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Influence of Various Cyclodextrins on the Stability of Hydrocortisone 17-Butyrate in Aqueous Solution

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Abstract □ The stabilizing effects of α-, β-, γ- and dimethyl-β-cyclodextrins (α-, β-, γ- and DM-β-CyDs) on the degradation of hydrocortisone 17-butyrate (HC-17B) in aqueous solution was investigated. HC-17B underwent a facile hydroxide ion-catalyzed rearrangement to the less active 21-butyrate ester by the apparent first-order kinetics, and maximum stability of HC-17B was obtained at around pH 4.0. The stability of HC-17B was increased by inclusion complexation with α-, γ- and DM-β-CyD in the pH range of 2.0-8.0 examined, whereas β-CyD accelerated the degradation of HC-17B at the pH higher than 5.0. The effects of ionic strength, solvent, temperature and CyD concentration were also investigated. Stability constants and apparent degradation rate constants of HC-17B-γ-CyD and HC-17B-DM-β-CyD complexes were determined kinetically on the basis of 1 : 1 complexation. The results suggested that the inclusion complexation with γ-CyD or DM-β-CyD was most useful means to enhance the stability of the steroid.

Keywords □ Hydrocortisone butyrate, Cyclodextrins, Rearrangement, Stabilization, Inclusion complex, pH effect, Solvent, Ionic strength, Stability constant

Cyclodextrins (CyDs) are known to form inclusion complexes with drugs either in aqueous solution or in the solid phase, and have recently received considerable attention in improvement of physico- and bio-pharmaceutical properties such as poor aqueous solubility, low dissolution characteristics, chemical instability or low bioavailability of various drugs[1,2]. Although β-CyD is now commercially available and has been used widely, it has a limited solubility (1.85 g/100 m*l*, 25℃). Recently dimethyl-β-cyclodextrin (DM-β-CyD), which is a modified cyclodextrin, has received great attention due to its higher aqueous solubility (57 g/100 m*l*, 25℃) and complexing ability than the parent β-CyD[3].

Hydrocortisone 17-butyrate (HC-17B) is available in various formulations for topical application and it has been shown that the ester is many times more active than the parent alcohol, hydrocortisone (HC)[4]. Steroid-17-esters, however, is known to readily rearrange to the thermodynamically stable but topically less active 21-esters under nonideal conditions[5-8]. Previous studies showed that HC-17B underwent acyl migration to the 21-ester followed by the hydrolysis of this ester to HC[9,10]. From our previous study[11], it was found that HC-17B forms inclusion complexes with various CyDs in aqueous solution.

In this work, the effects of α-, β-, γ- and DM-β-CyDs on the degradation of HC-17B in the aqueous solution were investigated kinetically in an attempt to explore the possibility of improving chemical stability of the steroid by inclusion complexation. Furthermore, the effects of pH, ionic strength, solvent, storage temperature and CyD concentration on the degradation of HC-17B were also studied to gain insight into the stabilization mechanism of HC-17B by CyD complexation.

EXPERIMENTAL METHODS

Materials

HC-17B, HC and DM-β-CyD were purchased from Sigma Chemical Co. α-, β- and γ-CyDs were

obtained from Tokyo Kasei Co., Ltd. and used after drying at 105℃ to constant weight. Hydrocortisone 21-butyrate (HC-21B), which was synthesized according to the method of Kawano *et al.*,[10] was used. Water and methanol were HPLC grade (E. Merck), and all other materials were of analytical reagent grade.

Kinetic studies

HC-17B was dissolved in acetate buffer solutions having different pH values (pH 2.0-8.0) and adjusted to an ionic strength (μ) of 0.4 with sodium chloride. The final concentration of HC-17B in all solutions was 9.66×10^{-5} M. Five m*l* of HC-17B solution with and without 1.0×10^{-2} M CyDs were incubated at 25, 35 and 42℃. At timed intervals, 10 μ*l* of sample solution were withdrawn with microsyringe, and subjected to HPLC analysis after mixing with equal volume of methanolic butyl-*p*-hydroxybenzoate solution (10 μg/m*l*) as the internal standard. Apparent first-order rate constants (k_{obs}) for the disappearance of HC-17B were determined from the slopes of linear plots of the logarithm of residual ester with time.

HPLC analysis

For the separative analysis of HC-17B and its hydrolytic products, the HPLC method was newly established by modifying previous methods[12,13]. The chromatograph (Shimadzu LC-6A) was operated at flow rate of 1.0 m*l*/min, and the eluent was monitored spectrophotometrically at 254 nm. The separation utilized a column, Shim Pack (LC-ODS, 5 μm in 4.6 mm×15 cm), with the mixture (63 : 37 v/v) of methanol and water as a mobile phase. Components were quantitated by measuring the peak area and comparing the area with that of known amount of internal standard (IS). One typical separation of standard mixture of HC. IS, HC-17B and HC-21B using HPLC is shown in Fig. 1A and the chromatogram of sample solution (pH 8.0) stored at 42℃ for 3 hr is seen in Fig. 1B. HC, IS, HC-17B and HC-21B were satisfactorily separated in the HPLC at the retention times of 4.86, 5.82, 11.25 and 13.88 min, respectively. Calibration plots for HC-17B, HC-21B and HC gave good linearities (r= 0.999).

RESULTS AND DISCUSSION

Effects of pH and cyclodextrins

Fig. 2 shows the linear relationship of the logarithm of the residual HC-17B with time in aqueous buffered solutions (pH 2.0-8.0). For all solutions stu-

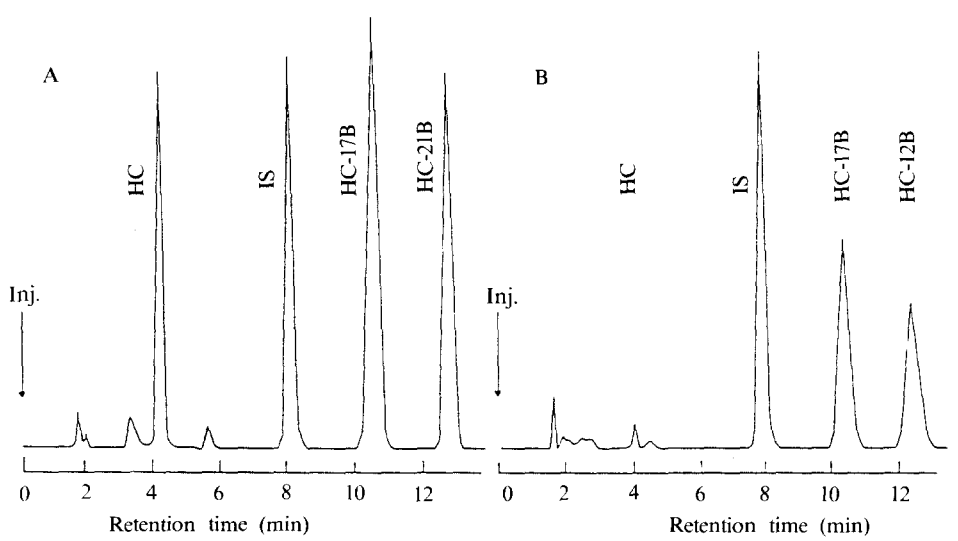


Fig. 1. HPLC chromatograms of the mixture containing hydrocortisone (HC), hydrocortisone 17-butyrate (HC-17B), hydrocortisone 21-butyrate (HC-21B) and butyl *p*-hydroxybenzoate (IS).
A : standard mixture, B : sample solution after 3 hr at pH 8.0 and 42℃.

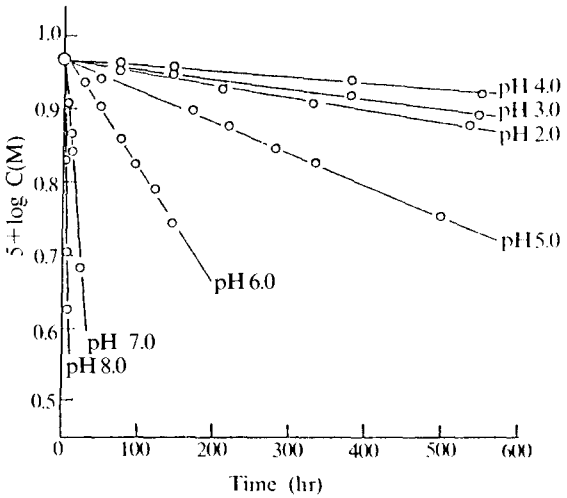


Fig. 2. Apparent first-order degradation of HC-17B in various buffered solutions at 35°C.

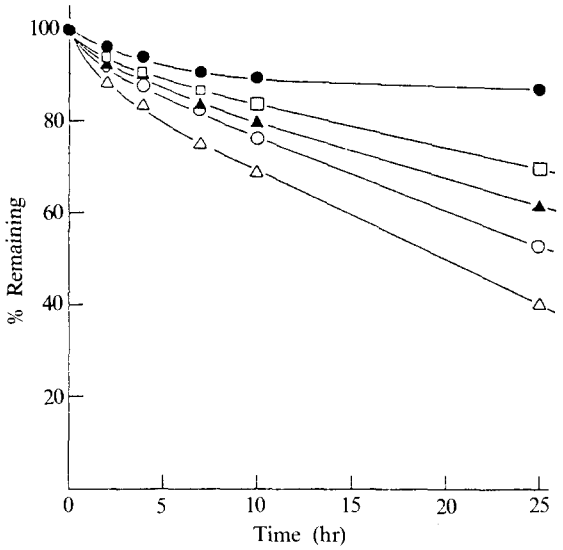


Fig. 3. Effect of CyDs on the degradation of HC-17B at pH 7.0 and 35°C.
○, HC-17B alone; ▲, α-CyD; △, β-CyD; □, γ-CyD; ●, DM-β-CyD.

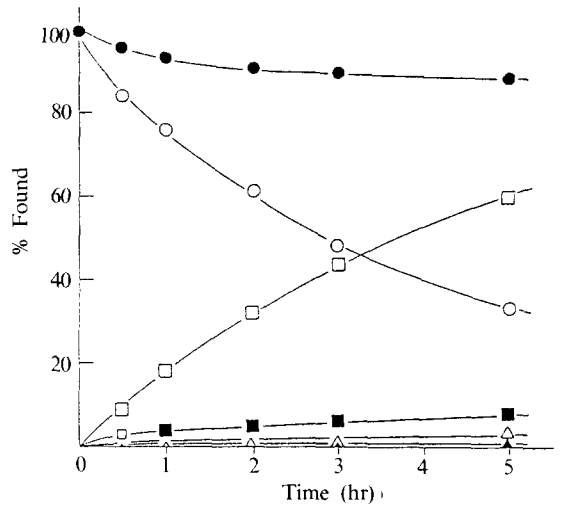


Fig. 4. Disappearance of HC-17B and formation of HC-21B and HC in the absence (open symbols) and presence (closed symbols) of DM-β-CyD at pH 8.0 and 42°C.
○, HC-17B; □, HC-21B; △, HC.

died, the disappearance of HC-17B displayed strict first-order kinetic behavior. Typical effects of α-, β-, γ and DM-β-CyDs on the degradation of HC-17B at pH 7.0 are shown in Fig. 3. The degradation of HC-17B at pH 7.0 was suppressed by α-, γ- and DM-β-CyDs, but accelerated by β-CyD. The decrease of HC-17B in neutral and alkaline buffer solutions was in all cases accompanied by the formation of HC-21B and HC in stoichiometric amounts as revealed by HPLC analysis. An example is shown in Fig. 4. Thus the complexation with CyDs did not seem to influence the degradation mechanism of HC-17B, this being exclusively a 17→21 acyl group migration as described previously.

The logarithms of apparent first-order rate constants in the absence and presence of CyDs were plotted against pH as shown in Fig. 5. In the absence of CyDs, the degradation rates of HC-17B increased with increasing pH in the neutral and alkaline regions, and with decreasing pH in the acidic region, showing the V-shaped pH-profile. The maximum stability was observed at around pH 4.0. This result was in good agreement with other reports[9,10]. In the presence of CyDs, the degradation rates decreased in the order of α->γ->DM-β-CyD at the pH lower than 5.0. However, in the pH region above 5.0, β-CyD accelerated the degradation instead, as examplified in Figs. 3 and 5. The stabilization extent of HC-17B with three CyDs at the pH lower than 5.0 became smaller in the order of DM-β-CyD>γ->α-CyD. The different stabilization effect of CyDs might be due to their different modes of inclusion with HC-17B.

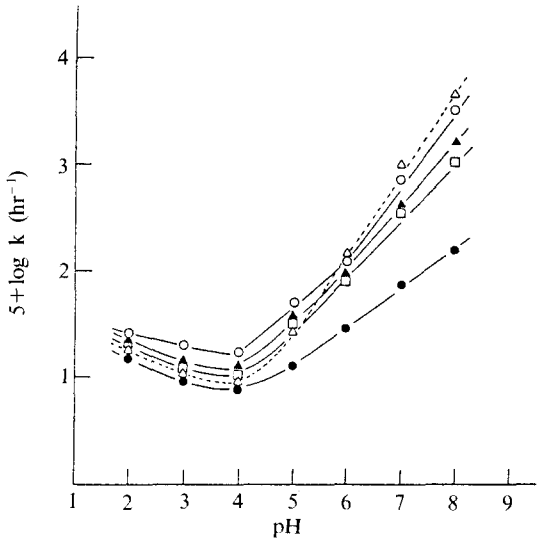


Fig. 5. Log k-pH profiles for the degradation of HC-17B in the absence and presence of CyDs at 25°C. ○, HC-17B alone; ▲, α-CyD; △, β-CyD; □, γ-CyD; ●, DM-β-CyD.

Acidic Region:

HC-17B $\xrightarrow{k_1}$ HC-21B $\xrightarrow{k_2}$ HC $\xrightarrow{k_3}$ Unknown Substances

HC-17B $\xrightarrow{k_4}$ Unknown Substances

Neutral Region:

HC-17B $\xrightarrow{k_1}$ HC-21B $\xrightarrow{k_2}$ HC $\xrightarrow{k_3}$ Unknown Substance

Alkaline Region:

HC-17B $\underset{k_1}{\overset{k_5}{\rightleftharpoons}}$ HC-21B $\xrightarrow{k_2}$ HC $\xrightarrow{k_3}$ Unknown Substances

HC-17B $\xrightarrow{k_6}$ Unknown Substances

Scheme 1. Degradation pathway and extent of HC-17B in acidic, neutral and alkaline regions (Lit. 10).

In our previous study[11] using ^{1}H-NMR, it was suggested that DM-β-CyD includes the butyl ester at C_{17} of HC-17B molecule and γ-CyD includes over C_{21} and C-ring of the steroid, whereas β-CyD includes preferentially A-ring of HC-17B. In contrast, α-CyD did not exhibit significant interaction with HC-17B, based on circular dichroism studies. These results support that γ-CyD as well as DM-β-CyD seem to have a pronounced stabilizing effect against the degradation of HC-17B, since the migration of butyl group at C_{17} to C_{21}, which is known to be the major degradation pathway in the neutral and alkaline regions, is inhibited by the inclusion of this butyl group. When the butyl ester of HC-17B is included into the cavity of CyDs, the nucleophilic attack on the ester will be hindered and the degradation will be retarded. The less effect of α-CyD on the stability of HC-17B may most likely be ascribed to its poor complexing ability.

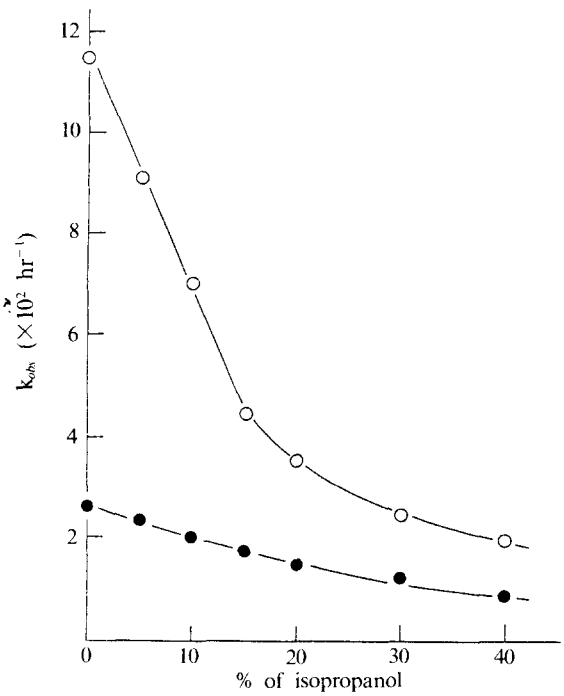
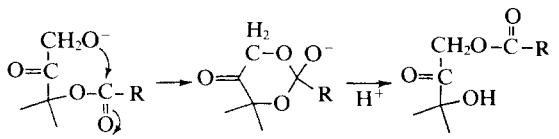


Fig. 6. Effect of isopropanol concentration on the rate of degradation of HC-17B in the absence (○) and presence (●) of DM-β-CyD at pH 8.0 and 35°C.

As shown in Scheme 1[10], in the acidic region HC-17B is degraded into unknown products in the faster rate than that in which HC-17B migrates into HC-21B. Therefore the inclusion of A-ring by β-CyD seems to protect HC-17B from the degradation into unknown products in the acidic region, and to facilitate the rearrangement of HC-17B to the 21-butyrate ester in the neutral and alkaline regions[10], resulting in the acceleration of degradation reaction.

Based on solubility studies[11], we recently determi-



Scheme 2. Possible intermediate step for the rearrangement.

ned the apparent 1 : 1 complex formation constants for HC-17B to be 282 (α-CyD), 1782 (β-CyD), 2561 (γ-CyD) and 6122 (DM-β-CyD) M^{-1} at 37℃. Apparently the larger the cavity of natural CyDs, the more favorable is the fit of the steroid ester. The strength of the inclusion complexes is, however, not the only factor influencing the stability of HC-17B. Thus the rate effect of β-CyD is quite opposite to those of γ-CyD and DM-β-CyD. The accelerating (non-covalent catalytic) or decelerating effect observed upon CyD inclusion complexation may arise from a microsolvent effect derived from the relatively apolar properties of the microscopic CyD cavity and/or conformational effects derived from the geometrical requirements of the inclusion process.

Effect of solvent and ionic strength

To evaluate the importance of the microsolvent effect, the rate of degradation of HC-17B in the absence and presence of DM-β-CyD was determined in acetate buffer containing varing amounts of isopropanol. Under these experimental conditions, the pH and ionic strength were maintained constant (pH 8.0, μ=0.4). As seen from Fig. 6, isopropanol causes a marked rate deceleration. In the absence of CyD, the apparent rate constant of HC-17B decreased markedly, and in the presence of DM-β-CyD decreased gradually with increasing isopropanol in aqueous acetate buffer solution of pH 8.0, indicating a decrease of the rate as the polarity of the solution decreased. Consequently, a microsolvent effect may be implicated but evidently, this does not explain the reversal in the effect of β-CyD and DM-β-CyD. A plausable mechanism of the acyl group migration involves a cyclic ortho ester as an intermediate, formed by a nucleophilic attact by a C_{21}-alkoxide ion upon the C_{17}-ester carbonyl moiety (Scheme 2). From this mechanism a number of different ways can be imagined by which the inclusion complexation may affect the rate of ester rearrangement, e.g., via a conformational effect

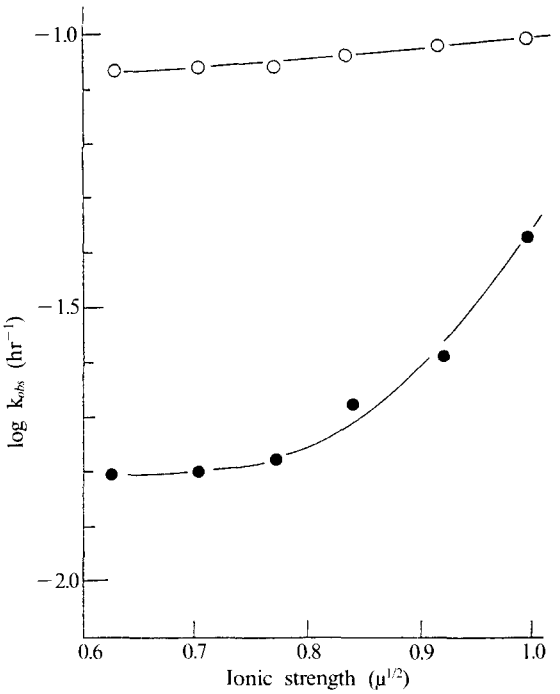


Fig. 7. Effect of ionic strength on the rate of degradation of HC-17B in the absence (○) and presence (●) of DM-β-CyD at pH 8.0 and 35℃.

which orients the C_{21}-hydroxymethyl group in a position facilitating attack at the C_{17} acyl moiety or, in the case of the deceleration, inclusion of the C_{17} acyl group within the CyD cavity affording protection against the attack from the C_{21} hydroxyl group. The former explanation may support the acceleration of degradation of HC-17B by β-CyD and the latter may deal with the stabilizing effect of DM-β-CyD. Similar rearrangement and reversal in the effect of CyDs were reported for the betamethasone-17-valerate[14].

The effect of ionic strength on the degradation rate is shown in Fig. 7. In the absence of DM-β-CyD, only a small effect was noted presumably due to the overriding buffer catalysis. In the presence of DM-β-CyD, however, the rate constants became hyperbolically greater and approached the rate constant of uncomplexed HC-17B with increasing ionic strength. This indicates that the increase of ionic strength primarily accelerated the dissociation of HC-17B-DM-β-CyD complex, resulting in the increase of uncomplexed HC-17B. However, the possibility that salt effect upon the hydroxide ion-cataly-

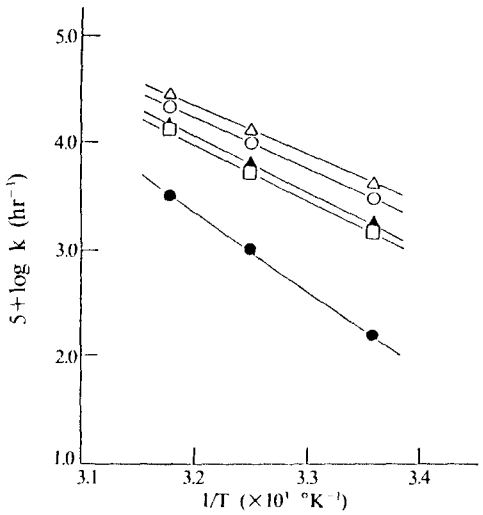


Fig. 8. Arrhenius plots for the degradation of HC-17B in the absence and presence of CyDs at pH 8.0.
○, HC-17B alone; ▲, α-CyD; △, β-CyD; □, γ-CyD; ●, DM-β-CyD.

Table I. Thermodynamic activation energies (E_a) for the degradation of HC-17B in various acetate buffer solutions in the absence and presence of CyDs

pH	E_a (kcal/mole)				
	With no CyD	α-CyD	β-CyD	γ-CyD	DM-β-CyD
6.0	21.5	24.0	20.5	24.0	24.2
7.0	29.1	32.4	31.1	33.1	33.9
8.0	30.3	300.8	28.3	30.1	33.9

zed rearrangement may be promoted by the inclusion complexation should be considered.

Effect of temperature

The effects of storage temperature on the degradation rate were investigated. Fig. 8 shows Arrhenius plots of the degradation rates of HC-17B at pH 8.0 in the absence and presence of CyDs over a temperature range of 25-42℃. Similarly, the Arrhenius relationship held well at pH 6.0 and 7.0 over the same temperature range. Table I summarizes the thermodynamic activation energies, where the activation energy change by the inclusion is not significant, which may also support the absence of the specific degradation mechanism in the complexed ester[15].

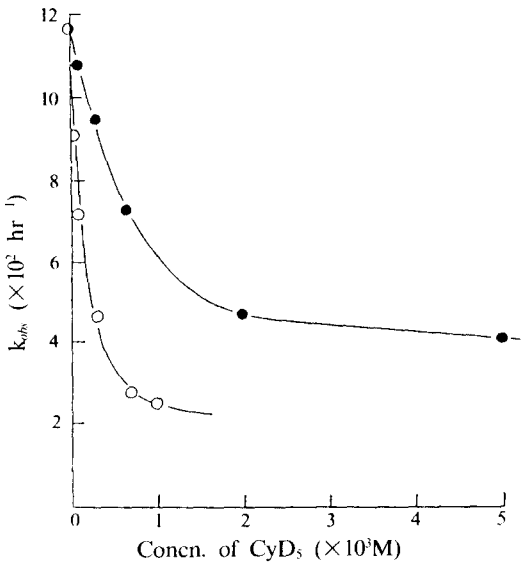


Fig. 9. Observed rate constants for the degradation of HC-17B as a function of CyD concentration in pH 8.0 acetate buffer at 35°C.
◑, HC-17B alone; ●, γ-CyD; ○, DM-β-CyD.

Effect of CyD concentration

Fig. 9 shows the effects of γ- and DM-β-CyD concentrations on the observed disappearance rate constant (k_{obs}) of HC-17B in the acetate buffer (pH 8.0, μ=0.4) at 35℃. The degradation rates decreased hyperbolically with increasing CyD concentration, showing characteristic saturation kinetics. The rate constant asymtotically approaches a minimum value with increasing CyD concentration. This saturation behavior is characteristic of reactions which proceed through a complex prior to the rate-determining step. The dependency of k_{obs} on the CyD concentration was quantitatively treated by Eq. (1)[16] to obtain the apparent stability constant (K_c) and the rate constant (k_c) of the complex, assuming the 1 : 1 complexation scheme (Scheme 3), where k_o and $[CyD]_t$ are the apparent rate constant in the absence of CyDs and the total concentration of CyD, respectively.

$$\frac{[CyD]_t}{k_o - k_{obs}} = \frac{1}{k_o - k_c}[CyD]_t + \frac{1}{K_c(k_o - k_c)} \quad (1)$$

Fig. 10 shows the plots of the data derived from Eq. (1) based on the data of Fig. 9. A linear re-

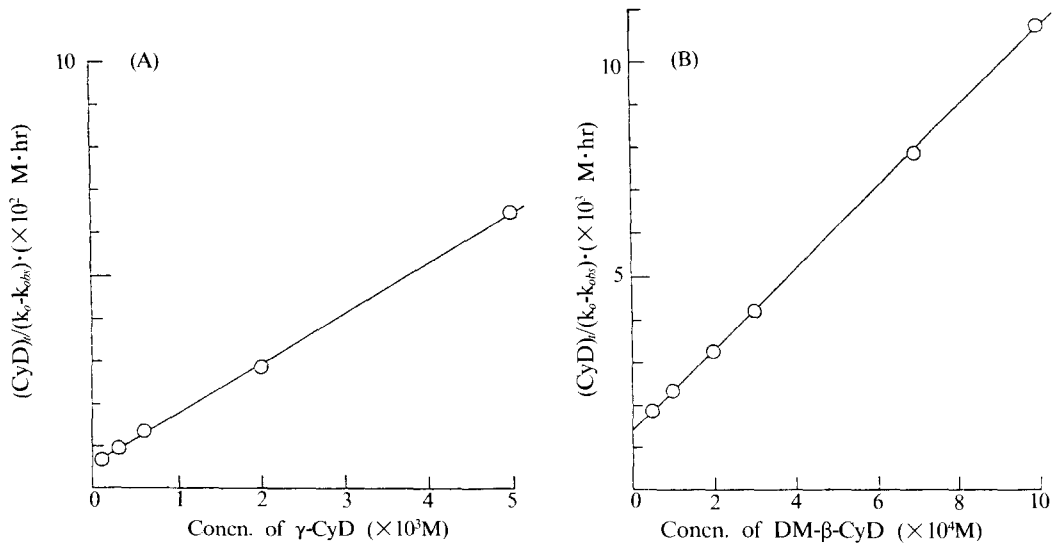
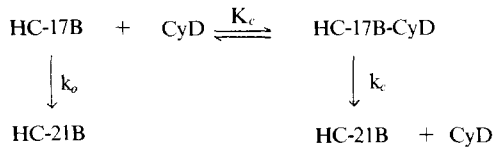


Fig. 10. Determination of K_c and k_c for HC-17B-γ-CyD (A) and HC-17B-DM-β-CyD (B) complexes by plotting kinetic data (Fig. 9) according to Eq. (1).



Scheme 3. Reaction mechanism of the degradation of HC-17B-CyD complex system.

Table II. Apparent first-order rate constants (hr^{-1}) and stability constants (K_c, M^{-1}) of HC-17B-CyD systems at pH 8.0 and 35°C

System	k_o	k_c	k_c/k_o	K_c
HC-17B alone	0.117	–	–	–
HC-17B-γ-CyD	–	0.033	0.285	2410
HC-17B-DM-β-CyD	–	0.011	0.009	6640

lationship having a correlation coefficient (r) of over 0.99 was obtained for the HC-17B-γ-CyD and HC-17B-DM-β-CyD systems, confirming 1 : 1 complexation (Scheme 3). From the slope and intercept of those plots, values of K_c and k_c were calculated. Table II summarizes the results on k_o, k_c, k_c/k_o, and K_c. The K_c values were found for γ- and DM-β-CyD complexes to be 2410 and 6640 M^{-1}, respectively. These values were fairly comparable with the previous results obtained from the solubility and spectroscopic methods[11]. The different K_c values with CyDs indicate that the spacial relationship between the host and guest molecules, together with the hydrophobicity of the binding sites, may play an important role in the complexation. On comparison with the k_c/k_o values, the deceleration effect of DM-β-CyD on the rearrangement of HC-17B was found to be three times larger than that of γ-CyD, indicating a higher affinity of HC-17B to DM-β-CyD. In the presence of DM-β-CyD, the degradation rate of HC-17B decreased above 13 times, comparing with that of HC-17B alone.

In conclusion, the results obtained demonstrate that the base-catalyzed rearrangement of HC-17B to the 21-butyrate ester in aqueous solution can be suppressed by α-, γ- and DM-β-CyDs. Due to the strong complexation with the γ- and DM-β-CyDs, the rate-deceleration effect is pronounced even at very low CyD concentrations. Therefore inclusion complexation of HC-17B with these CyDs may be a potentially useful means of increasing the stability of the steroid.

ACKNOWLEDGEMENT

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