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The effect of cyclodextrins on the rate of intramolecular lactamization of gabapentin in aqueous solution

A.S. Kearney, S.C. Mehta and G.W. Radebaugh

Department of Pharmaceutics, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Morris Plains, NJ 07950 (U.S.A.)

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Summary

The effect of various cyclodextrins on the intramolecular lactamization of 3,3-pentamethylene-γ-aminobutyric acid, **1**, in solution was investigated. Baseline studies in the absence of cyclodextrins were conducted under accelerated conditions to obtain reaction rates that could be followed over a shorter time interval. In aqueous buffered solutions at 80 °C and $\mu = 0.5$ M, **1** undergoes an intramolecular aminolysis to yield a stable, cyclized lactam product, 3,3-pentamethylene-γ-butyrolactam, **2**, over the pH range of 1.4–11.1. The buffer-independent pH-rate profile was described by two reaction pathways: a specific acid- and specific base-catalyzed lactamization of the uncharged species. Acetate and phosphate buffers were found to catalyze the rate of lactam formation, whereas borate had no apparent catalytic effect. Acetate appeared to be acting as a general-acid catalyst, whereas phosphate appeared to be acting as a general-acid and general-base catalyst. Next, the effect of various cyclodextrins on the lactamization rate was investigated over the pH range of 4.1–7.1. In the pH region defined as specific-acid catalyzed lactamization of the uncharged species, α- and γ-cyclodextrin had minimal effect on the rate, whereas β- and hydroxypropyl-β-cyclodextrin accelerated the lactamization rate. While in the pH region defined as specific-base catalyzed lactamization of the uncharged species, all four cyclodextrins catalyzed the reaction rate (β- > hydroxypropyl-β- > α- ≈ γ-cyclodextrin). Interestingly, the catalytic efficiency of acetate buffer varied depending on the cyclodextrin involved. The catalytic efficiency was the greatest in the presence of β-cyclodextrin which was followed by hydroxypropyl-β-cyclodextrin. In 100 mM phosphate buffer of pH 7 and in the presence of varying concentrations of the cyclodextrins, the rate of lactamization of **1** exhibited Michaelis-Menten-type kinetics. The data were consistent with relatively weak drug-cyclodextrin complex formation and with **1** being more chemically labile as complexed than uncomplexed drug. The enhanced rate observed in the presence of cyclodextrins was attributed to complexation-induced, conformational changes in the reactive moieties of **1**.

Introduction

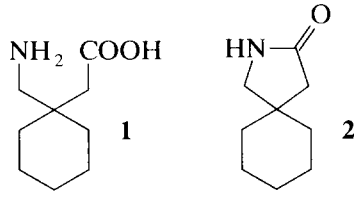
Gabapentin (3,3-pentamethylene-γ-aminobutyric acid; **1**) is an effective anticonvulsant which is an analog of γ-amino-*n*-butyric acid (GABA). The development of an aqueous solution dosage form for pediatric use is of interest; however, in aqueous solutions, gabapentin degrades via an intramolecular aminolysis to yield the corresponding 2-pyrrolidone derivative, 3,3-pentamethylene-γ-butyrolactam, **2**.

It is well known that intramolecular reactions

Correspondence: A.S. Kearney, Department of Pharmaceutics, Warner-Lambert Company, 170 Tabor Road, Morris Plains, NJ 07950, U.S.A.

generally have higher reaction rates than comparable intermolecular reactions due, in part, to the inherent proximity of the reacting groups (Kirby and Fersht, 1971; Kirby, 1980). Only a few studies have dealt with the effect of inclusion by cyclodextrins on the chemical reactivity of a compound which degrades by an intramolecular reaction involving a cyclic intermediate (e.g. Griffiths and Bender, 1973a; Andersen and Bundgaard, 1984). Interestingly, the reactivity of such compounds may vary depending on the type of cyclodextrin involved; one cyclodextrin may catalyze the reaction rate, whereas a different cyclodextrin oligomer may retard the reaction rate.

The present study was undertaken to investigate the intramolecular lactamization of **1** and the effect of different cyclodextrins on the lactamization of **1**.



1 **2**

Materials and Methods

Materials

1 and **2** were synthesized by the Chemistry Department, Parke-Davis Pharmaceutical Research. β-Cyclodextrin (β-CD) was obtained from Sigma Chemical Co. (St. Louis, MO); α-cyclodextrin (α-CD) and γ-cyclodextrin (γ-CD) were purchased from Aldrich Chemical Co. (Milwaukee, WI); and hydroxypropyl-β-cyclodextrin (HPβ-CD), having a degree of molar substitution of 0.38, was obtained from Janssen (Olen, Belgium). All other chemicals were of reagent or analytical grade. All experiments were conducted with deionized and distilled water.

HPLC analysis

The HPLC system consisted of an HP 1090 Liquid Chromatograph equipped with a diode array detector which was operated at a fixed wavelength of 210 nm. The column was a Beckman Ultrasphere® (4.6 mm × 25 cm) 5 μm ODS column. The mobile phase was composed of 575 ml of an aqueous phase (prepared by dissolving 1 g of the sodium salt of 1-decanesulfonic acid, 5.75 g of ammonium phosphate, 5 ml of phosphoric acid and 9 ml of triethylamine in water), 390 ml of methanol, and 110 ml of acetonitrile. The injection volume was 20 μl, and the eluent flow rate was 1.0 ml/min. The retention times, in the absence of cyclodextrin, were approx. 7.3 and 9.0 min for **1** and **2**, respectively. The retention time of **1** was found to decrease slightly with increasing concentration of the respective cyclodextrin until a limiting concentration of the cyclodextrin was attained where the retention time remained constant (this behavior was consistent with the formation of drug-cyclodextrin complex (Seno et al., 1990)).

Determination of apparent ionization constants

The apparent dissociation constants of **1** were determined at 80 °C by potentiometric titration using an Accumet pH meter 925 and an Orion semimicro-Ross combination glass electrode. Two separate aqueous solutions of **1** (0.01 M), having an ionic strength of 0.5 M with NaCl, were prepared and equilibrated at 80 °C. One was titrated with 5 ml of 0.1 N HCl in 0.5 ml increments, whereas the other was titrated with 5 ml of 0.1 N NaOH in 0.5 ml increments to obtain, by standard calculation methods (Albert and Serjeant, 1971), the apparent ionization constants of the carboxyl and the amino group, respectively.

Kinetic methods

Since the cyclization of **1** at ambient temperatures was relatively slow, the kinetics of degradation of dilute aqueous solutions of **1** (2.92×10^{-3} M) were determined at 80 °C. The reaction was studied at a fixed ionic strength ($\mu = 0.5$ M with NaCl) as a function of pH and buffer concentration in the absence and presence of the various cyclodextrins (8.76×10^{-3} M on an anhydrous weight basis). Kinetic experiments were initiated by adding 100 μl of a stock solution of **1** (50 mg/ml) to a 10 ml volumetric flask of the buffered medium that was temperature equilibrated in a

circulating water bath. The pH values of the reaction mixtures were determined at the experimental temperature with an Accumet pH meter 925 that was equipped with a Ross combination glass electrode.

At appropriate time intervals, samples were withdrawn, quenched in an ice-water bath, and assayed for the drug and the degradant by HPLC. The observed, pseudo, first-order rate constants, k_{obs}, were obtained either by following the disappearance of the peak area of the parent compound for at least 1.5 half-lives (pH > 10) or by following the initial rate of appearance of the product for about 5% of total reaction. The product concentrations were calculated from the slope of the linear regression of standard curves.

The observed rate constant was determined at four different buffer concentrations (i.e., 25, 50, 75, 100 mM) at a fixed pH value. Where appropriate, the intercepts of plots of k_{obs} vs the total buffer concentration yielded the first-order, buffer-independent rate constant, k_0, whereas the slopes of these plots yielded the second-order, buffer-dependent rate constant, k_{buff}.

For a given cyclodextrin, the kinetics of lactamization of **1** were also studied as a function of cyclodextrin concentration in 100 mM phosphate buffer at pH 7.0, 80 °C and $\mu = 0.5$ M. The concentration ranges of cyclodextrins investigated were as follows: α-CD, 2.92×10^{-3}–4.68×10^{-2} M; β-CD, 7.30×10^{-4}–4.68×10^{-2} M; γ-CD, 2.92×10^{-3}–9.36×10^{-2} M; and HPβ-CD, 1.46×10^{-3}–4.68×10^{-2} M.

Results and Discussion

Examination of the pH-rate profile

In the absence of cyclodextrin, the kinetics of lactamization of **1** were studied in dilute aqueous solutions as a function of pH and buffer concentration at 80 °C and constant ionic strength (μ = 0.5 M with NaCl). The dependence of the buffer-independent rate constant, k_0, on pH is shown in Fig. 1. The pH-rate profile exhibited a minimum at about pH 6.0 and pH-independent regions at low pH (pH < 3) and at high pH (pH > 10). The plateau in the basic pH region occurred at a k_0 value which was approx. 27-fold greater than the corresponding k_0 value in the acidic pH region.

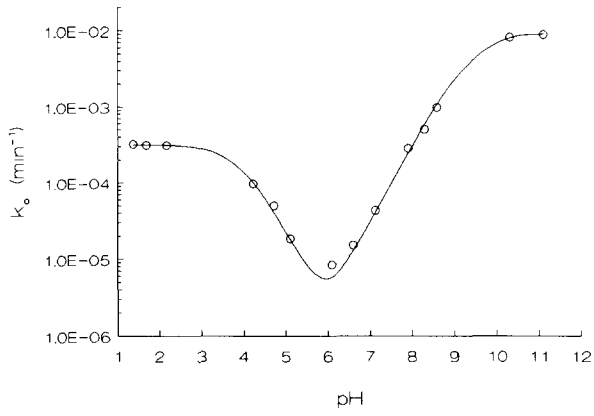
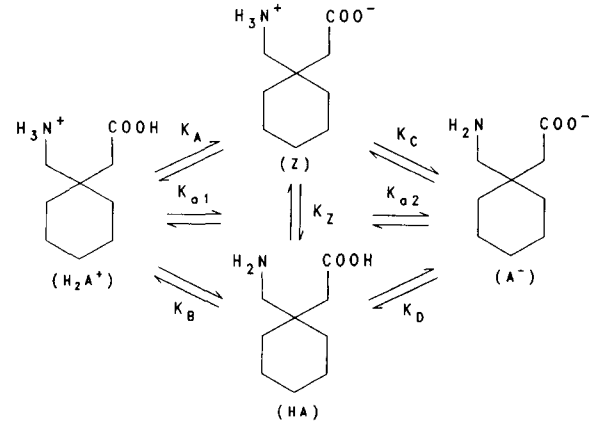


Fig. 1. pH-rate profile for the lactamization of **1** (2.92×10^{-3} M) at 80 °C and $\mu = 0.5$ M. The line represents the theoretical profile generated with non-linear regression of the experimental data (○) using Eqn 7.

The interpretation of the kinetics of lactamization of **1** in aqueous solutions is complicated by the fact that **1** can exist as a cationic, uncharged, zwitterionic, and/or anionic form, depending on the pH of the solution. The ionization processes for **1** are depicted in Scheme 1 (Albert and Serjeant, 1971).

At a fixed pH the ampholyte concentration, [N], is equal to the sum of the concentrations of the zwitterion, [Z], and uncharged species, [HA] (i.e., [N] = [Z] + [HA]). The ampholyte can be treated as a single species, since the equilibrium



Scheme 1.

between the zwitterion and the uncharged species, denoted by K_Z in Scheme 1, is independent of pH. The macroscopic equilibrium constants, K_{a1} and K_{a2}, are related to the microscopic constants shown in Scheme 1 by the following equations (Albert and Serjeant, 1971).

$$K_{a1} = K_A + K_B \tag{1}$$

$$\frac{1}{K_{a2}} = \frac{1}{K_C} + \frac{1}{K_D} \tag{2}$$

At a fixed pH, the fractions of the ionic species of **1** can be obtained by the following relationships:

$$F_{H_2A^+} = \frac{[H^+]^2}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \tag{3}$$

$$F_N = \frac{K_{a1}[H^+]}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \tag{4}$$

$$F_{A^-} = \frac{K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \tag{5}$$

where $F_{H_2A^+}$, F_N, and F_{A^-} are the fractions of the cationic, ampholytic, and anionic species, respectively, and $[H^+]$ is the hydrogen-ion activity. In this study, no attempt was made to determine the microscopic equilibrium constants; hence, F_{HA} and F_Z were not calculated.

In aqueous solutions over the pH range of 1.4–11.1, the degradation of **1** was found to proceed by an irreversible cyclization to a lactam product, **2**, which was stable under the reaction conditions. A minimal reaction scheme which can account for this observation is shown in Scheme 2 where the mechanism of lactamization can be described by a sequential, two-step mechanism involving a tetrahedral intermediate. The first step involves nucleophilic attack by the amine functionality at the neighboring carbonyl moiety resulting in the formation of a tetrahedral intermediate which subsequently dehydrates to form the lactam product, **2**. This simplified mechanism for ring closure has been previously proposed for the intramolecular aminolysis of structurally analogous compounds, 3-(2-aminophenyl)propionic acids (Camilleri et al., 1979) and 4-(arylamino) butanoic acids (Abdallah and Moodie, 1983).

The rate of lactam formation over the pH range investigated (Fig. 1) can be described by the following relationship:

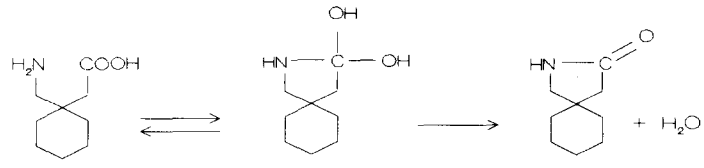
$$k_{obs} = k_1[H^+]F_N + k_2[^-OH]F_N \tag{6}$$

where $[^-OH]$ is the hydroxide-ion activity, k_1 denotes the apparent, second-order rate constant for the hydronium-ion catalyzed lactamization of the ampholyte of **1**, k_2 is the apparent, second-order rate constant for the hydroxide-ion catalyzed lactamization of the ampholyte of **1**, and F_N and $[H^+]$ represent the same quantities as defined above (Eqn 4). From Eqns 4 and 6, the following expression, relating the buffer-independent rate constant to the hydrogen-ion activity, can be derived:

$$k_0 = \frac{k_1[H^+]^2K_{a1} + k_2K_{a1}K_w}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \tag{7}$$

where K_{a1} and K_{a2} are the macroscopic dissociation constants of the carboxyl and amino groups of **1**, respectively, and K_w is the ion product of water at 80 °C which is 2.34×10^{-13} (Martin et al., 1983).

Eqn 7 assumes that the ampholyte form of **1** is reactive and that the cationic species is unreac-



Scheme 2.

tive. The latter species is assumed to be unreactive since, the amino group exists as the non-nucleophilic protonated form. Hence, the zwitterionic species should also be unreactive. Based on this reasoning, the rate constants, k_1 and k_2, would actually represent the specific acid- and specific base-catalyzed lactamization of the uncharged form of **1** instead of the ampholyte. However, the k_1 and k_2 terms as present in Eqns 6 and 7 should be proportional to the rate constants involving the uncharged species since this species comprises a fixed fraction of the ampholyte. Additionally, the k_2 term may also represent the kinetically equivalent term involving spontaneous or water-catalyzed lactamization of the anionic species.

The theoretical profile in Fig. 1 was constructed with the following values which were generated by non-linear regression analysis (PCNONLIN) of the experimental data using Eqn 7: $k_1 = 2.46\ M^{-1}\ min^{-1}$; $k_2 = 13.3\ M^{-1}\ min^{-1}$; $K_{a1} = 1.29 \times 10^{-4}$; and $K_{a2} = 3.44 \times 10^{-10}$. These kinetically determined pK_a values of 3.89 and 9.46 at 80 °C are in good agreement with the values 3.74 and 9.56 determined by potentiometric titration at 80 °C and $\mu = 0.5$ M.

Effect of buffers

The formation of the lactam product, **2**, was found to be catalyzed by acetate (pH 4.2–5.1) and phosphate (pH 6.1–7.1) buffers, whereas borate (pH 7.9–8.6) buffers had no apparent effect on the reaction rate. Shown in Fig. 2 are plots of the effect of phosphate buffer concentration on the observed rate constant. Similar plots were observed with the acetate buffers. The observed rate constant, k_{obs}, for the lactamization in the presence of a catalytic buffer species can be described by the following equation:

$$k_{obs} = k_0 + k_{buff}[B]_t \qquad (8)$$

where k_0 is the apparent first-order, buffer-independent rate constant, k_{buff} denotes the second-order, buffer-dependent rate constant for a given buffer species, and $[B]_t$ is the total buffer concentration. Based on the assumption that the ampholyte of **1** was the only chemically reactive species, the k_{buff} term can be described by Eqn 9:

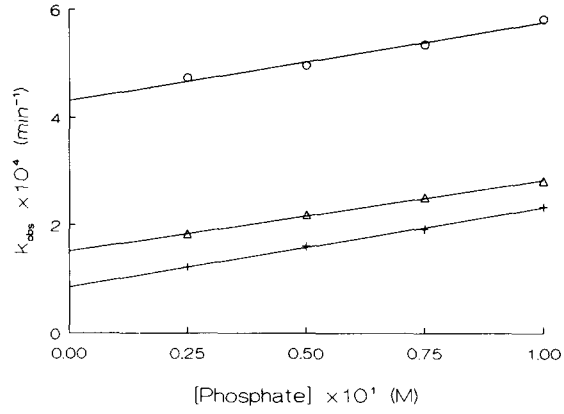


Fig. 2. Catalysis of the lactamization of **1** by phosphate buffers [(○) pH 7.12; (△), pH 6.60; (+), pH 6.10] at 80 °C and $\mu = 0.5$ M.

$$k_{buff} = F_N(k_{GA}F_{HB} + k_{GB}F_{B^-}) \qquad (9)$$

where k_{GA} and k_{GB} are the rate constants for general acid- and general base-catalysis of the lactamization reaction, respectively, F_{HB} and F_{B^-} are the fractions of the given buffer in the acidic and basic forms, respectively, and the other variables are the same as previously defined.

The dependence of the ratio, k_{buff}/F_N, on the fraction of the acetate buffer existing in the free

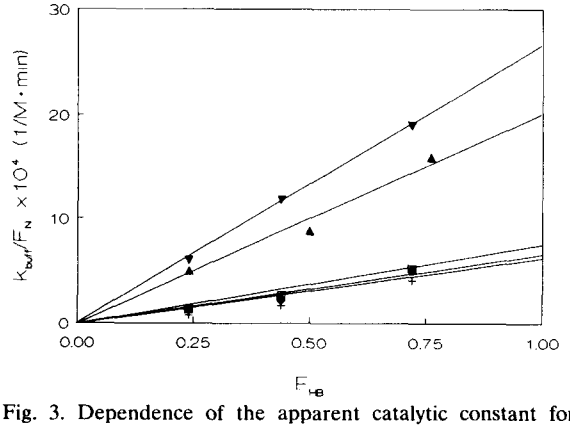


Fig. 3. Dependence of the apparent catalytic constant for acetate catalyzed lactamization of **1** on the buffer composition at 80 °C and $\mu = 0.5$ M. (+) No additive; (○) α-CD (8.76×10^{-3} M); (▼) β-CD (8.76×10^{-3} M); (■) γ-CD (8.76×10^{-3} M); (▲) HPβ-CD (8.76×10^{-3} M).

acid form, F_{HB}, is shown in Fig. 3. The y-intercept, which represents the apparent rate constant for catalysis by the basic component of the buffer, was not significantly different from zero. This finding was consistent with the lack of a general-base catalyzed pathway. Therefore, the plot was forced through zero at $F_{HB} = 0$, resulting in $k_{GA} = 4.99 \times 10^{-4}$ M^{-1} min^{-1}. The observance of general-acid catalysis for the lactamization of **1** in the acidic pH regions (or in the regions described by the k_1 term) is consistent with that found for the cyclization of 3-(2-aminoaryl)propanoic acids (Camilleri et al., 1979) where the results were interpreted as rate-determining, general-acid catalyzed breakdown of the neutral tetrahedral intermediate (step 2 of Scheme 2). By analogy, the rate-limiting step for the lactamization of **1** would involve breakdown of the tetrahedral intermediate, and general-acid catalysis could occur via the addition of a buffer proton to the leaving hydroxyl group.

The dependence of k_{buff}/F_N on the fraction of the phosphate buffer existing in the free acid form was non-linear, and extrapolation of the data to determine general-acid/base rate constants could not be performed. However, the data suggested that phosphate might have been acting as both a general-acid and a general-base catalyst. A similar explanation was alluded to for the phosphate-catalyzed lactamization of 3-(2-aminophenyl)propionic acids (Camilleri et al., 1979). General acid-base catalysis by the buffer can occur via proton transfer to one of the oxygen atoms and proton transfer from the other oxygen atom of the tetrahedral intermediate of **1**, since this would enhance water expulsion and carbonyl formation, respectively.

Effect of cyclodextrins

As can be seen in Fig. 4, the addition of various cyclodextrins to the reaction media had either a minimal or a pronounced effect on the rate of lactam formation over the pH range of about 4–7. In the pH range of 4.1–5.1 (denoted by k_1 in Eqn 7), α-CD and γ-CD had little effect on the lactamization rate, whereas β-CD and HPβ-CD accelerated the rate. In contrast, in the pH range of 5.9–7.1 (denoted by k_2 in Eqn 7), all

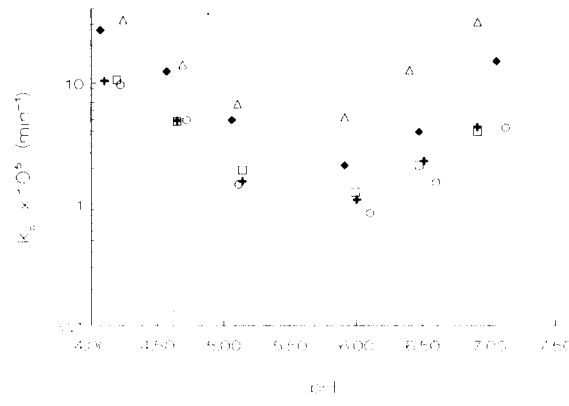


Fig. 4. Effect of cyclodextrins (8.76×10^{-3} M) on the pH-rate profile for the lactamization of **1** (2.92×10^{-3} M) at 80 °C and $\mu = 0.5$ M. (○) No additive; (+) α-CD; (△) β-CD; (□) γ-CD; (◆) HPβ-CD.

four cyclodextrins catalyzed the reaction rate. Over these pH ranges the interaction of **1** with the various cyclodextrins did not alter the mechanism of lactamization since the lactam product, **2**, was still the only product formed and the pH-rate profiles had similar shapes in all cases.

The cyclodextrin-dependent k_1 and k_2 terms, which will be designated as k_{1c} and k_{2c}, can be calculated utilizing the kinetic data generated in the presence of the various cyclodextrins, the kinetically determined K_{a1} and K_{a2} (i.e., 1.29×10^{-4} and 3.44×10^{-10}, respectively), and Eqn 7. No attempt was made to determine the K_a values in the presence of the cyclodextrins; however, at the cyclodextrin concentrations studied, the change in the K_a of the carboxyl group would probably be small (Connors and Lipari, 1976). Table 1 lists the calculated rate constants along with kinetic terms that give an indication of the catalytic efficiencies of the cyclodextrins in the two different reaction pathways. These catalytic efficiencies were calculated by dividing a given, cyclodextrin-dependent rate constant, k_{1c} or k_{2c}, by the comparable rate constant determined in the absence of cyclodextrins, k_1 or k_2 (2.46 and 13.3 M^{-1} min^{-1}, respectively). The catalytic efficiencies of the various cyclodextrins for both reaction pathways were found to decrease in the following order: β-CD > HPβ-CD > α-CD ≈ γ-CD.

TABLE 1

Comparison of the two cyclodextrin-dependent rate constants and the catalytic efficiencies of the cyclodextrins in the two different reaction pathways determined at 80 °C and $\mu = 0.5$ M

Cyclodextrin	k_{1c} (M^{-1} min^{-1})	k_{1c}/k_1	k_{2c} (M^{-1} min^{-1})	k_{2c}/k_2
α-	2.17 ± 0.09	~1	23.9 ± 2.37	1.8
β-	8.14 ± 0.33	3.3	169.3 ± 7.37	12.7
γ-	2.48 ± 0.08	~1	22.0 ± 1.94	1.6
HPβ-	5.29 ± 0.08	2.2	57.3 ± 1.74	4.3

The error values quoted for the k_{1c} and k_{2c} terms are the standard deviations.

In order to determine whether the catalysis of the lactamization reaction rate by the cyclodextrins was due to their decomposition to glucose under the experimental conditions, the lactamization of **1** was studied at a pH of 4.2 in the presence of D-glucose. If glucose is formed, it should be present to the greatest extent in the more acidic pH regions since the majority of acetals, the labile chemical moiety of the cyclodextrin molecule, are subject to only specific-acid catalysis (Jencks, 1987). Hence, an acidic pH (4.2) in the presence of the cyclodextrins was chosen for the glucose study. A concentration of 6.13×10^{-2} M glucose was used since this would be the molar concentration of glucose produced by the total decomposition of 8.76×10^{-3} M β-CD. A k_0 value of 8.78×10^{-5} M, which was in close agreement with the value found in the absence of β-CD, was obtained. These results indicate that it is the intact β-CD molecule and not the breakdown product, glucose, which catalyzes the rate of lactamization.

In the presence of the cyclodextrins as in their absence, the formation of the lactam product, **2**, was found to be catalyzed by acetate (pH 4.2–5.1) and phosphate (pH 6.1–7.1) buffers. As described in Eqn 9, the dependencies of the ratio, k_{buff}/F_N, on the fraction of the acetate buffer existing in the free acid form, F_{HB}, were linear in the presence of the various cyclodextrins (Fig. 3). Once again, acetate appeared to be acting as a general-acid catalyst, and the apparent general-acid catalyzed rate constants were determined after the plots were forced through zero at $F_{HB} = 0$. These apparent second-order rate constants for the catalysis of the lactamization of **1** by acetate buffer are listed in Table 2. The catalytic efficiencies of the buffers in the presence of the various cyclodextrins were calculated by dividing the k_{GA} term found in the presence of a given cyclodextrin by the corresponding term found in its absence, $k_{GA,un}$ (i.e., 4.99×10^{-4} M^{-1} min^{-1}). Interestingly, these efficiencies followed the same trend that was observed for the enhancement in the rate of lactamization due to the cyclodextrins: β-CD > HPβ-CD > α-CD ≈ γ-CD.

Assuming that complexation is occurring between **1** and the various cyclodextrins, the degradation reaction in the presence of a cyclodextrin should be described by the following equation (Griffiths and Bender, 1973b):

$$k_{obs} = \frac{k_S + k_{SL} K_{1:1}[CD]}{1 + K_{1:1}[CD]} \qquad (10)$$

which assumes that **1** can decompose either as the free or complexed species, that a 1:1 complex between **1** and the cyclodextrin of interest is formed, and that competitive complexation by the evolving lactam product, **2**, is not occurring to a significant extent. In Eqn 10, [CD] is the molar concentration of the cyclodextrin, k_S represents the rate constant for lactamization of the uncomplexed substrate, k_{SL} is the rate constant for lactamization of the **1**:cyclodextrin complex, and $K_{1:1}$ denotes the apparent complex formation (or stability) constant for the 1:1 complex.

Fig. 5 is a representative plot that shows the effect of β-CD concentration on the rate constant for lactam formation in 100 mM phosphate

TABLE 2

*The apparent, general acid-catalyzed rate constants for acetate buffer and the catalytic efficiencies in the presence of various cyclodextrins for the lactamization of **1** at 80 °C and $\mu = 0.5$ M*

Cyclodextrin	k_{GA} (M^{-1} min^{-1})	$k_{GA}/k_{GA,un}$
α-	6.21×10^{-4}	1.2
β-	2.67×10^{-3}	5.4
γ-	6.73×10^{-4}	1.3
HPβ-	2.00×10^{-3}	4.0

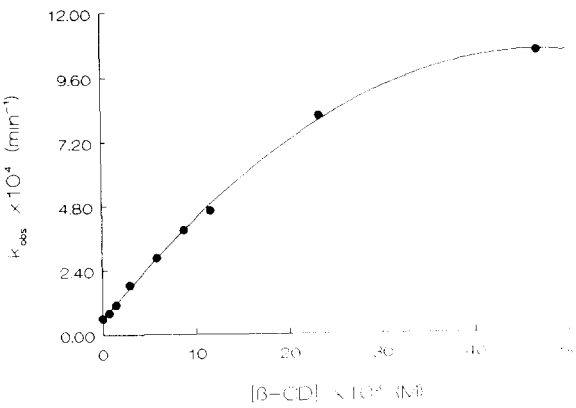


Fig. 5. Effect of β-CD concentration on the observed lactamization rate constant in 100 mM, pH 7 phosphate buffer at 80 °C and $\mu = 0.5$ M.

buffer at pH 7.0, 80 °C, and $\mu = 0.5$ M. The plot follows the trend predicted by Eqn 10 (i.e., Michaelis-Menten-type kinetics) and is consistent with complex formation prior to the rate-limiting step (although a change in the rate-determining step of the lactamization reaction, as the concentration of β-CD is increased, cannot be ruled out based solely on this data). Table 3 lists the kinetic parameters and complexation constants determined in the presence of the various cyclodextrins. These values were obtained by non-linear regression analysis (i.e., PCNONLIN) of the experimental data using Eqn 10, and were consistent with relatively weak drug-cyclodextrin complex formation and with **1** being more chemically labile as complexed than uncomplexed drug (i.e., $k_{SL} > k_S$). The complexed drug was found to be between 5- and 43-fold more reactive.

TABLE 3

The rate constants and complex formation constants for 1 and the various cyclodextrins at pH 7, 80 °C and $\mu = 0.5$ M

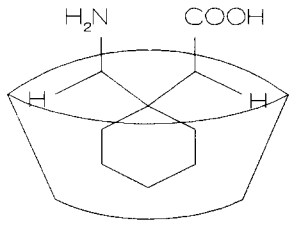
Cyclodextrin	k_{SL} (min^{-1})	$K_{1:1}$ (M^{-1})	n
α-	$2.76(\pm 0.07)\times 10^{-4}$	17.8 ± 1.0	7
β-	$1.85(\pm 0.16)\times 10^{-3}$	28.2 ± 4.9	9
γ-	$5.18(\pm 0.49)\times 10^{-4}$	3.9 ± 0.5	9
HPβ-	$8.47(\pm 0.43)\times 10^{-5}$	22.4 ± 2.2	7

The mean value of the k_S term (±SE) found with all of the cyclodextrins was 4.72×10^{-5} ($\pm 3.44\times 10^{-6}$) min^{-1}. The error values quoted are the standard errors, and n is the number of different cyclodextrin concentrations studied.

The inclusion of a guest molecule within the cyclodextrin cavity is dependent both on structural factors which include the size and shape of the guest molecule as well as the dimensions of the cyclodextrin cavity and on the extent of the non-covalent, attractive interactions between the guest molecule and the atoms comprising the interior of the cyclodextrin cavity (i.e., the glycosidic oxygen atoms and C-H groups). Hence, inclusion should allow for maximum interaction between the hydrophobic complexing moiety of the guest molecule and the apolar cavity of the cyclodextrin and for the maximum interaction between the hydrophilic functionalities of the guest molecule and the polar exterior of the cyclodextrin molecule as well as the solvent.

The low values of the complex formation constants (Table 3) indicate that relatively weak interactions occur between **1** and the various cyclodextrins. However, the trend observed with these constants (i.e., β- > HPβ- > α > γ-CD) is consistent with the inclusion of **1** by β-CD being energetically the most favorable. Of the cyclodextrin series studied, the steric compositions of **1** and β-CD allow for the maximum attractive interactions between the hydrophobic and hydrophilic portions of both entities upon inclusion. As expected, HPβ-CD, which has the most similar cavity dimensions to β-CD, had the second highest complex formation constant. In contrast, the smaller cavity diameter of α-CD may not allow for the same depth of penetration of the complexation-important moiety of **1** resulting in less attractive interactions between **1** and cyclodextrin, whereas the larger cavity diameter of γ-CD should allow for a greater depth of penetration. However, for γ-CD, the larger cavity size may not allow for the same degree of interaction between the complexation-important moiety of **1** and the atoms comprising the cyclodextrin cavity. Similar observations, where β-CD was found to have superior complexing ability, have been made with other compounds such as indomethacin (Backensfeld et al., 1990), salbutamol (Cabral Marques et al., 1990), and xanthone (Barra et al., 1990).

Non-covalent catalysis by cyclodextrins can occur via two mechanisms (Griffiths and Bender,



Scheme 3.

1973b): conformational effects and microsolvent effects. The rate enhancements observed for the intramolecular lactamization of **1** in the presence of the cyclodextrins can be explained by conformational effects where the geometrical requirements imposed upon **1** as a result of the inclusion process may facilitate the approach of the amino group to the carboxyl carbon by effectively locking **1** into a reactive conformation. A simplified depiction is shown in Scheme 3. The catalysis arises when the cyclodextrins preferentially complex with a reactive orientational conformer and, thereby, shift the equilibrium between the various orientational conformers of **1** in favor of the most reactive conformer since it can now exist in a free and in a cyclodextrin-bound state. Based on this reasoning, the magnitude of the rate acceleration due to complexation with the cyclodextrins may be accounted for, to a large extent, by the free energy change associated with the freezing of an internal rotation which is about 0.90 kcal/mol for the formation of a five-membered ring (Page and Jencks, 1971). This would account for a rate change of about 4-fold at 80 °C which is in reasonable agreement with many of the observed rate enhancement factors shown in Table 1. If more than one internal rotation is lost then the degree of rate enhancement would be even greater.

Another possibility is that **1** existing as the complex may facilitate the breakdown of the tetrahedral intermediate to the lactam product since the microscopic rate constants associated with both steps of the lactamization mechanism (Scheme 2) would comprise the experimentally observed, macroscopic rate constant if the second step is truly rate-limiting.

Conformational catalysis has been used to explain the changes in reaction rates seen with the intramolecular transesterification of betamethasone-17-valerate (Andersen and Bundgaard, 1984) and 2-hydroxymethyl-4-nitrophenyl trimethylacetate (Griffiths and Bender, 1973a) in the presence of cyclodextrins. The former compound exhibited an increased rate in the presence of β-CD, a decreased rate in the presence of γ-CD, and an unchanged rate in the presence of α-CD, whereas the latter compound exhibited a 6-fold increase and a 5-fold decrease in the rate of transesterification in the presence of α- and β-CD, respectively.

In the case of **1**, a microsolvent effect, which is derived from the relatively apolar properties of the CD cavity, might also play a role in the cyclodextrin-induced catalysis. However, the fact that the cyclodextrin-catalyzed reaction was pH-dependent negates an exclusive microsolvent effect (Griffiths and Bender, 1973b). Also, over the pH range studied, covalent participation by the cyclodextrin hydroxyl groups would not be expected to play a major role in the observed catalysis (Griffiths and Bender, 1973b).

The differences observed in the extent of the rate enhancement due to the various cyclodextrins (i.e., β-> HPβ-> α-$\approx$ γ-CD) can be attributed to differences in the degree of complex formation between **1** and the various cyclodextrins as denoted by $K_{1:1}$ of Eqn 10 and to differences in the dimensions of the cyclodextrin cavities which might affect the depth of inclusion, the orientation of the reactive moieties, and ultimately, the k_{SL} term of Eqn 10. The results (Table 3) suggest that the dimensions of the β-cyclodextrin and the comparably sized HPβ-cyclodextrin cavities may be close to the ideal size for catalysis to occur since both $K_{1:1}$ and k_{SL} were the largest for these two cyclodextrins.

The differences observed in the catalytic efficiencies of the acid form of the acetate buffer in the presence of the various cyclodextrins (Table 2) followed the same trend as the lactamization rate enhancement due to the various cyclodextrins (i.e., β-> HPβ-> α-$\approx$ γ-CD). These results may suggest that the reactive conformer of **1** which preferentially complexed with each of the

cyclodextrins had a more favorable orientation for catalysis by the buffer or that the buffer may also interact (e.g. via hydrogen bonding with the circumferential hydroxyl groups) with the cyclodextrin thereby localizing the two reactive molecules, **1** and the buffer.

Conclusion

Although attempts were made to slow the rate of intramolecular aminolysis of gabapentin by the formation of an inclusion complex with various cyclodextrins, none of the cyclodextrins investigated stabilized the compound. The results of our studies, where the rate of lactamization of gabapentin in the presence of various cyclodextrins was only accelerated, could be explained by the conformational catalysis of an inclusion complex.

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References

Abdallah, J.M. and Moodie, R.B., Kinetics and equilibria of ring closure through an amide linkage. 2. 1-Aryl-2-pyrrolidones. *J. Chem Soc. Perkin Trans.*, II (1983) 1243–1249.

Albert, A. and Serjeant, E.P., *Ionization Constants of Acids and Bases*, 2nd Edn, T.&A. Constable, Edinburgh, 1971.

Andersen, F.M. and Bundgaard, H., The influence of cyclodextrin complexation on the stability of betamethasone-17-valerate. *Int. J. Pharm.*, 20 (1984) 155–162.

Backensfeld, T., Muller, B.W., Wiese, M. and Seydel, K., Effect of cyclodextrin derivatives on indomethacin stability in aqueous solution. *Pharm. Res.*, 7 (1990) 484–490.

Barra, M., Bohne, C. and Scaiano, J.C., Effect of cyclodextrin complexation on the photochemistry of xanthone. Absolute measurement of the kinetics for triplet-state exit. *J. Am. Chem. Soc.*, 112 (1990) 8075–8079.

Cabral Marques, H.M., Hadgraft, J. and Kellaway, I.W., Studies of cyclodextrin inclusion complexes. I. The salbutamol-cyclodextrin complex as studied by phase solubility and DSC. *Int. J. Pharm.*, 63 (1990) 259–266.

Camilleri, P., Ellul, R, Kirby, A.J. and Mujahid, T.G., The spontaneous formation of amides. The mechanism of lactam formation from 3-(2-aminophenyl)propionic acid. *J. Chem. Soc. Perkin II* (1979) 1617–1620.

Connors, K.A. and Lipari, J.M., Effect of cycloamyloses on the apparent dissociation constants of carboxylic acids and phenols: Equilibrium analytical selectivity induced by complex formation. *J. Pharm. Sci.*, 65 (1976) 379–383.

Griffiths, D.W. and Bender, M.L., Orientational catalysis by cyclohexaamylose. *J. Am. Chem. Soc.*, 95 (1973a) 1679–1680.

Griffiths, D.W. and Bender, M.L., Cycloamyloses as catalysts. *Adv. Catal.*, 23 (1973b) 209–261.

Jencks, W.P., *Catalysis in Chemistry and Enzymology*, Dover, New York, 1987, p. 212, 497.

Kirby, A.J. and Fersht, A.R., Intramolecular catalysis. *Prog. Bioorg. Chem.*, 1 (1971) 1–82.

Kirby, A.J., Effective molarities for intramolecular reactions. *Adv. Phys. Org. Chem.*, 17 (1980) 183–278.

Martin, A., Swarbrick, J. and Cammarata, A., *Physical Pharmacy*, 3rd Edn, Lea & Febiger, Philadelphia, 1983, p. 194.

Page, M.I. and Jencks, W.P., Entropic contributions to rate accelerations in enzymic and intramolecular reactions and the chelate effect. *Proc. Natl. Acad. Sci. USA*, 68 (1971) 1678–1683.

Seno, M., Lin, M. and Iwamoto, K., Chromatographic behaviour of cyclodextrin complexes of nucleotides, nucleosides and their bases. *J. Chromatogr.*, 523 (1990) 293–299.