Prediction of Stability in Pharmaceutical Preparations XVII: Design of a Nonprecipitating Injectable Potassium Canrenoate Solution

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Abstract □ The pharmaceutical stability of a solution of potassium canrenoate, potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionate, is a function of the solubility of the lactone canrenone, 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone, at equilibrium and the pH-dependent thermodynamic equilibrium of the reversible lactonization and hydrolysis processes. General-base catalysis by carbonate ions does displace the equilibrium to the canrenoate ion, but the pH is too high for a proper injectable solution. Potential general-base catalysts such as citrate, tartrate, and succinate ions, as well as creatinine and imidazole, which could operate at lower pH values, did not exert any significant effect on canrenone hydrolysis to displace the equilibrium toward the canrenoate salt. Solubility studies of canrenone in various aqueous mixed solvent solutions with ethanol, propylene glycol, and polyethylene glycol 400 showed that aqueous ethanol was the solvent of choice to maintain equilibrated canrenone in solution at a minimum pH value. The rate constants for lactonization of canrenoate and hydrolysis of canrenone, as well as the equilibrium constants for the canrenoate–canrenone equilibrium, were determined for various apparent pH values in the mixed solvents. The minimum pH values for various mixed solvent solutions were predicted that would maintain nonprecipitating potassium canrenoate solutions for desired injectable concentrations of the drug.

Keyphrases □ Potassium canrenoate—design of a nonprecipitating injectable solution, stability factors □ Stability, pharmaceutical preparations—design of nonprecipitating potassium canrenoate solution □ Canrenoate–canrenone—lactonization, hydrolysis, equilibrium constants, solubility studies □ Injectable solutions—design of a nonprecipitating potassium canrenoate solution, solubility and stability factors □ Solubility studies, canrenone—for development of a nonprecipitating potassium canrenoate injectable solution

The potassium salt[1] of canrenoic acid, 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid, I, has been proposed to be used in an injectable solution as a sophisticated formulation of a steroidal diuretic agent which acts through the medium of aldosterone antagonism. The reversible processes of lactonization and hydrolysis take place in an aqueous solution of the canrenoate salt (1). The mechanisms of lactonization include hydrogen-ion attack on the undissociated canrenoic acid molecule and pH-independent closure of the canrenoate anion. The resultant lactone can be hydrolyzed by specific hydroxyl-ion catalysis back to the anion (1).

The canrenone[2], 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone, II, formed on lactonization tends to precipitate from a 2% injection solution of the potassium salt within a few days, even at pH 10 (1). Canrenone, II, has been found as a metabolite of spironolactone (2) and potassium canrenoate, I (3), and is believed to possess similar biological properties as an aldosterone antagonist.

The pharmaceutical stability of a solution of the canrenoate salt, I, is a function of the solubility of the lactone and the pH-dependent thermodynamic equilibrium of the reversible lactonization and hydrolysis processes. The canrenone lactone, II, precipitates when its concentration in equilibrium with the canrenoate anion and the undissociated canrenoic acid at a given pH value exceeds its solubility. The canrenone solubility was found to be poor in water (1).

This paper considers the plausible designs of injectable canrenoate solutions of relatively high concentrations which maintain their homogeneity and minimal amounts of canrenone in solution. One possible way of effecting this purpose would be to displace the equilibrium between the lactone and the acid anion in favor of the latter by finding a catalyst promoting hydrolysis and not lactonization. An alternative procedure would be the choice of a solvent system satisfactory for injection that minimizes canrenone formation and maintains all formed canrenone in solution.

EXPERIMENTAL

Materials and Reagents—Soldactone [potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionate], canrenone[2] [3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone], spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone], polyethylene glycol 400[3], alcohol USP, and propylene glycol USP were used. All other chemicals were of reagent grade.

A canrenone lot used in solubility determinations in the presence of different polyethylene glycol 400 concentrations was purified because it appeared to be excessively yellowish brown. The procedure was to extract a chloroform solution of the dissolved canrenone by an aqueous carbonate buffer (pH 10.4). The separated chloroform layer was evaporated *in vacuo*, and the residual oil was crystallized from 50% ethanol. Light gray-yellow crystalline needles were obtained. These needles were dried after separation at room temperature. TLC testing of this product showed only the presence of canrenone. The conditions of use of the spectrophotometer[4] were as previously stated (1).

Determination of Partition Properties of Potassium Canrenoate between Chloroform and Various Solvent Mixtures—An aliquot (1.00 ml.) of a 10^{-3} *M* solution of aqueous potassium canrenoate was diluted to 25.00 ml. with required volumes of nonaqueous solvents and water to obtain 4.00×10^{-5} *M* solutions in 20% propylene glycol; in 20.0 and 40% ethanol; and in 10.0, 20.0, 40.0, 50.0, and 65.0% polyethylene glycol 400 by volume. Aliquots (4.00 ml.) of each solution were extracted with 5.00 ml. of chloroform after addition of 3.00 ml. of an appropriate buffer. The buffers used were formate ([HCOOH] = 0.16 *M* and [HCOONa] = 0.04

[1] SC 14266, G. D. Searle and Co., Skokie, Ill.
[2] SC 9376, G. D. Searle and Co., Skokie, Ill.
[3] Union Carbide IC-636 463 MH-4-40E, New York, N. Y.
[4] Model DU-2, Beckman Instruments, Fullerton, Calif.

Table I—Apparent First-Order Rate Constants (in sec.$^{-1}$) for the Hydrolysis of Canrenone in pH 9.1 Carbonate Buffer[a] at 70°

Addend	Imidazole		Creatinine		Tartaric Acid		Citric Acid	Succinic Acid	
$10^4 \times$ Molarity of Addend	0.500	2.00	2.00	20.0	0.100	1.00	1.00	1.00	10.0
$10^4 k$	1.32	1.32	1.23	1.33	1.41	1.36	1.20	2.01	1.93
$10^4 k_h$	1.22	1.25	1.20	1.29	1.28	1.27	1.13	1.93	1.80

[a] Final solutions for all but succinic acid cases were 2.4×10^{-3} *M* in Na_2CO_3 and 2.76×10^{-2} *M* in $NaHCO_3$. The carbonate buffer concentration was four times this value in the succinic acid cases, and the final pH was 9.3.

M) at pH 3 and carbonate ($[CO_3^{-2}] = 0.15$ *M* and $[HCO_3] = 0.05$ *M*) at pH 10.4. The spectral absorbances of the chloroform layers were read at 282 nm. against an appropriate chloroform blank previously equilibrated with solvent mixture–buffer. The spectral absorbance of the solvent system mixture layers were read at 292 nm. against an appropriate chloroform-saturated solvent mixture–buffer blank.

Reevaluation of the Canrenone Assay Procedure in the Presence of Nonaqueous Solvents—The validity of the assay previously described for canrenone in an aqueous solution (1) had to be reevaluated for the mixed solvent systems to be used.

A filtered aqueous saturated solution of canrenone (about 4.6×10^{-5} *M* in this case) was used to prepare 0.47, 1.34, 2.13, 2.5, and 3.38×10^{-5} *M* solutions of canrenone in aqueous 20% propylene glycol solutions. In a similar manner, five different concentrations, in the range $0.5–2.3 \times 10^{-5}$ *M* solutions, of canrenone were prepared in aqueous 20 and 30% polyethylene glycol 400 (by volume) solutions. A 10^{-3} *M* canrenone–40% ethanol solution was used to prepare five different solutions in the concentration range $0.8–3.4 \times 10^{-5}$ *M* of canrenone in 5.0, 10.0, 15.0, 20.0, 30.0, 50.0, and 70.0% ethanol (by volume). Similarly, a filtered saturated solution of canrenone in aqueous 20% polyethylene glycol 400 solution, after 10-fold dilution with the same solvent system, was used to prepare various concentrations in the range $0.5–2.3 \times 10^{-5}$ *M* solutions of canrenone in aqueous 50% polyethylene glycol 400 solutions; and 0.7, 1.3, and 1.9×10^{-5} *M* solutions of canrenone in aqueous 70% polyethylene glycol 400 solution. Calibration curves were prepared for absorbance at 280 nm. of the canrenone extracted into the chloroform phase against the concentration of canrenone in a given mixed solvent system. The analytical procedure was given previously in detail (1) and consisted of the extraction of 4.00 ml. of canrenone solution, admixed with 3.00 ml. of pH 10.4 carbonate buffer, by 5.00 ml. of chloroform. The separated chloroform phase was read against an apparent buffer and mixed solvent saturated blank on the spectrophotometer. It was necessary to wait approximately 2 hr. to allow complete separation of the phases in the cases of solvent mixtures containing the highest concentrations of polyethylene glycol and ethanol.

Solubilities of Canrenone in Mixed Solvent Systems—Aqueous solutions that were 0, 5, 10, 15, and 20% by volume in ethanol, propylene glycol, and polyethylene glycol 400 were prepared. Additional solutions that were 30, 50, and 70% in polyethylene glycol 400 and 35, 50, and 70% in ethanol were also prepared. The solutions with low nonaqueous solvent concentration (0–20%) were 60% v/v in pH 9.2 carbonate buffer (0.016 *M* in Na_2CO_3 and 0.18 *M* in $NaHCO_3$). The solutions with the higher nonaqueous solvent concentration were 20% v/v in the pH 9.2 carbonate buffer. An appropriate amount of 2 *N* NaCl was added to maintain an ionic strength of 0.154 in all solutions. The various solutions were put into capped 60-ml. (2-oz.) prescription bottles and agitated with excess canrenone on an automatic shaker at room temperature. Samples were removed after 4, 5, 6, and 7 days and filtered. The filtrate was diluted with a known amount of the appropriate mixed solvent system and analyzed for canrenone by the chloroform extraction procedure described previously (1), using the calibration curves prepared in the manner stated for the separate solvent mixtures. Solubilities were also obtained in 50% ethanol with an ionic strength of 0.050 and in water without added sodium chloride.

Kinetic Studies on Canrenone Hydrolysis in Mixed Solvent Systems—The hydrolyses of canrenone at pH 10 at 45.0 ± 0.10° in various mixed solvent systems were followed with time by the analysis of the remaining nonhydrolyzed canrenone, using the individual calibration curves established for the chloroform extraction procedure. An aliquot (5 ml.) of a pH 10 carbonate buffer, so that the final concentrations were 5.5×10^{-3} *M* in Na_2CO_3 and 4.5×10^{-3} *M* in $NaHCO_3$, was placed in a 100-ml. volumetric flask. Also, 6.7 ml. of 2.00 *N* NaCl and appropriate volumes of nonaqueous solvents were added to obtain the desired final percent of nonaqueous solvent. These solvents were made up to volume with a filtered aqueous solution of saturated canrenone, and the apparent pH was measured on a pH meter[5] using a combination electrode[6].

Similar studies of the hydrolysis of canrenone were made at pH 8.0 in 20% propylene glycol and 20% ethanol with an ionic strength of 0.154 at 60.0, 70.0, 80.0, and 85.4°. Additional studies were made in 10, 30, and 40% ethanol at 80.0°. Stock phosphate buffer solution (5.00 ml.) was added to the 100-ml. volumetric flasks so that the final solution had the composition of 5.5×10^{-4} *M* KH_2PO_4 and 9.45×10^{-3} *M* Na_2HPO_4.

Kinetic Studies on Canrenoate Lactonization in Mixed Solvent Systems—The lactonizations of canrenoic acid at pH 3.5 at 45.0° in aqueous 20% propylene glycol, ethanol, and polyethylene glycol 400 mixed solvent systems of ionic strength 0.154 were followed with time by monitoring the appearance of canrenone by the previously stated methodology (1) and using the individual calibration curves developed for the chloroform extraction of that particular solvent mixture. The solutions were prepared from 1 ml. of 2×10^{-3} *M* potassium canrenoate, 7.65 ml. of 2 *N* NaCl, 20 ml. of the respective nonaqueous solvent, and 5 ml. of pH 3.1 phosphate buffer (so that the final composition of the solution was 8.84×10^{-3} *M* in KH_2PO_4 and 7.17×10^{-3} *M* in H_3PO_4), and were made up to 100 ml. with distilled water in volumetric flasks. If canrenoic acid precipitated from the prepared mixture, it was filtered before following the kinetics.

TLC Procedures—Silica gel GF_{254}[7] (30 g.) mixed with 60 ml. of water was used to coat five 0.25-mm. thick 20.4 × 20.4-cm. glass plates. These plates were dried at room temperature and then activated at 110° for 1 hr. The solutions were spotted and developed by a 1:1 chloroform–methanol mixture for 45 min., with 19 cm. of travel for the solvent front. The spots were visualized by UV

Table II—Absorbances, A_λ, of the Separated Phases after Chloroform Extraction of Aqueous Mixed Solvent Solutions of Potassium Canrenoate[a]

	pH of Aqueous Buffer			
	10.4		3.0	
Percent Nonaqueous	Aqueous Phase	Chloroform Phase	Aqueous Phase	Chloroform Phase
20% Ethanol	0.620	−0.005	0.015	0.860
40% Ethanol	0.610	0.010	0.010	1.01
10% Polyethylene glycol 400	0.530	0.010	—	—
20% Polyethylene glycol 400	0.535	0.030	—	—
40% Polyethylene glycol 400	0.535	0.090	—	—
50% Polyethylene glycol 400	0.535	0.105	—	—
65% Polyethylene glycol 400	0.530	0.170	0.020	0.820
20% Propylene glycol	0.415	0.005	0.005	0.630

[a] Chloroform (5.00 ml.) was used to extract a mixture of 4.00 ml. of 4.00×10^{-5} *M* potassium canrenoate in an aqueous mixed solvent system and 3.00 ml. of aqueous buffer at the stated pH value.

[5] Beckman.
[6] Sargent pH combination electrode S30070-10, Birmingham, Ala.
[7] E. Merck AG, Darmstadt, Germany.

fluorescence or by use of the hydroxamic acid–ferric-ion reagent (4) useful in identifying esters, lactones, lactams, and thiolactones.

The following solutions and the equal volume chloroform extracts of acidified samples of them were investigated by these procedures: 1 ml. of an aqueous 5% w/v solution of potassium canrenoate mixed with: (*a*) 10 ml. of pH 3.6 phosphate buffer, (*b*) 10 ml. of pH 7.9 phosphate buffer, and (*c*) 10 ml. of pH 10.4 carbonate buffer. Similar solutions (*d*, *e*, and *f*, respectively) were also prepared, except that each contained 2 ml. of ethanol, *i.e.*, were 20% ethanol by volume. Solutions *a*, *c*, *d*, and *f* were maintained at 70° for 19 hr. before 0.05 ml. of each was chromatographed. Solutions *b* and *e* were maintained at 80° for 19 hr. before 0.05 ml. of each was chromatographed. Aliquots of the chloroform extracts of each were also spotted. Standard solutions (0.01 ml.) of 1% canrenone in chloroform, 0.5% canrenoic acid in 1:1 ethanol–chloroform, 1% potassium canrenoate in ethanol and in water, and 1% spironolactone in chloroform were also spotted.

Kinetic Studies on Influence of Various Compounds on Canrenone Solvolysis and Its Equilibrium with Canrenoic Acid—Aqueous solutions were prepared in 100-ml. volumetric flasks; they consisted of 15 ml. of carbonate buffer stock solution (pH 9.2), appropriate amounts of 1.0 *M* KCl to maintain a constant ionic strength of 0.1, and desired amounts of imidazole, creatinine, and citric and tartaric acids. The final buffer concentrations were 2.4×10^{-3} *M* in Na_2CO_3 and 2.76×10^{-2} *M* in $NaHCO_3$. These solutions were made up to volume with a filtered aqueous solution of saturated canrenone. In the special case of succinic acid, which was added as a 10^{-3} *M* solution, 30 ml. of a carbonate buffer, twice as concentrated as the one already described, was added and appropriate 1.0 *M* KCl was added to maintain an ionic strength of 0.15. The temperature baths were maintained at 70 ± 0.1°. Samples were removed at 20-min. intervals and analyzed for lactone by the established assay (1) after equilibrium had been effected at about 10 half-lives.

RESULTS AND DISCUSSION

The pharmaceutical stability of the canrenoic acid salt in aqueous media was shown (1) to be a function of: (*a*) the pH-dependent thermodynamic equilibrium of its reversible lactonization to canrenone with the hydrolysis of this lactone and (*b*) the solubility of the lactone. The lactone canrenone precipitated where its concentration in equilibrium at a given pH value exceeded its solubility. These solubilities were unfortunately very low, so even a dilute solution of potassium canrenoate (0.02%) in water at pH 8.0 gave a precipitate in less than 1 month. It was proposed (1) that two possible methods of preparing a lasting homogeneous solution of potassium canrenoate could be: (*a*) to add an addend which increased the hydrolysis rate of the lactone without affecting the rate of lactonization, and thus to lower the amount of canrenone produced, or (*b*) to prepare the potassium canrenoate in mixed solvent systems so that the small amount of lactone in the equilibrium would remain completely solubilized.

Influence of Various Compounds on Canrenone Solvolysis—Since the carbonate dianion had shown a catalytic effect on canrenone solvolysis, it was hypothesized that other negative ions with multiple charge, which would possess their charge at more reasonable lower pH values than the carbonate dianion, may have similar catalytic effects. The anions of tartaric acid, citric acid, and succinic acid were considered to be suitable addends that could perform this action. Generally, two levels of concentrations of these compounds were added to separate solutions of equivalent pH, and the rates of hydrolysis of canrenone were followed at 70° in the manner described previously (1). The overall first-order rate constant, k, and the derived ratio constant for hydrolysis, k_h, given in Table I show that there is no significant enhancement of the rate of canrenone hydrolysis with increased concentrations of the compounds. Similar studies were made with several concentrations of potential general-base catalysts such as imidazole and creatinine. In neither of these cases was there any significant increase of the hydrolysis rate. Within the errors of measurement, the rate constants for lactonization and the derived equilibrium constants were of the same order of magnitude. The increased concentration of carbonate buffer in the succinic acid case clearly demonstrated a 1.3-fold enhancement of hydrolysis rate, again indicating that this buffer does exert a specific catalytic effect. However, the magnitude of this effect and the high pH necessary for its full utilization were not of practical significance for use in formulation.

Table III—Apparent Absorptivity Constants[a], $\epsilon = A_{CHCl_3}/[\text{Canrenone}]_{aq.}$, in the Chloroform Extract of Canrenone in Mixed Solvents on the Addition of pH 10.4 Carbonate Buffer

Nonaqueous (v/v)	ϵ
0%	23,200
20% Propylene glycol	23,290
5% Ethanol	22,800
13% Ethanol	21,600
20% Ethanol	21,000
30% Ethanol	20,480
50% Ethanol	19,520
70% Ethanol	17,240
20% Polyethylene glycol 400	23,600
30% Polyethylene glycol 400	22,160
50% Polyethylene glycol 400	19,520
70% Polyethylene glycol 400	15,700

[a] The absorbance, A_{CHCl_3}, of the chloroform phase at 280 nm. was read spectrophotometrically after 5.00 ml. of $CHCl_3$ extracted 4.00 ml. of the canrenone solution of concentration $[\text{canrenone}]_{aq.}$ to which had been added 3.00 ml. of pH 10.4 buffer. The apparent absorptivity constant, ϵ, for a given canrenone solvent system was obtained as the slope of the straight line formed from the plot of A_{CHCl_3} against $[\text{canrenone}]_{aq.}$. Such plots passed through the origin. The range of canrenone concentration studied was generally up to 3.6×10^{-5} *M*.

Reevaluation of Assay Procedures in the Presence of Nonaqueous Solvents—The use of a spectrophotometric analysis (1) of chloroform extracts of aqueous mixed solvent solutions of canrenone in the presence of canrenoic acid and its salt had to be reevaluated to determine if the procedure would assay canrenone when a pH 10.4 carbonate buffer was added previously to the aqueous mixed solvent phase and if it would assay canrenone and total canrenoic acid when a pH 3.0 phosphate buffer was added previously to the aqueous mixed solvent phase. The data given in Table II demonstrate clearly that chloroform extraction of an aqueous mixed solvent phase containing up to 40% ethanol and 20% propylene glycol after addition of pH 10.4 buffer does not extract potassium canrenoate from such mixed solvent systems for all practical purposes, since negligible absorbances were obtained in the chloroform layer. A similar extraction of such solutions, after adjustment of the aqueous mixed solvent phase with pH 3.0 buffer, showed that

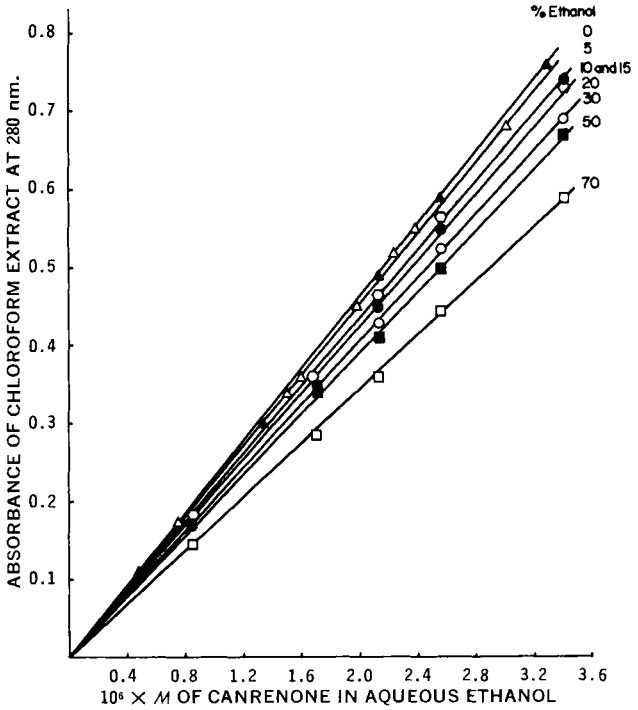

Figure 1—*Calibration curves for canrenone in various aqueous ethanol solutions. The spectral absorbance is of a 3.00-ml. chloroform extract of the 4.00 ml. of canrenone solution of the stated concentration mixed with 3.00 ml. of pH 10.4 carbonate buffer.*

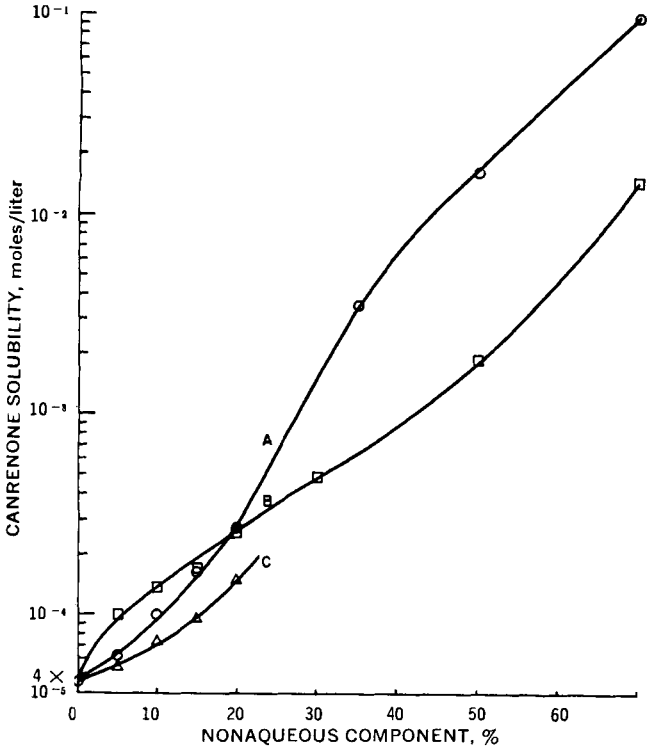

Figure 2—*Semilogarithmic plot of canrenone solubility in various percent nonaqueous components with pH 9.2 carbonate buffer at 25° with an ionic strength of 0.154. The curves and nonaqueous components were: (A) ethanol, (B) propylene glycol, and (C) polyethylene glycol 400.*

the chloroform extract contained significantly all of the canrenoic acid and negligible amounts remained in the aqueous mixed solvent phase. Although similar conclusions may be stated for mixed solvent solutions of canrenoate up to 20% polyethylene glycol 400, this is apparently not true for higher percent polyethylene glycol 400 solutions because a significant amount of potassium canrenoate is extracted into chloroform from such pH 10.4 adjusted quasi-aqueous phases.

The apparent absorptivity constants for canrenone in the chloroform extracts of the various mixed solvent solutions of canrenone are given in Table III. They were obtained as the slope of the straight line formed from the plots of the absorbances at 282 nm. in the separated $CHCl_3$ phase against the original molar concentration of canrenone in the specific mixed solvent solutions before the addition of pH 10.4 carbonate buffer. A typical set of such plots is given in Fig. 1 for mixed solvent solutions containing various percent ethanol concentrations. These plots were straight lines and passed through the origin to demonstrate the validity of Beer's law. Under the conditions of these extractions, no significant hydrolysis of the lactone could take place. No significant absorbance at 292 nm. was observed in the separated aqueous phase after chloroform extraction. Thus, it could be concluded that all of the canrenone was extracted into the chloroform phase under such experimental conditions. In addition, the fact that no significant canrenoate was extracted under similar conditions into the chloroform phase for all mixed solvent systems (except those in excess of 20% polyethylene glycol 400) validated the use of this analytical procedure for canrenone concentration in the stated mixed solvent systems when the appropriate apparent absorptivity (Table III) or when the appropriate calibration curve (Fig. 1) would be used.

The fact that the apparent absorptivity values for canrenone in water and 20% propylene glycol were similar argues for the negligible extraction of this aqueous alcoholic phase into the chloroform. The decrease in apparent absorptivity values with increasing percentages of ethanol and polyethylene glycol 400 (Table III and Fig. 1) argues for increased extraction of the components of the miscible aqueous and nonaqueous solvents into the spectrally analyzed chloroform phase. However, this does not interfere with the reliability of the analytical method for those stated mixed solvent systems if the appropriate apparent absorptivity value is used.

Solubilities of Canrenone in Aqueous Mixed Solvent Systems—The solubilities of canrenone in the aqueous mixed solvent systems studied are plotted in Fig. 2 against the percent of nonaqueous solvent (v/v) for an ionic strength of 0.154. These solvent systems were chosen as being representative of nontoxic solutions which could be parenterally or intravenously administered (5).

A reduction of ionic strength to one-third of its value only increased the solubility 1.1-fold in 50% ethanol. The solubility in a

Table IV—Apparent First-Order Rate Constants ($10^5 k$ with k in sec.$^{-1}$) for Canrenone Hydrolysis at 45° in pH 10[a] Carbonate Buffer at an Ionic Strength of 0.154 in Various Aqueous Mixed Solvent Systems

Nonaqueous Component, %	Propylene Glycol	Ethanol	Polyethylene Glycol 400
0.0	10.3	10.3	10.3
5.0	10.7	10.8	7.7
10.0	10.7	10.4	7.5
15.0	11.1	9.2	4.6
20.0	10.2	8.4	3.3

[a] The observed apparent pH values of the propylene glycol and ethanol solutions were between 10.1 and 10.3. The observed apparent pH values of the polyethylene glycol 400 solutions were 10.0. In a 65% polyethylene glycol 400 solution buffered similarly but with an apparent pH of 8.9, there was no apparent degradation of canrenone at 70° from the spectral assay of the chloroform extract. An absolute conclusion as to complete inhibition of hydrolysis by this concentration of polyethylene glycol 400 is not possible since a significant amount of potassium canrenoate can be extracted into chloroform from this solvent mixture.

Table V—Apparent First-Order Rate Constants[a] (in sec.$^{-1}$), Equilibrium Constants (K)[b], and Arrhenius Parameters[c] for the Transformation of Canrenone in Various Solvent Systems Admixed with pH 8.0 Phosphate Buffer

	Aqueous	20% Propylene Glycol	20% Ethanol
		85.0°	
$10^4 k$	1.18	1.26	0.88
$10^4 k_h$	0.80	0.75	0.50
$10^4 k_l$	0.38	0.52	0.38
K	2.1	1.4	1.4
		80.0°	
$10^5 k$	7.89	9.50	5.50
$10^5 k_h$	5.22	4.98	3.36
$10^5 k_l$	2.67	4.52	2.14
K	2.0	1.1	1.6
		70.0°	
$10^5 k$	2.44	2.44	2.42
$10^5 k_h$	1.66	1.30	1.45
$10^5 k_l$	0.78	1.14	0.97
K	2.1	1.2	1.5
		60.0°	
$10^6 k$	9.87	9.40	8.45
$10^6 k_h$	6.70	5.30	4.93
$10^6 k_l$	3.17	4.10	3.52
K	2.1	1.3	1.4

	ΔHa	log P	ΔHa	log P	ΔHa	log P
k_h	24.0	10.57	25.4	11.37	23.2	9.84
k_l	24.0	10.21	25.4	11.28	21.8	8.85

[a] The overall first-order rate constant, k, which is equal to the sum of apparent first-order hydrolysis and lactonization rate constants, $k_h + k_l$, was obtained from the slope of a plot of the logarithm of the difference between the amount of canrenone at any time and the amount at final equilibrium against time. The separate values of k_h and k_l were obtained from the known values of $k = k_h + k_l$ and the determined value of the equilibrium constant, $K = k_h/k_l$. [b] The values of K were determined from the canrenone concentration of the solution at equilibrium, *i.e.*, $[\text{canrenone}]_{eq.}$ where $K = ([\text{canrenone}]_o - [\text{canrenone}]_{eq.})/[\text{canrenone}]_{eq.}$ and $[\text{canrenone}]_o$ was the initial concentration of canrenone. [c] The ΔHa and log P values were obtained from the Arrhenius plots of log k_h and log k_l values against the reciprocal of the absolute temperature (T) in accordance with $\log k_i = \log P - (\Delta Ha/2.303R)(1/T)$, where R is 1.987 cal. deg.$^{-1}$ mole^{-1} and ΔHa is given in the table in kcal./mole.

solution of 0.154 ionic strength was increased only 1.2-fold in the absence of added salt.

Hydrolysis and Lactonization of Canrenone and Canrenoate in Mixed Solvent Systems—The kinetic studies of the hydrolysis of canrenone at pH 10 in mixed solvent systems demonstrated decreasing rate constants with increasing ethanol and polyethylene glycol 400 concentrations (Table IV). The rate constant decreased 20% in 20% ethanol and 70% in 20% polyethylene glycol 400 over purely aqueous systems. The amount of lactone remaining at final equilibrium was not experimentally significant at this pH value. A 65% polyethylene glycol 400 solution showed no significant hydrolysis at 70° for 25 hr. in contrast to an 80% decrease at 45° in 40% ethanol for only 5 hr. and complete (90%) hydrolysis in water within 3 hr. However, an increased percentage of propylene glycol to up to 20% had no significant effect on canrenone hydrolysis (Table IV). Since it is preferred that the canrenone–canrenoic acid equilibrium be shifted to the open form, so that the canrenone content is minimized at equilibrium, aqueous polyethylene glycol 400 does not appear to be the solvent mixture of choice for formulation.

The rates of lactonization of canrenoic acid at pH 3.5 at 45° in aqueous solution and in aqueous 20% solutions of propylene glycol and polyethylene glycol 400 were the same, *i.e.*, 1.75×10^{-4} sec.$^{-1}$, whereas the value in 20% ethanol appeared to be somewhat less, *i.e.*, 1.50×10^{-4} sec.$^{-1}$. The amount of canrenoic acid remaining at final equilibrium was not experimentally significant at this pH value. These data are indicative of the fact that the hydrogen-ion-catalyzed lactonization of canrenoic acid is relatively insensitive to the nonaqueous component, at least up to a content of 20%.

The apparent rate constants for the alkaline hydrolysis of canrenone and the pH-independent lactonization of canrenoate ion were determined from studies of canrenone hydrolysis to equilibrium conditions in aqueous, 20% propylene glycol, and 20% ethanol solutions buffered with pH 8.0 phosphate buffer (Table V). The methods of calculation were given previously and are summarized in the footnotes of Table V. The apparent equilibrium constants, $K = k_h/k_1$, are reasonably constant with respect to temperature and are reduced in the 20% nonaqueous solution to about 1.4 from the 2.1 of the purely aqueous solution.

In the 20% propylene glycol case, this reduction is largely due to the relative increase in the rate constant for lactonization, k_1. This is consistent with the prior observation (Table IV) that the apparent first-order rate constant for canrenone hydrolysis was relatively invariant with increasing propylene glycol concentrations.

In the 20% ethanol case, this decrease in K was due to the decrease in the rate constant for hydrolysis, k_h. This is consistent with the prior observation (Table IV) that the apparent first-order rate constant for canrenone hydrolysis decreased with an increasing alcohol percentage of the solution.

TLC Studies—The main purpose of the TLC studies was to determine that the only components of the solutions other than the solvents were canrenone and canrenoic acid and that the alcohol content of the solvent system did not form significant amounts of the ethyl esters of canrenoic acid. In the system used, the R_f values of canrenone and canrenoate were 0.94 and 0.10, respectively, and no other spot that could be attributed to an ethyl ester was observed.

An additional spot was observed at R_f 0.5 in both the reacted aqueous and mixed solvent solutions of canrenone. However, an unreacted solution of potassium canrenoate also demonstrated the same component, which could then be assigned to a contaminant. For this reason, canrenone was purified by the method given in the *Experimental* section.

Prediction of Optimum Compositions of Homogeneous Potassium Canrenoate Solutions—The inability of certain addends such as tartaric, citric, and succinic acid anions, as well as imidazole and creatinine, to accelerate canrenone hydrolysis left the use of aqueous mixed solvents to solubilize the small amounts of canrenone in equilibrium with potassium canrenoate as the only plausible alternative formulation of a nonprecipitating homogeneous solution of potassium canrenoate. Only those water-miscible nonaqueous solvents that were deemed satisfactory for parenteral use (5) were considered.

The enormous decrease in the rate of canrenone hydrolysis with increased concentrations of polyethylene glycol 400 (Table IV) argued against this solvent, since the equilibrium would be displaced in the direction of the unwanted lactone. The fact that canrenone was significantly less soluble in aqueous propylene glycol than in a comparable percent ethanol solution (Fig. 2) argued for ethanol–water solutions as solvents of choice.

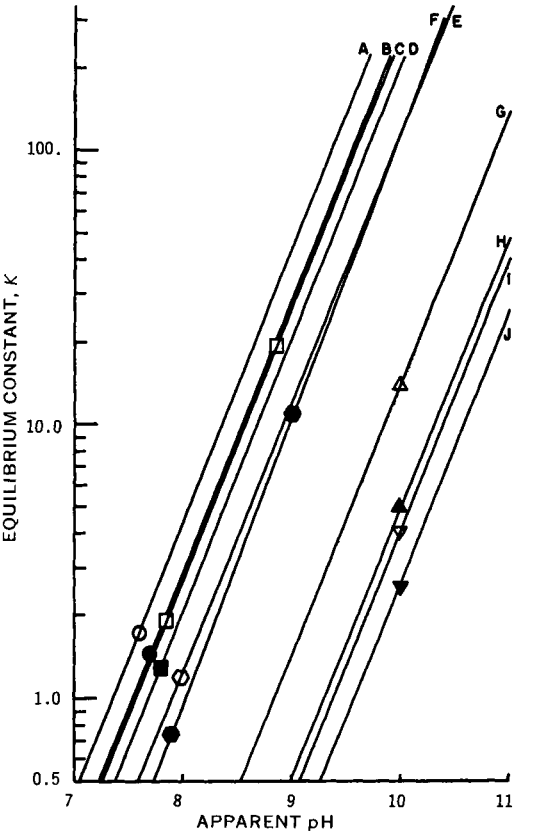

Figure 3—*Semilogarithmic plots of the apparent equilibrium constant, K, for the canrenoate–canrenone equilibrium as a function of apparent pH in various aqueous mixed solvents. The plots are drawn in accordance with log K = pH + intercept and have a slope of positive unity through the experimental points. The curves and their respective compositions are: (A) 10% ethanol, (B) 20% ethanol, (C) water, (D) 30% ethanol, (E) 20% propylene glycol, (F) 40% ethanol, (G) 5% polyethylene glycol 400, (H) 10% polyethylene glycol 400, (I) 15% polyethylene glycol 400, and (J) 20% polyethylene glycol 400.*

It was shown previously (1) that the dependence of the constant for the canrenone–canrenoate-ion equilibrium in the alkaline region could be expressed by:

$$K = k_h/k_1 = k_{OH}[OH^-]/k_1 \qquad \text{(Eq. 1)}$$

where k_1 is the apparent first-order rate constant for the pH-independent lactonization of canrenoate ion, and k_h is the apparent first-order rate constant for the hydroxide-ion-catalyzed solvolysis of canrenone at a given hydroxyl-ion concentration. It thus follows that:

$$\log K = \log (k_{OH}/k_1) - \text{pKw} + \text{pH}$$
$$= \text{pH} + \text{intercept} \qquad \text{(Eq. 2)}$$

and a plot of the logarithm of the equilibrium constant against pH should be linear with a slope of positive unity in a given solvent system at a given temperature where the intercept log (k_{OH}/k_1) − pKw is a function of the stated constants.

The fact that the apparent equilibrium constant, K, in a given solvent system was relatively invariant with temperature (Table V) permitted the construction of a log K *versus* pH plot for all temperatures from the K values obtained at 80° in about pH 7.8 phosphate buffer of ionic strength 0.154. Although only one value at a known apparent pH is necessary for the construction of the plots with unit positive slopes for the several mixed solvent solutions given in Fig. 3, additional data were obtained in several instances at higher pH values. These data are also plotted in Fig. 3 and confirm

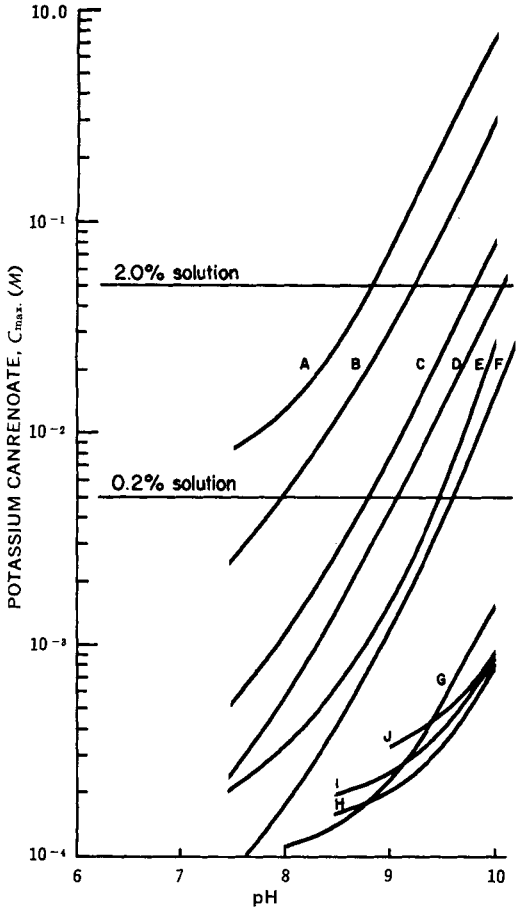

Figure 4—*Semilogarithmic plot of the maximum molar concentration, $C_{max.}$, of potassium canrenoate that will not give a precipitate of canrenone as a function of pH in various aqueous mixed solvents. The plots are drawn in accordance with log $C_{max.} = (1 + K)$ [canrenone]$_{sol.}$, where K is the equilibrium constant for the canrenone–canrenoate equilibrium at the given pH, and [canrenone]$_{sol.}$ is the canrenone solubility at 25° in the stated aqueous mixed solvent system. The curves and their respective components are: (A) 40% ethanol, (B) 30% ethanol, (C) 20% ethanol, (D) 10% ethanol, (E) 20% propylene glycol, (F) water, (G) 5% polyethylene glycol 400, (H) 10% polyethylene glycol 400, (I) 15% polyethylene glycol 400, and (J) 20% polyethylene glycol 400.*

the validity of the use of a unit slope of such log K–pH plots for purposes of predicting K values at other pH values.

The equilibrium constant, K, can be defined in terms of the original canrenoate concentration of the formulation, *i.e.*, [canrenoate]$_o$, and the canrenone concentration, *i.e.*, [canrenone]$_{eq.}$, after equilibrium has been achieved:

$$K = \frac{[\text{canrenoate}]_o - [\text{canrenone}]_{eq.}}{[\text{canrenone}]_{eq.}} \qquad \text{(Eq. 3)}$$

It follows that the canrenoate concentration in the formulation which will maintain an homogeneous solution will be a function of the solubility of the canrenone produced at equilibrium, *i.e.*:

$$[\text{canrenoate}]_o = (1 + K)[\text{canrenone}]_{eq.} \qquad \text{(Eq. 4)}$$

Substitution of the known canrenone solubility in a given mixed solvent at 25° (Fig. 2) and the pertinent K for a given pH value (Fig. 3) permits the calculation from Eq. 4 of the canrenoate concentrations, [canrenoate]$_o$, that may be formulated at those pH values that will not give precipitates at 25°. Such [canrenoate]$_o$ values are given in Fig. 4 as a function of apparent pH values for several mixed solvent systems.

The plots of Fig. 4 clearly show that 20% ethanol is better than 20% polyethylene glycol 400 and 20% propylene glycol in permitting the highest concentration of canrenoate to be formulated at a given pH value. Increased concentrations of ethanol also permit higher concentrations of canrenoate to be formulated at any given pH value.

If the formulation of a 2% (5.0×10^{-2} M) solution of potassium canrenoate at 25° is desired, Fig. 4 gives typical percentages of ethanol, the apparent pH values of their aqueous solutions, and the molarity of the canrenone at equilibrium, respectively, as: 40%, 8.8, 6.6×10^{-3}; 30%, 9.2, 1.5×10^{-3}; 20%, 9.8, 2.74×10^{-4}; and 10%, 10.05, 1×10^{-4}. The molarity of canrenone at equilibrium was obtained from the solubility of canrenone in the requisite solvent, as given in Fig. 2.

Similarly, if the formulation of a 0.2% (5.0×10^{-3} M) solution of potassium canrenoate is desired, typical percentages of nonaqueous solvent, apparent pH values of their aqueous solutions, and the molarity of the canrenone at equilibrium would be, respectively: 40% ethanol, 7.95, 5.6×10^{-3}; 20% ethanol, 8.8, 2.6×10^{-3}; 0.0% ethanol, 9.60, 4.5×10^{-5}; and 20% propylene glycol, 9.45, 1.4×10^{-4}.

It follows that a concentration of an aqueous mixed solution of potassium canrenoate can be chosen that fits the desired minimum percent nonaqueous solvent and minimum pH dependent on the amount of equilibrated canrenone in solution that can be tolerated.

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