DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 4(1), 31-52 (1978)

REACTION KINETICS OF SOME CATECHOLAMINES IN SODIUM BISULFITE SOLUTIONS

Glenn A. Portmann and Neil H. Brown
PRODUCT DEVELOPMENT DIVISION,
STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, NEW YORK 12144

ABSTRACT

Decreases in isoproterenol and N-t butylnor-isoproterenol concentrations as a function of time have been measured for solutions with different sodium bisulfite concentrations. The pseudo first-order constants are dependent upon the sodium bisulfite concentration and the solution pH. The activation energy for isoproterenol and levarteronol in sodium bisulfite solutions was calculated as 24.4 and 25.0 kilocalories per mole. A minimum rate constant as a function of pH was demonstrated and the general equation was derived for this relationship.

INTRODUCTION

To prevent oxidation of catecholamine drugs such as isoproterenol, epinephrine or levoarterenol, sodium bisulfite or metabisulfite are commonly used but their concentration and pH of the solution must be carefully chosen to decrease sulfonation of the catecholamine. The mechanism for the formation of 1-(3,4-dihydroxyphenyl)-2-methylaminoethane sulfonic acid from epinephrine and bisulfite has been studied in the pH range of 4 to 7 where

the epinephrine:bisulfite molar ratio was 1.4 to 4.3(1). It was suggested that two parallel reactions occur whereby sulfite ion reacts directly with epinephrine by second order kinetics and by a very fast reaction with the carbonium ion which is the intermediate during racemization. The agreement between experimental and predicted bisulfite half-lives was good within the pH range studied. However it is necessary to study this sulfonation reaction at the lower pH's of many commercial products where the racemization rate of the catecholamine becomes a large factor in the overall reaction. Also the catecholamine bisulfite molar ratio in many commercial products is much less than unity and in local anesthetic solutions with epinephrine the molar ratio is extremely low, the bisulfite molarity being 200 times larger than that of the catecholamine. The pH for optimum stability should be a function of bisulfite concentration. This pH for minimum reaction has not been determined for any catecholamine. The research reported herein is an attempt to develop some more kinetic information about this important reaction which could be used for determining optimum conditions for stabilization.

EXPERIMENTAL

A. Isoproterenol Solutions (3,4-Dihydroxy-α-[(isopropylamino) methyl]benzyl alcohol):

Isoproterenol HCl, 230 mcg/ml (Isuprel HCl, Winthrop Labs) was in solution with 0.18% sodium lactate, 0.012% lactic acid and 0.7% sodium chloride. Sodium bisulfite (reagent grade) was added in 0.1%, 0.080%, 0.050% or 0.025% concentration. These solutions were gassed with nitrogen and filled into 5 ml flint glass ampules autoclaved at 121°C for 15 minutes and stored in constant temperature ovens (Hotpack Co.) at temperatures of 90, 76, 62 and 50°C. At various time intervals

ampules were removed and the solution pH recorded. A kinetic study was also conducted at 121°C using non-autoclaved 5 ml ampules. The isoproterenol and sodium bisulfite were assayed by the following procedures.

Isoproterenol - An aliquot of the sample to be assayed containing about 400 mcg of isoproterenol was pipetted into a 50 ml beaker which contained 5.0 ml of 0.1N hydrochloric acid. This was heated on a hot plate regulated at low heat until the volume of the solution was reduced to 3 to 5 ml. This was cooled to room temperature and quantitatively transferred with approximately 4.0 ml of water to a 25 ml volumetric flask.

A standard solution was prepared containing 100 mcg/ml of isoproterenol in 0.1N hydrochloric acid. This solution should be made fresh before each assay. Zero, 2.0, 3.0 and 4.0 ml of the standard solution was pipetted into a series of 25 ml volumetric flasks. The volume in each flask was then made to 5.0 ml with 0.1N hydrochloric acid.

To the flasks containing the standards and samples, 5.0 ml of potassium ferricyanide-sodium acetate solution was added. The potassium ferricyanide-sodium acetate solution was prepared by dissolving 50 mg of potassium ferricyanide (reagent grade) in 100 ml of 10% sodium acetate (16.65 gm $NaC_2H_3O_2 \cdot 3H_2O$, reagent grade, per 100 ml water). Exactly two minutes after the addition of the ferricyanide-acetate solution to each flask, the reaction was quenched with 10.0 ml of alkaline ascorbate.

The ascorbate solution was prepared by mixing 135 ml of 10% sodium hydroxide (reagent grade) with 15 ml of 2% ascorbic acid. The 2% ascorbic acid should be made fresh before each assay using a freshly opened

bottle of ascorbic acid (reagent grade). This insures stability of the alkaline ascorbate solution for up to 60 minutes.

Each flask was diluted to volume with water and mixed. A reaction time of 20 minutes was allowed before the absorbance of each solution was determined at 410 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. The color is stable for up to one hour. The amount (mcg/ml) of isoproterenol was determined from a standard curve obtained from a plot of the absorbances of the standard solutions.

Sodium Bisulfite - To an aliquot of the sample containing 1 to 4 mg of sodium bisulfite, 10.0 ml of 6N hydrochloric acid and 5.0 ml of a starch indicator (0.2% aqueous starch solution) was added. This was titrated to a blue end point with 0.01N iodine. Each ml of 0.01N iodine is equivalent to 0.52 mg of sodium bisulfite.

B. Levarterenol Bitartrate Solutions (3,4-Dihydroxy-α-(aminomethyl)benzyl alcohol):

L-arterenol bitartrate monohydrate, 2.3 mg/ml (Levophed, Winthrop Labs) was dissolved in water for injection containing 2 mg/ml sodium bisulfite and 8 mg/ml sodium chloride. The water was purged with carbon dioxide before and during preparation of the solution. Each 4 ml flint glass ampule was gassed with nitrogen during the filling operation and the ampules were autoclaved at 104°C for 5 minutes. They were stored in constant temperature ovens at 90°, 80° and 70°C and removed at varying time periods depending upon the reaction rate at that temperature. Duplicate assays for levarterenol bitartrate were done using the USP XVIII procedure for injections containing 2 mg per ml(2). The sodium bisulfite concentrations were

determined using the procedure described in Section A and the solution pH was recorded.

C. 3,4-Dihydroxy-α-[(t-butylamino)methyl]benzyl alcohol methanesulfonate monohydrate Compound A Solutions:

Solutions containing 800 mcg/ml of Compound A were prepared in distilled water containing 7.2 mg/ml of sodium lactate (reagent grade). Sodium bisulfite (reagent grade) was added in 1, 2, 3, 4, and 5 mg/ml concentrations and the pH adjusted to 4 with 0.1N HCl. At the 4 mg/ml sodium bisulfite concentration adjustment also was made to pH 2.4, 2.7, 3.0, 3.5 and 4.5. These solutions were gassed with nitrogen, filled into 30 ml flint ampules and stored at 90°C. At various time intervals ampules were removed and the solution pH recorded. The catecholamine concentration was determined by the procedure described in section A for isoproterenol, the only exception being a three minute reaction period after the addition of the ferricyanide-acetate solution.

RESULTS AND DISCUSSION

A. Isoproterenol Kinetic Study - As shown in figures 1 to 3 psuedo first-order reaction kinetics can be applied to isoproterenol changes in solutions containing excess sodium bisulfite. A small decrease in pH was observed over the course of the experiment, the maximum decrease was from 3.96 to 3.83. For the solution studied at 90°, 76°, 62° and 50°C the molar ratio of sodium bisulfite (0.1%) to isoproterenol hydrochloride was 11 and for the solution studied at 121°C the molar ratio was 9. As a result of this large excess of bisulfite ion the reaction kinetics show first-order dependence on isoproterenol.

According to the following reaction model proposed by Higuchi and Schroeter(1) to describe sulfonation

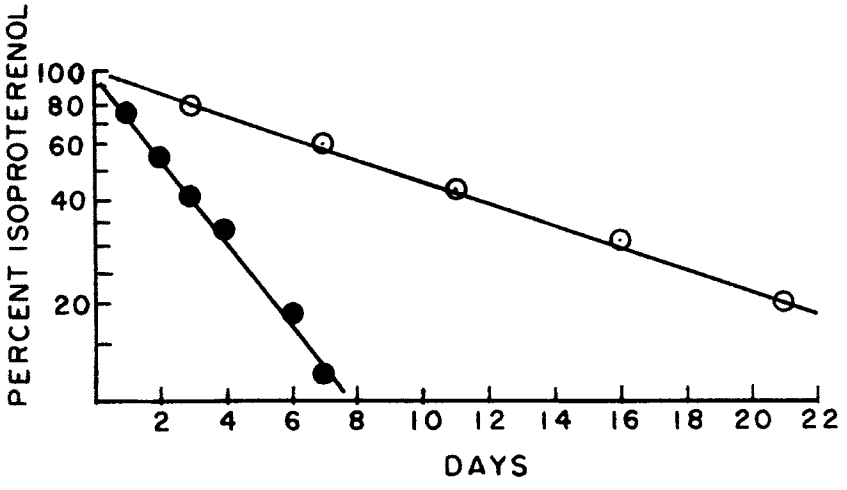


FIGURE 1

Semilogarithmic Plot of Percent Isoproterenol Unreacted Against Time At 90°C. (●) and 76°C (⊙). Initial Isoproterenol Hydrochloride, 0.0216%; Sodium Bisulfite, 0.10%; pH 3.96. Least-squares Regression Lines.

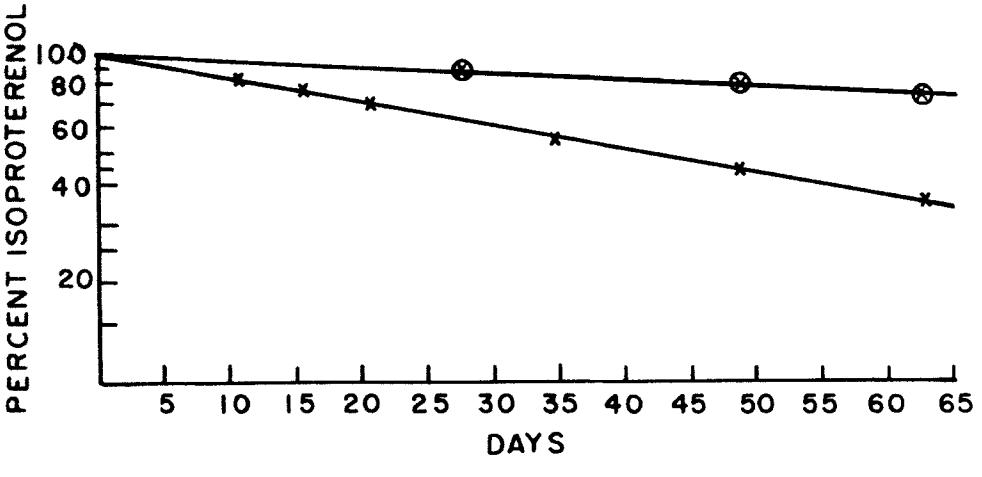


FIGURE 2

Semilogarithmic Plot of Percent Isoproterenol Unreacted Against Time at 62°C (X) and 50°C (⊗). Initial Isoproterenol Hydrochloride, 0.0216%; Sodium Bisulfite, 0.10%; pH 3.96. Least-squares Regression Lines.

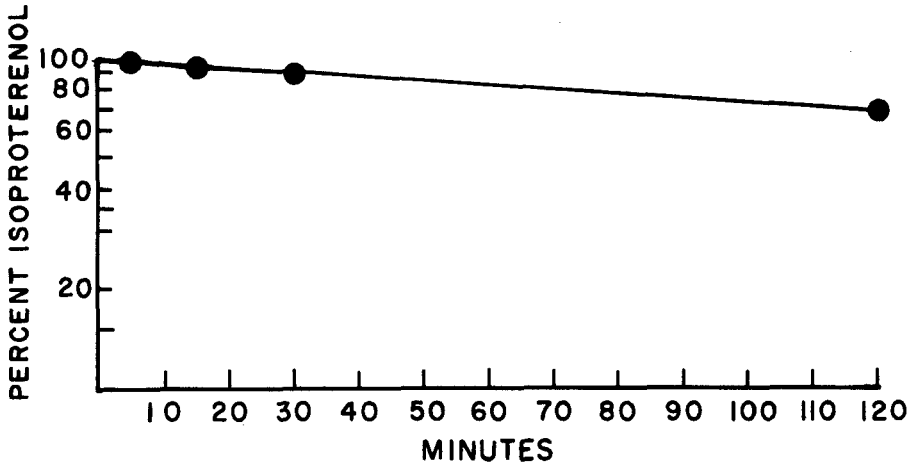
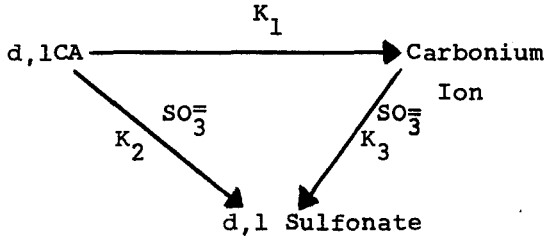


FIGURE 3

Semilogarithmic Plot of Percent Isoproterenol Unreacted Against Time at 121°C. Initial Isoproterenol Hydrochloride, 0.028%; Sodium Bisulfite, 0.10%; pH 3.96. Least-squares Regression Line.

kinetics with epinephrine the carbonium ion responsible for racemization in the absence of bisulfite(3) reacts very rapidly with sulfite (K_3) relative to the rate of formation of the activated state (K_1) or the reformation of the catecholamine. The second order reaction rate constant (K_2) represents the reaction of sulfite ion directly with the catecholamine (CA).



$$-\frac{d(CA)}{dt} = K_2(CA)(SO_3^{=}) + K_1(CA) \qquad (1)$$

One way to simplify the determination of rate constants for parallel first and second order reactions is to increase the concentration of the reactant which is not common to both rate expressions to the point where the concentration at the end of the reaction is not greatly different from the initial concentration. When the change in sulfite ion is very small due to a large molar excess of bisulfite, equation (1) can be further simplified to:

$$\frac{-d(CA)}{dt} = K_4(CA) \qquad (2)$$

where:
$$K_4 = K_2(SO_3^{=}) + K_1 \qquad (3)$$

or:
$$K_4 = K_2 \frac{(NaHSO_3)(K_A)}{(H+)} + K_1 \qquad (4)$$

where K_A is the second ionization constant for sulfurous acid. It is not necessary to consider the presence of pyrosulfite ions since dilute solutions of sodium bisulfite contain very little pyrosulfite if the ionic strength is low as shown by Bourne et al(4) for bisulfite solutions of pH 3 to 5. It has also been shown that the effect of ionic strength on sulfonation of epinephrine at pH 3.63 and pH 5.0 is very small(5).

An Arrhenius plot of the rate constants, K_4, is shown in figure 4. The apparent heat of activation for formation of isoproterenol sulfonate calculated from the regression equation is 24.4 kilocalories per mole which is the same as that determined for epinephrine sulfonation, 24 kilocalories per mole(1). Also the activation energy for epinephrine sulfonation in lidocaine solutions has been reported as 24.2 and 24.5 kilocalories per mole at pH 4.0 and 4.4(6). This similarity in activation energies would be expected

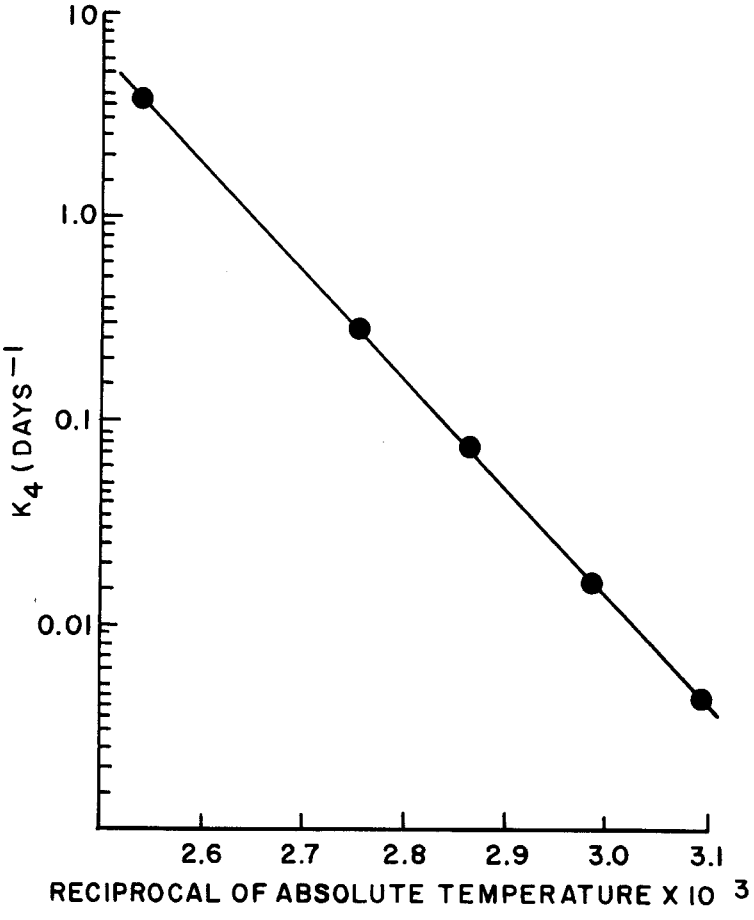


FIGURE 4

Arrhenius Plot For Isoproterenol Sulfonation. Least-squares Regression Line. Log $K_4 = 14.03-5,292/_T$.

since epinephrine and isoproterenol are so structurally similar differing only in the amine substitution (methyl and isopropyl).

The effect of sulfite concentration at pH 4.0 is shown in Table I and figure 5 where there is a 1.4

TABLE I

PSEUDO FIRST ORDER RATE CONSTANTS
(K_4) FOR ISOPROTERENOL AT pH 4

$K_4(days^{-1}) \times 10^3$

Sodium Bisulfite mg/ml	Molar Ratio ($NaHSO_3$/Isoproterenol)	90°C Experimental	50°C Theoretical	50°C Experimental	25°C Theoretical
0.80	8.6	290			0.188
0.46	4.9	238	3.69	3.42	0.155
0.22	2.4	201	3.12	2.89	0.131

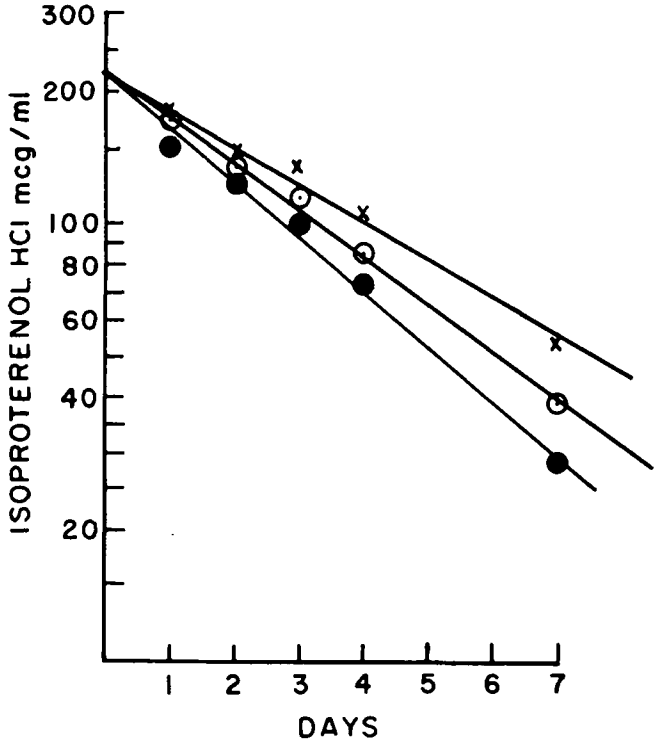


FIGURE 5

Semilogarithmic Plot of Isoproterenol Hydrochloride Concentration (mcg/ml) Against Time At 90°C With 0.080% (•), 0.046% (⊙) and 0.022% (X) Sodium Bisulfite at pH 4.0. Least-squares Regression Lines.

fold increase in rate constants between 0.22 and 0.80 mg/ml of sodium bisulfite. Using the 24.4 Kcal./mole activation energy and the rate constants for 90°C the theoretical rate constants for 50°C were calculated for the 0.46 and 0.22 mg/ml sodium bisulfite solutions. The prediction is excellent since the theoretical and experimental constants at 50°C differ by only 7%. By carrying the prediction further to 25°C the practical

advantage of a reduction in sodium bisulfite concentrations becomes evident. Quantities of catecholamines above the label claim as high as 15% are permitted in formulations. Considering a change from 110% to 90% of the labeled amount of isoproterenol the time would be extended from 2.9 years to 4.2 years with a change in sodium bisulfite from 0.08% to 0.025%. The improved stability of l-epinephrine solutions by decreasing bisulfite concentrations has been attributed to a lower sulfonation rate which is stated to be directly proportional to the concentration of bisulfite(7). Also the lower sodium bisulfite concentrations are adequate for prevention of oxidation since no color developed in all of the solutions during the 90°C study. No color was evident in ampules stored in a light cabinet (Envira-Lite) at 2000 foot-candles for 14 days. However with solutions containing very low (0.007%) or no sodium bisulfite a pink coloration developed rapidly at 90°C and with no bisulfite a black precipitate was formed by 14 days. However the degradation of isoproterenol was much less rapid than with higher concentrations of bisulfite.

The racemization rate constant (K_1) and K_2K_A for isoproterenol at pH 4.0 and 90°C is 0.168 $days^{-1}$ and 15.8 x 10^{-4} $days^{-1}$ determined according to equation (4). The contribution of the second order reaction to the overall reaction rate constant (K_4) at 90°C can be calculated and varies from 42% to 17% for 0.080% and 0.022% sodium bisulfite respectively.

It is interesting that a racemization rate constant (K_1) was determined according to the kinetic model with the racemic mixture of isoproterenol hydrochloride. The rate of racemization of d-isoproterenol observed experimentally in the absence of bisulfite at

pH 3.85 and 90°C was 0.133 $days^{-1}$ which is very close to the value of 0.168 $days^{-1}$ estimated with kinetic data on sulfonation of racemic isoproterenol at pH 4.0 and 90°C. At 84.5°C and pH 4.0 the racemization rate constant for epinephrine has been reported as 0.151 $days^{-1}$(1). Recently (8) it has been shown that the racemization rate of l-noradrenaline bitartrate is greatly decreased when the solvent polarity is slightly decreased, e.g. a 2 fold reduction in rate was observed in 17% ethanol in water compared to an aqueous solution both at pH 1.5. This reduction in racemization rate should also lower the overall sulfonation rate for catecholamine. The effect of decreasing polarity should be greater at low bisulfite concentrations since the contribution of the racemization rate is greater as the bisulfite levels are decreased.

B. Levarterenol Bitartrate Kinetic Study - Since there is a 2.8 fold molar excess of sodium bisulfite to levarterenol pseudo first order kinetics is expected as shown in figure 6. The rate constants are 0.379, 0.159 and 0.0506 reciprocal days at 90°, 80°, and 70°C respectively. As expected these rate constants are higher than those obtained with the isoproterenol solutions since the pH is lower and the bisulfite concentrations are higher with the l-norepinephrine solutions. The activation energy is 25.0 kilocalories per mole for levarterenol sulfonation which is comparable to that for isoproterenol (24.4 kilocalories). Also the rate constants based on bisulfite loss due to sulfonation are similar to those for levareterenol loss and the activation energy was calculated as 25.4 kilocalories per mole. A reduction in bisulfite concentration would be expected to decrease the rate of levarterenol loss. Using the Arrhenius equation the predicted rate constant at 25°C

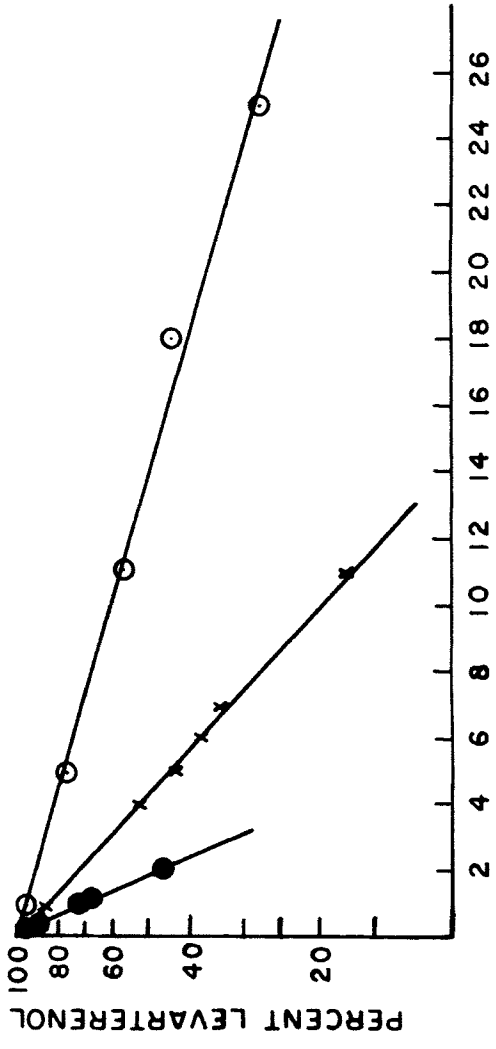


FIGURE 6

Semilogarithmic Plot of Percent Levarterenol Unreacted Against Time At 90°C. (•), 80°C. (✕) and 70°C (◎). Initial Levarterenol Bitartrate, 2.3 mg/ml; pH 3.5. Least-squares Regression Lines.

is 2.106 x 10^{-4} reciprocal days and the time for a change from 115% to 90% of the labeled amount is 3.2 years for the solution containing 2 mg/ml sodium bisulfite.

C. Compound A Sulfonation Study - This study was designed to evaluate the product K_2K_A by following rates with solutions of constant pH but variable sodium bisulfite concentrations. Then K_1 can be evaluated by determining the overall rate constant (K_4) for solutions at variable pH's. The pH for optimum stability of Compound A can then be calculated.

For the solutions containing from 1 to 5 mg/ml of sodium bisulfite the molar excess of bisulfite to catecholamine ranges from 4.1 to 20.5 and therefore pseudo first order kinetics would be expected. According to equation (4) these rate constants (K_4) should be a linear function of the sodium bisulfite concentrations at constant pH. This relationship is shown in figure 7. From the slope the product K_AK_2 can be obtained and is equal to 10.8 x 10^{-4} $days^{-1}$. The racemization constant (K_1) at pH 4.1 and 90°C was calculated from the regression line as 0.172 $days^{-1}$. Both of these constants are very similar to those obtained with isoproterenol as would be expected because of the structural similarity (N-t-butyl and N-isopropyl groups). As the sulfite concentration increases the contribution of the second order reaction (K_2) to the overall sulfonation rate increases from 43% at 1 mg/ml bisulfite to 79% at 5 mg/ml bisulfite as shown in Table II.

From the K_4 rate constants obtained with the solutions at variable pH's the K_1 constants can be calculated according to equation (4) using K_2K_A equal to 10.8 x 10^{-4} $days^{-1}$. The linear correlation between

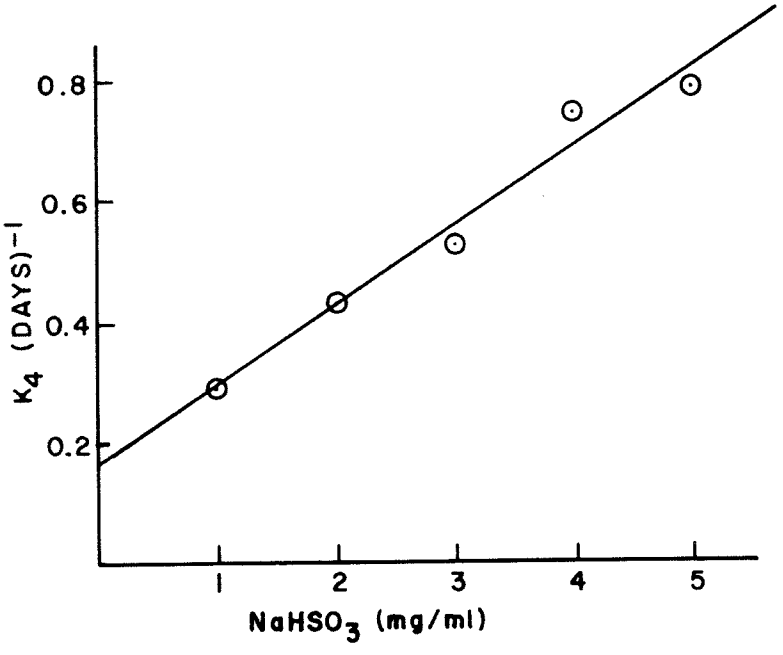


FIGURE 7

Rate Constant For Compound A Reaction (K_4, days^{-1}) Against Sodium Bisulfite Concentration (mg/ml) At 90°C. and pH 4.1. Least-squares Regression Line — Correlation Coefficient of 0.98.

TABLE II

FIRST ORDER RATE CONSTANTS (K_4) FOR COMPOUND A AT pH 4.1 AND 90°C.

$NaHSO_3$ mg/ml	Molar Ratio $NaHSO_3$/5563-3	K_4 (days^{-1})	% Contribution of Second Order Reaction
1	4.07	0.295	43
2	8.14	0.437	60
3	12.2	0.538	70
4	16.3	0.756	75
5	20.4	0.787	79

the logarithm of K_1 versus pH is shown in figure 8. Racemization rate constants for epinephrine have been determined at low pH's and the logarithm is similarly correlated with the pH (9). The value for the second ionization constant of sulfurous acid (K_A) at 90°C does not have to be known to calculate the contribution of the second order reaction since the product $K_A K_2$ can be determined from the experimental data.

The overall reaction rate constant (K_4) can now be calculated as a function of pH at any sodium bisulfite concentration. For a sodium bisulfite level of 4 mg/ml which was used experimentally the curve is shown in figure 9. Good agreement was obtained with the

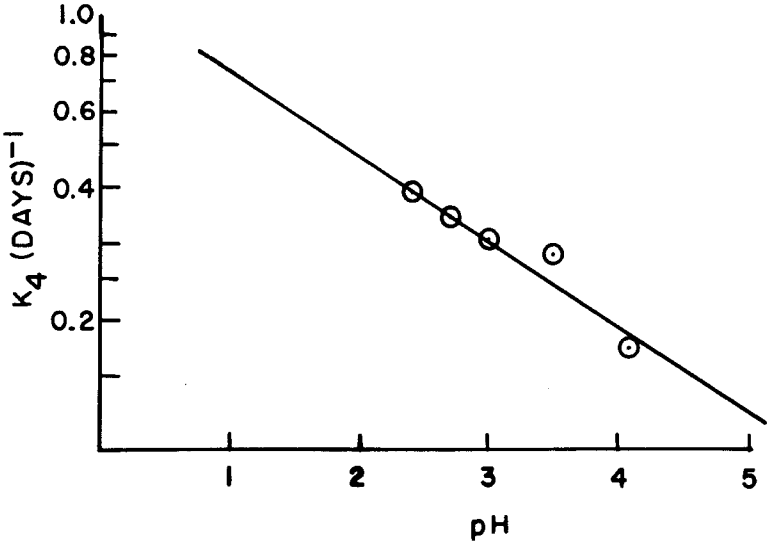


FIGURE 8

Semilogarithmic Plot of K_1 (days^{-1}) For Compound A Against pH At 90°C-Sodium Bisulfite-4 mg/ml. Least-squares Regression Line — Correlation Coefficient of 0.96. Log K_1 = -0.193 pH + 0.0666.

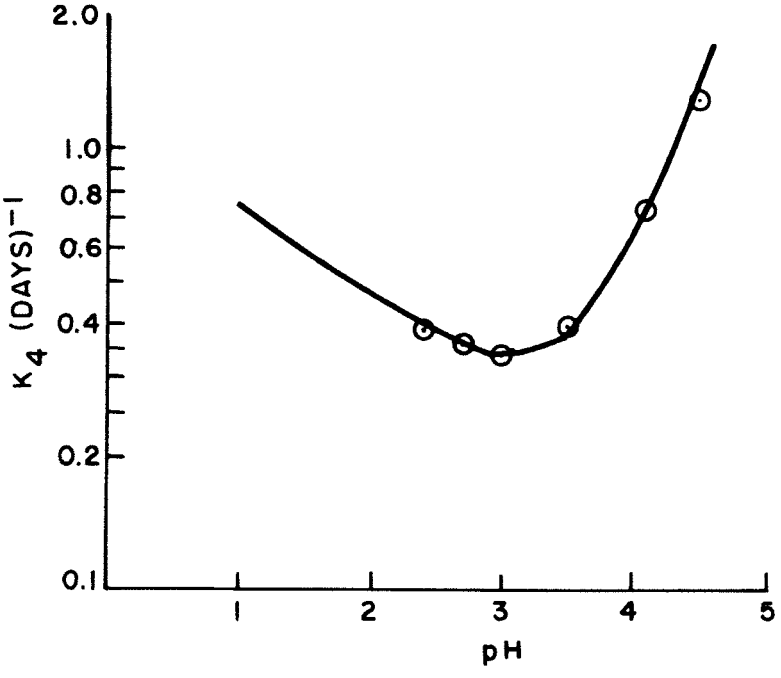


FIGURE 9

Semilogarithmic Plot Of K_4 (days^{-1}) For Compound A Against pH At 90°C.-Sodium Bisulfite-4 mg/ml. Calculated Line, ——; Experimental Values, ●.

experimental rate constants and those calculated with equation (4) using K_2K_A equal to 10.8 x 10^{-4} days^{-1} and K_1 calculated from the regression equation from figure 8 at each pH. The optimum pH for maximum stability is 3.1.

Since this optimum pH will change if the sodium bisulfite concentration is varied it is of practical importance to obtain an expression of this dependency when various formulations or dosage forms have different bisulfite requirements. Rearrangement of equation 4 gives the following expression:

$$\log (K_4-K_1) = pH + \log K_2K_A + \log (NaHSO_3) \quad (5)$$

Since log K_1 is linearly related to pH (figure 8) equation 5 can be expressed as:

$$K_4 = 10^{C} + 10^{-ApH+B} \qquad (6)$$

where $C = pH + \log K_2K_A + \log (NaHSO_3)$

differentiating with respect to pH yields

$$\frac{dK_4}{dpH} = Ln10\ (10^{C-A}10^{-ApH+B}) \qquad (7)$$

A minimum exists when dK_4/dpH is equal to zero. Therefore:

$$C = ApH + B + \log A \qquad (8)$$

Substituting for C and simplifying yields:

$$pH = \frac{B + \log \left(\frac{A}{K_2K_A}\right) - \log (NaHSO_3)}{1+A} \qquad (9)$$

This general expression relates the pH for maximum stability as a function of sodium bisulfite concentration. Substituting numerical values for each constant gives the following expression for Compound A.

$$pH = 1.95 - 0.838 \log (NaHSO_3) \qquad (10)$$

As shown in figure 10 the maximum stability pH varies for 2.8 to 3.6 for sodium bisulfite concentrations of 10 and 1 mg/ml respectively. West found that the optimum pH was 3.5 for noradrenaline solutions (1:1000) containing 1 mg/ml sodium metabisulfite by determining the great difference in activity of six solutions between pH 1.5 and 6.5 after exposure to 115°C for 6 hours(10). Also the optimum pH for adrenaline was 3.6 as determined

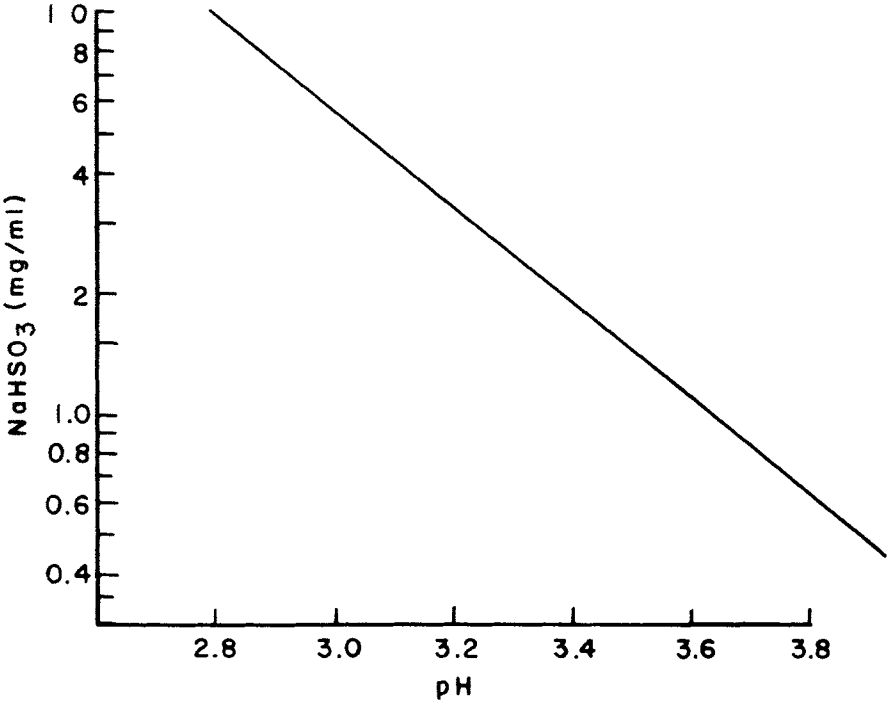


FIGURE 10

Semilogarithmic Plot Of Sodium Bisulfite Concentration (mg./ml.) Against pH For Maximum Stability.

by activity measurements with solutions containing 1 mg/ml sodium metabisulfite(11). These results are in excellent agreement with the predicted pH for maximum stability of Compound A which is structurally similar to the adrenalines.

SUMMARY

It has been clearly demonstrated that the rate for sulfonation of isoproterenol and compound A (N-t-butylnorisoproterenol) is dependent upon the sodium

bisulfite concentration and pH of the solution. From Arrhenius plots the activation energy for isoproterenol and levarterenol sulfonation is 24.4 and 25.0 kilocalories per mole which is similar to that reported for epinephrine (24.0 kilocalories). The practical significance of a longer shelf-life for catecholamine solutions by appropriately adjusting the pH and sodium bisulfite concentrations has been demonstrated. An equation was derived for calculating the pH for minimum reaction in the presence of different concentrations of sodium bisulfite.

ACKNOWLEDGMENTS

The authors wish to acknowledge Gary Drumm for the Compound A analyses and Beverly Bondley for the levarterenol assays.

REFERENCES

1. Takeru Higuchi and Louis C. Schroeter, J. Amer. Chem. Soc. 82, 1904 (1960).
2. "The United States Pharmacopeia", Eighteenth Revision, Mack, Easton, PA., 1970 p. 363.
3. L. C. Schroeter, T. Higuchi and E. Schuler, J. Am. Pharm. Assoc., 47, 723 (1958).
4. David W. A. Bourne, T. Higuchi and Ian H. Pitman, J. Pharm. Sci., 63, 865 (1974).
5. B. R. Hajratwala, J. Pharm. Sci. 64, 45 (1975).
6. B. R. Hajratwala, Drug Develop. Ind. Pharm 3, 65 (1977).

7. Walter Borisenok, Canadian Patent, 981182 (1976).
8. A. E. M. El-Nimr, A. A. Kassem and M. A. Kassem, Pharmazia, 30, 216 (1975).
9. L. C. Schroeter and Takeru Higuchi, J. Am. Pharm. Assoc. 47, 426 (1958).
10. G. W. West, J. Pharm. Pharmacol., 4, 560 (1952).
11. G. W. West, ibid, 2, 864 (1950).