Carbenicillin Prodrugs: Stability Kinetics of α-Phenyl and α-Indanyl Esters in Aqueous Solution

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Abstract □ Both ester and β-lactam degradations of α-esters of carbenicillin disodium, carbenicillin indanyl sodium, and carbenicillin phenyl sodium in aqueous solution at 35° and at 0.5 ionic strength were investigated. The reactions were followed by spectrophotometric assay, reversed-phase high-pressure liquid chromatography, and colorimetric assay. The degradation pathways were established, and the rate–pH profiles for ester and β-lactam cleavage reactions are given for pH 1–11. Below pH 3, the β-lactam degradation of the prodrugs proceeded exclusively. Above pH 7, the degradation was superseded by the ester hydrolysis to carbenicillin. β-Lactams of both prodrugs are around three times more stable than carbenicillin disodium at pH 1, six times at pH 2, and 17 times at pH 3. The half-lives for carbenicillin production were predicted to be 17 hr for carbenicillin indanyl sodium and 8.5 hr for carbenicillin phenyl sodium at pH 7.0 and 37°.

Keyphrases □ Carbenicillin—prodrugs, stability in aqueous solutions, pH effects, degradation kinetics □ Carbenicillin indanyl sodium—prodrug, stability in aqueous solutions, pH, degradation kinetics □ Carbenicillin phenyl sodium—prodrug, stability in aqueous solutions, pH, degradation kinetics □ Antibiotics—carbenicillin, prodrugs, stability in aqueous solutions, pH effects, degradation kinetics

Intravenous or intramuscular carbenicillin is effective against serious infections caused by *Pseudomonas aeruginosa* and other Gram-negative bacteria (1). This antibiotic is unstable in acidic solutions (2, 3) and gastric juice (4, 5) and is poorly absorbed from the GI tract after oral administration (1).

Several α-carboxyl esters of carbenicillin were synthesized to increase its oral bioavailability (5, 6). Carbenicillin indanyl sodium (carindacillin) and carbenicillin phenyl sodium (carfecillin) have been marketed as oral carbenicillin prodrugs.

This paper describes the kinetics of carbenicillin indanyl sodium and carbenicillin phenyl sodium in aqueous solution at various pH values. Their good stability at pH 2 in gastric juice has been well known, but complete kinetics for β-lactam cleavage and hydrolysis of the ester moiety to produce carbenicillin have not been reported.

EXPERIMENTAL

Materials—Carbenicillin disodium[1] (799 μg/mg), carbenicillin indanyl sodium[1] (704 μg/mg as carbenicillin free acid), and carbenicillin phenyl sodium[2] (979 μg/mg) were used as received.

All other chemicals were analytical grade and were used without further purification except imidazole, which was purified as described in the literature (7).

Reagents—The imidazole reagent was prepared according to the literature (7). The acidic reagent was 0.2 *M* acetate buffer solution adjusted to pH 4.

Prodrug Hydrolysis—Kinetic studies were carried out, unless otherwise stated, at 35° and an ionic strength of 0.5. Each antibiotic was dissolved in the buffer or in 0.5 *M* KCl, maintained at the desired pH by a pH-stat[3], to the final concentration of 5×10^{-5}–5×10^{-3} *M*, depending on the solubility. At appropriate intervals, aliquots were withdrawn, cooled, and analyzed. The pseudo-first-order rate constants, k_{obs}, were calculated by the least-squares analysis of the slopes of starting antibiotic concentration logarithm *versus* time plots.

Analytical Procedure—*Simultaneous Determination of Carbenicillin and Its Prodrug*—Carbenicillin and its prodrug were determined simultaneously by a spectrophotometric assay. This analytical method, utilizing the difference in the acid stability between carbenicillin and the prodrug, is based on the imidazole method (7).

If necessary, the sample was diluted with distilled water. Four equal samples of 1 ml each, A_1, A_2, B_1, and B_2, of the test solution were pipetted into separate test tubes. Five milliliters of imidazole reagent was added to A_1 and 5 ml of distilled water was added to B_1, followed by incubation at 60° for 25 min. After cooling, absorbances, E_{A1} and E_{B1}, were measured[4] at 325 nm. To samples of A_2 and B_2, 1 ml of acidic reagent was added, and the samples were incubated in a water bath at 60° for exactly 25 min and cooled. After addition of 5 ml of the imidazole reagent to A_2 and 5 ml of distilled water to B_2, these samples were incubated in a water bath at 60° for 25 min and cooled; their absorbances, E_{A2} and E_{B2}, were measured at the same wavelength.

The concentrations of each compound can be calculated from Eqs. 1 and 2 using the difference in absorbance between A and B; *i.e.*, E_1 ($= E_{A1} - E_{B1}$) and E_2 ($= E_{A2} - E_{B2}$):

$$E_1 = S_{p1}C_p + S_{c1}C_c \qquad \text{(Eq. 1)}$$

$$E_2 = S_{p2}C_p + S_{c2}C_c \qquad \text{(Eq. 2)}$$

where subscripts *c* and *p* refer to carbenicillin and its prodrug, respectively; *C* is the concentration of the antibiotic in sample solution, and *S* is the slope of the calibration curve of the antibiotics for respective reaction periods of 0 and 25 min with the acidic reagent.

A good straight-line relation between the absorbance and the concentration of each antibiotic was obtained within 0–3×10^{-4} *M* of the antibiotic in a sample solution. Both S_{c1} and S_{p1} were 3.95×10^3, and S_{p2} was 3.19×10^3 and 1.83×10^3 for two prodrugs and carbenicillin, respectively. The carbenicillin molar absorptivity, which could be calculated from S_{c1} and the final concentration in the analytical solution, was 2.37×10^4, comparable to the 2.67×10^4 reported previously (7). Synthetic mixtures of carbenicillin and its prodrug were analyzed in this way, and entirely satisfactory results were obtained (Table I).

High-Pressure Liquid Chromatography (HPLC)—To ensure the accuracy of the spectrophotometric method, the same samples in some reactions were determined also by reversed-phase HPLC. The liquid

Table I—Recovery of Carbenicillin Phenyl Sodium (I) and Carbenicillin Disodium (II) from a Synthetic Mixture in 0.1 *M* Phosphate Buffer at pH 6.54 and Ionic Strength 0.5 [a]

Added (10^3 *M*)		Found (10^3 *M*)		Recovery, %	
I	II	I	II	I	II
1.00	0.00	1.01	0.00	101.1	100.0
0.80	0.20	0.78	0.21	97.5	105.0
0.40	0.60	0.38	0.59	95.0	98.3
0.20	0.80	0.20	0.78	100.0	97.5
0.00	1.00	0.00	0.99	100.0	99.0

[a] The mixture was diluted fivefold with distilled water and analyzed according to the described procedure.

[1] Taito Pfizer Co., Tokyo, Japan.
[2] Beecham Yakuhin Co., Tokyo, Japan.
[3] pH-Stat titrator assembly consisting of TTT2 titrator and ABU12b autoburet, Radiometer, Copenhagen, Denmark.
[4] Double-beam spectrophotometer, UV-200S, Shimadzu, Kyoto, Japan.

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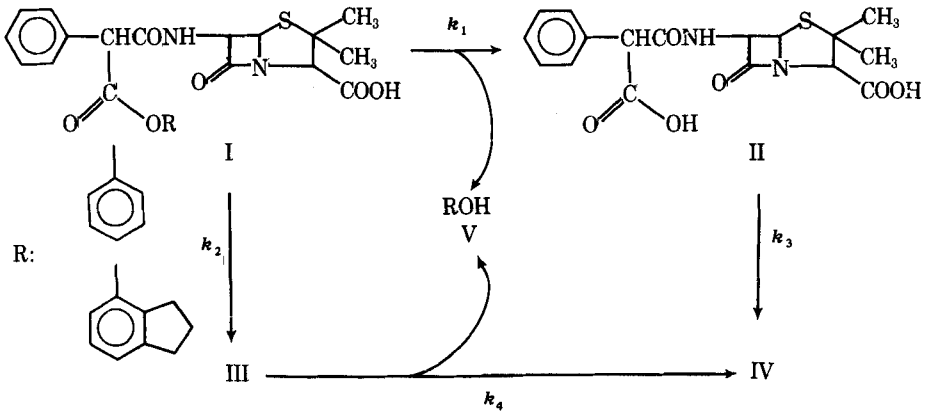

Scheme I—Prodrug degradation pathway in aqueous solution

chromatograph[5] was equipped with a UV detector[6] set at 254 nm. The stationary phase was octadecylsilane chemically bonded on totally porous silica gel, prepacked into a 300-mm stainless steel column[7] (4-mm i.d.). The mobile phase was 20% acetonitrile–0.01 *M* ammonium acetate. The instrument was operated at ambient temperature at a flow rate of 2 ml/min to produce the pressure of 110 kg/cm^2. Samples were injected through a 100-μl loop injector on flow. Peak heights were used for quantification.

Colorimetric Determination of Phenol—Phenol liberated from carbenicillin phenyl sodium was measured by the colorimetric method (8). The sample was diluted with distilled water, if necessary, and placed in a 20-ml volumetric flask. Then 1 ml of 0.3% 4-aminoantipyrine, 2 ml of 1% ferricyanide, and 5 ml of 0.2 *M* borate buffer (pH 9.2) were added consecutively, and the absorbance of the solution, adjusted to 20 ml with distilled water, was measured at 510 nm. The calibration curve was prepared with the standard phenol solution, and the concentration of phenol liberated was calculated.

RESULTS

Prodrug Degradation Pathway—Degradation of carbenicillin esters may proceed as depicted in Scheme I. In this scheme, I represents carbenicillin indanyl sodium and carbenicillin phenyl sodium, II represents carbenicillin, III represents β-lactam-cleaved products of I, IV represents the sum of products resulting from β-lactam cleavage of II and from ester hydrolysis of III, and V represents indanol or phenol.

Figures 1–3 show time courses of molar fractions of I–V during I degradation. Compounds I and II were determined by the UV spectrometric method developed in this study. As seen in Fig. 1, these concentrations were in good agreement with those determined by HPLC. Phenol (V) liberated from carbenicillin phenyl sodium (I) and III was determined by colorimetry. Concentrations of III and IV, therefore, were calculated from Eqs. 3 and 4 for carbenicillin phenyl sodium degradation:

$$[III] = [I]_0 - [I] - [V] \quad \text{(Eq. 3)}$$

$$[IV] = [V] - [II] \quad \text{(Eq. 4)}$$

where the molar concentration of all species is expressed with brackets and $[I]_0$ is the initial I concentration.

By solving the differential equation for Scheme I, the rate expression for the molar fraction of each species may be written as:

$$[I]/[I]_0 = e^{-(k_1+k_2)t} \quad \text{(Eq. 5)}$$

$$[II]/[I]_0 = \frac{k_1}{k_1+k_2-k_3}(e^{-k_3t} - e^{-(k_1+k_2)t}) \quad \text{(Eq. 6)}$$

$$[III]/[I]_0 = \frac{k_2}{k_1+k_2-k_4}(e^{-k_4t} - e^{-(k_1+k_2)t}) \quad \text{(Eq. 7)}$$

$$[IV]/[I]_0 = 1 - \frac{k_3k_4 - k_1k_3 - k_2k_4}{(k_1+k_2-k_3)(k_1+k_2-k_4)}e^{-(k_1+k_2)t} - \frac{k_2}{k_1+k_2-k_4}e^{-k_4t} - \frac{k_1}{k_1+k_2-k_3}e^{-k_3t} \quad \text{(Eq. 8)}$$

$$[V]/[I]_0 = 1 - \frac{k_1-k_4}{k_1+k_2-k_4}e^{-(k_1+k_2)t} - \frac{k_2}{k_1+k_2-k_4}e^{-k_4t} \quad \text{(Eq. 9)}$$

To evaluate k_3, degradation of II was performed under similar conditions (Fig. 3). The rate constants k_1, k_2, k_3, and k_4 to best fit all experimental points were obtained by the nonlinear least-squares regression analysis according to Eqs. 5–9. The resulting rate constants are listed in Table II, providing calculated curves fitting the observed values (Figs. 1–3).

In most cases, rate constants k_1 and k_4 for the ester hydrolysis of both I and III were almost identical, suggesting that β-lactam cleavage had no effect on the rate for α-ester hydrolysis.

Kinetics of Apparent First-Order Rate Constants—By plotting the logarithm of $[I]/[I]_0$ against time, the apparent first-order rate con-

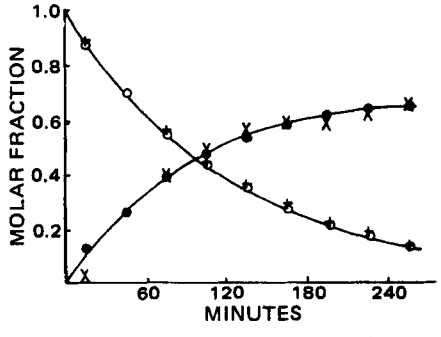

Figure 1—*Time course for carbenicillin phenyl sodium (○, separation assay; +, HPLC) and carbenicillin (●, separation assay; ×, HPLC) during degradation at pH 6.35 (0.2 M phosphate buffer), 60°, and ionic strength 0.5. The lines are the least-squares fits of the experimental points consistent with Scheme I on the assumption of* $k_1 = k_4$ ($k_1 = k_4 = 42.8 \times 10^{-2}\ hr^{-1}$, $k_2 = 5.58 \times 10^{-2}\ hr^{-1}$, *and* $k_3 = 6.38 \times 10^{-2}\ hr^{-1}$).

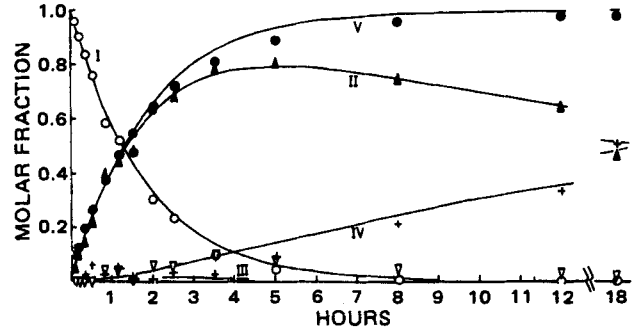

Figure 2—*Time course for carbenicillin phenyl sodium (I) (○), II (▲), III (▽), IV (+), and V (●) during degradation at pH 6.60 (0.1 M phosphate buffer), 60°, and ionic strength 0.5. The lines are the least-squares best fits to the experimental points consistent with Scheme I on the assumption of* $k_1 = k_4$ ($k_1 = k_4 = 53.9 \times 10^{-2}\ hr^{-1}$, $k_2 = 2.05 \times 10^{-2}\ hr^{-1}$, *and* $k_3 = 3.88 \times 10^{-2}\ hr^{-1}$). *The compound numbers are defined in the text.*

[5] Model FLC-A700, Japan Spectroscopic Co., Tokyo, Japan.
[6] Model UVIDEC-100, Japan Spectroscopic Co., Tokyo, Japan.
[7] μBondapak C_{18}, Waters Associates, Milford, Mass.

Table II—Rate Constants [a] for Carbenicillin Phenyl Sodium Degradation at pH 6.60 (60°, 0.1 *M* Phosphate Buffer), pH 6.35 (60°, 0.2 *M* Phosphate Buffer), and pH 4.60 (35°, 0.2 *M* Acetate Buffer) and Ionic Strength 0.5

pH	$k_1 \pm SD$, $10^2\ hr^{-1}$	$k_2 \pm SD$, $10^2\ hr^{-1}$	$k_3 \pm SD$, $10^2\ hr^{-1}$	$k_4 \pm SD$, $10^2\ hr^{-1}$
6.60	53.9 ± 1.27	2.05 ± 1.16	3.88 ± 0.31	53.9 ± 1.27 [b]
6.35	42.8 ± 0.78	5.58 ± 0.89	6.38 ± 0.96	42.8 ± 0.78 [b]
4.60	0.84 ± 0.05	0.50 ± 0.05	5.12 ± 0.20	0.66 ± 0.17

[a] The values were evaluated by the nonlinear least-squares analysis of experimental points in Figs. 1–3. [b] The calculation was carried out on the assumption of $k_1 = k_4$.

Table III—Various Rate Constants [a] for the Degradation of Prodrugs at 35° and Ionic Strength 0.5

Antibiotic	$(k_2)_H$, $M^{-1}\ hr^{-1}$	$10^2(k_2)_0$, hr^{-1}	$10^2(k_1)_H$, $M^{-1}\ hr^{-1}$	$10^3(k_1)_0$, hr^{-1}	$10^{-5}(k_1)_{OH}$, $M^{-1}\ hr^{-1}$
Carbenicillin disodium	52.2 [b]	183 [b]	—	—	—
Carbenicillin phenyl sodium	17.9 ± 1.1	9.79 ± 1.12	4.98 ± 1.40	3.72 ± 0.48	3.09 ± 0.35
Carbenicillin indanyl sodium	24.4 ± 0.3	8.22 ± 0.22	—	3.53 ± 0.49	1.46 ± 0.08

[a] Rate constants are defined in Eqs. 15 and 16, and they were calculated using the nonlinear regression analysis of the experimental points in Fig. 7. [b] Reference 15.

stants k_{obs} for overall degradation of I was obtained from the slopes, *i.e.*:

$$k_{obs} = k_1 + k_2 \qquad \text{(Eq. 10)}$$

The typical first-order plots obtained from the UV spectrophotometric separation assay are given in Fig. 4 for both prodrugs.

On the assumption that $k_1 = k_4$, as verified for carbenicillin phenyl sodium, Eq. 9 may be simplified to:

$$[V]/[I]_0 = 1 - e^{-k_1 t} \qquad \text{(Eq. 11)}$$

Good first-order plots were obtained for carbenicillin phenyl sodium, consistent with the prediction of Eq. 11 when $\log (1 - [V]/[I]_0)$ was plotted against time (Fig. 4).

During the kinetic runs above pH 7, the degradation of both prodrugs resulted in a gradual increase of the characteristic UV absorbance at 270–290 nm. Plots of $\log (E_\infty - E_t)$ at λ_{max} (268 nm for carbenicillin phenyl sodium and 280 nm for carbenicillin indanyl sodium) *versus* t become reasonably linear according to the first-order expression:

$$\log (E_\infty - E_t) = \log (E_\infty - E_0) - \frac{k_{obs}}{2.303} t \qquad \text{(Eq. 12)}$$

where E_t, E_0, and E_∞ are the absorbances at times t, zero, and infinity, respectively. All reactions were followed for one to three half-lives, and values for the pseudo-first-order rate constant k_{obs} were obtained by plots of Eq. 12 or by the method of Guggenheim (9). Figure 4 shows typical plots of the logarithm of $(E_\infty - E_t)/(E_\infty - E_0)$. The k_{obs} values obtained by UV spectrophotometry were equal to $(k_1 + k_2)$ determined by the separation assay. For carbenicillin phenyl sodium, the rate constants above pH 7 were almost in agreement with the k_1 values determined by the colorimetric method.

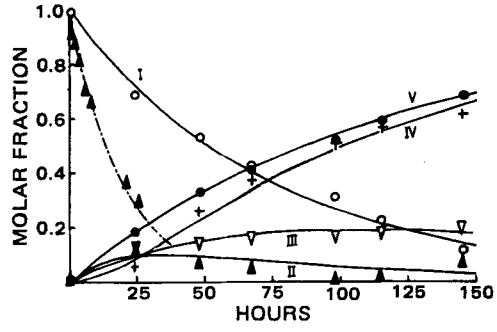

Figure 3—*Time course for carbenicillin phenyl sodium (I) (○), II (▲), III (▽), IV (+), and V (●) during degradation at pH 4.60 (0.2 M acetate buffer), 35°, and ionic strength 0.5. The lines are the least-squares best fits to the experimental points consistent with Scheme I ($k_1 = 0.84 \times 10^{-2}\ hr^{-1}$, $k_2 = 0.50 \times 10^{-2}\ hr^{-1}$, $k_3 = 5.12 \times 10^{-2}\ hr^{-1}$, and $k_4 = 0.66 \times 10^{-2}\ hr^{-1}$). Carbenicillin (▲) on the broken line was determined by degradation of II under the same conditions. The compound numbers are defined in the text.*

Buffer Concentration Effect on Degradation Rates—At constant pH and in the presence of excess buffer, the rate constants k_{obs} for the total loss of I and k_1 for the ester hydrolysis were affected by general acid–base catalysis by buffer components. Typical plots for the catalytic effect of various buffers on k_{obs} and k_1 are shown in Figs. 5 and 6, respectively, giving a reasonably straight line at constant pH in all cases. Extrapolation of such plots to zero buffer concentration provides, as intercepts, the values of the pseudo-first-order rate constants $(k_{obs})_{pH}$ and $(k_1)_{pH}$, corresponding to the non-buffer-catalyzed degradation of the β-lactam and of the ester bond of the prodrug nucleus. For ester linkage hydrolysis, the rate constant may be defined in the phosphate buffer system as:

$$k_1 = (k_1)_{pH} + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] \qquad \text{(Eq. 13)}$$

Equation 13 can be written in terms of the total buffer concentration, $[B]_T$:

$$k_1 = (k_1)_{pH} + [k_{H_2PO_4^-} + (k_{HPO_4^{2-}} - k_{H_2PO_4^-}) f_{HPO_4^{2-}}][B]_T \qquad \text{(Eq. 14)}$$

where $f_{HPO_4^{2-}}$ is the fraction of monohydrogen phosphate ion. By employing pKa = 6.59 at 35° and ionic strength 0.5 for phosphate (10), plots of the slopes at pH 6.93, 6.38, and 5.87 against $f_{HPO_4^{2-}}$ provided the catalytic constant of $k_{HPO_4^{2-}} = 0.240\ M^{-1}\ hr^{-1}$ for the HPO_4^{2-} buffer component. No significant catalytic effect could be assigned to the $H_2PO_4^-$ buffer component.

Log k_{pH}–pH Profiles—The k_{pH} values were obtained by extrapolation of k_{obs} to zero buffer concentration. These values also were determined by the pH-stat.

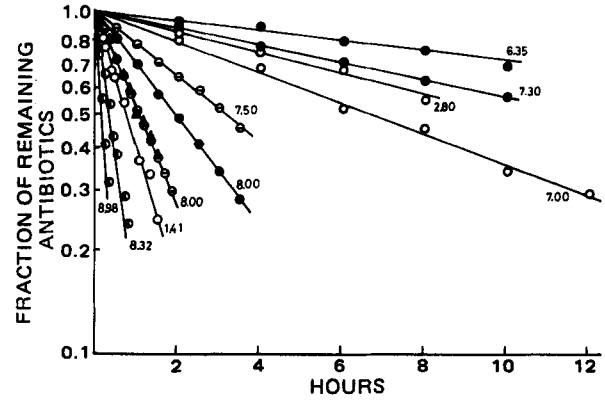

Figure 4—*Apparent first-order plots followed by spectrophotometric assay for degradation of carbenicillin indanyl sodium (●, separation assay; ◐, UV method) and carbenicillin phenyl sodium (○, separation assay; ⊖, colorimetric method; ▲, UV method) at various pH values, 35°, and ionic strength 0.5. The 0.5 M KCl solution was maintained at each pH (8.98, 8.00, 7.50, 7.30, 7.00, 2.80, and 1.41) by means of a pH-stat; the 0.2 M borate buffer was at pH 8.32, and the 0.2 M phosphate buffer was at pH 6.35.*

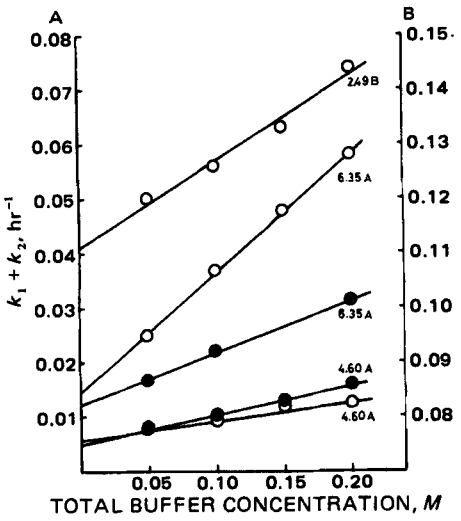

Figure 5—*Pseudo-first-order rate constants,* $k_1 + k_2$, *versus total buffer concentration for degradation of carbenicillin indanyl sodium* (●) *and carbenicillin phenyl sodium* (○) *in various buffer systems at 35° and ionic strength 0.5. The rate constants were determined from the spectrophotometric assay. The phosphate buffer was at pH 2.49 and 6.35; the acetate buffer was at pH 4.60.*

In Fig. 7 is plotted log $(k_{obs})_{pH}$ and log $(k_1)_{pH}$ *versus* pH for carbenicillin phenyl sodium degradation at 35° and ionic strength 0.5. The pH dependency of the ester hydrolysis indicated a simple and U-shaped curve fitted by:

$$(k_1)_{pH} = (k_1)_H a_{H^+} + (k_1)_0 + (k_1)_{OH} K_w / a_{H^+} \qquad \text{(Eq. 15)}$$

where a_{H^+} is the hydrogen-ion activity as determined by glass electrodes; K_w is the autoprotolytic constant for water; $(k_1)_H$ and $(k_1)_{OH}$ are the second-order rate constants for the hydrogen-ion catalyzed- and hydroxide-ion-catalyzed ester hydrolysis, respectively; and $(k_1)_0$ is the first-order rate constant for spontaneous or water-catalyzed hydrolysis.

At pH >6, $(k_{obs})_{pH}$ was almost identical with $(k_1)_{pH}$, indicating that ester hydrolysis to produce carbenicillin proceeds exclusively in this pH region. Below pH 3, $(k_{obs})_{pH}$ was 10–100 times larger than $(k_1)_{pH}$. This finding suggests that β-lactam degradation rather than ester hydrolysis proceeds predominantly in carbenicillin phenyl sodium. It is obvious, therefore, that the rate constant $(k_{obs})_{pH}$ below pH 3 is approximately equal to $(k_2)_{pH}$.

For the acid degradation of the β-lactam of penicillin, $(k_2)_{pH}$ obeys the general rate law (10):

$$(k_2)_{pH} = [(k_2)_H a_{H^+} + (k_2)_0] \frac{a_{H^+}}{K_a + a_{H^+}} \qquad \text{(Eq. 16)}$$

where K_a is the dissociation constant of I, and $(k_2)_{pH}$ and $(k_2)_0$ represent hydrogen-ion-catalyzed and spontaneous or water-catalyzed degradation rates, respectively, of undissociated I.

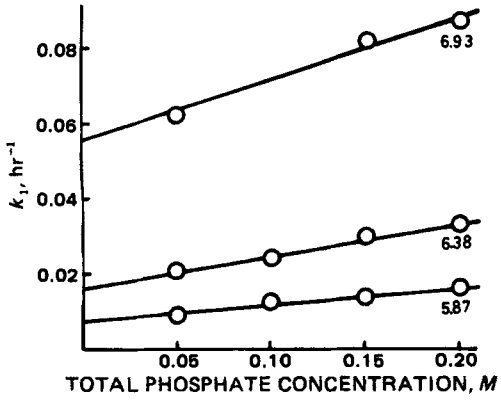

Figure 6—*Pseudo-first-order rate constants* k_1 *for the liberation of phenol from carbenicillin phenyl sodium* versus *total phosphate concentration at 35° and ionic strength 0.5. The rate constants were determined by colorimetry.*

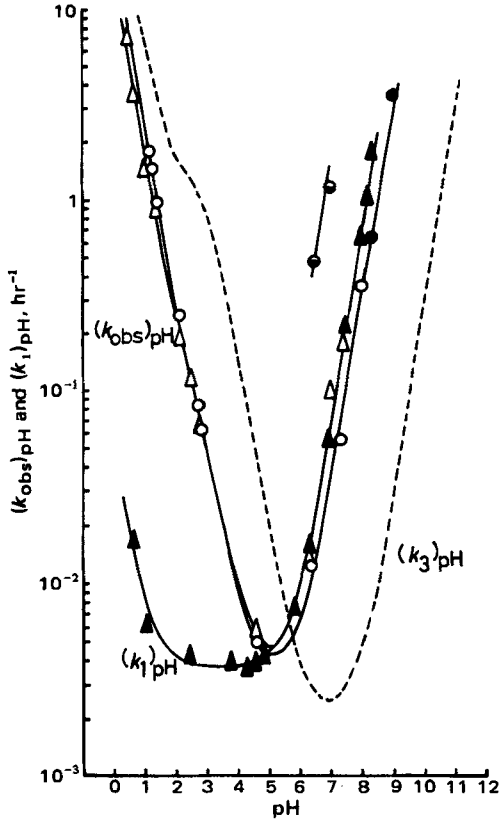

Figure 7—*Log* $(k_{obs})_{pH}$–*pH profiles for the degradation and log* $(k_1)_{pH}$–*pH profiles for the ester hydrolysis of carbenicillin indanyl sodium* (○) *and carbenicillin phenyl sodium* (△) *at 35° and ionic strength 0.5. The lines represent the curves calculated from Eqs. 15–17 and the constants in Table III; the points are experimental values. Open symbols are data determined by the separation assay, and closed ones are data determined by the colorimetric and/or UV method. The degradation of carbenicillin phenyl sodium* (●) *was determined at 60° in 0.2 M phosphate buffer. The broken line represents the log* $(k_3)_{pH}$–*pH profile of carbenicillin* (2).

Log $(k_{obs})_{pH}$–pH profiles for carbenicillin indanyl sodium degradation are shown in Fig. 7, providing a similar shape to those for carbenicillin phenyl sodium. Therefore, Eqs. 15 and 16 apply to both compounds.

By employing the pKa values (11) of 2.94 for carbenicillin indanyl sodium and 2.91 for carbenicillin phenyl sodium and $K_w = 2.09 \times 10^{-14}$ (12), these specific rate constants that produced the best fits of the observed points above pH 7 and below pH 3 are listed in Table III. The calculated curves for $(k_{obs})_{pH}$ were generated from Eq. 17, corresponding to the sum of Eqs. 15 and 16 by the use of appropriate parameters, and were in reasonable agreement with all experimental values:

$$(k_{obs})_{pH} = (k_1)_H a_{H^+} + (k_1)_0 + [(k_2)_H a_{H^+} + (k_2)_0] \frac{a_{H^+}}{K_a + a_{H^+}} + (k_1)_{OH} K_w / a_{H^+} \qquad \text{(Eq. 17)}$$

To compare the β-lactam stability between the prodrugs and carbenicillin, the log $(k_3)_{pH}$–pH profiles for carbenicillin degradation under the same conditions are redrawn in Fig. 7 from the literature (2). It can be seen that the β-lactam of both carbenicillin indanyl sodium and carbenicillin phenyl sodium is more stable than that in carbenicillin by about three times at pH 1, six times at pH 2, and 17 times at pH 3. The half-lives at 35° and ionic strength of 0.5 were ~20 min, 2.6 hr, and 13 hr at pH 1, 2, and 3, respectively.

Temperature Effect on Degradation Rate—Arrhenius plots of the observed first-order rate constants k_{obs} for I degradation in buffer solutions of two different pH values are illustrated in Fig. 8. The enthalpies of activation, $\Delta H^{\neq}$, calculated from the slope of the line at pH 3.87 (in 0.2 *M* acetate buffer), were 17.7 and 18.1 kcal/mole for carbenicillin indanyl sodium and carbenicillin phenyl sodium, respectively; the enthalpy of activation at pH 6.98 (in 0.2 *M* phosphate buffer) was 16.6 kcal/mole for carbenicillin phenyl sodium. Because of the small catalytic effect of acetate buffer, activation enthalpy at pH 3.87 may be regarded

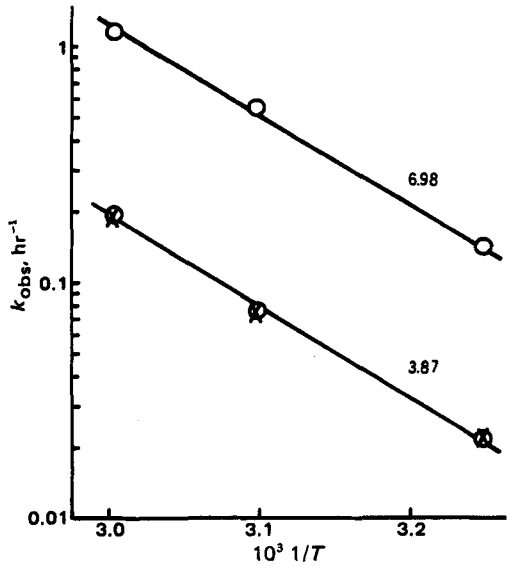

Figure 8—*Arrhenius plots for the apparent first-order rate constants k_{obs} for carbenicillin indanyl sodium (×) and carbenicillin phenyl sodium (○) at pH 6.98 (0.2 M phosphate buffer) and pH 3.87 (0.2 M acetate buffer) and ionic strength 0.5.*

as that of spontaneous degradation of the β-lactam of undissociated I, comparable with enthalpies in the 18–21-kcal/mole range for other penicillin antibiotics (2).

The apparent activation energy of 17.2 kcal/mole at pH 6.98 may include the energies for hydroxide-ion-catalyzed and diphosphate-ion-catalyzed hydrolysis and also for heat of ionization of water. If this energy were to be employed to predict the hydrolysis rate of the I ester linkage at constant pH in nonbuffered solution, half-lives at 37° for production of H from carbenicillin indanyl sodium and carbenicillin phenyl sodium would be 17 and 8.5 hr, respectively, at the small intestinal pH of 7.0 and 7 and 3.5 hr, respectively, at the body fluid pH of 7.4.

DISCUSSION

The degradation of the two prodrugs of II proceeds through a total cleavage of the β-lactam ring and of the ester bond. In acidic media below pH 3, the β-lactam degradation pathway contributes >92%, and ester hydrolysis contributes <8%. Susceptibility of the β-lactam of penicillins to acid-catalyzed degradation is attributed not only to the strained ring (13) but also to the intramolecular attack of the side-chain amide on the β-lactam carbonyl (14). The intramolecular reaction results in the acceleration of the rates by electron-donating substituents, while electron-withdrawing substituents reduce the β-lactam cleavage rate. Enhanced stability of I compared to II may be due to the increased inductive effect of indanyl and phenyl esters compared to the carboxylate anion. The magnitude of the corresponding reaction rate constants $(k_2)_H$ and $(k_2)_0$ for both prodrugs was similar to those of phenoxy and isoxazolyl derivatives of orally effective penicillins (2, 15).

Previous studies (16) clarified that, in an environment below pH 5, GI absorption of penicillins, excluding amphoteric derivatives such as ampicillin, follows the common lipoidal membrane transport mechanism of the undissociated species permeating through the aqueous diffusion layer barrier. This mechanism predicts that the increased lipophilicity of the undissociated penicillin molecules enhances the GI absorption rate according the quantitative relationship described previously (17). The partition coefficient of the undissociated species, P_u, of orally effective penicillins in an octanol–water system as the lipophilic index is above 90 (11, 18). The corresponding values of carbenicillin indanyl sodium and carbenicillin phenyl sodium are 5900 and 910, respectively (11), suggesting that these prodrugs would be absorbed well from the GI membrane. In fact, this possibility was substantiated previously; intestinal absorption of carbenicillin indanyl sodium at pH 4.0 is 4.7 and 2.2 times faster than absorption of penicillin V and propicillin, respectively (19).

Above pH 7, on the other hand, I degradation is superseded in importance by ester hydrolysis to produce II. The second-order rate constant of the β-lactam hydrolysis by nucleophilic hydroxide-ion attack is $\sim 10^3$ M^{-1} hr^{-1} at 35° and $\mu = 0.5$, independent of the electronic nature of the side chain at position 6 (2, 10, 20, 21). The present kinetic data indicate that the ester hydrolysis of I by the nucleophilic hydroxide-ion attack is ~100 times faster than the β-lactam hydrolysis. However, such chemical degradation of I ester bonds is almost negligible, as predicted, under intestinal pH conditions between pH 5 and 7.

A nonspecific esterase present on the GI lumen surface and/or in solution may facilitate ester hydrolysis producing II, as verified previously in recirculating intestinal perfusion experiments in rats (19). Intestinal absorption of ionized penicillins follows the transport mechanism almost independently of the lipoidal nature of penicillin molecules above pH 6 and is very slow (17). These results suggest that a slow prodrug release above pH 5–6 from oral tablets may result in the significant bioavailability reduction because of the decreased concentration of well-absorbed undissociated prodrug species and the rapid yield of poorly absorbed II by the possible action of intestinal esterase rather than by the chemical hydrolysis before I absorption.

In summary, the two prodrugs investigated have appropriate acid stability and lipoidal solubility to serve as oral dosage forms. The two prodrugs are well designed, from the pharmaceutical viewpoint, to improve II absorption and acid lability, to exhibit sufficient ester bond chemical stability before absorption, and to yield II rapidly by the action of tissue and serum esterase after absorption (6). If the two prodrugs were to be controlled for sufficient dissolution below pH 4 by a suitable pharmaceutical technique, the dissolved prodrugs might be absorbed rapidly both in the stomach, to a small extent, and in the upper small intestine, which has relatively low pH values and a large surface area, to a larger extent.

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