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Prediction of Stability in Pharmaceutical Preparations XVI: Kinetics of Hydrolysis of Canrenone and Lactonization of Canrenoic Acid

EDWARD R. GARRETT and CHONG MIN WON

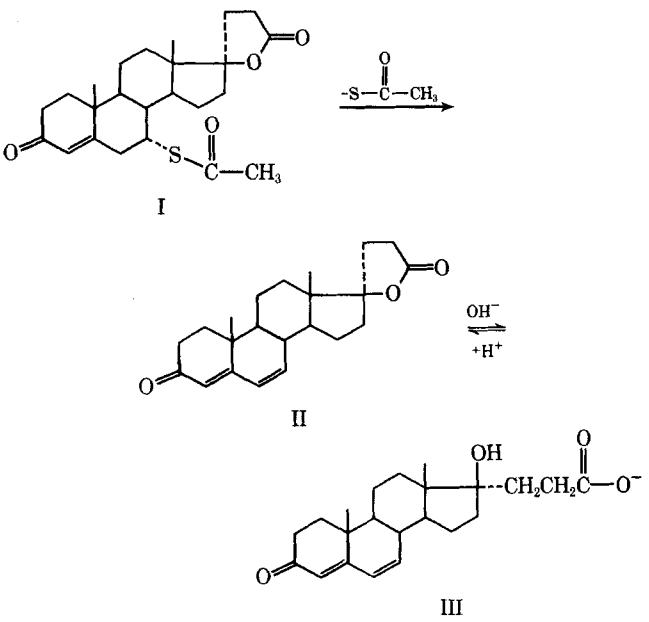
Abstract □ The kinetics of the hydrolysis of the lactone canrenone, 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone, and the lactonization of its corresponding canrenoic acid salt, potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionate, were studied by spectrophotometrically analyzing the chloroform-extracted lactone from canrenoic acid solution. The log *k*–pH profiles in the pH range of 1–12 in various buffer solutions at 25.0, 37.5, 45.0, 60.0, 70.0, and 79.9° show that the kinetics of lactonization include hydrogen-ion attack on the undissociated canrenoic acid molecule and a pH-independent closure of the canrenoate anion. In addition to the hydroxide-ion-catalyzed hydrolysis of the lactone, general base-catalyzed hydrolysis in carbonate buffer solution was observed and attributed to carbonate-dianion attack. The rate constants, equilibrium constants, pKa′ values, solubilities, and Arrhenius' parameters were obtained. Maximum concentrations of canrenoic acid salt to maintain elegant pharmaceutical preparations are given as a function of pH.

Keyphrases □ Canrenone, canrenoic acid—hydrolysis–lactonization kinetics, pH effect, appropriate pharmaceutical preparations □ Pharmacokinetics—canrenone hydrolysis, canrenoic acid lactonization, pH effect □ Hydrolysis—canrenone □ Lactonization—canrenoic acid □ UV spectrophotometry—analysis, canrenone and canrenoic acid

Spironolactone (I), 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, has been widely used in the treatment of edema that has not responded properly to treatment with conventional diuretics (1). Spironolactone exhibits a specific antagonism to the tendency of the adrenal steroid aldosterone to increase the reabsorption of sodium by reversibly competing with aldosterone at the receptor sites and thus modifying the sodium-retaining electrolyte excretion pattern, which is said to be the mechanism responsible for the production and maintenance of edema (2–4).

Canrenone (II), aldadiene [3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone], may be the conjugated diene steroid found in the plasma after the oral administration of spironolactone (5). It may be formed by the elimination of the thioacetate group of spironolactone and may possess similar biological properties as an aldosterone antagonist (Scheme I). As is common with γ-lactones, canrenone (II) is stable in acid and hydrolyzes in alkali to the corresponding canrenoic acid salt (III), potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionate. This study was undertaken to determine the quantitative transformations of canrenone in aqueous solution as a function of pH to supply the basic information necessary for preparing stable aqueous pharmaceutical preparations of canrenone and canrenoic acid.

In addition, it is important to know the ease of the acid–lactone transformation to anticipate the species that may appear in the body under GI and other physiological conditions. As yet, it has not been clarified whether the lactone or the acid, or a metabolite derived from one or the other, is the pharmacologically active compound. The two species also might show different tendencies for protein binding and, hence, influence the distribution pattern of the drug in the



Scheme I

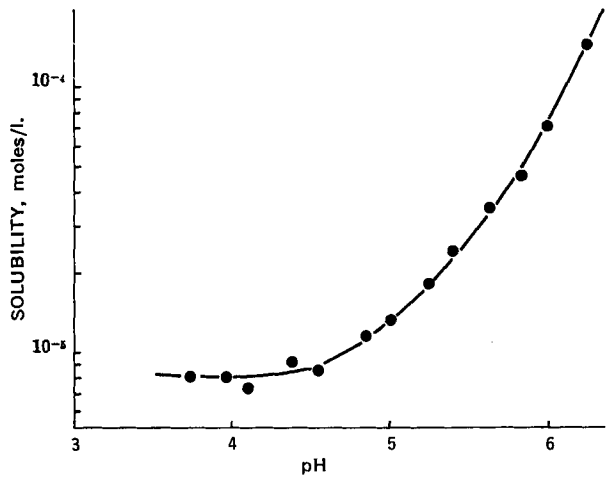


Figure 1—*Semilogarithmic plot of the molar solubility of canrenoic acid as a function of pH at 25.0°.*

body. Thus, this study on the interconversion of lactone and acid can serve as background for biopharmaceutical and pharmacokinetic studies of canrenone and canrenoic acid and as a basis for optimal design of appropriate dosage forms.

EXPERIMENTAL

Materials and Reagents—Canrenone (II), 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone, and the potassium salt of canrenoic acid, potassium canrenoate (III), potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionate, were used in this study[1]. All other chemicals were of reagent grade.

Kinetic Procedures—A filtered aqueous solution of canrenone (about 9×10^{-6} *M*) was used as a stock solution for the kinetic studies on lactone hydrolysis. An aqueous stock solution of potassium canrenoate (about 4×10^{-3} *M*) was used for the kinetic studies on lactonization. The kinetic studies of hydrolysis and lactonization of canrenone and canrenoate anion, respectively, were made on final solutions in the concentration range of $1.0\text{–}3.6 \times 10^{-5}$ *M*.

For slow reactions, *i.e.*, half-lives in days, 5–20 ml. of stock buffer solutions and appropriate amounts of 1 *M* KCl to maintain an ionic strength of 0.1 (0.16 in phosphate buffer) were added to appropriately diluted volumes of the stock solutions. The first

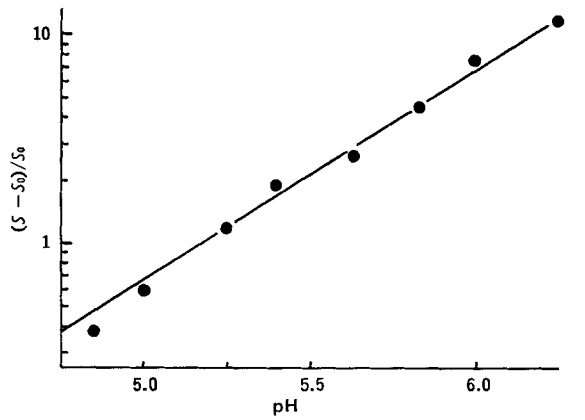


Figure 2—*Semilogarithmic plot of the solubility function* $(S - S_0)/S_0$ *against pH at 25.0° in accordance with* $\log(S - S_0)/S_0 = pH - pKa'$, *where* S *is the total molar solubility of canrenoic acid and its anion at a given pH, and* S_0 *is the intrinsic solubility of the undissociated canrenoic acid. The slope is unity, and the intercept permits the calculation of pKa' as 5.20.*

analyses of lactone concentration were made after the reacting solutions equilibrated in the temperature bath.

For the fast reactions, *i.e.*, half-lives in hours or minutes, 99.0 ml. of appropriately diluted stock solutions of the reacting substances, adjusted to appropriate ionic strengths, was preheated in the temperature bath prior to the addition of 1.0 ml. of the acid or alkali used to accelerate the reaction. The bath temperatures were maintained within ±0.1° for 79.9° and within ±0.05° for all other temperatures.

The compositions of the buffer solutions at the various temperatures studied are given in Table I. Samples of the reacting solutions were taken at various times, cooled to room temperature, and analyzed for the lactone. The fast reactions were followed for 5–6 half-lives and the slow ones for at least 2–3 half-lives. The concentration of the lactone at infinite time was obtained after the reaction went 10 half-lives.

Assay Procedure—Cooled aliquots (4.00 ml.) of the reacting solutions were put into a 30-ml. separator, which contained 5.00 ml. of chloroform and 3.00 ml. of carbonate buffer ($[CO_3^{-2}] = 0.15$ *M*, $[HCO_3^-] = 0.05$ *M*), pH 10.4. The funnel was shaken vigorously for 1 min., the chloroform layer was separated, and the absorbance of the extracted lactone was read at 280 nm. with a slit width of 0.2 mm. against a buffer-saturated chloroform blank in a spectrophotometer[2]. Within the significance of the spectral assay, all of the lactone was extracted into the chloroform layer under these conditions, and none could be spectrophotometrically observed in the aqueous layer. However, all of the potassium canrenoate remained in the aqueous layer under these conditions, and none could be spectrophotometrically observed in the chloroform layer.

Determination of Equilibrium Constants—The equilibrium constants were determined at different pH values at an ionic strength of 0.1 by analyzing the concentrations of the lactone and the acid in the sample after the reaction reached an equilibrium. Measurements also were made after the rate studies confirmed the achievement of this equilibrium. The assay procedure was used to determine the lactone concentration. The total acid and lactone concentration was obtained by partitioning both acid and lactone into 5.00 ml. of the chloroform layer by shaking 4.00 ml. of the sample solution together with the chloroform and 3.00 ml. of formate buffer ([HCOOH] = 0.16 *M*, [HCOONa] = 0.04 *M*), pH 3.0. The absorbance of the chloroform layer was read against a buffer-saturated chloroform blank at 280 nm. with a slit width of 0.2 mm. The molar absorptivity (ϵ = 29,000) at the wavelength of maximum absorption (280 nm.) of the lactone canrenone and canrenoic acid in the chloroform was the same. The acid concentration was then calculated from the observed difference of the two assay procedures.

pKa' Determination—The pKa' (apparent pKa) of canrenoic acid (III) was determined by measuring the solubilities of the acid as a function of pH. A series of acetic acid–acetate, monohydrophosphate–dihydrophosphate solutions, ionic strength 0.1, ranging in pH values from 3.7 to 6.2, was prepared. Potassium canrenoate solution (1.00 ml. of 2.8×10^{-2} *M*) was added to 99.0 ml. of each buffer solution. This addition produced a precipitate of canrenoic acid whose solubility was exceeded at these pH values. The solutions were shaken at 25.0° in a mechanical shaker. After the solutions equilibrated, they were filtered. After adjustment of an aliquot with carbonate buffer to pH 10.4, as previously described for the lactone assay procedure, the resultant canrenoic acid solution was extracted with chloroform to remove the lactone; the separated buffered aqueous layers were spectrophotometrically assayed at $\lambda_{max.}$ 294 nm. (ϵ = 25,000) at pH 10.4 for the sum of the canrenoic acid and its anion concentrations in the original solution at the various pH values. From these values, the total solubility of canrenoic acid and its anion could be calculated at the several pH values. The pKa' values at higher temperatures were determined from similar solubility determinations at the two pH values of 4.0 and 5.2 (acetate buffer). The intermittently shaken solutions were maintained in a constant-temperature water bath.

RESULTS AND DISCUSSION

Estimation of pKa'—The low solubility of canrenoic acid (III) in water necessitated the use of the solubility method for the

[1] Obtained from G. D. Searle & Co. as SC-9376 and SC-14266' respectively.

[2] Beckman DU.

Table I—Apparent First-Order Rate Constants, k^a, in sec.$^{-1}$ for the Achievement of Equilibrated Lactonization of Canrenoic Acid and Hydrolysis of Canrenone as a Function of pH[b] at Various Temperatures

Medium[c]	25.0° pH	25.0° k	37.5° pH	37.5° k	45.0° pH	45.0° k
[HCl]						
0.100	1.10	6.91×10^{-3}	—	—	—	—
0.070	1.23	4.77×10^{-3}	—	—	—	—
0.050	1.38	3.55×10^{-3}	—	—	—	—
0.040	1.47	2.80×10^{-3}	—	—	—	—
0.010	2.04	7.45×10^{-4}	2.04	1.62×10^{-3}	2.04	3.44×10^{-3}
[HCOOH] [NaOH]						
0.050 0.010	3.05	1.01×10^{-4}[d]	3.00	2.00×10^{-4}[l]	3.00	4.46×10^{-4}[l]
[CH_3COOH][CH_3COONa]						
0.040 0.010	4.00	9.98×10^{-6}[e]	4.00	2.65×10^{-5}[m]	4.00	4.43×10^{-5}[u]
0.010 0.040	5.23	1.08×10^{-7}[f]	—	—	5.15	4.11×10^{-6}[v]
[Na_2HPO_4] [KH_2PO_4]						
0.005 0.020	6.28	1.13×10^{-7}[g]	—	—	—	—
0.016 0.004	—	—	7.42	5.94×10^{-7}[n]	—	—
0.018 0.002	—	—	7.75	6.91×10^{-7}[o]	—	—
[H_3BO_3] [NaOH]						
0.010 0.003	8.80	7.73×10^{-7}[h]	—	—	8.70	8.71×10^{-6}[w]
[Na_2CO_3] [$NaHCO_3$]						
0.0020 0.0040	9.60	6.93×10^{-6}[i]	9.65	2.50×10^{-5}[p]	9.60	4.80×10^{-5}[x]
0.0035 0.0045	—	—	10.00	5.20×10^{-5}[q]	—	—
0.0060 0.0060	—	—	—	—	10.05	1.23×10^{-4}[y]
0.0045 0.0035	—	—	10.15	7.70×10^{-5}[r]	—	—
0.0053 0.0027	10.25	2.25×10^{-5}[j]	10.30	9.10×10^{-5}[s]	—	—
[NH_2CH_2COOH][NaOH]						
0.0104 0.0096	10.65	4.30×10^{-5}[k]	—	—	—	—
[NaOH]						
0.005	11.6	5.43×10^{-4}	—	—	—	—
0.010	12.0	1.20×10^{-3}	—	—	—	—
0.015	12.1	1.89×10^{-3}	—	—	—	—
0.020	12.2	2.35×10^{-3}	—	—	—	—
[CH_3COOH][CH_3COONa]						
0.040 0.010	—	—	4.00	1.89×10^{-4}[z]	—	—
0.010 0.015	—	—	4.80	3.28×10^{-5}	—	—
0.010 0.040	5.20	5.72×10^{-6}	5.20	1.10×10^{-5}[a']	5.20	2.90×10^{-5}[a']
[Na_2HPO_4] [KH_2PO_4]						
0.005 0.020	—	—	6.20	1.05×10^{-5}[b']	6.20	2.75×10^{-5}[f']
0.006 0.004	7.00	4.68×10^{-6}	7.00	9.60×10^{-6}	7.00	2.81×10^{-5}[g']
0.016 0.004	7.40	6.86×10^{-6}	7.40	1.39×10^{-5}[c']	7.40	3.91×10^{-5}
0.018 0.002	7.80	9.14×10^{-6}	7.80	2.05×10^{-5}[d']	7.80	6.56×10^{-5}
[H_3BO_3] [NaOH]						
0.010 0.003	—	—	8.70	8.75×10^{-5}	—	—

[a] The rate constants are the sums of the hydrolysis and the lactonization rate constants. They are the values obtained by extrapolation to zero buffer concentration whenever a buffer catalysis was observed. [b] The pH values in HCl and NaOH were calculated from pH = $-\log \gamma_{HCl}$[HCl] and pH = pKw − pOH, where pOH = $-\log \gamma_{NaOH}$[NaOH] where the activity coefficients, γ_{HCl} and γ_{NaOH}, and pKw values were obtained from the literature (10). All other pH values were measured by a Radiometer pH meter equipped with a Corning semimicro combination electrode (temperature range $-5 \sim 100°$). The pH meter was standardized with standard buffers (Sargent-Welch) of pH 4.0, 7.0, and 10.0 at the same temperature. [c] KCl was added to maintain the ionic strength, μ, at 0.1 (0.16 in phosphate buffer). [d] The 10^4k for multiples of the stated buffer concentration were: 1×, 0.98; 2×, 0.96; 3×, 1.10; and 4×, 1.00. [e] The 10^5k for multiples of the stated buffer concentration were: 1×, 1.07; 2×, 1.07; 3×, 1.14; and 4×, 1.19. [f] The 10^7k for multiples of the stated buffer concentration were: 1×, 1.13; 2×, 1.46; 3×, 1.68; and 4×, 1.92. [g] The 10^7k for multiples of the stated buffer concentration were: 1×, 1.32; 2×, 1.38; 3×, 1.48; and 4×, 1.57. [h] The 10^7k for multiples of the stated buffer concentration were: 1×, 7.73; 2×, 7.73; 3×, 7.73; and 4×, 7.74. [i] The 10^6k for multiples of the stated buffer concentration were: 1×, 7.14; 1.5×, 7.33; 3.5×, 7.90; and 5.0×, 8.04. [j] The 10^5k for multiples of the stated buffer concentration were: 0.75×, 2.32; 1.13×, 2.68; 2.64×, 3.02; and 3.77×, 3.19. [k] The 10^5k for multiples of the stated buffer concentration were: 1×, 4.77; 2×, 6.17; 3×, 7.00; and 4×, 7.45. [l] The 10^4k for multiples of the stated buffer concentration were: 2×, 2.15; 3×, 1.95; and 4×, 1.91. [m] The 10^5k for multiples of the stated buffer concentration were: 1×, 2.84; 2×, 3.03; 3×, 3.34; and 4×, 3.23. [n] The 10^7k for multiples of the stated buffer concentration were: 1×, 5.94; 1.5×, 5.94; 2.0×, 5.94; and 2.5×, 5.94. [o] The 10^7k for multiples of the stated buffer concentration were: 1×, 6.91; 1.5×, 6.91; 2.0×, 6.91; and 2.5×, 6.91. [p] The 10^5k for multiples of the stated buffer concentration were: 1×, 2.57; 1.5×, 2.92; 3.5×, 3.42; and 5.0×, 3.50. [q] The 10^5k for multiples of the stated buffer concentration were: 1×, 5.48; 2×, 5.97; 3×, 6.17; and 4×, 6.66. [r] The 10^5k for multiples of the stated buffer concentration were: 1×, 7.63; 2×, 8.44; 3×, 9.29; and 4×, 9.59. [s] The 10^5k for multiples of the stated buffer concentration were: 1×, 9.44; 2×, 10.3; 3×, 11.0; and 4×, 11.1. [t] The 10^4k for multiples of the stated buffer concentration were: 1×, 4.46; 2×, 4.46; 3×, 4.46; and 4×, 4.46. [u] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 4.61; 1.5×, 5.22; 2.0×, 5.49; and 2.5×, 5.57. [v] The 10^6k for multiples of the stated buffer concentration were: 1×, 4.11; 2×, 4.11; 3×, 4.11; and 4×, 4.11. [w] The 10^6k for multiples of the stated buffer concentration were: 1×, 8.61; 2×, 8.97; 3×, 8.38; and 4×, 8.90. [x] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 5.20; 1.5×, 5.43; 3.5×, 6.14; and 5.0×, 6.95. [y] The 10^4k for multiples of the stated buffer concentration were: 1×, 1.23; 2×, 1.23; 3×, 1.23; and 4×, 1.23. [z] The 10^4k for multiples of the stated buffer concentration were: 1.0×, 2.14; 1.5×, 2.26; 2.0×, 2.46; and 2.5×, 2.59. [a'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 1.39; 1.5×, 1.39; 2.0×, 1.54; and 2.5×, 1.51. [b'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 1.15; 1.5×, 1.30; 2.0×, 1.38; and 2.5×, 1.39. [c'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 1.34; 1.5×, 1.28; 2.0×, 1.47; and 2.5×, 1.47. [d'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 2.38; 1.5×, 2.38; 2.0×, 2.62; and 2.5×, 2.71. [e'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 2.82; 1.5×, 2.78; 2.0×, 3.27; and 2.5×, 2.84. [f'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 2.65; 1.5×, 2.66; 2.0×, 2.99; and 2.5×, 3.10. [g'] The 10^5k for multiples of the stated buffer concentration were: 1.0×, 2.84; 1.5×, 2.84; 2.0×, 2.81; and 2.5×, 2.77.

determination of the ionization constant. If S_0 is the intrinsic solubility of the undissociated acid, [HA], the ionization constant:

$$K_a' = [H^+][A^-]/[HA] \quad \text{(Eq. 1)}$$

can be expressed in terms of the solubilities (6) as:

$$K_a' = [H^+](S - S_0)/S_0 \quad \text{(Eq. 2)}$$

$$\log [(S - S_0)/S_0] = \text{pH} - \text{pKa}' \quad \text{(Eq. 3)}$$

where S is the total solubility of the undissociated canrenoic acid in equilibrium with its anion at a given pH.

When the logarithm of $(S - S_0)/S_0$ is plotted against pH, a straight line is produced with a slope of unity and the negative intercept of pKa'. The analytical procedure was presumed to remove any lactone formed during the equilibrium process, and lactonization was presumed to be a slower process than the equilibration between the solid canrenoic acid and its saturated solution. The solid phase of the equilibrated mixture was confirmed to be canrenoic acid by

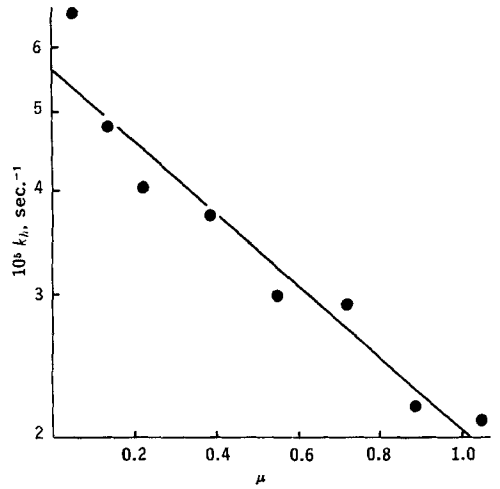


Figure 3—*Semilogarithmic plot of the apparent first-order rate constants in the hydrolysis of canrenone as a function of ionic strength, μ, in carbonate buffer (pH 10.5) at 25.0°.*

the fact that it was instantaneously soluble in mildly alkaline aqueous solution.

As would be expected from the relations of Eqs. 2 and 3, the solubility approaches the constant value S_0 as the pH decreases (Fig. 1). The plot of log $[(S - S_0)/S_0]$ *versus* pH is shown in Fig. 2. The intercept estimates a pKa′ value of 5.20 at 25.0° (Eq. 3). No pKa′ dependence on temperature was observed within experimental error. The experimental pKa′ values at the various temperatures are: 25.0°, 5.20; 37.5°, 5.21; 45.0°, 5.25; and 60.0°, 5.02.

Effect of Ionic Strength on Hydrolysis Rate—The hydrolysis of canrenone in carbonate buffer of pH 10.5 was tested for a salt effect with KCl up to ionic strengths of 1.25. A negative salt effect was observed (Fig. 3) since the logarithms of the hydrolysis rate constant significantly and linearly decreased with ionic strength. At higher ionic strengths (7, 8), the activity coefficient of an ion in aqueous solution at 25° adheres to:

$$\log \gamma_i = -0.509 z_i^2 \sqrt{\mu} + b_i \mu \qquad \text{(Eq. 4)}$$

where z_i is the charge on species i. It can be shown by transition-

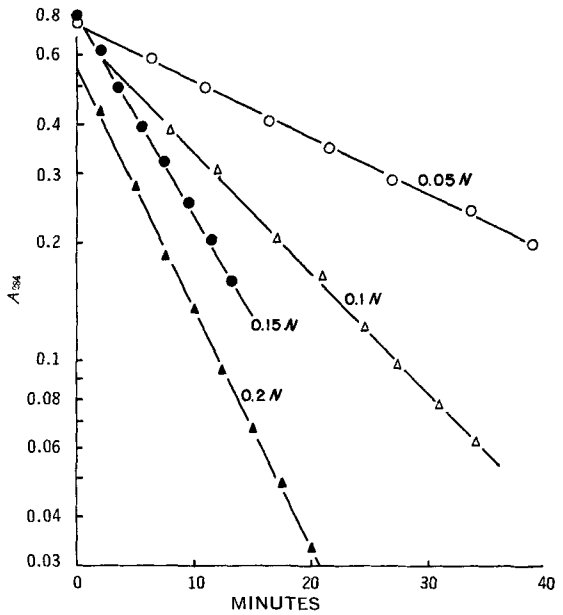


Figure 4—*Typical first-order plots for hydrolysis of canrenone in various NaOH concentrations at 25.0°. The absorbance values, A, of the unreacted lactone were measured at 284 nm. in the chloroform extract.*

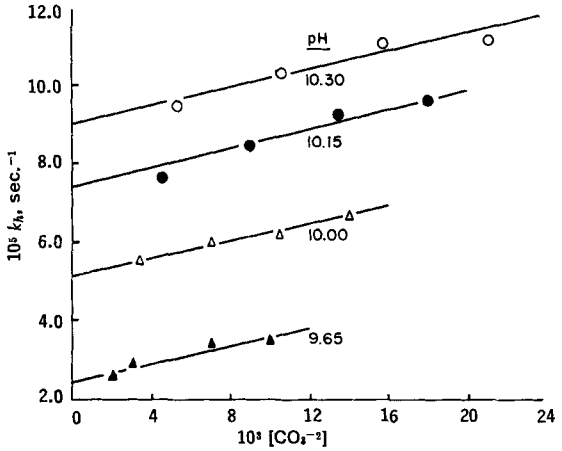


Figure 5—*Apparent first-order rate constants in hydrolysis of canrenone in carbonate buffer at 37.5° as a function of pH and the concentration of buffer anion, [CO_3^{-2}].*

state theory (9) that:

$$\log k = \log k_0 + (b_0 + b_i - b\ddagger)\mu \qquad \text{(Eq. 5)}$$

where b_i is the coefficient of ionic strength for hydroxide ion, b_0 is for the lactone, and $b\ddagger$ is for the activated complex, when k_0 is the rate constant at zero ionic strength. The conformity of the data of Fig. 3 to Eq. 5 indicates that the ionic concentration has a greater effect on the activated complex formed from hydroxide ion and lactone than it does on the two separated species. Of course, the possibility does exist that the activity of the lactone itself is decreased with increased ionic strength.

Rate Constants—The apparent first-order rate constants (Table I) were estimated from the slopes of appropriately plotted experimental data in accordance with:

$$\log |A_{eq} - A| = -kt/2.303 + \log |A_{eq} - A_0| \qquad \text{(Eq. 6)}$$

or by the method of least squares using the Wang 700 calculator. There was no significant difference between the statistical and graphical methods. The A_{eq} values were the final absorbances of the reaction, the A_0 values were the initial readings, and the absorbance values A were read at any given time t. At any constant pH, semilogarithmic plots in accordance with Eq. 6 increased or decreased linearly with time to provide confidence in k as an apparent first-order rate constant. Typical first-order plots for various NaOH concentrations are given in Fig. 4.

The separation of the apparent first-order lactonization (k_l) and hydrolysis (k_h) rate constants was achieved with the use of the equilibrium constants, since the apparent first-order rate constant for the achievement of an equilibrium is the sum of the forward and backward rate constants (Eq. 7) and the equilibrium constant is the quotient (Eq. 8):

$$k = k_h + k_l \qquad \text{(Eq. 7)}$$

$$K = k_h/k_l \qquad \text{(Eq. 8)}$$

Thus, k_h and k_l can be obtained from the experimental k and K values obtained at a given pH value.

General Acid–Base Catalysis—The rate constants were independent of buffer concentration at constant pH and ionic strength for formate and borate buffers and gave no evidence of general acid–base catalysis. The buffer catalysis by acetate and phosphate buffers was insignificant. However, significant buffer catalysis was observed with carbonate and glycine buffers in the hydrolysis of canrenone. The rate constants for the reactions studied in different buffer concentrations are given in the footnotes of Table I.

The possible catalytic contributions to the apparent first-order rate constant in the carbonate buffer region where only complete hydrolysis of the lactone was observed, on the assumption of no water attack, may be expressed by:

$$k_h = k_{OH}[OH^-] + k_{HCO_3}[HCO_3^-] + k_{CO_3}[CO_3^{-2}] \qquad \text{(Eq. 9)}$$

where k_h is the apparent first-order rate constant for the hydrolysis in the alkaline region, and k_{OH}, k_{HCO_3}, and k_{CO_3} are the respective catalytic rate constants. Since, when K_{HCO_3} is the dissociation constant of HCO_3^-:

$$[HCO_3^-]/[CO_3^{-2}] = [H^+]/K_{HCO_3} \quad \text{(Eq. 10)}$$

$$k_h = k_{OH}[OH^-] + (k_{HCO_3}[H^+]/K_{HCO_3} + k_{CO_3})[CO_3^{-2}] \quad \text{(Eq. 11)}$$

or:

$$k_h = k_{OH}[OH^-] + (k_{HCO_3} + k_{CO_3}K_{HCO_3}/[H^+])[HCO_3^-] \quad \text{(Eq. 12)}$$

the plots of k_h against $[HCO_3^-]$ or $[CO_3^{-2}]$ at constant $[H^+]$ should be linear and of positive slopes with intercept values of ($k_{OH}[OH^-]$). When the slopes, S_1, of Eq. 11:

$$S_1 = k_{HCO_3}[H^+]/K_{HCO_3} + k_{CO_3} \quad \text{(Eq. 13)}$$

are plotted against the hydrogen-ion activity, the resultant slope is k_{HCO_3}/K_{HCO_3} and the resultant intercept is k_{CO_3}. Similarly, from the slopes, S_2, of Eq. 12 against $1/[H^+]$:

$$S_2 = k_{HCO_3} + k_{CO_3}K_{HCO_3}/[H^+] \quad \text{(Eq. 14)}$$

k_{HCO_3} and k_{CO_3} can be evaluated separately when K_{HCO_3} is known.

The apparent rate constant, k_h, was plotted against $[CO_3^{-2}]$ (Fig. 5) and $[HCO_3^-]$ (Fig. 6). The fact that the slopes (S_1) in Fig. 5 are independent of pH within experimental error indicates the nonvariance of ($k_{HCO_3}[H^+]/K_{HCO_3} + k_{CO_3}$) with $[H^+]$ and, thus, the lack of a catalytic rate constant, k_{HCO_3}, in carbonate buffer-catalyzed hydrolysis of the lactone. The catalytic rate constant, k_{CO_3}, was 1.1×10^{-3} sec.$^{-1}$ at 37.5°. The identical value of k_{CO_3} can be obtained from the slope of plots of the slopes (S_2) of Fig. 6 against $1/[H^+]$ in accordance with Eq. 12. The intercept of zero confirms the fact that $k_{HCO_3} \sim 0$.

The intercepts of the plots in Figs. 5 and 6 vary and are consistent with hydroxyl-ion catalysis; *i.e.*, the intercepts ($k_{OH}[OH^-]$) *versus* $[OH^-]$ give a linear plot of slope k_{OH}. The line passes through zero and confirms the fact that there is no water hydrolysis. The k_{OH} value estimated by such a method was 0.21 sec.$^{-1}$ at 37.5°. The rate constants were extrapolated to zero buffer concentrations, and the determined $k_{OH}[OH^-]$ values were used in the log k–pH profiles.

Specific Acid–Base Catalysis—Plots of the apparent first-order rate constants against HCl and NaOH (Fig. 7) in strongly acidic and alkaline regions were linear and with zero intercept in accordance with:

$$k_l = k_{HCl}[HCl] \quad \text{(Eq. 15)}$$

$$k_h = k_{NaOH}[NaOH] \quad \text{(Eq. 16)}$$

In these highly acidic regions, all canrenoic acid was converted to the lactone canrenone within the precision of the experimental method. In these highly alkaline regions, all of the lactone canrenone was converted to the canrenoic acid within the precision of the experimental method. Both lines (Fig. 7) had zero intercepts to indicate the lack of water reaction.

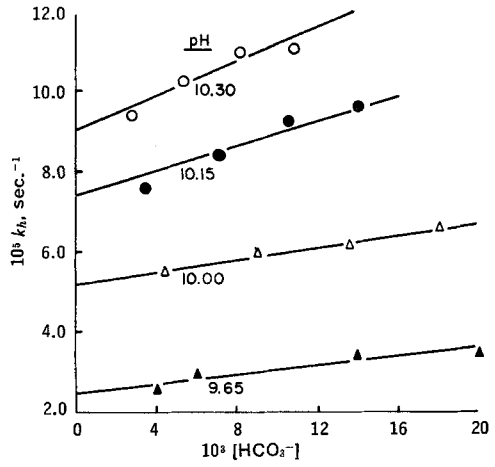


Figure 6—*Apparent first-order rate constants in hydrolysis of canrenone in carbonate buffer at 37.5° as a function of pH and the concentration of buffer acid, [HCO_3^-].*

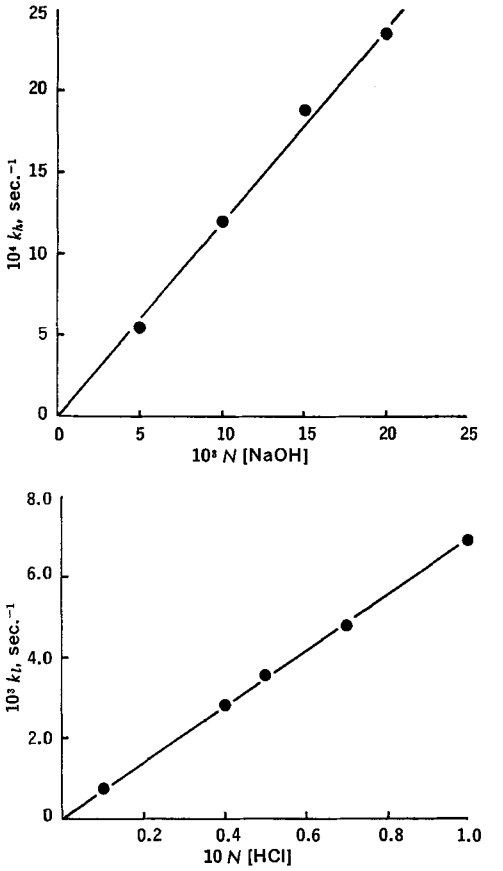


Figure 7—*Apparent first-order rate constant dependence on [NaOH] for hydrolysis of canrenone (top) and on [HCl] for lactonization of canrenoic acid (bottom) at 25.0°.*

Equilibrium Constants—The equilibrium constants at any pH value can be defined as the ratio of apparent first-order hydrolysis

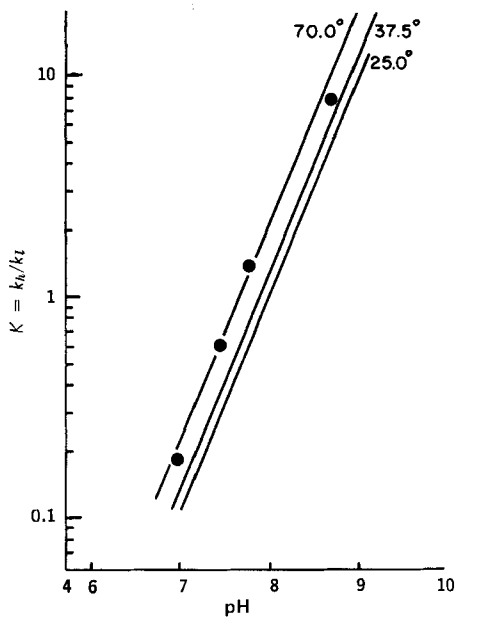


Figure 8—*Semilogarithmic plot of equilibrium constants, K ($= k_h/k_l$), as a function of pH. The drawn lines at 25.0 and 37.5° were calculated from the microscopic rate constants with the use of Arrhenius plots.*

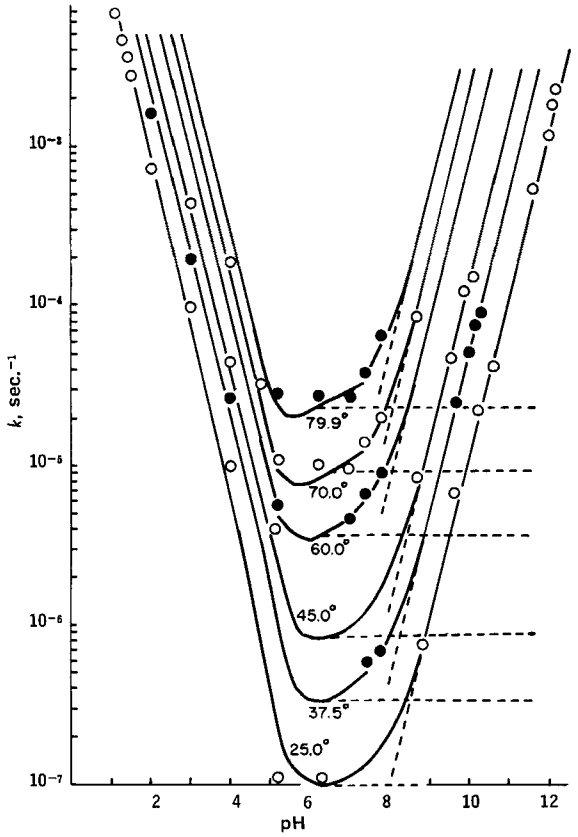


Figure 9—*Log* k–*pH profile for the lactonization of canrenoic acid and hydrolysis of canrenone at 25.0, 37.5, 45.0, 60.0, 70.0, and 79.9°. The drawn lines were calculated from the derived microscopic rate constants. The dashed lines were the separated log* k–*pH profiles for lactonization and hydrolysis.*

and lactonization constants. Consequently, it is a function of hydrogen- and hydroxyl-ion activity. The experimental equilibrium constants ($K = k_h/k_l$) at 70.0° are plotted semilogarithmically in Fig. 8. Experimental values of K obtained at 79.9° were not significantly different.

Log *k*–pH Profile—The log k–pH profiles were constructed from the first-order rate constants (Table I) and pH values at 25.0, 37.5, 45.0, 60.0, 70.0, and 79.9°. The sum of the rate constants of the lactonization and the hydrolysis as a function of pH are plotted in Fig. 9. The separated log k–pH profiles for lactonization and hydrolysis are shown in this figure by the dashed lines. The pH values of the strongly acidic or strongly alkaline solutions were calculated from:

$$\text{pH} = -\log \gamma_{\text{HCl}}[\text{HCl}] \qquad \text{(Eq. 17)}$$

$$\text{pH} = \text{pKw} - \text{pOH} = \text{pKw} + \log \gamma_{\text{NaOH}}[\text{NaOH}] \qquad \text{(Eq. 18)}$$

where pKw and the activity coefficients, γ_{HCl} and γ_{NaOH}, were obtained from the data in the literature (10).

The logarithmic transformations of Eqs. 15 and 16 are:

$$\log k_l = \log k_{\text{H}} - \text{pH} \qquad \text{(Eq. 19)}$$

$$\log k_h = \log k_{\text{OH}} - \text{pKw} + \text{pH} \qquad \text{(Eq. 20)}$$

where the specific bimolecular rate constants, k_{H} and k_{OH}, are now defined with respect to hydrogen- and hydroxide-ion activities rather than their respective molarities, as were the k_{HCl} and k_{NaOH} of Eqs. 19 and 20. The separated k_l and k_h values were calculated on the basis of the observed overall first-order rate constants of change (Table I and Fig. 9) and the determined equilibrium constants (Fig. 8). The bases for this separation were given previously in Eqs. 7 and 8.

The log k_h decreases with decreased pH with slope of unity in accordance with Eq. 20 (Fig. 9). The apparent lactonization rate constant conforms to the dependency of Eq. 21 below a pH of 3

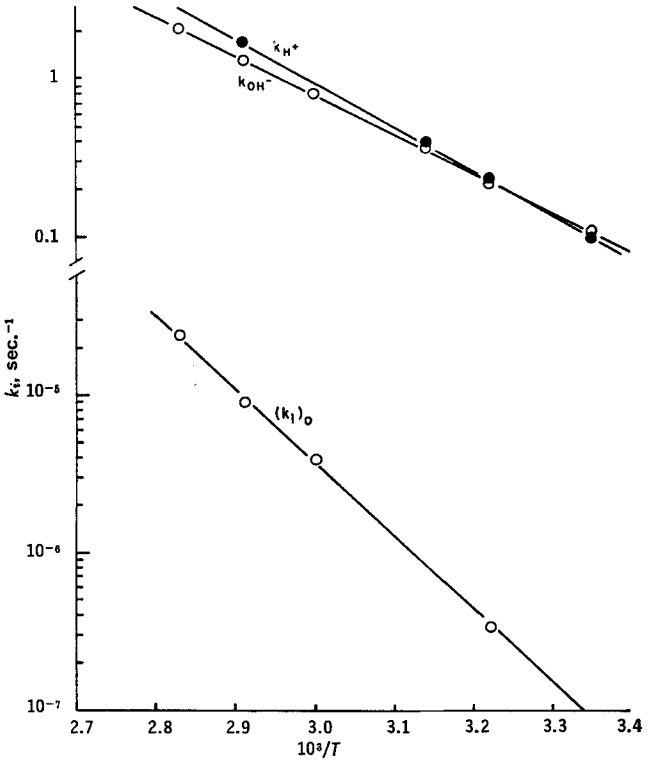


Figure 10—*Arrhenius plots of the microscopic rate constants for lactonization of canrenoic acid,* k_H *and* $(k_l)_0$, *and hydrolysis of canrenone,* k_{OH}.

where essentially all the acid molecules are undissociated. The apparent first-order lactonization rate constant tends to be independent of pH as the fraction of dissociated acid present increases (as pH tends to exceed the pKa′). This indicates a pH-independent lactonization of the anion. Hence, the overall lactonization can be formulated as:

$$k_l = k_{\text{H}}[\text{H}^+]f_{HA} + (k_l)_0 f_A = k_{\text{H}}[\text{H}^+]^2/([\text{H}^+] + K_a) + (k_l)_0 K_a'/([\text{H}^+] + K_a') \qquad \text{(Eq. 21)}$$

where f_{HA} and f_A are the fractions of undissociated and dissociated acids, respectively (11), and $(k_l)_0$ is the rate constant for a pH-independent lactonization of the anion. The solid drawn lines in the log k–pH profile (Fig. 9) were derived from the sums of the first-order rates constants of Eqs. 20 and 21.

The fact that a plot of log K *versus* pH (Fig. 8) is linear and of positive slope of unity in the pH regions where the amounts of canrenone and canrenoic acid assayed were significant indicates that:

$$K = k_h/k_l = k_{\text{OH}}[\text{OH}^-]/(k_l)_0 \qquad \text{(Eq. 22)}$$

Table II—Microscopic Rate Constants[a], Arrhenius Parameters[b], and Entropies of Activation[c] for Hydrolysis of Canrenone and Lactonization of Canrenoic Acid

Temperature	k_{H}, sec.$^{-1}$	k_{OH}, sec.$^{-1}$	$10^8(k_l)_0$, sec.$^{-1}$
25.0°	0.10	0.11	—
37.5°	0.24	0.21	34.0
45.0°	0.40	0.36	—
60.0°	—	0.80	400
70.0°	1.70	1.32	900
79.9°	—	2.12	2400
E_a, kcal.	12.84	11.42	21.23
log A	8.40	7.38	8.50
$\Delta S^{\ddagger}$, e.u.	−20.0	−24.7	−19.5

[a] Where $k_h = k_{\text{OH}}[\text{OH}^-]$, $k_l = k_{\text{H}}[\text{H}^+]f_{HA} + (k_l)_0 f_A$. [b] Where $\log k = -E_a/2.303RT + \log A$. [c] Where $\Delta S^{\ddagger}/R = 2.303\,[\log A - \log(kT/h)] - 1$.

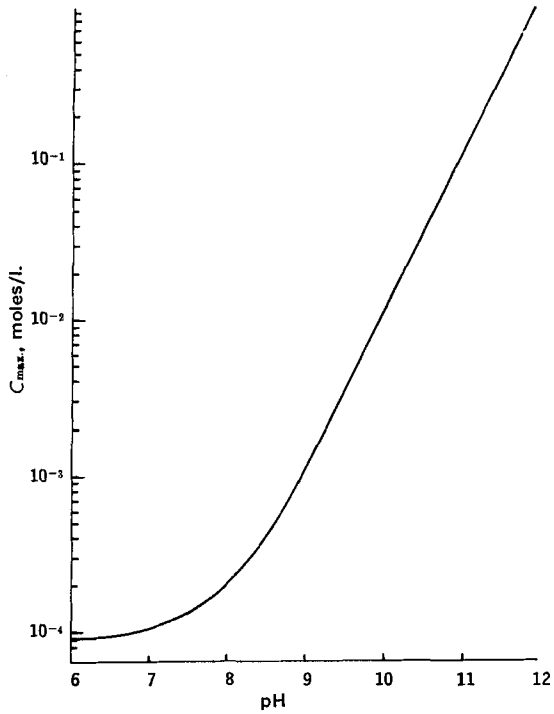


Figure 11—*Semilogarithmic plot of the maximum molar concentration, $C_{max.}$, of stabilizable potassium canrenoate in aqueous preparations as a function of pH at 25.0°.*

so that:

$$\log K = \log [k_{OH}/(k_l)_0] - pKw + pH \qquad \text{(Eq. 23)}$$

where the intercept $\log [k_{OH}/(k_l)_0] - pKw = 7.96$ at 25.0°. Thus, in the pH region where significant amounts of both species are present in equilibrium, the hydrolysis rate must be hydroxide ion dependent and the lactonization rate must be pH independent.

If this was not the case and if hydrolysis was $[OH^-]$ dependent and lactonization was $[H^+]$ dependent:

$$K = k_h/k_l = k_{OH}[OH^-]/k_H[H^+] \qquad \text{(Eq. 24)}$$

It would follow that:

$$\log K = \log (k_{OH}K_w/k_H) + 2pH \qquad \text{(Eq. 25)}$$

and the plot of Fig. 8 would be linear with positive slope of 2, which is not the case.

If lactonization of undissociated acid was pH dependent and that of dissociated acid was pH independent, and hydrolysis was hydrogen-ion as well as hydroxyl-ion catalyzed, then:

$$K = k_h/k_l = (k_H'[H^+] + k_{OH}[OH^-])/\{k_H[H^+]f_{HA} + (k_l)_0 f_A\} \qquad \text{(Eq. 26)}$$

Thus, it would follow that at high alkalinity when $[H^+]$ is negligible, the log K is the function of pH as given in Eq. 23 and justified by the plot of Fig. 8. This would also be true in the neutral pH region of Fig. 8 if $k_H[H^+]f_{HA}$ and $k_H'[H^+]$ were negligible. At higher acidity, *i.e.*, where $[H^+] \gg [OH^-]$, it would follow that $K = k_H'/k_H$ so that the equilibrium constant would tend to become invariant with pH at low pH values and the plot of log K *versus* pH of Fig. 8 would tend to flatten at lower pH values. This could not be concluded within the experimental error. Although the acid-catalyzed hydrolysis of γ-lactones was observed in the hydrolysis of γ-butyrolactone (12), γ-substituted γ-lactones (13), and pilocarpine (14), one could not conclude that it was significant in the case of canrenone. At least the acid-catalyzed hydrolysis rate cannot be any more than 5% as large as the acid-catalyzed lactonization rate, *i.e.*, the equilibrium constant in highly acidic solution cannot be greater than 0.05.

Microscopic Rate Constants—The values of the microscopic rate constants, k_H and k_{OH}, at all temperatures were estimated directly from the best fit of the log k–pH profile (Fig. 9) in low and high pH regions, respectively. The bimolecular rate constants of pH-independent lactonization of the dissociated acid were estimated from the best fit of the profile in the pH 7–8 region where essentially all the acid is in the dissociated form. The microscopic rate constants at different temperatures are given in Table II. The solid lines in the log k–pH profile (Fig. 9) were calculated from the k_i values in Table II.

Rate Dependency on Temperature—The Arrhenius equation gives the quantitative relation of rate constants and temperature. The logarithmic form of the equation is:

$$\log k = -E_a/2.303RT + \log A \qquad \text{(Eq. 27)}$$

where E_a is the activation energy, and A is the frequency factor. The Arrhenius plots of the logarithms of the various catalytic rate constants are shown in Fig. 10 for: k_{OH}, the specific base catalytic hydrolysis rate constant; k_H, the specific acid catalytic lactonization rate constant; and $(k_l)_0$, the pH-independent lactonization rate constant of the dissociated acid. The Arrhenius parameters were calculated from the plots in accordance with Eq. 27. The values of E_a, log A, and $\Delta S‡$ (entropy of activation) are included in Table II.

Since the rate of achievement of an equilibrium in the neutral pH regions at lower temperatures is an extremely slow process, the equilibrium constants at lower temperatures were calculated from the ratio of Eqs. 20 and 21. The microscopic rate constants given in these equations were based on the values of the constants estimated from the use of their Arrhenius parameters (Eq. 27 and Table II). Similar plots of such calculated equilibrium constants as a function of pH are given in Fig. 8 for 25.0 and 37.5°.

Mechanism for Hydrolysis of Canrenone and Lactonization of Canrenoic Acid—Long and Friedman (15), by using ^{18}O-labeled water, showed that acyl oxygen scission of a γ-lactone occurs in both the acid- and base-catalyzed reactions and that the hydrolysis passes through an intermediate with enough lifetime to allow

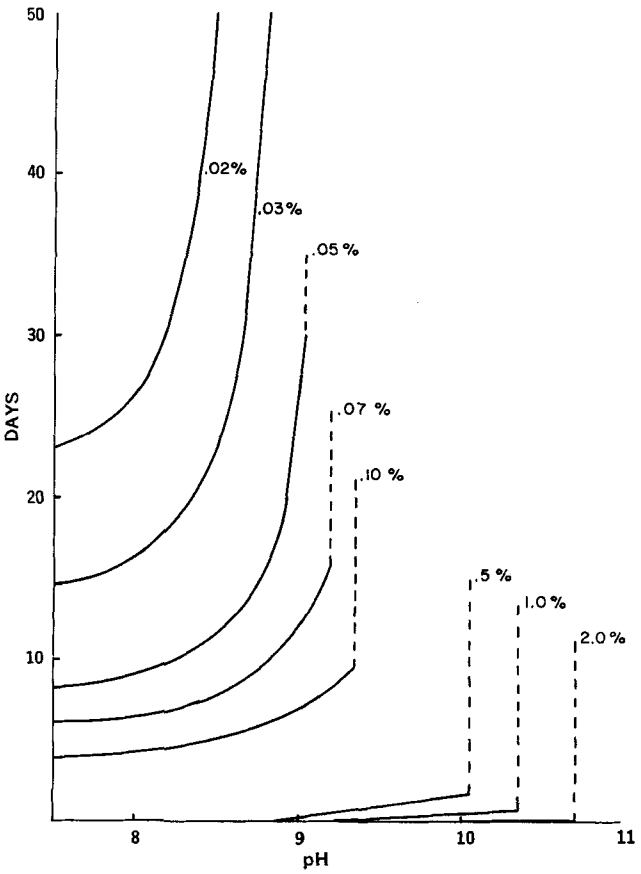
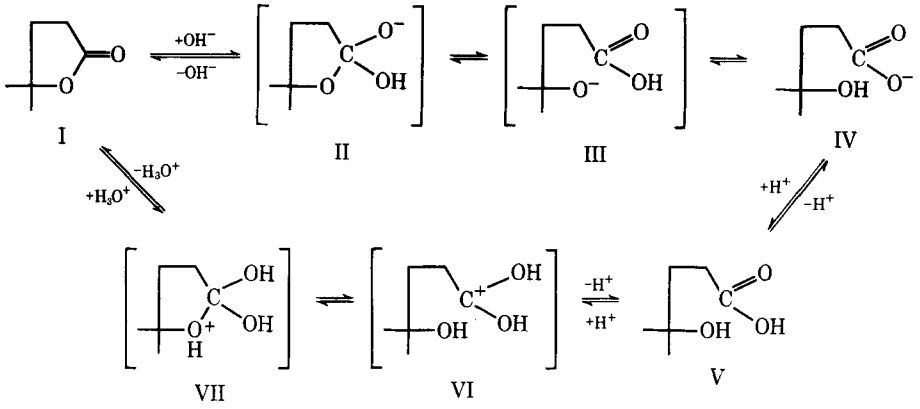


Figure 12—*Time required to start forming precipitates of canrenone in aqueous preparations of potassium canrenoate as a function of pH and concentration of potassium canrenoate at 25.0°.*



Scheme II

appreciable oxygen exchange, such as that found in the hydrolysis of simple esters. Long *et al.* (12) also studied the kinetics of the acid-catalyzed hydrolysis of γ-butyrolactone in strongly acidic solutions and found that the rate of hydrolysis is proportional to the concentration of hydrogen ion, but the rate of the lactonization is proportional to h_0 (Hammett's acidity function). This indicated that the activated complex consisted not only of a molecule of lactone and hydrogen ion but also of a water molecule [Hammett–Zucker hypothesis (16)].

Scheme II is consistent with these mechanisms for lactonization and hydrolysis of γ-lactones. The sequence I → IV explains the base-catalyzed hydrolysis of canrenone and is consistent with a rate dependency of the apparent first-order rate constant on $k_{OH}[OH^-]$. It is also consistent with the pH-independent lactonization of the canrenoic acid monoanion, which may be initiated by an intramolecular general base attack on the alcoholic group (IV → III) (possibly mediated by a molecule of water), which then results in the intramolecular nucleophilic attack of the resultant alcoholate anion on the carbonyl carbon to produce the common activated complex, II.

The sequence V → VI → VII → I explains the acid-catalyzed lactonization of undissociated canrenoic acid and is consistent with a rate dependency of the apparent first-order rate constant on $k_H[H^+]f_{HA}$. The possible reverse reaction, the acid-catalyzed hydrolysis of canrenone, does not appear to be large enough to affect significantly the equilibrium for all practical purposes. This was also true for the acid-catalyzed hydrolysis of pilocarpine (14), although the acid-catalyzed hydrolysis of γ-butyrolactone was significant (12). Bruice and Pandit (17) showed that alkyl substitutions on the carbons of structures amenable to intramolecular reaction also favor the cyclization.

Prediction of Stability—The pH-independent lactonization occurs in the alkaline region at all pH values, as shown in the log k–pH profile (Fig. 9). The extent of its formation is a function of its equilibrium with the hydroxide-ion-catalyzed hydrolysis. The lactone canrenone precipitates when the concentration of the lactone in equilibrium at a given pH value exceeds its solubility. Thus, the pharmaceutical stability of the canrenoic acid salt is a function of the solubility of the lactone and the pH-dependent thermodynamic equilibrium of the reversible lactonization and hydrolysis processes. The solubilities of the lactone, canrenone (S_L), in water were found to be: 25.0°, 9.4×10^{-5} *M*; 37.5°, 7.79×10^{-4} *M*; and 60.0°, 2.43×10^{-3} *M*. Substitution of the ratio of canrenoate anion (at pH values much higher than 5.2 where the acid concentration is negligible) to canrenone concentrations for the equilibrium constant K in Eq. 23 results in:

$$\log K = \log([\text{canrenoate}]/[\text{canrenone}]) = \log[(C_{max.} - S_L)/S_L] = \log[(C_{max.} - 9.4 \times 10^{-5})/9.4 \times 10^{-5}] = \text{pH} - 7.96 \quad \text{(Eq. 28)}$$

where $C_{max.}$ in moles/l. is the maximum concentration of canrenoic acid salt that can be prepared at a given pH in solution at 25.0° and yet will not give an ultimate precipitate of equilibrated canrenone. A plot of $C_{max.}$ calculated from Eq. 28 for various pH values is given in Fig. 11 and clearly shows the maximum concentrations of canrenoate solution that can be prepared at any specific pH value to maintain a true solution at 25.0°.

The time required to reach that point where the lactone will precipitate from a canrenoic acid salt solution is a function of the concentration of the canrenoic acid salt as well as the pH, since both the rate and equilibrium constants are pH dependent. The lactone concentration, L_{eq}, in moles/l. at equilibrium may be expressed by:

$$L_{eq} = C_0/(1 + K) \quad \text{(Eq. 29)}$$

where C_0 is the initial concentration in moles/l. of the canrenoic acid salt and $K = (C_0 - L_{eq})/L_{eq}$. The apparent first-order transformation of canrenoate anion to the lactone, canrenone, at a constant pH may be expressed by:

$$\log(C - C_{eq}) = \log(C_0 - C_{eq}) - kt/2.303 \quad \text{(Eq. 30)}$$

where C is the concentration of nonlactonized canrenoic acid or anion at any time t, and k is the apparent first-order rate constant (Table I) to achieve an equilibrium. If the concentration, C, of canrenoate is limited so that precipitates of the lactone cannot occur, then:

$$C = C_0 - S_L \quad \text{(Eq. 31)}$$

and, from Eq. 29:

$$C_{eq} = KL_{eq} = KC_0/(1 + K) \quad \text{(Eq. 32)}$$

Thus, substitution of Eqs. 31 and 32 into Eq. 30 and subsequent rearrangement result in

$$t_{S_L} = (2.303/k)\log[C_0/\{C_0 - S_L(1 + K)\}] \quad \text{(Eq. 33)}$$

and the time, t_{S_L}, when the lactone, canrenone, of solubility S_L precipitates from a solution of C_0 (moles/l.) concentration may be calculated at any temperature when the apparent first-order rate constant, k (Table I), for the achievement of the equilibrium and the equilibrium constant, K (Fig. 8), are known. These times, t_{S_L}, required for precipitation of the lactone for various concentrations of the canrenoic acid salt are given in Fig. 12. Even a dilute solution of potassium canrenoate (0.02%) at pH 8.0 gives a precipitate in less than a month. Thus, if it is still desirable to have a soluble preparation of potassium canrenoate, either adjuncts should be added to increase the hydrolysis rate of the lactone without affecting the rate of lactonization or mixed solvents should be considered so that a small amount of lactone in the equilibrium will be completely solubilized.

Biopharmaceutical Aspects of Spironolactone, Canrenone, and Canrenoic Acid—Gantt *et al.* (18) reported originally that the addition of polysorbate 80 enhanced the absorption of spironolactone. Subsequently, they revised (19) their conclusion to state that the addition of polysorbate 80 does not contribute directly to the previously observed increased absorption. This revision was due to their observation that the smaller particle size of spironolactone was preserved when the drug was encapsulated with the surfactant; thus they attributed the improved absorption to the

particle size rather than to the addition of the surfactant. The smaller particle-sized spironolactone was as well absorbed as the one with polysorbate 80. Their observation was supported by several clinical and blood level studies (20–22). Venning (23) argued against this dependence of absorption on particle size by claiming that blood level studies demonstrated that conventional tablets with an average particle size of 1 μ were as poorly absorbed as those of 10 μ. He concluded that "absorption of spironolactone is dependent upon some more complex function of the physical state within the finished tablets." The fact of the matter was that these two sets of tablets had different formulation factors. However, dissolution studies by Levy (24) showed that the dissolution rates were faster with the more readily absorbed new tablet formulations than with the old ones. He assigned this discrepancy to the dissolution rate-limited absorption of spironolactone.

If canrenoic acid salt were administered orally, the drug would be transformed readily to its undissociated form and undoubtedly precipitate as such in the stomach. Lactonization would be anticipated to occur prior to absorption at the pH of the stomach (pH 1) since the half-life of the lactonization is only 30 sec. The overall absorption process should be limited by the rate of dissolution and the intrinsic solubility of the canrenoic acid (5.7 mcg./ml. at 37.5°). Certainly, the oral administration of the canrenoic acid salt may give more rapid dissolution (25), wherein it would be precipitated as a fine, readily redissolvable particle. Thus, this could serve as an alternative method of orally administering the lactone. In the less acidic conditions of the intestine, canrenoic acid would be more soluble and the absorption process would be facilitated if the dissolution is rate determining. Since the rate of lactonization is much slower at pH 5.3 at the absorption surface of the intestine (half-life 12 days), the major portion of the drug would be absorbed as the acid species rather than as the lactone. Once the drug is absorbed, either as canrenoic acid or as canrenone, it should remain as it is in the body fluids (hydrolysis or lactonization is slow at pH 7.4 with a half-life of 24 days, provided that enzymic processes do not catalyze lactonization or hydrolysis). Therefore, the rate of transfer of the drug from the stomach to the intestine may not only determine the overall rate of absorption but may also determine the ratio of the acid and the lactone absorbed. These predictions are based on the assumption that the drug transforms with the same rate in the body fluids as in aqueous solution. A recent study of plasma levels of potassium canrenoate in man by Karim *et al.* (26) showed that the plasma levels of canrenoic acid *and* canrenone were about equal 3 hr. after intravenous administration of potassium canrenoate. This finding indicates that there are enzymatic processes and, hence, an *in vivo* equilibrium is probably established much faster.

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