Examination of Arrhenius Kinetics for an Antiarrhythmic Compound

Tugrul T. Kararli,[1,2] Thomas Catalano,[1] and Richard M. Bittman[1]

Received November 9, 1989; accepted July 17, 1990
KEY WORDS: nonlinear; Arrhenius; degradation; stability.

INTRODUCTION

During the preformulation stage of drug development, the stability of a drug molecule is investigated to find conditions for a stable product. The use of accelerated temperature stability studies using the Arrhenius relationship is routine for estimating the stability of a drug at room temperature.

Compound I (+trans-4[2-[bis[1-methylethyl]-4,4a,5,6,7,8 hexahydro-1-methyl-4-phenyl-3h-pyrido[1,2-clpyrimidin-3-one) is an antiarrhythmic compound under development. Since it is intended to be formulated as a parenteral product, its solution state stability was investigated in low temperature studies as a function of pH (1.0 to 9.0) and temperature (5 to 70°C). Further, in a high-temperature study (77, 80, and 95°C), the degradation mechanism of Compound I was determined at pH 4. Using the kinetic information generated from the high-temperature study, an Arrhenius plot for Compound I was constructed and the shelf life of the drug was estimated at 30°C.

MATERIALS AND METHODS

Chemicals. All Compound I lots were synthesized at Searle & Co., Skokie, IL. Their purities were estimated to be greater than 98%.

Degradation Products. A 100-mg/ml solution of Compound I was degraded in a pH 5.0 medium (initial pH was controlled by HCl) for 10 days at 100°C. The products of the reaction were separated by HPLC and characterized by NMR, mass, and UV spectroscopic techniques. The identified reaction products were then synthesized and purified to more than 99% purity. The chemical structures of these products are presented in Fig. 1.

Preparation of Stability Samples. The stability samples in the studies at pH 1.0 to 9.0 and temperatures from 5 to 70°C (low-temperature study) were prepared at a concentration of 0.5 mg/ml. Samples were packaged in 10-ml sealed glass ampoules. The fill volume was 5 ml. Acetate buffer (10 m*M*) was used to produce pH 4.0 and 5.0; 10 m*M* phosphate buffer was used to produce pH 6.0 and 7.0; and 10 m*M* sodium bicarbonate buffer was used to produce pH 9.0. The pH of samples at pH 1.0, 2.0, and 3.0 were adjusted by HCl. The ionic strength of the solutions was adjusted to 0.1 *M* by additions of NaCl. At each time point duplicate samples were submitted for analysis of the percentage of initial Compound I remaining.

The stability samples for a study at 77, 80, and 95°C (high-temperature study) were prepared at 0.5 mg/ml concentration in 10 m*M* acetate buffer. Samples were filled and packaged as in the low-temperature study. The ionic strength of the solutions was not adjusted further. Duplicate samples were analyzed at each time point. An additional sample was submitted at every other time point for analysis by TLC.

The stabilities of the reaction products, Compound II (α-[2-[bis(1-methylethyl)amino]ethyl]benzenacetic acid, monohydrochloride) and Compound III (α-[2-[bis(1-methylethyl)amino]ethyl]benzenacetamide), were determined at 95°C in the same acetate buffer solution employed above. Their initial concentrations were also 0.5 mg/ml.

Sample Analysis. In the high-temperature study the levels of Compound I and degradation products except diisopropylamine (DIPA) and Compound IV (2-piperidineacetamide, monohydrochloride) were analyzed by an HPLC method. The liquid chromatography was performed with a Waters 6000A solvent delivery system equipped with a Waters 710B (Wisp) autosampler and a Waters 481 UV detector. The column used was a Supelco LC18 DB, 5 μm, 25 cm × 4.6-mm i.d. The mobile phase consisted of a mixture of 10% acetonitrile and 90% triethylamine phosphate, pH 3.0 (v/v). The flow rate of the mobile phase was 2.0 ml/min at ambient column temperature. The injection volume was 20 μl. The detection of the column effluent was performed by UV absorption at 210 nm and peak area measurements were used for quantitation. Analysis of Compound IV and diisopropylamine (DIPA) was performed using quantitative TLC. In the TLC studies methylene chloride/ethanol/ammonium hydroxide (65/30/5, v/v/v) was used as the solvent and Woelm silica gel 60, 250 μm, with fluorescent indicater activated at 254 nm was used as the adsorbent. The visualization was achieved by using t-butyl hypochlorite/starch KI.

Samples from the low-temperature studies were analyzed for Compound I, utilizing the same HPLC conditions, except for the following: UV detection was at 254 nm; the mobile phase composition was a mixture of 8% acetonitrile and 92% triethlyamine phosphate (v/v), pH 3.0; and the flow rate was 1.5 ml/min.

Kinetic Models

In all the model fittings, rate constants and other kinetic parameters were obtained using the NLIN procedure of the Statistical Analysis System (SAS) (1). This procedure estimates parameters by nonlinear least squares and also calculates asymptotic standard errors of these estimates.

Low-Temperature Study. The stability results collected from the low-temperature study at pH values from 1.0 to 9.0 and temperatures from 5 to 70°C were analyzed with a first-order degradation model (non-Arrhenius model):

$$[\text{Compound I}] = [\text{Compound I}]_{o,T} \exp(-k_T t) \quad (1)$$

[1] Searle Research and Development, Pharmaceutical Development, 4901 Searle Parkway, Skokie, Illinois 60077.
[2] To whom correspondence should be addressed.

0724-8741/91/0100-0123$06.50/0

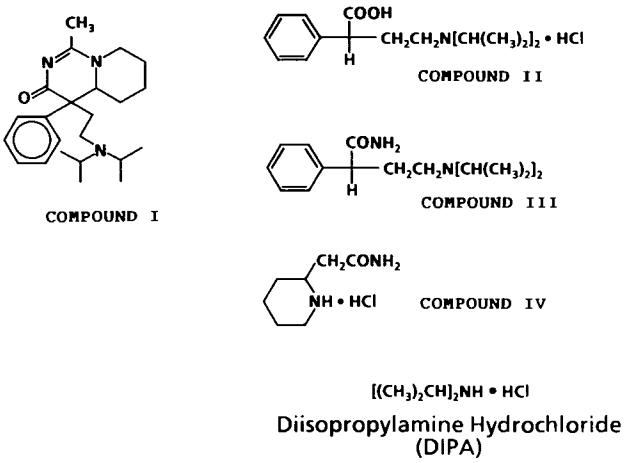
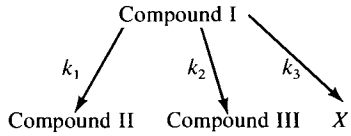


Fig. 1. The chemical structures of Compound I and its degradation products.

where [Compound I] is the concentration at time t and temperature T, [Compound I]$_{o,T}$ is the initial concentration of the samples stored at temperature T, k_T is the overall rate constant of degradation, and exp denotes the exponential function.

For a given pH, these models were fit simultaneously for all temperatures.

High-Temperature Study. Concentrations of both Compound I and its decomposition products, Compound II, Compound III, and unmeasured unknown(s) designated as Compound X, were modeled simultaneously using a three-parallel pathways mechanism (2).



Here k_1, k_2, and k_3 are the first-order rate constants for the indicated steps at a given temperature.

As in Eq. (1), the degradation of Compound I is first order, and additionally, Arrhenius temperature dependence is included in the modeling, in that the rate constants of degradation for each pathway are expressed as

$$k_{T,i} = k_{\text{ref},i} \exp\left[-E_i\left(\frac{1}{T} - \frac{1}{T_o}\right)\right] \quad (i = 1,2,3) \qquad (2)$$

where $k_{T,i}$ is the rate constant for pathway i at temperature T, E_i is the activation energy divided by the gas constant for pathway i, and $k_{\text{ref},i}$ is the rate constant for pathway i at reference temperature T_o.

In a three-parallel pathways mechanism, the overall rate constant of degradation for the parent compound at temperature T is the sum, k_T, of the rate constants of degradation for each pathway.

$$k_T = k_{T,1} + k_{T,2} + k_{T,3} \qquad (3)$$

The appearance of each product also follows first-order kinetics:

$$[\text{Product}] = \frac{k_{T,i}}{k_T}[\text{Compound I}]_{o,T}\{1 - \exp(-k_T t)\} \qquad (4)$$

where product is Compounds II, III, and X.

In model fittings, the concentration–time data at temperatures 77, 80, and 95°C for Compound I, Compound II, and Compound III were the input data. Note that for Compound X, the parameter $k_{\text{ref},3}$ was estimated by subtracting the sum of $k_{\text{ref},1} + k_{\text{ref},2}$ from k_T.

pH–Rate Constant Profile. The rate constants estimated without the Arrhenius assumption at 55 and 70°C were fit to the equation

$$k = k_o + k_m \frac{K_a}{K_a + [H^+]} \qquad (5)$$

where k is the rate constant at a given pH, k_o and k_m are the intrinsic rate constants of degradation for the protonated and unprotonated species of Compound I, and K_a is the dissociation constant of the relevant ionizable group.

RESULTS

The time courses of Compound I degradation in the low-temperature study at pH 4.0 and temperatures 5, 30, 55, and 70°C are shown in Fig. 2, along with predictions from the non-Arrhenius model [Eq. (1)]. In Fig. 3, the pH–rate constant profiles of Compound I are shown for the 55 and 70°C data. The corresponding pK_a values were estimated to be 4.9 ± 0.1 and 4.5 ± 0.1 for the 55 and 70°C data, respectively. From the potentiometric titration, the pK_a of Compound I was measured as 4.7 ± 0.3 at room temperature.

In Fig. 4, the time courses for the disappearance of Compound I, the appearance of Compound II and Compound III, and the mass balance at 95°C, measured in the high-temperature studies, are given (the data at 77 and 80°C are similar in shape to Fig. 4). In the same figure the predicted concentrations of the compounds based on the three-parallel pathways mechanism are also plotted. The residuals from the model fitting did not show large under- or overprediction.

The stabilities of Compound II and Compound III were examined at pH 4.0. After 8 days of storage at 95°C Compound II showed no degradation and Compound III degraded by approximately 1.1% to Compound II.

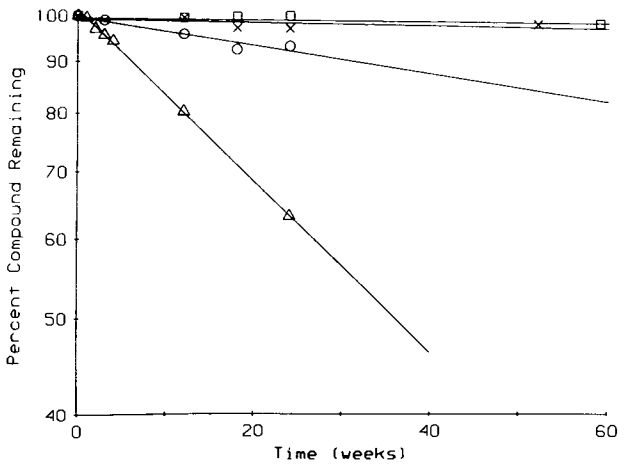


Fig. 2. The time courses of degradation of Compound I at pH 4.0 and temperatures of (□) 5°C, (x) 30°C, (0) 55°C, and (△) 70°C.

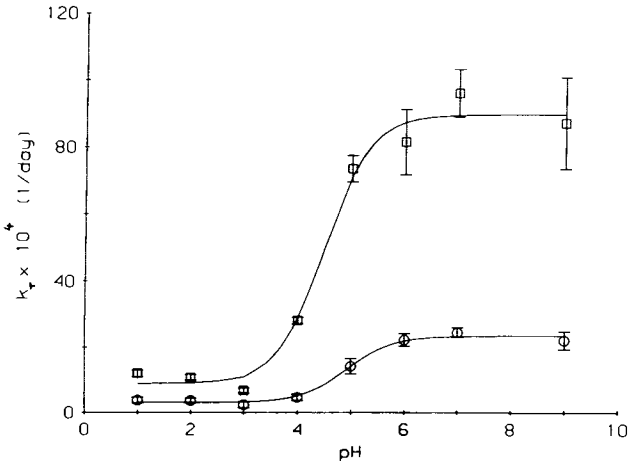


Fig. 3. The first-order rate constant of degradation of Compound I at 55°C (○) and 70°C (□) as a function of the pH of the medium. The bars indicate standard errors associated with the estimates.

The estimates of the first-order rate constants at the reference temperature, 77°C, and of E_a values for each pathway for the three-parallel pathways mechanism are presented in Table I.

Using estimates of the E_a values and the degradation rate constant for individual pathways in Eqs. (2) and (3), the overall degradation rate constant of Compound I, k_T, was estimated as a function of temperature. The logarithms of these k_T values are plotted in Fig. 5 as a function of the reciprocal of temperature.

DISCUSSION

In the low-temperature study it was not possible to obtain an accurate estimate of the shelf life of the compound at pH 4.0 (Fig. 5).

Further studies were conducted at 77, 80, and 95°C to determine the degradation mechanism of the compound at pH 4.0. The purpose of the studies was to build a kinetic

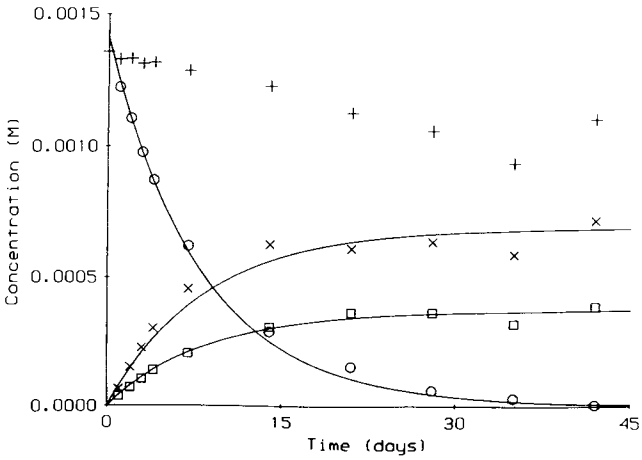


Fig. 4. The time courses of the disappearance of Compound I (0) and the appearance of Compound II (x) and Compound III (□) with the mass balance (+) (sum of assay values for Compound I, Compound II, and Compound III) at pH 4.0 and 95°C.

Table I. Activation Energies (E_a) and Rate Constants of Degradation at 77°C for Each Pathway in the Three-Parallel Pathways Mechanism

Rate constant	k (±SE) ($10^3 \times$ day^{-1})	E_a (±SE) (kcal/mol)
k_1 for Compound II	4.67 (±0.21)	35.68 (±0.88)
k_2 for Compound III	3.45 (±0.20)	31.61 (±1.20)
k_3 for unknown product, X	3.19 (±0.58)	32.33 (±3.61)

model and estimate the room temperature shelf life of the compound from the Arrhenius plot. In Fig. 4 the ratio of the concentrations of the two major degradation products, Compound II and Compound III, is approximately constant over time, suggesting a parallel-pathways degradation mechanism with at least two separate pathways. Further, the drop in mass balance as a function of storage time (Fig. 5) indicates the need for more than two pathways.

Two possibilities for additional pathways were considered. The first was that at least one of the two major degradation products degrades further. The degradation studies of Compound II and Compound III at pH 4.0 and 95°C showed negligible degradation. Thus, the first possibility was not pursued further. The second possibility was that there are three or more parallel pathways. This possibility was pursued by model fitting.

As shown in Fig. 4, a three-parallel pathways mechanism showed a reasonable fit to the data. Further, the presence of the identified (Fig. 1) and unidentified compounds (data not shown) suggested that there ought to be more than two major degradation pathways. The degradation product, diisopropylamine (DIPA), was shown not to be a by-product of either Compound II or Compound III. Also, the level of DIPA, 10% after 21 days, is consistent with the value of k_3

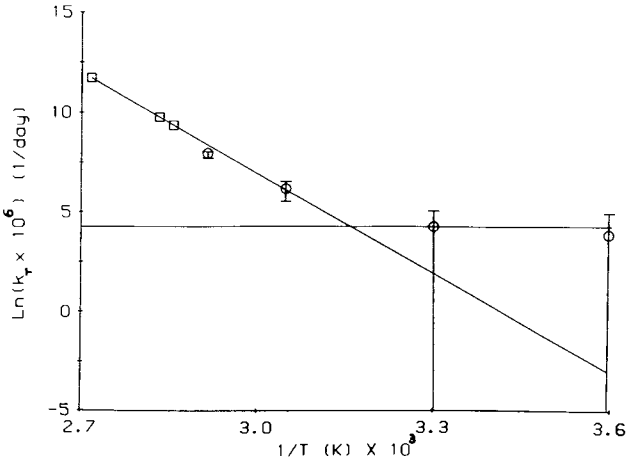


Fig. 5. The natural logarithm of the overall degradation rate constants of Compound I plotted as a function of the reciprocal of the temperature. (0) The results from the low-temperature study (pH 4.0). (□) The predicted values of k_T. The line represents the calculated values of k_T based on Eqs. (2) and (3). The horizontal line represents the value of the first-order rate constant of degradation that would result in 95% remaining in 2 years. The vertical bars represent the 95% confidence intervals.

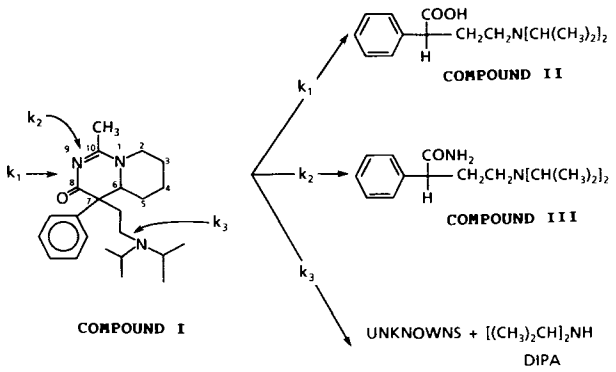


Fig. 6. The chemical degradation mechanisms of Compound I.

estimated from the three-parallel pathways mechanism (Table I). Therefore, this compound might be the result of at least another major degradation reaction.

A parallel-pathways mechanism with significantly different activation energy for each pathway can cause curvature in Arrhenius plots (3–5). In Fig. 5, almost no curvature is apparent in the Arrhenius plot for Compound I over a wide temperature range since the activation energies for the individual pathways were almost equal (Table I).

The overall degradation rate constant of Compound I at 55 and 70°C, estimated from the low-temperature study, lies close to the line calculated using the high-temperature study. The possibility of another reaction pathway with very low activation energy at temperatures below 55°C was ruled out since the analysis of some stability samples stored at temperatures below 55°C did not show any major degradation products other than Compounds II and III. Overall, these results argue that the use of the Arrhenius model based on the high-temperature data may be valid in the estimation of the room temperature shelf life of the compound. As shown in Fig. 5, the room-temperature shelf life of the compound in 2 years is much less than 5%.

A chemical degradation mechanism of Compound I based on the three-parallel pathways model is proposed in general terms in Fig. 6. A more detailed mechanism cannot be given here because of the lack of knowledge about the degradation intermediates. However, it is suggested that in order to produce Compound II and Compound III, the bonds at the 6–7, 8–9, and 9–10 positions of the molecule must be cleaved.

In conclusion, the results suggest that the Arrhenius plot for Compound I is practically linear and a pH 4.0 solution of Compound I should be stable as a parenteral product.

ACKNOWLEDGMENTS

The technical assistance of Mrs. L. Marsan and Mrs. V. Gupta in preparing the stability samples and of Mr. R. Patel in analyzing the stability samples is acknowledged. Valuable discussions with Drs. H. Gaud, M. Beno, and J. Dygos are acknowledged.

REFERENCES

1. SAS Institute Inc. *SAS User's Guide: Statistics, Version 5 Edition,* SAS Institute Inc., Cary, NC, 1985.
2. K. Kowalski, M. Beno, C. Bergstrom, and H. Gaud. *Drug Dev. Ind. Pharm.* 13:2823–2838 (1987).
3. S. P. Eriksen and H. Stelmach. *J. Pharm. Sci.* 54:1029–1034 (1965).
4. J. T. Carstensen. *Theory of Pharmaceutical Systems,* Academic Press, New York and London, 1972.
5. P. Salomaa. *Acta Chem. Scand.* 11:239–246 (1957).