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DECOMPOSITION OF ANTHRALIN IN ALKALINE SOLUTIONS

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ABSTRACT

Using conventional absorbance spectra and a first-derivative transformation technique anthralin was shown to undergo apparent first-order decomposition. The pH-log rate profile for the rate of disappearance of anthralin from aqueous solutions was determined at 25°C in the pH range of 7.74 to 10.02 at an ionic strength of 0.5 M. The profile indicated a maximum near the pK_a of anthralin. Measured values of the half life of anthralin decreased from 43.9 min at pH 7.74 to 14.8 min at pH 9.44 and then increased to 18.4 min at pH 10.02. No primary kinetic salt effect was observed. Since it was thought likely that trace amounts of metal ions could catalyze the degradation of anthralin, the effect of disodium ethylenediaminetetraacetic acid addition was also investigated; this chelating agent did not influence the rate. Thermodynamic parameters were calculated from the temperature dependency of the decomposition.

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INTRODUCTION

Anthralin (1,8,9-anthracenetriol) is an effective antipsoriatic agent which has been used for over sixty years.[1,2] The treatment of psoriasis with commercial anthralin preparations suffers from two side effects: staining of skin and clothing and irritation of surrounding normal skin.[3] It is well documented[1] that anthralin is unstable in solution and, depending upon the exposure conditions, can form the corresponding quinone (1,8-dihydroxy-9,10-anthraquinone) and/or the corresponding dimer (1,8,1´,8´-tetrahydroxy-10,10´-dianthrone). Despite reports[3] that the therapeutic effect observed stems from anthralin only and not from its decomposition products, little data on the chemical stability of anthralin in aqueous solutions has been published. Thus the aim of the present work was to investigate these kinetics and to determine the factors affecting them.

It has been reported[3-6] that anthralin could be quantitated using spectral detection techniques after separation from its degradation products. In kinetic studies of labile drugs, however, it is desirable to make measurements both rapidly and in situ. In this study a microprocessor-controlled, parallel-detection, photodiode array spectrophotometer was employed which permitted acquisition of absorbance data over the full 220-800 nm wavelength range in one second. Proper selection of analytical wavelength avoided interference problems and both conventional zero-order absorbance and first-derivative techniques yielded consistent results.

MATERIALS AND METHODS

Anthralin and quinone were obtained from Aldrich and were used as received. Dimer was prepared according to the method of Kinget.[7]

All of these solids were stored in the dark and refrigerated in a desiccator until needed. Tris(hydroxymethyl)aminomethane was of primary standard grade and was obtained from Sigma Chemical Company. Certified hydrochloric acid solution (1N) and potassium chloride (certified A.C.S.) were purchased from Fisher Scientific Company. Disodium ethylenediaminetetraacetic acid (dihydrate) was from J.T. Baker Chemical Company, and 95% ethanol (USP grade) was purchased from Pharmco Products Incorporated. All buffer solutions were prepared in double-distilled water (all glass distillation apparatus). UV cells (Spectrocell) with 10 mm light paths and 3.5 ml volumes were utilized in this study. The pH values of the aqueous solutions were measured with a Model 611 digital pH meter (Orion Research) and a Ag/AgCl glass electrode (Orion Research) in a 50 ml water-jacketed beaker maintained at the appropriate temperature ± 0.1°C.

All absorbance spectra were obtained with an HP8450A parallel-detection, photodiode array UV-VIS spectrophotometer. This spectrophotometer had a microprocessor which allowed rapid calculation of first-derivative spectra. Three (3.0) ml of buffer solutions in UV cuvettes were placed into a desiccator which was alternately evacuated and flushed with nitrogen gas (four cycles). These cuvettes were then placed in the reference and sample positions of the spectrophotometer. The balance operation (automatic base-line correction) was then performed. Upon completion of the cell balancing procedure, 50 µl of an anthralin solution in 95% ethanol were added to the sample cuvette and the contents mixed by inversion of the cuvette. The kinetic measurements were initiated as per the pre-programmed time intervals. The laboratory lights were turned off during the kinetic runs and the UV radiation itself illuminated the

sample only when an absorbance measurement was being made. The absorption spectrum of the reaction mixture at each measurement time was recorded from 220-800 nm employing three to five second integration times. This integration time provided an average of six to ten individual spectra for each measurement. All of the spectra were stored on magnetic diskettes for retrieval and analysis. The ionic strengths of the buffer solutions were maintained constant by the addition of appropriate amounts of potassium chloride.

The HP89100A temperature control accessory provided the spectrophotometer with sample temperature control which was remotely programmable. This unit utilized thermoelectric heating and cooling (Peltier principle) and could maintain control over a -10°C to +105°C temperature range with 0.1°C resolution. The validity of Beer's law for the analysis of anthralin concentration was confirmed at pH 7.54 and 10.00 (25°C and an ionic strength, μ, of 0.5 M).

The amount of dissolved oxygen in the buffer solutions was determined by a colorimetric procedure employing a dissolved oxygen test kit (Model O-12; CHEMetrics, Incorporated). The test method involved breaking a CHEMET reagent tube in the sample to be measured and comparing the resultant color with liquid color standards. The CHEMET tube contained an indigo-carmine color forming reagent in acid solution and the procedure was claimed by the manufacturer to be free from the chemical interferences normally encountered in titrimetric procedures.

All of the kinetic data were computer analyzed using a linear least squares regression program that employed an iterative procedure for determining the best A_∞ value.

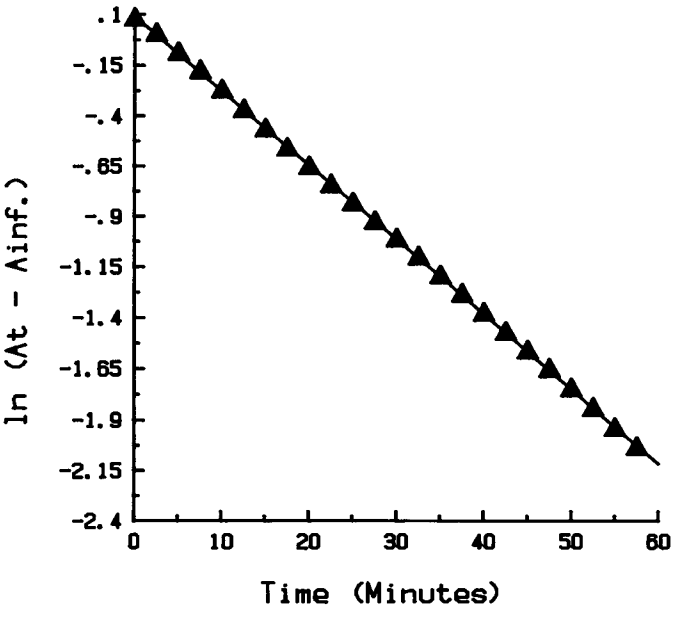

Figure 1. Plot showing the overall first-order decomposition of anthralin (pH = 10.02, μ = 0.5 M) at 25°C. Correlation coefficient = 0.9999.

RESULTS

Spectrophotometry and Order of Reaction

Although it was evident from the spectroscopic work early in this study that the presence of quinone would interfere with the analysis of anthralin at 258 nm, it was also apparent from the first-derivative spectra that analysis of anthralin could be accomplished at 263 nm. The plots of ln $(A_t - A_\infty)$ versus time and ln $[(dA/d\lambda)_t - (dA/d\lambda)_\infty]$ versus time (Figs. 1 and 2) showed excellent linearity for a minimum of three half-lives. Additionally, alteration of the initial anthralin concentration did not affect the rate constant. The plots of anthralin decomposition in aqueous solution

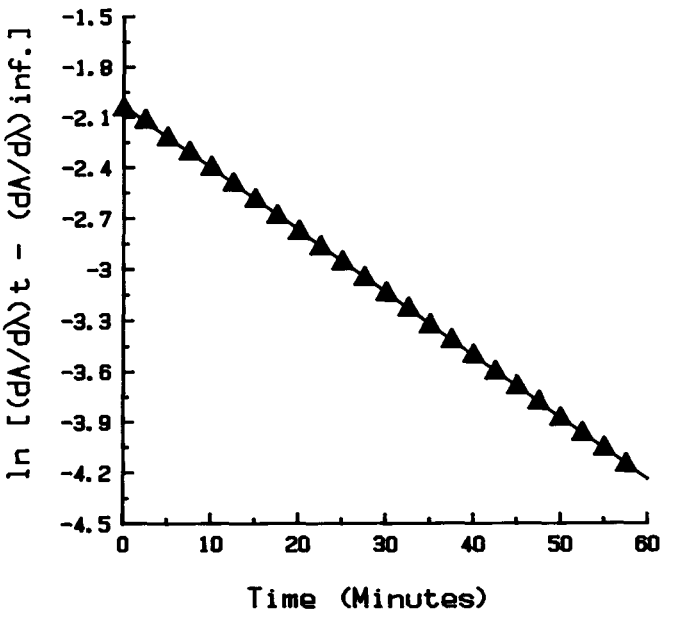

Figure 2. Plot of the first derivatives of the absorbance data of Figure 1 showing the overall first-order decomposition of anthralin. Correlation coefficient = 0.9999.

demonstrated apparent first-order behavior under all experimental conditions.

pH-Rate Profile

Figure 3 shows the experimental pH-log rate profile for anthralin. A decrease in half life from 43.9 min at pH 7.74 to 14.8 min at pH 9.44 was noticed with a subsequent increase to 18.4 min as pH was further elevated to pH 10.02.

Effect of Ionic Strength

No primary salt effect was observed at pH 10.02 and 45°C as indicated by the ratio of the rate constants, k(μ=0.50)/k(μ=0.05), being 1.06.

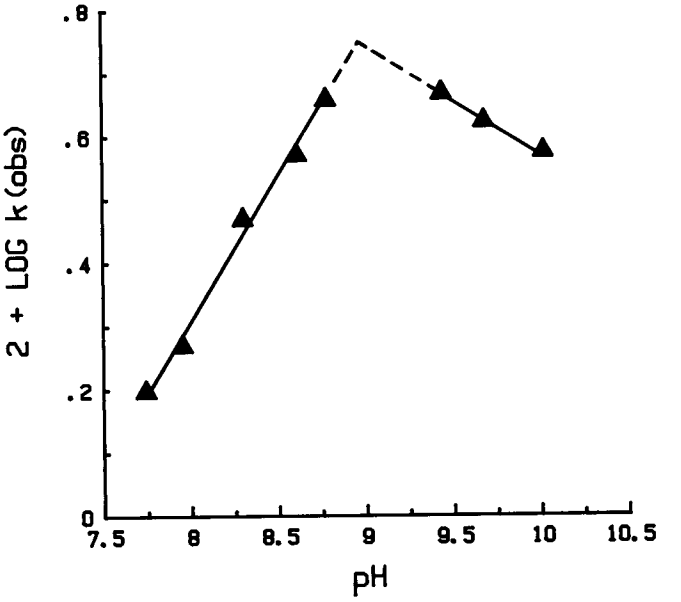

Figure 3. pH-rate profile of anthralin decomposition at 25°C.

Effect of Chelating Agent Addition

The addition of 0.1% disodium ethylenediaminetetraacetic acid did not affect the rate constant for decomposition of anthralin. Both in the absence and presence of the chelating agent, a half life of 4.23 min was obtained at pH 10.02 (45°C, μ=0.5).

Arrhenius Plot

An activation energy of 13.7 Kcal/mole was calculated from the Arrhenius plot (Fig. 4). A similar plot yielded an enthalpy of activation of 13.1 Kcal/mole and an entropy of activation of -29.3 e.u. The free energy of activation was determined to be 21.8 Kcal/mole.

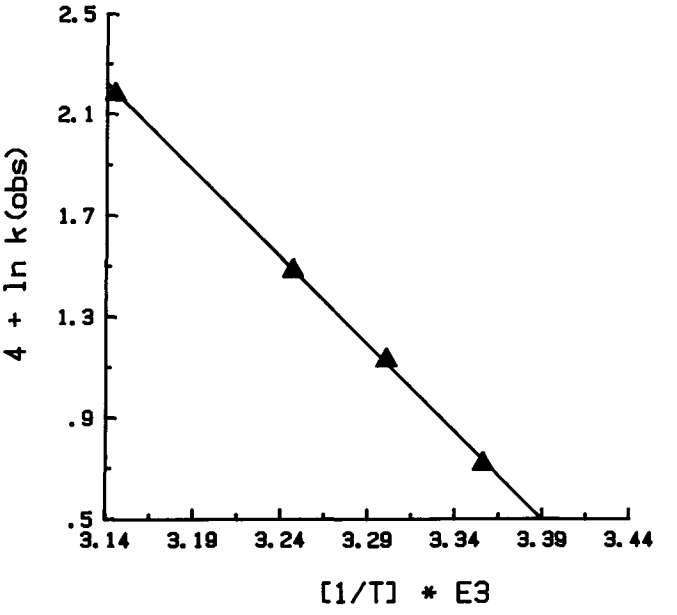

Figure 4. Arrhenius plot demonstrating the temperature dependence of anthralin decomposition (pH = 10.02). Correlation coefficient = 0.9998.

DISCUSSION

Order of Reaction

An increase in the initial concentration of anthralin from approximately 1 μg/ml to 5 μg/ml did not affect the half life for anthralin decomposition. This independence of half life on the initial concentration of reactant is characteristic of first-order reactions. Since, for a first-order reaction, any property that bears a functional relationship to concentration can be used to investigate the kinetics of that reaction, the first derivatives of the absorbance data at 263 nm were plotted versus time (Fig. 2). This analysis yielded a half life of 18.4 min as calculated from the slope of the linear least-squares regression line. At

this wavelength quinone and dimer made no contribution to the overall absorbance thus indicating that a simpler procedure utilizing absorbance directly could be employed even in the presence of quinone and dimer. This was confirmed by a plot of absorbance versus time (Fig. 1) that yielded the same half life ($t_{\frac{1}{2}}$ = 18.4 min).

Effect of Ionic Strength

The effect of ionic strength on the rate of decomposition of anthralin was investigated at a pH of 10.02 and a temperature of 45°C in tris buffer. The solutions were adjusted to ionic strengths of 0.05 M and 0.5 M by the addition of potassium chloride. The theory of Brønsted and Bjerrum predicts, for low ionic strengths, a linear relationship between the logarithm of the rate constant and the square root of the ionic strength when two charged species react. The absence of a primary salt effect at this pH thus suggests that the predominant process is either a reaction between a neutral species and a charged species or a reaction between two neutral species.

Effect of Chelating Agent Addition

The possibility of metal ion catalysis of anthralin decomposition was evaluated by also studying the decomposition in the presence of 0.1% disodium ethylenediaminetetraacetic acid. The rate constant obtained duplicated that found in the absence of a chelating agent. However, the particular trace metal ions present as impurities might not be effective in catalyzing anthralin decomposition, or if catalytic, their concentrations might be too low to have a measurable effect on a drug this reactive.

Effect of Dissolved Oxygen

Oxygen was found to be a participant in the reaction. Although the procedure of alternate evacuation and nitrogen gas purging did

not remove oxygen completely, the procedure helped to bring the dissolved oxygen to a relatively constant concentration. The oxygen to anthralin molar ratio in this study was found to be 17:1.

pH-Rate Profile

The pH-rate profile (Fig. 3) was constructed from the observed first-order rate constants at 25°C and the indicated pH values (μ=0.5 M). The rate constant increased with pH up to the pK_a[8] of anthralin and then declined slowly. The increasing slope indicates that the ionized form of anthralin reacts more rapidly than the unionized form although it has been reported[9] that both forms can react with dissolved oxygen. Since the rate constant is increasing, it is apparent that the reaction does not involve hydrogen ion concentration except as it affects the ionization of anthralin. The decrease in rate constant above the pK_a cannot be satisfactorily explained at this time but it does indicate that the reaction is also kinetically independent of hydroxide ion concentration.

Assuming that both forms of anthralin react with dissolved oxygen, that the concentration of dissolved oxygen remains essentially constant, and that ionized and unionized anthralin each react via a single mechanism, then the rate equation for the oxidation of anthralin can be written as follows:

$$\text{Rate} = k_1[HA] + k_2[A^-] \qquad \text{Eqn. 1}$$

where k_1 and k_2 are the apparent rate constants for HA and A^-, respectively. Expressions for the fractions of HA and A^- present at any given pH can readily be developed from the standard equilibrium relationship:

$$HA \overset{K_a}{\rightleftarrows} H^+ + A^- \qquad \text{Eqn. 2}$$

where HA represents the neutral anthralin molecule, A^- represents ionized anthralin, and K_a is the acid-base equilibrium constant. This equilibrium constant is defined by the equation:

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{Eqn. 3}$$

The fractions of undissociated and dissociated anthralin can be written as:

$$f_{HA} = \frac{[HA]}{[HA] + [A^-]} \qquad \text{Eqn. 4}$$

and

$$f_{A^-} = \frac{[A^-]}{[HA] + [A^-]} \qquad \text{Eqn. 5}$$

respectively. Substitution of Eqn. 3 into Eqn. 4 and of Eqn. 3 into Eqn. 5 leads, with simplification, to:

$$f_{HA} = \frac{[H^+]}{[H^+] + K_a} \qquad \text{Eqn. 6}$$

and

$$f_{A^-} = \frac{K_a}{[H^+] + K_a} \qquad \text{Eqn. 7}$$

respectively.

Employing Equations 4, 5, 6, and 7 and allowing the summation of the ionized and unionized drug concentrations to be represented by $[A_T]$, the original rate expression (Equation 1) becomes:

$$\text{Rate} = \left\{\frac{k_1[H^+] + k_2 K_a}{[H^+] + K_a}\right\} [A_T] \qquad \text{Eqn. 8}$$

or

$$\text{Rate} = -\frac{d}{dt}[A_T] = k_{obs}[A_T] \qquad \text{Eqn. 9}$$

where

$$k_{obs} = \frac{k_1[H^+] + k_2K_a}{[H^+] + K_a} \qquad \text{Eqn. 10}$$

Equation 9 represents the experimentally observed first-order loss of total anthralin and Equation 10 can be used to calculate the individual apparent rate constants for the unionized and ionized anthralin reactions. Values of log k_{obs} (Table 1) were linearly regressed against their corresponding pH values (for pH < pK_a) and expected k_{obs}, pH data pairs were taken from the fitted line (r^2 = 0.993). The pK_a of anthralin had been previously determined[8] to be 9.06 at similar experimental conditions (same solvent, temperature, and ionic strength). Solution for k_1 and k_2 by means of simultaneous equations should now yield stable estimates of these constants if the only influence of pH on the system is the ionization of anthralin. Calculated values of k_1 ranged from 0.0093

TABLE 1

Apparent First Order Rate Constants for Anthralin Decomposition at Various pH Values (μ = 0.5M)

pH	Observed k (min^{-1})	Log k_{obs}
7.74	0.0158	-1.800
7.95	0.0187	-1.728
8.30	0.0296	-1.529
8.61	0.0373	-1.428
8.78	0.0458	-1.339
9.44	0.0468	-1.330
9.68	0.0422	-1.375
10.02	0.0377	-1.423

min^{-1} to 0.0150 min^{-1} as pH was increased while calculated values of k_2 ranged from 0.149 min^{-1} to 0.104 min^{-1}. Since the variation observed in these calculated values is systematic it is possible that an additional reaction mechanism or reaction condition becomes significant at higher pH values. Presumably this mechanism or condition is also responsible for the unexpected decline in the pH-rate profile above the pK_a.

In the presence of excess oxygen, the following reactions might a priori be considered reasonable for the major routes of anthralin decomposition:

$$HA \xrightarrow{k_1} P \qquad (A)$$

$$A^- \xrightarrow{k_2} P \qquad (B)$$

$$A^- + OH^- \xrightarrow{k_3} P \qquad (C)$$

$$HA + H^+ \xrightarrow{k_4} P \qquad (D)$$

$$HA + OH^- \xrightarrow{k_5} P \qquad (E)$$

The observed pH-rate profile rules out reactions C and D. For reaction D to hold the observed rate constant should decrease with increasing pH in the pH region below the pK_a. If reaction C were to hold, k_{obs} should increase with pH in the pH region above the pK_a. Additionally, reaction C predicts a positive primary salt effect which was not observed. Reactions B and E are kinetically indistinguishable since both lead to rate laws which are interconvertible by substitution of the appropriate equilibrium expressions for the ionization of the reactant. Thus reactions A and B are presumed to be important in explaining the observed rate

phenomena. Both anthralin and the anthroxide ion appear to react with oxygen, as previously reported.[9] Since oxygen concentration was essentially constant in this study (large excess), it does not appear in the rate law (Equation 1). Both reactions are consistent with the data in that neither predicts an ionic strength effect.

Arrhenius Plot

The Arrhenius plot (Fig. 4) was constructed from the observed first-order rate constants (pH 10.02, μ=0.5 M) at four temperatures. The Arrhenius equation quantitatively describes the temperature dependence of a reaction. The activation energy was graphically evaluated according to the equation:

$$\ln k = \ln A - Ea/RT \qquad \text{Eqn. 11}$$

where A is the frequency factor and the remainder of the terms have their customary definitions. The rate constant for a reaction can also be expressed in terms of the enthalpy of activation and the entropy of activation as given by the equation:

$$k = \frac{RT}{N_o h} (e^{\Delta S^{\neq}/R})(e^{-\Delta H^{\neq}/RT}) \qquad \text{Eqn. 12}$$

where N_o is Avogadro's number, h is Planck's constant, $\Delta S^{\neq}$ is the entropy of activation, and $\Delta H^{\neq}$ is the enthalpy of activation. Division of both sides of the equation by T followed by logarithmic transformation yields:

$$\ln \frac{k}{T} = \ln(\frac{R}{N_o h}) + \frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{RT} \qquad \text{Eqn. 13}$$

A plot of ln(k/T) versus 1/T allows the enthalpy of activation to be calculated from the slope of the line and the entropy of activation to be calculated from the y-intercept. The thermodynamic

TABLE 2

Apparent First Order Rate Constants at Different Temperatures and Accompanying Extra-Thermodynamic Parameters (pH = 10.02, μ = 0.5 M)

Quantity	Result
T (°C)	Observed k (x 10^4, sec^{-1})
25	6.29
30	9.48
35	13.49
45	27.11
E_a	13.7 Kcal/mole
ln A	19.85 sec^{-1}
$\Delta H^{\neq}$	13.1 Kcal/mole
$\Delta G^{\neq}$	21.82 Kcal/mole
$\Delta S^{\neq}$	-29.3 e.u.

parameters calculated according to Equations 11 and 13 are listed in Table 2. Normally the midpoint of the data set is chosen for calculating these parameters. In this case, however, the temperatures employed included 25°C, the temperature of most general interest, and thus the derived parameters were calculated at this temperature.

The rather low activation energy observed for anthralin would be expected for a compound this reactive. The entropy of activation, which is of help in characterizing the transition state, was substantially negative. This finding is consistent with the bimolecular reactions proposed for anthralin.

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