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Research Article

Potential Improvement in the Shelf Life of Parenterals Using the Prodrug Approach: Bacampicillin and Talampicillin Hydrolysis Kinetics and Utilization Time

Ngoc-Anh T. Nguyen,[1] Lobna M. Mortada,[2] and Robert E. Notari[3,4]

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The utilization time for a parenteral prodrug solution with a bioavailable fraction of unity was defined as the time during which the *total* of the prodrug concentration and the drug concentration equals or exceeds 90% of the initial prodrug concentration. This utilization time was calculated as a function of pH, buffer, and temperature using the experimentally determined rate expressions for bacampicillin and talampicillin. The results were compared to the shelf life of ampicillin solutions under identical storage conditions. First-order rate constants were determined for conversion of the prodrugs to ampicillin (k_c), for β-lactam degradation of the prodrugs (k_{nc}), for the overall loss of prodrugs (k_{sum}), and for β-lactam degradation of ampicillin (k_h) in aqueous solutions at 25.0 to 60.0°C, $\mu = 0.5$, in the pH range 0.90 to 8.4. Loss of bacampicillin proceeded primarily by degradation at pH levels below 4 but was due predominantly to conversion at pH levels above 5. Loss of talampicillin was due primarily to conversion throughout the entire pH range. While the prodrug utilization times were approximately twice the shelf life of ampicillin in acidic solutions, ampicillin was significantly better in neutral solutions. The results illustrate the potential for increased prodrug storage periods when utilization time is defined on the basis of the bioactivity rather than on the prodrug concentration alone.

KEY WORDS: bacampicillin; talampicillin; ampicillin; stability; prodrugs; shelf life; hydrolysis; chemical kinetics; pH profiles; storage; parenteral; degradation kinetics; utilization time.

INTRODUCTION

The expiration date for a drug in solution is conventionally based on the time during which the formulation maintains 90% of its labeled concentration (T_{90}). The application of this criterion to a prodrug solution may underestimate the time during which the formulation maintains 90% of its bioactivity. Consider, for example, an intravenous solution of a prodrug which is rapidly and completely bioconverted to drug in the blood. Using the T_{90} convention, the solution would expire if 10% of the prodrug hydrolyzed to the drug during storage. However, if drug degradation is neglible, intravenous administration of this expired solution would provide a drug plasma concentration time course that is bioequivalent to the original solution. Schwartz and Hayton (1) have defined the true utilization time (UT) as the time during which the *total* concentration of prodrug and drug equals or exceeds 90% of the original prodrug concentration. The current paper is the first report which examines how the use of that UT definition can influence the prodrug pH–shelf-life profiles.

In the above example, the hypothetical prodrug hydrolyzed exclusively to drug without degrading to inactive products. In practice, the degree to which the T_{90} underestimates the UT for such a solution depends upon the rate of prodrug conversion to drug relative to the competing rates for degradation to inactive products during storage. These processes are illustrated in Scheme I using two ampicillin prodrugs where the rate constant, k_c, represents conversion to ampicillin; k_{nc} and k_h represent β-lactam hydrolysis to inactive products.

These ampicillin esters, bacampicillin and talampicillin (Scheme I), were studied as model prodrugs for two reasons. (i) Based on the *in vitro* reactivity of other penicillin prodrugs (2–4), these esters were expected to reduce the β-lactam hydrolysis rate (k_{nc}) in acid and increase this rate in base while simultaneously undergoing conversion to ampicillin (k_c). Thus, UT can be assessed under both favorable and adverse conditions. (ii) These two prodrugs undergo such rapid and complete bioconversion to ampicillin in the blood (5–9) that intravenous prodrug solutions would be bioequivalent to solutions of ampicillin itself.

In vitro kinetic studies on the hydrolysis of bacampicillin and talampicillin are limited (5,9,10). In this report, the kinetics of hydrolysis of these prodrugs and ampicillin were studied as a function of pH, temperature, and buffer composition. These data were then used to calculate prodrug UT values based on the *total* concentration of the prodrug and ampicillin remaining as a function of time. Subsequently,

[1] Present address: Pharmaceutical Development, Glaxo Inc., Research Triangle Park, North Carolina 27709.

[2] Present address: Department of Pharmaceutics, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

[3] Lloyd M. Parks Hall, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

[4] To whom correspondence should be addressed.

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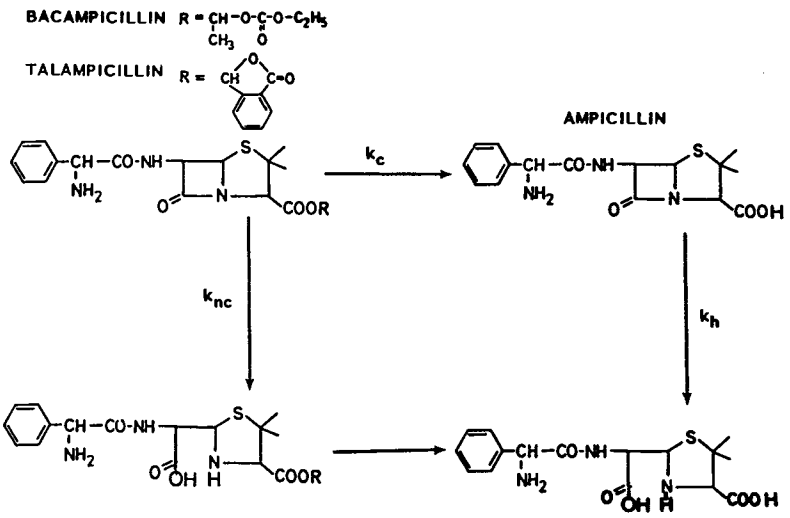

Scheme I

these UT values were compared to the T_{90} values calculated from the concentration of prodrug alone. The potential for gaining a storage advantage by using a solution containing prodrug in place of ampicillin was also examined by comparing the prodrug UT values to the ampicillin T_{90} values.

The specific aims of this research were:

- to determine the kinetics for degradation and conversion of these selected prodrugs as a function of pH, buffer, and temperature;
- to determine the kinetics of ampicillin degradation under identical experimental conditions;
- to calculate the UT for these prodrug solutions as a function of pH and temperature using the experimentally determined rate expressions;
- to compare the prodrug UT versus pH profiles to the prodrug T_{90} versus pH profiles and also to the ampicillin T_{90} versus pH profiles;
- to quantify the influence of the rates in Scheme I on the extent of the observed differences between a prodrug UT and a prodrug T_{90} and between a prodrug UT and an ampicillin T_{90} under identical conditions; and
- to provide the experimental basis for a subsequent paper on the kinetic requirements for optimizing prodrug UT.

MATERIALS AND METHODS

Chemicals. All chemicals and solvents were reagent or high-performance liquid chromatographic (HPLC) grade except the prodrugs and ampicillin, which were used as received from suppliers: bacampicillin (Pfizer), talampicillin (Yamanouchi Pharmaceutical, Tokyo), and ampicillin (Wyeth).

Kinetics. Reaction conditions are described in Tables I–III. The ionic strength (μ) was maintained at 0.5 using NaCl and the pH values were measured at reaction temperatures. Samples (~0.8 ml) were removed as a function of time from the constant-temperature reactions, then cooled, and 0.5-ml aliquots were diluted 10-fold to a final pH of 5–6 and refrigerated to quench the reaction. No loss of bacampicillin or ampicillin was observed after refrigeration for 4 days. Talampicillin dilutions were assayed on the same day as the kinetic experiment without loss of prodrug during storage and assay.

HPLC Analysis. Convenient retention times could be attained only by using separate HPLC conditions for the prodrugs and ampicillin (11). The isocratic assays were carried out at a flow rate of 1 ml/min using a liquid chromatograph system (Model 332, Beckman Instruments, Inc.) equipped with an Altex 20-μl injection loop, reverse-phase columns for bacampicillin and ampicillin (Altex Ultrasphere-ODS, 5 μm, 4.6 × 250 mm) and for talampicillin (μ-Bondapak C18, Waters Associates), together with a UV detection wavelength of 220 nm (Model 1040A, Hewlett Packard) and a recorder (Model 3090A, Hewlett Packard). The mobile phases contained aqueous 0.005 *M* NaH_2PO_4 and 0.001 *M* Na_2HPO_4 with an acetonitrile concentration of 60% (v/v) for bacampicillin, 52% (v/v) for talampicillin, and 12% (v/v) for ampicillin. The aqueous phase was filtered through a type HA 0.45-μm membrane filter (Millipore Corp.), degassed under vacuum, mixed with the acetonitrile, and deaerated by sonication. Following assays for ampicillin in mixtures, the columns were cleaned of bacampicillin or talampicillin by elution with 60% (v/v) acetonitrile. The retention times, detection ranges, and coefficients of variation (%CV) were as follows: ampicillin—7.5 min, (0.10–10.0) × 10^{-5} *M*, and 4%; bacampicillin—7.0 min, (1.0–10.0) × 10^{-5} *M*, and 2.6%; and talampicillin—8.1 min, (0.25–5.0) × 10^{-5} *M*, and 1.5%.

RESULTS

Kinetics of Transformations. Ampicillin degradation in aqueous solution proceeds through two competing rate processes, hydrolysis and polymerization. The balance depends upon the ampicillin concentration and the pH of the solution. Hou and Poole (12) studied the hydrolysis of ~7.3 × 10^{-3} *M* ampicillin. Bundgaard (13) reported simultaneous hydrolysis and polymerization in concentrated solutions (0.054–0.673 *M*) in the pH range 8.1 to 9.1. At a constant ampicillin concentration, polymerization decreased with increasing pH values. At 0.054 *M*, for example, polymerization decreased from 59 to 13% during an increase in pH from 8.1 to 9.1. At a constant pH, polymerization increased with the

Table I. Experimental Conditions and First-Order Rate Constants (min^{-1}) for Overall Loss of Bacampicillin (k_{sum}), Conversion to Ampicillin (k_c), and Hydrolysis of Ampicillin (k_h)[a]

°C	pH	Buffer concentration (M)			10^3k_{sum}	10^3k_c	10^3k_h
		HCOOH	HCOONa	NaCl			
50.5	2.62	0.800	0.100	0.40	1.71	1.59	12.8
	(±0.02)	0.600	0.075	0.44	1.33	1.46	9.73
		0.400	0.050	0.45	1.19	1.16	7.12
		0.200	0.025	0.48	0.715	0.367	4.29
		CH_3COOH	CH_3COONa	NaCl			
35.0	3.52	0.384	0.0384	0.46	0.203	0.0301	—
	(±0.02)	0.288	0.0288	0.47	0.188	0.0310	0.326
		0.192	0.0192	0.48	—	—	0.251
		0.096	0.0096	0.49	0.148	0.0340	0.173
50.5	3.52	0.384	0.0384	0.46	0.797	0.148	1.61
	(±0.02)	0.288	0.0288	0.47	0.670	0.119	1.29
		0.192	0.0192	0.48	0.602	0.0825	1.06
		0.096	0.0096	0.49	0.515	0.0566	0.760
	4.64	0.400	0.400	0.10	2.38	0.586	1.53
	(±0.04)	0.300	0.300	0.20	—	—	1.21
		0.200	0.200	0.30	1.50	0.272	0.850
		0.100	0.100	0.40	0.936	0.160	0.500
	5.64	0.040	0.400	0.10	2.35	0.682	0.320
	(±0.05)	0.030	0.300	0.20	2.09	0.607	0.270
		0.020	0.200	0.30	1.57	0.462	0.210
		0.010	0.100	0.40	1.09	0.366	0.170
60.0	3.52	0.384	0.0384	0.46	1.65	0.388	—
	(±0.02)	0.288	0.0288	0.47	1.54	0.340	3.21
		0.192	0.0192	0.48	—	—	2.60
		0.096	0.0096	0.49	1.10	0.204	1.92
	5.64	0.040	0.400	0.10	—	—	0.810
	(±0.04)	0.030	0.300	0.20	—	—	0.720
		0.020	0.200	0.30	—	—	0.570
		0.010	0.100	0.40	—	—	0.400
		NaH_2PO_4	Na_2HPO_4	NaCl			
35.0	7.52	0.0150	0.120	0.12	8.26	2.62	0.390
	(±0.04)	0.0096	0.077	0.26	5.91	2.39	0.260
		0.0048	0.038	0.38	3.48	1.44	0.170
50.5	6.56	0.058	0.058	0.27	12.0	2.15	0.900
	(±0.04)	0.048	0.048	0.31	10.4	1.91	0.750
		0.029	0.029	0.38	6.87	1.65	0.510
		0.019	0.019	0.42	4.91	1.42	0.390
	7.52	0.0150	0.120	0.12	33.1	12.9	1.45
	(±0.04)	0.0096	0.077	0.26	26.4	10.1	1.02
		0.0072	0.058	0.32	19.9	8.77	0.860
		0.0048	0.038	0.38	—	—	0.600
		0.0024	0.0192	0.44	12.8	6.34	0.530
60.0	7.52	0.0150	0.120	0.12	92.5	33.3	3.60
	(±0.06)	0.0096	0.077	0.26	—	—	2.63
		0.0072	0.058	0.32	56.5	25.2	2.28
		0.0048	0.038	0.38	47.8	23.5	1.90
		Boric acid	Sodium borate	NaCl			
25.0	8.40	0.100	0.0125	0.49	6.29	3.37	0.106
	(±0.05)	0.0693	0.00867	0.49	6.29	4.10	0.099
		0.0500	0.00625	0.49	6.28	4.23	—
35.0	8.40	0.100	0.0125	0.49	19.3	12.7	0.370
	(±0.05)	0.0693	0.00867	0.49	16.1	11.5	0.346
		0.0500	0.00625	0.49	14.0	10.1	0.351
50.5	8.40	0.100	0.0125	0.49	84.0	47.2	1.93
	(±0.05)	0.0693	0.0087	0.49	76.4	47.6	1.62
		0.0500	0.0062	0.49	74.0	47.7	1.50

[a] All initial concentrations were 1.0×10^{-3} M except for bacampicillin (0.5×10^{-3} M) at pH 7.52 and 8.40.

Table II. First-Order Rate Constants (min^{-1}) for Overall Loss of Bacampicillin (k_{sum}), Conversion to Ampicillin (k_c), and Ampicillin Hydrolysis (k_h) in HCl and in the pH Range 2.60–8.40[a]

°C	HCl (*M*)	pH	10^3k_{sum}	10^3k_c	10^3k_h
35.0	0.15	0.89	1.36	0.309	3.32,3.60[b]
	0.10	1.04	1.05	0.141	—
	0.06	1.18	0.630	0.0986	—
	0.04	1.38	0.480	0.0443	1.30, 1.36[b]
50.5	0.15	0.89	4.84	0.991	14.1
	0.10	1.04	4.06	0.345	9.41
	0.06	1.18	2.35	—	6.48
	0.04	1.38	1.86	0.260	4.76
	0.01	1.98	0.710	0.0662	2.42
60.0	0.15	0.89	11.5	3.12	32.4
	0.10	1.04	9.54	0.895	21.0
	0.06	1.18	5.72	—	13.7
	0.04	1.38	3.75	0.556	11.3
35.0		3.52	0.129	0.0353	0.0978
		7.52	1.31	1.03	0.0646
		8.40	8.80	7.72	0.356
50.5		2.62	0.458	0.152	1.45
		3.52	0.418	0.024	0.434
		4.64	0.493	—	0.156
		5.64	0.596	0.237	0.115
		6.56	1.48	1.10	0.130
		7.52	9.00	5.08	0.240
		8.40	63.5	47.5	1.05
60.0		3.52	0.880	0.143	1.27
		5.64	—	—	0.248
		7.52	25.7	18.5	1.08
25.0		8.40	6.29	5.19	0.103

[a] Based on intercept values for k_{sum}, k_c, or k_h vs buffer concentration. Initial concentrations were 1.0×10^{-3} *M* except for bacampicillin (0.5×10^{-3} *M*) at pH 7.52 and 8.40. [b] Reported by Hou and Poole (12).

ampicillin concentration. When the concentration was increased from 0.054 to 0.673 *M* at pH 9.1, polymerization increased from 13 to 85%. Equations reported by Bundgaard (13) indicate that polymerization would be insignificant and that ampicillin loss can be attributed to hydrolysis under the present conditions. While the prodrug degradation products have not been elucidated here or elsewhere (10), β-lactam hydrolysis is the expected route for competing loss.

Determination of Rate Constants. At a constant pH, in buffers or dilute hydrochloric acid, loss from a solution of ampicillin or a prodrug was described by $-dC/dt = kt$, which integrates to

$$\mathrm{Ln}[C]_t = \mathrm{Ln}[C]_o - kt \quad (1)$$

where $[C]_o$ is the initial concentration of ampicillin or prodrug, $k = k_h$ for ampicillin, and $k = k_{sum} = k_{nc} + k_c$ for prodrug. The apparent first-order rate constants obtained from linear plots based on Eq. (1) are reported in Tables I–III.

In accordance with Scheme I, the concentration of the prodrug [PD] and drug [D] as a function of time, *t*, may be written

$$[PD]_t = [PD]_0 e^{-(k_{nc} + k_c)t} \quad (2)$$

$$[D]_t = \frac{[PD]_0 k_c}{[k_h - (k_{nc} + k_c)]} [e^{-(k_{nc} + k_c)t} - e^{(-k_h)t}] \quad (3)$$

where $[PD]_0$ is the initial prodrug concentration.

Based on Eqs. (2) and (3), simultaneous nonlinear regression (14) was applied to the ampicillin and prodrug concentration time course data to estimate k_{nc} and k_c, while k_h was independently determined from Eq. (1) using data for ampicillin degradation under identical conditions (Fig. 1). The k_c values were verified using a published method (15), whenever reliable estimates for the area under the curve for formation and loss of ampicillin from $t = 0$ to ∞ could be obtained.

pH–Rate Profiles. The apparent first-order rate constants in the absence of general acid–base catalysis were used to construct pH–rate profiles for prodrug conversion (k_c), prodrug degradation (k_{nc}), overall loss of prodrug (k_{sum}), and hydrolysis of ampicillin (k_h). The rate constants were obtained either in dilute hydrochloric acid or from the intercepts of plots for *k* versus buffer concentration at a constant pH. When values were too small for reliable intercept estimates, k_{nc} was determined from the difference between the intercept values for the overall rate constant and the conversion constant, $k_{sum} - k_c$. In each case, rate constants were defined in terms of pH-dependent expressions containing pH-independent rate constants which were estimated using nonlinear regression.

The equation reported by Hou and Poole (12) was employed to describe ampicillin hydrolysis as a function of pH

$$k_h = k_{H1}a_H f_{1d} + k_{H2}a_H f_{2d} + k_s(f_{2d} + f_{3d}) + k_{OH}(K_w/a_H)f_{3d} \quad (4)$$

where a_H is the hydrogen ion activity; K_w is the autoprotolytic constant for water at the reaction temperature; k_{H1} and k_{H2} are catalytic constants for hydrogen ion; k_{OH} is the catalytic constant for hydroxide ion; k_s is the first-order rate constant for spontaneous solvolysis of the zwitterionic and anionic species; and f_{1d}, f_{2d}, and f_{3d} are the cationic, zwitterionic, and anionic fractions calculated from the dissociation constants, K_1 and K_2, reported for ampicillin (16).

The pH–rate profiles for loss of bacampicillin and talampicillin were described by

$$k_{sum} = k_H a_H f_{1p} + k_s(f_{1p} + f_{2p}) + k_{OH1}(K_w/a_H)f_{1p} + k_{OH2}(K_w/a_H)f_{2p} \quad (5)$$

where k_{OH1} and k_{OH2} are catalytic rate constants for hydroxide ion, k_H is the catalytic constant for hydrogen ion, k_s is the first-order rate constant for spontaneous solvolysis, and f_{1p} and f_{2p} are the fractions of protonated and unprotonated prodrug. These fractions were calculated from the dissociation constants for bacampicillin determined from partial potentiometric titrations owing to alkaline instability ($\mu = 0.5$): 25°C, $pK_a = 7.20$; 35°C, $pK_a = 6.91$; 50.5°C, $pK_a = 6.58$; and 60°C, $pK_a = 6.35$. Talampicillin could not be titrated due to extreme alkaline instability. Its pH–rate profile data were adequately described by assigning a pK_a value equal to that for the pK_2 of ampicillin. The catalytic rate constants which produced the curves representing k_h and

Table III. First-Order Rate Constants (min^{-1}) for Overall Loss of Talampicillin (k_{sum}) and Conversion to Ampicillin (k_c)

°C	pH	Concentration (M)			10^3k_{sum}	10^3k_c
		HCl	NaCl			
50.5	0.89	0.15	0.35		8.46	5.53
	1.18	0.06	0.44		4.68	2.72
	1.38	0.04	0.46		3.94	2.32
	1.68	0.02	0.48		3.84	2.31
	1.98	0.01	0.49		3.09	2.27
		CH_3COOH	CH_3COONa	NaCl		
50.5	3.52	0.288	0.0288	0.47	4.08	3.01
	(±0.01)	0.19	0.019	0.48	3.62	2.49
		0.096	0.0096	0.49	2.98	2.21
		0.038	0.0038	0.50	2.75	1.96
			Intercept[a]		2.51	1.79
50.5	4.64	0.400	0.400	0.10	20.0	16.2
	(±0.04)	0.300	0.300	0.20	16.3	12.6
		0.200	0.200	0.30	11.2	8.64
		0.100	0.100	0.40	7.33	5.79
			Intercept[a]		2.93	2.01
50.5	5.67	0.040	0.400	0.10	24.3	16.8
	(±0.03)	0.030	0.300	0.20	20.4	14.5
		0.020	0.200	0.30	14.9	10.8
		0.010	0.100	0.40	9.95	7.75
			Intercept[a]		5.24	3.75
		NaH_2PO_4	Na_2HPO_4	NaCl		
50.5	6.56	0.058	0.058	0.27	51.1	38.8
	(±0.01)	0.048	0.048	0.31	48.6	37.1
		0.029	0.029	0.38	35.9	29.0
		0.019	0.019	0.42	32.7	27.3
			Intercept[a]		24.7	20.7
50.5	7.52[b]	0.0096	0.077	0.26	212	195
	(±0.05)	0.0072	0.058	0.32	180	158
		0.0048	0.0385	0.38	158	138
		0.0024	0.019	0.44	124	114
			Intercept[a]		97.9	86.1
		HCl	NaCl			
25.0	0.89	0.15	0.35		1.27	0.350
		CH_3COOH	CH_3COONa	NaCl		
25.0	3.53	0.288	0.0288	0.47	0.726	0.462
	(±0.01)	0.192	0.0192	0.48	0.635	0.455
		0.038	0.0038	0.49	0.507	0.414
			Intercept[a]		0.473	0.409
		NaH_2PO_4	Na_2HPO_4	NaCl		
25.0	7.52[b]	0.0096	0.077	0.26	14.5	11.2
	(±0.05)	0.0072	0.058	0.32	14.0	9.61
		0.0048	0.038	0.38	13.4	11.4
			Intercept[a]		12.3	10.6[c]

[a] Obtained from plots of k_{sum} or k_c versus total buffer concentration.
[b] Initial concentration was 2.5×10^{-4}; all others were 5.0×10^{-4} M.
[c] Average of the k_c values listed.

Table IV. Catalytic Constants and Energetics for Overall Loss (k_{sum})[a] of Talampicillin and Bacampicillin and for Hydrolysis of Ampicillin (k_h)[b,c]

	10^2k_{H1}	k_{H2}	10^4k_s	$10^{-3}k_{OH1}$	$10^{-3}k_{OH2}$
Ampicillin					
35.0°C	1.69	0.336	0.116	0.0414	
50.5°C	7.23	1.02	1.15	0.0709	
60.0°C	13.6	3.05	2.90	0.235	
E_a (kcal/mol-deg)	17.1	17.6	26.5	13.4	
ln$A$[d]	24.1	27.7	32.2	25.6	
Bacampicillin					
35.0°C	8.88		1.24	1.95	1.59
50.5°C	33.4		4.14	6.66	4.51
60.0°C	79.4		8.38	10.5	7.52
E_a (kcal/mol-deg)	17.8		15.5	13.9	12.7
ln$A$[d]	24.4		16.5	30.4	28.3
Talampicillin					
50.5°C	3.66		25.7	140.	441.

[a] Defined by Eq. (5).
[b] Defined by Eq. (4).
[c] Units are M^{-1} min^{-1} except k_s = min^{-1}.
[d] Units for A = M^{-1} min^{-1}.

k_{sum} in Eqs. (4) and (5) as shown in Figs. 2, 3, and 4 are listed in Table IV.

Values for k_c as a function of pH in the absence of general acid–base catalysis are listed in Tables II and III. The pH–rate profiles for conversion were described by the following equation:

$$k_c = k_H a_H f_{1p} + k_s(f_{1p} + f_{2p}) + k_{OH1}(K_w/a_H)f_{1p} + k_{OH2}(K_w/a_H)f_{2p} \quad (6)$$

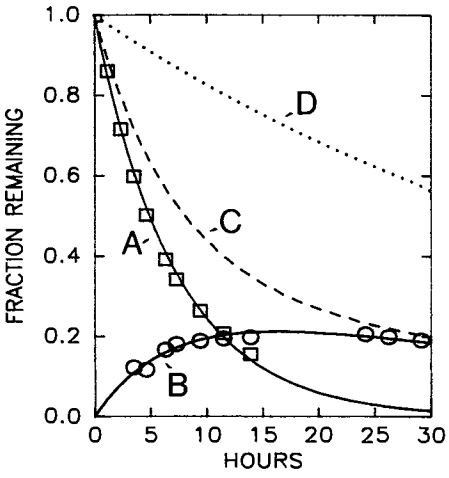

Fig. 1. The loss of bacampicillin (□) and formation of ampicillin (○) in 0.44 M acetate buffer at pH 5.64, μ = 0.5, 50.5°C, plotted as the fraction of the initial bacampicillin concentration as a function of time. Curves A and B, joining the data points, are computer fits based on Eqs. (2) and (3). Curve C represents the *total* of bacampicillin and ampicillin. Curve D is the independently observed loss of ampicillin under identical conditions.

The pH–rate profile for non-drug-forming degradation of prodrug was described by

$$k_{nc} = k_H a_H f_{1p} + k_s(f_{1p} + f_{2p}) + k_{OH}(K_w/a_H)f_{2p} \quad (7)$$

The components of Eqs. (6) and (7) are defined under Eq. (5). Values for the individual catalytic rate constants defining k_c and k_{nc} in Eqs. (6) and (7) are listed in Table V. The curves representing k_c and k_{nc} as a function of pH are shown in Figs. 4 and 5.

Temperature Dependence. Linear plots of k_T versus $1/T$, based on the Arrhenius relationship

$$\ln k_T = \ln A - E_a/RT \quad (8)$$

where R = 1.987 kcal/mol-deg and T is the absolute temperature, were used to determine the activation energies (E_a) and preexponential constants (A) for the first- and second-

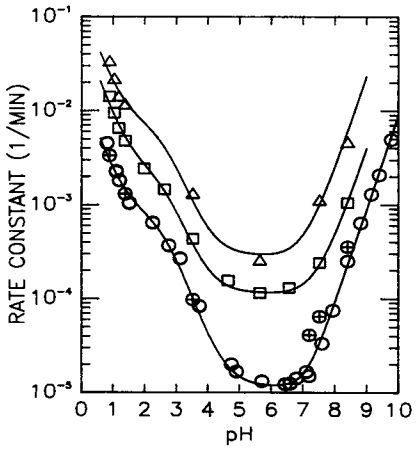

Fig. 2. The pH–rate profiles for the observed first-order hydrolysis (k_h) of ampicillin in the absence of general acid–base catalysis, μ = 0.5, at 60°C (△), 50.5°C (□), and 35°C using observed (⊕) and reported (○) values (12). The curves are calculated using Eq. (4).

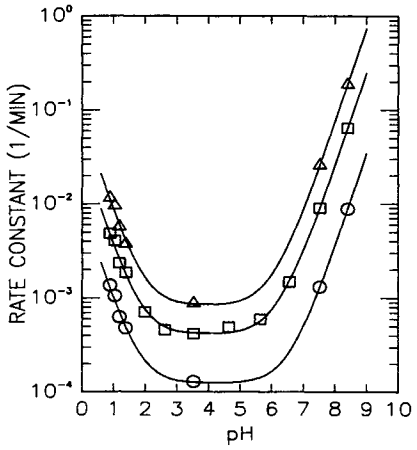

Fig. 3. The pH–rate profiles for the observed first-order loss (k_{sum}) of bacampicillin in the absence of general acid–base catalysis, μ = 0.5, at 60°C (△), 50.5°C (□), and 35°C (○). The curves are calculated using Eq. (5).

order catalytic rate constants, k_T, as a function of temperature (Table IV).

DISCUSSION

Kinetic Scheme. Although the *in vivo* conversion of bacampicillin and talampicillin proceeded through a stepwise process to form ampicillin (17–19), there was no evidence for accumulation of intermediates in the present study if, indeed, they were formed. The observed rate processes were kinetically equivalent to Scheme I. There was no lag time in the ampicillin formation time course. Furthermore, when ampicillin was relatively stable, linear first-order plots using ampicillin data showed no induction period. Based on similar observations using pivampicillin and bacampicillin, Bundgaard (10) reported that any intermediate which may have formed spontaneously decomposed to ampicillin.

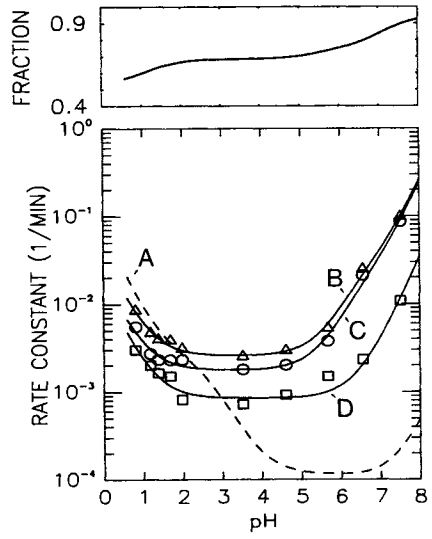

Fig. 4. Top: The fraction of talampicillin converted to ampicillin as a function of pH at 50.5°C, μ = 0.5. Bottom: the pH–rate profiles at 50.5°C, μ = 0.5, for (A) ampicillin degradation, k_h (---); (B) overall loss of talampicillin, k_{sum} (△); (C) talampicillin conversion to ampicillin, k_c (○); and (D) talampicillin degradation, k_{nc} (□). The curves are calculated from Eqs. (4), (5), (6), and (7), respectively.

Table V. Catalytic Constants Defining Conversion (k_c)[a] and Degradation (k_{nc})[b] of Talampicillin and Bacampicillin at 50.5°C[c]

	10^3k_H	10^5k_s	$10^{-3}k_{OH1}$	$10^{-3}k_{OH2}$
Bacampicillin				
k_c	5.73	1.93	8.68	2.66
k_{nc}	0.295	29.9	1.13	
Talampicillin				
k_c	19.6	176.	107.	41.8
k_{nc}	16.6	78.2	6.05	

[a] Defined by Eq. (6).
[b] Defined by Eq. (7).
[c] Units are M^{-1} min^{-1} except k_s = min^{-1}.

Relative Rates of Conversion and Degradation. At 50.5°C, ampicillin degradation was 2.7 times faster than the overall loss (k_{sum}) of bacampicillin at pH 0.9, approximately equal to pH 3.5, and 1/40 as fast at pH 7.5 (Fig. 5). The conversion of bacampicillin to ampicillin, which was less than 20% in acid, increased above pH 4 and passed through a maximum of 75% at pH 6.5 (Fig. 5, top). Thus, ester hydrolysis yielding ampicillin (k_c) predominated near pH 6.5, whereas k_{nc} was nearly equal to k_{sum} below pH 4 (Fig. 5, bottom). At pH 0.9, bacampicillin was 3.3 times more stable to degradation (k_{nc}) than ampicillin (Fig. 5, bottom). Degradation rates were equal at pH 3.5 but ampicillin was 15 times more stable at pH 7.5.

Ampicillin degradation at 50.5°C was 1.6 times faster than the overall loss of talampicillin at pH 0.9, approximately equal at pH 2, 1/6 as fast at pH 3.5, and 1/400 as fast at pH 7.5 (Fig. 4). Unlike bacampicillin, the fraction of talampicillin converted to ampicillin increased as a function of pH, having a minimum of 0.6 at pH 0.9 and reaching a maximum value of 0.93 at pH 8 (Fig. 4, top). Consequently, the overall loss of talampicillin at 50.5°C was due primarily to conversion throughout the entire pH range.

The individual rate constants and fractions converted were also compared at 25°C using the three pH values, 0.9, 3.5, and 7.5, which represent three unique regions in the pH–rate profiles. Talampicillin constants were experimentally determined at 25°C (Table III), while the remaining constants were determined by extrapolation from higher temperatures using Eq. (8). The extrapolated rate constants (10^3k in min^{-1}) at 25°C, for pH values of 0.9, 3.5, and 7.5, are as follows: k_h = 1.21, 0.0306, and 0.0170 for ampicillin; k_{sum} = 0.519, 0.0546, and 0.352; and k_c = 0.105, 0.0189, and 0.274 for bacampicillin. The corresponding fractions converted are as follows: bacampicillin = 0.20, 0.35, and 0.78; and talampicillin = 0.28, 0.86, and 0.88.

The ratios of the reactivities of the prodrugs relative to ampicillin hydrolysis at 25°C were similar to those at the higher temperature. Ampicillin degradation was two times faster than overall bacampicillin loss (k_{sum}) at both pH 0.9 and pH 3.5 but 1/21 as fast at pH 7.5. Relative to overall

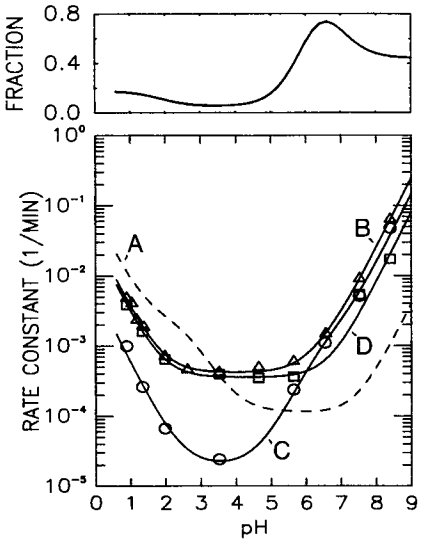

Fig. 5. Top: The fraction of bacampicillin converted to ampicillin as a function of pH at 50.5°C, μ = 0.5. Bottom: the pH–rate profiles at 50.5°C, μ = 0.5, for (A) ampicillin degradation, k_h (---); (B) overall loss of bacampicillin, k_{sum} (△); (C) bacampicillin conversion to ampicillin, k_c (○); and (D) bacampicillin degradation, k_{nc} (□). The curves are calculated from Eqs. (4), (5), (6), and (7), respectively.

talampicillin loss, ampicillin degradation was similar at pH 0.9, 1/15 as fast at pH 3.5, and 1/700 as fast at pH 7.5.

At 25°C, bacampicillin was three times more stable to degradation (k_{nc}) than ampicillin at pH 0.9 and approximately equal at pH 3.5. At pH 7.5, ampicillin was five times more stable to degradation. Talampicillin was 1.3 times more stable to degradation (k_{nc}) than ampicillin at pH 0.9, one-half as stable at pH 3.5, and 1/100 as stable at pH 7.5.

Page *et al.* (2,3) have studied the effect of substituents at the C-3 carboxylate position in penicillins on β-lactam reactivity. Methyl esters stabilized benzylpenicillin and penicillanic acid to degradation under acidic conditions while enhancing degradation under basic conditions. In the present study, the slight reduction in the k_{nc} values relative to the observed k_h values for hydrolysis of ampicillin was similar to that observed for the methyl ester prodrugs reported by Page *et al.* This reduction can be attributed to the electron-donating effect of the ester group, relative to the hydrogen on an unsubstituted penicillin, stabilizing the protonated nitrogen atom and reducing the rate of C–N fission.

Page *et al.* also reported that alkaline hydrolysis of penicillins involved β-lactam opening via expulsion of a weak amine. The rates of alkaline hydrolysis of β-lactams showed an inverse dependency on the pK_a of the amine-leaving group. The alkaline hydrolysis rate for the β-lactam of the methyl ester of benzylpenicillin was about 20 times faster than that of benzylpenicillin. This was attributed to a decrease of 2 units in the pK_a of the amine-leaving group. The pK_a values for the amine-leaving groups in bacampicillin and talampicillin are not known but would be expected to show a reduction relative to ampicillin as observed for the benzylpenicillin methyl ester relative to benzylpenicillin.

Storage Stability: A Comparison of UT to T_{90} Values

For a first-order degradation having a rate constant, k, the time during which a solution maintains 90% of its initial concentration is defined as $T_{90} = 0.105/k$. Figure 6 shows T_{90} values as a function of pH at 50.5°C for ampicillin (curve A; $T_{90} = 0.105/k_h$), bacampicillin (curve C; $T_{90} = 0.105/k_{sum}$), and talampicillin (curve E; $T_{90} = 0.105/k_{sum}$).

Since bacampicillin and talampicillin convert rapidly and completely to ampicillin in the blood (5–9), the sum of the prodrug and drug concentration in an intravenous solution constitutes the bioactivity. The UT can therefore be defined as the time during which the sum of the concentration of prodrug $[PD]_t$ and ampicillin $[D]_t$ exceeds 90% of the initial prodrug concentration $[PD]_o$. Equations (2) and (3) were used to describe $[PD]_t$ and $[D]_t$ as a function of time in aqueous solutions where the observed values for k_{sum} were substituted for $(k_{nc} + k_c)$. The rate constants k_{sum}, k_c, k_{nc}, and k_h were characterized as a function of hydrogen ion activity in Eqs. (4)–(7). The utilization time for a solution of bacampicillin and talampicillin was calculated by using Eqs. (2) and (3) to determine reiteratively the time at which the sum of $[PD]_t$ and $[D]_t$ decreased to 90% of $[PD]_o$. The UT values as a function of pH are shown in Fig. 6 for bacampicillin (curve B) and talampicillin (curve D).

As illustrated in Fig. 6 using 50.5°C data, prodrug UT values are alwasy equal to or greater than prodrug T_{90} values. In addition, both the bacampicillin and the talampicillin UT values showed a two- to threefold storage advantage over ampicillin at pH values below 3. The UT values were equal to the ampicillin T_{90} value at pH 3.0 and 3.5. Above pH 3.5, the T_{90} for ampicillin exceeded the UT values and became 8 times larger than that for bacampicillin and 18 times larger than that for talampicillin at pH 7.5.

The rate constants at 25°C were used to compare the prodrug UT values to the T_{90} values for ampicillin at pH values of 0.9, 3.5, and 7.5. The UT/T_{90} ratios comparing bacampicillin to ampicillin at pH 0.9 and 3.5 at 25°C were similar to those at 50.5°C (Table VI). The UT/T_{90} ratio at pH 7.5

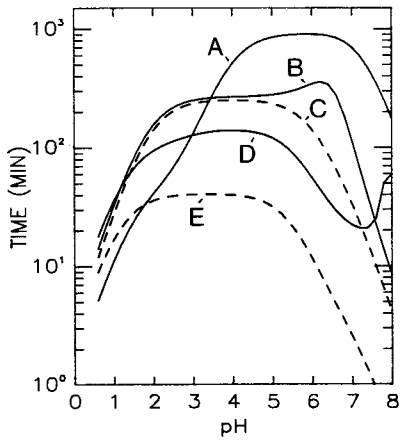

Fig. 6. Comparison of the utilization time (UT) and the time for 10% first-order loss (T_{90}) as a function of pH. Curves represent (A) ampicillin T_{90} (B) bacampicillin UT, (C) bacampicillin T_{90}, (D) talampicillin UT and (E) talampicillin T_{90} at 50.5°C.

Table VI. Utilization Times (UT in Hours) for Bacampicillin (BAC) and Talampicillin (TAL), T_{90} (Hours) for Ampicillin (AMP), and UT/T_{90} Ratios[a]

		T_{90}	UT		UT/T_{90}	
°C	pH	AMP	BAC	TAL	BAC/AMP	TAL/AMP
25.0	0.89	1.45	4.15	2.14	2.86	1.48
	3.52	57.2	49.4	32.9	0.864	0.575
	7.52	103.	26.6	5.65	0.258	0.0548
50.5	0.89	0.124	0.44	0.476	3.55	3.84
	3.52	4.03	4.46	2.57	1.11	0.638
	7.52	7.29	0.481	0.287	0.066	0.0394

[a] Initial concentrations: AMP, 1×10^{-3} *M*; BAC, 1×10^{-3} *M* except at pH 7.52 (which is 0.5×10^{-3} *M*); and TAL, 5×10^{-4} *M* except at pH 7.52 (which is 2.5×10^{-4} *M*).

and 25°C increased fourfold relative to that at 50.5°C because the k_{nc}/k_h ratio decreased threefold at 25°C. In addition, since the bacampicillin β-lactam is less stable at both temperatures, the larger fraction converted at 25°C increased the relative concentration of the more stable ampicillin.

The UT/T_{90} ratios comparing talampicillin to ampicillin at pH 3.5 and 7.5 were similar at 25 and 50.5°C (Table VI). At pH 0.9 and 25°C, the k_{nc} value was nearly equal to k_h so that the UT/T_{90} ratio was close to unity. The greater advantage for talampicillin at pH 0.9 and 50.5°C resulted from the fact that $k_{nc} \simeq 0.2\ k_h$. The observed 2.3-fold increase in the fraction converted at 50.5 relative to 25°C was insufficient to offset this 5-fold increase in β-lactam stability.

Although bacampicillin and talampicillin did not provide a dramatic improvement in the storage stability of ampicillin *via* prodrug formation, these results illustrate the potential for increased prodrug storage periods when utilization time is based on the sum of the prodrug and the drug remaining in solution. This approach is applicable to intravenous solutions of bioequivalent prodrugs since their potential for storage improvement depends only on their conversion rate and their rate of degradation relative to the drug itself. A detailed determination of the effects of the relative bioavailability and the rate constants, k_h, k_{nc}, and k_c, all of which influence the utilization time of oral prodrug solutions, is presented in a subsequent paper.

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