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Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase

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Summary

Inclusion complexation of 18 steroid hormones with 3 cyclodextrins (α-, β- and γ-CyDs) in water and in solid phase were studied by the solubility method, spectroscopies (UV, CD, IR and ^{1}H-NMR), X-ray diffractometry and thermal analysis, and their modes of interactions were assessed. A spatial relationship between host and guest molecules was clearly reflected in the magnitude of the stability constant (γ-> β-> α-CyD) and in the stoichiometry of the inclusion complexes. The ^{1}H-NMR studies including spin-lattice relaxation time and chemical shift measurements suggested that the A-ring of the steroid molecule was predominantly included in the cavity of CyDs. The solid complexes of some steroids with β- and γ-CyDs were obtained generally in the molar ratios of 1:2 and 2:3, respectively, and their dissolution behaviors were examined. The results indicated that the CyD complexes may have a great utility as a rapidly dissolving form of steroids in water.

Introduction

Cyclodextrins (CyDs) have received considerable attention in pharmaceutical fields because of improved aqueous solubility, chemical stability and bioavailability of various drug molecules through inclusion complex formation (Uekama, 1979; Saenger, 1980). The solubilization of poorly soluble steroid hormones by macromolecule adjuvants has been investigated by several authors (Lach et al., 1966; Thakkar et al., 1968; Tomida et al., 1978; Lundberg et al., 1979). Testosterone and cortisone

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acetate reportedly form inclusion complexes with β-CyD by means of solubility analysis (Lach and Pauli, 1966). Preliminary studies in this laboratory also showed that some steroid hormones, including cortisone acetate, form soluble complexes with β- and γ-CyDs in various molar ratios. The present study deals, in detail, with the cavity size effect of CyDs (α-, β- and γ-CyDs) on the inclusion complexes of 18 steroid hormones in water and in the solid state. Solubility analysis, spectroscopies (ultraviolet (UV), circular dichroism (CD), infrared (IR) and proton magnetic resonance (^{1}H-NMR)), X-ray diffractometry and thermal analysis were employed. In addition the dissolution behaviors of some solid inclusion complexes in water were examined.

Materials and methods

Materials

The natural and synthetic steroids (see Table 1) were re-crystallized from ethanol–water before use. Compounds 1, 2, 3, 4, 5 and 6 were obtained from Nakarai Chemicals (Kyoto, Japan). Compounds 7, 8, 9, 10, 11, 12, 14, 15, 16, 17 and 18 were kindly donated by Mitsubishi Yuka Pharmaceuticals (Ibaraki, Japan). Compound 13 was a gift of Showa Yakuhin Kako (Kawasaki, Japan). The α-, β- and γ-CyDs were purchased from Nippon Shokuhin Kako (Tokyo, Japan), and re-crystallized twice from water. All other materials were of analytical reagent grade. Deionized, double-distilled water was used throughout the study.

Solubility studies

Solubility measurements were carried out according to Higuchi and Lach (1954). Excess amounts of steroid were added to aqueous solutions containing various concentrations of CyDs and were shaken at 25 ± 0.5°C. After equilibration was attained (approximately 10 days), an aliquot was centrifuged and pipetted through a cotton filter. A portion of the sample (0.5 ml) was then diluted with 50% v/v ethanol–water and analyzed spectrophotometrically. An apparent stability constant, K_c, was calculated from the initial straight line portion of phase solubility diagrams according to the following equation (Higuchi and Connors, 1965).

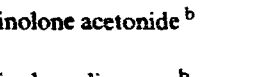
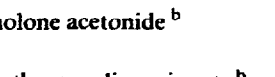
$$K_c = \frac{\text{slope}}{\text{intercept} \cdot (1 - \text{slope})} \quad (1)$$

Preparation of solid complexes

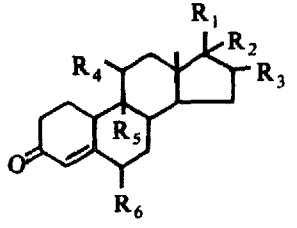
The solid complexes were derived by mixing appropriate amounts of the CyD and the steroid in water. Amounts were calculated from the descending curvature of the phase solubility (see Fig. 1). For example, 0.45 g of cortisone acetate and 6.8 g of β-CyD were added in 300 ml water, sealed in a flask, and the mixture was stirred with magnetic stirrer at 25°C for 7 days. The complex, which precipitated as a micro-crystalline powder, was filtered and dried under vacuum at 60°C for 48 h.

TABLE I

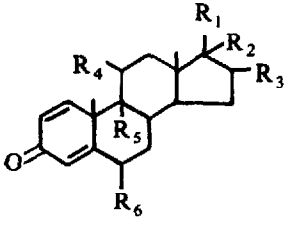
STEROIDS USED IN THIS STUDY

Compound	Structure					
	R_1	R_2	R_3	R_4	R_5	R_6
(1) Hydrocortisone [a]	$COCH_2OH$	α-OH	H	β-OH	H	H
(2) Cortisone [a]	$COCH_2OH$	α-OH	H	=O	H	H
(3) Hydrocortisone acetate [a]	$COCH_2OCOCH_3$	α-OH	H	β-OH	H	H
(4) Cortisone acetate [a]	$COCH_2OCOCH_3$	α-OH	H	=O	H	H
(5) Progesterone [a]	$COCH_3$	H	H	H	H	H
(6) Testosterone [a]	OH	H	H	H	H	H
(7) Prednisolone [b]	$COCH_2OH$	α-OH	H	β-OH	H	H
(8) Prednisolone acetate [b]	$COCH_2OCOCH_3$	α-OH	H	β-OH	H	H
(9) Triamcinolone [b]	$COCH_2OH$	α-OH	α-OH	β-OH	α-F	H
(10) Triamcinolone acetonide [b]	$COCH_2OH$			β-OH	α-F	H
(11) Triamcinolone diacetate [b]	$COCH_2OCOCH_3$	α-OH	α-$OCOCH_3$	β-OH	α-F	H
(12) Dexamethasone [b]	$COCH_2OH$	α-OH	α-CH_3	β-OH	α-F	H
(13) Betamethasone [b]	$COCH_2OH$	α-OH	β-CH_3	β-OH	α-F	H
(14) Dexamethasone acetate [b]	$COCH_2OCOCH_3$	α-OH	α-CH_3	β-OH	α-F	H
(15) Betamethasone-17-valerate [b]	$COCH_2OH$	α-$OCOC_4H_9$	β-CH_3	β-OH	α-F	H
(16) Paramethasone [b]	$COCH_2OH$	α-OH	α-CH_3	β-OH	H	α-F
(17) Fluocinolone acetonide [b]	$COCH_2OH$			β-OH	α-F	α-F
(18) Beclomethasone dipropionate [b]	$COCH_2OCOC_2H_5$	α-$OCOC_2H_5$	β-CH_3	β-OH	α-Cl	H

(a)



(b)



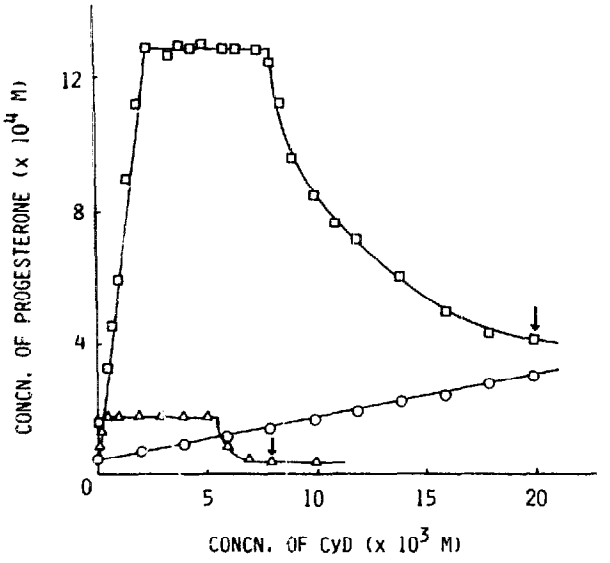


Fig. 1. Phase solubility diagrams of progesterone–CyD systems in water at 25°C. ○, α-CyD; △, β-CyD; □, γ-CyD. An arrow showing experimental condition of the preparation of solid complex (see text).

This powder corresponded to a 1:2 cortisone acetate–β-CyD complex which had a molecular weight of 2672 ± 5%.

Spectroscopic studies

The CD and UV spectra were taken by a Jasco J-40 S recording spectropolarimeter (Tokyo, Japan) and Hitachi 556S double-beam spectrophotometer (Tokyo, Japan), respectively, at 25 ± 0.5°C. The CD spectra were expressed as molar ellipticities (θ) (deg · cm^2 · $dmol^{-1}$). The IR spectra were measured as KBr disc, using a Jasco DS-702 double-beam spectrophotometer (Tokyo, Japan).

^{1}H-NMR spectra were taken on a JEOL JNM-FX 200 spectrometer (200 MHz) (Tokyo, Japan) equipped with a JEOL Fourier-transform computer system (22 K memory), using a 10 mm sample tube. The concentrations of hydrocortisone and CyDs in D_2O solvent were 1.1×10^{-3} M and 4.0×10^{-3} M, respectively. In general, an average of 5000 accumulations with 8000 data points was made at a sweep-width of 2000 Hz. The ^{1}H-chemical shifts were referenced to external 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS) with an accuracy of ±0.0012 ppm. ^{1}H spin-lattice relaxation times (T_1) were measured by the inversion-recovery method (Freeman and Hill, 1970) using a ($-180° - t - 90° - T -$) pulse sequence with $T > 5T_1$ for protons being measured. The T_1 values were obtained by least-squares analysis of $\ln(A_\infty - A_t)$ vs t, where A_∞, A_t and t are the peak intensity at time ∞, the peak intensity at time t, and pulse interval time t in ms, respectively. The slope of the line was taken as $-1/T_1$, with accuracy of ±2%.

X-Ray diffractometry

The powder X-ray diffraction patterns were taken by a Rigaku Denki Geiger Flex-2012 diffractometer (Tokyo, Japan). The operation conditions were as follows:

X-ray: Ni-filtered Cu-Kα radiation, voltage: 30 kV, current: 20 mA, time constant: 2 s, scanning speed: 1°/min.

Differential thermal analysis (DTA)

All measurements were carried out using a scanning rate of 10°/min on a Shimadzu DT-20B thermal analyzer (Tokyo, Japan).

Dissolution studies

The sample powder was compressed into a cylindrical tablet (diameter 10 mm) at a pressure of about 200 kg/cm^2. The release of steroid was measured using a rotating disc apparatus (Nogami et al., 1966) in 25 ml water at 91 rpm and 25°C. At appropriate intervals, 1 ml samples were removed from the flask, diluted with 75% ethanol–water solution and assayed spectrophotometrically at the UV maximum of the steroid. Corrections were applied for cumulative dilution caused by replacement of sample by equal volumes of the original medium. The tablets maintained a constant shape throughout the measurement.

Results and Discussion

Inclusion complexation in water

Solubility studies

Fig. 1 shows the phase solubility diagrams obtained for progesterone with 3 CyDs, as a typical example, where the difference in solubility curves was clearly noted. In the case of α-CyD, the solubility of progesterone increased linearly as a function of α-CyD concentration and the solubility curve can be generally classified as type A_L (Higuchi and Connors, 1965). On the other hand, β- and γ-CyD systems showed B_S-type solubility curves with micro-crystalline complexes precipitating at the higher CyD concentrations. The stoichiometries of the complexes in the solid phase were analyzed on the basis of data in the plateau region of the solubility diagrams, and were estimated to be 1:2 for progesterone–β-CyD and 2:3 for progesterone–γ-CyD, respectively. The results were in good agreement with those obtained by isolation and analysis of the solid complexes. In sharp contrast, no precipitation was observed for the α-CyD system. This might be due to the smaller cavity size of α-CyD, therefore allowing very little penetration of the bulky steroid molecule.

The apparent stability constants, K_c, were calculated from the initial straight line portion of the solubility diagrams. The results of the solubility studies and the partition coefficients of steroids are listed in Table 2. In all cases, K_c values increased in the order of γ-> β-> α-CyD, suggesting that the larger the CyD cavity, the more favorable is the fit of steroid molecules. The hydrophobic guest molecules such as progesterone, triamcinolone acetonide, dexamethasone acetate and fluocinolone acetonide exhibited the greatest binding (largest K_c values), as would be expected from their partition coefficients. However, there was no linear correlation

TABLE 2

STABILITY CONSTANTS (K_c, M^{-1}), STOICHIOMETRIES, TYPES OF PHASE SOLUBILITY DIAGRAMS AND PARTITION COEFFICIENTS (P.C.) FOR STEROID–CyD SYSTEMS AT 25°C

Compound	P.C. [a]	α-CyD system		β-CyD system			γ-CyD system		
		K_α	Type [c]	K_β	Molar [b] ratio	Type [c]	K_γ	Molar [b] ratio	Type [c]
(1) Hydrocortisone	35.7	57	A_L	1720	1:2	B_S	2240	2:3	B_S
(2) Cortisone	26.2	63	A_L	2300	1:2	B_S	2170	2:3	B_S
(3) Hydrocortisone acetate	154	88	A_L	3250	1:2	B_S	2270	2:3	B_S
(4) Cortisone acetate	126	86	A_L	4150	1:2	B_S	2470	2:3	B_S
(5) Progesterone	7410	145	A_L	13300	1:2	B_S	24000	2:3	B_S
(6) Testosterone	1960	134	A_L	7540	2:3	B_S	16500	1:2	B_S
(7) Prednisolone	414	298	A_L	3600	1:2	B_S	3240	2:3	B_S
(8) Prednisolone acetate	250	274	A_P	5770	–	A_P	3880	2:3	B_S
(9) Triamcinolone	10.8	121	A_L	2370	1:2	B_S	9920	2:3	B_S
(10) Triamcinolone acetonide	205	256	A_L	3230	–	A_L	26100	2:3	B_S
(11) Triamcinolone diacetate	83.7	300	A_L	3530	1:2	B_S	12100	2:3	B_S
(12) Dexamethasone	67.8	169	A_L	4660	1:2	B_S	26600	2:3	B_S
(13) Betamethasone	87.7	223	A_L	5420	1:2	B_S	21600	2:3	B_S
(14) Dexamethasone acetate	806	316	A_P	9560	–	A_L	37300	2:3	B_S
(15) Betamethasone-17-valerate	307	302	A_L	2990	1:2	B_S	9850	2:3	B_S
(16) Paramethasone	580	489	A_P	2540	–	A_L	8310	2:3	B_S
(17) Fluocinolone acetonide	1120	297	A_L	3000	–	A_L	31900	2:3	B_S
(18) Beclomethasone dipropionate	3340	354	A_P	1120	–	A_L	6300	1:2	B_S

[a] The partition coefficient (*n*-octanol/water) of steroid taken from Tomida et al. (1978).

[b] Steroid:CyD

[c] The type of phase solubility diagrams was defined according to Higuchi and Connors (1965).

between the K_c values and the partition coefficients, which may be due in part to the heterogeneous nature of the steroid substituents. The importance of the spatial relationship between host and guest molecules is most clearly noted in the stoichiometries of the solid complexes. The β- and γ-CyDs generally formed 1:2 and 2:3 complexes with steroids, respectively, although there were some exceptions. These findings suggest that steric and hydrophobic factors of the host and guest molecules were responsible for these interactions.

UV and CD studies

The solubility studies showed that the steroid hormones will form soluble complexes with the 3 CyDs. These interactions in aqueous solution were further examined by UV and CD spectroscopy. Hydrocortisone was selected for this study

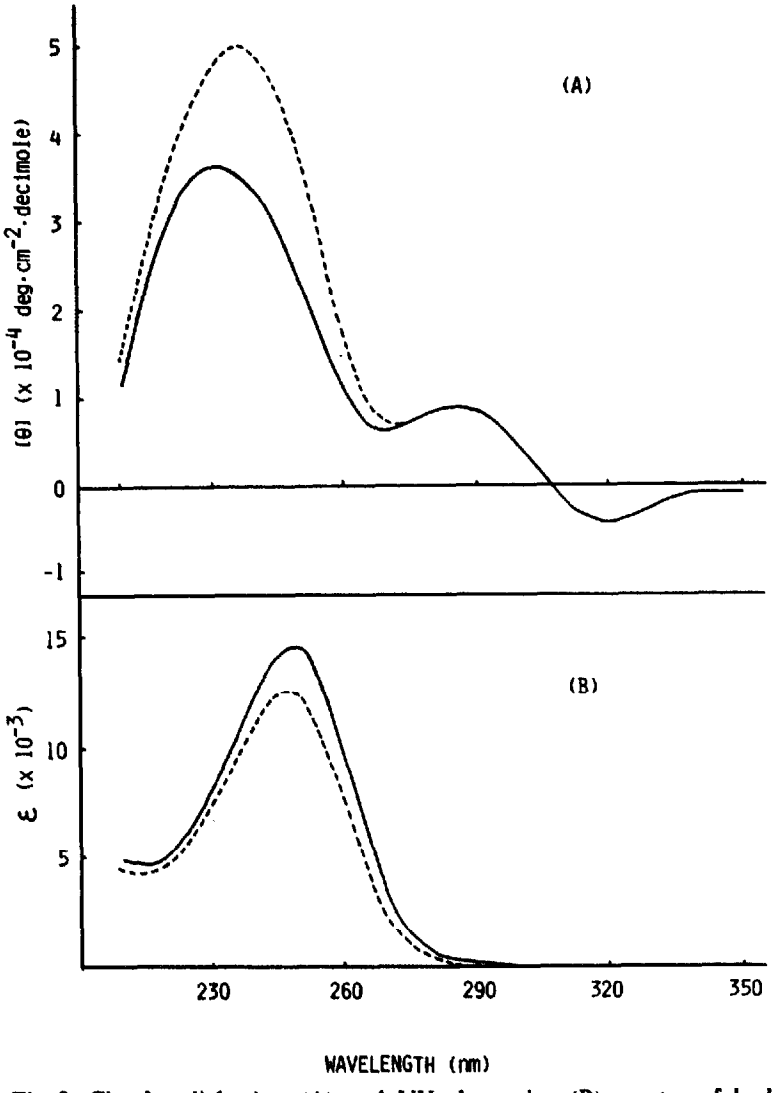


Fig. 2. Circular dichroism (A) and UV absorption (B) spectra of hydrocortisone in the absence and presence of β-CyD in water at 25°C. Hydrocortisone, 9.45×10^{-5} M; β-CyD, 5.0×10^{-3} M. ————, hydrocortisone; - - - - - -, hydrocortisone + β-CyD.

because it is relatively water-soluble and its spectral data are easily analyzed.

Fig. 2 shows the UV and CD spectra of hydrocortisone in the absence and presence of β-CyD in water. Hydrocortisone exhibited an intense UV maximum around 250 nm due to a π-π^* transition of the conjugated carbonyl group and showed at least two Cotton effects between 220 and 400 nm associated with the π-π^* and n-π^* carbonyl transitions (Crabbe, 1972), respectively. On the addition of β-CyD, the positive and negative CD peaks shifted to a longer wavelength with an increase in optical activity, accompanying the decrease in absorbance in the UV spectrum. When hydrocortisone was dissolved in a 50% methanol–water solution, similar spectral changes were observed. These facts suggest that the chromophores of the steroid molecule may be located in the hydrophobic CyD cavity.

NMR studies

^{1}H-NMR technique (200 MHz) was employed to gain insight into the inclusion mode of the hydrocortisone–CyD complexes in aqueous solution. The assignments for hydrocortisone (Hampel and Kraemer, 1966) and CyDs (Demarco and Thakkar, 1970) were further confirmed by a decoupling experiment in this study. The effects of hydrocortisone on ^{1}H-chemical shifts of CyDs are summarized in Table 3. It was expected that if inclusion does indeed occur, protons located within or near the cavity (e.g. H-3, H-5 or H-6) should be strongly shielded by aromatic moiety of guest molecule (Demarco and Thakkar, 1970). Alternatively, if association takes place at the exterior of the torus, H-1, H-2 and H-4 should be more strongly affected. As can be seen in Table 3, all protons experience a shielding effect, indicating that hydrocortisone may interact both on the exterior surface at the CyD cavity as well as in the

TABLE 3

HYDROCORTISONE-INDUCED CHEMICAL SHIFT CHANGES OF CyDs

Proton	$\Delta\delta$ (ppm)		
	α-CyD	β-CyD	γ-CyD
H_1	−0.006	−0.009	−0.012
H_2	−0.002	−0.002	−0.005
H_3	−0.017	−0.039	−0.032
H_4	−0.004	−0.006	−0.006
H_5	−0.007	−0.042	−0.042
H_6	−0.006	−0.020	−0.016

$\Delta\delta$ is difference in chemical shifts of CyDs in the presence and absence of hydrocortisone. Negative sign indicated the upfield displacement. Accuracy of chemical shift is ±0.0012 ppm.

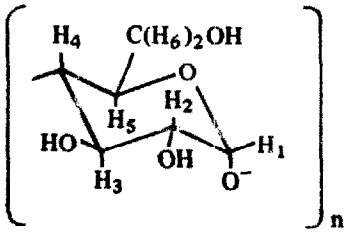
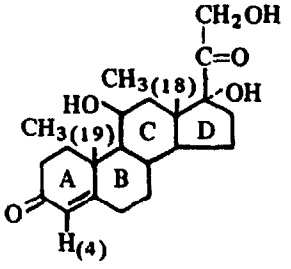


TABLE 4

CyD-INDUCED CHEMICAL SHIFT CHANGES OF HYDROCORTISONE

Proton	Δδ (ppm)		
	α-CyD	β-CyD	γ-CyD
$H_{(4)}$	0.137	0.092	0.044
$CH_{3(18)}$	0.011	0.087	0.031
$CH_{3(19)}$	0.032	0.142	0.065

Δδ is the difference in chemical shifts of hydrocortisone in the presence and absence of CyDs.



interior. When attention was directed toward the interior protons (H-3 and H-5), the magnitude of the upfield shift decreased in the order of β-> γ-> α-CyD. The upfield shift of H-5 in α-CyD was significantly smaller than the other CyDs. These data suggest that the hydrocortisone molecule is located at the entrance of the α-CyD cavity, it could penetrate further into the β-CyD cavity, and is loosely bound to γ-CyD. Table 4 summarizes the effects of CyDs on some ^{1}H-chemical shifts (C_4-H, C_{18}-methyl, and C_{19}-methyl) of hydrocortisone. Unfortunately, the other proton signals were too weak to be quantitatively analyzed under the experimental conditions used. In the presence of CyDs, all the signals moved downfield, probably due to the steric perturbation through inclusion complex formation (Cheney, 1968; Suzuki and Sasaki, 1979). The smaller the CyD cavity size, the larger the deshielding effect of the C_4-proton was observed. The downfield shifts of the C_4-H and C_{19}-methyl protons were apparently greater than that of C_{18}-methyl protons, particularly for β-CyD. These facts suggest that the A-ring of hydrocortisone may strongly interact with β-CyD. The molecular motions of the inclusion complex between hydrocortisone and β-CyD was further examined by means of ^{1}H-nuclear

TABLE 5

EFFECT OF β-CyD ON SOME ^{1}H SPIN-LATTICE RELAXATION TIMES OF HYDROCORTISONE

Proton	T_1 (s)		
	Without β-CyD (I_0)	With β-CyD (I)	I_0/I
$H_{(4)}$	1.44	0.336	4.29
$CH_{3(18)}$	0.410	0.230	1.78
$CH_{3(19)}$	0.498	0.194	2.57

relaxation measurements. Table 5 summarizes the effects of β-CyD on the spin-lattice relaxation times (T_1) of some hydrocortisone protons. In the presence of β-CyD, the T_1 values decreased by a factor of about 3; the most significant decrease in T_1 occurred for the C_4 proton. This suggests that the molecular motion of hydrocortisone, particularly of the A-ring was reduced as a consequence of the coupling of its motion to that of β-CyD. This is consistent with observation made with the space-filling Corey-Pauling-Koltun molecular models of the inclusion complexes.

Inclusion complexation in solid state

Microcrystalline complexes of the steroids with the β- and γ-CyDs were examined by X-ray diffractometry, IR spectroscopy and DTA measurement, and compared with the corresponding physical mixtures in the same molar ratio.

Fig. 3 shows the powder X-ray diffraction patterns of the hydrocortisone–CyD complexes and their physical mixtures. The diffraction patterns of the physical

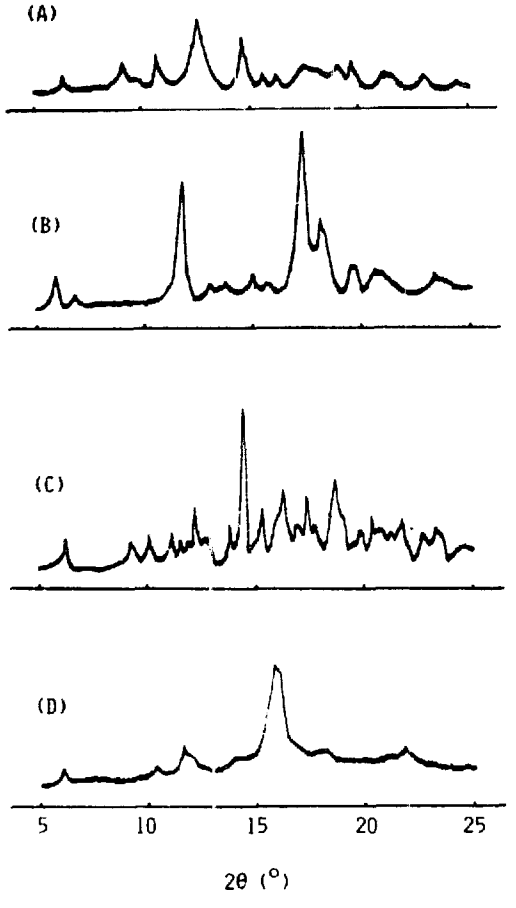


Fig. 3. Powder X-ray diffraction patterns of hydrocortisone-CyD system. (A) physical mixture of hydrocortisone and β-CyD; (B) complex of hydrocortisone with β-CyD; (C) physical mixture of hydrocortisone and γ-CyD; (D) complex of hydrocortisone with γ-CyD.

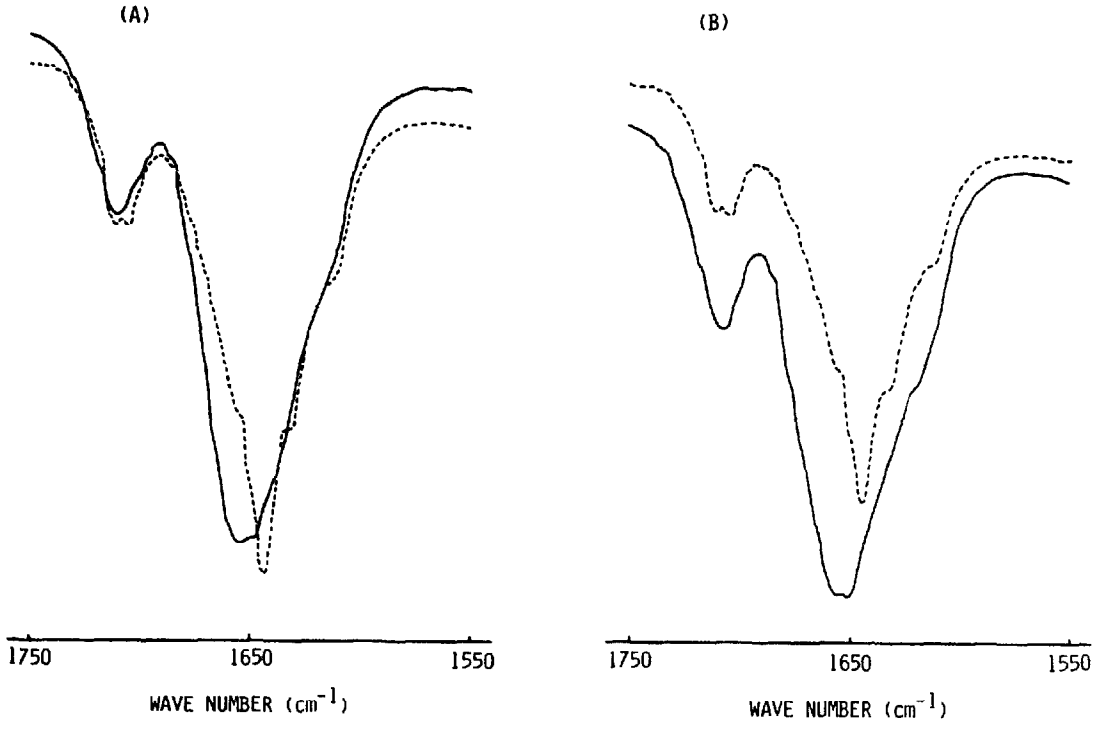


Fig. 4. IR spectra of hydrocortisone–CyD system, measured by KBr disc method. (A) β-CyD system; (B) γ-CyD system. - - - - - -, physical mixture of hydrocortisone and CyD; ————, complex of hydrocortisone with CyD.

mixtures were simply the superposition of each component, while those of CyD complexes were apparently different from each constituent and constitute a new solid phase. The CyD complexes gave somewhat diffuse diffraction patterns, suggesting that they are much less crystalline than the physical mixtures. It was difficult to determine the crystal packing of the complexes because the diffraction patterns were too complicated to be reliably indexed by the powder method (Takeo and Kuge, 1969).

Fig. 4 shows IR spectra of hydrocortisone–CyD complexes and their physical mixtures, in the carbonyl-stretching regions. The peaks at 1643 cm^{-1} and 1710 cm^{-1} are assigned to carbonyl-stretching bands of C_3 and C_{20} in hydrocortisone, respectively (Dence, 1980). In the cases of β- and γ-CyD complexes, the 1643 cm^{-1} band shifted to 1655 cm^{-1} and 1653 cm^{-1}, respectively, while no appreciable shift was observed for the 1710 cm^{-1} band. These spectral changes can be explained by the dissociation of the intermolecular hydrogen bonds of hydrocortisone (Weeks et al., 1973) through inclusion complexation.

Fig. 5 shows the DTA thermograms of the testosterone–CyD systems. The physical mixtures showed an endothermic peak around 155° corresponding to the melting temperature of testosterone. The interactions of testosterone with both β- and γ-CyDs were accompanied by the disappearance of this endothermic peak, as would be expected (Uekama et al., 1979). A similar trend in the DTA thermograms

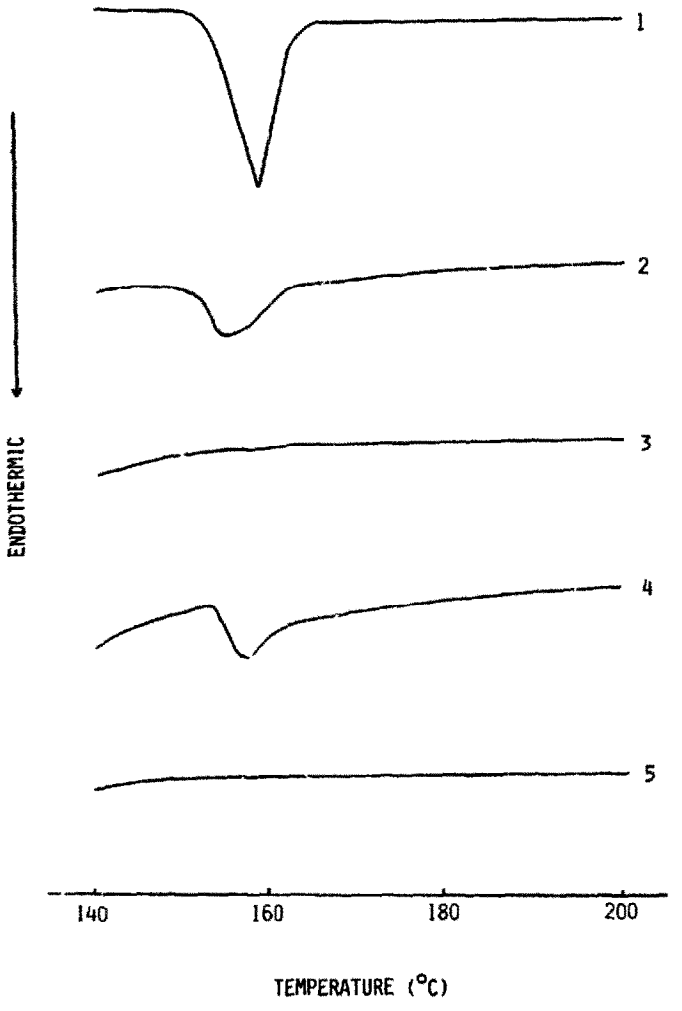


Fig. 5. DTA thermograms of testosterone–CyD systems. (1) testosterone; (2) physical mixture of testosterone and β-CyD; (3) complex of testosterone with β-CyD; (4) physical mixture of testosterone and γ-CyD; (5) complex of testosterone with γ-CyD.

was observed for all other steroid–CyD systems. The above results clearly indicate that the steroid–CyDs (β- and γ-CyDs) complexes exist in the solid state.

Dissolution behaviors of the complexes

The typical dissolution curves obtained for progesterone and its β- and γ-CyD complexes in water are shown in Fig. 6. It is evident that the dissolution rate of progesterone was significantly improved by complex formation, particularly by the γ-CyD complex. Table 6 summarizes the apparent dissolution rates (k_0, k_β and k_γ) and solubilities (S_0, S_β, and S_γ) of steroids and their β- and γ-CyD complexes. The S_β and S_γ values were estimated from the downward curvatures of the B_S-type phase solubility diagrams obtained for the β- and γ-CyD systems, respectively. The apparent dissolution rates were found to correlate well with the apparent solubilities

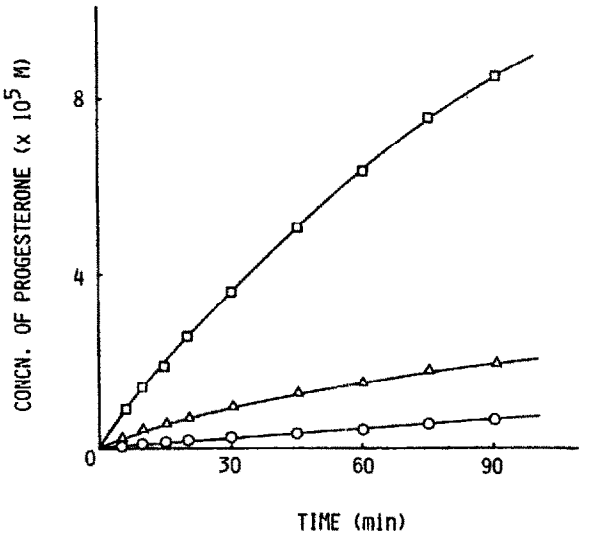


Fig. 6. Dissolution profiles of progesterone and its CyD complexes in water at 25°C by rotating disc method. ○, progesterone; △, β-CyD complex; □, γ-CyD complex.

of the solid sample, as shown in Table 6. The apparent dissolution rate of the complex is known to be dependent upon various factors such as solubility, diffusion coefficient and the dissociation of the complex in the dissolution medium (Donbrow and Touitou, 1978). An attempt was made to analyze the dissolution rates of the complexes in terms of their solubility and dissociation constant ($1/K_c$), using the method of multiple regression. Good correlations were found for both β- and γ-CyD systems with a correlation coefficient, $r > 0.95$ at the 99% confidence level.

$$\log k_\beta = 0.536 \log S_\beta + 0.322 \log(1/K_c) - 2.59 \quad (\beta\text{-CyD system:}$$

$$n = 7, r = 0.986, s = 0.075) \qquad (2)$$

TABLE 6

APPARENT DISSOLUTION RATES (k) AND SOLUBILITIES (S) OF STEROID–CyD SYSTEMS IN WATER AT 25°C

Compound	Steroid		β-CyD complex		γ-CyD complex	
	k_0 ($\times 10^7$ M/min)	S_0 ($\times 10^4$ M)	k_β ($\times 10^7$ M/min)	S_β ($\times 10^5$ M)	k_γ ($\times 10^6$ M/min)	S_γ ($\times 10^4$ M)
Hydrocortisone	22.8	18.5	34.8	57.2	5.34	9.35
Cortisone	12.6	8.67	9.00	4.38	5.35	4.81
Hydrocortisone acetate	0.362	0.625	33.0	37.3	2.74	1.75
Cortisone acetate	1.01	0.784	12.0	6.82	1.77	1.50
Progesterone	0.797	0.532	4.39	3.14	1.60	1.44
Testosterone	2.70	1.29	7.97	7.82	8.80	12.1
Betamethasone	5.60	2.38	34.8	7.63	5.43	5.56

$$\log k_\gamma = 0.692 \log S_\gamma + 0.053 \log(1/K_c) - 2.87 \quad (\gamma\text{-CyD system:}$$

$$n = 7, r = 0.959, s = 0.097) \tag{3}$$

This suggests that both the solubility and the dissociation of the complexes are responsible for the apparent dissolution rates of the complexes. Further investigations should be made to elucidate the dissolution mechanism of the CyD complexes, since the wettability and diffusion coefficient of the complexes should not be excluded. This kind of knowledge will provide both a rational basis for formulation design and a means for enhancing the bioavailability of poorly soluble steroids.

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