparent first-order rate constant at a given pH; k_H and k_{OH} are the second-order rate constants for the hydrogen-ion-catalyzed degradation and hydroxide-ion-catalyzed degradation, respectively; k_0 is the first-order rate constant for spontaneous or water-catalyzed degradation; K_W is the dissociation constant of water; and a_H is the hydrogen-ion activity as measured by the glass electrode. The least-squares reactivities for each reaction at the two temperatures were determined, according to Eq. 1, by nonlinear regression. The calculated pseudo-first-order rate constants, k_{pH}, were in good agreement with the observed values (Table I). The observed k_{pH} data points are shown in Fig. 3 with the calculated k_{pH} curves. The least-squares reactivities of hydrogen ion, water, and hydroxide ion and the Arrhenius activation energies are listed in Table II.

Stability of II at High Concentration—Slow pseudo-first-order reaction of II in the nonnucleophilic, inert buffers had been observed at initial II concentrations of 1 and 2 mg/ml. Any competitive second-order reaction of II must have been very slow relative to the first-order reaction. Therefore, to detect any second-order reaction, the initial II concentration was varied under conditions at which the pseudo-first-order reaction was minimal, pH 7.0 at 25° and pH 6.75 at 40°.

Table II—Calculated Reactivities and Activation Energies in Nonnucleophilic Buffers

Parameter	k_H, M^{-1} hr^{-1}	k_0, hr^{-1}	k_{OH}, M^{-1} hr^{-1}
Least-squares reactivity at 40°	9730	0.01565	10,300
Least-squares reactivity at 25°	2780	0.00403	4,150
E_a, kcal/mole	15.5	16.8	11.2

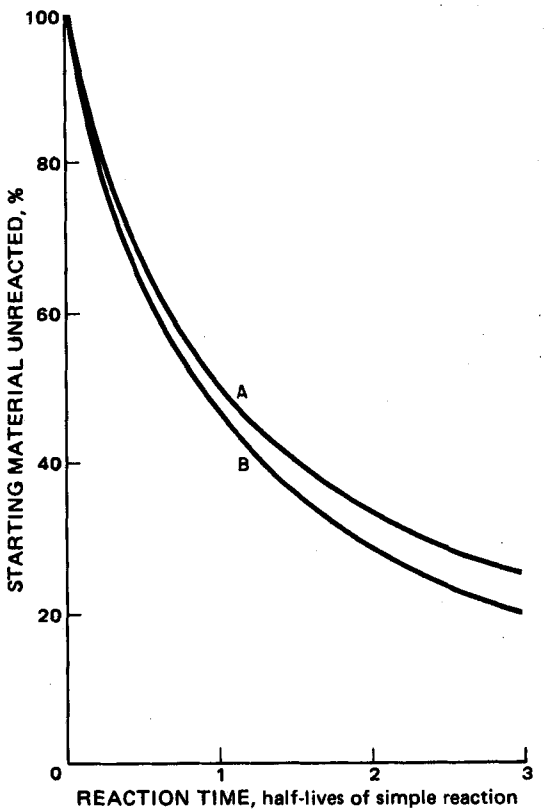


Figure 4—*Calculated time course of second-order reactions. Key: A, simple second-order reaction; and B, second-order reaction involving oligomers.*

At each temperature, a series of kinetic runs was performed with initial II concentrations of 1.8, 3, 6, 15, and 30 mg/ml (0.006, 0.01, 0.02, 0.05, and 0.1 *M*, respectively) in 1 *M* 3-(*N*-morpholino)propanesulfonic acid. In each series, the decomposition rates, as shown by plots of percent II unreacted *versus* reaction time, were progressively more rapid at higher initial concentration. Furthermore, the product UV spectra, measured at pH 7, included progressively more high wavelength absorbance (peak wavelength at ~365 nm, which is similar to that observed with low initial II concentrations at pH 5–6) for runs at higher initial concentration. This trend of product compositions was not due to pH shifts. The pH of all reaction solutions remained the same as the pH of the buffer used.

Thus, competitive first- and second-order reactions of II were observed. The second-order reaction was treated as dimerization in that the second-order rate was evaluated according to:

$$-dA/dt = k_{pH}A + k_2A^2 \qquad \text{(Eq. 2)}$$

where A represents the II concentration. The reaction product, however, was not identified.

The assumed dimerization of II is complex in that oligomers higher than a dimer can form. Presumably, this reaction involves attack by the amidine group of one II molecule on the β-lactam group of another. Since the resulting dimer has one amidine group and one β-lactam group, it could react further; a trimer, tetramer, *etc.*, could form. However, solvent attack on the β-lactams of these oligomers would hinder their propagation.

Results of kinetic studies of condensation polymerization reactions show that the chemical reactivity of a functional group usually does not depend on the size of the molecule to which it is attached (6). At any stage of polymerization, the reactivity of every like functional group is the same. Furthermore, the rate constant for a given reaction step usually remains constant throughout polymerization. Consequently, polymerization can be regarded as a reaction between functional groups, of constant reactivity, without differentiation in regard to molecular size.

Accordingly, a second-order oligomerization was simulated in which all products had the reactivity of the monomeric starting material toward each other and toward unreacted monomer. The concentration dependence of the starting material on reaction time is shown in Fig. 4 with the

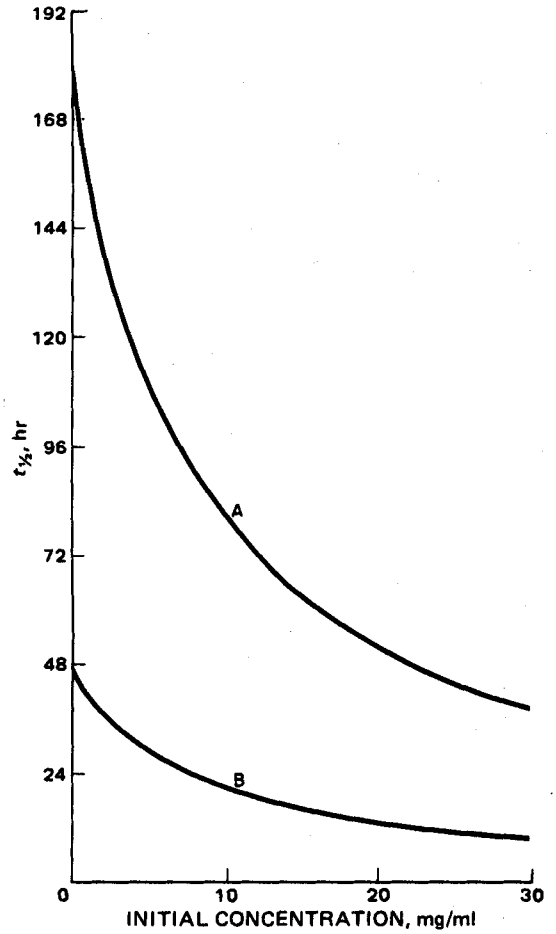


Figure 5—*Half-life at neutral pH* versus *initial II concentration. Key: A, reactions at 25° and pH 7.00; and B, reactions at 40° and pH 6.75.*

corresponding curve for a simple second-order dimerization.

The initial reaction rates are equal since only monomer initially is present in both reactions. The calculated effect of oligomers on the monomer is slight. For example, 53% of the monomer reacts in the complex reaction in the same time that 50% reacts in the simple reaction. Therefore, Eq. 2 is a good approximation.

The change of variable $y = 1/A$ reduces Eq. 2 to linear form:

$$dy - k_{pH}y\,dt = k_2\,dt \qquad \text{(Eq. 3)}$$

The integrating factor $e^{-k_{pH}t}$ affords integration to (7):

$$\left(\frac{1}{A} + \frac{k_2}{k_{pH}}\right) e^{-k_{pH}t} = \left(\frac{1}{A_0} + \frac{k_2}{k_{pH}}\right) \qquad \text{(Eq. 4)}$$

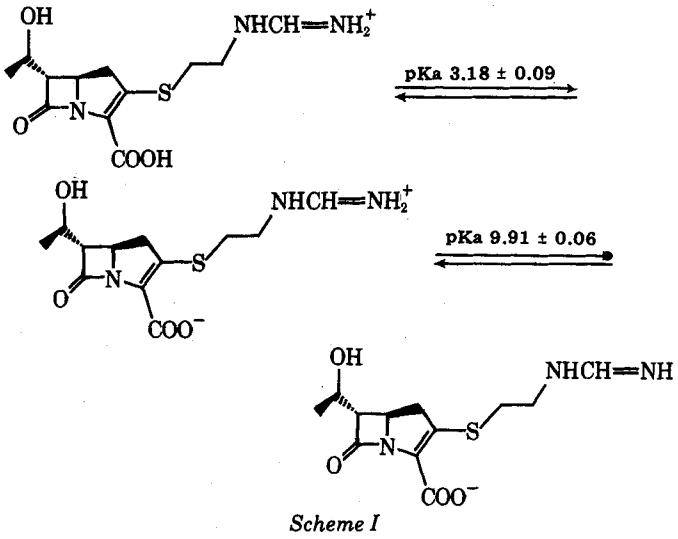
where A represents the II concentration and A_0 represents the initial concentration.

For each series of runs in which the initial II concentration was varied, the pseudo-first-order rate constant value, k_{pH} (observed at low initial concentration), was substituted in Eq. 4. Then the second-order rate constant value, k_2, was adjusted to fit the UV assay data.

The dimer, the first oligomerization product, predominated among the oligomers for several half-lives of the monomer in the simulated oligomerization with all rate constants being equal. As already mentioned, when two monomers react, one of the two β-lactams remains intact. Consequently, the rate measured by UV assay should be very nearly one-half of the actual rate. Therefore, the k_2 value was calculated as twice the value corresponding to the UV data.

The k_2 values so calculated were 0.2 M^{-1} hr^{-1} at pH 7.0 and 25° and 0.8 M^{-1} hr^{-1} at pH 6.75 and 40°. The corresponding Arrhenius activation energy was about 17 kcal/mole. The effect of the second-order reaction on II stability is shown by the plots of half-life *versus* initial concentration (Fig. 5).

Two more series of runs were performed similarly at 25° with initial II concentrations of 1.8–30 mg/ml in 1 *M* 2-(*N*-morpholino)ethanesulfonic acid (pH 6) and in 1 *M* 3-(*N*-morpholino)propanesulfonic acid (pH



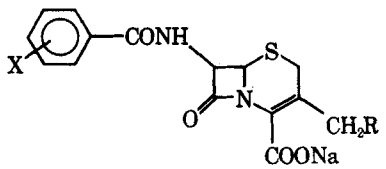
Scheme I

8). The second-order rate constant of ~0.2 M^{-1} hr^{-1}, the value found at pH 7 and 25°, also was found at pH 6 and 8. The product UV spectra of the runs at pH 6 were all similar to curve C in Fig. 1, and those at pH 8 were all similar to curve B in Fig. 1. (All UV spectra were measured at pH 7.) In the runs at high initial II concentration, these different products formed largely from the second-order reaction, whose rate was pH independent. This fact supports the earlier conclusion that rate-determining β-lactam opening, even in the absence of oxygen, precedes the rapid formation of several products whose composition is pH dependent (see *Reaction Products*).

Compound II exists in ionic equilibria (Scheme I) for which the pKa values, obtained by analyzing titration curves, are as shown. According to these pKa data, about 0.01, 0.1, and 1% of the amidine nitrogen are unprotonated at pH 6, 7, and 8, respectively. One might expect the sec-

Table III—Reactivities with Hydrogen Ion, Water, and Hydroxide Ion at 35°

β-Lactam Antibiotic	k_H, M^{-1} hr^{-1}	10^3k_0, hr^{-1}	$10^{-2}k_{OH}$, M^{-1} hr^{-1}
II	6500	10.1	77
Penicillins			
Penicillin G	601	0.90	11.9
Carbenicillin	52.2	2.04	12.1
Cloxacillin	35.6	0.94	13.4
Propicillin	30.7	0.89	17.3
Cyclacillin	4.61	2.49	11.0
Ampicillin	1.82	0.75	25.7
Cephalosporins			
Cephalothin	0.172	10.9	10.6
III*a*	0.238	21.0	9.08
III*b*	0.206	18.7	8.56
III*c*	0.186	11.4	8.45
III*d*	0.265	7.06	9.55
IV*a*	—	0.270	1.43
IV*b*	—	—	1.33
IV*c*	—	0.270	1.56
IV*d*	—	—	1.52



III*a*: R = $OCOCH_3$, X = 4-CH_3
III*b*: R = $OCOCH_3$, X = H
III*c*: R = $OCOCH_3$, X = 4-Cl
III*d*: R = $OCOCH_3$, X = 4-NO_2
IV*a*: R = H, X = 4-CH_3
IV*b*: R = H, X = H
IV*c*: R = H, X = 4-Cl
IV*d*: R = H, X = 4-NO_2

Table IV—Reactivities with Monobasic Phosphate Anion

β-Lactam Antibiotic	$k_{HPO_4^{2-}}$, M^{-1} hr^{-1}	Temperature	Reference
II	0.58	23°	—
Ampicillin	0.166	35°	11
Carbenicillin	0.064	35°	11
Cloxacillin	0.092	35°	11
Cyclacillin	0.355	35°	11
Methicillin	0.087	35°	11
	2.0	75°	12
Oxacillin	0.073	35°	11
Penicillin G	0.078	35°	11
	0.82	60°	13
Phenethicillin	0.094	35°	11
Propicillin	0.128	35°	11
Sulbenicillin	0.078	35°	11
6-(α-Toluenesulfonamido)-penicillanic acid	0.030	35°	11

ond-order reaction to involve attack by the nucleophilic unprotonated amidine nitrogen of one II molecule on the β-lactam of another. The fact that the second-order rate constant is pH independent rules out predominant attack by this nucleophile and is consistent with attack by the protonated amidine nitrogen. However, this fact allows the reactivity of the unprotonated amidine to be the greater. The apparent second-order rate still could be essentially independent of pH because of the relatively large amounts of protonated amidine present at pH 6–8.

Stability Comparison of II and Other β-Lactam Antibiotics—The stability of penicillins and cephalosporins in aqueous solution has been described extensively. Stabilities at 35° and $\mu = 0.5$ were summarized by Yamana and Tsuji (8) in terms of Eq. 1. The II reactivities are shown in Table III with some penicillin and cephalosporin data. (The reactivities of II at 25 and 40°, shown in Table II, were interpolated to 35° by means of the Arrhenius equation.)

The reactivities of II with hydrogen ion, water, and hydroxide ion are all significantly greater than those of penicillin G. The other penicillins listed in Table III have smaller k_H values than penicillin G since they are designed to avoid intramolecular rearrangement in acidic solution. Their reactivities with water and hydroxide ion are about the same as those of penicillin G.

Cephalothin and the other cephalosporins with the 3-acetoxymethylene group are less reactive with hydrogen ion than are the penicillins, but they exhibit reactivities with water comparable to that of II and with hydroxide ion comparable to those of the penicillins. The reactivities of these cephalosporins, however, are due mainly to the 3-substituent (for which II and the penicillins have no counterpart), as shown by the lower reactivities of the corresponding deacetoxycephalosporins.

Furthermore, II is more reactive with phosphate than the penicillins, and the cephalosporins are essentially unreactive with phosphate. Phosphate buffers have been used as inert media for rate studies of cephalosporins (9). Moreover, the apparent first-order rate constant for the degradation of cefoxitin at 25°, measured in this laboratory at pH 7 and $\mu = 0.5$ in agreement with the published value of 0.00246 hr^{-1} (10), was the same with nonnucleophilic 3-(*N*-morpholino)propanesulfonic acid buffers as with phosphate buffers of 0.01–0.25 *M*.

Table V—Σ_N and *D* Values Determined from X-Ray Crystallographic Data

Compound	Σ_N	*D*, Å	Reference
N-Acetylthienamycin methyl ester	325.9°	0.49	14
Penicillin V	337°	0.40	14
Ampicillin	339°	0.38	14
6-Aminopenicillanic acid	343°	0.32	14
Cephalosporin C	345°	0.32	15
Cephaloridine	350.7°	0.24	14
7-Phenylacetamido-7-methoxy-3-methyl-3-cephem-*tert*-butyl ester	356.7°	0.15	14
Δ^2-Cephem	359.3°	0.06	15

The reactivity of II with phosphate at 23° (Fig. 2) is compared with literature data for various penicillins at $\mu = 0.5$ and at 35° and higher temperatures in Table IV. In the present study, the reactivity of II with phosphate was measured only at pH 7.2 and 23°, using 0.50 *M* phosphate ($\mu = 1$) or 1.00 *M* phosphate ($\mu = 2$). The same rate constant was observed at the two phosphate concentrations. The reactivity of II with HPO_4^{2-} (Table IV) was calculated from the observed rates with the assumption that $H_2PO_4^-$ is inert or much less reactive than HPO_4^{2-}. (This assumption holds true for the penicillins, according to the references given in Table IV.)

The degradations of the penicillins and cephalosporins in aqueous solution are complex and, consequently, cannot be compared easily with the degradation of II in general terms. Nevertheless, the reactivities in Tables III and IV fall into three distinct groups. Compound II is more reactive than the penicillins which, in turn, are more reactive than the deacetoxycephalosporins. This distinction reflects the relative strains of the nuclei as shown by the X-ray crystallographic data in Table V. The table includes values for the sum of the bond angles about the β-lactam ring nitrogen, Σ_N, and the distance of the nitrogen atom from the plane of the three attached carbon atoms, *D*.

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