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The Amino Acid Nature of Ampicillin and Related Penicillins

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Abstract □ The amphoteric penicillins, ampicillin and Wy-4508 (cyclacillin), possess properties similar to the alicyclacillinphatic amino acids. At a pH equal to the isoelectric point (pI) they exist essentially as zwitterions (dipolar ions), and in this form are most stable and least soluble in water. The aqueous solubility of ampicillin changes only slightly with a change in ionic strength unless a nonpolar solvent is added. In water at 25°, the carboxyl groups of all penicillins appear to have the same pK_1 (2.6–2.7), while the amino groups of the amphoteric penicillins vary in the pK_2 values over a wide range (7.24–7.65), probably being influenced by the adjacent side chin groups. A change in the dielectric constant affects the pK_1 more than the pK_2; a change in temperature does the opposite. The formation of ampicillin zwitterions from its uncharged species is an exothermic reaction, and the heat of formation is about 10.7 kcal./mole.

Keyphrases □ Ampicillin, related penicillins—amino acid similarities □ pH–stability profile—penicillins □ Amphoteric penicillins in solution—apparent stability □ Physicochemical properties, penicillins—biological activity relationship

Ampicillin (6-[2-amino-2-phenylacetamido]penicillanic acid) was first prepared by Doyle *et al.* (1) in 1961. After extensive antimicrobial and pharmacological evaluations, this antibiotic was shown to be very acid-stable (2), well absorbed (3–5), and effective at low minimal inhibitory concentration (MIC) against a wide variety of Gram-negative as well as Gram-positive organisms (5–7). Wy-4508, an aminoalicyclic penicillin prepared by Grant *et al.* (8, 9), also has a broad antibacterial spectrum, and in addition has a higher ratio of *in vivo* to *in vitro* activity than ampicillin. Part of this superiority may be due to its high, prolonged blood concentrations and low serum binding (10). Ampicillin apparently owes its activity and stability to the presence of the free amino group at the α-position of the *N*-acyl side chain of the penicillin nucleus, since when this group is substituted or derivatives are made, the activity reverts to a more Gram-positive and lipophilic type (1). Recently, Cieslak and Wasilewa (11) reported that the presence of a free α-amino group, although required, is apparently insufficient for broad antimicrobial activity; the chemical nature of the penicillin side chain is also an essential factor.

Commonly used penicillins, both natural and semi-synthetic, such as pencillins benzyl-and phenoxymethyl, methicillin, dicloxacillin, and nafcillin are salts of monobasic *N*-substituted penicillanic acids. Ampicillin and Wy-4508, however, are ampholytes, *i.e.*, they behave as both acids and bases since they carry both carboxyl and amino groups at the ends of the molecules.

Austin *et al.* (12) reported the formation of ampicillin hydrates. Grant and Alburn (13) found the monohydrate form of ampicillin less stable than the anhydrate. Poole and Bahal (14) further studied the anhydrate-trihydrate phase transition. The authors recently reported on the chemical kinetics of ampicillin in solution (15). Now using ampicillin as a model, these studies have been extended to the solubility characteristics, dissociation behavior, and apparent heat of dissociation (ionization) in solution of these amphoteric penicillins.

EXPERIMENTAL

Penicillins—All the penicillins were products or new compounds of Wyeth Laboratories. Their purity was greater than 98%. The following were used: ampicillin (anhydrate), 6-(2-amino-2-phenylacetamido)penicillanic acid, Lot C-10575 and Lot 10959, m.p., 202-203° (dec.); Wy-4508 6-(1-aminocyclohexanecarboxamido)-penicillanic acid, Lot C-10789 (anhydrate) m.p., 182–183° (dec.); Wy-7953, 6-(1-aminocyclopentanecarboxamido)penicillanic acid, Lot C-10785, m.p., 188–189° (dec.); Wy-8542, 6-(1-amino-3-methylcyclopentanecarboxamido)penicillanic acid, m.p., 164-165° (dec.), (new compound); 6-APA, 6-aminopenicillanic acid, Lot C-11093, m.p., 187-189° (dec.); dicloxacillin sodium monohydrate, 6-[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido] penicillanic acid, sodium salt·H_2O, Lot C-10651; nafcillin sodium monohydrate, 6-(2-ethoxy-1-naphthamido) penicillanic acid, sodium salt·H_2O.

All other chemicals were commercially available reagent grade. The water used for penicillin solutions, titrants, and buffers was deionized, distilled and freshly boiled. The pH was 6.75 at 25°.

Procedures—To determine the solubility, an excess of each drug (about four times the amount needed for saturation) was added to each of a series of 120-ml. (4-oz.) bottles, followed by adding 50 ml. of solvent. The bottles were capped, placed in a constant-temperature bath, mechanically rotated for 2 hr., and allowed to stand about 30 min. at the appropriate temperature. Samples were taken from the supernatants and filtered through Millipore filters,[1] diluted with

[1] Millipore filters were type HA (aqueous solvent) and type LC (aqueous-organic solvent mixture).

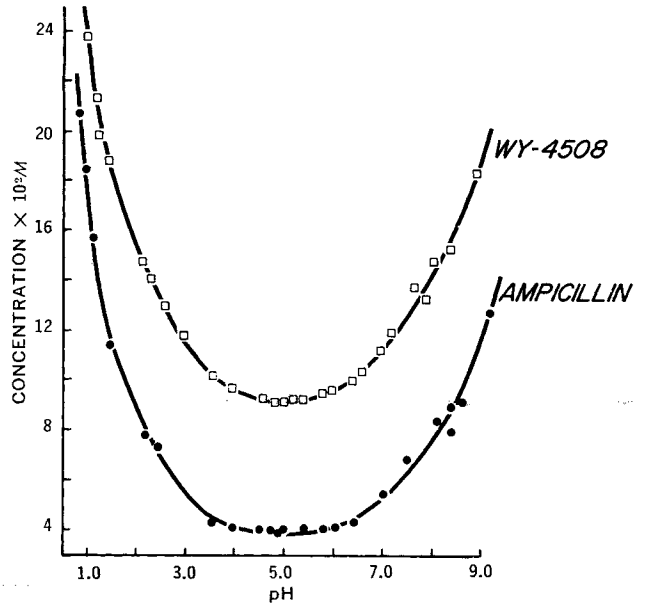


Figure 1—*The pH-apparent solubility profiles of ampicillin and Wy-4508 at 25° in buffers of total ionic strength 0.5.*

water to make final concentrations of about 1–4 mg./ml., and then assayed for penicillin content.

For this assay, a modified iodometric procedure (16) was used. At room temperature, a 2-ml. sample was transferred into each of two stoppered conical flasks. To one flask was added 5 ml. of 1.0 *N* NaOH and 15 min. later, 5 ml. of 1.2 *N* HCl, 5 ml. of pH 4 citrate buffer, and 15 ml. of 0.01 *N* iodine solution. The mixture was allowed to stand another 15 min. and the excess of iodine was titrated with 0.01 *N* sodium thiosulfate solution. As the end point was approached about 0.1 g. of thyodene (indicator) was added, and the titration was continued until the blue color disappeared. To the second flask the same amount of buffer and iodine solution was added and the titration was completed immediately. The difference between the two titers represented the actual amount of iodine being consumed by the intact penicillin. The conversion factors used were: 1 ml. of 0.01 *N* iodine equals 0.398 mg. of ampicillin, or 0.388 mg. of Wy-4508.

For the pKa determinations, a potentiometric titration technique was used. The penicillins were dried in a desiccator over P_2O_5 under reduced pressure before use. Fresh solutions of the penicillins were prepared at 25°, and proper corrections for the purity of the drug were also made. The KOH solution was prepared and stored in a carbonate-free condition as described by Albert and Serjeant (17). The titration vessel was a water-jacketed, semiclosed container with inlets for electrodes, nitrogen gas, and a buret. The temperature of the solution was kept constant throughout the titration by circulating water from a constant temperature bath which was maintained at ±0.1° by a Haake thermostat. Nitrogen gas saturated with moisture was gently bubbled on the top of the solution, and a magnetic stirrer was used for fast equilibrium during titration. The titrant was added in 0.1-ml. portions from a buret calibrated in 0.01-ml. divisions. The pH changes during titrations were measured by a Corning model 12 research pH meter equipped with a glass-SC electrode system.

The calibration equations of Glasstone and Hammel (18) were used for computing the dissociation constants of the amphoteric penicillins, with elimination of the activity coefficient terms of the drug:

$$pK_1 = pH - \log\left(\frac{C}{A - c_{H^+}} - 1\right) \qquad \text{(Eq. 1)}$$

$$pK_2 = pH + \log\left(\frac{C}{B - c_{OH^-}} - 1\right) \qquad \text{(Eq. 2)}$$

In these equations, pH is defined as $-\log\alpha_{H^+}$, c_{H^+} and c_{OH^-} are the concentrations of hydrogen and hydroxyl ions in a solution consisting of C moles of penicillin to which A moles of acid or B moles of base have been added. The values of c_{H^+} and c_{OH^-} are derived from the pH measurements by assuming the activity coefficients of the hydrogen and hydroxyl ions to be equal to the mean values for hydrochloric acid or potassium hydroxide, respectively, at the same ionic strength. Within the pH range of 5–7, however, the term of c_{OH^-} may be neglected in Eq. 2. The activity coefficients for hydrochloric acid given by Robinson and Harned (19) were used. The concentration terms were properly corrected to account for the change in volume due to the addition of titrant.

RESULTS AND DISCUSSION

pH-Solubility Profile—At constant temperature (25°) and an ionic strength of 0.5, the pH-apparent solubility profiles of ampicillin and Wy-4508 were U-shaped as is shown in Fig. 1. With both of these amphoteric compounds there was a marked effect of pH on solubility. The minimum solubilities of ampicillin (3.98×10^{-2} *M*) and Wy-4508 (9.09×10^{-2} *M*) occurred at pH 4.9 and 5.0, respectively, and these pH values should represent the isoelectric points. Within the isoelectric region the ampholytes exist essentially as electrically neutral zwitterions (dipolar ions), and in the solid state their crystal lattice forces would be at a maximum, resulting in the least solubility. At lower or higher pH's however, the molecules exist mainly as ions (cations or anions), and the repulsive forces between such charged ions would be higher, thus increasing the solubility. Wy-4508 had more than twice the aqueous solubility of ampicillin over the entire pH range investigated, and this is believed due to the inborn structure or chemical nature of the former compound.

Effect of Salt and Organic Solvent on Solubility—The solubility of ampicillin at 25° in water and in mixtures of 80% of water and 20% of either methanol, ethanol, *n*-propanol, isopropanol, acetone, dimethylsulfoxide, *p*-dioxane or tetrahydrofuran (THF) in the presence of a varied amount of potassium chloride was investigated. Figure 2 shows that the solubility in water was 1.39 g./100 ml., but that at low and high salt concentrations a "salting-in" and "salting-out" effect occurred. Due to the presence of 20% organic solvents (particularly the alcohols), a relatively low intrinsic solubility was shown, but the solubility was noticeably increased with an increase in salt concentration, particularly in the presence of *p*-dioxane and THF.

At low salt concentrations, the attraction between the hydrated salt ions and the ionic groups of ampicillin increases, resulting in a "salting-in" effect. This interaction was shown to be enhanced in the solvent medium of low dielectric constant. At high salt concentrations, however, there is an opposite "salting-out" effect. In addition, the relatively low intrinsic solubility of ampicillin in water-organic solvent mixtures is probably related to a dehydration effect, *i.e.*, the water molecules surrounding the ionic groups of ampicillins interact mainly with the salt ions or organic solvent molecules, thereby decreasing the solute-solvent interaction. Thus the solubility of ampicillin would decrease.

Ampicillin is insoluble in almost all organic solvents (20). Its EtOAc/H_2O partition is essentially constant (coefficient, 0.044) and

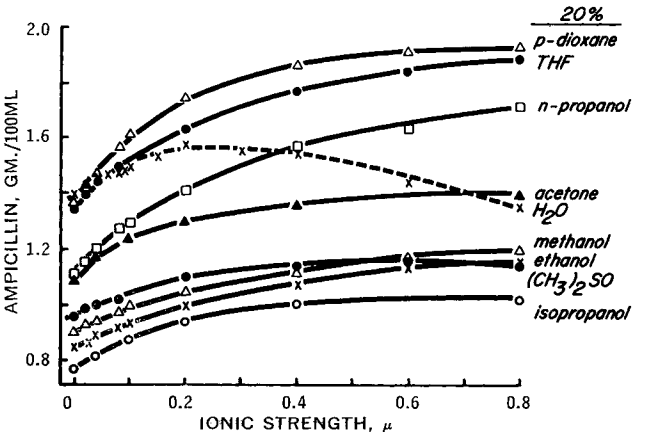
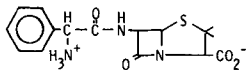
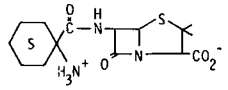
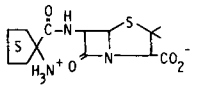
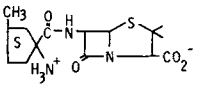
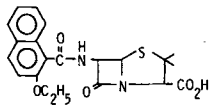
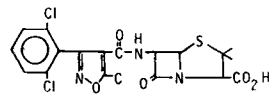


Figure 2—*Effect of ionic strength on the apparent solubility of ampicillin in 20% (v/v) organic solvent mixtures and in water (dotted line) at 25°.*

Table I—Dissociation Constants of Some Penicillins in Water at 25 ± 0.1°

Compd.	Structure	Concn., *M*	pK_1	pK_2	pI[a]
Ampicillin		6.52×10^{-3} 8.00×10^{-3}	2.66 ± 0.03 2.64 ± 0.05	7.25 ± 0.03 7.24 ± 0.02	4.95
Wy-4508		8.53×10^{-3}	2.68 ± 0.04	7.50 ± 0.02	5.09
Wy-7953		8.00×10^{-3} 8.00×10^{-3}	2.62 ± 0.05 2.61 ± 0.06	7.60 ± 0.05 7.62 ± 0.04	5.11
Wy-8542		5.86×10^{-3}	—	7.65	—
Nafcillin		6.00×10^{-3}	2.65[b]		
Dicloxacillin		6.12×10^{-3}	2.67[b]		

[a] pI = pH at isoelectric point. [b] The pK value was obtained by extrapolation to zero alcohol concentration from several pK values in water–ethanol mixtures.

is not significantly affected by pH. This is in contrast to the behavior of the monobasic type of penicillins. The solubility and partition behavior clearly indicate that ampicillin is more hydrophilic than lipophilic, behaving more like ionic than organic molecules in comparison with the monobasic penicillins.

Apparent Stability of Amphoteric Penicillins in Solution—The zwitterionic species of ampicillin and Wy-4508 are apparently the most stable toward acids and bases. This was done by measuring the remaining concentrations of intact penicillin iodometrically at constant temperature and pH and at an ionic strength of 0.5 (15). The initial rate for both ampicillin and Wy-4508 follow a pseudo-first-order kinetics, and the maximum stabilities lie on essentially the respective isoelectric points, pI = $(pK_1 + pK_2)/2$[2]. Figure 3 illustrates the pH-apparent stability profiles of ampicillin at 35° and Wy-4508 at 25°, respectively, in buffers of pH 2–8. The minimum rate for ampicillin lies on a pH of 4.85, which gives a half-life of

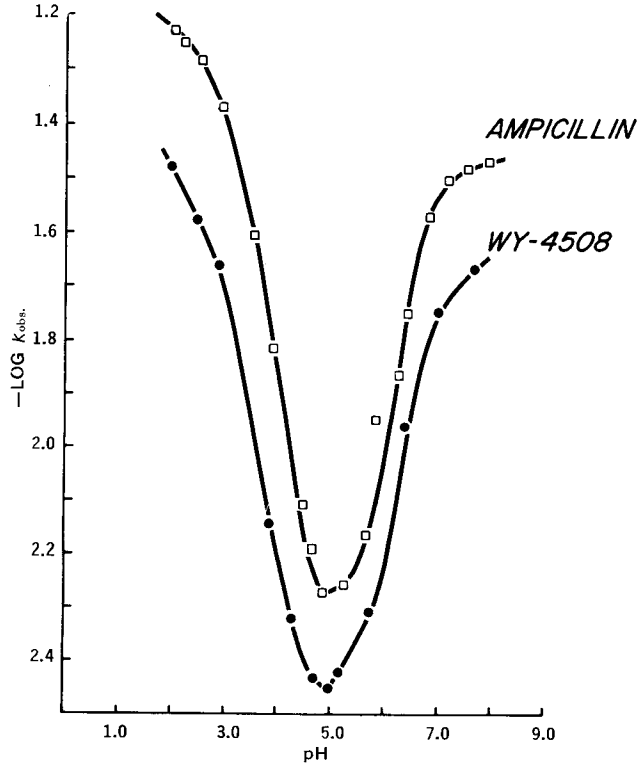


Figure 3—*The pH-apparent rate profiles of ampicillin at 35° and Wy-4508 at 25° in buffers of total ionic strength 0.5.*

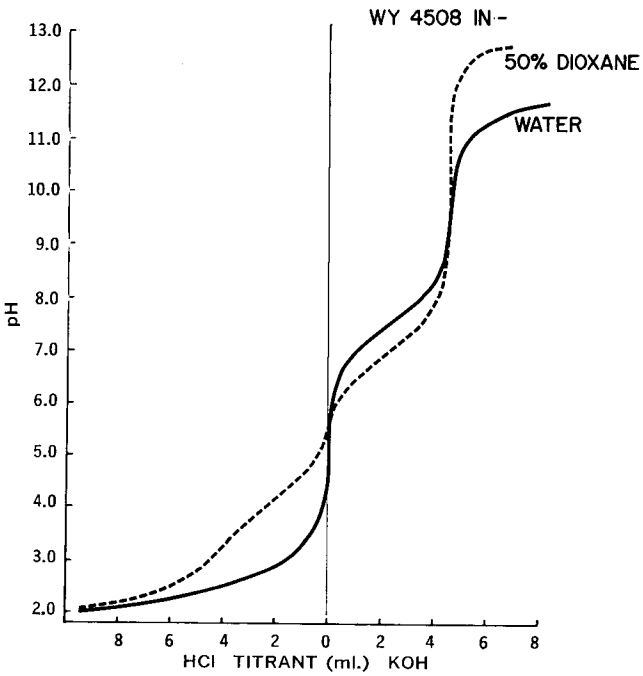


Figure 4—*Titration curve of Wy-4508 in water (solid line) and in 50% p-dioxane–water mixture (dashed line) at 25°.*

[2] The pI was 4.82 for ampicillin at 35° (Table V) and was 4.93 for Wy-4508 at 25° in water in the presence of 0.5 *M* KCl. The latter was determined by a solubility technique.

Table II—Determination of pK_1 of Ampicillin in Water at 25 ± 0.1°[a]

Titrant, ml.	pH	Concn., A × 10^3 M	γ[b]	$\log\left(\frac{C}{A - c_{H^+}} - 1\right)$	pK_1
1.0	3.278	2.067	0.954	0.626	2.652
1.1	3.240	2.272	0.949	0.574	2.666
1.2	3.195	2.476	0.947	0.530	2.665
1.3	3.160	2.680	0.945	0.485	2.675
1.4	3.120	2.883	0.944	0.447	2.673
1.5	3.073	3.086	0.942	0.415	2.658
1.6	3.043	3.288	0.940	0.378	2.665
1.7	3.020	3.490	0.939	0.338	2.681
1.8	2.955	3.692	0.937	0.322	2.643
1.9	2.943	3.893	0.936	0.287	2.656
2.0	2.920	4.094	0.935	0.254	2.666
2.2	2.862	4.495	0.932	0.202	2.660
2.4	2.820	4.894	0.929	0.156	2.673
2.6	2.771	5.291	0.926	0.095	2.676
2.8	2.730	5.687	0.924	0.060	2.680
3.0	2.695	6.082	0.922	−0.002	2.697

Av. = 2.66 ± 0.03 (pK_1)

[a] The initial ampicillin concentration was 8.0 × 10^{-3} M. The titrant was 0.2088 N HCl. [b] The activity coefficient for HCl was taken from *Reference* 19.

Table III—Determination of pK_2 of Ampicillin in Water at 25 ± 0.1°[a]

Titrant, ml.	pH	Concn., B × 10^3 M	$\log\left(\frac{C}{B} - 1\right)$	pK_2
1.0	6.750	2.039	0.460	7.210
1.1	6.800	2.241	0.403	7.203
1.2	6.855	2.443	0.349	7.204
1.3	6.912	2.644	0.298	7.210
1.4	6.965	2.844	0.246	7.214
1.5	7.040	3.044	0.201	7.241
1.6	7.095	3.244	0.154	7.249
1.7	7.140	3.443	0.108	7.248
1.8	7.175	3.642	0.063	7.238
1.9	7.220	3.840	0.018	7.238
2.0	7.270	4.039	−0.026	7.244
2.1	7.317	4.237	−0.071	7.246
2.2	7.360	4.434	−0.116	7.244
2.3	7.410	4.631	−0.162	7.248
2.4	7.462	4.828	−0.209	7.253
2.5	7.515	5.024	−0.257	7.258
2.6	7.567	5.220	−0.307	7.260
2.7	7.624	5.415	−0.358	7.265
2.8	7.680	5.611	−0.413	7.267
2.9	7.726	5.805	−0.459	7.256
3.0	7.800	6.000	−0.531	7.269

Av. = 7.24 ± 0.03 (pK_2)

[a] The initial ampicillin concentration was 8.0 × 10^{-3} M. The titrant was 0.206 N KOH.

130 hr., while that for Wy-4508 lies on a pH of 4.96, which gives a half-life of approximately 200 hr.

Surprisingly enough, 6-aminopenicillanic acid (6-APA) was shown to behave quite differently from ampicillin and Wy-4508. At low pH's (1–3), the initial rates for 6-APA were shown to follow a first-order kinetics, but this was not the case at pH > pK_2 (4.92) and no satisfactory pseudo-first-order kinetic rates were obtained. The difference may be due to the polymerization of 6-APA initiated by the 6-amino group towards the β-lactam ring of a second molecule. The degradation and polymerization behavior of 6-APA at higher pH's has been reported by Grant *et al.* (21) and Dennen (22). The maximum stability at pH of 8 (22) could be due to the polymerized form of 6-APA.

Dissociation Constants of Penicillins—The dissociation constants of ampicillin, Wy-4508, Wy-7953, and Wy-8542 were determined potentiometrically in water at 25°. The results are shown in Table I. Each of the pK values is an average of 16 to 21 individual values corresponding in most cases to 25–75% of neutralization. The pI (isoelectric point) for monocarboxylic and monoamino amopholytes is defined as $(pK_1 + pK_2)/2$.

In the present investigation, the dissociation constants were shown to be uniform, never deviating more than ± 0.05 pK unit. Tables II and III present one typical set of computed data on ampicillin in water at 25°. In several cases the apparent pK_1 of ampicillin was calculated simply by using the pH value as the hydrogen ion concentration in Eq. 1. The average pK values obtained in this manner were shown to be only 0.002 to 0.03 higher than the true values.

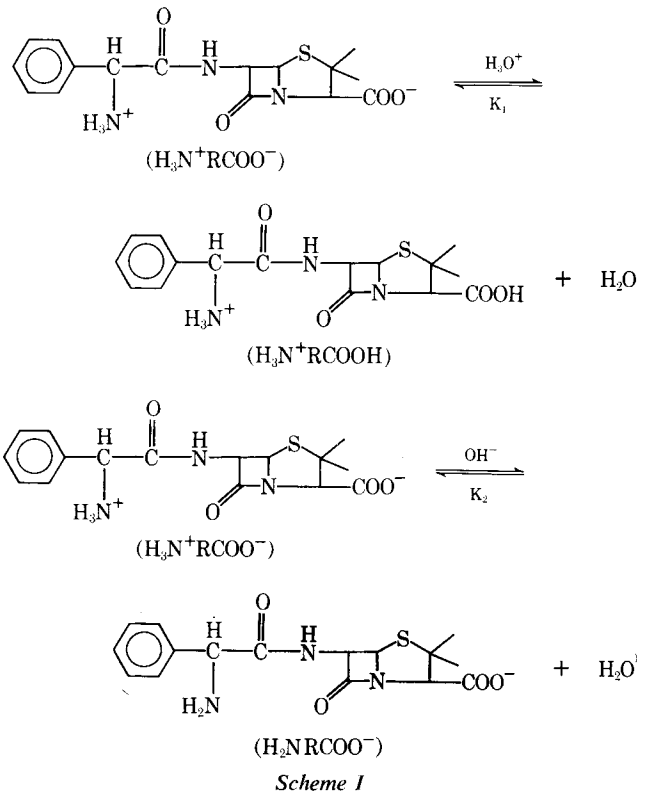
Figure 4 is a titration curve obtained for Wy-4508. The titration with acid is a process of proton association (proton gain) from zwitterion to cation; while that with base is a process of proton dissociation (proton lost) from zwitterion to anion, as is shown in Scheme I. The corresponding dissociation constants K_1 and K_2 may thus be expressed as:

$$K_1 = \frac{(H^+)\,(H_3N^+RCOO^-)}{(H_3N^+RCOOH)} \qquad \text{(Eq. 3)}$$

$$K_2 = \frac{(H^+)\,(H_2NRCOO^-)}{(H_3N^+RCOO^-)} \qquad \text{(Eq. 4)}$$

Data obtained in these laboratories and those in the literature (23, 24), indicate that regardless of the side chain group, all penicillins possess a pK_1 (carboxyl group) 2.65 ± 0.1 in water at 25°. This was believed due to the large distance (> 6 atoms) between the side chain group and the carboxyl ion of penicillins. The dissociation constant of the amino groug of amphoteric penicillins, however, is definitely influenced by the adjacent nonpolar side-chain group. The pK_2 value in water at 25° increases from 7.24 for ampicillin to 7.65 for an alkyl-substituted aminoalicyclic penicillin (Wy-8542). Conceivably this difference is due to the positive inductive effect of the side chain, which seems to correlate with the pK_2 increases, as is shown in the following order (see Scheme II).

Effect of Organic Solvent on Dissociation Constants—The dissociation constants of ampicillin in 50% *p*-dioxane and of Wy-4508 in 50% each of *p*-dioxane, dimethylformamide (DMF) and *n*-propanol at 25° are listed in Table IV. A decrease in the dielectric con-



Scheme I

Table IV—Dissociation Constants of Ampicillin and Wy-4508 in 50% Organic Solvents at 25°

Compd. (solvent)	Original Concn., *M*	pK_1	pK_2	ΔpK_1[a]	ΔpK_2[b]
Ampicillin in 50% *p*-dioxane	6.52×10^{-3}	4.15 ± 0.03	6.97 ± 0.02	1.49	−0.27
Wy-4508 in 50% *p*-dioxane	8.53×10^{-3}	4.16 ± 0.03	7.04 ± 0.03	1.48	−0.46
50% dimethylformamide	8.53×10^{-3}	4.14 ± 0.03	7.22 ± 0.02	1.46	−0.28
50% *n*-propanol	8.53×10^{-3}	3.70 ± 0.03	6.86 ± 0.02	1.02	−0.64

[a] Difference between pK_1 in solvent mixture and in water. [b] Difference between pK_2 in solvent mixture and in water.

stant of the reaction mixture resulted in a decrease of both the acidity and basicity of ampicillin and Wy-4508, that is the pK_1 increased and the pK_2 decreased in both instances. Also, the organic solvent had a greater effect on the pK_1 than on the pK_2. Accordingly, the ΔpK_1 values were large and positive while the ΔpK_2 values were small and negative in all the solvents investigated.

Figure 4 depicts the titration curve obtained for Wy-4508 in 50% *p*-dioxane and in water. A large difference is observed in the acid side compared to that noted in the base area. For ampicillin, the titration curve in these solvents was similar.

The addition of 10 and 20% of formaldehyde (HCHO) to an aqueous solution of ampicillin caused essentially no change of the titration curve (or pH) on the acid side, but a considerably different type of curve resulted in the course of neutralization by base, as shown in Fig. 5. It is reasonable to assume that formaldehyde must strongly interact with the amino group of ampicillin to form methylol derivatives. Such interaction is common in the amino acids. In such an instance the titration by base no longer involves a neutralization of the charged amino group.

The dissociation constants for salts of monobasic penicillins (nafcillin and dicloxacillin) were also determined. They were obtained by titrations with HCl in water-ethanol mixtures at 25° and calculating the corresponding pK_1 values in water by extrapolating in the usual manner to zero alcohol concentration (25). The respective values for nafcillin and dicloxacillin were 2.65 and 2.67 (Table I).

Effect of Temperature on the pK Values of Ampicillin—The data on the pK_1 and pK_2 values of ampicillin in water at several temperatures are listed in Table V. Within the experimental temperature range of 5 to 45° the pK_1 was virtually temperature-insensitive but a significant temperature coefficient was observed for the pK_2. The apparent heat of reaction, $\Delta H°$, calculated from Eq. 5

$$\Delta H° = \frac{R \cdot \ln K}{1/T} \qquad \text{(Eq. 5)}$$

was −0.8 ± 0.04 kcal./mole for the carboxyl group and +11.5 ± 0.05 kcal./mole for the charged amino group. For amino acids and peptides the apparent heat of dissociation reactions is reported from −1300 to +1800 cal. for reaction $RCOO^- + H^+ = RCOOH$ and from 10000 to 12800 cal. for reaction $RNH_3^+ = RNH_2 + H^+$ (26). Accordingly, the values obtained for ampicillin were within the range of heats of reaction of amino acids. Also, from the above data, the formation of ampicillin zwitterions from uncharged species[3] is an exothermic reaction and the net heat of reaction is approximately 10.7 kcal./mole.

Physicochemical Properties and Biological Activity of Ampicillin and Wy-4508—The high m.p. (with decomposition) of ampicillin (202–203°) and Wy-4508 (182–183°), along with the previously discussed solubility behavior, dissociation (ionization) reaction, and the heat of dissociation of ampicillin, indicate the close relation of these drugs to the aliphatic amino acids (26). Regardless of the penicillin nucleus and the side chain group, ampicillin, Wy-4508 and possibly other similar types of amino penicillins exist essentially as zwitterions at neutral pH. Recently, James and Hall (28) reported that ampicillin exists as a zwitterion even in the crystalline state. The observed biological activity of ampicillin and Wy-4508 accordingly is no doubt directly related to their zwitterionic nature.

The mode of action of the penicillins is by no means simple. Knox (29) hypothesized that the Gram-negative bacteria have a receptive surface which is attacked only when in close contact with one of the active sites of the penicillin side chain (presumably the NH_2 group in ampicillin), while the Gram-positive bacteria are attacked only by the carboxyl group. According to this "close contact" hypothesis, the Gram-negative activity of all the amino penicillins may depend on the attachment of susceptible sites in the organisms to charged amino groups, while the Gram-positive activity of monobasic and amphoteric penicillins may depend similarly on attachment to the β-lactam and ionic carboxyl groups. This

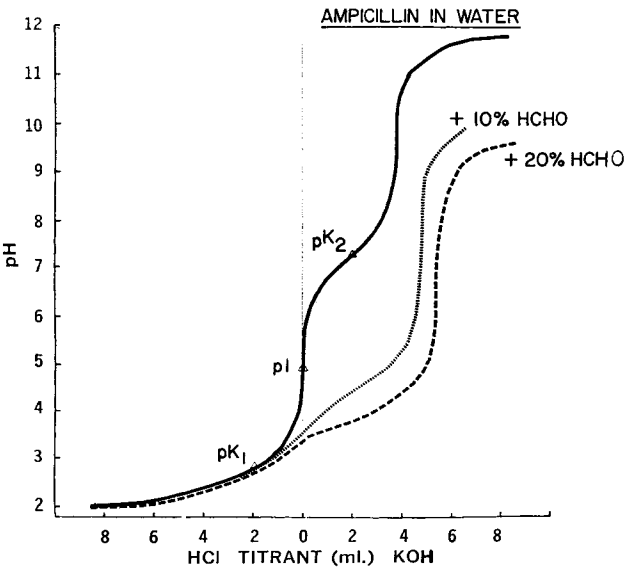
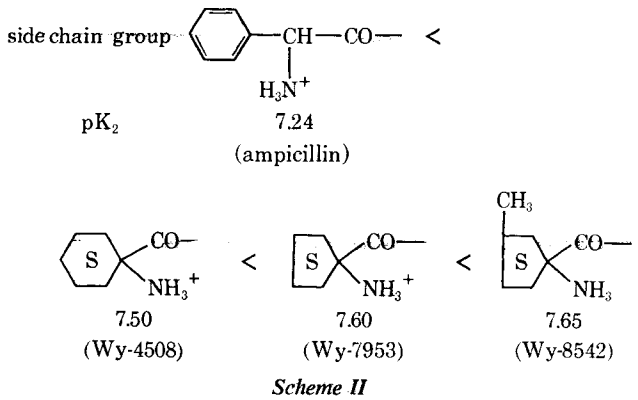


Figure 5—*Titration curves of ampicillin at 25° in water (solid line), 10% formaldehyde (dotted line), and 20% formaldehyde (dashed line).*



Scheme II

[3] Normally amino acids zwitterions are in equilibrium with their uncharged species in solution at a neutral pH. A rigorous treatment of this matter is given by Edsall and Blanchard (27).

Table V. Effect of Temperature of pK_1 and pK_2 Values of Ampicillin in Water[a]

Temperature, °C	pK_1	pK_2
5	2.62 ± 0.04	—
10	—	7.67 ± 0.04
15	2.63 ± 0.03	—
20	—	7.39 ± 0.03
25	2.66 ± 0.03	7.24 ± 0.02
	2.64 ± 0.05	7.25 ± 0.03
30	2.67 ± 0.03	—
35	2.68 ± 0.04	6.96 ± 0.04
	2.69 ± 0.05	
45	2.73 ± 0.05	6.72 ± 0.05
35 (u = 0.5)	2.60 ± 0.02	7.05 ± 0.04

[a] The original ampicillin concentration used for these runs was 8.00×10^{-3} *M*. The titrants were 0.2088 *N* HCl and 0.2060 *N* KOH.

type of drug-bacteria interaction presumably leads to a drug-substrate complex formation which destroys the normal metabolic function of the bacteria. The stereospecificity and electron density of the side chain group, as well as the mutagenic activity of a particular drug, are also important factors for overall penicillin activity.

In any case, the broad antimicrobial activity of the amphoteric penicillins is probably due in a large degree to the zwitterionic nature of these compounds. Rolinson and Stevens (7) demonstrated that ampicillin is about 10 times more active in a pH 5.5 than in a pH 8 buffer toward strains of *Escherichia coli* and *Streptococcus faecalis*, suggesting that the zwitterion is the most active molecular species. Although Wy-4508 has relatively high *in vitro* MIC values, the high solubility and lower serum binding result in an efficient *in vivo* performance (30, 31). Rosenman *et al.* (10) found that after a single oral dose, Wy-4508 gave about four times higher mean blood serum concentrations than ampicillin at 30 min. and about five times higher after 1 hr. This rapid attainment of a higher blood level, probably related to the solubility of the drug, may be an important therapeutic advantage *in vivo*.

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