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STABILIZATION OF BITOLTEROL BY THE USE OF MIXED SOLVENT SYSTEMS

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ABSTRACT

Analytical methods using thin-layer and high pressure liquid chromatographic techniques were developed and utilized for measuring decreases in bitolterol concentrations as a function of time. Pseudo first-order hydrolysis rate constants were determined and shown to be strongly dependent upon pH and solvent polarity for this catecholamine diester. The activation energy was calculated as 18 kilocalories per mole for bitolterol in aqueous buffer solution both with or without 25% alcohol. Large reductions in rate constants as a function of dielectric constant were demonstrated and illustrate that solvent polarity effects may be important for drug stabilization.

INTRODUCTION

Bitolterol, the methane sulfonate salt of the di-p-toluate ester of N-t-butylarterenol, is an active beta-adrenoceptor agonist which is being evaluated

clinically in aerosol and oral formulation. The bronchodilator activity after oral administration in the dog has been reported (1,2). Bitolterol was selected from several mono and diesters of N-t-butylarterenol whose synthesis and pharmacological activity have recently been described (2). Esterification of the catechol groups resulted in a greater duration of bronchodilation activity, reduction of tachycardia and greater bioavailability. The prolonged bronchodilator activity was due to a depot of bitolterol in lung tissue which hydrolyzes to N-t-burylarterenol as reported by Shargel and coworkers (3,4).

Since catechol esters are readily hydrolyzed a challenge is presented in the formulation of aqueous solutions or suspensions. It is known that catechol esters or esters of phenol with a pK_A of greater than 8 are hydrolyzed by general base catalysis (5,6). The hydrolysis rates for monoesters of catechol are much greater than those for the diesters or for phenolic esters. For example, the hydrolysis rate in alkaline solution for catecholmonoacetate is about 126 times greater than that for the diacetate ester (7). Also the rate constant for catecholmonoacetate hydrolysis is about 700 times larger than that of the phenol ester; phenylacetate. Relatively high hydrolysis rates for cyclic succinoylcatechol were shown compared to noncyclic analogs (8). The hydrolysis rates for a cate-

cholamine diester (bitolterol), α-(tert-butylaminomethyl)-3',4'-dihydroxybenzyl alcohol 3',4'-bis-(4-methylbenzoate) methanesulfonate salt, in aqueous-organic solvents as a function of pH and dielectric constant are described in this study.

EXPERIMENTAL

A. Stability vs pH study - Solutions containing 200 mcg/ml of bitolterol were prepared in buffer solutions (0.2 molar) containing 25% methanol (reagent grade). These solutions were filled into 30 ml flint ampules and stored in a constant temperature oven (Hotpack Co.) at 80°C. At various time intervals ampules were removed, cooled to 25°C, the pH recorded and bitolterol assayed by the following thin-layer chromatographic procedure.

TABLE 1

Bitolterol·Hydrolysis Rate Constants and Dielectric Constants For Different Solvents at pH 3.5

Solvent (% by Volume)	Dielectric Constant 25°C	Dielectric Constant 90°C*	Rate Constant, At 90°C ($Days^{-1}$)
Water	78.6	57.8	1.30
25% Glycerine	64.9	51.2	0.693
20% Ethanol	66.3	50.3	0.0951
25% Propylene Glycol	63.9		0.153
25% Ethanol	63.4	48.2	0.0493
30% Ethanol	61.2	46.1	0.0317
25% Polyethylene Glycol-400	65.4		0.0878

*Values for 90°C were obtained from reference (9) by calculation from log Dielectric constant and temperature linear correlations.

Fifteen ml of 1.0 N hydrochloric acid was added to 50 ml of the bitolterol solutions which were then extracted with three 25 ml portions of chloroform. The chloroform extracts were pooled and evaporated under a stream of nitrogen. The bitolterol residue was dissolved in methanol and quantitatively transferred to a 10 ml volumetric flask and brought to volume with methanol. Five microliters of the methanol solution containing 50 mcg of bitolterol was spotted on a TLC plate having as an absorbent, silica gel F-254 (Merck). For quantitation a bitolterol standard in methanol (10 mg/ml) was spotted in amounts ranging from 10 to 100% of the amount spotted with the stressed sample. The plate was developed with the system:chloroform:methanol:isopropylamine (94:3:3). For detection the plate was viewed with a lamp emitting short wavelength ultraviolet light.

B. Arrhenius Studies - A bitolterol solution of 10 mg/ml concentration was prepared in 0.05 molar acetate buffer of pH 3.5 and filled into 10 ml flint ampules which were then stored in constant temperature ovens at 50°, 70° and 90°C. A solution (10 mg/ml) was also prepared with 0.05 molar acetate buffer (pH 3.5) in 25% ethanol (reagent grade) and filled into 10 ml ampules. These ampules were kept at 50°, 70°, 80° and 90°C in constant temperature ovens. Periodically the ampules were removed, cooled to 25°C, the pH recorded and bitolterol assayed by the thin-layer chromatographic pro-

cedure described in Section A, the only exception being that 5 mcl of the solutions was spotted directly on the plate.

C. Hydrolysis Study With Aqueous Buffer - Organic Solvent Mixtures - Bitolterol solutions (10 mg/ml) were prepared with 0.05 molar acetate buffer (pH 3.5) containing by volume 25% polyethylene glycol 400, 25% propylene glycol, 25% glycerin, or 20% and 30% ethanol. Ten ml flint ampules containing these solutions were kept at 90°C in an oven. The thin-layer chromatographic procedure described in Section B was used for the measurement of bitolterol.

D. Stability Study With Aqueous Citric Acid - Organic Solvent Mixtures - Bitolterol solutions (1 mg/ml) containing sorbitol, propylene glycol and ethanol in various concentrations (Tables 2 and 3) with 0.3% citric acid were prepared and sealed in 10 ml flint ampules. Periodically ampules were removed from the 90°C oven and assayed by the following procedure.

A 1 ml aliquot of the bitolterol solution was diluted to 10 ml with 0.3% citric acid. Bitolterol was assayed by high pressure liquid chromatography using a Dupont Model 840[1] equipped with a Zorbax ODS column. Thin-layer chromatography was not used because of inter-

[1]Dupont Co.; Instrument Products Division; Wilmington, Delaware

TABLE 2

Bitolterol Hydrolysis Rate Constants and Dielectric Constants For Sorbitol Solutions At pH 2.6-2.8

Solvent (% w/v in water)*	Dielectric Constant (25°C)	Rate Constant At 90°C ($Days^{-1}$)
63.4% Sorbitol	68.2	0.181
72.5% Sorbitol	65.4	0.174
81.5% Sorbitol	63.4	0.159
90.6% Sorbitol	60.4	0.134
81.5% Sorbitol-10% Ethanol	57.1	0.0903
72.5% Sorbitol-20% Ethanol	53.7	0.0283
72.5% Sorbitol-10% Ethanol - 10% Propylene Glycol	53.7	0.0570

*Ethanol and Propylene Glycol - % v/v in water
Citric acid (3 mg/ml) was added to all solvents
Sorbitol solutions were prepared by dilution of Sorbo (ICI America) with water

TABLE 3

Bitolterol Hydrolysis Rate Constants and Dielectric Constants For Different Solvents At pH 2.9-3.0

Solvent (% v/v in Water)*	Dielectric Constant (25°C)	Rate Constant At 90°C ($days^{-1}$)
Water	78.6	0.240
25% Ethanol	63.4	0.0320
10% Ethanol-10% Propylene Glycol	64.8	0.0570
20% Ethanol-20% Propylene Glycol	59.6	0.0212
25% Ethanol-25% Propylene Glycol	53.5	0.0133
30% Ethanol-30% Propylene Glycol	47.0	0.0102

*Citric acid (3 mg/ml) was added to all solvents.

pH was measured at 25°C after diluting 1:1 with distilled water.

ference caused by the presence of the citric acid. The solvent system used was acetonitrile:5% monosodium citrate in 5% citric acid:water (65:5:30). Good resolution was obtained using a flow rate of 0.32 ml/min. A 5 mcl aliquot of the diluted solution was injected on the column and compared to a 5 mcl (0.5 mcg of bitolterol) aliquot of a 0.1 mg/ml standard solution. Peak heights of standard and unknown solutions were compared for quantitation.

E. Determination of Dielectric Constant - Measurements were made with a Sargent Chemical Oscillometer[2] at 25°C. The cell constant and the ratio of constants were derived by measurements with liquids of known dielectric constants: carbon tetrachloride and methanol.

RESULTS AND DISCUSSION

Psuedo first order decreases for percent bitolterol in 25% methanol at different pH's are presented in Figure 1. A minimum in the pH vs hydrolysis rate constant curve is shown about pH 3 (Figure 2). Based on the hydrolysis profile of bitolterol the following equation can be applied where K_T is the total hydrolysis rate

$$K_T = K_A(H^+) + K_W + K_B\ (OH^-)$$

constant and K_A, K_W and K_B are the rate constants for

[2] E.H. Sargent and Co., 4647 W. Foster Ave., Chicago, Illinois

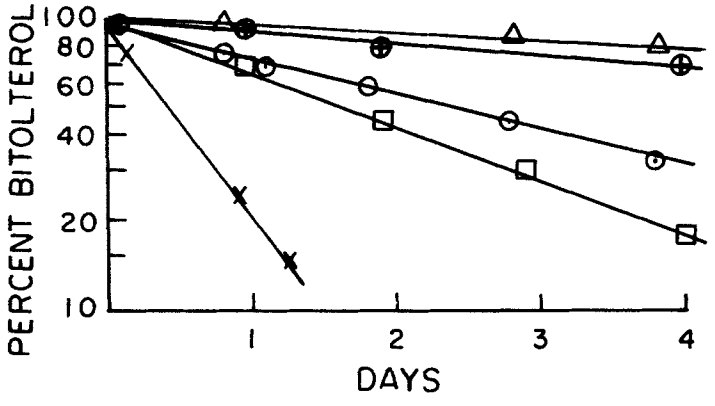

FIGURE 1

Semilogarithmic Plot of Percent Bitolterol vs Time (Days) At 80°C For 0.20 mg/ml Solutions In 25% Methanol At pH 2.60 (Δ); pH 3.45 (⊕); pH 4.60 (⊙); pH 1.45 (□) and pH 5.50 (X). Least-Squares Regression Lines

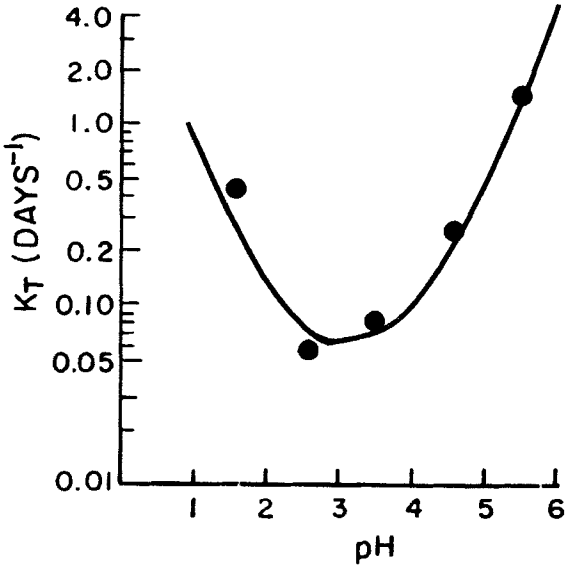

FIGURE 2

Semilogarithmic Plot of K (Days^{-1}) For Bitolterol Hydrolysis vs pH. Bitolterol Solutions (0.20 mg/ml) At 80°C In 25% Methanol.

hydronium ion, water and hydroxyl ion hydrolysis respectively. A good fit of experimental data is obtained using a K_A of 9 $days^{-1}$, K_W of 0.05 $days^{-1}$ and a K_B of 40 x 10^7 $days^{-1}$ as shown by the calculated curve shown in Figure 2. The strong dependency of reaction rate on the solution pH is evident by the rapid increases in hydrolysis rate on both sides of the minimum. Apparently the hydrolytic rate for the monoester is much more rapid than that for the diester since only the completely hydrolyzed catecholamine and no monoester was observed during the course of the reaction at all pH's. This is in accord with other studies with catechol mono and diesters (7).

The methanol was added in order to obtain sufficient solubility at all of the pH's for a preformulation study to determine the optimum pH for stability. In a subsequent kinetic study with bitolterol dissolved in an aqueous acetate buffer at pH 3.5 the rate constant was about 8 times larger than that obtained with the 25% methanol solution at the same pH indicating a significant solvent effect on the reaction which is much greater than the influence of the small difference in water content.

From the Arrhenius plots shown in Figure 3 the activation energy was calculated as 18 kilocalories per mole for bitolterol hydrolysis in aqueous buffer both with and without 25% ethanol. The presence of ethanol

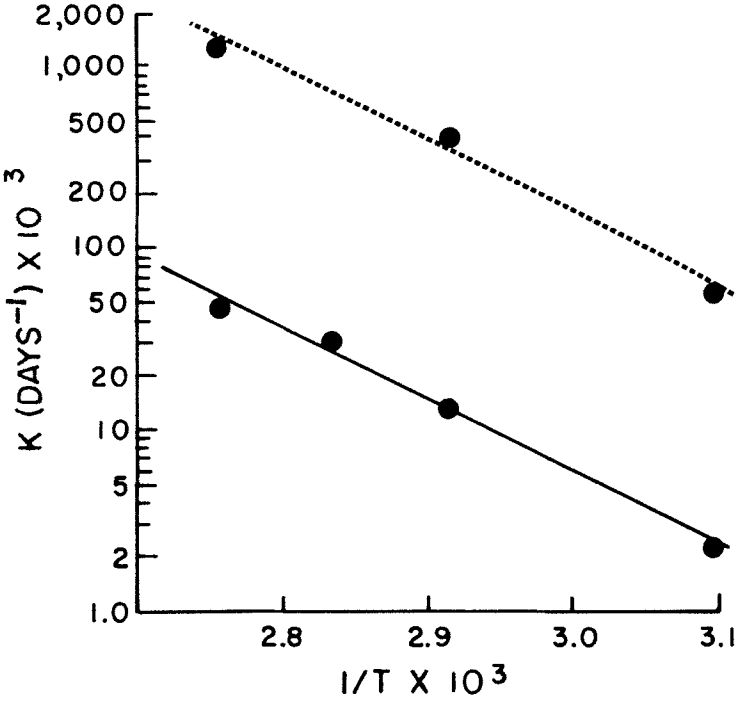

FIGURE 3

Arrhenius Plots For Bitolterol Hydrolysis In Buffer Solutions Without (•----•) and With 25% Ethanol (•—•) At pH 3.4-3.5. Least-Squares Regression Lines.

has a large effect on the reaction rate but does not cause a detectable change in the activation energy. Hydrolysis rate constants obtained with the 25% ethanol were about 26 times smaller than those without the alcohol at the same pH. The predicted bitolterol half-life at room temperature (25°C) is 8.4 years in 25% ethanol solution compared to 0.34 years in buffer both at pH 3.5.

Because of this pronounced effect of ethanol on hydrolysis rates the stability of bitolterol was determined in a variety of solvents. Large differences in reaction rates were observed for 25% solutions of various glycols as shown in Figure 4. For dipolar molecule reactions the logarithm of the rate constant should be negatively proportional to the inverse of the solvent dielectric constant as derived by Amis (13). This reduction in hydrolysis rate constant with a decreasing solvent dielectric constant is shown in Figure 5 and Table 1. The experimental values for the dielectric constants at 25°C are similar to those reported by others (9-12). Similar slopes are obtained for the relationship between the logarithm of the rate constant at 90°C and the reciprocal of the dielectric constant both at 25°C

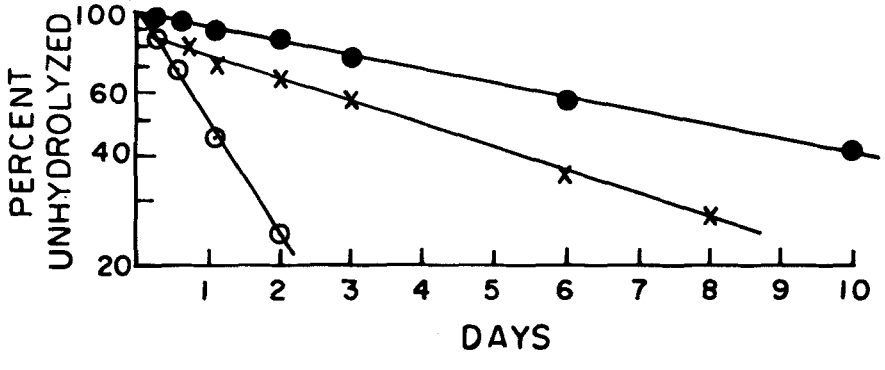

FIGURE 4

Semilogarithmic Plot of Percent Unhydrolyzed Bitolterol vs Time (Days) At 90°C For 10 mg/ml Solutions In 25% Polyethylene Glycol 400 (●); 25% Propylene Glycol (X) and 25% Glycerin (©). Least-Squares Regression Lines.

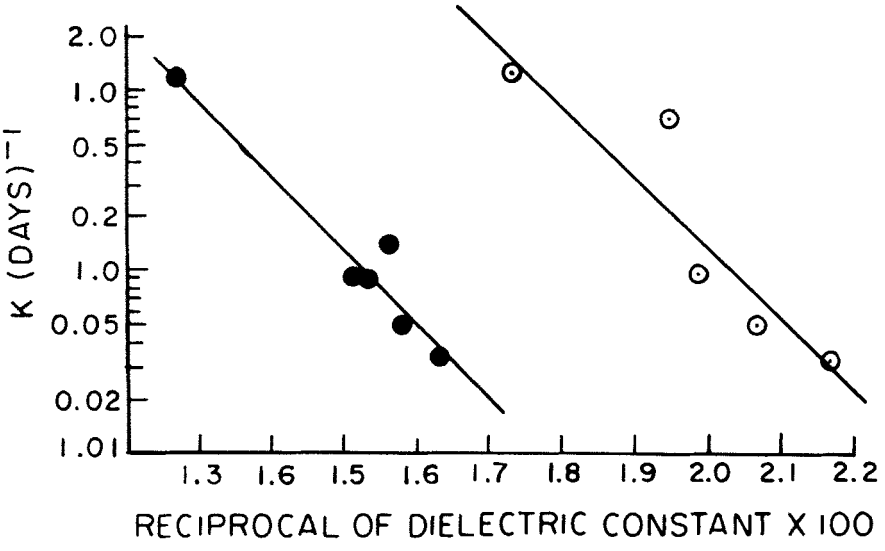

FIGURE 5

Semilogarithmic Plot of Hydrolysis Rate Constant (Days^{-1}) At 90°C vs 100/Dielectric Constant At 25°C (●) And 90°C (⊙). Least-Squares Regression Lines - Correlation Coefficients Of 0.96 and 0.92-pH 3.5.

and 90°C. This solvent effect on rates is very large as evidenced by the 40 fold rate reduction by the use of 30% ethanol.

Rates were also measured with sorbitol solutions of bitolterol and are shown in Table 2. A linear relationship exists between the reciprocal of the dielectric constant (25°C) and the hydrolysis rate constant (90°C) for the Sorbitol solutions in water as shown in Figure 6. It is apparent that as the sorbitol is increased in concentration the rate constant decrease is

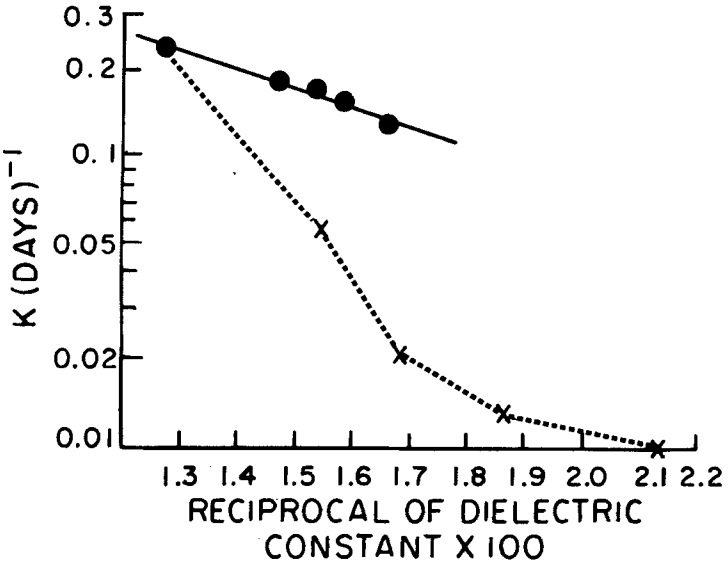

FIGURE 6

Semilogarithmic Plot of Hydrolysis Rate Constant (Days^{-1}) At 90°C vs 100/Dielectric Constant At 25°C For Sorbitol Solutions At pH 2.6-2.8 (•——•) and Ethanol-Propylene Glycol Solutions At pH 2.9-3.0 (X---X).

approximately equal to the decrease in water content. However a disproportionate decrease in rate constant occurs when small amounts of alcohol are present. This effect is many fold in excess of that due to the small decrease in water content. For the ethanol or ethanol-propylene glycol solvents the effect on rates is very pronounced as seen in Figure 6 and Table 3. By reducing the dielectric constant from 78.3 to 47 the rate constant was decreased 24 times. Curvature of the line occurs below a dielectric constant of about 60. This

may be due to selective solvation and the difference between the observed solvent dielectric constant and the microscopic dielectric constant around the reacting species or transition state as discussed by Amis (13). This deviation is, as predicted, in the direction of that obtained with the higher dielectric constant solvent, water.

The rate of a reaction may increase or decrease with a change of solvents dependent upon the reaction mechanism. Few studies have appeared on the influence of solvent dielectric constant on reaction rates for drug or drug formulations with pharmaceutically acceptable solvents. The hydrolysis rates for chloramphenicol (14), pancuronium bromide (15) and the thermal degradation of reserpine (16) are accelerated by lowering of the solvent dielectric solvent. Participation of the solvent in solvolysis must always be considered in this type of study. Only small changes in reaction rate constants have been observed for these compounds with relatively large changes in dielectric constants of the solvent media. The great reductions in hydrolysis rates that have been demonstrated for bitolterol illustrate that dielectric constant effects may be important for stabilization of drugs. This stabilization also has been shown for a clinical aerosol formulation and an inhalation solution of bitolterol where the low dielectric

constant of the solvent has resulted in a very stable product.

SUMMARY

Stability-indicating analytical methods using thin-layer and high pressure liquid chromatographic techniques were developed and applied to bitolterol. The hydrolysis rates for this catecholamine diester were measured as a function of pH and dielectric constant in water and in aqueous-organic solvent mixtures. A strong rate dependency was shown for each of these parameters. By appropriate adjustment of pH and solvent dielectric constant the reaction rate can be greatly reduced. A 40 fold reduction in the rate constant resulted from the use of 30% ethanol at pH 3.5 as compared to a buffer solution at the same pH. Solvent polarity, an often neglected factor, should be considered whenever possible for the stabilization of drugs.

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