Solid-State Stability Studies of 13-*cis*-Retinoic Acid and All-*trans*-Retinoic Acid Using Microcalorimetry and HPLC Analysis

Xinyi Tan,[1] Noel Meltzer,[2] and Siegfried Lindenbaum[1,3]

Received July 16, 1991; accepted February 26, 1992

The solid-state stabilities of 13-*cis*-retinoic acid and all-*trans*-retinoic acid in the presence and absence of oxygen were investigated. The samples were first evaluated using microcalorimetry. The rate laws of different samples under different conditions were deduced from the shapes of the heat flow curves, and the activation energies of the reactions were determined from Arrhenius plots. Under an air atmosphere, the decomposition of 13-*cis*-retinoic acid is an autocatalytic reaction, while all-*trans*-retinoic acid undergoes a zero-order process. The degradation of the two compounds at a selected elevated temperature was also determined utilizing HPLC analysis. This technique confirmed the decomposition kinetics. Hence, their half-lives and shelf lives at room temperature could be calculated. Under a nitrogen atmosphere, the microcalorimetric experiment showed a first-order phenomenon for both samples, but HPLC analysis showed no degradation, suggesting that the two samples, in the absence of oxygen, undergo only a physical change.

KEY WORDS: solid-state stability; all-*trans*-retinoic acid; 13-*cis*-retinoic acid; microcalorimetry; HPLC analysis.

INTRODUCTION

Retinoic acids are currently used in dermatological formulations. Naturally occurring all-*trans*-retinoic acid (Trans) has been used for treatment of acne in several topical dosage forms. 13-*cis*-Retinoic acid (Cis) is a synthetic retinoid with fewer adverse effects and enhanced biological activity compared to Trans. Cis is also commercially available as gelatin capsules containing a suspension of the drug for oral administration. Retinoids are unstable compounds, being sensitive to oxygen, heat, and light. Hence, their stability is of pharmaceutical interest, but their decomposition is expected to be complex on the basis of their molecular structures. In this work, we studied the decomposition kinetics in the presence and absence of oxygen.

Microcalorimetry is useful for testing drug stability (1–4). With this method, one can follow the time course of a chemical or physical change from a single sample, determine the rate law of the reaction, and obtain its activation energy. However, calorimetry is a technique based on macrophenomena; it does not directly indicate the mechanism of the reaction. Other methods, such as HPLC, are also needed for the characterization of decomposition kinetics.

THEORY

Solid-state decomposition kinetics are complex. Various empirical formulas have been used to describe experimental data (5). A general equation has been put forward by Ng which includes the principles of most of the other formulas (6):

$$d\alpha/dt = k\alpha^{1-x}(1 - \alpha)^{1-y} \quad (1)$$

where α is the fraction of the reaction which has occurred to time t, $\alpha = 0$ at $t = 0$, and $\alpha = 1$ at $t = \infty$; k is the rate constant; and x and y are constants characteristic of the reaction rate law. When $x = y = 1$, the reaction is zero order; if $x = 1$ and $y = 0$, the reaction is first order; in many cases, x and y are fractions and the reaction is autocatalytic.

Equation (1) may be expressed in logarithmic form:

$$\ln(d\alpha/dt) = \ln k + (1 - y)[a \ln \alpha + \ln(1 - \alpha)] \quad (2)$$

in which a is a constant defined as the ratio of $1 - x$ to $1 - y$ and its value can be calculated from α_m:

$$a = (1 - x)/(1 - y) = \alpha_m/(1 - \alpha_m) \quad (3)$$

where α_m is the value of α when the rate of the reaction is maximum. Thus a plot of $\ln(d\alpha/dt)$ vs $[a \ln \alpha + \ln(1 - \alpha)]$ allows calculation of the constants k, x, and y.

From Eq. (1), another equation can be derived:

$$kt_\alpha = k't'_\alpha = \text{constant} = B \quad (4)$$

Here t_α or t_α' is the time corresponding to α and may be expressed as half-life or shelf life. To calculate half-life, $B = \ln 2$ for a first-order reaction and $B = 0.5$ for a zero-order reaction; in the case of an autocatalytic reaction, B is an unspecified constant.

Measurement of degradation is usually conducted at an elevated temperature, where k and t_α are measurable by direct chemical analysis. The most relevant information for pharmaceutical products is the shelf life (time for 10% decomposition) or half-life at room temperature, and to calculate this, the rate constant at room temperature, k', is needed. The k' may be calculated from k and the activation energy. In this work, activation energies are determined by microcalorimetry.

The heat of the reaction, h, is related to α by the following equation:

$$h = -\Delta H\beta D_0\alpha \quad (5)$$

Here ΔH is the enthalpy of the reaction, D_0 is the amount of sample, and β is the fraction of the drug which will ultimately react. The quantity directly measured by microcalorimetry is the rate of heat production q,

$$q = dh/dt = -\Delta H\beta D_0 \, d\alpha/dt \quad (6)$$

Substituting for Eq. (1) yields the following equation, which describes the solid-state decomposition kinetics in terms of the calorimetrically determined quantity, q/D_0:

$$q/D_0 = -\Delta H\beta k \alpha^{1-x}(1 - \alpha)^{1-y} \quad (7)$$

[1] Department of Pharmaceutical Chemistry, Malott Hall, The University of Kansas, Lawrence, Kansas 66045.
[2] Roche Dermatologics, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, New Jersey 07110.
[3] To whom correspondence should be addressed.

0724-8741/92/0900-1203$06.50/0

Because α is a function of time, q/D_0 must be a function of time. This function is the reaction curve that is to be measured by microcalorimetry. Examples of standard reaction curves characteristic of different reaction rate laws are given by Hansen and co-workers (2). After a reaction curve is measured, the rate law of the reaction may be judged by comparing the shape of the reaction curve with those of the model curves. Values of the activation energy can be determined by measuring reaction curves as a function of temperature.

MATERIALS AND METHODS

Materials

The Cis (Lot No. 63875.909045) and Trans (Lot No. 904008) samples were supplied by Hoffmann-La Roche, Inc. (Nutley, NJ). They were stored at −70°C in amberized bottles under nitrogen atmosphere. All organic solvents were of HPLC grade. Purified water was prepared by a Millipore system.

Microcalorimetry

An LKB 2277 thermal activity monitor (TAM) was used in this study (1). For this measurement, it is important to ensure that the heat being measured is indeed due only to the reaction of interest. Previous studies have shown that both stainless-steel and rubber-stoppered glass vials can produce heat signals independent of the sample. The steel vessel is subject to leakage and is sensitive to the reactant or products due to the corrosion of the vessel material; in the glass vial, heat measurements are always accompanied by stress relaxation in the rubber disk (2–4). To avoid the interferences from the sample cell, a combination of a glass ampoule and a steel vessel was used in this study. The glass ampoule (Fisher Catalog Number 01-215A or 01-215B) used as the sample container fitted closely into the interior of the steel vessel. The steel vessel was used only as a holder and was therefore not sealed. The glass ampoules were sealed or left open to the air, depending upon the experimental design. For the experiment under air atmosphere, the ampoules were left open to the atmosphere during the entire measurement, as the samples were not hygroscopic. This maintained a constant oxygen tension and avoided the problem of oxygen depletion (2). For the study under a nitrogen atmosphere, the ampoule was flushed with nitrogen before loading the sample. The ampoule neck was prenarrowed to allow a rapid flame sealing after loading the sample. The ampoule top was then cooled with a stream of cold air to prevent heat conductance to the sample. The sample size varied depending on the magnitude of the heat effect. For the study in the presence of oxygen, between 0.02 and 0.2 g of Cis and between 0.06 and 0.5 g of Trans were used; for the study in the absence of oxygen, about 0.3-g samples of both Cis and Trans were used. The reference cell was always an empty steel vessel. In most cases, measurements were run in duplicate.

HPLC Analysis

The separation of the Cis and Trans isomers has been the subject of several investigations (7,9–11). Even though these two isomers were separately analyzed in this study, their separation was essential in order to detect their isomerization. In this study, a Waters Associate HPLC apparatus (including a Model 6000A solvent delivery system, a U6K injector, and a Model 440 UV detector at 280 nm) was used to determine degradation. A C_{18} reversed-phase column, Hypersil ODS (4.6 mm × 15 cm, 10 μm), and a guard column were used. The mobile phase consisted of 95% (v/v) acetonitrile and 5% (v/v) of a 1% (w/v) aqueous ammonium acetate solution delivered at a flow rate of 1.1 ml/min. The degradation studies were carried out using samples of about 10 mg of Cis or Trans, weighed in small glass tubes and placed in a 70°C oven. The tubes were opened to air. At specific intervals, samples were withdrawn and stored in a refrigerator. On the day of HPLC analysis, the samples were washed into 25-ml amberized volumetric flasks with the mobile phase. In the chromatogram resulting from this HPLC system for a mixture of Cis and Trans samples, Cis elutes at a retention time of 7.2 min and Trans at 11.0 min. The resolution for these two isomers is better than 3. In the chromatograms of samples subjected to thermal exposure, the decomposition products elute early as a broad band; the peaks of both Cis and Trans components are well separated from those of the decomposition products and are used for quantitative determination. Quantitation was accomplished using the external standard method. A computer was connected to the detector for data acquisition and peak area and retention time calculation. Figure 1 shows typical chromatograms for a mixture of fresh Cis and Trans, a degraded Cis sample, and a degraded Trans sample. No isomerization of the two retinoic acids was found in the solid state. All operations were performed in a darkened room illuminated with yellow light.

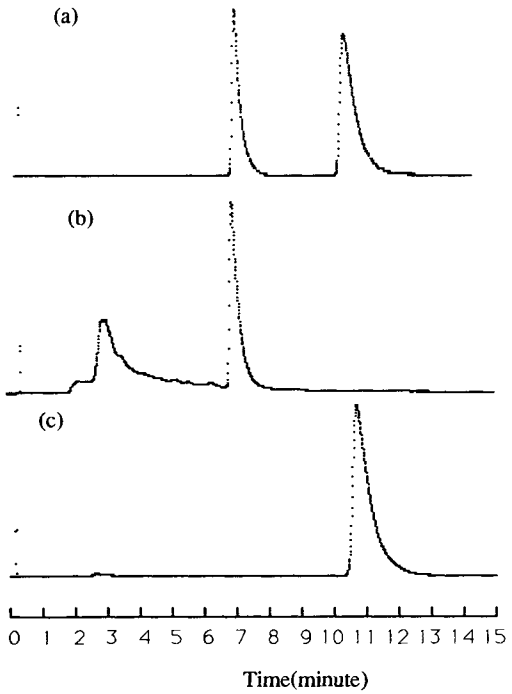

Fig. 1. Typical HPLC chromatograms of a mixture of fresh Cis and Trans samples (a), a degraded Cis sample (b), and a degraded Trans sample (c).

RESULTS AND DISCUSSION

A DSC Check for the Samples

Differential Scanning Calorimetry (DSC) thermograms of the Cis and Trans samples were measured in order to check sample purities. The thermogram for Trans has a small peak in addition to the melting peak, which is due to the crystalline transition from a monoclinic to a triclinic structure. The thermogram for Cis has only one melting peak; Cis does not have a crystalline transition. This is in agreement with literature observations (12). But the crystalline transition of Trans occurs at 150°C rather than at 120°C as reported previously (12). From the DSC analysis, the melting points were found to be 175.6°C for Cis and 182.4°C for Trans, consistent with reported values. The sample purities were estimated from the melting peaks to be 98.2% for Cis and 98.8% for Trans.

Cis Decomposition Kinetics Under an Air Atmosphere

Microcalorimetric Study. The reactions of Cis samples under an air atmosphere were measured with the TAM at different temperatures. The reaction curves are shown in Fig. 2. Each reaction curve first increases and after reaching a maximum decreases. The shape of the reaction curves is characteristic of an autocatalytic reaction at all temperatures studied. Only the rate of heat evolution at the maxima is needed for the purpose of evaluating the activation energy. Each maximum point consists of two parameters: the ordinate Q_m and the abscissa t_m. The activation energy can be obtained from the variation of each of these parameters with temperature. Applying Eq. (7) to the these maxima,

$$Q_m = -\Delta H \beta \alpha_m^{1-x} (1 - \alpha_m)^{1-y} = A''k = A'''e^{-E/RT} \quad (8)$$

$$\ln Q_m = \ln A''' - E/RT \quad (9)$$

where T is the absolute temperature. If A'' is a constant, a plot of $\ln Q_m$ vs $1/T$ will be a straight line, with a slope of $-E/R$. The linear regression of the Q_m data in Fig. 2 according to Eq. (9) yields a straight line: $\ln Q_m = 17.6 - 5.35 \times 10^3/T$ ($r^2 = 0.983$, $s_b = 4.08 \times 10^2$, s_b is the standard error of the slope). The activation energy calculated from the slope is 10.6 ± 0.8 kcal/mol.

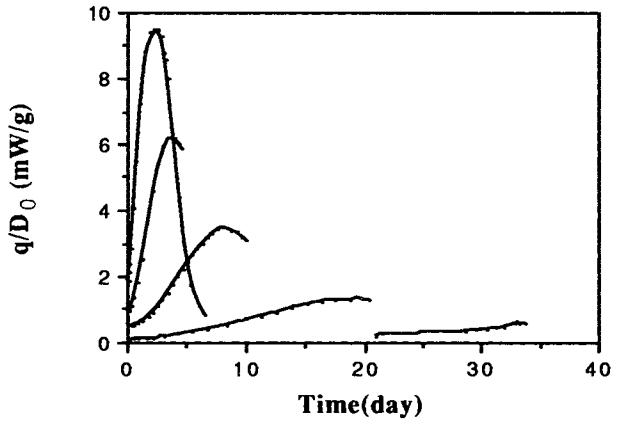

Fig. 2. Reaction curves of solid 13-*cis*-retinoic acid in the presence of oxygen at different temperatures measured by microcalorimetry. They are at 80, 65, 50, 37, and 25°C, with the maximum points from left to right.

For an autocatalytic reaction, the activation energy can be evaluated from t_m data as well. Applying Eq. (4) to the maximum points,

$$kt_m = B \quad (10)$$

$$t_m = B/k = (B/A)e^{E/RT} \quad (11)$$

$$\ln t_m = \ln (B/A) + E/RT \quad (12)$$

If B/A is a constant, a plot of $\ln t_m$ versus $1/T$ will be a straight line, with a slope of E/R. The five t_m data in Fig. 2 according to Eq. (12) fall on the straight line $\ln t_m = -11.1 + 5.29 \times 10^3/T$ ($r^2 = 0.996$, $s_b = 1.87 \times 10^2$). The t_m data are also in good agreement with the theory. The activation energy calculated from the slope is 10.5 ± 0.3 kcal/mol, in agreement with that obtained from Q_m data.

The advantage of using Eq. (12) for the calculation of activation energy lies in the use of time to reach a maximum rather than the value of Q_m itself. Time measurement is usually easier and more accurate than other quantities; accordingly, the correlation coefficient and slope standard error obtained from t_m values are better than those from Q_m values. In addition, if values of t_m at two temperatures are known, the value at any other temperature can be estimated. Accurate determination can then be accomplished by preheating the sample in an oven for the requisite length of time and using the instrument for only the time needed to ascertain the exact value of t_m. In this way, the time necessary for the use of TAM is minimized.

This example demonstrates the utility of the microcalorimetric method for the stability studies of an autocatalytic reaction. At each temperature, only one sample is needed to produce one reaction curve. The activation energy can be evaluated from the reaction curves at different temperatures. The same shape of the reaction curve at lower temperatures as those at higher temperatures suggests the same kinetics. Whereas a rate constant cannot be determined calorimetrically, a knowledge of t_m is useful for planning a rate study using conventional techniques. Determination of degradation in the vicinity of t_m should be emphasized in order to obtain a more reliable rate constant.

HPLC Analysis. The degradation of Cis under an air atmosphere was performed at 70°C. The degradation curve of γ, the fraction of the sample decomposed, versus time has a sigmoidal shape (Fig. 3) and supports the conclusion from the microcalorimetric experiment that the degradation of Cis in the presence of oxygen is autocatalytic. The samples after thermal exposure were found to increase in weight by 10%, which may be explained by the addition of oxygen to the compounds in the degradation process and suggests that this reaction is an autocatalytic oxidation. The plateau in the curve represents the fraction, β, equal to 0.805 to which the Cis sample ultimately decomposed. This suggests that 20% of the initial amount of the sample is not subject to decomposition.

The kinetic equation may be established by the degradation curve. The γ data divided by β are α values required for Eq. (1). The α data may be plotted against time in order to derive $d\alpha/dt$ data (Table I). The α_m is found to be 0.420. The value of the constant a is 0.724 using Eq. (3).

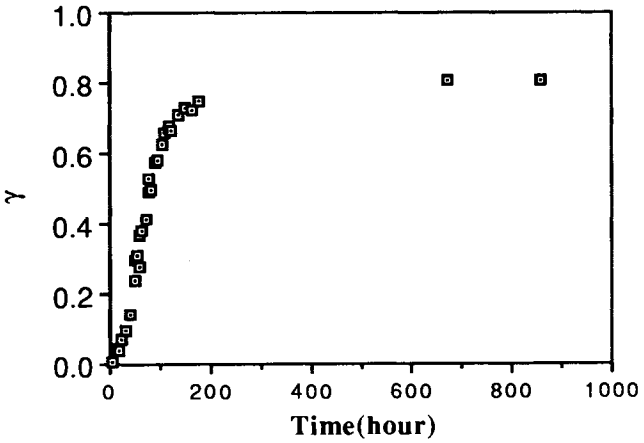

Fig. 3. The degradation fraction of 13-*cis*-retinoic acid in the presence of oxygen at 70°C determined with HPLC.

The following equation may be derived from Eq. (2): $\ln(d\alpha/dt) = -2.225 + 1.85[a \ln\alpha + \ln(1-\alpha)]$ ($r^2 = 0.984$, $s_b = 0.060$, $s_a = 0.093$, s_a is the standard error of the intercept). From the intercept, the rate constant is calculated to be 0.108 ± 0.010/hr; from the slope, $1 - y = 1.85 \pm 0.06$, and $1 - x$ is calculated to be 1.34. The solid-state decomposition kinetics for Cis in the presence of oxygen can then be described by the following equation:

$$d\alpha/dt = k\,\alpha^{1.34}(1-\alpha)^{1.85}$$

Calculated values of $d\alpha/dt$ are listed in Table I and are in good agreement with the experimental values.

Since the rate constant at 70°C and the activation energy are known, the rate constant at any other temperature can be readily calculated. The rate constant at 25°C is 0.0103/hr, and the half-life for the degradation of Cis under an air atmosphere can be calculated by Eq. (4). At the half-life, $\gamma = 0.5$ and $\alpha = 0.62$, from the α – t curve, $t_{0.62}^{70°C} = 78$ hr, then $t_{0.62}^{25°C} = 818$ hr.

The time for 10% degradation to occur can be calculated as follows: $\gamma = 0.1$, $\alpha = 0.12$, from the α – t curve, $t_{0.12}^{70°C} = 31$ hr, then $t_{0.12}^{25°C} = 325$ hr.

The enthalpy of this reaction, ΔH, is calculated to be −193 kcal/mol from the maximum point data at 70°C using Eq. (6). This value suggests that the mechanism of degradation involves the formation of a double bond. However, as shown in Fig. 1, the actual decomposition process is not well understood because the decomposition results in a large number of products. The identification of these products and a detailed determination of the reaction pathways were not attempted in this study.

Guillory and Higuchi studied the stabilities of various vitamin A derivatives and found a relationship between the stabilities and the melting points of the compounds (13). The straight line of $\ln t_m$ versus $1/T$ obtained here extends to approximately zero at the melting point of the Cis substance. This hints at a relationship between the melting point and t_m. The fact that the samples became lumpy after thermal exposure suggests that the sample liquefies first and degradation then occurs in the liquid phase (14).

Table I. The $d\alpha/dt$ Data Derived from the α–t Curve

α	*t* (hr)	$d\alpha/dt$	$d\alpha/dt^{cal}$
0.050	16.3	0.00210	0.00177
0.100	26.0	0.00475	0.00406
0.150	34.2	0.00640	0.00629
0.200	42.0	0.00740	0.00827
0.250	47.0	0.00900	0.00990
0.300	52.0	0.0115	0.0111
0.350	56.0	0.0122	0.0119
0.400	60.0	0.0131	0.0123
0.450	64.0	0.0129	0.0122
0.500	67.5	0.0124	0.0118
0.550	71.5	0.0115	0.0111
0.600	76.0	0.00980	0.0100
0.650	82.0	0.00790	0.00870
0.700	89.5	0.00630	0.00722
0.750	97.0	0.00575	0.00565
0.800	107.0	0.00400	0.00408
0.850	125.5	0.00250	0.00260
0.900	152.0	0.00115	0.00132

Trans Decomposition Kinetics Under an Air Atmosphere

Microcalorimetric Study. Trans samples under an air atmosphere were measured with TAM at different temperatures. The reaction curves are shown in Fig. 4. Some of the measurements were extended to several days; the maximum time shown here is 40 hr. The shape of the reaction curve is different from that in Fig. 2. Each reaction curve reaches a plateau after about 20 hr. Based on the shape of the reaction curves after 20 hr, the reaction of Trans in the presence of oxygen follows a zero-order rate law. The value of the plateau is denoted Q_p. For a zero-order reaction, $x = y = 1$, Eq. (7) takes the following form:

$$Q_p = -\Delta H\beta\, k = A' e^{-E/RT} \quad (13)$$

$$\ln Q_p = \ln A' - E/RT \quad (14)$$

If A' is a constant, a plot of $\ln Q_p$ vs $1/T$ will be a straight line, with a slope equal to $-E/R$. The Q_p data in Fig. 4 according to Eq. (14) fall on a straight line: $\ln Q_p = 18.5 - 7.02 \times 10^3/T$ ($r^2 = 0.992$, $s_b = 3.64 \times 10^2$). The data are in good agree-

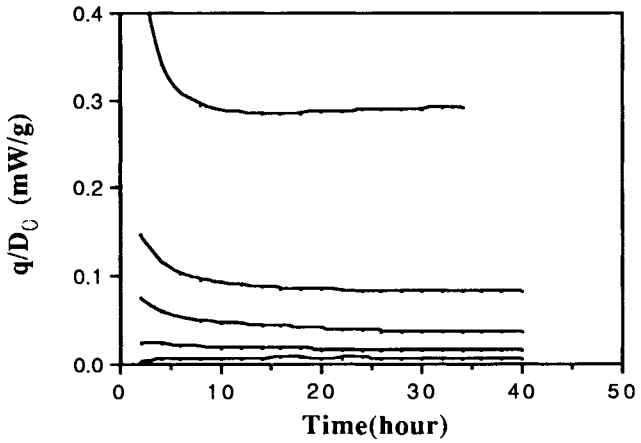

Fig. 4. Reaction curves of solid all-*trans*-retinoic acid in the presence of oxygen at different temperatures measured by microcalorimetry. They are at 80, 65, 50, 37, and 25°C from top to bottom.

ment with the theory. The activation energy is 13.9 ± 0.7 kcal/mol.

HPLC Analysis. A degradation determination for Trans under an air atmosphere was also performed at 70°C (Fig. 5). Compared to Cis, the degradation of Trans was slow, as expected from the microcalorimetric data, which also showed a smaller heat effect for Trans than for Cis. The degradation experiment extended to 40 days, and only 10% loss was measured. At each time point, four samples were prepared and analyzed. The standard deviations are also shown in Fig. 5.

The degradation behavior throughout the experimental range can be treated by a zero-order model, in agreement with the conclusion drawn from the microcalorimetric experiment. A straight line is obtained and its slope is the rate constant for Trans degradation under an air atmosphere at 70°C, $k_{70°C}$ = 0.00283/day. If β is assumed to be 1, the decomposition kinetics for Trans under air atmosphere can be expressed as

$$d\alpha/dt = k$$

Since the activation energy has been determined by microcalorimetry, the rate constants at other temperatures can be calculated. The rate constant at 25°C is 1.30×10^{-4}/day, corresponding to a shelf life of 769 days.

Stability Study Under a Nitrogen Atmosphere

The stabilities of both Cis and Trans samples under nitrogen atmosphere were measured on TAM at several temperatures. Under the conditions studied, both compounds exhibit the same behavior. However, the reaction curves are quite different from that of either Cis or Trans under air atmosphere. Figure 6 shows the reaction curves for Cis and Trans at 44°C. The reaction curves at other temperatures have the same shape. The shape is similar to that of a first-order reaction. For a first-order reaction, $x = 1$ and $y = 0$, and Eq. (7) reduces to Eq. (15):

$$q/D_0 = -\Delta H\beta\, k(1 - \alpha) = A'e^{-kt} \quad (15)$$

$$\ln (q/D_0) = \ln A' - kt \quad (16)$$

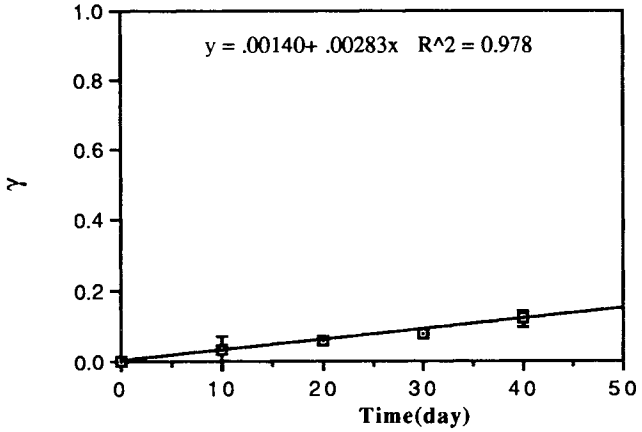

Fig. 5. The degradation fraction of all-*trans*-retinoic acid in the presence of oxygen at 70°C determined with HPLC and its linear regression.

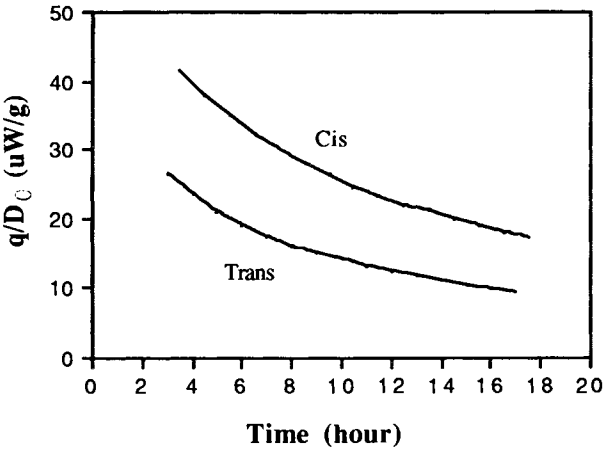

Fig. 6. Reaction curves of solid 13-*cis*-retinoic acid and all-*trans*-retinoic acid in the absence of oxygen at 44°C measured by microcalorimetry.

The slope of a plot of $\ln(q/D_0)$ vs time is the rate constant. A first-order reaction is the only case in which the rate constant can be measured directly by microcalorimetry. Besides this straight line, a plot of logarithm of the rate constants vs $1/T$ is also a straight line:

$$\ln k = \ln A - E/RT \quad (17)$$

Its slope yields the activation energy. Experimentally, the semilogarithms of the reaction curves are straight lines. The rate constants are the slopes of the straight lines. Table II lists the measured rate constants for the reaction of Cis and Trans under a nitrogen atmosphere at different temperatures. The plots of these rate constants listed in Table II according to Eq. (17) against $1/T$ are good straight lines. The activation energies of the reactions under a nitrogen atmosphere are 13.5 ± 0.10 kcal/mol for Cis and 9.06 ± 0.5 kcal/mol for Trans. The rate constants at 25°C are then calculated to be 0.0150/hr for Cis and 0.0269/hr for Trans, and the half-lives for Cis and Trans are 46 and 26 hr, respectively.

Comparing half-lives under a nitrogen atmosphere and those under an air atmosphere, it appears that both Cis and Trans undergo much faster reactions under a nitrogen atmosphere. The reaction curve of a long-term experiment at 50°C did descend to the baseline after about 3 days and remain at baseline afterward. However, it does not seem probable that retinoic acids decompose faster in the absence of oxygen than in the presence of oxygen. Accordingly, the samples which had been subjected to thermal stress in the calorimeter under nitrogen atmosphere were analyzed by HPLC. No significant degradation was observed. In addition, the reactions under nitrogen atmosphere have a much smaller heat

Table II. Rate Constants of the Reaction of Retinoic Acids in the Absence of Oxygen

T	13-*cis*-Retinoic acid	All-*trans*-retionic acid
50°C	0.0825/hr	0.0875/hr
44°C	0.0612/hr	0.0705/hr
37°C	0.0328/hr	0.0507/hr
31°C	0.0235/hr	0.0364/hr

Table III. Stability Characteristics of the Solid Retinoic Acids

	Air atmosphere		Nitrogen atmosphere	
	Cis	Trans	Cis	Trans
Nature of reaction	Chemical decomposition		Physical change	
Rate law	Autocatalytic	Zero-order	First-order	First-order
Activation energy (kcal/mol)	10.5	13.9	13.5	9.06
Rate constant at 25°C (/day)	0.247	0.000130	0.369	0.646
Shelf life (days)	13.5	769	—	—
Half-life (days)	34.1	3846	—	—

effect than the reactions in the presence of oxygen. (The ordinate in Fig. 6 is in units of microwatts per gram, while those in Figs. 1 and 3 are milliwatts per gram). The physical appearance of a sample before and after thermal stress was determined using electron microscopy. The surface of the sample changed from smooth to rough after thermal stress. These observations suggest a premelting process for both Cis and Trans under a nitrogen atmosphere. This physical change also occurs in the presence of air and probably precedes the degradation reactions. It is the first process before their degradations. This would account for the descending phenomenon on the initial portions (before the first 20 hr) of the reaction curves for Trans (Fig. 4) as well as Cis (barely seen in Fig. 2).

ACKNOWLEDGMENTS

Support for this work by Hoffmann-La Roche Inc. and Marion Merrell Dow is gratefully acknowledged.

REFERENCES

1. R. Oliyai and S. Lindenbaum. Stability testing of pharmaceuticals by isothermal heat conduction calorimetry: Ampicillin in aqueous solution. *Int. J. Pharm.* 73:33–36 (1991).
2. L. D. Hansen, E. A. Lewis, D. J. Eatough, R. G. Bergstrom, and D. D. Johnson. Kinetics of drug decomposition by heat conduction calorimetry. *Pharm. Res.* 6:20–27 (1989).
3. M. J. Pikal and K. M. Dellerman. Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25°C: Cephalosporins in the solid and aqueous solutions states. *Int. J. Pharm.* 50:233–252 (1989).
4. M. Angberg and C. Nystrom. Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. *Acta Pharm. Suec.* 25:307–320 (1988).
5. S. R. Byrn. *Solid-State Chemistry of Drugs,* Academic Press, New York, 1982. pp. 59–74.
6. W.-L. Ng. Thermal decomposition in the solid state. *Aust. J. Chem.* 28:1169–1178 (1975).
7. C. Eckhoff and H. Nau. Identification and quantitation of all-*trans*- and 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid in human plasma. *J. Lipid. Res.* 31:1445–1454 (1990).
8. D. J. Elbaum. Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. *J. Am. Acad. Dermatol.* 19:486–491 (1988).
9. Y. M. Peng, M. J. Xu, and D. S. Albert. Analysis and stability of retinol in plasma. *J. Natl. Cancer Inst.* 78:95–99 (1987).
10. C. A. Frolik, T. E. Tavela, and M. B. Spor. Separation of the natural retinoids by high-pressure liquid chromatography. *J. Lipid Res.* 19:32–37 (1987).
11. A. P. De Leenheer, W. E. Lambert, and M. G. M. De Ruyter. *Modern Chromatographic Analysis of the Vitamins,* Marcel Dekker, New York, 1985.
12. M. I. Dawson, P. D. Hobbs, R. Chan, K. Derdzinski, C. T. Helms, W. Chao, E. Meierhenry, and L. J. Schiff. Conformational restrictions of the retinoid skeleton. In *Ciba Foundation Symposium 113, Retinoids, Differentiation and Disease,* Pitman, London, 1985, pp. 6–28.
13. J. K. Guillory and T. Higuchi. Solid state stability of some crystalline vitamin A compounds. *J. Pharm. Sci.* 51:100–105 (1962).
14. K. A. Connors, G. L. Amidon, and V. J. Stella. *Chemical Stability of Pharmaceuticals,* John Wiley & Sons, New York, 1986, pp. 115–134.