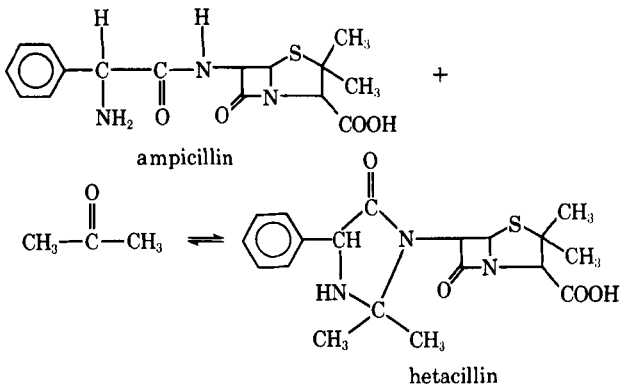
Relative Stability of Hetacillin and Ampicillin in Solution

MICHAEL A. SCHWARTZ[▲] and WILLIAM L. HAYTON*

Abstract □ Sodium ampicillin in solution loses almost 10% of its activity in 1 hr. at room temperature at a concentration of 250 mg./ml.; but at 30 mg./ml., it requires 8 hr. for the same loss of activity. Hetacillin, an acetone adduct of ampicillin, loses somewhat less than 10% activity in 6 hr. at a concentration of 250 mg./ml. under the same conditions. Hetacillin has been shown to convert to ampicillin with a half-life of only 20–30 min. at room temperature and, therefore, should lose more than 10% activity in 6 hr. An explanation of these inconsistent data is offered herein by adopting a model reaction scheme which incorporates the concentration dependence of both ampicillin degradation and the hetacillin–ampicillin equilibrium. The consistency of the model is shown using literature data, and the properties of the model are discussed.

Keyphrases □ Hetacillin solutions—stability dependence on hetacillin–ampicillin equilibrium and ampicillin degradation □ Ampicillin and hetacillin in solution—stability, equilibrium, ampicillin degradation

Hetacillin, a derivative of ampicillin, is prepared by treating the latter with excess acetone in an aqueous medium (1) (Scheme I). Both hetacillin and ampicillin are marketed as powders for reconstitution for parenteral use with a limited utilization time[1] because of relatively rapid degradation in solution. Table I indicates the utilization times specified by manufacturers (2, 3). Two apparent inconsistencies appear in these data: (*a*) ampicillin is much more stable in dilute solution than in concentrated solution, and (*b*) hetacillin, in concentrated solution, is much more stable than ampicillin. If hetacillin is rapidly converted to ampicillin, as has been shown (4, 5), with a half-life of 20–30 min. at room temperature, it would be expected that the rates of degradation of hetacillin and ampicillin would be much closer than indicated. The purpose of this paper is to offer an explanation of these data based on a proposed mechanism of hetacillin and ampicillin degradation.



Scheme I

Table I—Product Utilization Times

Drug	Concentration, mg./ml.	Utilization Time, hr.
Ampicillin	250	1
Ampicillin	30	8
Ampicillin	2	8
Hetacillin	250	6

THEORY

From the data in Table I, it appears that the rate of degradation of ampicillin in solution is highly dependent upon concentration. Additional, more direct evidence for a concentration dependence is available from a study of ampicillin stability in frozen solutions and at low temperature in the liquid state (6)[2].

A reasonable explanation for this concentration dependence may be formulated from the known formation of polymers from ampicillin in solution at pH 8–9 (sodium salt) (7). For the ampicillin polymers, Structure I was suggested by the data. This structure could arise from aminolysis of ampicillin at the β-lactam by the free amino group of a second molecule and the process continued. A rate law for overall degradation of ampicillin can be developed from the series of reactions shown in Scheme II, in which A represents ampicillin, A_2 a "dimer," A_3 a "trimer," *etc.* The rate law would be:

$$\frac{-dA}{dt} = k_1(A) + k_2(A)^2 + k_3(A_2)(A) + \ldots \qquad \text{(Eq. 1)}$$

This probably would be limited to the first two terms, especially in the initial phase of the reaction, since the concentration of A_2 would be very small. Thus, one would not expect first-order loss of ampicillin in solution at high concentrations where the k_2 term would be a major contributor, but one might expect to observe first-order loss at low concentrations where only the term in k_1 would be significant. This scheme would also explain the relatively rapid loss of ampicillin in frozen solutions (6) where there is effectively a high concentration of the drug.

With hetacillin, the equilibrium between the drug and ampicillin and acetone must be taken into account. There are two reports dealing with the rate of conversion of hetacillin to ampicillin (4, 5),

$$A \xrightarrow[k_1]{H_2O} \text{product}$$

$$A + A \xrightarrow{k_2} A_2$$

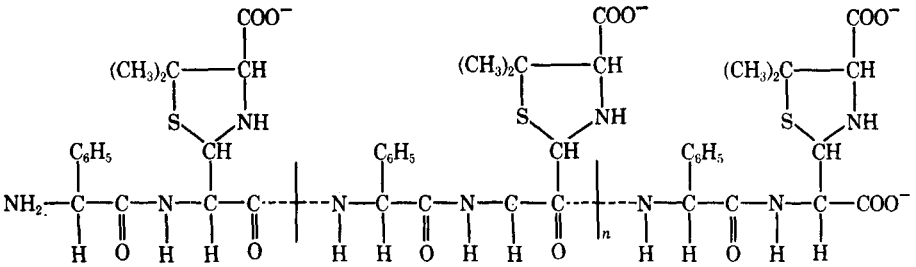
$$A_2 + A \xrightarrow{k_3} A_3$$

$$\ldots$$

Scheme II

[1] The term "utilization time" indicates the period after reconstitution during which there will be less than 10% loss in biological activity.

[2] The authors thank Mr. D. R. Savello and Dr. R. F. Shangraw for a prepublication copy of their paper.



I

and both were conducted at low initial concentrations of hetacillin. Smith and Hamilton-Miller (5) found that the β-lactam in hetacillin was not subject to nucleophilic attack by hydroxylamine; therefore, it appears that conversion to ampicillin is the only major route of hetacillin degradation. At the concentrations used in these studies, the equilibrium was apparently far to the right (more than 95% ampicillin) and the authors were justified in treating the reaction as virtually unidirectional and calculating first-order rate constants.

The overall model for hetacillin degradation appears to be as shown in Scheme III. where H represents hetacillin, A is ampicillin, B denotes acetone, and P_1 is the product of degradation of ampicillin at low concentration.

Test of Model—To test this model, it is necessary to show that when reasonable values are taken for the rate constants it is possible to produce a set of resulting data consistent with the known utilization times for these drugs. Values for the rate constants were obtained as follows.

k_1—Hou and Poole (8) studied rates of ampicillin degradation as a function of pH at low drug concentration (0.0073 M) where the squared term in Eq. 1 would not be significant. They found the specific alkaline hydrolysis rate constant to be 1945 M^{-1} hr.$^{-1}$ at 35° and determined the heat of activation to be 9.2 kcal./mole. Using these data, we estimated k_1 to be in the range 0.00325–0.013 hr.$^{-1}$ between pH 8.4 and 9.0. From the data of Savello and Shangraw (6), we calculated a rate constant of 0.00354 hr.$^{-1}$, which falls in the range expected, and decided to use this value in subsequent calculations.

k_2—From Eq. 1, in which only the first two terms were used, the following equation was derived:

$$\ln \frac{A(k_1 + k_2A_0)}{A_0(k_1 + k_2A)} = -k_1t \qquad \text{(Eq. 2)}$$

Substituting appropriate values for k_1, A_0, and A, we calculated a value of k_2 which would give both less than 10% loss in 1 hr. at A_0 = 0.717 M (250 mg./ml.) and less than 10% loss in 8 hr. at A_0 = 0.086 M (30 mg./ml.). The best value thus obtained was k_2 = 0.11 M^{-1} hr.$^{-1}$.

Determination of Rate Constants (k_f and k_r)—Examination of the data from the earlier studies (4, 5) of rate of conversion of hetacillin to ampicillin revealed that well over 90% of the hetacillin was converted to ampicillin. These studies were conducted at low concentrations (2–5 mg./ml.). To obtain reasonable values for k_f and k_r, we first assumed a value for the extent of the reaction. From this, values for k_f and k_r were calculated (9). These values were then combined with the values already obtained for k_1 and k_2 in order to calculate the time necessary for 10% loss. The latter calculations were carried out by numerical integration of the differential equations on the digital computer using the MIMED program (10). The results of these operations are depicted in Table II.

$$H \underset{k_r}{\overset{k_f}{\rightleftharpoons}} A + B$$

$$A \xrightarrow{k_1} P_1$$

$$A + A \xrightarrow{k_2} A_2$$

Scheme III

It seems clear from these data that to obtain a utilization time for the concentrated hetacillin solution of about 6 hr., the values k_f = 0.70 and k_r = 1.75 should apply; these were utilized in further computations.

Thus, a set of constants were obtained which, when used in the model, generate concentration–time patterns consistent with observations of stability of ampicillin and hetacillin. Table III summarizes the results.

DISCUSSION

The model presented here for ampicillin and hetacillin degradation is unique in that it embodies a combination of equilibrium and rate steps, both of which are concentration dependent. If the system were allowed to come to equilibrium without decomposition of ampicillin, the following would be observed. At an initial hetacillin concentration of 0.00515, 0.077, and 0.64 M, the percent ampicillin at equilibrium would be 98.7, 80.0, and 54, respectively. Thus, at higher concentrations there will be a slower net rate of conversion of hetacillin to ampicillin. Figures 1–3 show the concentrations of both hetacillin and ampicillin and the sum of the two (bioactivity) as a function of time at the concentrations of 0.64, 0.077, and 0.00515 M, respectively, as determined from the computer simulation. In each case, it can be seen that the system rapidly approaches a pseudoequilibrium state, after which loss of biological activity parallels the loss of ampicillin. Whereas at the two higher concentrations there is a significant steady-state concentration of hetacillin remaining, at the lowest concentration hetacillin approaches zero and the activity is essentially all due to ampicillin.

The latter system is essentially an example of a simple sequence of reactions with the second step being slower; thus we see little difference in rate between ampicillin and hetacillin at the very low concentration (Table III).

Table II—Calculated Rates and Equilibrium Constants

Percent Ampicillin at Equilibrium in Dilute Solution	k_f, hr.$^{-1}$	k_r, M^{-1} hr.$^{-1}$	K_{eq}, M	Time for 10% Loss of Activity[a], hr.
95	0.75	8.1	0.092	10.2
98	0.72	3.0	0.24	8.0
98.7	0.70	1.75	0.4	6.1

[a] Starting with 250 mg./ml. hetacillin and k_1 = 0.00354 hr.$^{-1}$, k_2 = 0.11 M^{-1} hr.$^{-1}$.

Table III—Summary of Simulated Results

Drug and Initial Concentration, mg./ml.	Initial Concentration, M	Time for 10% Loss of Activity, hr.
Ampicillin, 250	0.717	1.3
Ampicillin, 30	0.086	8.4
Ampicillin, 2	0.0057	25.5
Hetacillin, 250	0.646	6.1
Hetacillin, 30	0.077	13.2
Hetacillin, 2	0.00515	27.7

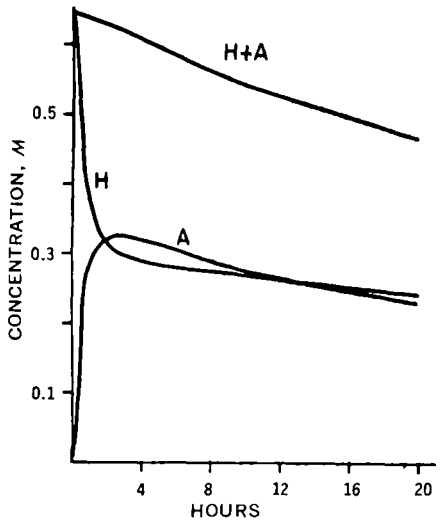


Figure 1—*Concentrations of ampicillin (A), hetacillin (H), and bioactivity (H + A) as a function of time obtained from computer simulation when H_0 = 0.646* M.

At the highest concentration (Fig. 1), there is an initial acceleration in rate of loss of biological activity which is due to the increasing predominance of the $k_2(A)^2$ term (Eq. 1) as the concentration of ampicillin builds up. The ampicillin concentration becomes maximal at only about 50% of the total initial hetacillin; thus the rate of loss is slower than would be expected when the initial ampicillin concentration is 0.717 *M*. The difference in rate is close to fourfold since the $k_2(A)^2$ term predominates.

At the intermediate concentration (Fig. 2), the pseudoequilibrium occurs when ampicillin is about 85% of the original hetacillin; thus the difference in rates is intermediate. At this concentration, both terms in Eq. 1 are significant. At the lowest concentration (Fig. 3), the apparent equilibrium occurs when hetacillin is very close to zero concentration and has essentially no effect on the rate of loss of activity.

Figure 4 shows semilog plots of the computer-simulated biological activity as a function of time at the various initial hetacillin concentrations. While at the lowest concentration the loss of biological activity is close to first order, there is considerable curvature at

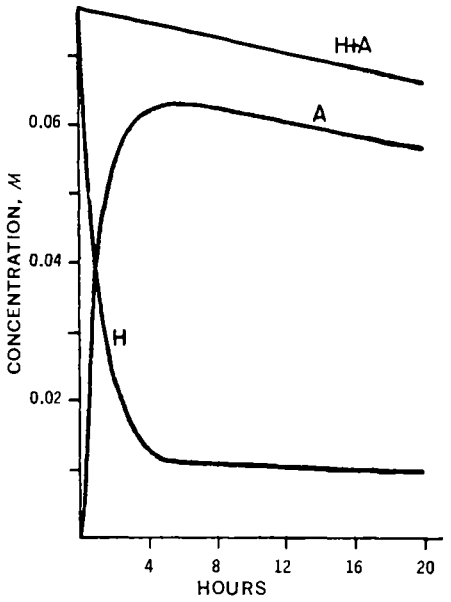


Figure 2—*Concentrations of ampicillin (A), hetacillin (H), and bioactivity (H + A) as a function of time obtained from computer simulation when H_0 = 0.077* M.

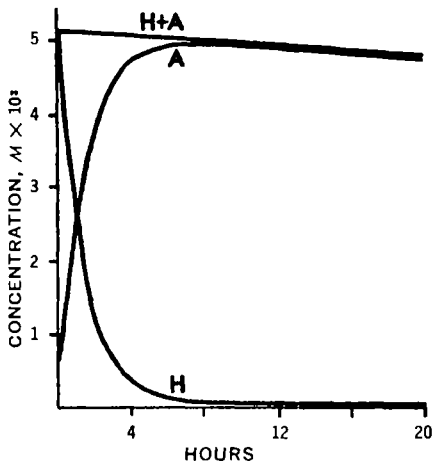


Figure 3—*Concentrations of ampicillin (A), hetacillin (H), and bioactivity (H + A) as a function of time obtained from computer simulation when H_0 = 0.00515* M.

the higher concentrations, reflecting the second-order term in the rate law.

From the curve for the 0.646 *M* hetacillin solution, it can be observed that the rate is slower after 80% loss than for the initial stages of the curve for the 0.077 *M* solution. This may be explained by consideration of the equilibrium. The remaining residual acetone, which becomes in excess as ampicillin degrades, effectively shifts the equilibrium to the left and thus slows the loss of activity. In contrast, as shown in Fig. 5 which depicts the computer-simulated loss of ampicillin from solutions on a semilog plot, the rate is only a function of drug concentration at any given moment in time.

Sufficient data were not available to determine temperature effects on rate, but it is apparent that a simple relationship will not hold, especially at the higher concentrations. For purposes of prediction of stability, account must be taken of the effect of temperature on all rate constants.

Finally, it should be noted again that the data utilized in this treatment are approximations and were employed only to illustrate

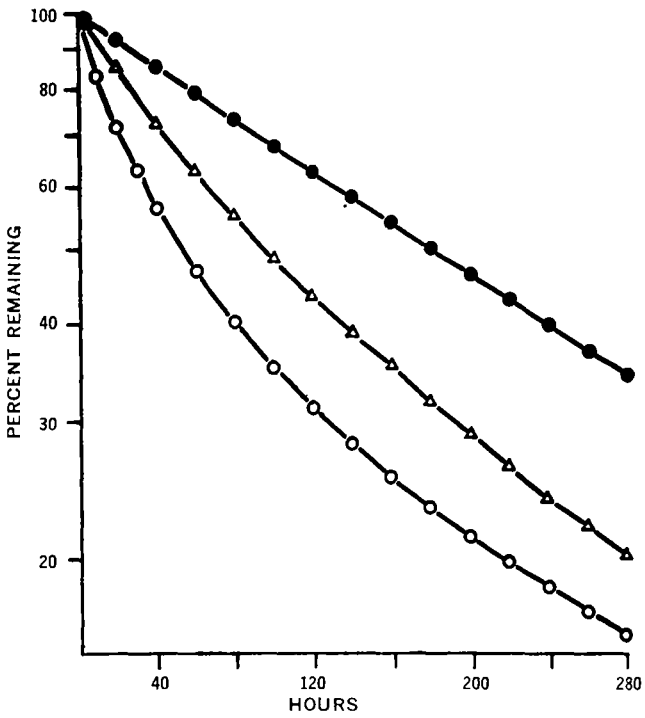


Figure 4—*Plot of log bioactivity* versus *time obtained from computer simulation for various initial concentrations of hetacillin. Key:* ○, H_0 = 0.646 M; △, H_0 = 0.077 M; *and* ●, H_0 = 0.00515 M.

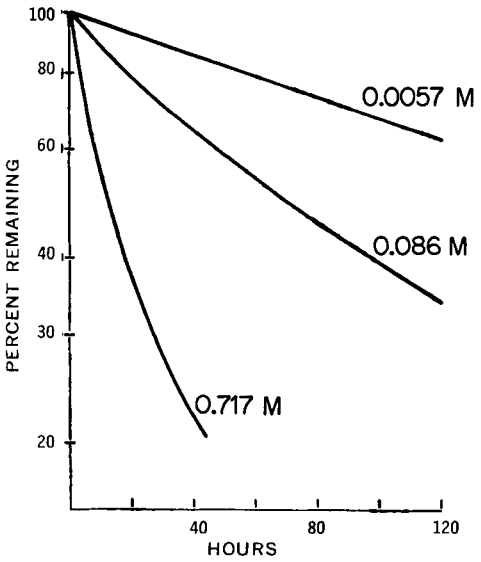


Figure 5—*Plot of log bioactivity* versus *time obtained from computer simulation for various initial concentrations of ampicillin.*

the model. The kinetic model seems sufficient to explain presently available results but awaits experimental confirmation, as do the actual values for the rate constants.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 2, 1971, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication February 17, 1972.

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Molecular Orbital Calculations on Some Nitrogen Derivatives of Conjugated Hydrocarbons: Base Strength of Benzacridines and Their Amino Derivatives

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Abstract □ The relations which exist between the electronic structure and base strength of benzacridines and their amino derivatives were investigated using semiempirical molecular orbital calculations. The calculations are complicated by a number of factors which affect equilibria in solution. The results indicate that the energy to protonate a nitrogen derivative of a conjugated hydrocarbon may be divided into the terms ΔE_σ and ΔE_π for changes in localized and delocalized electron energies, respectively, an energy term for solvation, and a term for steric hindrance to protonation. When the term ΔE_π alone is used to determine the pKa values, it yields a linear relationship within each family of derivatives. The term ΔE_{solv} appears to depend primarily on the size of the molecule and may be calculated by the use of a modified Born equation. The combination of ΔE_π and ΔE_{solv}, as a representation of ΔE, yields a single relationship when plotted against pKa for a number of benzacridines and their amino derivatives as well as the derivatives of pyridine, isoquinoline, quinoline, and acridine, which were reported previously. The deviation from this relationship for some of the compounds appears to be due to structural factors which depend on the ΔE_{ster} and ΔE_σ terms.

Keyphrases □ Benzacridines and amino derivatives—relationship between electronic structure and base strength, molecular orbital calculations □ Base strength of benzacridines and amino derivatives—relationship to electronic structure, molecular orbital calculations □ Molecular orbital calculations—used to determine the relationship between electronic structure and base strength of benzacridines and amino derivatives

The relationships which exist between the electronic structure and the base strength of organic molecules were studied by a number of investigators, and reference to several of their reports was made by Peradejordi (1).

THEORETICAL

The equilibrium reaction occurring in solution between a neutral organic base B and its positively charged acid BH^+, which is said to be conjugated to the base, may be written as Scheme I. The con-