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COMPLEXATION OF 6-ACYL-O-β-CYCLODEXTRIN DERIVATIVES WITH STEROIDS - EFFECTS OF CHAIN LENGTH AND SUBSTITUTION DEGREE

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ABSTRACT

This study was designed to test a hypothesis that the esterification of the primary 6-hydroxyl groups on β-cyclodextrin (β-CD) may disrupt its crystallinity, thereby enhancing its aqueous solubility and increasing its ability to solubilize water-insoluble compounds. 6-acyl-o-β-CD derivatives with different chain length and substitution degree were synthesized by esterifying the primary hydroxyl groups at 6-position of β-CD with various acyl chlorides. X-ray diffraction patterns confirmed the existence of 6-acyl-o-β-CD derivatives as amorphous solid. Effects of chain length and substitution degree of the acyl groups on 6-acyl-o-β-CD/steroid complexation and solubilization were investigated with the phase solubility method. The results indicated that the solubilities of 6-acyl-o-β-CD derivatives were significantly higher than that of β-CD itself, which resulted in an increase in their ability to solubilize poorly water-soluble drugs such as steroids. The addition of side chains to β-CD did not change the stoichiometric ratio (2:1) of β-CD/steroid complexes. The increase in chain length can enhance the association of 6-acyl-o-

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β-CDs with steroids as it was evidenced from the apparent stability constants. A solubility study utilizing different combinations of 6-acyl-o-β-CDs, α-CD and 2,6-dimethyl-o-β-CD suggested a competitive complexation process among the family of acyl-β-CD derivatives. A non-competitive steroid complexation between 6-acyl-o-β-CDs and α-CD and between 6-acyl-o-β-CDs and 2,6-dimethyl-o-β-CD was observed.

INTRODUCTION

The utility of β-cyclodextrin (β-CD) as a solubilizing agent to improve the solubility of poorly water-soluble compounds is often limited due to its low intrinsic aqueous solubility. The search for better water-soluble β-CD derivatives has therefore received much attention. It has led to the discovery of several β-CD-based solubilizers such as 6-hydroxypropyl-o-β-CD, glucosyl-o-β-CD and 2,6-dimethyl-o-β-CD (1-6). The surprisingly low solubility displayed by β-CD is due to the fact that β-CD has a rather rigid molecular structure consisting of sevenfold axis of symmetry, making it more inclined to crystallization than the other CDs (α-CD and γ-CD). The overall molecular dipole of β-CD is greater than that of the other CDs as a result of its odd number of glucopyranose units, which enhance the intermolecular interaction of β-CD molecules in solid state and thus require higher free energy to break the molecular association, thereby imparting a lower aqueous solubility (7). Therefore, it is conceivable that the interruption of β-CD's crystallinity can reduce the intermolecular interaction and in turn increase its aqueous solubility.

One β-CD molecule contains seven primary hydroxyl groups located at the outside of the torus and fourteen secondary hydroxyl groups in the torus which can be used to modify its chemical structure. In the β-CD torus, the secondary hydroxyl group on C-2 position of each glucopyranose forms a hydrogen bond with its neighboring hydroxyl group on C-3 of the adjacent glucopyranose unit. The substitution of the secondary hydroxyl groups on β-CD generally leads to the change in its complexation capability. The complete substitution of all hydroxyl groups on β-CD could produce a molecular geometry similar to β-CD itself and might not be desirable. The partial substitution of the hydroxyl groups on β-CD may interrupt β-CD's crystallinity and yield a less crystalline solid (2). Since the primary hydroxyl groups are located at the outside of the torus, the substitution of

the hydroxyl group at 6-position of β-CD could make a more significant change in its physical properties and less significant change in its complexation properties. This concept can be applied to examine the effect of substituent and substitution degree on β-CD's crystallinity and aqueous solubility.

This report describes the effect of the chain length and substitution degree of acyl groups on the solubilities of 6-acyl-o-β-CD derivatives. Our previous study has characterized the mechanism of β-CD/steroid complexation using the apparent stability constants (8). The present study focuses on the role of the substituents on the aqueous solubility, complexation and solubilization of steroids, which were chosen as model drugs. The aim is to investigate any correlation between the aqueous solubilities of 6-acyl-o-β-CD derivatives and their capabilities to solubilize water-insoluble drugs as well as to delineate the effects of CD structure on association equilibria.

MATERIALS AND METHODS

MATERIALS

Progesterone, acetyl, propionyl, and butyryl chlorides were provided by Aldrich Chemical Company (Milwaukee, WI.). β-CD, α-CD, 2,6-dimethyl-o-β-CD, testosterone, cortisone and hydrocortisone were purchased from Sigma Chemical Company (St. Louis, MO). Pyridine, dimethylformamide (DMF) and ethanol were of reagent grade. Phenol and sulfuric acid were of analytical grade. Dimethyl-d6-sulphoxide (D6-DMSO) obtained from MSD Isotopes Division of Merck Frosst Canada Inc., Montreal, Canada contained a minimum isotopic purity 99.9% of atom D. All chemicals were used as received. Deionized double distilled water was used throughout the study.

METHODS

Synthesis of 6-Acyl-o-β-CD Derivatives

To a stirred solution of 5.65 grams β-CD in 50 ml of pyridine: DMF (1:1), required amount acyl chloride was added in a dropwise manner. The reaction mixture was placed in an ice-bath during the addition of acyl chloride and throughout the reaction period. After about 4-8 hours, five ml of water was added

to terminate the reaction. The solvent was removed by an evaporating rotator at a temperature of 55-70°C. A syrup-like residue was obtained. Ten ml of 95% ethanol was added to the residue after it was allowed to cool to room temperature. The ending mixture appeared to be a solution or a suspension depending on the amount and type of acyl chloride initially added. Immediately the reaction mixture was neutralized to pH 7.0 with 1 N sodium hydroxide solution. It was then placed in an ice-bath and an adequate amount of 95% ethanol or ethyl acetate was added to precipitate the product. The compound was subsequently crystallized twice more. White amorphous powders were obtained after filtration. The solid powders were dried *in vacuo* at 60-80°C overnight over phosphorus pentaoxide.

Characterization of 6-Acyl-β-CD Derivatives

The fast atom bombardment mass spectra (FABMS) of 6-acyl-o-β-CD derivatives was obtained with the Kratos MS-50 sector mass spectrometer (Kratos Analytical, Urmston Manchester, UK) using glycerin as sample matrix. A Varian XL-200 NMR instrument was used to obtain the proton NMR spectra for the derivatives utilizing D_6-DMSO as the solvent. The average substitution degree was estimated utilizing the change in the proton integral value of the primary hydroxyl groups on CD's prior to and after esterification. The average molecular weight was calculated according to the average substitution degree. The solubilities of 6-acyl-o-β-CD derivatives were determined by a colorimetric method (9) with a Beckman-DU7 spectrophotometer (Beckman Instruments, Westbury, NY) equipped with a data handing computer. IR spectra were obtained from a Nicolet FTIR spectrometer (Nicolet Analytical Instruments, Madison, WI) using KBr pellet method. The X-ray diffraction studies were performed with an automated Siemens Powder Diffractometer by using copper tube at an accelerated voltage of 40 kV and an electric current of 20 mA. The melting endotherm was examined with a differential scanning calorimeter (DSC). DSC was performed with a Perkin Elmer DSC-4 at a heating rate of 20°C/min., and indium was utilized as a standard indicator of the melting point.

Phase Solubility Study

The procedure adopted in the phase solubility study was described in our previous report (8). Briefly, the experiments were performed with 10 ml liquid scintillation vials (Research Product International Corp., Mount Prospect, IL) immersed in a thermostated water-bath (MGW Lauda, Postfech, Germany) at 30 ±

0.5 °C. An adequate amount of steroid powder was weighed into a scintillation vial to which five milliliters of a solution containing a known concentration of 6-acyl-o-β-CD was added. After equilibrium was reached, the remaining solid was filtrated off using a 0.4-mm polycarbonated membrane (Nucleopore, Pleasanton, CA). The filtrate was collected, appropriately diluted with water, and the absorbance was determined at 243 nm with a Beckman-DU7 spectrophotometer. The assay of steroid in presence of 6-acyl-o-β-CD derivatives was validated with a recovery study.

Preparation and Characterization of Solid Complexes

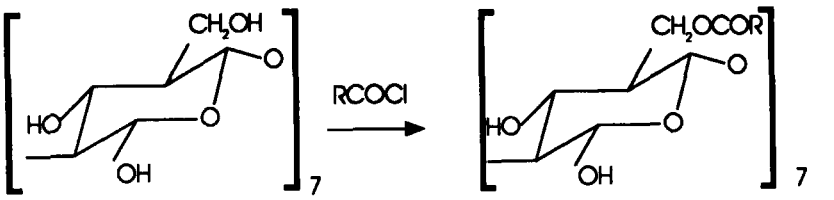
The pure 1:2 complexes were obtained by collecting solid residue from the descending portion of the solubility phase diagram where no solid drug exists. The procedure was described in a previous report (8). The stoichiometric ratio of complexes was estimated with FABMS, and was further verified with solubility phase diagrams and chemical analysis of pure solid complexes.

Solubilization Study

The solubilization experiments were carried out in the same manner as the phase solubility study described previously (8). The solubilizing ability of 6-acyl-o-β-CD derivatives was compared by measuring the solubility of progesterone in the presence of a 10 mM 6-acyl-β-CD derivative and an excess amount of progesterone solid powder. The combined effect of two different 6-acyl-o-β-CDs on solubilization was evaluated by measuring the solubility of progesterone in an aqueous solution, containing two different 6-acyl-o-β-CD compounds at a concentration of 10 mM each. The solubilizing ability of solutions containing two 6-acyl-o-β-CD compounds with the same types of substituents but different substitution degree, or with the different type of substituent were also determined. Studies utilizing different species of CDs such as α-CD and 2,6-dimethyl-o-β-CD and their combination with 6-acyl-o-β-CDs were also performed in order to clarify the mechanism of additive solubilization for given a binary component system.

RESULTS AND DISCCUSSION

6-acyl-o-β-CD derivatives with different chain length (acetyl, propionyl, and butyryl) were obtained by acylation of the primary hydroxyl groups at 6-position of



R	Compound
-CH3	ACD
-CH2CH3	PCD
-(CH2)2CH3	BCD

Scheme 1: Acylation Reaction of β-CD

β-CD using acetyl, propionyl , and butyryl chloride. For the sake of clarity of presentation, 6-acetyl, propionyl, and butyryl-o-β-CDs will be expressed as ACD, PCD, and BCD, respectively (Scheme 1). The derivatives with the different substitution degrees were prepared by controlling the initial molar ratios of acyl chloride to β-CD in the esterification reaction. Subscript numbers have been used to denote the initial molar ratio between acyl chloride and β-CD.

The esterification mainly occurs at the primary hydroxyl groups as was shown by the proton NMR spectra. The NMR spectrum of β-CD using D6-DMSO as solvent yields a chemical shift value of 5.88 ppm for the hydroxyl proton at 6-position. The hydrogens of secondary hydroxyl groups at 2 and 3-positions have a chemical shift at 7.16 and 7.08 ppm, respectively. A reduction in hydrogen integral value associated with the primary hydroxyl groups was detected post-esterification, which suggests that the acylation mainly takes place at 6-position. A minor reduction at 2-position was also observed when a large amount of acyl chloride was added to the reaction. This, however, is not significant. The average substitution degree was estimated by dividing the hydrogen integral value of the primary hydroxyl group post-esterification with that obtained from β-CD only. IR spectra showed an increase in the intensity at a wavenumber of 1734 cm^{-1} post-acylation, which indicated the addition of ester groups to β-CD. X-ray powder diffraction

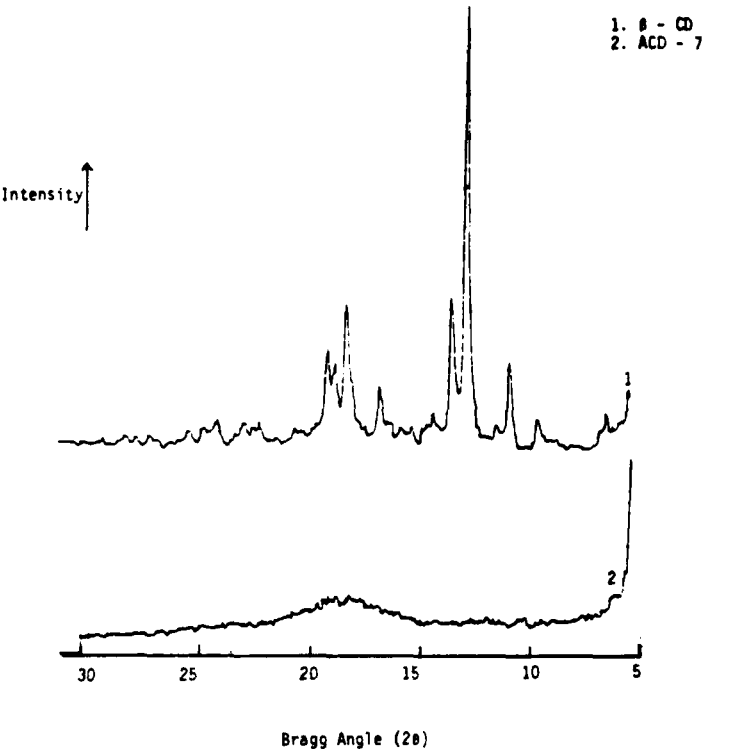


Figure 1
X-Ray Powder Diffraction Patterns of β-CD and ACD-7

patterns demonstrated that 6-acyl-o-β-CD solids are amorphous in nature, whereas β-CD powders exhibit a crystalline form, as shown in Fig. 1. None of the CD derivatives including β-CD showed any sharp melting endotherm on a DSC thermogram.

The aqueous solubility studies revealed that the solubilities of the derivatives (Table I) varies with the chain length and substitution degree. An increase in the substitution degree for a given acyl group tends to increase the solubility, which may be due to the fact that greater interruption of original crystallinity by more of the substituent gives rise to a higher solubility. The solubilities, however, decrease with increasing carbon chain length of substituent which implies that the hydrophobic side chain plays an important role in determining the aqueous

TABLE I

Physical Properties of 6-Acyl-o-β-CD Derivatives

Compound	Substitution* Degree (%)	Solubility at 30°C (mg/ml)	pH (5 %)	m.p.** (°C)
β-CD	0	18	-	255
ACD-1	3.33	70	5.64	-
ACD-3	7.33	110	5.80	-
ACD-5	16.60	230	5.82	-
ACD-7	22.00	460	5.47	240
PCD-3	6.97	100	5.60	-
PCD-5	12.30	170	5.73	-
PCD-7	21.70	240	5.70	240
BCD-7	-	150	6.12	-

* substituted percentage of primary hydroxyl groups on CD

** decomposed at this temperature

solubility. The stability of the derivatives in solution was determined by monitoring the change in pH at different time intervals. The derivatives in an aqueous solution was stable over at least a period of 48 hours at 30 °C.

The solubility phase diagrams obtained from acyl-β-CD/steroid complexation are defined as the B-type complexation, as shown in Figs.2 and 3. The stoichiometric ratio of the pure complexes is estimated from the solubility phase diagrams and the chemical analysis of the pure solid complexes. The result suggests that the stoichiometric ratio of the pure solid complexes is the same as that of β-CD, which is 2:1 (CD:steroid). X-ray powder diffraction pattern demonstrated that the solid complexes were less crystalline than that of drug itself, which may increase the dissolution rate of the drug. FABMS spectrum of ACD-7/progesterone complex seems to indicate that all species containing different number of substituents are able to form complexes with progesterone molecules at the same stoichiometric ratio of 2:1 (Fig.4).

The solubilization of steroids by the derivatives is dependent on the solubilities of the derivatives themselves, as depicted in Fig.5. For instance, ACD-7

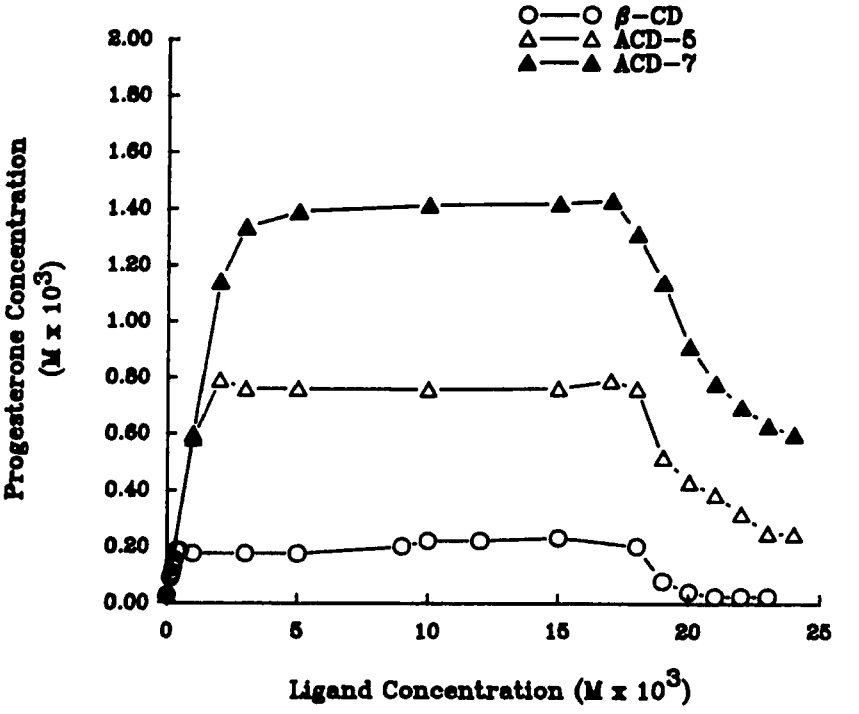


Figure 2
Phase Solubility Diagrams Obtained from Complexation of Progesterone with β-CD and ACDs

shows the highest aqueous solubility as well as solubilization power whereas β-CD exhibits the lowest. A previous report has described a solubilization study employing hydroxypropyl-o-β-CD with different substitution degree and indicated that an increase in substitution degree may increase its surface tension in aqueous solution (10). Therefore, the solubilizing ability decreases with an increase in the substitution degree. Our results suggest that the solubility of the complex is highly dependent on the solubility of the ligand.

The solubilization of progesterone given by a system consisting of two different CD derivatives demonstrate that an additive effect can be obtained if one of the components exhibits the A-type complexation (Fig.6). Complexation of α-CD and 2,6-dimethyl-o-β-CD with progesterone were defined as A-tpye (11). Both compounds were able to produce an additive solubilizing effect when combined

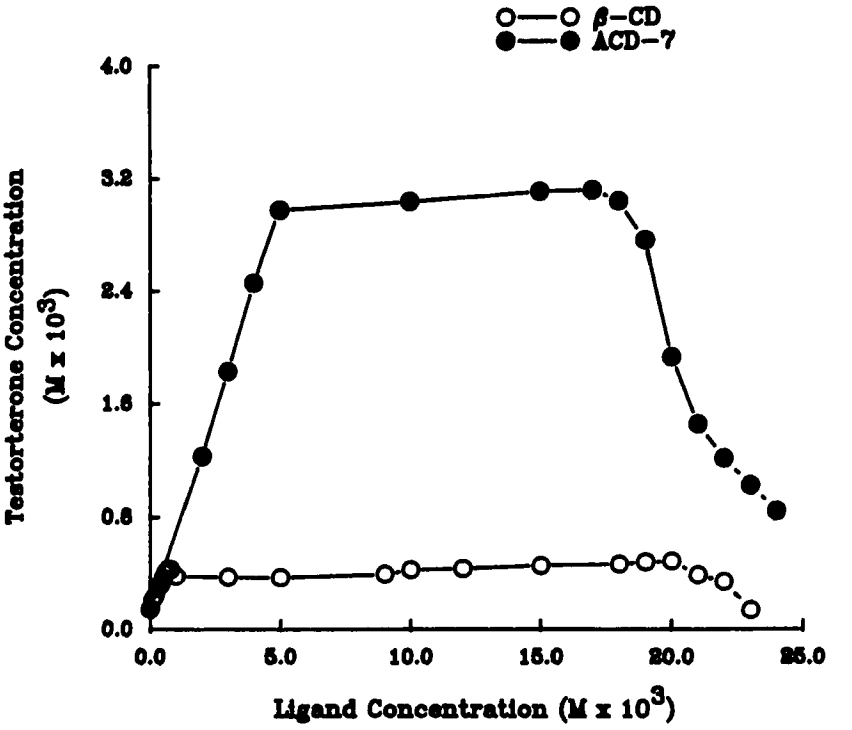


Figure 3

Phase Solubility Diagrams Obtained from Complexation of Testosterone with β-CD and ACD-7

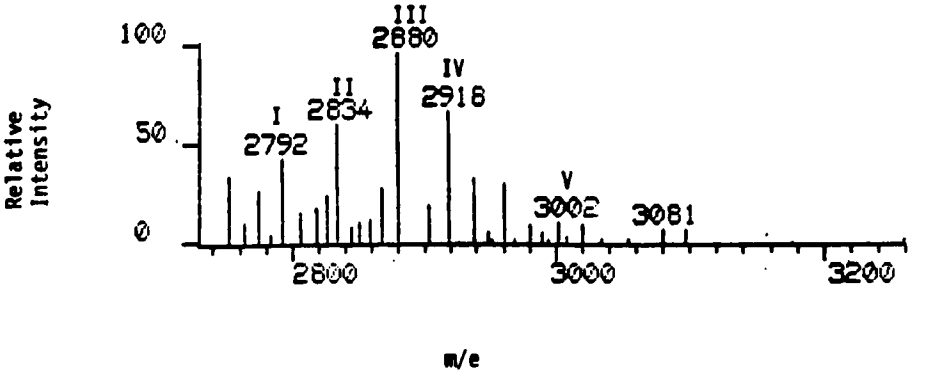


Figure 4

FAB Mass Spectrum of Pure ACD-7/Progesterone Complex:
I. the Complex Ion of $P(CD)_2(AC)_5$, i.e., One Progesterone Molecule (P) and Two β-CD Molecules, $(CD)_2$ Containing Five Acetyl Groups $(AC)_5$, II. $(CD)_2(AC)_6$, III. $P(CD)_2(AC)_7$, IV. $P(CD)_2(AC)_8$, and V. $(CD)_2(AC)_{10}$

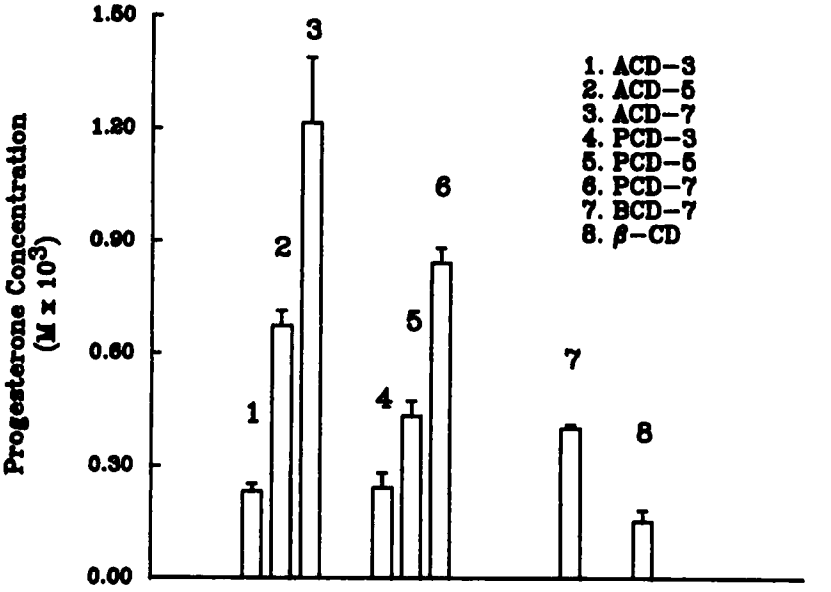


Figure 5
Solubilization of Progesterone by Different CD Derivatives at a Concentration of 10 mM

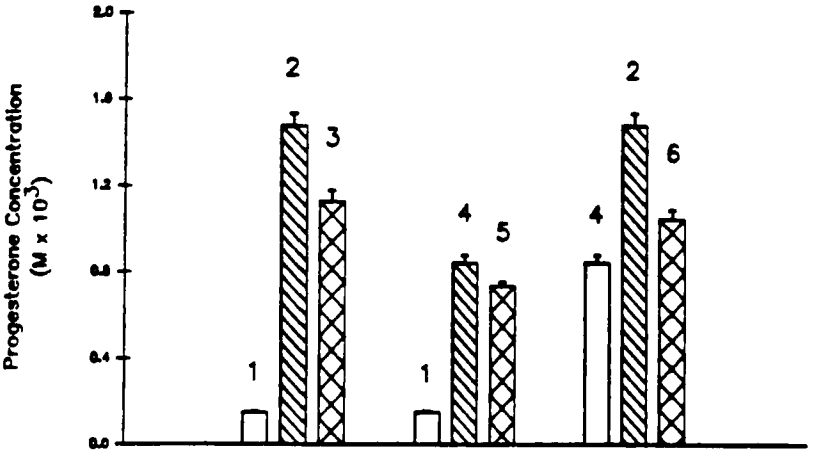


Figure 6
Solubilization of Progesterone by Single Component and a Binary Combination of CD Derivatives: 1. 10 mM β-CD, 2. 10 mM ACD-7, 3. 1+2, 4. 10 mM PCD-7, 5. 1+4, and 6. 2+4

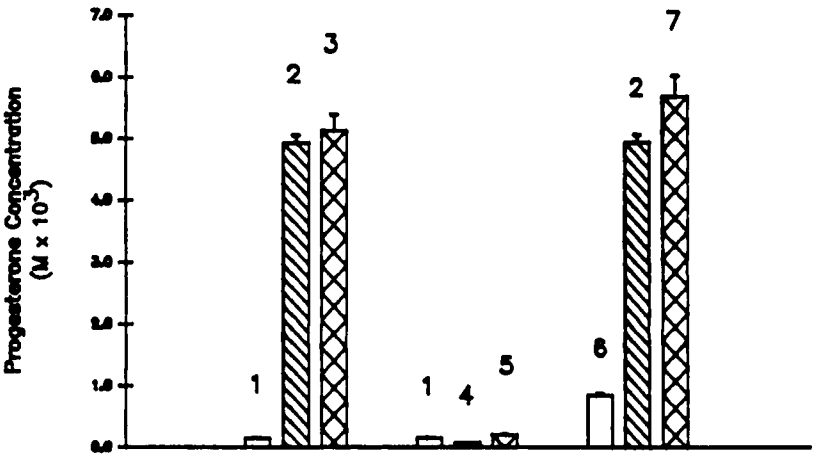


Figure 7

Solubilization of Progesterone by Single Component and Binary Combination of CD Derivatives: 1. 10 mM β-CD, 2. 10 mM 2,6-o-dimethyl-CD, 3. 1+2, 4. 10 mM a-CD, 5. 1+4, 6. 10 mM PCD-7, and 7. 6+2

with 6-acyl-o-β-CDs. This might be due to the fact that the complex formed by the A-type ligand does not reach its saturation solubility, thereby not generating a significant "salting out" effect on the other complex in solution. An antagonistic effect was observed when two B-type ligands such as 6-acyl-o-β-CDs were combined to solubilize progesterone (Fig.7). In a B-type complexation, when two complexes formed from two different ligands reach their saturated concentrations, the solution is very sensitive to the "salting out" effect . Both complex molecules tend to compete for water molecules each other and result in an antagonistic solubilization effect.

The ability of the derivatives to complex with steroids was evaluated by the determination of the apparent stability constants generated by the derivatives /progesterone complexation. The apparent stability constants for 1:1 complexation were calculated from the initial portion of the phase diagram (Fig.8 and Table II). The results indicate that the ability of the derivatives to form initial complex increases with the chain length. The implication is that the side chain may be

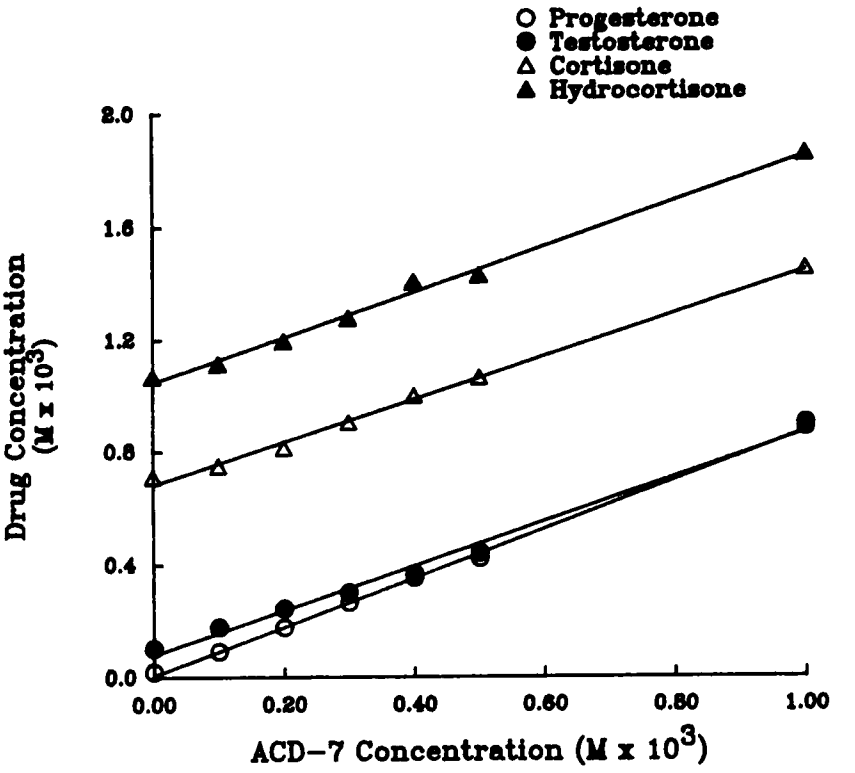


Figure 8
Initial Solubility Phase Diagrams Obtained from Complexation of Steroid with ACD-7

TABLE II
Apparent Stability Constants for the 1:1 Complexes

Compound	$K_{1:1}(M^{-1})$			
	β-CD	ACD-7	PCD-7	BCD-7
Progesterone	24705	41546	50137	53880
Testosterone	5058	16529	18255	-
Cortisone	2683	4574	5769	-
Hydrocortisone	2632	3920	5630	-

involved in the stabilization of complex, which has been discussed in a previous report (7). It is known that the substituent at different position of CD can change its complexation properties (7). However, the influence of the number of substituent is still unclear.

In summary, the interruption of the crystallinity of β-CD can give rise to higher solubility, thereby producing an improved solubilization power. The chain length and substitution degree play an important role in the solubilities of the derivatives. An additive solubilization can be achieved by combining the derivatives with a A-type CD-based solubilizer. An increase in the hydrophobicity of the side chain may enhance the formation of initial complex.

ACKNOWLEDGMENTS

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