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Effect of Ionic Strength on Chemical Stability of Potassium Penicillin G

ROBERT E. LINDSAY* and STANLEY L. HEM▲

Abstract □ Ionic strength up to 0.5 does not affect the chemical stability of potassium penicillin G unless one of the ions contributing to the ionic strength is also catalytic. Thus, ionic strength adjusted with NaCl had no effect on the chemical stability of 0.01 *M* potassium penicillin G solutions in 0.06 *M* citrate buffer at pH 7.0 where the major buffer species was the noncatalytic citrate ion. However, in penicillin solutions buffered with 0.06 *M* citrate buffer, the ionic strength had a direct effect at pH 6.5 or lower. The magnitude of the ionic strength effect was inversely related to pH. The monohydrogen citrate and dihydrogen citrate ions were found to be catalytic. At 45°, the catalytic rate constants were 0.272 and 1.92 l. mole^{-1} hr.$^{-1}$, respectively. At pH's where either of these catalytic species was present, an increase in the ionic strength by the addition of NaCl caused an increased rate of degradation.

Keyphrases □ Penicillin G, potassium—effect of ionic strength on chemical stability □ Ionic strength—effect on chemical stability of potassium penicillin G □ Degradation, penicillin—effect of ionic strength on chemical stability □ Potassium penicillin G—effect of ionic strength on chemical stability

The chemical instability of penicillin has led to the development of reconstitutable liquid dosage forms which demonstrate limited shelflife after reconstitution but possess the advantages of oral or parenteral liquid dosage forms. These dosage forms normally have a relatively high ionic strength due to the buffers, preservatives, and chelating agents which are required components of the formula. Most reports involving penicillin stability adjust the ionic strength to a constant value and study the effect of variables such as pH and temperature. The authors are aware of no studies which have examined the effect of ionic strength on the chemical stability of penicillins, although both Finholt *et al.* (1) and Hou and Poole (2) suggested that it may affect the chemical stability.

Carstensen (3) recently reviewed the applications of the primary salt effect to pharmaceuticals. He pointed out 14 drugs including penicillin that are affected by ionic strength.

This variable may have an important effect because liquid penicillin dosage forms have various ionic strengths depending on the individual formulation. In most cases, pharmaceutical products have a relatively high ionic strength. In addition, the ionic strength of gastric juice is reported to be 0.09–0.24 (4). The effect of ionic strength may be pH dependent, and a different effect may occur at the low pH of the stomach compared to the more neutral pH of liquid dosage forms.

The objective of this study was to investigate the effect of ionic strength on the chemical stability of potassium penicillin G with the aim of increasing the understanding and ability to control the degradation of penicillin.

EXPERIMENTAL

Materials—Potassium penicillin G[1] was used. All other chemicals were reagent grade.

Methods—The chemical stability of 0.01 *M* potassium penicillin G solutions at 45° in 0.06 *M* citrate buffer was studied at pH values from 4.0 to 7.0 and at ionic strengths of 0.30 to 0.50. The pKa values for citric acid at 60° and ionic strength 0.50 are 2.72, 4.30, and 5.47 (1). These values were used to calculate the concentration of each citrate species present in the solutions studied (Table I) because they were determined at temperature and ionic strength conditions very similar to the experimental conditions of this study.

The ionic strength of the 0.01 *M* potassium penicillin G solutions with 0.06 *M* citrate buffer was calculated at each pH from the data in Table I and from the reported pKa for potassium penicillin G of 2.78 at 60° and ionic strength 0.5 (1). The concentration of NaCl needed to obtain ionic strengths of 0.30, 0.35, 0.40, 0.45, and 0.50 was determined at each pH. Solutions were prepared using 0.01 *M* potassium penicillin G, 0.06 *M* citric acid, and the calculated concentration of NaCl. The volume of 1 *N* NaOH needed to adjust each solution to the desired pH was determined, and a final small correction in ionic strength was made to include the concentration of sodium ion used in adjusting pH. The final composition of each solution is given in Table II.

Stability samples were prepared by adding the calculated amounts of citric acid, NaCl, and 1 *N* NaOH to doubly distilled water. One hundred milliliters of these solutions was placed in a 45° water bath, and 0.001 mole of potassium penicillin G was added when the solution reached 45°. An initial sample was taken immediately after the addition of the potassium penicillin G to the 45° solution.

[1] Eli Lilly and Co.

Table I—Molar Concentration of Citrate Species

	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Citric acid	0.00204	0.00036	0.00006	—	—	—	—
Dihydrogen citrate ion	0.03820	0.02160	0.00780	0.00177	0.00027	0.00003	—
Monohydrogen citrate ion	0.01910	0.03432	0.03848	0.02790	0.01370	0.00510	0.00172
Citrate ion	0.00066	0.00366	0.01300	0.03000	0.04600	0.05480	0.05830
Total	0.0600	0.0600	0.0600	0.0600	0.0600	0.0600	0.0600

Table II—Composition of Potassium Penicillin G Solutions (Molar)

μ	Citric Acid	Potassium Penicillin G	pH 4.0 NaCl	pH 4.5 NaCl	pH 5.0 NaCl	pH 5.5 NaCl	pH 6.0 NaCl	pH 6.5 NaCl	pH 7.0 NaCl
0.30	0.06	0.01	0.230	0.211	0.132	0.093	0.049	0.033	0.023
0.35	0.06	0.01	0.280	0.261	0.182	0.143	0.099	0.083	0.073
0.40	0.06	0.01	0.330	0.311	0.232	0.194	0.149	0.133	0.123
0.45	0.06	0.01	0.380	0.361	0.282	0.243	0.198	0.183	0.173
0.50	0.06	0.01	0.430	0.411	0.332	0.293	0.249	0.233	0.223

Further samples were taken at appropriate intervals depending on the rate of degradation. Due to the high rate of degradation at pH 4.0 and 4.5, the samples at these pH's were immediately assayed. Stability samples at other pH's were collected and frozen until the assays were performed.

The concentration of intact penicillin G was determined by the modified iodometric assay (5). Rate constants were calculated by least-squares analysis.

The pH of each solution was determined initially and after the last sample was withdrawn. A maximum change in pH of 0.15 occurred at pH 7.0. The other solutions showed changes of less than 0.10 pH unit.

RESULTS AND DISCUSSION

The degradation of potassium penicillin G in all solutions studied followed apparent first-order kinetics. The stability data obtained at pH 5.0 (Fig. 1) are typical of the data at other pH's. The apparent first-order rate constant, $k_{obs.}$, was calculated for each solution (Table III). Inspection of Table III indicates that the ionic strength generally causes an increase in $k_{obs.}$.

The direct effect of ionic strength on $k_{obs.}$ is seen in Figs. 2 and 3. The linear nature of the relationship is significant because it allows extrapolation of ionic strength effects. The effect of ionic strength seems to be pH dependent. The greatest effect was observed at pH 4.0. The effect diminishes as the pH increases until no effect is observed at pH 7.0.

The apparent first-order rate constant at zero ionic strength, k_0, can be obtained by extrapolation from Figs. 2 and 3. These values are given in Table IV and agree very well with the pH profile for potassium penicillin G reported by Schwartz and Buckwalter (6). The pH of maximum stability occurs in a narrow region at pH 6.5.

The Brønsted-Bjerrum equation describes the effect of ionic strength on reaction rate:

$$\log k = \log k_0 + 2QZ_AZ_B\sqrt{\mu} \qquad \text{(Eq. 1)}$$

where k is the observed rate constant at ionic strength μ, $2Q$ is a constant equal to 1.07 at 45°, and Z_A and Z_B are charges of the reacting species. Potassium penicillin G is present predominantly as the penicillin anion (Z_A) at all pH's studied; Z_B represents the valence of any reacting ionic species.

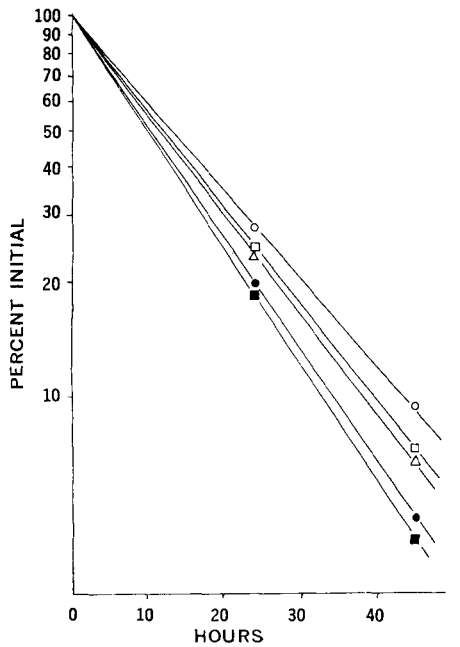

Figure 1—*Effect of ionic strength on the chemical stability of potassium penicillin G at pH 5.0 and 45°. Key: ○, μ = 0.30; □, μ = 0.35; △, μ = 0.40; ●, μ = 0.45; and ■, μ = 0.50.*

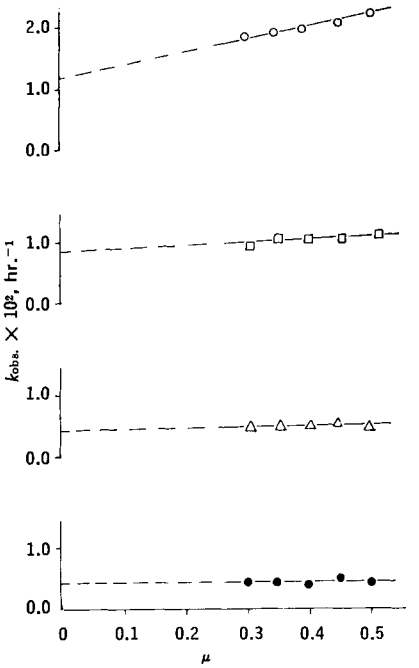

Figure 2—*Effect of ionic strength on the observed first-order rate constant at 45°. Key: ○, pH 5.5; □, pH 6.0; △, pH 6.5; and ●, pH 7.0.*

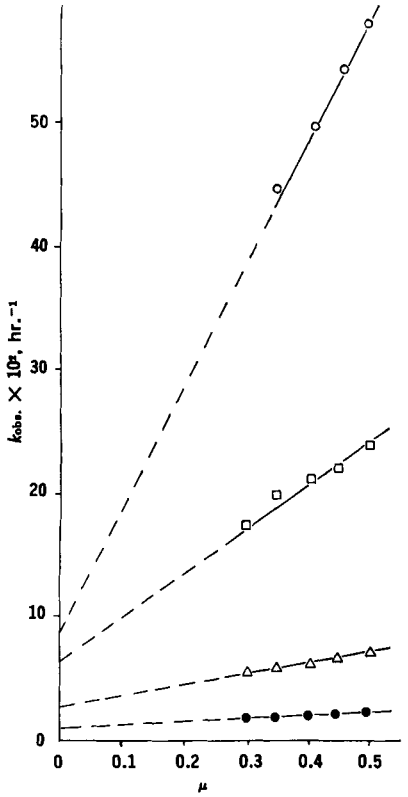

Figure 3—*Effect of ionic strength on the observed first-order rate constant at 45°. Key:* ○, *pH 4.0;* □, *pH 4.5;* △, *pH 5.0; and* ●, *pH 5.5.*

A plot of log k/k_0 *versus* $\sqrt{\mu}$ is given in Fig. 4. A straight line passing through the origin was obtained at each pH. The slopes of the lines are given in Table V.

The Brønsted–Bjerrum equation would predict integer slopes. However, the slopes obtained from Fig. 4 are not integers and range from 0 to 1.15. Carstensen (3) recently proposed a modification of the Brønsted–Bjerrum equation based on the modified Debye–

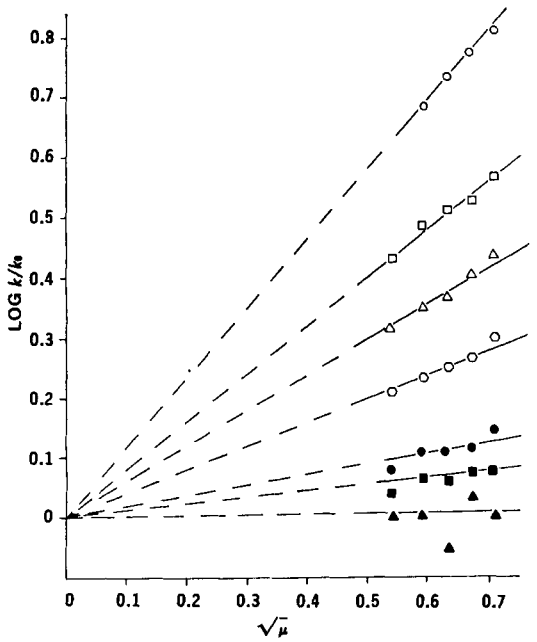

Figure 4—*Effect of ionic strength as described by the Brønsted–Bjerrum equation. Key:* ○, *pH 4.0;* □, *pH 4.5;* △, *pH 5.0;* ○, *pH 5.5;* ●, *pH 6.0;* ■, *pH 6.5; and* ▲, *pH 7.0.*

Table III—Apparent First-Order Rate Constants at 45°

pH	μ	$k_{obs.} \times 10^2$, hr.$^{-1}$ (2σ = 8%)
4.0	0.30	50.2
	0.35	45.3
	0.40	48.5
	0.45	53.8
	0.50	57.5
4.5	0.30	17.5
	0.35	20.0
	0.40	21.0
	0.45	21.5
	0.50	23.7
5.0	0.30	5.35
	0.35	5.85
	0.40	6.12
	0.45	6.78
	0.50	7.18
5.5	0.30	1.86
	0.35	1.96
	0.40	2.04
	0.45	2.09
	0.50	2.25
6.0	0.30	0.945
	0.35	1.06
	0.40	1.04
	0.45	1.06
	0.50	1.11
6.5	0.30	0.445
	0.35	0.485
	0.40	0.480
	0.45	0.502
	0.50	0.386
7.0	0.30	0.445
	0.35	0.445
	0.40	0.383
	0.45	0.480
	0.50	0.445

Huckel equation. This equation as given here is expected to be more accurate at higher ionic strengths:

$$\log k = \log k_0 + 2QZ_AZ_B \frac{\sqrt{\mu}}{1 + \sqrt{\mu}} \qquad \text{(Eq. 2)}$$

Noninteger slopes were also obtained when log k/k_0 was plotted *versus* $\sqrt{\mu}/(1 + \sqrt{\mu})$. Thus, it appears that neither Eq. 1 nor 2 adequately describes the observed effect of ionic strength.

A good deal of insight can be obtained by studying the effect of ionic strength as indicated by the slopes obtained from Fig. 4. The

Table IV—Apparent First-Order Rate Constants at Zero Ionic Strength and 45°

pH	$k_0 \times 10^2$, hr.$^{-1}$ (2σ = 8%)
4.0	9.0
4.5	6.5
5.0	2.65
5.5	1.15
6.0	0.83
6.5	0.425
7.0	0.445

Table V—Apparent Effect of Ionic Strength from the Brønsted–Bjerrum Equation

pH	Slope from Fig. 4
4.0	1.15
4.5	0.79
5.0	0.59
5.5	0.40
6.0	0.18
6.5	0.09
7.0	0.00

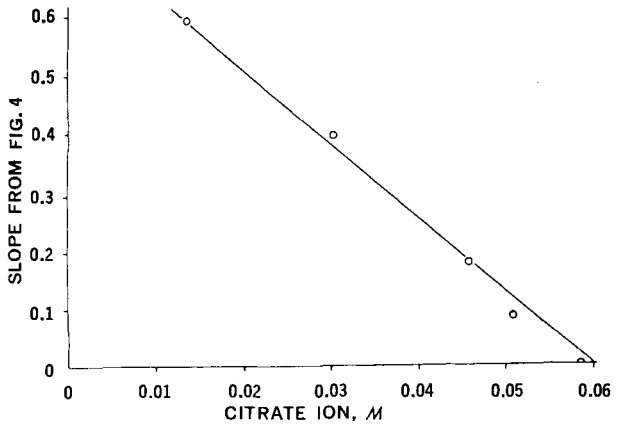

Figure 5—*Effect of citrate ion on the ionic strength effect obtained from the Brønsted–Bjerrum equation.*

slopes are positive, which indicates that the reaction responsible for the effect of ionic strength involves the penicillin anion and another anion.

The slopes are also pH dependent. However, at pH 7.0 a slope of zero was obtained, indicating no ionic strength effect at this pH. Since an increasing ionic strength effect was observed as the pH decreased, the observed effect of ionic strength may be due to the catalytic effect of a pH-dependent anionic species.

The anions present in the solution, in addition to the penicillin anion, are the citrate ion, the monohydrogen citrate ion, the dihydrogen citrate ion, the chloride ion, and the hydroxyl ion. The hydroxyl ion is present to a significant extent only at pH 6.5 and 7.0 where virtually no ionic strength effect was observed.

The chloride ion appears to have no effect. The data in Table II indicate that at pH 7.0 the concentration of chloride ion in the 0.5 ionic strength solution is 10 times greater than in the 0.3 ionic strength solution. However, no ionic strength effect was observed at pH 7.0.

The citrate-ion concentration is inversely related to the slope obtained from Fig. 4. Figure 5 indicates that as the concentration of citrate ion increased, a decreasing ionic strength effect was observed. The figure predicts a slope of zero if all of the citrate species in the system were present as citrate ion. Therefore, the citrate ion appears to be noncatalytic.

A plot (Fig. 6) of the concentration of monohydrogen citrate ion *versus* the slope obtained from Fig. 4 appears to have two components. The concentration of monohydrogen citrate ion is directly related to the slope from pH 5.0 to 7.0. Similar monohydrogen citrate-ion concentrations occur at pH 4.5 and 4.0, but at these pH values the slope from Fig. 4 is larger than expected. Inspection of Table I reveals that citrate ion and monohydrogen citrate ion are the predominant citrate species present at pH 5.5–7.0. At lower pH's, monohydrogen citrate ion and dihydrogen citrate ion predominate.

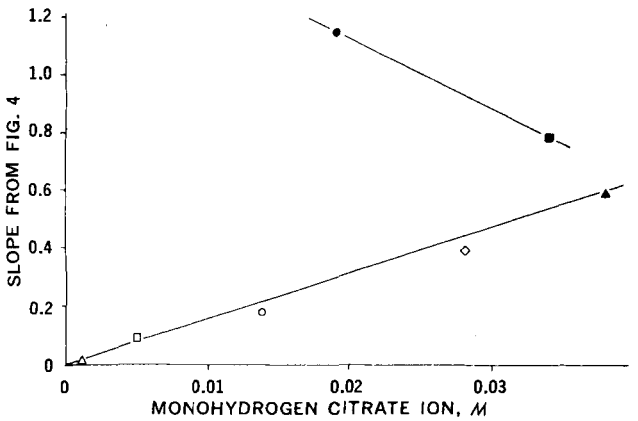

Figure 6—*Effect of monohydrogen citrate ion on the ionic strength effect obtained from the Brønsted–Bjerrum equation. Key: △, pH 7.0; □, pH 6.5; ○, pH 6.0; ◇, pH 5.5; ▲, pH 5.0; ■, pH 4.5; and ●, pH 4.0.*

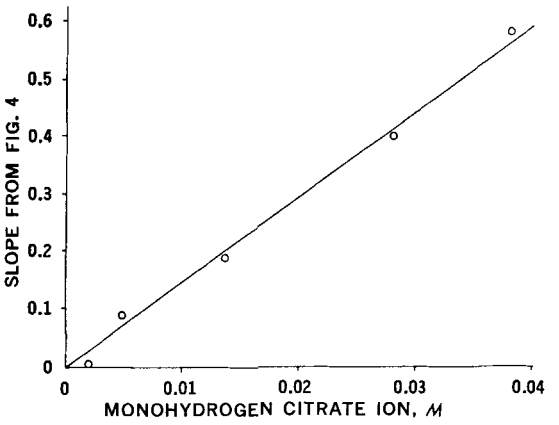

Figure 7—*Effect of monohydrogen citrate ion on the ionic strength effect obtained from the Brønsted–Bjerrum equation in the pH range 5.0–7.0.*

The slope at pH 4.0 and 4.5 is greater than can be accounted for by monohydrogen citrate ion. However, if dihydrogen citrate ion were also catalytic, an additive effect of these two ions on the slope would be expected.

The catalytic action of both monohydrogen citrate ion and dihydrogen citrate ion can be seen by dividing Fig. 6 into components. The catalytic effect of monohydrogen citrate ion is seen in Fig. 7 where the slope from Fig. 4 is directly related to the concentration of monohydrogen citrate ion in the pH range where this ion is present with the noncatalytic citrate ion. As seen in Fig. 7, a straight line passing through the origin is obtained. A plot of the concentration of dihydrogen citrate ion at pH 4.0–5.0 (where this ion predominates) *versus* slope (Fig. 8) also yields a direct linear relationship.

Thus, the observed effect of ionic strength appears to be due to catalysis by two of the ions which contribute to the ionic strength. This finding is confirmed by the absence of an ionic strength effect at pH 7.0 where the postulated catalytic ions are absent. Finholt *et al.* (1) also observed the catalytic effect of monohydrogen and dihydrogen citrate ions when they studied the catalytic effects of buffers on the degradation of penicillin.

The catalytic effect of monohydrogen and dihydrogen citrate ions can be quantified by analysis of k_0, the extrapolated rate constant at zero ionic strength. According to Schwartz and Buckwalter (6), potassium penicillin G degrades by specific acid-catalyzed hydrolysis below pH 6.5 and specific base-catalyzed hydrolysis above pH 6.5. The presence of the catalytic ions below pH 6.5 requires the expression of k_0 as:

$$k_0 = k_{H^+}(H^+) + k_{C_6H_6O_7^{-2}}[C_6H_6O_7^{-2}] + k_{C_6H_7O_7^{-1}}[C_6H_7O_7^{-1}] \quad \text{(Eq. 3)}$$

Values of k_{H^+}, $k_{C_6H_6O_7^{-2}}$, and $k_{C_6H_7O_7^{-1}}$ can be calculated if appropriate values at pH 4.0, 5.0, and 5.5 for k_0, (H^+), $[C_6H_6O_7^{-2}]$, and $[C_6H_7O_7^{-1}]$ are substituted into Eq. 3. The equations were solved by third-order determinants. The following values were obtained for each rate constant at 45°:

$$k_{H^+} = 149 \text{ l. mole}^{-1} \text{ hr.}^{-1}$$

$$k_{C_6H_6O_7^{-2}} = 0.272 \text{ l. mole}^{-1} \text{ hr.}^{-1}$$

$$k_{C_6H_7O_7^{-1}} = 1.92 \text{ l. mole}^{-1} \text{ hr.}^{-1}$$

To test the reliability of this conclusion, k_0 at pH 4.5 was calculated to be 5.6×10^{-2} hr.$^{-1}$ using Eq. 3, the above rate constants, and the appropriate concentration terms from Table I. This value agrees very well with the extrapolated value of 6.5×10^{-2} hr.$^{-1}$ reported in Table IV.

Rate constants can also be calculated for the pH region where virtually no monohydrogen or dihydrogen citrate-ion catalysis occurs. Based upon the pH profile of potassium penicillin G (6) and the results of this study, the degradation at pH 7.0 is completely base catalyzed. Thus, k_0 can be expressed as:

$$k_0 = k_{OH^-}(OH^-) \quad \text{(Eq. 4)}$$

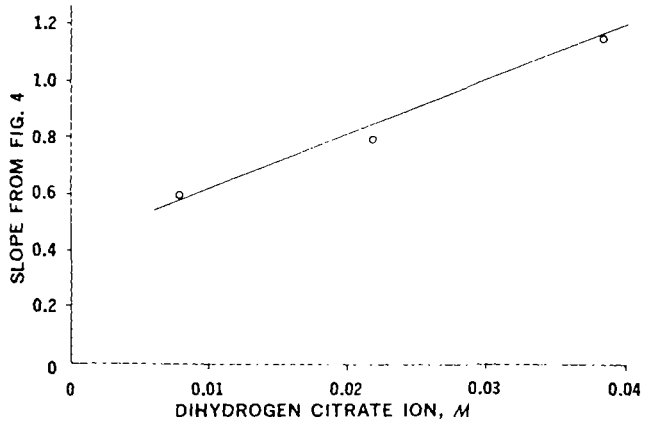

Figure 8—*Effect of dihydrogen citrate ion on the ionic strength effect obtained from the Brønsted–Bjerrum equation in the pH range 4.0–5.0.*

At 45°, pKw is 13.4 (7). Thus, (OH^-) in the pH 7.0 solution is 4×10^{-7}. Using this value and k_0 at pH 7.0 from Table IV, it is possible to calculate that k_{OH^-} is 1.1×10^4 l. mole^{-1} hr.$^{-1}$.

Specific acid-catalyzed hydrolysis, specific base-catalyzed hydrolysis, and monohydrogen citrate-ion-catalyzed hydrolysis all contribute to k_0 at pH 6.5. Thus, k_0 at 45° can be expressed as:

$$k_0 = k_{H^+}(H^+) + k_{OH^-}(OH^-) + k_{C_6H_6O_7^{-2}}[C_6H_6O_7^{-2}] \quad \text{(Eq. 5)}$$

The calculated value of 2.83×10^{-3} hr.$^{-1}$ for k_0 at pH 6.5 also agrees well with the observed value of 4.25×10^{-3} hr.$^{-1}$.

The effect of ionic strength has direct application to the formulation of parenteral and oral liquid penicillin dosage forms. Although it was found that the citrate buffer system provided excellent control over the pH, it contributes to the observed rate of degradation if used below pH 6.5. The buffer system must be selected on the basis of adequate control over pH as well as the absence of catalytic effects.

The ionic strength of the solution has an effect on the chemical stability if a catalytic ion is present. Thus, if a citrate buffer system must be used below pH 6.5, the ionic strength of the solution should be minimized to reduce the catalytic effects of monohydrogen and dihydrogen citrate ions. The minimum effective concentration of such ionic adjuvants as chelating agents, preservatives, and salts used for palatability should be determined to minimize the effect of ionic strength.

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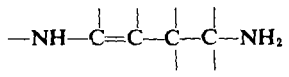
Oxidative Fragmentation of 9-Aminomethylacridan

GEORGE A. DIGENIS*▲ and H. W. WHITLOCK, Jr.†

Abstract □ 9-Aminomethylacridan (*Ia*) and *N,N*-dimethyl-9-aminomethylacridan (IV*a*) were subjected to sodium 1,2-naphthoquinone-4-sulfonate and nitrous acid. Fragmentation of the aminomethyl side chain during oxidation to produce acridine was observed with *Ia* but not with IV*a*. A mechanism based on electrophilic attack at the primary amine nitrogen of the side chain is proposed for the oxidation of *Ia* and similar compounds. The synthesis and purification of the new compounds, *Ia* and its acetamide, IV*a*, 9-hydroxymethylacridan, and the acetamide of 9-aminomethylacridine are described.

Keyphrases □ 9-Aminomethylacridan—synthesis, oxidative fragmentation, acetamide formation □ *N,N*-Dimethyl-9-aminomethylacridan—synthesis, oxidative fragmentation, acetamide formation □ Acridine—formation by oxidative fragmentation of 9-aminomethylacridan □ Acridans—synthesis, oxidative fragmentation □ Sodium 1,2-naphthoquinone-4-sulfonate—oxidation of acridans □ Nitrous acid—oxidation of acridans □ 9-Hydroxymethylacridan—synthesis

It is believed that the biosynthesis of the alkaloid gramine (IX) from tryptophan involves an oxidative fragmentation of the amino acid's side chain by a pyridoxal-dependent enzyme (1). Since tryptophan and 9-aminomethylacridan[1] (*Ia*) have the common structual unit:



the behavior of *Ia* toward pyridoxal hydrochloride was studied. When *Ia* was treated with pyridoxal hydrochloride, it was found to suffer an oxidative amine

[1] 9,10-Dihydroacridines are referred to as derivatives of the acridan nucleus throughout this paper.