Ionization Constants of Cephalosporin Zwitterionic Compounds

WILLIAM H. STRENG ˣ, H. E. HUBER, JOYCE L. DeYOUNG, and M. A. ZOGLIO

Abstract □ The microionization constants for two zwitterionic compounds were determined by incorporating two experimental techniques. These compounds have chromophoric changes dependent upon the solution pH. By combining the spectrophotometric measurements with potentiometric measurements, all four microionization constants were calculated. The method used is completely general and is applicable to all diprotic compounds that exhibit this spectrophotometric behavior. The observed pKa's had differences of at most 1.2 units for either compound and were in the 1–4 range. A comparison of the results with each compound and similar compounds indicates that the values are reasonable.

Keyphrases □ Ionization constants—cephalosporin zwitterionic compounds, calculated by combining spectrophotometric and potentiometric measurements □ Cephalosporin zwitterionic compounds—ionization constants calculated by combining spectrophotometric and potentiometric measurements □ Zwitterionic compounds, cephalosporins—ionization constants calculated by combining spectrophotometric and potentiometric measurements

The ionization constants of acids and acid salts have been determined by several methods and investigators since the late 1800's. Albert and Serjeant (1) discussed the potentiometric, spectrophotometric, and conductometric methods. Compounds containing one acidic proton generally have uncomplicated calculations, and determination of the ionization constants is a simple matter using any of the three methods.

BACKGROUND

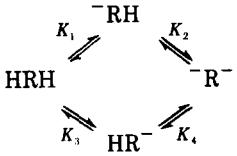
If two acidic protons are present, four equilibria need to be considered instead of two (Scheme I) and the determination becomes more complex. If HRH is a symmetrical molecule, K_1 and K_3 will be equal as will K_2 and K_4; this is not the case for unsymmetrical molecules, and elucidating the constants becomes a problem. Most methods result in the calculation of two constants instead of four (2). It is easy to show that these macroscopic constants are related to the microscopic constants as follows:

$$K_{13} = K_1 + K_3 \qquad \text{(Eq. 1)}$$

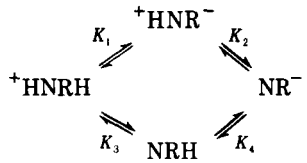
$$\frac{1}{K_{24}} = \frac{1}{K_2} + \frac{1}{K_4} \qquad \text{(Eq. 2)}$$

where K_{13} and K_{24} are the macroscopic constants and K_1, K_2, K_3, and K_4 are the microscopic constants. Obviously, if K_1 is much larger than K_3 or if K_2 is much smaller than K_4, the macroscopic constant K_{13} will equal K_1 and K_{24} will equal K_2. In this case, there will be little $[HR^-]$ in solution at any pH. However, if K_1 is of similar magnitude to K_3 and if K_2 is of similar magnitude to K_4, $[HR^-]$ and $[^-RH]$ will both be appreciable. Under these conditions, it is necessary to determine each microscopic constant to calculate individual species concentrations.

Included among the diacidic compounds represented by Scheme I are amphoteric compounds, *i.e.*, those that can behave as either a



Scheme I



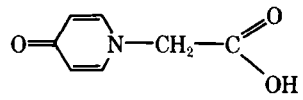
Scheme II

proton acceptor or donor. As depicted in Scheme II, these compounds can simultaneously contain positive and negative charges and are, as such, zwitterions. The relative concentrations of zwitterion and uncharged molecules will depend on the magnitudes of K_1 and K_3 or K_2 and K_4, *i.e.*, $[^+HNR^-]/[NRH] = (K_1/K_3) = (K_4/K_2)$.

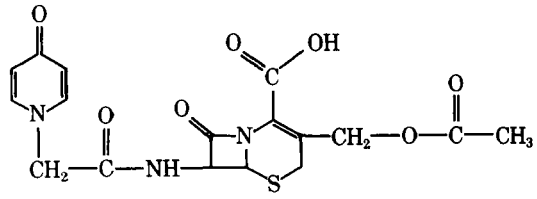
Several methods have been reported for determining the microscopic constants of zwitterionic compounds utilizing spectrophotometric (3–5) and potentiometric (6) techniques. Each method has its own restrictions, from requiring accurate measurements over wide pH ranges to preparing esters and assuming that they will not influence other equilibria.

As a means of circumventing some undesirable limitations, a method has been developed that combines spectrophotometric and potentiometric measurements to calculate the constants. Two constants can be obtained directly from calculations while the remaining two can be determined by either graphical techniques or further calculations utilizing one of the first two constants.

Measurements were made on two compounds which form zwitterions, pyridoneacetic acid (I) and the sodium salt of 3-[(acetyloxy)-methyl] -8-oxo-7- [[(4-oxo-1(4*H*)-pyridinyl)acetyl]amino] -5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (II). Compound II is a cephalosporin containing the pyridone moiety. Each behaves as an amphoteric compound for which the carboxylic acid group acts as a proton donor and the nitrogen in the pyridone ring acts as a proton acceptor.



I



II

EXPERIMENTAL

Apparatus and Equipment—Potentiometric measurements were made using the system diagrammed in Fig. 1. Four holes were cut into a number 13½ rubber stopper to hold two electrodes, a 50-ml buret, and a nitrogen tube. The two electrodes[1], a standard calomel electrode and a glass electrode, were attached to a pH meter[2] which could be operated in expanded scale mode. A glass extension (12 cm) was fused to a 50-ml buret so that the tip of the buret would be below the surface of the solution. Water-saturated nitrogen was bubbled into the so-

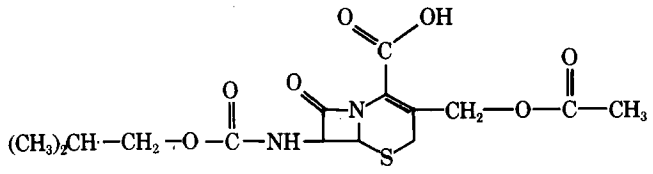
[1] Beckman calomel internal with quartz junction reference electrode 39400 and Beckman pH (glass) electrode 39301.
[2] Beckman Century SS-1.

lution to stir it and to keep other gases from being dissolved. The stopper was fit into a 250-ml beaker.

Spectrophotometric measurements were made with a UV–visible spectrophotometer[3].

Solution Preparation and Measurement—Solutions were prepared for the spectrophotometric measurements using 0.05 *M* ionic strength buffers in the pH range of 1.72–2.97 for II and 0.05 and 0.2 *M* ionic strength buffers in the pH range of 2.56–3.93 for I. The concentrations of II and I were approximately 4×10^{-5} *M*.

Two chromophores were assigned to the pyridone ring. A peak at $\lambda = 262$ nm was attributed to the unprotonated nitrogen; that at $\lambda = 245$ nm was attributed to the protonated nitrogen. Spectra were obtained over the 350–225-nm range using the appropriate buffer solution as a reference. Other studies have shown that, over the pH range used, the compounds are stable for the duration of the experiment. Furthermore, measurements on 3-[(acetyloxy)methyl]-7-[[(2-methylpropoxy)carbonyl]amino] -8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (III), which is similar to II but has only the β-lactam chromophore, indicated that the β-lactam ring absorbance at $\lambda = 262$ nm changes 3% at most over this pH range. Therefore, any absorbance changes can be considered to be due to changes in the concentration of the unprotonated nitrogen species.



III

Solutions of the sodium salt of II were prepared in the $1–2.3 \times 10^{-2}$ *M* concentration range; solutions of I were prepared at a concentration of 2×10^{-3} *M*. These solutions were titrated with hydrochloric acid solution to doubly protonate the molecules and then back-titrated with potassium hydroxide solutions as diprotic compounds. The potassium hydroxide solutions were standardized with potassium acid phthalate, and the hydrochloric acid solutions were standardized with potassium hydroxide solutions. For the titration of I, the acid and base concentrations were 0.023 *N* hydrochloric acid and 0.003 *N* potassium hydroxide; for II, both titrant solutions were 0.15 *N*.

THEORETICAL

In the following derivations, {}, [], *Y*, and *K* represent activity, concentration, activity coefficient, and equilibrium constant on a molar scale, respectively. The total concentration of base added as titrant, halide, and acid being titrated are given by [B], [X], and [A].

Exact Solution to Scheme II—An expression can be derived that relates the individual equilibrium constants in Scheme II to the total acid concentration, base concentration, and pH. This exact solution is as follows.

The equilibrium constants are given by:

$$K_1 = \frac{\{^+HNR^-\}\{H^+\}}{\{^+HNRH\}} = \frac{[^+HNR^-]\{H^+\}}{[^+HNRH]}\frac{Y_{^+HNR^-}}{Y_{^+HNRH}} \quad \text{(Eq. 3a)}$$

$$K_2 = \frac{\{NR^-\}\{H^+\}}{\{^+HNR^-\}} = \frac{[NR^-]\{H^+\}}{[^+HNR^-]}\frac{Y_{NR^-}}{Y_{^+HNR^-}} \quad \text{(Eq. 3b)}$$

$$K_3 = \frac{\{NRH\}\{H^+\}}{\{^+HNRH\}} = \frac{[NRH]\{H^+\}}{[^+HNRH]}\frac{Y_{NRH}}{Y_{^+HNRH}} \quad \text{(Eq. 3c)}$$

$$K_4 = \frac{\{NR^-\}\{H^+\}}{\{NRH\}} = \frac{[NR^-]\{H^+\}}{[NRH]}\frac{Y_{NR^-}}{Y_{NRH}} \quad \text{(Eq. 3d)}$$

$$K_\omega = \{H^+\}\{OH^-\} \quad \text{(Eq. 3e)}$$

The material balance for the acid is:

$$[A] = [^+HNRH] + [^+HNR^-] + [NRH] + [NR^-] \quad \text{(Eq. 4)}$$

and the electrical balance is:

$$[B] + [H^+] + [^+HNRH] = [OH^-] + [X] + [NR^-] \quad \text{(Eq. 5)}$$

[3] Beckman Acta III or Cary 17.

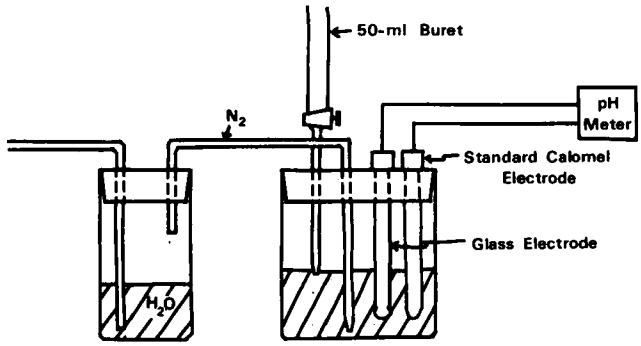


Figure 1—*Apparatus used for potentiometric measurements.*

By using the equilibrium constants, the material balance equation can be expressed in terms of $[NR^-]$:

$$[A] = \frac{[NR^-]\{H^+\}^2}{K_3K_4}\frac{Y_{NR^-}}{Y_{^+HNRH}} + \frac{[NR^-]\{H^+\}}{K_2}\frac{Y_{NR^-}}{Y_{^+HNR^-}} + \frac{[NR^-]\{H^+\}}{K_4}\frac{Y_{NR^-}}{Y_{NRH}} + [NR^-] \quad \text{(Eq. 6)}$$

In the same manner, the electrical balance equation can be solved for $[NR^-]$:

$$[NR^-] = \{[B] + [H^+] - [OH^-] - [X]\} \Big/ \left\{1 - \frac{\{H^+\}^2}{K_3K_4}\frac{Y_{NR^-}}{Y_{^+HNRH}}\right\} \quad \text{(Eq. 7)}$$

Substituting Eq. 7 into Eq. 6 for $[NR^-]$ and realizing that [X] will equal [A] for this type of compound result in:

$$[A] = \left\{\frac{[B] + [H^+] - [OH^-] - [A]}{K_3K_4 - \{H^+\}^2\frac{Y_{NR^-}}{Y_{^+HNR^-}}}\right\}\left\{K_3K_4 + K_3\{H^+\}\frac{Y_{NR^-}}{Y_{NRH}} + \frac{\{H^+\}}{K_2}\frac{Y_{NR^-}}{Y_{^+HNR^-}} + \frac{\{H^+\}^2}{K_3K_4}\frac{Y_{NR^-}}{Y_{^+HNRH}}\right\} \quad \text{(Eq. 8)}$$

Equation 8 can be rearranged to the form:

$$\alpha = K_1\frac{Y_{^+HNRH}}{Y_{^+HNR^-}}\beta + K_3\frac{Y_{^+HNRH}}{Y_{NRH}}\beta + K_3K_4\frac{Y_{^+HNRH}}{Y_{NR^-}}\gamma \quad \text{(Eq. 9)}$$

where:

$$\alpha = [B]\{H^+\}^3 + \frac{\{H^+\}^4}{Y_{H^+}} - \frac{K_\omega\{H^+\}^2}{Y_{OH^-}}$$

$$\beta = [A]\{H^+\}^2 - [B]\{H^+\}^2 - \frac{\{H^+\}^3}{Y_{H^+}} + \frac{K_\omega\{H^+\}}{Y_{OH^-}}$$

$$\gamma = 2[A]\{H^+\} - [B]\{H^+\} - \frac{\{H^+\}^2}{Y_{H^+}} + \frac{K_\omega}{Y_{OH^-}}$$

It is not possible to solve Eq. 9 directly for the equilibrium constants because the first two terms on the right have the same coefficient, β. If one equilibrium constant is known, it is then possible to use this equation to solve for the remaining constants.

Combining Measurements to Solve Scheme II—The individual constants (microconstants) in Scheme II can be determined if the compound has a chromophore dependent upon the pH. The spectrophotometric information can be combined with potentiometric data to obtain the constants. In this derivation, it is assumed that protonation or deprotonation of the nitrogen atom is responsible for the observed spectral changes. Therefore, from the spectrophotometric data, the total concentration of protonated nitrogen species, [Z], can be determined:

$$[Z] = [^+HNRH] + [^+HNR^-] \quad \text{(Eq. 10)}$$

Substituting Eq. 10 into the material balance equation, Eq. 4, and expressing [NRH] in terms of $[NR^-]$ result in:

$$[A] = [NR^-] + \frac{[NR^-]\{H^+\}}{K_4}\frac{Y_{NR^-}}{Y_{NRH}} + [Z] \quad \text{(Eq. 11)}$$

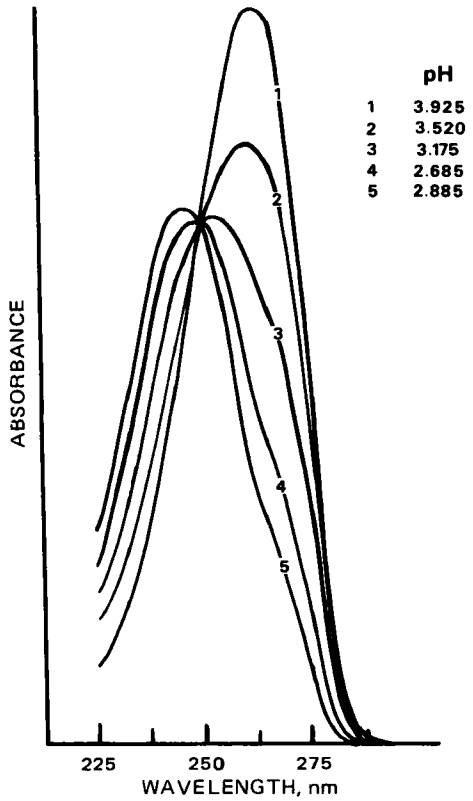


Figure 2—*Spectral curves for I.*

Inserting Eq. 7 into Eq. 11 for $[NR^-]$ gives:

$$[A] = \left\{ \frac{[B] + \{H^+\} - [OH^-] - [A]}{K_3K_4 - \{H^+\}^2 \frac{Y_{NR^-}}{Y_{+HNRH}}} \right\} \left\{ 1 + \frac{\{H^+\}Y_{NR^-}}{K_4 Y_{NRH}} \right\} + [Z] \qquad \text{(Eq. 12)}$$

Equation 12 can be rearranged as shown by Eq. 13 to obtain an expression that can be used to determine K_3 and K_4:

$$\{H^+\}^2 \frac{Y_{NR^-}}{Y_{+HNRH}} \{[Z] - [A]\} = K_4\{H^+\} \frac{Y_{NR^-}}{Y_{NRH}} \left\{ [B] + \frac{\{H^+\}}{Y_{H^+}} - \frac{K_\omega}{\{H^+\}Y_{OH^-}} - [A] \right\} + K_3K_4 \left\{ [B] + \frac{\{H^+\}}{Y_{H^+}} - \frac{K_\omega}{\{H^+\}Y_{OH^-}} - 2[A] + [Z] \right\} \qquad \text{(Eq. 13)}$$

Equation 13 can be solved either by graphical techniques or by simultaneous equations for K_3 and K_4. Therefore, Eq. 13 is expressed as follows:

$$\delta = K_4\epsilon + K_3K_4\xi \qquad \text{(Eq. 14)}$$

A plot of δ/ξ *versus* ϵ/ξ will be linear with a slope of K_4 and δ/ξ will equal K_3K_4 at $\epsilon/\xi = 0$. Also, the value of ϵ/ξ at $\delta/\xi = 0$ will equal $-K_3$.

Equation 14 may be solved using simultaneous equations by utilizing titrimetric and spectrophotometric data obtained at each of two pH values. By labeling the two sets of data as 1 and 2, K_4 and K_3 can be calculated according to:

$$K_4 = \frac{\frac{\delta_1}{\xi_1} - \frac{\delta_2}{\xi_2}}{\frac{\epsilon_1}{\xi_1} - \frac{\epsilon_2}{\xi_2}} \qquad \text{(Eq. 15)}$$

$$K_3 = \frac{\delta_1 - K_4\epsilon_1}{K_4\xi_1} \qquad \text{(Eq. 16)}$$

Substituting the value of K_3 or K_4, calculated from Eq. 13, into Eq. 9 will permit the determination of K_1 and K_2. Again, K_1 and K_2 can be determined using either graphical or simultaneous equation techniques. A plot of α/γ *versus* β/γ should be linear with an intercept at $\beta/\gamma = 0$ of K_3K_4 while the slope will equal $K_1 + K_3$. Combining K_4 with K_3K_4 will result in K_3, which can then be used to obtain K_1 from the slope.

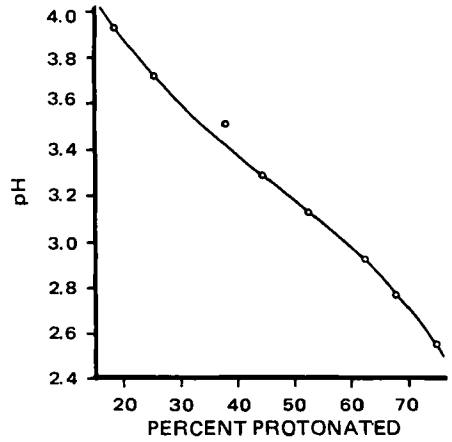


Figure 3—*Percentage of species containing a protonated nitrogen for I.*

There is a relationship between the four equilibrium constants, and it is easy to show that K_1K_2 must equal K_3K_4. Therefore, when knowing K_1, K_3, and K_4, it is possible to determine K_2 according to the relationship $K_2 = K_3K_4/K_1$. Solving Eq. 9 using simultaneous equations results in equations similar to Eqs. 15 and 16. The values calculated are the same as those obtained by the graphical method; *i.e.*, the product K_3K_4, the sum $K_1 + K_3$, and the individual constants can be determined as before.

RESULTS AND DISCUSSION

Since the results for both compounds are similar, only those for I will be used to demonstrate the procedure. In Fig. 2, the spectral curves as a function of pH are shown for the solution of 0.2 *M* ionic strength; these curves are not affected by ionic strength changes over the 0.05–0.2 *M* range. It can be seen that the chromophore occurring at $\lambda = 262$ nm is a basic form while that at $\lambda = 245$ nm is an acidic form. From these curves, the concentration of the protonated nitrogen species can be determined as a function of pH. A plot of the percent protonated nitrogen species *versus* pH is given in Fig. 3.

A typical potentiometric titration curve for I is given in Fig. 4. From this curve, it can be seen that there is no break at the first equivalence point and that there is a very large break at the second. An excess of hydrochloric acid was added to the solution prior to the titration with potassium hydroxide to assure that the I was entirely in its diprotic form. Therefore, an initial volume of base is needed to titrate the excess acid and the titration of I does not start until the volume at the mark indicated on the figure is reached.

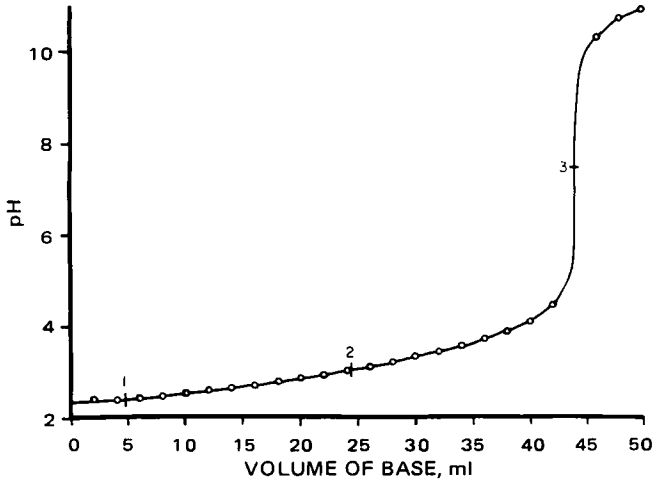


Figure 4—*Potentiometric titration curve for I. Key: 1, titration of I starts here (4.76 ml); 2, first equivalence point (24.38 ml); and 3, second equivalence point (43.99 ml).*

Table I—Ionization Constants of I and II

	$\frac{K_1}{Y_{+-}}$	$K_2\ Y_{+-}$	K_3	K_4
		I		
	1.065×10^{-2}	4.89×10^{-4}	4.57×10^{-3}	1.14×10^{-3}
pKa	1.97	3.31	2.34	2.94
		II		
	2.01×10^{-2}	1.45×10^{-3}	1.04×10^{-2}	2.79×10^{-3}
	2.00×10^{-2}	1.54×10^{-3}	9.96×10^{-3}	3.10×10^{-3}
	1.97×10^{-2}	1.76×10^{-3}	1.17×10^{-2}	2.97×10^{-3}
Average	1.99×10^{-2}	1.58×10^{-3}	1.07×10^{-2}	2.95×10^{-3}
pKa	1.70	2.80	1.97	2.53

	$[^+HNR^-]/[NRH]$
I	2.330
II	1.864

From the potentiometric titration, values of the pH for a given volume of base addition (taking account of the excess) were obtained and combined with the spectrophotometric data to calculate K_3 and K_4 using Eq. 14. The most consistent values using simultaneous equations were those for K_4. The remaining equilibrium constants could then be determined by Eq. 9. A plot of α/γ *versus* β/γ for I is shown in Fig. 5. It can be seen that the relationship is linear and can be treated as explained, resulting in values for K_1, K_2, and K_3.

In performing the calculations, an attempt was made to correct for activity effects by utilizing the Davies equation (7) to approximate the single ion activities:

$$-\log Y_i = AZ_i^2 \left\{ \frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I \right\} \qquad \text{(Eq. 17)}$$

where Y_i is the single ion activity coefficient, A is a parameter whose value at 25° is 0.5092 on a molar scale, Z_i is the charge of ion i, and I is the molar ionic strength. The advantage of this equation over the extended Debye–Hückel law is that it does not require the use of any ion size parameters.

An approximation was not made for the activity of the zwitterionic species because there is no expression comparable to Eq. 17. It was not expected that Y_{+-}[4] would deviate greatly from the ideal value of 1. It also was assumed that the uncharged species had an activity coefficient of 1, a reasonable assumption within the limits of the experiment.

The calculated values for the equilibrium constants are listed in Table I. All calculations were performed on a computer[5]. A computer program was written which calculated δ, ϵ, and ξ in Eq. 14 and α, β, and γ in Eq. 9 for 10 sets of data. The values of δ, ϵ, and ξ were combined according to Eqs. 15 and 16 to determine K_4 and K_3; α, β, and γ were output to determine the remaining constants graphically. The consistency of the calculations for several concentrations can be seen in the data for II.

A comparison of the K's with literature values is not directly possible because there have been no ionization constants of comparable compounds determined in a manner resulting in the microscopic constants. It would appear that they are reasonable if the macroscopic constants for I, as given by Eqs. 1 and 2, are compared with those listed as proton gained and proton lost for the pyridinecarboxylic acids in Ref. 1[6]. This work, originally reported by Lumme (8), gave values of about 2 and 4.8 for these pKa's, whereas the pK_{13} and pK_{24} values for I are 1.8 and 3.5.

One review article gave a value of 1.75 for the carboxylic acid group on 7-aminocephalosporanic acid (9). This value is in good agreement with the carboxylic acid equilibrium constant, pK_1, for II, which is 1.70. From structural considerations, it would be expected that the pKa's associated with the deprotonation of the nitrogen should be nearly the same for I and II. As shown in Table I, the corresponding K_a values differ by only a factor of two, which is in agreement with this assumption.

By knowing the equilibrium constants, species profiles can be calculated:

$$\%[^+HNRH] = 100 \Big/ \left\{ 1 + \frac{K_1}{[H^+]} + \frac{K_3}{[H^+]} + \frac{K_3K_4}{[H^+]^2} \right\} \qquad \text{(Eq. 18a)}$$

$$\%[NRH] = 100 \Big/ \left\{ 1 + \frac{[H^+]}{K_3} + \frac{K_4}{[H^+]} + \frac{K_1}{K_3} \right\} \qquad \text{(Eq. 18b)}$$

$$\%[^+HNR^-] = 100 \Big/ \left\{ 1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]} + \frac{K_3}{K_1} \right\} \qquad \text{(Eq. 18c)}$$

$$\%[NR^-] = 100 \Big/ \left\{ 1 + \frac{[H^+]}{K_2} + \frac{[H^+]}{K_4} + \frac{[H^+]^2}{K_3K_4} \right\} \qquad \text{(Eq. 18d)}$$

The equations are written in their most general form. It is possible to express the percentages of one species as percentage functions of the other species (10) to simplify these equations.

The species profiles given in Figs. 6 and 7 were obtained using Eqs. 18a–18d to calculate the percent of species. According to the results in Table I, K_1 is larger than K_3 for both compounds. Consequently,

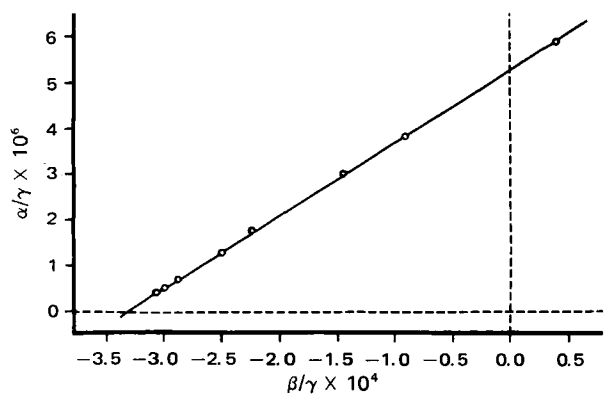


Figure 5—*Plot of* α/γ versus β/γ *for I.*

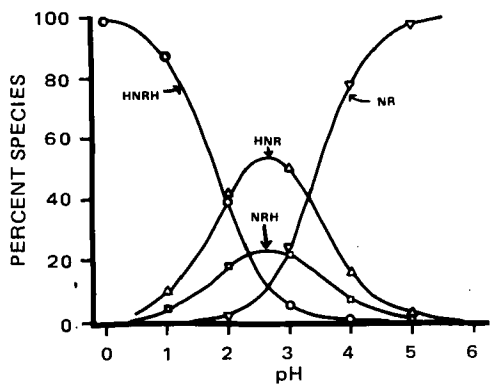


Figure 6—*Species profile for I.*

[4] The activity coefficient for the zwitterionic species.
[5] Digital Equipment Corp. RSTS-PDP-11.
[6] See Table 8.13.

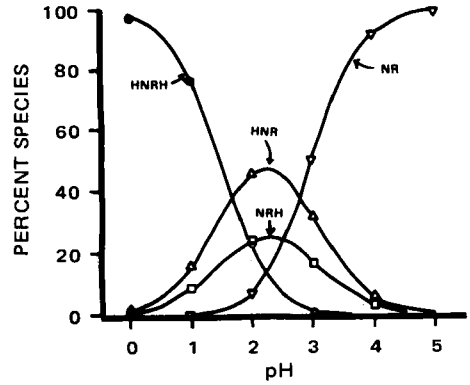


Figure 7—*Species profile for II.*

more zwitterions than unchanged molecules will be present in solution. This can be seen in the species profiles for I and II, for which the ratios of zwitterions to uncharged molecules are 2.33 and 1.864, respectively. The maximum concentration of zwitterionic species occurs at a pH equal to the average of pK_1 and pK_2 or pK_3 and pK_4, *i.e.*:

$$pH = \frac{pK_1 + pK_2}{2} = \frac{pK_3 + pK_4}{2} \qquad \text{(Eq. 19)}$$

For I, the maximum concentration occurs at pH 2.64; for II, it occurs at pH 2.25.

CONCLUSION

The ionization constants calculated for these compounds are reasonable when compared with each other and literature values for similar compounds. The approach used to calculate the individual equilibrium constants involves two methods, one that measures the total influence of all species upon the pH and one that measures only the sum of the concentrations of two species. Although neither method is suitable by itself, their combination enables one to calculate all four constants. Any two methods can be used provided that they are interrelated but not equivalent and one method gives the total influence of all species.

REFERENCES

(1) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N.Y., 1962.

(2) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed., 5th rev., Butterworth and Co., London, England, 1970, p. 348.

(3) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **71**, 2431(1955).

(4) J. T. Edsall, R. B. Martin, and B. R. Hollingworth, *Proc. Nat. Acad. Sci. USA*, **44**, 505(1958).

(5) S. Riegelman, L. A. Strait, and E. Z. Fischer, *J. Pharm. Sci.*, **51**, 129(1962).

(6) M. J. Nye and W. P. Tang, *Tetrahedron*, **28**, 463(1972).

(7) J. N. Butler, in "Ionic Equilibrium, a Mathematical Approach," Addison-Wesley, Reading, Mass., 1964, p. 437.

(8) P. O. Lumme, *Suom. Kemistil.*, **30B**, 173(1957).

(9) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **60**, 503(1971).

(10) J. N. Butler, in "Ionic Equilibrium, a Mathematical Approach," Addison-Wesley, Reading, Mass., 1964, pp. 210–212.

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Pharmacokinetic Studies of Pentylenetetrazol in Dogs

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Abstract □ Pharmacokinetic profiles of pentylenetetrazol in the dog were studied following rapid intravenous and oral administrations of a convulsant dose (15–20 mg/kg). Plasma level–time curves after a rapid intravenous injection showed biexponential decline, indicating that the disposition of this drug in the dog follows a two-compartment body model. Pharmacokinetic parameters were calculated from the intravenous data. After oral administration of the solution dose, the peak plasma level appeared at about 30 min postdose, indicating that the absorption occurs rapidly. Areas under the oral plasma level–time curves showed that the drug was absorbed completely and that the first-pass metabolism effect was minimal. The ligation studies of the kidney and the liver suggested that the main elimination pathway of this drug was biotransformation in the liver. The average plasma half-life was 1.4 hr. At steady state, the volume of distribution was approximately equivalent to the volume of the total body water.

Keyphrases □ Pentylenetetrazol—pharmacokinetic studies following intravenous and oral administrations, dogs □ Pharmacokinetics—pentylenetetrazol, intravenous and oral administrations, dogs □ Stimulants—pentylenetetrazol, pharmacokinetic studies following intravenous and oral administrations, dogs

Pentylenetetrazol[1] is being used for various clinical purposes in humans and animals. The primary use of this drug in humans is as a central nervous system (CNS) stimulant for the therapeutic management of chronic depression and confusion in mental patients. In animals, this drug is also being used as a CNS stimulant for respiratory failure or collapse during surgical anesthesia.

Although the properties of biological disposition of pentylenetetrazol were studied (1, 2), pharmacokinetic profiles have not been reported. Recently, a sensitive and reproducible GLC determination of this compound in biological fluids was described (3); the method allowed the determination of pharmacokinetic properties of pentylenetetrazol in the dog as reported in this study.

EXPERIMENTAL

Conditions of Dogs—Seven healthy dogs (four beagle and three mongrel breeds), 8–13 kg, were used. Four dogs received a single pentylenetetrazol dose of 15 or 20 mg/kg iv and po on separate occasions. Three dogs were utilized in the liver and kidney ligation studies. When two or more blood level studies were performed on the same dog, at least 2 weeks was allowed between experiments. During the blood level studies, dogs were anesthetized with pentobarbital sodium.

Assay Procedures—Plasma concentrations of pentylenetetrazol were determined by the GLC technique recently developed (3). The

[1] Metrazol, Knoll Pharmaceutical Co., Whippany, N.J.