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Report

Influence of Temperature and Hydrophobic Group-Associated Icebergs on the Activation Energy of Drug Decomposition and Its Implication in Drug Shelf-Life Prediction

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The E_a values of aspirin hydrolysis, as a result of hydronium-ion catalysis, intramolecular-nucleophilic catalysis, and hydroxyl-ion catalysis, were significantly different from each other when determined in the 30–40, 45–55, and 60–70°C ranges. The different E_a values were attributed to differences in both ΔH^* and ΔS^*, which could be accounted for by the various activated complexes formed in the hydrolysis of aspirin for each mechanism and the disruptive effect of temperature on the iceberg structures of water present around the phenyl group and the methyl group of aspirin at 42 and 58°C, respectively. A linear relationship observed between the calculated "differential" enthalpy and entropy values, with a slope (compensation temperature) value of about 307° K, supported a role for icebergs associated with hydrophobic groups in the formation of the activated complexes. This study illustrates that the predicted shelf life of a drug at room temperature could be erroneous if estimated from a single E_a value which is calculated from the decomposition rate constants determined at widely spaced temperatures in the range of 10–70°C, using the Arrhenius relationship.

KEY WORDS: aspirin hydrolysis; temperature-dependent activation energies; thermodynamic activation parameters; hydrophobic group-associated icebergs; temperature-sensitive icebergs; compensation temperature.

INTRODUCTION

Rate constants of drug decomposition are predicted on the basis of the energy of activation of their decomposition reactions calculated from the Arrhenius relationship. This energy is determined from the slope of the semilogarithmic plot of the rate constant of decomposition versus the reciprocal of absolute temperature, where the rate constants are generally determined at only a few widely spaced temperatures in the 20–70°C range. An apparent linearity of the Arrhenius plot permits the assumption that the energy of activation of the chemical reaction is relatively constant over the temperature range studied. However, iceberg structures of water are formed around the aromatic and aliphatic hydrophobic groups of compounds in aqueous solution, and such structures are more intense around the aliphatic groups than around the aromatic groups. Further, as predicted by Nemethy and Scheraga (1–3) on the basis of a physical model, the icebergs around the aromatic and aliphatic groups are disrupted at 42 and 58°C, respectively, with positive enthalpy and entropy changes. This Nemethy–Scheraga prediction was indirectly verified by Nagwekar and co-workers (4,5). Since both the release of water molecules from reactants in the formation of an activated complex and the restructuring of solvating water molecules around the activated complex contribute to the overall enthalpy and entropy of activation of reactions, the enthalpies and entropies of organic compounds in aqueous phase can be different from each other at temperatures below 42°C, in the 42–58°C range, and above 58°C. This deviation is particularly expected if the hydrophobic groups of a compound are involved in the formation of an activated complex. Therefore, the reaction rate constants should be determined at small temperature intervals over a certain range (e.g., 30–70°C).

We investigated the kinetics of hydrolysis of acetylsalicylic acid (aspirin) in the temperature ranges of 30–40, 45–55, and 60–70°C, by using small temperature intervals in each temperature range, at pH 1.2, 6.8, and 9.1. Aspirin was a suitable model because its hydrolysis occurs by several mechanisms, namely, the hydronium-ion catalysis (represented at pH 1.2), the hydroxyl-ion catalysis (represented at pH 9.1), and the intramolecular-nucleophilic catalysis (represented at pH 6.8), all involving the aliphatic and/or aromatic groups of the drug (6–8). The steps involved and the activated complexes formed in these reactions are shown in Scheme I (8), Scheme II (7,8), and Scheme III (7,8).

MATERIALS AND METHODS

Chemicals. Aspirin purchased from J. T. Baker Chem-

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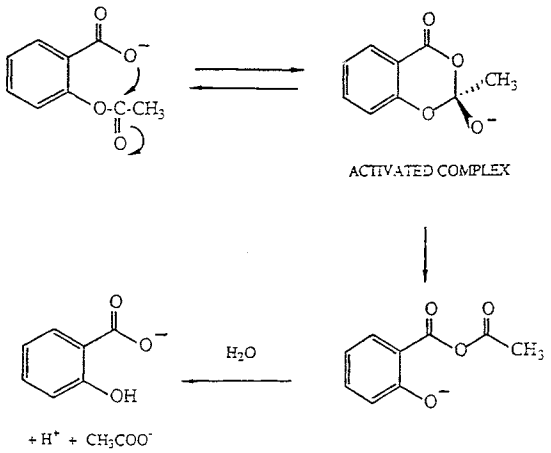
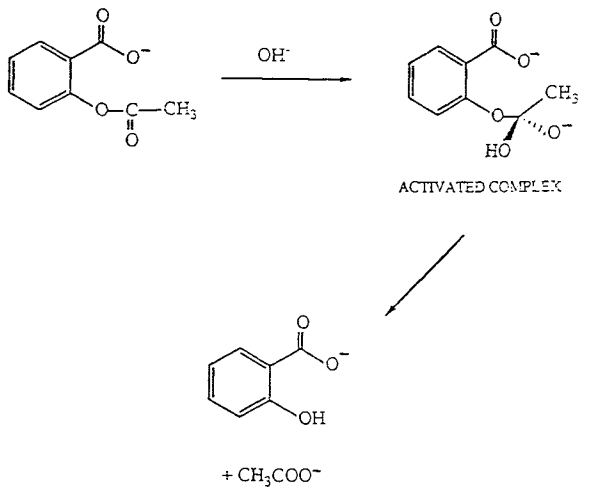


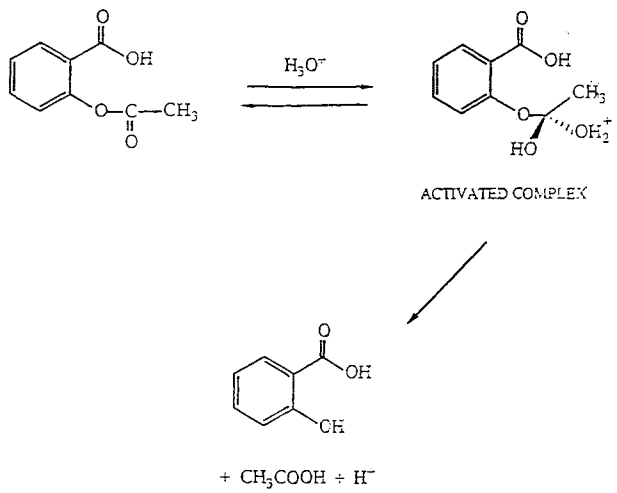
Scheme I. Intramolecular nucleophile-catalyzed aspirin hydrolysis.

ical Co. contained a negligible amount of salicylic acid. The following analytical-grade reagents were used: sodium phosphate monobasic, sodium phosphate dibasic, sodium chloride, hydrochloric acid, ferric nitrate, boric acid, salicylic acid, and sodium hydroxide.

Kinetics. The kinetics of aspirin hydrolysis were studied at pH 1.2, 6.8, and 9.1 in buffer solutions whose compositions are listed in Table I. The ionic strength of each buffer solution was 0.4. The hydrolysis reactions were studied in the temperature ranges of 30–40, 45–55, and 60–70°C, at a 1–3°C interval in each temperature range. The hydrolysis at a given temperature and pH was studied at three different initial concentrations of aspirin (1.0, 1.5, and 2.0 mg/ml). The reaction mixtures were as follows: three 50-ml test tubes, equipped with screw caps, were set up in a water bath maintained at a given temperature. To each tube, 20 ml of a buffer solution of a given pH was added. After the buffer solution attained the desired temperature, accurately weighed 20, 30, and 40 mg of aspirin were transferred to the respective tubes to yield the initial concentrations of 1.0, 1.5, and 2.0 mg/ml of aspirin. The drug was rapidly dissolved by shaking the tubes in the water bath. The zero-time sample (1 ml) was withdrawn for the analysis of preexisting salicylic acid, which was usually negligible. Only one sample of 1 ml was



Scheme II. Hydroxyl ion-catalyzed aspirin hydrolysis.



Scheme III. Hydronium ion-catalyzed aspirin hydrolysis.

withdrawn from a given reaction mixture at an appropriate predetermined time when 8 to 20% of aspirin was hydrolyzed. The samples were quantitatively analyzed for salicylic acid, using the spectrophotometric method (9). The absorbance was measured at 530 nm on a Bausch and Lomb Spectronic 20 spectrophotometer. A fresh standard curve for salicylic acid was prepared daily.

Treatment of Data. We used the initial-rate method (10) for the determination of the kinetic order and the rate constants of the reactions. The data were treated according to the following equation:

$$(dc/dt)_{\text{initial}} = k_{\text{obs}}C_o^n \quad (1)$$

or

$$\log (dc/dt)_{\text{initial}} = \log k_{\text{obs}} + n \log C_o \quad (2)$$

where $(dc/dt)_{\text{initial}}$ is the initial rate of formation of salicylic acid (which corresponds with the initial rate of aspirin hydrolysis), C_o is the initial concentration of aspirin, k_{obs} is the rate constant of aspirin decomposition, and n is the order of reaction. The rate of aspirin hydrolysis at the predetermined time (T) was referred to as the initial rate of hydrolysis. Although the values of T were varied according to the pH and temperature of the reaction mixture, the T value was maintained constant for all three initial concentrations of aspirin at a given pH and temperature. The range of T values and that of the percentage of aspirin hydrolyzed in a given temperature range at a given pH are listed in Table I. The linear regression analysis carried out for the data of log (initial rate) vs log (initial concentration) obtained at each pH and temperature yielded the slope values (i.e., values of n), which were close to 1 (0.99 ± 0.05), indicating that all reactions followed an apparent first-order process.

The apparent first-order rate constant of aspirin hydrolysis under each experimental condition was calculated using the following equation:

$$k_{\text{obs}} = 1\text{n } (C_o/C_T)/T \quad (3)$$

where C_T is the concentration of unhydrolyzed aspirin at time T.

Table I. Composition of Buffer Systems of Different pH Employed in the Study and the Ranges of Temperature, Reaction Time Intervals (T), and Percentages of Aspirin Hydrolyzed at the Respective pH's

pH	Buffer composition	Temperature range (°C)	Range of T (hr)	Range of % aspirin hydrolyzed
1.2	Hydrochloric acid (0.104 *M*)	30–40	4.0–8.0	8–15
	Sodium chloride (0.306 *M*)	45–55	0.75–3.0	16–21
		60–70	0.75–1.0	10–20
6.8	Sodium phosphate monobasic (0.02 *M*)	30–40	3.5–7.0	10–17
	Sodium phosphate dibasic (0.02 *M*)	45–55	1.0–2.5	15–17
	Sodium chloride (0.32 *M*)	60–70	0.5	14–23
9.1[a]	Boric acid (0.094 *M*)	30–40	2.0–7.0	34–40
	Sodium hydroxide (0.076 *M*)	45–55	0.33–0.5	15–20
	Sodium chloride (0.230 *M*)	60–70	0.167	19–30

[a] As stated in the text, 9.1 was the pH of the borate buffer at 30°C.

The following equations, (4), (5), and (6), describe k_{obs} at pH 1.2, 6.8, and 9.1 (or any alkaline pH), respectively:

$$k_{obs} = k_{H_3O^+}(H_3O^+) \quad (4)$$

$$k'_{obs} = k_I \quad (5)$$

$$k''_{obs} = k_{OH^-}(K_w/H_3O^+) + k_I \quad (6)$$

where k_I is the apparent first-order rate constant of intramolecular nucleophile-catalyzed reaction, $k_{H_3O^+}$ is the apparent second-order rate constant of hydronium ion-catalyzed reaction, k_{OH^-} is the apparent second-order rate constant of hydroxyl ion-catalyzed reaction, and K_w is the apparent dissociation constant of water. Since the pH's of solutions of the buffer systems used at pH 1.2 and 6.8 are negligibly affected in the temperature range of 30–70°C (11), $k_I = k'_{obs}$ and $k_{H_3O^+} = k_{obs}/(H_3O^+)$, where the hydronium ion concentration is that of pH 1.2.

Since temperature affects the dissociation constant of water (12), the pH of 9.1 at 30°C of the borate buffer solutions is expected to decrease by 0.2 pH unit with the increase in temperature to 70°C (11). Our analysis of the pH versus temperature data described by Bates (11) for the pH 9.14 borax solution indicated that the pH of the borate buffer would decrease by approximately 0.0061 pH unit per degree in the 30–55°C temperature range and by 0.0042 pH unit per degree in the 55–70°C temperature range. Our analysis of the K_w versus temperature data (12) indicated that the enthalpy of water dissociation in the 30–70°C temperature range is 12.17 kcal/mol. Therefore, the hydronium ion concentrations and water dissociation constants were determined at respective temperatures and were used to calculate the apparent k_{OH^-} as follows:

$$k_{OH^-} = (k''_{obs} - k_I)(H_3O^+)/K_w \quad (7)$$

Edwards (6) has demonstrated that, at a given temperature, k_I remains constant at any pH above 5.

To determine the energy of activation (E_a) of the reaction using the Arrhenius equation, $\log k = \log A - (E_a/2.303RT)$ (13), the semilogarithmic plots of k vs $1/T$ were prepared for the data obtained for each temperature range of 30–40, 45–55, and 60–70°C for each reaction (Figs. 1 and 2); E_a for each temperature range was calculated from the respective slope of the straight line obtained by linear regression using the Minitab (14) computer program. The standard deviations of E_a were calculated from the standard deviation of slopes provided by the computer program.

The free energies of activation (ΔF^*) were calculated using the equation (13), $\Delta F^* = -RT \ln K^*$, where K^* is the equilibrium constant for the formation of the activated complex of the reaction and R is a gas constant. The value of K^* was calculated using the following equation (13): $K^* = kNh/RT$, where k is the reaction rate constant, N is Avogadro's number, and h is Planck's constant.

The enthalpies of activation (ΔH^*) and the entropies of activation (ΔS^*) were calculated using the standard equations (13), $\Delta H^* = E_a - RT$ and $\Delta S^* = (\Delta H^* - \Delta F^*)/T$.

RESULTS AND DISCUSSION

Table II lists the values of E_a and Table III lists the values of ΔH^* and ΔS^*, which were calculated for various reactions and temperature ranges. The E_a values obtained in the 30–40, 45–55, and 60–70°C temperature ranges, designated E_a^{30-40}, E_a^{45-55}, and E_a^{60-70}, respectively, are significantly different from each other for each reaction (Table II). Further, E_a^{30-40} was the highest and E_a^{60-70} was the lowest for the intramolecular nucleophile-catalyzed and hydroxyl ion-catalyzed reactions. However, for the hydronium ion-catalyzed reaction, E_a^{45-55} was the highest, while E_a^{60-70} was still the lowest.

Table III shows that, for each reaction, the changes observed with temperature in the qualitative patterns of the magnitudes of ΔH^* and ΔS^* were identical to those noted for E_a (Table II). Accordingly, ΔH^* and ΔS^* were the highest in the 30–40°C range and the lowest in the 60–70°C range for the intramolecular nucleophile-catalyzed and hydroxyl ion-catalyzed reactions. For the hydronium ion-catalyzed reaction, ΔH^* and ΔS^* were the highest in the 45–55°C range and the lowest in the 60–70°C range. However, the overall ΔH^* was positive and ΔS^* was negative for the formation of the activated complexes at each temperature range for all reactions.

Physicochemical Basis of E_a *Differences.* The differ-

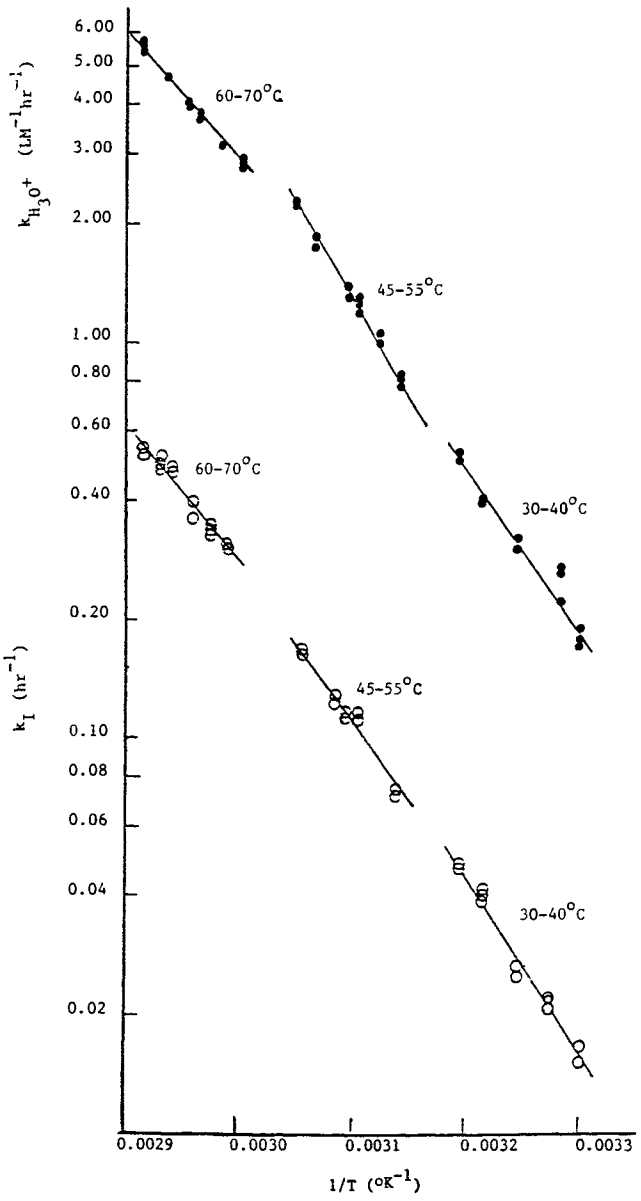


Fig. 1. Semilogarithmic Arrhenius plots of $k_{H_3O^+}$ vs $1/T$ (●) and k_I vs $1/T$ (○) obtained for the hydronium-ion catalyzed and the intramolecular nucleophilic catalyzed aspirin hydrolysis, respectively, in the 30–40°, 45–55° and 60–70°C temperature ranges.

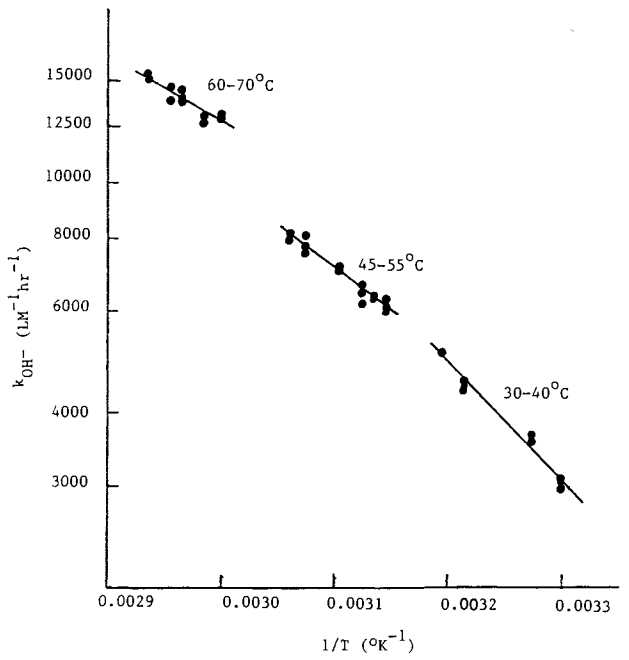


Fig. 2. Semilogarithmic Arrhenius plots of k_{OH^-} vs $1/T$ obtained for the hydroxyl ion-catalyzed aspirin hydrolysis in the 30–40, 45–55, and 60–70°C temperature ranges.

ences observed in the magnitudes of ΔH^* and ΔS^* in Table II can arise from several factors: (a) the mechanisms involved for the hydrolysis of aspirin in each reaction studied, (b) the effect of the charge at the reaction center of the activated complex and that of temperature on the iceberg structures of water present around the methyl and phenyl groups of aspirin molecules, and (c) the water of hydration associated with the hydroxyl ions, the hydronium ions, and the carboxylate group involved in catalyzing the hydrolysis of aspirin.

With the decrease in temperature, the ΔH^* values became more positive and the ΔS^* values became less negative for the intramolecular nucleophile-catalyzed reaction and for the hydroxyl ion-catalyzed reaction (Table III). This result indicates that, besides those processes involved in the 60–70°C range, additional processes, which are accompanied by the positive enthalpy (requiring supply of energy) and positive entropy (reflecting some disorder) effects, are also involved in the 45–55 and 30–40°C ranges during the formation of the activated complexes. The processes that contribute to the overall ΔH^* and ΔS^* in the 60–70°C range for the intramolecular nucleophile-catalyzed reaction may include the release of some water molecules of solvation of the carboxylate ion during the formation of the cyclic structure of the activated complex (Scheme I) and the association of some water molecules in solvating the activated complex. The processes that contribute to the overall ΔH^* and ΔS^* in the 60–70°C range for the hydroxy ion-catalyzed reaction are the release of water molecules of solvation of the hydroxyl ions (15) during the formation of the activated complex (Scheme II) and the association of some water molecules in solvating the activated complex.

It is noted in Schemes I and II that the newly created negative charge in the activated complexes is in close proximity to the methyl group. Therefore, a disruption of the iceberg structure around the methyl group can occur through an ion–dipole interaction (2,16) between the negative charge and the water molecules of the icebergs. Since the methyl group is devoid of iceberg structure above 58°C, the additional positive enthalpy and entropy contributions to the overall ΔH^* and ΔS^* can be expected only in the 45–55 and 30–40°C ranges. However, the ΔH^* and ΔS^* values for these reactions in the 30–40°C range are greater than those in the 45–55°C range, suggesting that the negative charge of the activated complex may also disrupt the phenyl group-associated iceberg structure. Indeed the icebergs associated with a phenyl group exist only up to 42°C (2), and the influence of a singly charged ion extends to 5 Å in water (17).

Table II. Activation Energies (±SD) Determined for Aspirin Hydrolysis Catalyzed by Various Reactions at Various Temperature Ranges

Catalytic reaction	Energy of activation (kcal/mol)			Statistical significance of difference (P)		
	E_a^{30-40}	E_a^{45-50}	E_a^{60-70}	E_a^{30-40} vs E_a^{45-55}	E_a^{45-55} vs E_a^{60-70}	E_a^{60-70} vs E_a^{30-40}
Hydronium ion	19.33 ± 1.17	20.26 ± 0.50	15.82 ± 0.49	<0.01	<0.01	<0.01
Intramolecular nucleophilic	20.87 ± 1.14	17.90 ± 1.55	16.17 ± 1.18	<0.01	<0.01	<0.01
Hydroxyl ion	9.11 ± 0.39	8.69 ± 0.48	7.37 ± 0.63	<0.01	<0.05	<0.01

Since icebergs exist mostly near the edges of the aromatic ring (2), the edge (carbon) of the phenyl ring of aspirin whose icebergs may be exposed to the influence of the charge is that within the 5-Å distance from the negative charge of the activated complex. Such an edge carbon would appear to be that attached to the acetyl group, because it is only 2.87 and 3.24 Å away from the charge of the reaction centers of the activated complexes in Scheme I and Scheme II, respectively. These distances were measured on a Silicon Graphics 4D 220GTX computer using the SYBYL molecular modeling software package (Tripos and Associates, St. Louis, MO); the structures for the proposed transition states were built and energy minimized using the Tripos force field equation.

The processes that contribute to the overall ΔH^* and ΔS^* of the hydronium ion-catalyzed reaction in the 60–70°C range are the release of water molecules of solvation of the hydronium ions (15) during the formation of the activated complex (Scheme III) and the association of water molecules in solvating the activated complex. Since the hydrophobic groups of aspirin are devoid of iceberg structures above 58°C, the additional positive enthalpy and entropy contributions to the overall ΔH^* and ΔS^* can be expected only in the lower temperature range. However, in the case of the hydronium ion-catalyzed reaction, unlike in the case of the intramolecular nucleophile- or hydroxyl ion-catalyzed reaction, the ΔH^* value is less positive and the ΔS^* value more negative in the 30–40°C range than in the 45–55°C range. Even though positive entropy and enthalpy contributions are expected from the release of water molecules of iceberg structures from the methyl group by interaction with the vicinal positive charge (Scheme III), an additional process is involved in the 30–40°C range, but not in the 45–55°C range, which is exothermic and imparts more order to the activated complex.

The structures of the activated complexes shown in Schemes I to III can account for the above phenomenon. (i) The carboxyl group of aspirin remains dissociated in Schemes I and II; it remains undissociated in Scheme III. (ii) The charge on the activated complex is positive in Scheme III; it is negative in Schemes I and II. (iii) In Scheme III, the $-OH_2^+$ group can interact with the carboxyl group through an ion–dipole interaction or H-bonding (because the distance between the two groups is only 2.96 Å). This interaction is not possible in Scheme I because of the formation of the cyclic structure and in Scheme II because of the repulsion between the negative charge at the reaction center of the activated complex and the negative charge of the carboxylate ion. Thus, while the charge at the reaction center in Schemes I and II may be available to disrupt the iceberg structure of the phenyl group as suggested previously, the charge at the reaction center in Scheme III may not be available for this purpose because of its possible involvement with the undissociated carboxyl group. If the latter interaction, which is more likely to be weak, is broken above 45°C, the absence of such exothermic process in the 45–55°C range would render the activation enthalpy more positive and the entropy less negative in this temperature range.

Diagnostic Test for the Role of Water. A reaction, which involves a single water structure-making or water structure-breaking process, is characterized by a linear relationship between its enthalpy and entropy values. Further, the value of the linear slope, which is referred to as the

Table III. Activation Enthalpies (ΔH^*) and Entropies (ΔS^*) Determined for Aspirin Hydrolysis Catalyzed by Various Reactions at Various Temperature Ranges[a]

Catalytic reaction	Temperature range					
	30–40°C		45–55°C		60–70°C	
	ΔH^* (kcal/mol)	ΔS^* (e.u.)	ΔH^* (kcal/mol)	ΔS^* (e.u.)	ΔH^* (kcal/mol)	ΔS^* (e.u.)
Hydronium ion	18.72	−16.44	19.62	−13.58	15.15	−27.52
Intramolecular nucleophilic	20.26	−16.19	17.26	−25.83	15.49	−31.06
Hydroxyl ion	8.50	−30.83	6.18	−38.19	4.83	−41.79

[a] These are the apparent mean values of a given activation parameter in the given temperature range. The mean value of a parameter was calculated by dividing the sum total of the parameter values determined at each temperature by the number of temperatures employed in the given temperature range. This approach was conveniently used because the parameter values fell within a very narrow range in a given temperature range; for example, in the 30–40°C range, the range of ΔH^* was 18.70 to 18.73, 20.25 to 20.27, and 8.49 to 18.52 kcal/mol for the hydronium ion-, intramolecular nucleophilic-, and hydroxyl ion-catalyzed reactions, respectively.

compensation temperature (T_C), generally lies in the 250–320 K range (18). If the differences in the E_a values noted in the present study were due to the proposed role the icebergs around the hydrophobic groups play in the formation of the activated complexes, it follows that there should exist a linear relationship between the differential enthalpy of activation ($\Delta\Delta H^*$) and the differential entropy of activation ($\Delta\Delta S^*$), representing the activation parameters due mainly to the net release of water molecules in the formation of the activated complexes. The $\Delta\Delta H^*$ and $\Delta\Delta S^*$ for each hydrolytic mechanism were calculated by subtracting its overall ΔH^* and ΔS^* values obtained in the 60–70°C range from the corresponding overall ΔH^* and ΔS^* values obtained in each temperature range of 30–40 and 45–55°C. As shown in Fig. 3, the plot of $\Delta\Delta H^*$ vs $\Delta\Delta S^*$ was found to be linear ($r = 0.999$) with T_c equal to 307°K.

In conclusion, this study demonstrated that E_a^{30-40}, E_a^{45-55}, and E_a^{60-70} determined for the hydrolysis of aspirin were significantly different from each other. E_a^{30-40} was greater than E_a^{60-70} by as much as 22 to 29% for these reactions, and the icebergs associated with hydrophobic groups can account for these differences. If the energy of activation value is calculated from rate constants determined in the entire temperature range of 30–70°C, as is customarily done, such a value can be erroneous. To illustrate this possibility, we deliberately calculated a E_a^{30-70} value for each reaction and compared it with the E_a^{30-40}, E_a^{45-55}, and E_a^{60-70} values for the respective reaction. We noted that the E_a^{30-70} values were 17.59 ± 0.37, 18.25 ± 0.14, and 8.98 ± 0.23 kcal/mol for the hydronium ion-catalyzed reaction, the intramolecular nucleophile-catalyzed reaction, and the hydroxyl ion-catalyzed reaction, respectively (incidentally, $r > 0.99$ in each case), and that all E_a values (Table II), except the E_a^{45-55} value for the intramolecular nucleophile-catalyzed reaction, were significantly different ($P < 0.01$) from the respective E_a^{30-70} value for a given reaction. Therefore, the shelf life of a drug predicted at room temperature on the basis of the energy of activation calculated from the widely spaced temperature data values in the range of 10–70°C or higher may result in an error. However, a more realistic shelf-life prediction of a drug at room temperature can be made on the basis of the energy of activation determined from its decomposition rate constants at temperatures below 42°C.

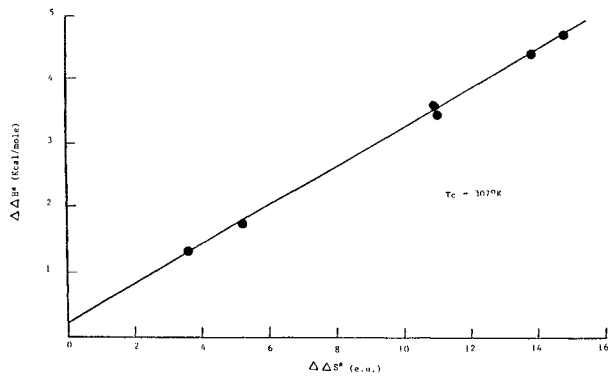


Fig. 3. A plot showing a linear relationship between the differential enthalpy ($\Delta\Delta H^*$) and the differential entropy ($\Delta\Delta S^*$) of activation of aspirin hydrolysis.

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