Catalytic Effect of Buffers on Degradation of Penicillin G in Aqueous Solution

By PER FINHOLT, GRETHE JÜRGENSEN, and HARALD KRISTIANSEN

The catalytic effect of general acids and bases on the degradation of penicillin G in aqueous solution at 60° has been studied. Dihydrogen citrate ion, monohydrogen phosphate ion, and borate ion are catalytic with respect to benzyl penicillinate ion, while acetic acid catalyzes the degradation of benzyl penicillinic acid. There is a positive primary salt effect in weakly alkaline solution but no primary salt effect in weakly acid solution.

THE DEGRADATION of penicillin G in aqueous solution has been studied extensively. The reaction has been found to be first order with respect to penicillin[1] in acid, neutral, and alkaline medium (1, 2). The rate depends on hydrogen-ion concentration. In alkaline medium, the rate is inversely proportional to the hydrogen-ion concentration at constant salt concentration and temperature (2). In acid solution, the rate increases with increasing hydrogen-ion concentration, but there is no strictly linear relation between rate and hydrogen-ion concentration. The reason for this is that benzyl penicillin, which has a pKa of about 2.8, exists as a mixture of free acid and ion in the pH range from about 1.5 to 4.5, and each of these species is inactivated at a different rate. This produces a curvature in the pH-rate profile around pH 2.8 (2).

The pH-rate profile of the hydrolysis of benzyl penicillin has a minimum at a pH of about 6.5. To obtain optimum stability of penicillin solutions, it is necessary to buffer at a pH of about 6.5. Phosphate and citrate have been used for this purpose.

Pratt (3) found phosphate buffers of low concentrations to exert a stabilizing action on solutions of penicillin. There was an increase in the stabilizing effect by increasing the phosphate concentration from 1 mmole per liter up to 5 mmoles per liter. This result indicates that the buffer solutions of the lower concentrations had too low a buffer capacity for the concentration of penicillin used. In other experiments, where both the phosphate and the penicillin concentration were varied, it was shown that maximum protection occurred when the ratio between the molar concentration of penicillin and of phosphate was approximately 1 to 1.

Clapham (4) used sodium citrate for stabilizing penicillin solutions and found that the optimum concentration of the anhydrous salt was 4.5% w/w for penicillin concentrations from 50,000 to 500,000 units/ml. For some samples of sodium penicillin, an increase in buffer concentration within certain limits was an advantage in the case of the more concentrated penicillin solutions. According to Macek *et al.* (5), the stabilizing effect of citrate and phosphate depends on their buffer action and not on a specific ion effect.

Some authors have compared stability of penicillin in citrate and phosphate buffers of the same pH. Hahn (6) found better stability in 0.0025 *M* citrate solution, pH 6.4 to 6.8, than in distilled water adjusted with phosphate buffer to pH 6.6. (The phosphate concentration is not given.) Hahn suggests that sodium citrate may have an inhibitory effect on the hydrolytic destruction of penicillin. He apparently does not realize that the reason for the difference in stability of penicillin in the two buffer solutions may be that phosphate catalyzes the decomposition of the drug, while citrate does not.

Kaern (7) showed that penicillin was more stable in 0.2 *M* citrate buffer than in 0.2 *M* phosphate buffer. The pH of both buffer solutions was 6.1. At this pH, the buffer capacity of citrate and phosphate was found to be identical. He suggests that the phosphate ion may have a catalytic effect on the hydrolysis of penicillin, but he does not try to prove this.

Fujiwara (8) studied the kinetics of the decomposition of procaine penicillin in aqueous solution and found that phosphate buffer pH 6.75 had a pronounced catalytic effect on the degradation. Citrate and acetate buffer of the same pH also had some effect, but less than the phosphate buffer. Procaine penicillin exists in aqueous solution partially in the undissociated state and partially as the dissociated species of procaine and penicillin (9).

Schwartz *et al.* (10) determined the degradation rates for potassium phenethicillin (α-phenoxyethyl-penicillin) in aqueous solution and found

Received August 26, 1964, from the Institute of Pharmacy, University of Oslo, Oslo, Norway.
Accepted for publication October 29, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.

[1] When the word "penicillin" is used in this paper, it means benzyl penicillin.

TABLE I.—INFLUENCE OF EXCESS IODINE ON RECOVERY AND PRECISION OF THE ANALYTICAL METHOD

Excess 0.01 *N* Iodine, ml.	Determinations, No.	Recovery, %	Coefficient of Variation, %
1.50–2.50	13	101.4	0.26
3.50–4.50	14	103.6	2.48
5.50–6.50	11	106.7	3.58

that secondary phosphate ion catalyzed the hydrolysis, while unprotonated citrate ion had no catalytic effect.

Surprisingly, no studies of the catalytic effect of buffers on the degradation of benzyl penicillin in aqueous solutions have appeared in the literature. The scope of the present work was to make such a study. In addition, the authors were interested in investigating the influence of the ionic strength on the degradation. Such studies have been undertaken by Brodersen (11) but only so far as the degradation in acid solution is concerned. From his experiments, Brodersen drew the conclusion that penicillin had no charge in strongly acid solutions. The effect of ionic strength on the reaction rate in neutral or alkaline solutions has not been studied earlier.

EXPERIMENTAL

Materials.—The benzyl penicillin used was benzyl penicillin sodium, Ph.N.V ("Pharmacopoea Norvegica," V ed. Addendum, 1957).

All reagents were of analytical grade. The water was distilled water redistilled from a neutral glass still, boiled, and cooled under oxygen-free nitrogen.

Analytical Method.—The residual penicillin concentration of the heated solutions was determined iodometrically according to Örtenblad's (12) modification of Alicino's (13) method.

From the heated solution, two equal samples, *A* and *B*, each containing approximately 3.5 mg. of benzyl penicillin sodium, are pipeted into separate 100-ml. conical flasks with glass stoppers. To *A*, 1.00 ml. of 1 *N* sodium hydroxide is added. After standing for 20 min. at room temperature, 5 ml. of phthalate buffer solution, pH 4.5, 1.00 ml. of 1 *N* hydrochloric acid, and *a* ml., usually 10.00 ml., of 0.01 *N* iodine are added. The flask is closed and kept for 20 min. in darkness at room temperature. Excess of iodine is titrated with 0.01 *N* sodium thiosulfate using 2 drops of starch mucilage as indicator. The amount (*a*) of 0.01 *N* iodine added to sample *A* has to be such that 1.5–2.5 ml. of 0.01 *N* sodium thiosulfate are consumed by the titration.

To *B*, 5 ml. of phthalate buffer solution, pH 4.5, and *a* ml. of 0.01 *N* iodine are added. The flask is closed and kept for 20 min. in darkness at room temperature, and the mixture is titrated with 0.01 *N* sodium thiosulfate.

The difference between the two titrations represents the amount of iodine equivalent to the penicillin present. Each milliliter of 0.01 *N* iodine is assumed to be equivalent to 0.4455 mg. of benzyl penicillin sodium.

Iodine, 0.01 N.—Iodine (1.27 Gm.) and 2.0 Gm. of potassium iodide are dissolved in sufficient water to produce 1000 ml.

Phthalate Buffer Solution, pH 4.5.—Potassium biphthalate (60 Gm.) is dissolved in about 500 ml. of water and mixed with 80 ml. of *1 N* sodium hydroxide. Sufficient water to produce 1000 ml. is added.

Kinetic Studies.—In most cases the following procedure was used. Benzyl penicillin sodium (0.001 mole) was dissolved in 100 ml. of the appropriate buffer solution containing a sufficient amount of sodium chloride to give the penicillin solution an ionic strength of 0.50. The solution was filled into 5-ml. ampuls, and the air in the ampuls was replaced by nitrogen. The ampuls were sealed and heated at 60.0° in a constant-temperature bath. At appropriate intervals, ampuls were taken from the bath, cooled on ice, and the solution analyzed.

In those cases where the half-life was less than 30 min., *i.e.*, at pH < 4, the following technique was used. The buffer solution (99 ml.) was heated at 60.0° in a 200-ml. volumetric flask in a constant-temperature bath, and 0.001 mole of benzyl penicillin sodium dissolved in 1 ml. of water was added. Five-milliliter samples were withdrawn at appropriate intervals, mixed with a sufficient amount of a concentrated solution of sodium hydroxide to yield a pH of about 6.5 in the mixture, and cooled on ice.

Determination of pKa of Benzyl Penicillin at 60° and Ionic Strength = 0.5.—The pKa of benzyl penicillin was determined by measuring the pH at 60° of solutions prepared by mixing known amounts of benzyl penicillin sodium with known amounts of 0.1 *N* hydrochloric acid and adding water and a sufficient amount of sodium chloride to obtain an ionic strength = 0.5. The pKa was calculated according to Eq. 1.

$$\mathrm{pKa} = \mathrm{pH} + \log \frac{(a/10) - [\mathrm{H^+}]}{c - (a/10) + [\mathrm{H^+}]} \quad \text{(Eq. 1)}$$

where c = moles of benzyl penicillin sodium added per 1000 ml. of the solution, and a = milliliters of 0.1 *N* hydrochloric acid added per milliliter of the solution. pKa = 2.78 was found.

Determination of the pKa of Some General Acids at 60° and Ionic Strength = 0.5.—To determine the catalytic effect of a buffer on the degradation of benzyl penicillin, a series of solutions of different buffer concentrations but the same pH and ionic

TABLE II.—DETERMINATION OF PENICILLIN IN SOLUTIONS CONTAINING DEGRADATION PRODUCTS

Penicillin (moles/L. × 10^3)	Acid-degraded Penicillin (moles/L. × 10^3)	Base-degraded Penicillin (moles/L. × 10^3)	Penicillin Found (moles/L. × 10^3)	Recovery, %
9.00	1.00	0	9.00	100.0
8.00	2.00	0	7.92	99.0
7.00	3.00	0	6.86	98.0
6.00	4.00	0	5.87	97.8
5.00	5.00	0	5.02	100.4
4.00	6.00	0	3.88	97.0
8.00	0	2.00	7.95	99.4
7.00	0	3.00	6.89	98.4
6.00	0	4.00	6.00	100.0
5.00	0	5.00	4.94	98.8
4.00	0	6.00	3.97	99.3
3.00	0	7.00	2.83	94.3

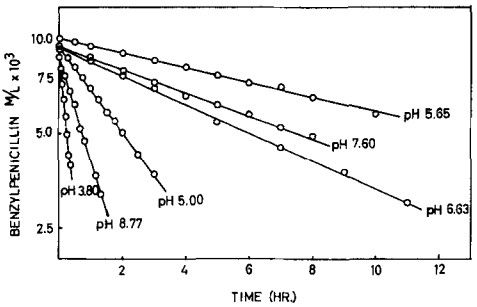

Fig. 1.—Plots showing the over-all first-order character of the degradation of penicillin G in aqueous solution at different pH values at 60°C. For the actual runs, 0.3 *M* acetate (pH 3.80 and pH 5.00), 0.06 *M* citrate (pH 5.65), 0.2 *M* phosphate (pH 6.63), 0.1 *M* phosphate (pH 7.60), and 0.1 *M* borate (pH 8.77) were used.

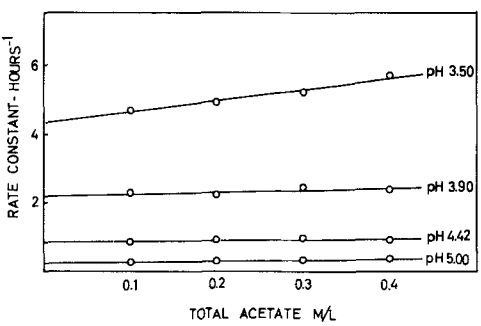

Fig. 2.—Effect of acetate concentration on the pseudo first-order rate constant of the degradation of penicillin G at fixed pH values (60°C., μ = 0.5).

strength had to be prepared. To calculate the composition of these solutions, the pKa of the acid component of the buffer solution was determined at 60° and an ionic strength = 0.5. The values obtained are:

Acid	pKa
Acetic acid	4.58
Boric acid	8.75
Phosphoric acid	6.54 (2nd)
Citric acid	2.72, 4.30, 5.47

RESULTS AND DISCUSSION

Precision of the Analytical Method.—Solutions containing known amounts of benzyl penicillin sodium (approximately 3.5 mg./ml.) were analyzed according to the analytical method. The amount (*a*) of 0.01 *N* iodine added was varied.

Table I shows that there is an increase in recovery and in coefficient of variation with increasing excess of iodine in sample *A*. The method gave the most precise results when the excess of 0.01 *N* iodine was 1.5–2.5 ml. Consequently, an amount of 0.01 *N* iodine was added so that 1.5–2.5 ml. of 0.01 *N* sodium thiosulfate was consumed in the titration of sample *A*.

Determination of Penicillin in Solutions Containing Degradation Products.—A solution containing the degradation products formed by the decomposition of benzyl penicillin in acid solution was prepared by keeping a 0.05 *M* solution of benzyl penicillin sodium to which hydrochloric acid had been added to pH 3.3 for 24 hr. at room temperature. No undegraded penicillin could be found in this solution by the iodimetric determination. Different amounts of this solution, containing 0.05 moles of acid-degraded penicillin per liter, were mixed with different amounts of freshly prepared 0.05 *M* benzyl penicillin solution and a sufficient amount of water to obtain various mixtures with a total concentration of undegraded and acid-degraded penicillin equal to 0.01. One-milliliter aliquots of the mixtures were analyzed (Table II).

A solution containing the degradation products formed by the decomposition of benzyl penicillin in alkaline solution was prepared in the following way. Benzyl penicillin sodium (0.00125 mole) was dissolved in a mixture of 5.0 ml. of 1 *N* sodium hydroxide and 20.0 ml. of water. After standing for 20 min. at room temperature, 20.0 ml. of phthalate buffer solution, pH 4.5, and 5.0 ml. of 1 *N* hydrochloric acid were added. Different amounts of this solution, containing 0.025 mole of base-degraded penicillin per liter, were mixed with different amounts of freshly prepared 0.025 *M* benzyl penicillin solution and a sufficient amount of water to yield various mixtures with a total concentration of undegraded and base-degraded penicillin equal to 0.01 *M*. One-milliliter aliquots of these mixtures were analyzed (Table II).

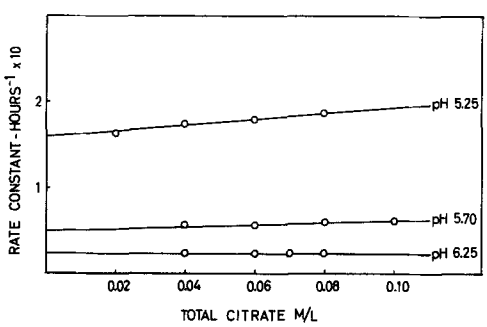

Fig. 3.—Effect of citrate concentration on the pseudo first-order rate constant of the degradation of penicillin G at fixed pH values (60°C., μ = 0.5).

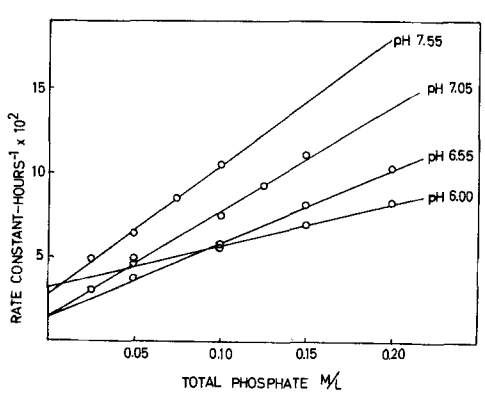

Fig. 4.—Effect of phosphate concentration on the pseudo first-order rate constant of the degradation of penicillin G at fixed pH values (60°C., μ = 0.5).

Table II shows that the analytical method gives satisfactory results for determination of penicillin in solutions containing degradation products.

Örtenblad (12) used this method for determination of penicillin in partially degraded solutions and found that it gave results that agreed well with those of biological determination.

That in all our kinetic experiments a strictly linear relationship between time and logarithm of residual penicillin concentration was found (*cf.* Fig. 1) strongly indicates that the analytical procedure was satisfactory for studies on the degradation of penicillin in aqueous solution.

Catalytic Effect of Buffers on the Degradation of Benzyl Penicillin.—The catalytic effect of a certain buffer was determined by experiments at constant pH, constant ionic strength ($\mu = 0.50$), and constant penicillin concentration, varying only the buffer concentration at a given pH. This was repeated at several pH values within the effective range of the buffer employed.

The results of these studies are summarized in Figs. 2–5, where the observed rate constants are plotted against buffer concentration.

Figure 2 shows the catalytic effect of acetate buffers pH 3.50 to pH 5.00. In this pH region, penicillin will exist as undissociated acid (HP) and penicillinate ion (P^-). The following reactions may occur:

$$HP \xrightarrow{k_1} \text{products}$$
$$P^- \xrightarrow{k_2} \text{products}$$
$$HP + HA \xrightarrow{k_3} \text{products}$$
$$HP + A^- \xrightarrow{k_4} \text{products}$$
$$P^- + HA \xrightarrow{k_5} \text{products}$$
$$P^- + A^- \xrightarrow{k_6} \text{products}$$

where HA = undissociated acetic acid, and A^- = acetate ion.

The over-all velocity for such a system would be equal to the sum of the rates of all these reactions.

$$-\frac{d[P_T]}{dt} = k_1[HP] + k_2[P^-] + k_3[HP][HA] + k_4[HP][A^-] + k_5[P^-][HA] + k_6[P^-][A^-] \quad \text{(Eq. 2)}$$

$$[P_T] = [HP] + [P^-] \quad \text{(Eq. 3)}$$

Because of the first-order character of the over-all reaction we have

$$-\frac{d[P_T]}{dt} = k[P_T] \quad \text{(Eq. 4)}$$

Combining Eqs. 2, 3, 4, and the equations

$$Ka_{acet} = \frac{[H^+][A^-]}{[HA]} \quad \text{(Eq. 5)}$$

$$Ka_{pen} = \frac{[H^+][P^-]}{[HP]} \quad \text{(Eq. 6)}$$

$$[A_T] = [HA] + [A^-] \quad \text{(Eq. 7)}$$

$$k_0 = \frac{k_1[HP] + k_2[P^-]}{[P_T]} \quad \text{(Eq. 8)}$$

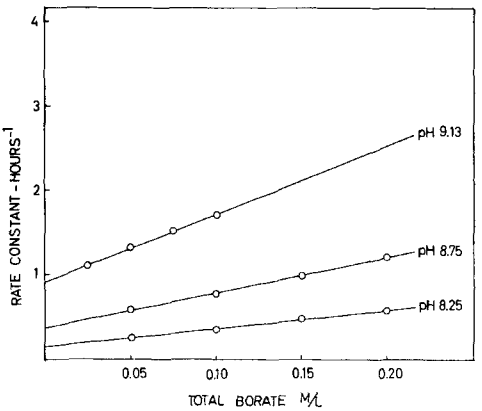

Fig. 5.—Effect of borate concentration on the pseudo first-order rate constant of the degradation of penicillin G at fixed pH values (60°C., $\mu = 0.5$).

TABLE III.—SLOPES ($S_{exp.}$) OF THE LINES IN FIG. 2 AND SLOPES ($S_{calcd.}$) CALCULATED FROM EQ. 10

pH	$S_{exp.}$	$S_{calcd.}$
3.50	3.3	3.3
3.90	0.7	1.3
4.42	0.3	0.3
5.00	0.3	0.1

we find

$$k = k_0 + \frac{[A_T](k_3[H^+]^2 + k_4Ka_{acet}[H^+] + k_5Ka_{pen}[H^+] + k_6Ka_{acet}Ka_{pen})}{([H^+] + Ka_{pen})([H^+] + Ka_{acet})} \quad \text{(Eq. 9)}$$

where k_0 is the rate constant at zero buffer concentration.

According to Eq. 9, a plot of rate constant against total acetate concentration should yield a straight line with

$$\text{slope} = \frac{k_3[H^+]^2 + k_4Ka_{acet}[H^+] + k_5Ka_{pen}[H^+] + k_6Ka_{acet}Ka_{pen}}{([H^+] + Ka_{pen})([H^+] + Ka_{acet})} \quad \text{(Eq. 10)}$$

By substituting $k_3 = 22$ hr.$^{-1}$ mole^{-1} liter, $k_4 = 0$, $k_5 = 0$, and $k_6 = 0$ into Eq. 10, slopes were calculated that agreed well with the slopes in Fig. 2 (*cf.* Table III).

Figure 3 shows the catalytic effect of citrate buffers, pH 5.25 to pH 6.25. In this pH region less than 0.1% of total penicillin exists as undissociated penicillinic acid, and less than 0.1% of total citrate exists as undissociated citric acid (H_3A). Therefore, it is reasonable to assume that Fig. 3 gives a picture of the catalytic effect of citrate ion ($A^{\equiv}$), monohydrogen citrate ion ($HA^{=}$), and dihydrogen citrate ion (H_2A^-) with respect to penicillinate ion.

At pH 6.25, 86% of total citrate exists as citrate ion. The zero slope of the line at this pH indicates that citrate ion is noncatalytic. The catalytic constants $k_{H_2A^-}$ and k_{HA^-} of dihydrogen and mono-

TABLE IV.—SLOPE ($S_{exp.}$) OF THE LINES IN FIG. 4 AND SLOPES ($S_{calcd.}$) CALCULATED FROM EQ. 20

pH	$S_{exp.} \times 10^2$	$S_{calcd.} \times 10^2$
6.00	24	25
6.55	44	44
7.05	62	63
7.55	76	76

TABLE V.—SLOPES ($S_{exp.}$) OF THE LINES IN FIG. 5 AND SLOPES ($S_{calcd.}$) CALCULATED FROM EQ. 22

pH	$S_{exp.}$	$S_{calcd.}$
8.25	2.2	2.2
8.75	4.2	4.6
9.13	8.1	6.6

hydrogen citrate ion have been calculated from the experiments at pH 5.25 and pH 5.70 using the following equations:

$$k = k_0 + k_{H_2A^-}[H_2A^-] + k_{H_2A^-}[H_2A^-] \quad \text{(Eq. 11)}$$

$$Ka_1 = \frac{[H^+][H_2A^-]}{[H_3A]} \quad \text{(Eq. 12)}$$

$$Ka_2 = \frac{[H^+][HA^-]}{[H_2A^-]} \quad \text{(Eq. 13)}$$

$$Ka_3 = \frac{[H^+][A^{\equiv}]}{[HA^-]} \quad \text{(Eq. 14)}$$

$$[H_3A] + [H_2A^-] + [HA^-] + [A^{\equiv}] = C \quad \text{(Eq. 15)}$$

where

k = the observed rate constant.
k_0 = the rate constant at zero buffer concentration = the intercept of the lines with the y axis.
$[H_3A]$ = concentration of undissociated citric acid.
$[H_2A^-]$ = concentration of dihydrogen citrate ion.
$[HA^-]$ = concentration of monohydrogen citrate ion.
$[A^{\equiv}]$ = concentration of citrate ion.

The following catalytic constants were found:

$$k_{H_2A^-} = 3.3 \text{ hr.}^{-1} \text{ mole}^{-1} \text{ liter}$$

$$k_{HA^-} = 0.2 \text{ hr.}^{-1} \text{ mole}^{-1} \text{ liter}$$

Figure 4 shows the catalytic effect of phosphate buffers, pH 6.00 to pH 7.55. The following equations are valid:

$$k = k_0 + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^-}[HPO_4^-] \quad \text{(Eq. 16)}$$

$$Ka_2 = \frac{[H^+][HPO_4^-]}{[H_2PO_4^-]} \quad \text{(Eq. 17)}$$

$$[H_2PO_4^-] + [HPO_4^-] = C \quad \text{(Eq. 18)}$$

Combining Eqs. 16, 17, and 18 gives

$$k = k_0 + C\frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^-}Ka_2}{[H^+] + Ka_2} \quad \text{(Eq. 19)}$$

A plot of observed rate constant *versus* total phosphate concentration should yield a straight line with

$$\text{slope} = \frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^-}Ka_2}{[H^+] + Ka} \quad \text{(Eq. 20)}$$

By substituting the k values mentioned below into Eq. 20, slopes were calculated that agreed well with the slopes of the lines in Fig. 4 (*cf.* Table IV).

$$k_{H_2PO_4^-} = 0.06 \text{ hr.}^{-1} \text{ mole}^{-1} \text{ liter}$$

$$k_{HPO_4^-} = 0.82 \text{ hr.}^{-1} \text{ mole}^{-1} \text{ liter}$$

It is obvious from Fig. 5 that borate buffers, pH 8.25 to pH 9.13, have a catalytic effect on the degradation of penicillin. If the assumption is made that boric acid in this pH region exists mainly as H_3BO_3 and $H_2BO_3^-$, an equation (Eq. 21) similar to Eq. 19 may be derived.

$$k = k_0 + C\frac{k_{H_3BO_3}[H^+] + k_{H_2BO_3^-}Ka}{[H^+] + Ka} \quad \text{(Eq. 21)}$$

A plot of observed rate constant *versus* total borate should yield a straight line with

$$\text{slope} = \frac{k_{H_3BO_3}[H^+] + k_{H_2BO_3^-}Ka}{[H^+] + Ka} \quad \text{(Eq. 22)}$$

By substituting $k_{H_3BO_3} = 0$, and $k_{H_2BO_3^-} = 9.3$ hr.$^{-1}$ mole^{-1} liter into Eq. 22, slopes were calculated that agreed well with the slopes of the lines in Fig. 5 (*cf.* Table V).

Primary Salt Effect.—A series of runs was made keeping pH, penicillin concentration, and buffer concentration constant in each series but varying the ionic strength by addition of different amounts of sodium chloride. The authors are aware that the hydrogen-ion concentrations of solutions of the same pH but of different ionic strengths will not be the same, but in the experiments the differences in hydrogen-ion concentrations were so small that the effects on the reaction rates were not significant.

The results of the experiments are shown in Fig. 6, where the logarithm of the rate constant, k, has been plotted against the square root of the ionic strength, μ. This plot shows that there is a primary salt effect at pH 6.80 and pH 8.75 but no effect at pH 4.50.

According to the theory of Brønsted and Bjerrum, there is a linear relationship between the logarithm of the rate constant and the square root of the ionic strength when two charged species react in dilute solution ($\sqrt{\mu} < 0.2$). At higher concentrations, the situation is more complex. Therefore, it is rather surprising that we find—at pH 6.80 and pH 8.75—a nearly linear relationship between log k and $\sqrt{\mu}$ at $\sqrt{\mu}$ values from 0.3 up to 0.7.

The positive primary salt effect at pH 6.80 and pH 8.75 indicates that the dominating processes at

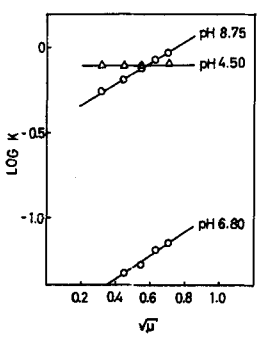

Fig. 6.—Effect of the ionic strength (μ) on the pseudo first-order rate constant (k) of the degradation of penicillin G at different pH values at 60°C.

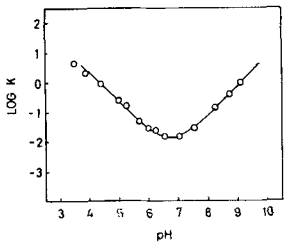

Fig. 7.—pH-rate profile of the degradation of penicillin G in aqueous solution at 60° C. Key: O, experimental results; —, corresponds to that expected theoretically from the three proposed reactions.

these pH values are reactions between two species with the same charge, positive or negative.

At pH 6.80, where 0.1 *M* phosphate was used as buffer, the dominating process is a reaction between the negatively charged penicillinate ion (P^-) and monohydrogen phosphate ion.

$$P^- + HPO_4^= \rightarrow \text{products}$$

Since a doubly charged ion is involved, it is not surprising that the log $k/\sqrt{\mu}$ curve at pH 6.80 is rather steep.

At pH 8.75, 0.1 *M* borate was used as buffer. Between pH 8 and 9, there is an increase in rate with increasing pH (Fig. 7). The dominating processes at pH 8.75 are presumably

$$P^- + OH^- \rightarrow \text{products}$$

$$P^- + H_2BO_3^- \rightarrow \text{products}$$

Both reactions will give positive primary salt effects.

At pH 4.50, there is no primary salt effect. The buffer used, 0.1 *M* acetate, has no catalytic effect. Since the rate decreases with increasing pH between pH 4 and 5, the rate-determining process at pH 4.5 must be a water attack on unionized penicillinic acid (HP)

$$HP \xrightarrow[H_2O]{} \text{products}$$

pH-Rate Profile of the Degradation of Benzyl Penicillin in Aqueous Solution at 60°.—The rate constants at zero buffer concentration can be picked easily from Figs. 2–5. In Fig. 7, the logarithms of these rate constants are plotted *versus* pH. The shape of the pH-rate profile may be explained by assuming the following reactions to occur in the pH region 4–9.

$$HP \xrightarrow{k_1} \text{products} \quad \text{(Reaction 1)}$$

$$P^- \xrightarrow{k_2} \text{products} \quad \text{(Reaction 2)}$$

$$P^- + OH^- \xrightarrow{k_3} \text{products} \quad \text{(Reaction 3)}$$

The reaction, $HP + H^+ \rightarrow$ products, is assumed to be of no significance at pH > 4.

The over-all velocity is equal to the sum of the rates of these reactions.

$$-\frac{d[P_T]}{dt} = k_1[HP] + k_2[P^-] + k_3[P^-][OH^-] \quad \text{(Eq. 23)}$$

Combining Eq. 23 with Eqs. 3, 4, and 6 gives

$$k = \frac{k_1[H^+] + k_2Ka_{pen} + k_3Ka_{pen}[OH^-]}{[H^+] + Ka_{pen}} \quad \text{(Eq. 24)}$$

$[H^+]$ and $[OH^-]$ may be calculated from the pH measurements using Eqs. 29 and 30. These equations were derived in the following way.

Harned and Hamer (14) have shown that

$$[H^+] \times [OH^-] = 1.94 \times 10^{-13} \quad \text{(Eq. 25)}$$

in solutions of potassium chloride at 60° and μ = 0.5. The same authors have found that

$$a_{H^+} \times a_{OH^-} = 9.61 \times 10^{-14} \quad \text{(Eq. 26)}$$

at 60°.

Combining Eqs. 25 and 26 with the equations

$$a_{H^+} = f[H^+] \quad \text{(Eq. 27)}$$

$$a_{OH^-} = f[OH^-] \quad \text{(Eq. 28)}$$

gives f = 0.7 and

$$\log[H^+] = -pH + 0.15 \quad \text{(Eq. 29)}$$

$$\log[OH^-] = pH - 12.94 \quad \text{(Eq. 30)}$$

From the experimental results, the following k values have been calculated

$$k_1 = 29.4 \text{ hr.}^{-1}$$

$$k_2 = 6 \times 10^{-3} \text{ hr.}^{-1}$$

$$k_3 = 6.38 \times 10^3 \text{ hr.}^{-1} \text{ mole}^{-1} \text{ liter}$$

The theoretical line in Fig. 7 has been calculated by substituting the above k values into Eq. 24.

The relatively good agreement of the experimental data and the theoretical profile does not prove that the proposed reactions *1–3* are the correct ones. Other reactions could lead to the same observed experimental dependency.

At pH 4–6, the following two reactions would give the same log k/pH dependency.

$$P^- + H^+ \rightarrow \text{products}$$

$$HP \rightarrow \text{products}$$

The lack of primary salt effect at pH 4.5 indicates that the dominating reaction cannot be $P^- + H^+ \rightarrow$ products, but HP → products.

At pH > 8, only the reaction $P^- + OH^- \rightarrow$ products will give the log k/pH dependency found.

The pH-rate profile has a minimum at pH 6.75.

$$k_{min.} = 1.41 \times 10^{-2} \text{ hr.}^{-1}$$

At 60°, Brodersen (2) found by calculation that $k_{min.} = 1.91 \times 10^{-2}$ hr.$^{-1}$ at log $[H^+] = -6.50$, *i.e.*, at pH = 6.65.

SUMMARY

The catalytic effect of general acids and bases on the degradation of penicillin G in aqueous solution at 60° has been studied. It has been found that dihydrogen citrate ion, monohydrogen phosphate ion, and borate ion catalyze the degradation of penicillinate ion; while acetic acid, acetate ion, monohydrogen citrate ion, unprotonated citrate ion, dihydrogen phosphate ion, and boric acid are noncatalytic or nearly noncatalytic. Acetic acid

catalyzes the degradation of benzyl penicillinic acid, while acetate ion has no effect.

There is a positive primary salt effect in neutral solution (phosphate buffer, pH 6.80) and in weakly alkaline solution (borate buffer, pH 8.75) but no primary salt effect in weakly acid solution (acetate buffer, pH 4.50).

In buffer-free solutions, pH 4–9, at least three separate reactions take place: (*a*) noncatalyzed cleavage of undissociated benzyl penicillinic acid, (*b*) noncatalyzed cleavage of benzyl penicillinate ion, and (*c*) hydroxyl ion catalyzed cleavage of benzyl penicillinate ion. Reaction *a* is about 5000 times faster than reaction *b*.

REFERENCES

(1) Benedict, R. G., *et al.*, *J. Bacteriol.*, **49**, 85(1945).
(2) Brodersen, R., *Acta Pharmacol. Toxicol.*, **3**, 345(1947).
(3) Pratt, R., THIS JOURNAL, **36**, 69(1947).
(4) Clapham, P. C. H. V., *Pharm. J.*, **165**, 126(1950).
(5) Macek, T. J., Hanus, E. J., and Feller, B. A., THIS JOURNAL, **37**, 322(1948).
(6) Hahn, L., *Biochim. Biophys. Acta*, **2**, 113(1948).
(7) Kaern, M., *Dansk Tidsskr. Farm.*, **29**, 93(1955).
(8) Fujiwara, H., *J. Pharm. Soc. Japan*, **75**, 681(1955).
(9) Swintosky, J. V., *et al.*, THIS JOURNAL, **45**, 34(1956).
(10) Schwartz, M. A., Granatek, A. P., and Buckwalter, F. H., *ibid.*, **51**, 523(1962).
(11) Brodersen, R., *Kgl. Danske Videnskab. Selskab Mat. Fys. Medd.*, **24** (No. 14), (1948).
(12) Örtenblad, B., *Acta Chem. Scand.*, **4**, 518(1950).
(13) Alicino, J. F., *Ind. Eng. Chem. Anal. Ed.*, **18**, 619 (1946).
(14) Harned, H. S., and Hamer, W. J., *J. Am. Chem. Soc.*, **55**, 2194(1933).

Anticholinergic Heterocyclic Ketoximino-Ethers and -Esters

By MAN M. KOCHHAR*, ROBERT G. BROWN, and JAIME N. DELGADO

The synthesis of a series of oximino-ethers and -esters was accomplished by *O*-alkylations and esterifications of 1-methyl-4-oximinopiperidine and 3-oximinotropane. In the course of this investigation, 13 new compounds were synthesized and evaluated for their anticholinergic activity.

ALTHOUGH MANY of the anticholinergic compounds are esters, it is known that the ester group is not absolutely essential for this bioaction (1, 2). Consequently, the medicinal chemist has studied how other groups affect the distribution characteristics and the intrinsic reactivity of compounds possessing the anticholinergic pharmacophores (3). The oximino group should certainly alter the partition (lipid-water) coefficient of the compound and thus affect its distribution in the body. Additionally, the oximino linkage should affect the intrinsic reactivity and stereochemistry of a given compound as well as its susceptibility toward metabolic degradation. In accordance with these considerations, it became of interest to study such structure-activity relationships by synthesizing oximino-esters and -ethers possessing the anticholinergic pharmacophores. Structure-activity studies of anticholinergic agents have led to the postulation of three pharmacophoric groups, *i.e.*, (*a*) a potential or actual cationic-nitrogen function to interact with the anionic region of the acetylcholine-receptor site, (*b*) a polar and/or polarizable group to interact with the esteratic region on this receptor site, (*c*) a large aryl or aralkyl moiety to provide the umbrellalike effect that appears to be necessary for effective blockade of the receptor surface (4, 5). The spatial relations among these groups appear to be critical factors that affect the activity. Accordingly, this work entails a study of structure-activity relationships among certain oximino-esters and oximino-ethers derivable from tropinone and 1-methyl-4-piperidone.

The oximes, starting materials for the synthesis of oximino-ethers and -esters, were prepared according to the method of Dickerman and Lindwall (6), with slight modification of reaction period. In the oximino-ethers, this work demonstrated that 1-methyl-4-oximinopiperidine and 3-oximinotropane can be alkylated to give *O*-alkyl ethers. The *O*-alkylation was effected by allowing a dialkylsulfate or an alkyl halide to react with the salt of the oxime, according to the method of Waters and Hartung (7) and French and Harrison (8). The use of a weak base (*e.g.*, tri-*n*-propylamine, pyridine, etc.) did not promote the reac-

Received August 26, 1964, from the Pharmaceutical Chemistry and Pharmacology Laboratories, College of Pharmacy, University of Texas, Austin.
Accepted for publication November 2, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
* Present address: School of Pharmacy, Auburn University, Auburn, Ala.