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Inclusion complexation of hydrocortisone butyrate with cyclodextrins and dimethyl-β-cyclodextrin in aqueous solution and in solid state

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Summary

Inclusion complexation of hydrocortisone butyrate (HB) with α-, β-, γ- and dimethyl-β-cyclodextrins (DM-β-CyD) in aqueous solution and in solid state was investigated to examine and compare the interactions of CyDs with HB, which is practically insoluble in water, and to improve solubility and dissolution rate. Complex formation of HB with CyDs in aqueous solution was studied by solubility analysis, circular dichroism, ultraviolet absorption and ^{1}H-NMR spectroscopy. In the study of solid state interaction of HB with CyDs, equimolar HB-CyD solid complexes were prepared by the solvent evaporation method and complexation was assessed by infrared spectroscopy and thermal analysis. To elucidate the inclusion complexation, the stoichiometric ratio, which was found to be 1 : 1, apparent stability constants and thermodynamic parameters for HB-CyD systems were determined. The solubility increase of HB in the presence of CyDs in water was in the rank order of DM-β-CyD ≫ γ-CyD > β-CyD > α-CyD. The spectroscopic data suggested a different inclusion mode of HB within various CyD cavities. The dissolution rate of DM-β-CyD solid complex was extremely rapid compared with those of α-, β- and γ-CyD solid complexes.

Introduction

Cyclodextrins (CyDs) are known to form inclusion complexes with many substances of appropriate molecular size and polarity, in particular, hydrophobic drug molecules. There are numerous reports and reviews describing improvements in pharmaceutically unfavorable properties of guest drugs on CyD complexation, such as solubilization and dissolution enhancement of poorly water-soluble drugs, stabilization of chemically labile drugs, reduction of irritation, masking of unpleasant odor and taste, reduction of volatility, and enhancement of bioavailability (Uekama, 1981; Duchêne et al., 1986).

Due to its more favorable complexing ability, industrial availability and price considerations,

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β-CyD has been studied most extensively, although it has limited aqueous solubility. Chemically modified CyDs have received increased attention due to their improved complexing abilities and aqueous solubilities (Müller and Brauns, 1985; Uekama et al., 1985; Green and Guillory, 1989).

Hydrocortisone butyrate (HB) is more potent than the parent alcoholic form, hydrocortisone (Ashurst, 1972). The presence of the ester function at carbon 17 was found to improve delivery of the corticosteroid to the site of action due to the increased lipophilicity, and to increase topical potency by imparting resistance to cutaneous metabolic degradation (O'Neil and Carless, 1980). HB is formulated in various formulations such as creams, gels and lotions for topical application (Krook, 1983; Giannetti et al., 1984). However, it has limited aqueous solubility. Recently, the increased topical availability of steroids is focused in considerable research projects. Although complexation and cogrinding of many steroids with CyDs have been described previously (Uekama et al., 1982, 1985c; Frank and Kavaliunas, 1983; Lin et al., 1988; Liu et al., 1990; Torricelli et al., 1991), complexation of HB with CyD has not been characterized previously. So far few investigations have dealt with the topical administration of steroids included in natural CyDs or their derivatives (Otagiri et al., 1984; Uekama et al., 1985b, 1987; Glomot et al., 1988).

The purpose of present study was to investigate the inclusion complex formation of HB with CyDs and dimethyl-β-cyclodextrin (DM-β-CyD) in order to gain insight into the mode of interaction in aqueous solution as well as in solid state.

Materials and Methods

Materials

HB was used as supplied by Pacific Pharmaceutical Co., Ltd (Ansung, Korea). α-, β- and γ-CyDs were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan) and DM-β-CyD from Sigma Chemical Co. (St. Louis, U.S.A.) and used without further purification. All CyDs were dried over phosphorus pentoxide under vacuum to constant weight. Sigma Chemical Co. reports that DM-β-CyD contains approx. 30% of DM-β-CyD and the remainder consists of its homologues. In this work, we assumed the average molecular weight of DM-β-CyD to be 1331 g/mol, which is the molecular weight of pure DM-β-CyD. All other materials and solvents were of analytical reagent grade. Double-distilled water was used throughout the study.

Solubility studies

Excess amounts of HB (0.05 g) were added to aqueous solutions (pH 4.0, adjusted with acetic acid) containing various concentrations of CyDs and the samples were shaken at 20, 30 and 37 ± 0.3°C, until solubility equilibria were reached. After equilibration, aliquots of the supernatant were filtered through a membrane filter (0.45 μm Acrodisk, Gelman Sciences). The filtrates were then suitably diluted with water and analyzed spectrophotometrically at 247 nm. Analysis of HB and its decomposition products, which might be formed during solubility studies and the preparation of solid complexes described below, were performed using a Perkin Elmer Series 410 (4-solvent pump) with an LC-90 variable UV detector (247 nm), an LCI-100 laboratory computing integrator, and a Pecosphere-3CR C18 column (0.46 × 8.3 cm, 3 μm). The mobile phase was composed of a mixture of methanol and water (64 : 36 v/v) at a flow rate of 0.8 ml/min. Calibration plots of peak area vs concentration of HB were found to be linear over the concentration range of 1–150 μg/ml. Apparent stability constants (K_c) were calculated from the initial linear portion of phase solubility diagrams according to Eqn 1 (Higuchi and Connors, 1965):

$$K_c = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})} \quad (1)$$

Circular dichroism (CD) and ultraviolet (UV) absorption studies

The CD and UV absorption spectra were recorded with a Jasco J-20C spectropolarimeter and a Perkin-Elmer Lambda 4A spectrophotome-

ter, respectively. All measurements were carried out at 25°C using water solutions. The molar ellipticity, $[\theta]$, and the molar absorption coefficients (ϵ) were calculated on the basis of total drug concentration. The optical anisotropy factor, g value, was calculated in the same manner as reported previously (Otagiri et al., 1976). The CD and UV absorption changes of HB with increasing concentrations of α-, β-, γ- and DM-β-CyDs were determined at the maximum wavelength due to complex formation. Apparent stability constants (K_c) of HB-CyD complexes by these spectroscopic methods were calculated according to Scott's equation (Eqn 2) (Scott, 1956; Otagiri et al., 1975):

$$\frac{[G]_t[CyD]_t}{\Delta A} = \frac{1}{\epsilon_c}[CyD]_t + \frac{1}{K_c\epsilon_c} \qquad (2)$$

where $[G]_t$ is the total concentration of HB, $[CyD]_t$ denotes the total concentration of CyD, ϵ_c is the difference of the molar absorptivities for free and complexed HB, and ΔA represents the change in absorbance or ellipticity of HB on the addition of CyD.

^{1}H-NMR studies

^{1}H-NMR spectra were acquired on a Brucker FT-NMR 200 spectrometer at an ambient temperature of 25 ± 0.5°C. ^{1}H chemical shifts were automatically calibrated with a precision of 0.001 ppm. HB and CyD were dissolved in a mixture of D_2O and CD_3OD (6:4 v/v) to result in a concentration of 1×10^{-2} M at the 1:1 molar ratio.

Preparation of solid complexes

The solid complexes were prepared by evaporating 50% (v/v) ethanolic solutions of HB and CyD at a 1:1 molar ratio (Glomot et al., 1988). The pH of an aqueous solution containing CyD was adjusted to pH 4.0 with acetic acid prior to combining with ethanolic HB solution. The mixture was evaporated at 50°C under reduced pressure using a rotary evaporator. The residue obtained was dried further in vacuo to constant weight at room temperature and passed through a 100-mesh screen. HB-CyD physical mixtures were prepared by triturating powders passed through the 100-mesh screen at a 1:1 molar ratio.

IR and DSC studies

The IR spectra were measured on KBr disks using an Alpha Centauri FT-IR spectrophotometer. For DSC all measurements were carried out at a scanning speed of 10°C/min on a Dupont DSC 2000 thermal analyzer.

Determination of dissolution rates

Dissolution rates of HB from HB-CyD systems were measured in 900 ml of water by the paddle method at 50 rpm and 37 ± 0.5°C. The amount of samples used was equivalent to 50 mg of HB. At predetermined time intervals, suitable aliquots were withdrawn through a filter tip, diluted with water and assayed spectrophotometrically at 247 nm. The experiments were run in triplicate.

Results and Discussion

Solubility studies

The phase solubility diagrams obtained for HB and CyDs are shown in Fig. 1. The solubility of HB increased linearly in the concentration range of α-, β- and DM-β-CyDs used, showing A_L-type phase behavior (Higuchi and Connors, 1965). However, the γ-CyD system showed a B_S-type behavior with microcrystalline complexes precipitated at higher concentrations. The HB-γ-CyD solid complex was found to have a stoichiometry of 2:3 molar ratio by analysis of the plateau region and chemical assay. Similar solubilization patterns of HB with CyDs were also observed at 20 and 30°C. The solubilities of HB in 0.01 M α-, β-, γ- and DM-β-CyD aqueous solutions were 3.5, 11.8, 14.8 and 28.8 times that of HB alone (1.97×10^{-4} M), respectively. Increased solute solubility with the addition of CyDs is generally considered to be mainly due to the formation of inclusion complexes.

The solubility of HB, which was markedly affected by DM-β-CyD, increased with elevation of temperature, resulting in an increase in the slope of the solubility curve. This temperature depen-

dency of HB solubility was also observed for α-, β- and γ-CyD systems. This increase in the slope of the solubility curve may be related to the liberation of water molecules bound in the cavity of CyDs on elevation of temperature (Shinoda and Fujihara, 1968).

The K_c values, assuming that a 1 : 1 complex is formed at the initial step, were calculated from the linear portion of the solubility diagrams in Fig. 1. The K_c values are summarized in Table 1. The K_c values were in the rank order of DM-β-CyD > γ-CyD > β-CyD > α-CyD, indicating that DM-β-CyD is the most suitable candidate for HB molecules to form a stable complex. DM-β-CyD showed a different complexing ability from that for the unmodified CyDs, in which the larger the CyD cavity, the more favorable was the fit of steroid molecules (Uekama et al., 1982). The methylation of hydroxyls of β-CyD has the effect of expanding the hydrophobic region of the CyD by capping the cavity and thus enhances substrate binding by means of a hydrophobic effect (Green and Guillory, 1989).

The decrease in K_c values with increasing temperatures indicates the exothermic nature of inclusion complexation. Typical van't Hoff plots conform fairly well to linear behavior over the temperature range of 20–37°C (Fig. 2). Thermodynamic parameters were determined from the dependency of the K_c values on temperature in water. As seen in Table 1, inclusion complexation is predominantly due to favorable enthalpy changes which could still compensate more for the unfavorable entropy changes. For complex formation between HB and DM-β-CyD, a positive value of ΔS was observed. This type of change is usually explained in terms of the hydrophobic effect, which involves the breakdown and removal of the structured water molecules inside the CyD cavity and around the nonpolar substrate (HB). The magnitude of ΔS is usually small due to restructuring of water molecules around exposed parts of the guest molecule, once included in the cavity of the CyD. In other cases, a negative value of ΔS was obtained, suggesting that more water molecules were bound to the product than reactants (Uekama et al., 1978). This indicates that hydrophobic interactions are not the predominant factor for the inclusion of HB with unmodified CyDs, but rather, other intermolecular forces such as hydrogen bonding and dipole-dipole interactions might be responsible.

CD and UV absorption studies

The CD spectra of HB (5×10^{-5} M)-CyD (1×10^{-3} M) systems are shown in Fig. 3. The observed CD spectra of HB-CyD systems are greater

TABLE 1

Thermodynamic parameters for the complexation of HB with CyDs in water

Host compound	Temperature (K)	Apparent stability constant (K_c)	ΔF (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/degree per mol)
α-CyD	293	321	−3.361		1.77
	303	282	−3.397	−2.841	1.83
	310	249	−3.399		1.80
β-CyD	293	2688	−4.598		−3.40
	303	1782	−4.508	−5.594	−3.58
	310	1442	−4.481		−3.59
γ-CyD	293	3297	−4.717		−0.68
	303	2561	−4.726	−4.916	−0.63
	310	2067	−4.703		−0.69
DM-β-CyD	293	8293	−5.254		1.52
	303	6122	−5.251	−4.808	1.46
	310	5273	−5.280		1.52

in magnitude than that of HB alone and show positive signs in the optical activity region of HB alone. However, on the addition of α-CyD, no significant spectral change was observed, suggesting very weak interaction due to its smaller cavity for inclusion. The positive peaks of HB in the presence of three CyDs shifted to shorter wavelength with increasing magnitude of optical activity at 262 nm in the order of β-CyD > DM-β-CyD > γ-CyD. The greater difference in the CD spectra shown in the three complexes may be due to the different orientation and/or disposition of HB within the CyD cavities. The larger induced CD with β-CyD apparently suggests that the chromophore of HB is located intimately within

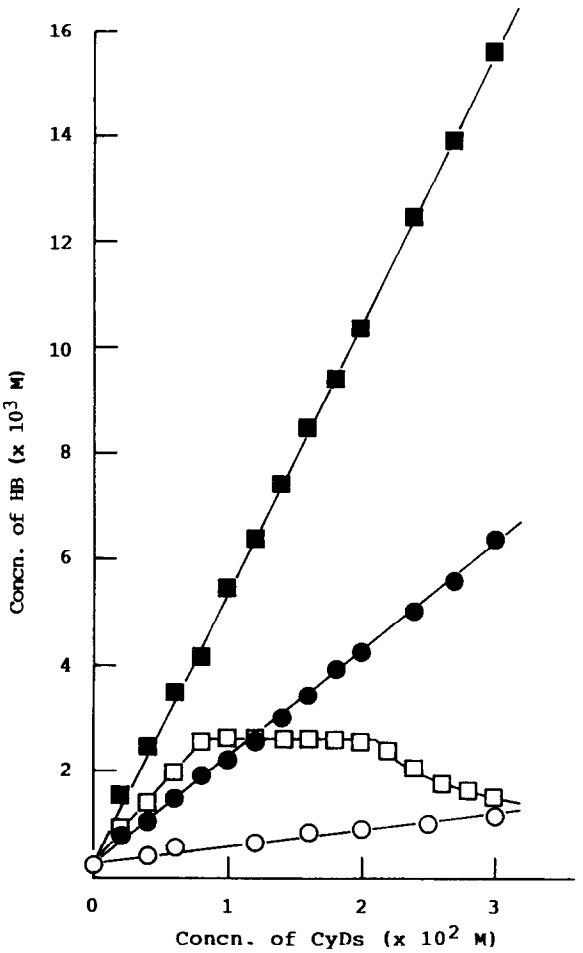

Fig. 1. Phase solubility diagrams of HB-CyD systems in water at 37°C. (○) α-CyD; (●) β-CyD; (□) γ-CyD; (■) DM-β-CyD.

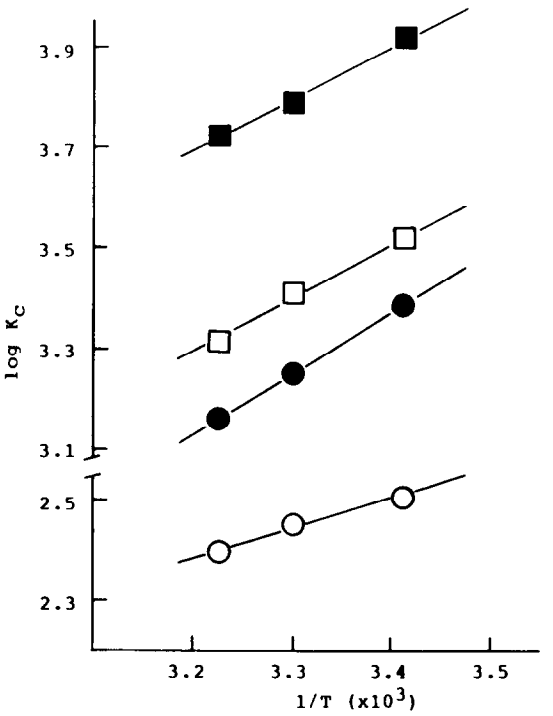

Fig. 2. Typical van't Hoff plots for apparent stability constants of HB-CyD complexes at 25°C. (○) α-CyD; (●) β-CyD; (□) γ-CyD; (■) DM-β-CyD.

the hydrophobic cavity of β-CyD, resulting in a more favorable fit.

Since intrinsic Cotton effects of CyDs are observed below 220 nm (Otagiri et al., 1975), these CD bands above 220 nm can be attributed to the optical activity of HB induced on the formation of inclusion complexes. The induced CD of the complexes is generally characterized by their sign, magnitude, and wavelength of the location. According to the symmetry rule (Schellman, 1968), the sign of the induced Cotton effect depends upon the spacial relationship between the asymmetric center and perturbed chromophore, whereas the magnitude of the extrinsic Cotton effect seems to depend on the rigidity of the complex formed (Uekama, 1978).

In order to obtain further quantitative information regarding complex formation from the CD curves generated, the effects of CyD concentration were investigated. Fig. 4 shows the effect

of β-CyD on the CD spectrum of HB (at 5×10^{-5} M), where the maximum wavelength and the magnitude of the induced CD changed concomitantly with increasing concentrations of β-CyD in the range of 7×10^{-4}–1×10^{-2} M. It is assumed that the asymmetric center of β-CyD is located in a region of the drug chromophore which makes a positive contribution to the Cotton effects. The resultant complexes are rigid enough to prevent the asymmetric center from entering a region of negative contribution. On the other hand, the HB-DM-β-CyD system showed a different tendency of the CD spectral change. As shown in Fig. 5, positively induced CD bands increased successively on increasing the DM-β-CyD concentration from 2.5×10^{-5} to 5×10^{-4} M, whereas they decreased markedly with concentrations greater than 5×10^{-4} M. In particular, on the addition of DM-β-CyD above 2×10^{-3} M,

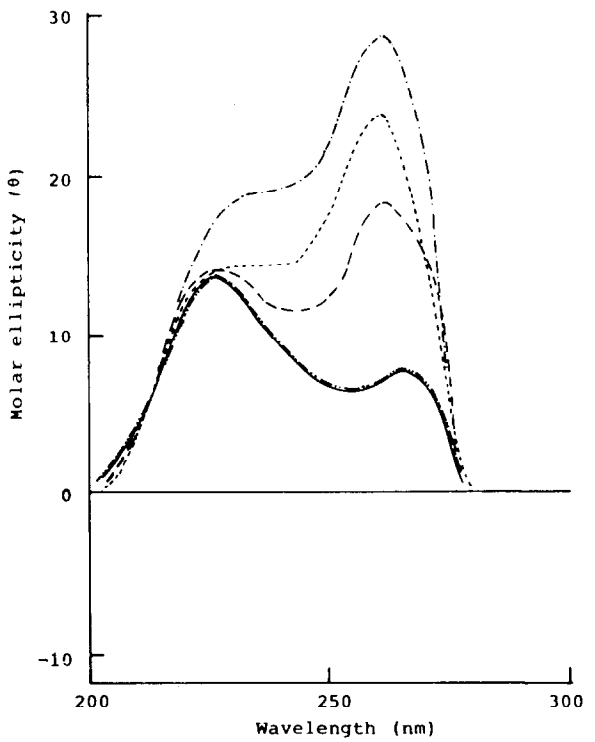

Fig. 3. CD spectra of HB in the absence and presence of CyDs in water at 25°C. The concentrations of HB and CyDs were 5×10^{-5} and 1×10^{-3} M, respectively. (———) Without CyD; (–·–·–) with α-CyD; (– – –) with γ-CyD; (------) with DM-β-CyD; (–·–·–) with β-CyD.

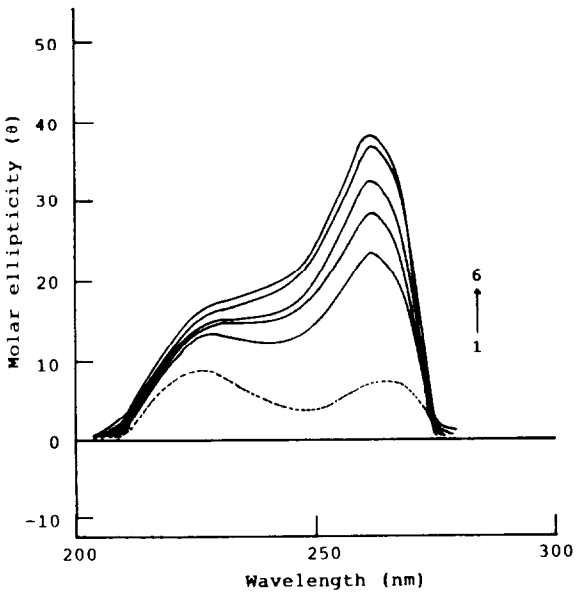

Fig. 4. Effect of β-CyD concentration on the CD spectra of HB (5×10^{-5} M) in water. The concentrations of added β-CyD were as follows: curve 1, 0 M; 2, 7×10^{-4} M; 3, 1×10^{-3} M; 4, 2×10^{-3} M; 5, 5×10^{-3} M; 6, 1×10^{-2} M.

HB exhibited an increasing negative peak around 277 nm and a decreasing positive peak around 258 nm. This fact may explain the distinctively different inclusion mode in the binding region apart from the HB-β-CyD system.

Similarly, a γ-CyD increase induced a CD spectrum with increasing γ-CyD concentration from 2.5×10^{-5} to 5×10^{-4} M, but decreased significantly above 5×10^{-4} M (Fig. 6). The CD spectral changes for HB-γ-CyD systems were far smaller than those obtained for HB-DM-β-CyD systems, and showed no negative sign.

In contrast to β-, γ- and DM-β-CyDs, α-CyD showed no appreciable CD change even at higher concentrations, indicating that the cavity size of α-CyD is not large enough to include the bulky guest molecule. However, the solubilization effect of α-CyD on HB might be due to interaction with HB through weak intermolecular hydrogen bonding from the outside or the entrance side of the cavity (Uekama et al., 1985c).

Table 2 summarizes spectral characteristics such as the λ_{max}, ϵ, $[\theta]$ and g values obtained for HB bound to CyDs, where the differences men-

tioned above are quantitatively shown. The optical anisotropy factor (g), which is proportional to the induced Cotton effect, decreases in the order of β-CyD > DM-β-CyD > γ-CyD. The order in the magnitude of g values was different from those of the solubilizing effects of CyDs (Fig. 1).

The effect of DM-β-CyD on the UV spectrum of HB was observed by increasing the concentration of DM-β-CyD, indicating that the absorption maximum of HB shifted to shorter wavelength and the absorption intensity of HB decreased concomitantly (Table 2). Similar UV spectral changes were also observed for HB-β-CyD and HB-γ-CyD systems. However, the HB-α-CyD system did not show any significant UV spectral change. This also demonstrates that the smaller cavity of α-CyD is insufficient for inclusion as described above for the CD studies.

From the continuous variation plots of UV absorbance change, it was found that HB forms a 1:1 complex with DM-β-CyD (Fig. 7). CD and UV absorption changes were used to calculate the K_c values. Fig. 8 illustrates a typical Scott plot of the ellipticity changes for HB-β-CyD and HB-DM-β-CyD systems. Table 3 summarizes the K_c values determined via CD and UV methods. In every case, the K_c values were similar to those found using the solubility method.

NMR studies on inclusion complexation

The chemical shift changes following the interaction between HB and CyDs were examined by high-resolution ^{1}H-NMR. ^{1}H-NMR peaks of both HB and CyDs were assigned according to the integration area and on the basis of previous work (Demarco and Thakkar, 1970; Ueda and Nagai, 1980; Florey, 1983). Table 4 summarizes the HB-induced ^{1}H chemical shifts of CyDs. It is clearly evident that HB caused an upfield displacement of all signals in β-CyD. In the case of γ-CyD, the signals of protons located around the interior of the CyD cavity (e.g., C_3-H, C_5-H or

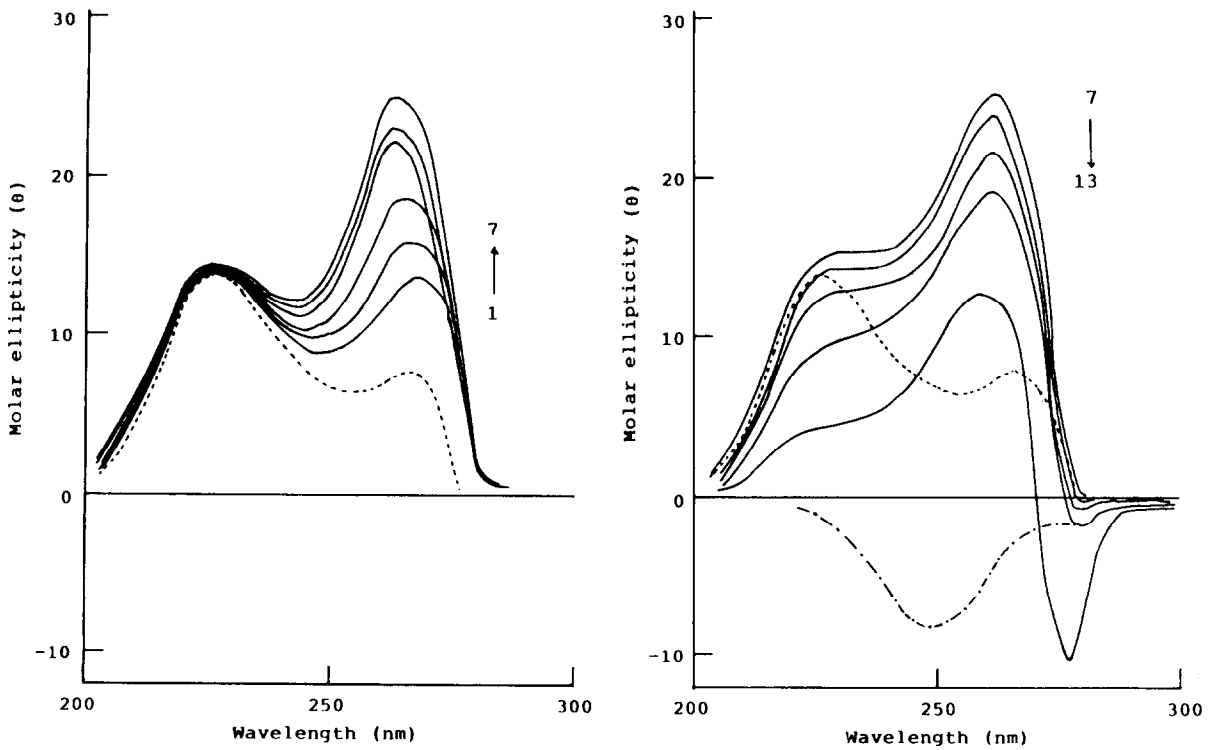

Fig. 5. Effect of DM-β-CyD concentration on the CD spectra of HB (5×10^{-5} M) in water. The concentrations of added DM-β-CyD were as follows: curves 1 and 12, 0 M; 2, 2.5×10^{-5} M; 3, 5×10^{-5} M; 4, 1×10^{-4} M; 5, 2×10^{-4} M; 6, 3×10^{-4} M; 7, 5×10^{-4} M; 8, 1×10^{-3} M; 9, 2×10^{-3} M; 10, 5×10^{-3} M; 11, 1×10^{-2} M; 13, DM-β-CyD alone (1×10^{-2} M).

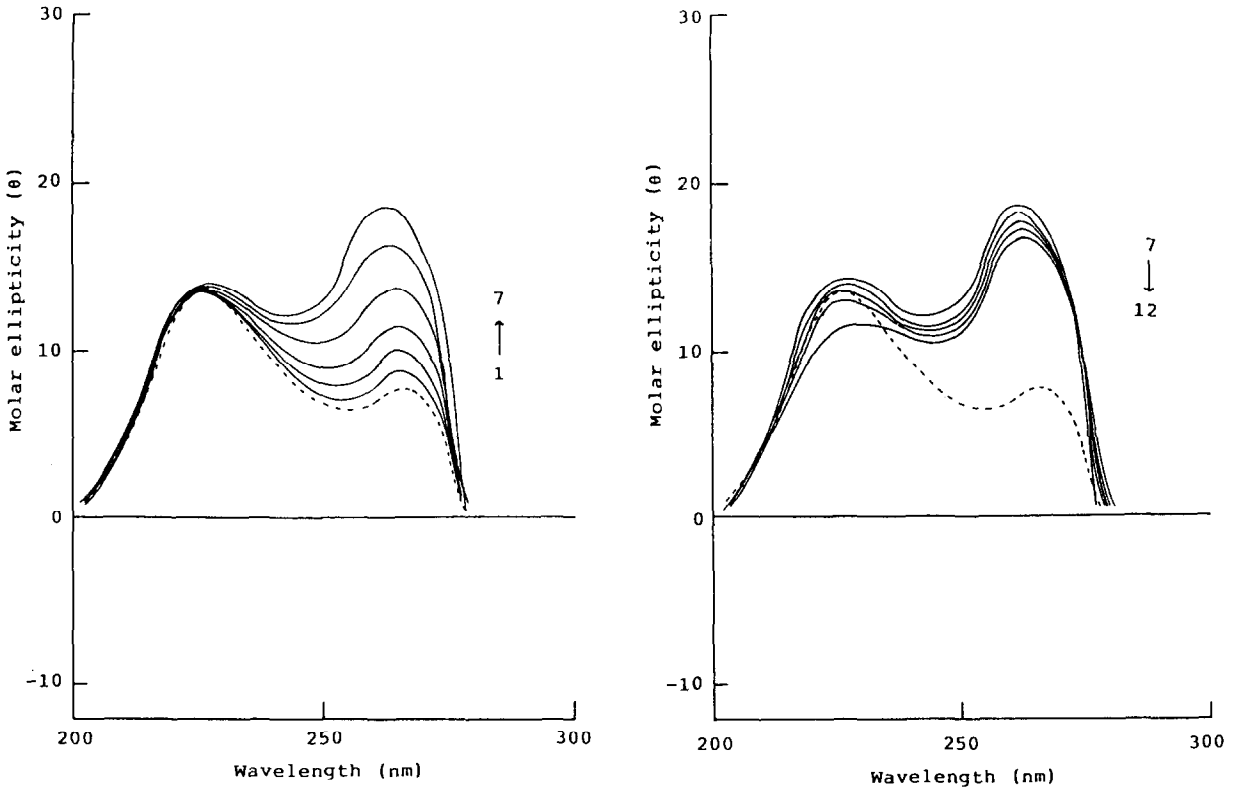

Fig. 6. Effect of γ-CyD concentration on the CD spectra of HB (5×10^{-5} M) in water. The concentrations of added γ-CyD were as follows: curves 1 and 12, 0 M; 2, 2.5×10^{-5} M; 3, 5×10^{-5} M; 4, 1×10^{-4} M; 5, 2×10^{-4} M; 6, 3×10^{-4} M; 7, 5×10^{-4} M; 8, 1×10^{-3} M; 9, 3×10^{-3} M; 10, 5×10^{-3} M; 11, 1×10^{-2} M.

C_6-H) shifted upfield, while the protons (C_2-H and C_4-H) on the exterior of the cavity shifted downfield, except for C_1-H. It should be noted that the extent of the upfield shifts of C_3-H and C_5-H in β-CyD was larger than that in γ-CyD. The upfield shift of C_5-H was significantly larger in comparison with that of C_3-H in both β- and γ-CyDs. Conversely, HB induced an upfield shift for all the peaks in DM-β-CyD except for that of C_2-CH_3, although the extent of the upfield shift was rather small as compared with β-CyD. The signals of protons such as C_3-H and C_5-H demon-

TABLE 2

Induced CD and UV absorption bands by the binding of HB to α-, β-, γ- and DM-β-CyDs [a]

Host compound	CD maximum			UV maximum	
	λ_{max}	$[\theta]$ [b]	g [c] ($\times 10^7$)	λ_{max}	$\epsilon(\times 10^{-4})$ [d]
α-CyD	not observed			247.1	1.632
β-CyD	262	21.5	9.12	246.2	1.503
γ-CyD	261	12.0	5.38	245.4	1.468
DM-β-CyD	261	16.7	7.67	245.9	1.470

[a] Concentrations of HB and CyDs were 5×10^{-5} and 1×10^{-3} M, respectively.
[b] Molar ellipticity (degree mol l^{-1}).
[c] Optical anisotropy factor.
[d] Apparent molar absorptivity.

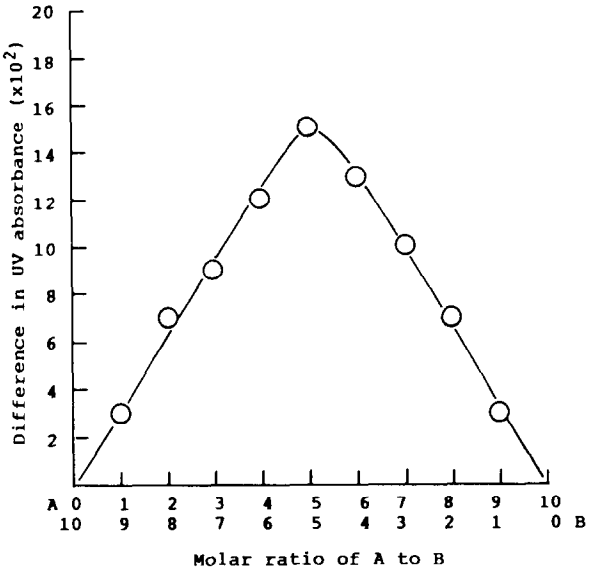

Fig. 7. Continuous variation plots for HB (A)-DM-β-CyD (B) system in water. The sum of the molar concentrations of HB and DM-β-CyD was 4.5×10^{-5} M.

strated a greater upfield shift compared with other signals.

These changes in chemical shift may be the

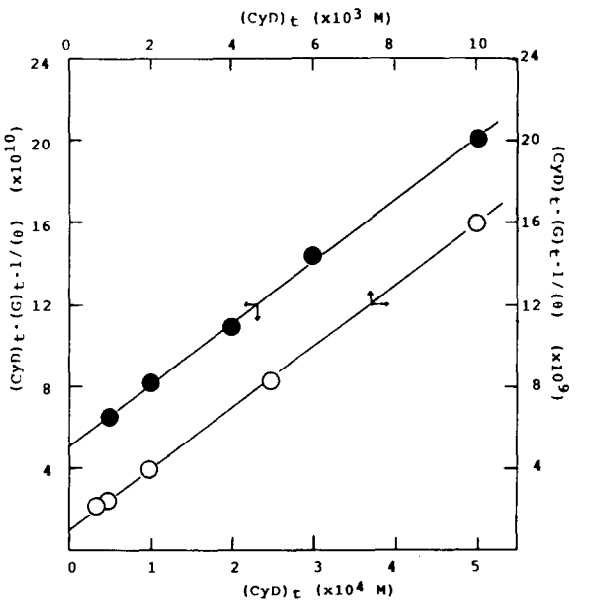

Fig. 8. Typical Scott plots for the interaction between HB and CyDs in water at 25°C. For notation on axes, see text. (○) HB-β-CyD complex; (●) HB-DM-β-CyD complex.

TABLE 3

Apparent stability constants of HB-CyD complexes according to three different measuring methods

Complex	Apparent stability constant (M^{-1})		
	Solubility method (30°C)	CD method (25°C)	UV method (25°C)
HB-α-CyD	282	– [a]	– [a]
HB-β-CyD	1782	1691	1456
HB-γ-CyD	2561	2037	1483
HB-DM-β-CyD	6122	6039	4834

[a] Could not be determined due to lack of significant spectral change.

reflection of a different mode of inclusion in the three complexes, as expected from CD studies. The results suggest that the HB molecule can penetrate into the cavity of β-CyD and is loosely bound to γ-CyD and DM-β-CyD.

Table 5 lists the effects of the three CyDs on the ^{1}H chemical shifts of HB. The other proton signals were too weak to be quantitatively analyzed under the present experimental conditions. On binding to β-CyD, the proton signal of C_4-H in the A ring shifted upfield, although most of the other proton signals moved downfield. This finding suggests that the A ring of HB may preferentially interact with β-CyD. In the case of the γ-CyD complex, most of the proton signals of HB shifted nonspecifically, and the magnitudes of the

TABLE 4

HB-induced ^{1}H chemical shift changes of CyDs

Proton	Δδ (ppm)		
	β-CyD	γ-CyD	DM-β-CyD
C_1-H	−0.042	−0.001	−0.006
C_2-H	−0.042	0.006	−0.001
C_3-H	−0.094	−0.021	−0.022
C_4-H	−0.027	0.042	−0.005
C_5-H	−0.065	−0.045	−0.017
C_6-H	−0.005	−0.021	−0.011
C_2-CH_3			0.064
C_6-CH_3			−0.004

Δδ is the difference in chemical shifts of CyDs in the presence and absence of HB. Negative signs indicate upfield displacement. The concentrations of both compounds in the mixture of D_2O and CD_3OD (6:4 v/v) were the same at 1×10^{-2} M.

displacements were rather small. This may be due to the loose inclusion of HB into γ-CyD. These results were in accord with those obtained for prednisolone (Uekama et al., 1985c). On the other hand, the proton signal of C_{17}-CH_3 only shifted upfield on binding of HB to DM-β-CyD, although the others were displaced downfield. This observation suggests that the butyl group of the ester may be included in the cavity of DM-β-CyD.

Stability of HB during the preparation of solid complexes

When the solid complexes were prepared using the aqueous phase, adjusted to pH 4.0 with acetic acid, the HPLC chromatograms showed a single peak of HB at a retention time of 7.12 min, indicating no recognizable degradation products. However, the solid complexes prepared without any acetic acid treatment revealed about 7% degradation of HB and a considerable amount of hydrocortisone 21-butyrate. This is the ester isomerized from HB, which was identified as the main rearrangement product at a retention time of 8.61 min. These results satisfactorily support a previous report (Kawano et al., 1981). Therefore, it is necessary that the pH of the aqueous phase be adjusted to the optimum pH prior to solvent evaporation.

Inclusion complexation in solid state

The DSC curves revealed some information on solid state interactions of HB with CyDs (Fig. 9).

TABLE 5

CyD-induced ^1H chemical shift changes of HB

Proton	Δδ (ppm)		
	β-CyD	γ-CyD	DM-β-CyD
C_4-H	−0.035	0.027	0.034
C_{11}-H	0.023	−0.021	0.045
C_{21}-H	0	−0.005	0.023
C_{17}-CH_3	−0.002	0.016	−0.017
C_{18}-CH_3	0.017	0.025	0.039
C_{19}-CH_3	0.038	0.017	0.060

Δδ is the difference in chemical shifts of HB in the presence and absence of CyDs. Negative signs indicate upfield displacement. The concentrations of both compounds in the mixture of D_2O and CD_3OD (6:4 v/v) were the same at 1×10^{-2} M.

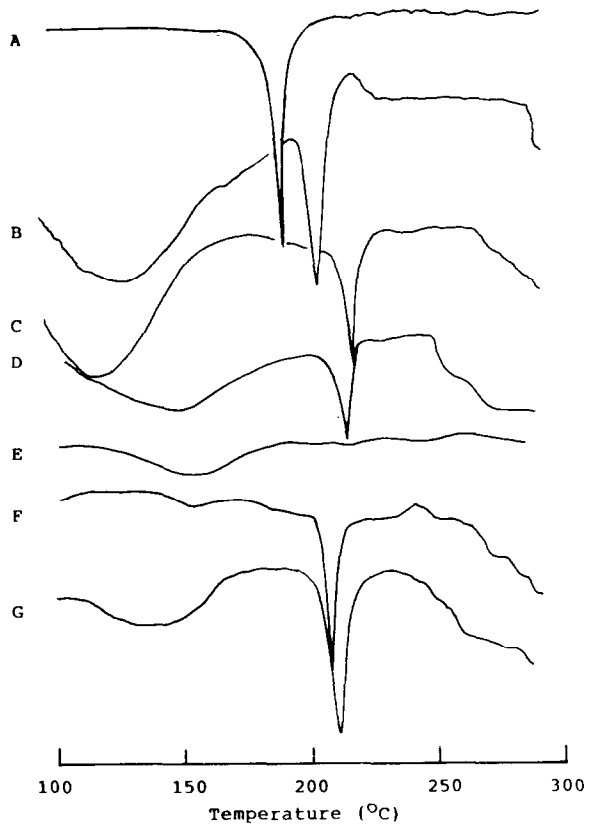

Fig. 9. DSC thermograms of HB-CyD systems. (A) HB alone; (B) HB-β-CyD physical mixture; (C) HB-β-CyD solid complex; (D) HB-γ-CyD physical mixture; (E) HB-γ-CyD solid complex; (F) HB-DM-β-CyD physical mixture; (G) HB-DM-β-CyD solid complex.

The characteristic thermal peak of HB, corresponding to its melting and decomposition, appeared at 190.8°C. In the HB-β-CyD systems, β-CyD exhibits a very broad thermal rise between 100 and 150°C, corresponding to the release of water. The HB-β-CyD mechanical mixture and solid complex showed a shift of endothermal peaks to 201.5 and 218.4°C, respectively. Similar changes of thermal rises were observed for the HB-DM-β-CyD physical mixture and solid complex, resulting in 18.0 and 22.4°C shifts of the endothermic peaks, respectively. These results differ from the observation that the endothermic peak, due to melting around 250°C, which was observed for prednisolone alone and for prednisolone-β-CyD physical mixtures, disappeared in

TABLE 6

Changes of IR absorption frequencies of HB in HB-CyD systems

Position	HB (cm^{-1})	HB-β-CyD		HB-γ-CyD		HB-DM-β-CyD	
		Physical mixture	Complex	Physical mixture	Complex	Physical mixture	Complex
νC=O (C_{20})	1735	−1	−9	0	−7	0	−5
νC=O (C_3)	1650	2	23	2	17	3	19
νC–C–C (A ring)	1272	1	−4	0	24	−1	−2
νC–O–C (ester, as)	1181	−1	−3	– [a]	−7	15	23
νC–O–C (ester, s)	1085	−3	−5	−5	−4	3	5

[a] Not observed. Negative signs indicate the changes to lower frequency.

the solid dispersion system (Fukuda et al., 1986). Thus, the greater temperature shift of the thermal peaks for HB-β-CyD and HB-DM-β-CyD systems proves that some interaction exists between HB and CyD for the physical mixture as well as for the solid complex. This corresponds to the formation of a complex with weak physical bonds (Glomot et al., 1988). The shifts of thermal rises for HB-CyD physical mixtures, compared with HB alone, may be mainly due to the occurrence of some interaction between HB and CyD during heating for DSC scanning. This is because inclusion complexation occurs by heating mixtures of a drug and CyD hydrate in a sealed container (Nakai et al., 1989). On the other hand, in the case of HB-γ-CyD systems, although the physical mixture showed an endothermic peak around 213.5°C, complete disappearance of the endothermic peak was observed for the solid complex.

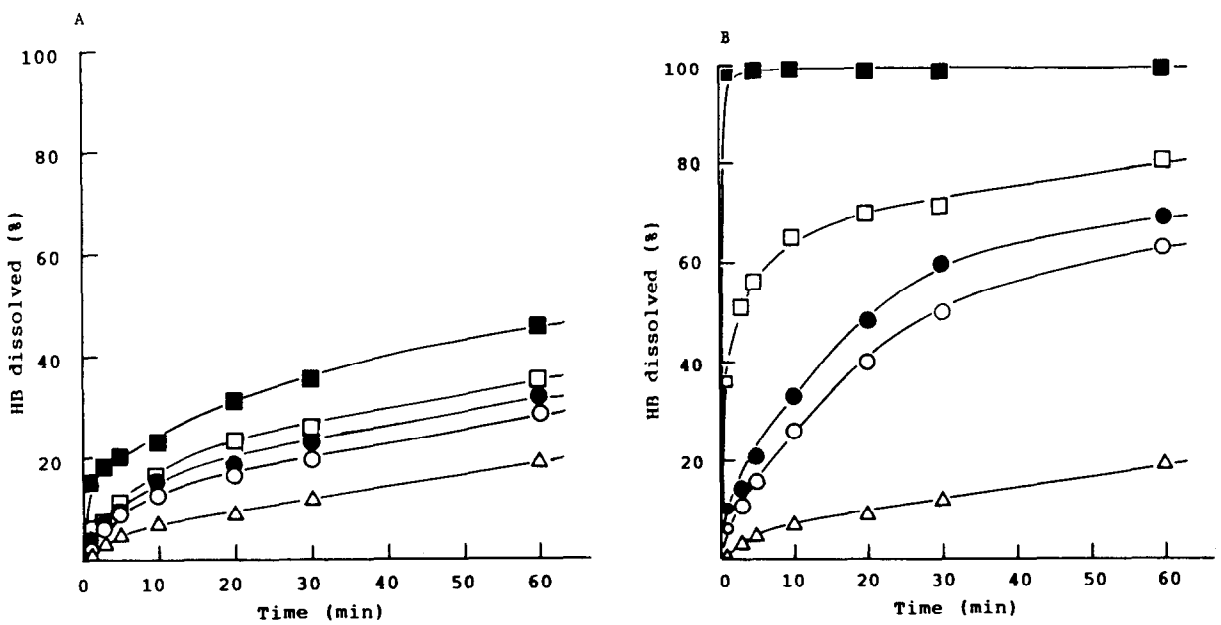

Fig. 10. Dissolution behavior of HB-CyD physical mixtures (A) and solid complexes (B) in water at 37°C. (△) HB alone; (○) HB-α-CyD system; (●) HB-β-CyD system; (□) HB-γ-CyD system; (■) HB-DM-β-CyD system.

The changes in IR absorption frequencies between HB itself and HB-CyD systems are summarized in Table 6. The peaks at 1650 and 1735 cm^{-1} are assigned to carbonyl stretching bands of C_3 and C_{20} in HB, respectively (Dence, 1980; Florey, 1983). There was no appreciable change in IR absorption frequencies for the physical mixtures. In the cases of complexes, however, the 1650 cm^{-1} band shifted to higher frequencies, while no appreciable shift was observed for the 1735 cm^{-1} band. These results may indicate monomolecular dispersion of HB in the CyD cavity and dissociation of the intermolecular hydrogen bonds of HB through inclusion complexation (Hibi et al., 1984). Similar spectral changes were also explained by the dissociation of intermolecular hydrogen bonds of hydrocortisone (Weeks et al., 1973) due to inclusion complexation.

Dissolution characteristics of CyD complexes

The dissolution behavior of HB alone, HB-CyD equimolar physical mixtures, and their complexes in water was examined by means of the dispersed amount method. As shown in Fig. 10A, the dissolution rates of the physical mixtures were considerably greater compared with that of HB alone, and followed the order of DM-β-CyD > γ-CyD > β-CyD > α-CyD, depending upon the magnitude of the K_c values (Table 1). These increased dissolution rates of HB from the physical mixtures, compared with drug alone, might be attributed to the formation of a readily soluble complex in the dissolution medium (Corrigan and Stanley, 1982). The dissolution rates of the complexes were markedly faster than those of HB alone and their physical mixtures (Fig. 10B). The rank order of the dissolution rates for the four complex systems was found to correlate well with the magnitude of the HB solubilities in the presence of CyDs (Fig. 1). In particular, for the DM-β-CyD complex, more than 98% dissolved within 1 min, indicating the formation of a very soluble complex. In other cases, the enhanced dissolution rates may be mainly due to the increased solubility, wettability, and the reduced drug particle size of the complex systems.

In conclusion, these results indicate that HB-CyD complexes, especially the DM-β-CyD complex, may be of considerable value for the rapid dissolution of HB in water. This kind of knowledge may prove useful for the development of improved dosage forms and provide a means for enhancing the topical bioavailability of the poorly soluble steroid.

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