Phenylbutazone Ionization Kinetics

V. J. STELLA ˣ and J. D. PIPKIN

Abstract □ Phenylbutazone has been associated with bioavailability problems and has shown nonclassical behavior in phase-transport studies. This nonclassical behavior has been attributed, in part, to the fact that phenylbutazone, as a carbon acid, undergoes noninstantaneous ionization kinetics. Instantaneous reaction is an assumption made in many diffusion-limited transport models involving a simultaneous ionization reaction. The ionization kinetics of phenylbutazone were determined at an ionic strength of 0.1 and 25° using a stopped-flow spectrophotometer. A log k_{obs} *versus* pH profile for the approach to the ionization equilibrium was determined, and a mechanism consistent with the profile was postulated. The percent enol *versus* the diketo form of phenylbutazone acid as well as pKa_{enol} and pKa_{diketo} was kinetically calculated. The protonation reaction was highly catalyzed by general acids while the deprotonation reaction was highly catalyzed by general bases. The general acid, water, was a poor proton donor to the anionic form (the so-called mesomeric anion) of phenylbutazone.

Keyphrases □ Phenylbutazone—ionization kinetics, relative concentrations and pKa's of enol and diketo forms calculated, effect of acids and bases □ Ionization kinetics—phenylbutazone, relative concentrations and pKa's of enol and diketo forms calculated, effect of acids and bases □ Anti-inflammatory agents—phenylbutazone, ionization kinetics

The anti-inflammatory phenylbutazone (I) has demonstrated nonclassical phase-transport characteristics (1–7). It was recently postulated that this nonclassical behavior of the carbon acid phenylbutazone might be accounted for by its relatively slow ionization rate (7). Noninstantaneous ionization is a characteristic of most carbon acids (8–10). In the design of any model involving phase-transport phenomena with simultaneous ionization, the assumption often is made that all ionizations are rapid (instantaneous) relative to diffusion processes, and the role of buffer components is often neglected. However, it is uncertain at this time what constitutes instantaneous ionization.

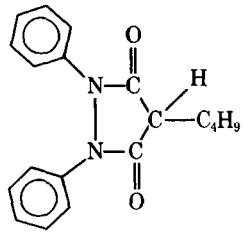
The ionization kinetics of phenylbutazone have not been studied previously, although the ionization kinetics of other carbon acids have been well documented (8–18). The immediate objective of this study was to determine the ionization kinetics of phenylbutazone. The ultimate objective is to use this information to help define when an ionization can be considered instantaneous or noninstantaneous in relation to phase-transport phenomena.

EXPERIMENTAL

Materials—Phenylbutazone[1] was used as supplied. All reactions and pKa determinations were carried out in glass-distilled water. The buffer components and sodium chloride used to adjust the ionic strength were of reagent or analytical reagent quality and were used without further purification.

pKa Determination—The pKa of phenylbutazone was determined spectrophotometrically[2] (19) at 265 nm and the same conditions of temperature and ionic strength as the kinetic studies, *i.e.*, 25 ± 0.2° and $\mu = 0.1$.

Kinetic Studies—The ionization kinetics of phenylbutazone were



I

determined at 25 ± 0.2° and $\mu = 0.1$ with sodium chloride using a stopped-flow spectrophotometer[3] at 265 nm and a pH jump technique (18). For example, when the ionization kinetics of phenylbutazone acid to phenylbutazone mesomeric anion were studied, phenylbutazone at 6×10^{-5} *M* and $\mu = 0.1$ in water, with the pH lowered to approximately 4 with very dilute hydrochloric acid, was placed in one syringe of the stopped-flow spectrophotometer. The desired buffer at double the desired final concentration ($\mu = 0.1$) was stored in the second syringe.

On activation, equal volumes of the two syringes were mixed and the change in transmission at 265 nm was recorded on the calibrated oscilloscope of the stopped-flow spectrophotometer. A permanent recording of the transmission change with time was recorded by photography[4]. Plots of log $(A_{eq} - A)$ *versus* t, where A_{eq} and A are the absorbances (calculated from the transmission data) at time infinity (or at equilibrium) and time t, respectively, were constructed to determine the observed rate constant. Least-squares analysis of the data was performed.

Because the system under study represents a reversible reaction, the observed rate constant, k_{obs}, is the sum of both the forward and reverse rate constants. Buffer concentrations were varied to allow for the calculation of k_{obs} at zero buffer concentration. At pH's where the primary reaction was the protonation of the mesomeric anion to phenylbutazone acid, phenylbutazone at pH ~7 was jumped to acid pH with the transmission as a function of time again being recorded at 265 nm.

RESULTS

Figure 1 shows the UV spectrum of phenylbutazone under basic

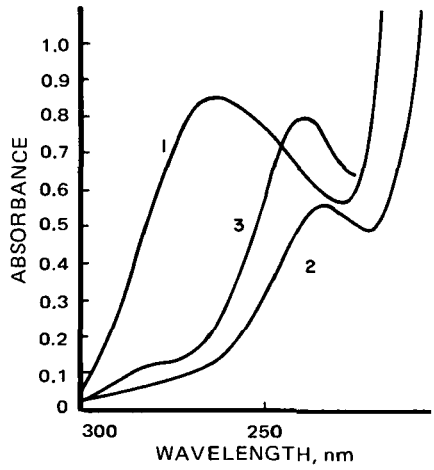


Figure 1—*UV spectrum of phenylbutazone (5.00×10^{-5} M) in 0.1 M NaOH (curve 1), 0.1 M HCl (curve 2), and dichloromethane (curve 3).*

[1] Ciba Geigy Corp.
[2] Perkin-Elmer 356 dual-beam spectrophotometer.
[3] Durrum, with a thermostated cell and syringes maintained at 25 ± 0.2°.
[4] Tetronix C-27 oscilloscope camera.

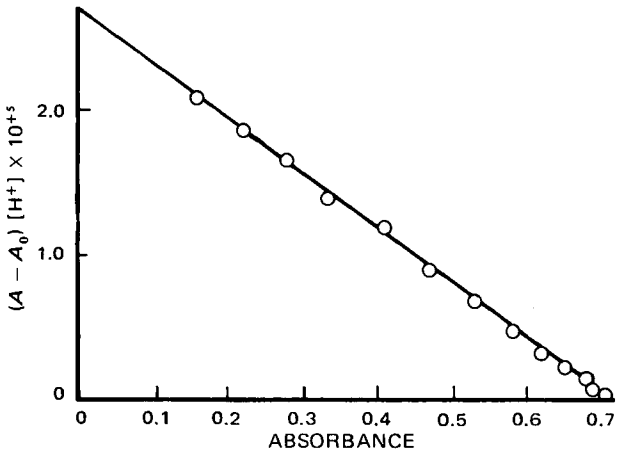


Figure 2—*Plot used to determine the macroscopic ionization constant of phenylbutazone at 25° and μ = 0.1 with sodium chloride.*

as well as acidic conditions. The equilibrium ionization constant of phenylbutazone could be determined by observing the change in absorbance at 265 nm as a function of pH using:

$$K_a = \frac{(A - A_0)[H^+]}{(A_\infty - A)} \qquad \text{(Eq. 1)}$$

where K_a is the macroscopic or observed ionization constant at 25° and μ = 0.1; A is the absorbance of phenylbutazone at a given hydrogen-ion concentration, $[H^+]$; A_0 is the absorbance of the phenylbutazone under acidic conditions, *i.e.*, the ionization completely suppressed (pH = 1.0 or 0.1 *M* HCl); and A_∞ is the absorbance of the phenylbutazone at pH's $\gg$ pKa (pH = 13 in 0.1 *M* NaOH).

Rearranging Eq. 1 allows the determination of K_a:

$$(A - A_0)[H^+] = K_a A_\infty - K_a A \qquad \text{(Eq. 2)}$$

from a plot of $(A - A_0)[H^+]$ *versus* A, with the ionization constant K_a being calculated from the slope. This approach is useful when an exact value of A_∞ is difficult to obtain. Figure 2 gives such a plot for a 4×10^{-5} *M* solution of phenylbutazone. The value of K_a under the experimental conditions was 3.71×10^{-5} (average of three determinations) with pKa calculated as 4.43.

Table I gives the observed rate constants for the ionization kinetics of phenylbutazone under various pH and buffer concentrations. All observed rate constants are the mean of at least three or more determinations. Also included in this table are the observed rate constants extrapolated to zero buffer concentration obtained from plots of k_{obs} *versus* total buffer concentration. Figure 3 is a plot of $\log k'_{obs}$ *versus* pH for the ionization of phenylbutazone where k'_{obs} is the buffer-independent observed rate constant.

DISCUSSION

A model for the ionization and ionization kinetics of relatively strong carbon acids is defined in Scheme I. The three possible species of phenylbutazone that can exist in solution are the diketo form of phenylbutazone, K, the enol form of phenylbutazone, E, and the so-called mesomeric anion or E^- (9). Therefore, the macroscopic ionization constant for phenylbutazone would be defined by:

$$K_a = \frac{[E^-][H^+]}{[K] + [E]} \qquad \text{(Eq. 3)}$$

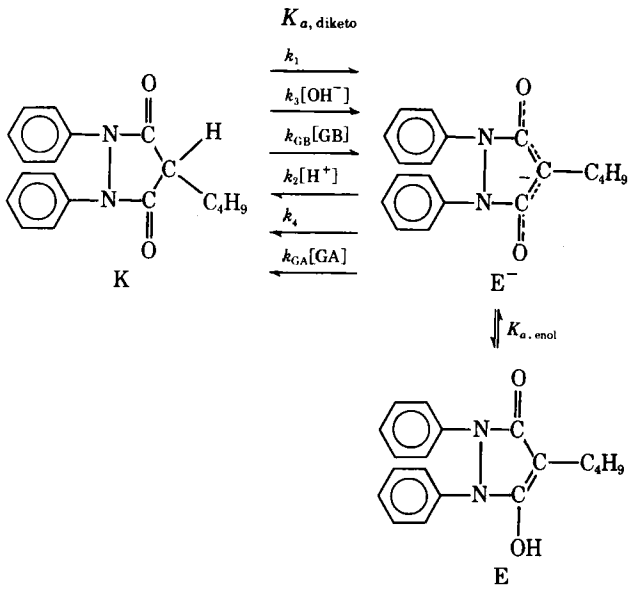
Rearranging gives:

$$\frac{1}{K_a} = \frac{1}{K_{a,\text{diketo}}} + \frac{1}{K_{a,\text{enol}}} \qquad \text{(Eq. 4)}$$

which shows the relationship of this macroscopic constant to the microscopic constants, $K_{a,\text{diketo}}$ and $K_{a,\text{enol}}$, which are the ionization constants for the diketo and enol forms of phenylbutazone, respectively.

In Scheme I, the assumption is made that the equilibrium defined by $K_{a,\text{enol}}$ is rapid and instantaneous[5]. This assumption appears valid

[5] The word instantaneous is used to describe phenomena taking place at rates near the diffusion-controlled limit of $\sim 2 \times 10^{10}\ M^{-1}\ \text{sec}^{-1}$.



Scheme I

in light of Eigen's studies (9) of the ionization rates of enolic compounds. The equilibrium represented by $K_{a,\text{diketo}}$ is considered to be the slow step (9). The rate constant k_1 represents the water-catalyzed deprotonation of phenylbutazone, k_3 is the hydroxide-ion-catalyzed deprotonation rate constant, and k_{GB} is the general base-catalyzed deprotonation rate constant. The constant k_2 represents the specific acid-catalyzed protonation constant of the mesomeric anion, k_4 is the water or spontaneous rate constant for the protonation of the mesomeric anion, and k_{GA} is the general acid-catalyzed protonation of the mesomeric anion.

Deriving the equation for the equilibrium ionization kinetics of phenylbutazone, assuming Scheme I and neglecting the buffer contribution to the kinetics, leads to:

$$k'_{obs} = k_1 + k_3[OH^-] + \frac{k_2[H^+]K_{a,\text{enol}}}{K_{a,\text{enol}} + [H^+]} + \frac{k_4 K_{a,\text{enol}}}{K_{a,\text{enol}} + [H^+]} \qquad \text{(Eq. 5)}$$

By referring to Fig. 1, it is obvious from the poor UV absorption of phenylbutazone under acid conditions in the 250–270-nm range that phenylbutazone exists primarily in the diketo form. This was confirmed by measuring the UV absorption spectrum of phenylbutazone

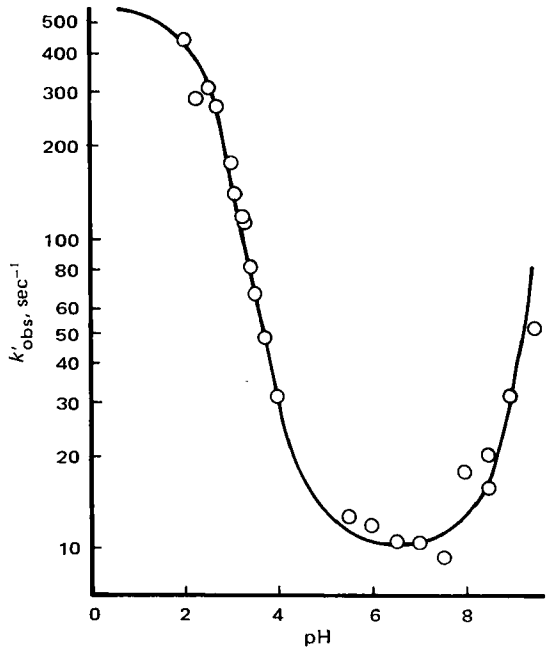


Figure 3—*Plot of log* k'_{obs} versus *pH for the establishment of an ionization equilibrium for phenylbutazone.*

Table I—Effect of pH and Buffer Concentrations on the Ionization Kinetics of Phenylbutazone at 25° and μ = 0.1 with Sodium Chloride

pH	Buffer	Buffer Concentration, *M*	k_{obs}, sec^{-1}	k'_{obs}, sec^{-1}
2.00	Hydrochloric acid	—	440 ± 56[a]	440 ± 56[a]
2.30	Hydrochloric acid	—	281 ± 18	281 ± 18
2.52	Hydrochloric acid	—	309 ± 23	309 ± 23
2.70	Hydrochloric acid	—	269 ± 10	269 ± 10
3.00	Hydrochloric acid	—	178 ± 7	178 ± 7
3.00	Hydrochloric acid	—	172 ± 4	172 ± 4
3.00	Hydrochloric acid	—	140 ± 6	140 ± 6
3.22	Hydrochloric acid	—	117 ± 3	47 ± 3
3.30	Hydrochloric acid	—	112 ± 7	112 ± 7
3.40	Hydrochloric acid	—	79.9 ± 1.1	79.9 ± 1.0
3.50	Acetate	0.02	81.6 ± 2.1	—
3.50	Acetate	0.03	93.6 ± 1.4	—
3.50	Acetate	0.04	100 ± 2	—
3.50	Acetate	0.05	107 ± 3	—
3.50	Acetate	0.00 (calc.)	—	66.6
3.70	Hydrochloric acid	—	47 ± 2	47 ± 2
4.00	Acetate	0.02	47.6 ± 1.1	—
4.00	Acetate	0.03	55.4 ± 0.5	—
4.00	Acetate	0.04	61.3 ± 2.9	—
4.00	Acetate	0.05	72.9 ± 0.1	—
4.00	Acetate	0.00 (calc.)	—	30.7
5.50	Acetate	0.005	16.4 ± 1.0	—
5.50	Acetate	0.02	33.4 ± 0.3	—
5.50	Acetate	0.03	45.1 ± 2.2	—
5.50	Acetate	0.04	51.9 ± 1.6	—
5.50	Acetate	0.05	62.6 ± 0.6	—
5.50	Acetate	0.00 (calc.)	—	12.5
6.00	Acetate	0.02	32.5 ± 1.0	—
6.00	Acetate	0.03	44.9 ± 1.4	—
6.00	Acetate	0.04	52.4 ± 1.2	—
6.00	Acetate	0.05	65.8 ± 0.8	—
6.00	Acetate	0.00 (calc.)	—	11.6
6.50	Succinate	0.0075	23.7 ± 0.9	—
6.50	Succinate	0.009	25.6 ± 0.8	—
6.50	Succinate	0.012	31.9 ± 1.0	—
6.50	Succinate	0.015	36.5 ± 1.4	—
6.50	Succinate	0.00 (calc.)	—	10.3
7.00	Succinate	0.0075	23.7 ± 0.5	—
7.00	Succinate	0.009	26.2 ± 0.8	—
7.00	Succinate	0.012	32.2 ± 1.8	—
7.00	Succinate	0.015	37.0 ± 0.5	—
7.00	Succinate	0.00 (calc.)	—	10.2
7.50	Phosphate	0.0075	39.5 ± 1.4	—
7.50	Phosphate	0.009	46.0 ± 2.0	—
7.50	Phosphate	0.012	59.4 ± 1.2	—
7.50	Phosphate	0.015	70.0 ± 4.0	—
7.50	Phosphate	0.00 (calc.)	—	9.2
8.00	Phosphate	0.0075	46.8 ± 1.6	—
8.00	Phosphate	0.009	54.2 ± 1.7	—
8.00	Phosphate	0.012	67.0 ± 2.1	—
8.00	Phosphate	0.015	77.4 ± 3.0	—
8.00	Phosphate	0.00 (calc.)	—	17.1
8.50	Phosphate	0.0075	50.5 ± 1.2	—
8.50	Phosphate	0.009	57.5 ± 1.7	—
8.50	Phosphate	0.012	69.4 ± 3.1	—
8.50	Phosphate	0.015	81.9 ± 2.1	—
8.50	Phosphate	0.00 (calc.)	0.00	19.7
8.50	Borate	0.02	23.7 ± 0.5	—
8.50	Borate	0.03	29.3 ± 0.2	—
8.50	Borate	0.04	32.6 ±	
8.50	Borate	0.05	37.2 ± 0.3	—
8.50	Borate	0.00 (calc.)	—	15.4
9.00	Borate	0.00	42.8 ± 0.9	—
9.00	Borate	0.03	50.2 ± 1.1	—
9.00	Borate	0.04	55.7 ± 0.7	—
9.00	Borate	0.05	61.9 ± 0.9	—
9.00	Borate	0.00 (calc.)	—	30.7
9.50	Borate	0.02	75.6 ± 5.4	—
9.50	Borate	0.03	91.0 ± 4.6	—
9.50	Borate	0.04	103.0 ± 1.6	—
9.50	Borate	0.05	114.0 ± 7.2	—
9.50	Borate	0.00 (calc.)	—	51.4

[a] Mean ± *SD*.

in an inert solvent such as dichloromethane where the formation of the more polar enol form of phenylbutazone would be suppressed [curve 3 of Fig. 1 (20)]. This means that $pKa_{diketo} \gg pKa_{enol}$ or that $K_{a,enol} \gg K_{a,diketo}$. Because of this observation, it can be concluded that $pKa_{diketo} \approx$ pKa and that $pKa_{enol} < 4.43$.

At pH's where the primary observed reaction is the *ionization of*

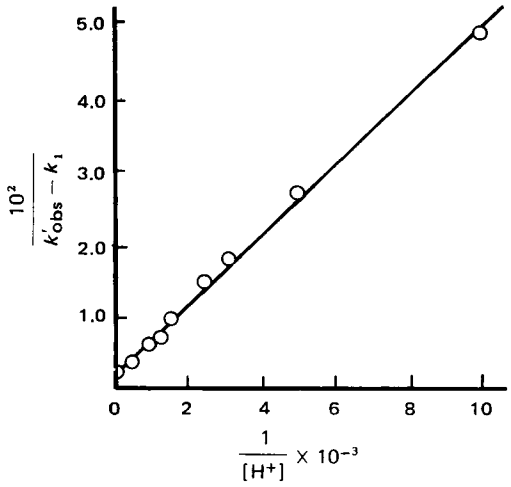


Figure 4—*Plot used to determine* k_2 *and* $K_{a,enol}$ *for the ionization of phenylbutazone. (Refer to Eq. 14 and Scheme I.)*

phenylbutazone acid to the mesomeric anion, *i.e.*, at pH ≫ pKa_{diketo}, the observed rate constant can be approximated to:

$$k'_{obs} = k_1 + k_3[OH^-] + \frac{k_4 K_{a,enol}}{K_{a,enol} + [H^+]} \quad \text{(Eq. 6)}$$

and since $K_{a,enol} \gg [H^+]$ at this pH:

$$k'_{obs} = (k_1 + k_4) + k_3[OH^-] \quad \text{(Eq. 7)}$$

$$k'_{obs} = k_0 + k_3[OH^-] \quad \text{(Eq. 8)}$$

where:

$$k_0 = k_1 + k_4 \quad \text{(Eq. 9)}$$

Equation 8 predicts that a plot of k'_{obs} *versus* $[OH^-]$ should give a straight line. However, as can be noted in Fig. 3, there was much scatter in the data in this pH range. Therefore, only an estimate could be made of k_3 ($\sim 2 \times 10^6\ M^{-1}\ sec^{-1}$), although a reasonably accurate estimate could be made of k_0 ($10.1\ sec^{-1}$).

Since $pKa_{diketo} \approx$ pKa, Eq. 10 can be derived:

$$\frac{K_{a,diketo}}{K_w} = \frac{k_3}{k_4} \sim \frac{K_a}{K_w} \quad \text{(Eq. 10)}$$

allowing an estimation of k_4 by knowing k_3, K_a, and K_w under the experimental conditions. This approach gives a value of k_4 of $5.4 \times 10^{-4}\ sec^{-1}$. It is obvious, therefore, that the constant k_0 is approximately equal to k_1:

$$k_0 = k_1 + k_4 \sim k_1 = 10.1\ sec^{-1} \quad \text{(Eq. 11)}$$

From Fig. 1, it can be noted that at pH < 8, $k_3[OH^-]$ can be neglected. Therefore:

$$k'_{obs} = k_1 + \frac{k_2[H^+]K_{a,enol}}{K_{a,enol} + [H^+]} + \frac{k_4 K_{a,enol}}{K_{a,enol} + [H^+]} \quad \text{(Eq. 12)}$$

and since $K_{a,enol}/(K_{a,enol} + [H^+])$ is always < 1 and k_4 is small, Eq. 12 collapses to:

$$k'_{obs} = k_1 + \frac{k_2[H^+]K_{a,enol}}{K_{a,enol} + [H^+]} \quad \text{(Eq. 13)}$$

which can be rearranged to:

$$\frac{1}{k'_{obs} - k_1} = \frac{1}{k_2[H^+]} + \frac{1}{k_2 K_{a,enol}} \quad \text{(Eq. 14)}$$

Equation 14 predicts that a plot of $1/(k'_{obs} - k_1)$ *versus* $1/[H^+]$ will give a straight line of slope $= 1/k_2$ and intercept $1/k_2 K_{a,enol}$, thus allowing the estimation of k_2 and $K_{a,enol}$. Such a plot is shown in Fig. 4. The rate constant k_2 was estimated to be $2.11 \times 10^5\ M^{-1}\ sec^{-1}$, and $K_{a,enol}$ was estimated to be 2.62×10^{-3} (pKa_{enol} 2.58).

The equilibrium ionization constant of the diketo form of phenylbutazone is defined as:

$$K_{a,diketo} = \frac{k_1}{k_2} = \frac{10.1}{2.11 \times 10^5} = 4.78 \times 10^{-5} \quad \text{(Eq. 15)}$$

Table II—Kinetic and Equilibrium Constants for the Ionization of Phenylbutazone at 25° and μ = 0.1 with Sodium Chloride

Parameter	Value
k_1	$10.1\ sec^{-1}$
k_2	$2.11 \times 10^5\ M^{-1}\ sec^{-1}$
k_3	$\sim 2 \times 10^6\ M^{-1}\ sec^{-1}$
k_4	$\sim 4.2 \times 10^{-4}\ sec^{-1}$
$K_{a,enol}$	2.62×10^{-3} ($pKa_{enol} = 2.58$)
$K_{a,diketo}$	4.78×10^{-5} ($pKa_{diketo} = 4.32$)
K_a	4.67×10^{-5} or pKa = 4.33 (kinetically determined) 3.71×10^{-5} or pKa = 4.43 (determined spectrophotometrically)
% enol	1.8

or $pKa_{diketo} = 4.32$. A value for K_a can be calculated (Eq. 4) from the kinetic data to be 4.67×10^{-5} or pKa can be kinetically calculated to be 4.33. This value compares favorably with the experimentally determined value of 4.43. Determination of $K_{a,diketo}$ specifically allows a reestimation of the value of k_4 ($4.2 \times 10^{-4}\ sec^{-1}$).

Table II is a summary of the kinetic and equilibrium constants. Included in this table is the percent enol present in phenylbutazone acid dissolved in solution. It can be shown that the percent enol can be calculated from Eqs. 16 and 17:

$$\% \text{ enol} = \frac{[E]}{[E] + [K]} \times \frac{100}{1} \quad \text{(Eq. 16)}$$

$$\% \text{ enol} = \frac{K_{a,diketo}}{K_{a,diketo} + K_{a,enol}} \times \frac{100}{1} \quad \text{(Eq. 17)}$$

The rate constants from Table II were placed back into Eq. 5 to help generate a theoretical curve for the ionization behavior of phenylbutazone, assuming the mechanism shown in Scheme I. The solid line drawn through the experimental points in Fig. 3 is the line generated by Eq. 5 using the constants summarized in Table II. As can be seen from the fit of these data, Eq. 5 and subsequently Scheme I do appear to define the ionization kinetics of phenylbutazone adequately.

Alternative mechanisms might be postulated to describe the ionization of phenylbutazone. For example, it might be postulated that a pathway exists that allows the direct conversion of the diketo phenylbutazone to the enol in a concerted acid–base mechanism. This mechanism appears unlikely because the plots of k_{obs} *versus* total buffer concentration were linear for a range of buffer species; *i.e.*, no second-order term in buffer concentration was noted. It is becoming increasingly evident that few, if any, concerted acid–base mechanisms exist in aqueous solvents (21).

A second mechanism that might be postulated is the conversion of the diketo phenylbutazone to the enolic form of phenylbutazone *via* an acid-catalyzed mechanism. But such a mechanism would not predict the type of curve given in Fig. 3. Moreover, protonation of the diketo phenylbutazone [estimated pKa of the diketone, acetylacetone, is −5 to −6 (22)] is so unfavorable that it would represent a case of an intermediate for which heightened reactivity fails to compensate for inferior concentration (23).

This discussion is not to say that alternative mechanisms such as these two do not exist. The point can be made, however, that Scheme I does appear to describe the ionization of phenylbutazone adequately.

As a result of the choice of buffers used in this study, accurate and meaningful values of k_{GA} and k_{GB} could not be determined from these data because species such as the monoanion of succinic acid, $H_2PO_4^-$, and HPO_4^{-2} could act both as acids or bases. The general acid–general base catalysis of phenylbutazone ionization is currently under investigation. The objective of this extended study is to define mechanistically the ionization kinetics of phenylbutazone. However, two observations can be made from the buffer data:

1. Relative to its pKa, water is a very weak proton donor to a carbanion [an observation consistent with other recent work (24)].

2. Relative to its pKa, borate ion is a very weak proton acceptor [an observation also consistent with earlier findings (25)].

In an earlier study (7), it was demonstrated that the phenylbutazone dissolution rate and phase-transport phenomena in general may be affected by the relatively slow ionization of phenylbutazone.

Constants k_1 through k_4 of Table II are substantially slower than the diffusion-limited constants for the ionization of normal or classical acids such as carboxylic, phenolic, and nitrogen acids (8, 9). The ionization kinetics generated in this work will be used to determine whether, in fact, the nonclassical phase-transport behavior of phenylbutazone (1–7) can be traced to this relatively slow ionization rate.

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Kinetic Analysis of Penicillin Degradation in Acidic Media

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Abstract □ Degradation of penicillin in acidic media (pH 2.7) was monitored by high-pressure liquid chromatography and UV spectroscopy. The effects of temperature, buffer concentration, and ionic strength were examined. A degradation pathway is proposed, and the apparent first-order rate constant and energy of activation were calculated for each reaction. One or more degradation products containing a sulfhydryl group, a functional group often suggested as having a major role in eliciting allergic responses to penicillin therapy, were present throughout the degradation scheme.

Keyphrases ◻ Penicillin—degradation in acidic media, kinetic analysis, monitored by high-pressure liquid chromatography and UV spectroscopy, effect of temperature, buffer concentration, and ionic strength ◻ Degradation—penicillin in acidic media, kinetic analysis, monitored by high-pressure liquid chromatography and UV spectroscopy, effect of temperature, buffer concentration, and ionic strength □ High-pressure liquid chromatography—used to monitor degradation of penicillin in acidic media, kinetic analysis □ UV spectroscopy—used to monitor degradation of penicillin in acidic media, kinetic analysis □ Antibacterial agents—penicillin, degradation in acidic media, kinetic analysis

It is estimated that between 1 and 10% of the population experiences allergic response after penicillin therapy, with 300 fatalities in the United States each year (1). Research on penicillin allergy has considered four factors which may be of importance: (*a*) direct reactions of the drug with protein *in vivo*, (*b*) degradation products of penicillin that can react with protein, (*c*) impurities other than degradation products that may be in the dosage form with penicillin, and (*d*) metabolites of penicillin that can react with protein (2).

One approach to the problem of penicillin allergy would be to determine the role of specific penicillin degradation products in producing an allergic response and, through dosage form design, to minimize the formation of degradation products showing significant potential as allergenic determinants. To use this approach, the time sequence for the presence of penicillin degradation products in aqueous media needs to be established.

Several reviews (2, 3) suggested schemes for the degradation of penicillin. It was proposed that penillic and penicilloic acids are the major degradation products when penicillin is aged in acidic solutions (4). The mechanism of penicillin degradation was studied in acidic solution, and it was proposed that penicillenic acid forms from the reaction of the penicillin ion with a proton or from the spontaneous rearrangement of undissociated penicillin (5). It was suggested also that penillic acid forms from penicillenic acid and that penilloic acid is the product from the acid catalysis of