Complexation of Steroid Hormones with Cyclodextrin Derivatives: Substituent Effects of the Guest Molecule on Solubility and Stability in Aqueous Solution

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Abstract □ The inclusion complexation of homologous derivatives of steroid hormones with cyclodextrins and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) was investigated with regard to underlying structure–interaction relationship. The interaction was studied by phase solubility analysis and stabilization effects of complex formation with 2-HP-β-CD. The solubilizing and stabilizing abilities of 2-HP-β-CD were generally more effective for testosterone derivatives than for estradiol esters. Within a homologous series of steroid hormones, the steepest linear solubility isotherms were found for 17-methyl and 3-methyl derivatives. The solubilization of steroid esters by 2-HP-β-CD depended on the structure and length of the ester side chain. The interaction of 2-HP-β-CD with the steroids was hindered by long-chain fatty acid ester groups. With increasing length of the side chain, a decline of the isotherms occurred and the phase solubility behavior changed from linear to exponential. Contrary to expectations, benzoylation of steroids considerably decreased the guest–host interaction. The observed rates of degradation of the steroid esters were significantly reduced by 2-HP-β-CD, depending on the chain length, and correlated well with the order found in phase solubility analysis. The degradation showed no deviations from pseudo-first-order kinetics, and the degradation mechanism was not changed because of complexation. The results suggest that interaction of 2-HP-β-CD with steroid esters involves the ester functions of the prodrugs and is more suitable for unsubstituted guest molecules.

The solubilizing properties of cyclodextrins are limited by their relatively low water solubility, particularly for β-cyclodextrin. The water solubility and, hence, the solubilizing power of the cyclodextrins can be considerably increased by partial etherification with alkyl or hydroxyalkyl groups.[1] 2-Hydroxypropyl-β-cyclodextrin (2-HP-β-CD) may be used as a parenteral drug carrier, in view of its weak hemolytic activity and its intrinsically amorphous character. Inclusion complexation by cyclodextrins involves molecular encapsulation of the guest molecule in the solid or liquid phase, with the result that physicochemical properties of the guest molecule (e.g., solubility, stability, and spectral characteristics) are altered.

The purpose of the present investigation is to determine the dependence of the guest–host interactions on the structure of the guest molecule, particularly steroid hormones. Homologous guest molecules differing only in their ester side chains were used for the systematic study of guest–host interactions. As an example of a sterically hindered steroid ester, cyproterone acetate, which antagonizes the effects of testosterone on humans and thus has antiandrogenic potency, was examined. To determine structural elements of the guest molecule that favor inclusion complexation, phase solubility and stability studies were carried out.

Testosterone is an α,β-unsaturated ketone with a secondary alcohol function in position 17. Estradiol can undergo ester formation in positions 3 and 17. Because of the aromatic A ring of estradiol, the 3-OH function possesses phenolic characteristics. In the therapeutic application of steroid hormones, the prodrug properties of their esters are used to prolong their effects to varying degrees[2] and to enhance the release and permeation rates in the case of transdermal administration.[3] It has not yet been possible to produce aqueous solutions of steroid hormones for administration by the parenteral route, because of growing objections to conventional solubilizing agents, such as PEG-hydrogenated castor oil (Cremophor RH 40), and polar components, such as 1,2-propylene glycol. Official and new parenteral formulations of steroid hormones with low solubility (Table I) include solid dosage systems and solutions with fixed oils (e.g., olive or arachis oil). However, fixed oils may cause allergic responses and are not feasible for the intravenous route because of the risk of lipid embolism. Inclusion complexes, especially with γ- and β-cyclodextrin, have been described for the parent molecules testosterone and estradiol.[4–8] Cyclodextrin derivatives, such as dimethyl-β-cyclodextrin and 2-HP-β-CD, are powerful solubilizing agents,[9,10] transdermal penetration enhancers,[11,12] and nasal absorption enhancers[13,14] for unsubstituted steroids; however, it is not known if these activities are valid for therapeutically relevant steroidal prodrugs.

Experimental Section

The steroids were purchased from the following manufacturers: testosterone cypionate (Tcy), testosterone (T), 17-methyltestosterone (MeT), testosterone acetate (Tac), testosterone propionate (Tpr), testosterone enanthate (Ten), cyproterone acetate (Cyp), estradiol (E), estradiol 17-valerate (Eva), estradiol 3-methyl ether (E3me), estradiol 17-enanthate (Een), estradiol 17-propionate (Epr), estradiol 17-undecylate (Eun), and estradiol 3-benzoate (E3be) from Schering AG, Berlin, Germany; and testosterone benzoate (Tbe) and estradiol 17-acetate (Eac) from Sigma Chemie GmbH, Deisenhofen, Germany. The identities of the steroids were checked by ^{1}H NMR spectroscopy.[15,16] β-Cyclodextrin (β-CD) and 2-HP-β-CD, having molar degrees

Table I—Classification of Parenteral Formulations of Steroid Hormone Derivatives

Formulation	Characteristics
Implants	Sterile pellets[a] and implants[b]
Sterile aqueous suspension	Particle size ≤ 150 μm[a,c]
Sterile injection	In suitable vegetable oil or ethyl oleate[a,b,c]
Therapeutic systems	Transdermal drug delivery systems; transdermal bioactivated hormone delivery device (3)
Sterile lipid emulsions	With egg lecithin or poloxamer, particle size ≤ 1 μm
Cyclodextrin inclusion compounds	For parenteral, nasal, and transdermal routes of administration

[a] The United States Pharmacopeia XXII, 1990. [b] British Pharmacopoeia, 1988. [c] Pharmacopoeia of Japan, 1986.

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of substitution (MS) of 0.47 (androgens) and 0.51 (estrogens), respectively, were obtained from Chinoin, Budapest, Hungary.

The chemical homogeneity of the partially substituted cyclodextrins was demonstrated with ^1H NMR. The water contents were determined with a Karl-Fischer apparatus (Metrohm AG, Herisau, Switzerland) and taken into account in the calculation of the sample weights: β-CD, 10.9%; 2-HP-β-CD, 4.1% (MS = 0.47) and 2.4% (MS = 0.51). Phase solubility studies were carried out as described previously.[17,18] Excessive amounts of the steroids (100–200 mg/vial) were weighed into 50-mL brown glass bottles for two series of measurements in each case, and 10-mL portions of aqueous cyclodextrin solutions of various concentrations were added. Freshly distilled water (pH 7.4; 0.01 mol · L^{-1} potassium phosphate buffer) was used for the preparations. The tightly stoppered bottles were agitated in a shaking thermostat (MS 20 S, Lauda, Lauda-Königshofen, Germany) at 25 ± 0.01 °C and treated at intervals in an ultrasonic bath (Laboson 200, Bender Hobein, Munich, Germany). When the complex formation had reached equilibrium (≈10 days), the solutions were filtered through 0.22-μm membrane filters (Millex-GS, Millipore Corp., Bedford, MA), and the absolute quantity of dissolved steroid in the clear filtrate was determined after suitable dilution with methanol (purified grade, Merck, Darmstadt, Germany). All percentages given for solutions are weight by volume.

The analyses, modified for both androgens[19–22] and estrogens,[23–25] were carried out by high-performance liquid chromatography (HPLC) with an HPLC instrument equipped with a UV–visible detector (Merck/Hitachi, Darmstadt, Germany). A 250 × 4.6-mm i.d. stainless steel column, packed with 5-μm particles of octadecyl silica (ODS Hypersil; Shandon, Runcorn, GB) was used. The column was thermostated at 30 °C. Samples were applied via autoinjection (autosampler, Merck) or alternatively with a microliter syringe (50 μL; Hamilton Bonaduz AG, Bonaduz, Switzerland) in a Rheodyne valve (Rheodyne Incorporated, Cotati, CA) with a 20-μL loop size. The mobile phase consisted of isocratic methanol (LiChrosolv, Merck) and water at a flow rate of 1 mL · min^{-1} and a pressure of 48–68 bar. The conditions for each steroid, given as eluant methanol:water (v/v), detection wavelength (nm), were as follows: MeT: 91:9, 240.0; T: 91:9, 241.0; Tac: 95:5, 241.0; Tpr: 97:3, 241.0; Ten: 100:0, 240.0; Tcy: 100:0, 240.0; Cyp: 89:11, 282.0; Tbe: 93:7, 234.3; E3me: 86:14, 278.3; E: 80:20, 280.0; Eac: 85:15, 280.8; Epr: 88:12, 280.7; Eva: 90:10, 280.0; E3be: 88:12, 230.0; Een: 95:5, 280.6; and Eun: 100:0, 280.7. The relation between the area under the curve of concentration versus time and the concentration was linear within the measurement range. A line passing through the origin, with a correlation coefficient of >0.999, was obtained in every case. The external-standard method was used for calibration. The precision, expressed as the relative standard deviation of the response factors, was 0.18–0.67% (n = 4). The absorption maxima were determined with a Lambda 5 UV–visible twin-beam spectrophotometer (Perkin-Elmer, Überlingen, Germany).

For kinetic measurements, aqueous cyclodextrin and methanolic solutions were prepared containing steroid at 300 mg · mL^{-1}. The minimum methanol contents required to dissolve the steroids, determined in advance, were 40% for Tac; 50% for Tpr, Eac, Epr, and Een; 58% for Tcy; 78% for Tbe; and 60% for E3be. The concentration of 2-HP-β-CD was varied between 2 and 10% (Chinoin, MS = 0.52; water content, 3.05%). After adjustment to pH 9 (borate buffer Titrisol, Merck), the solutions were aseptically filled into 2-mL clear glass ampules and bubbled with nitrogen, and the ampules were stored in thermostatically controlled ovens at 30, 40, 50, and 70 °C. Groups of three ampules were selected at fixed intervals, and their undecomposed steroid contents were determined. The pseudo-first-order rate constants were determined as described previously,[26] and the statistical analysis was according to the method of Sachs.[27]

Results and Discussion

The androgen derivatives were solubilized to a greater degree with 2-HP-β-CD than the estradiol derivatives, and within a given class of substances, the solubility of the complex was highest for the 17-methyl and 3-methyl derivatives (Figures 1 and 2). On esterification of the secondary alcohol group in position 17 with fatty acids, the isotherms became increasingly flat and changed to a different type. According to the Higuchi classification,[17] linear A_L isotherms were found for MeT, T, Tac, Tpr, E3me, and E, whereas the

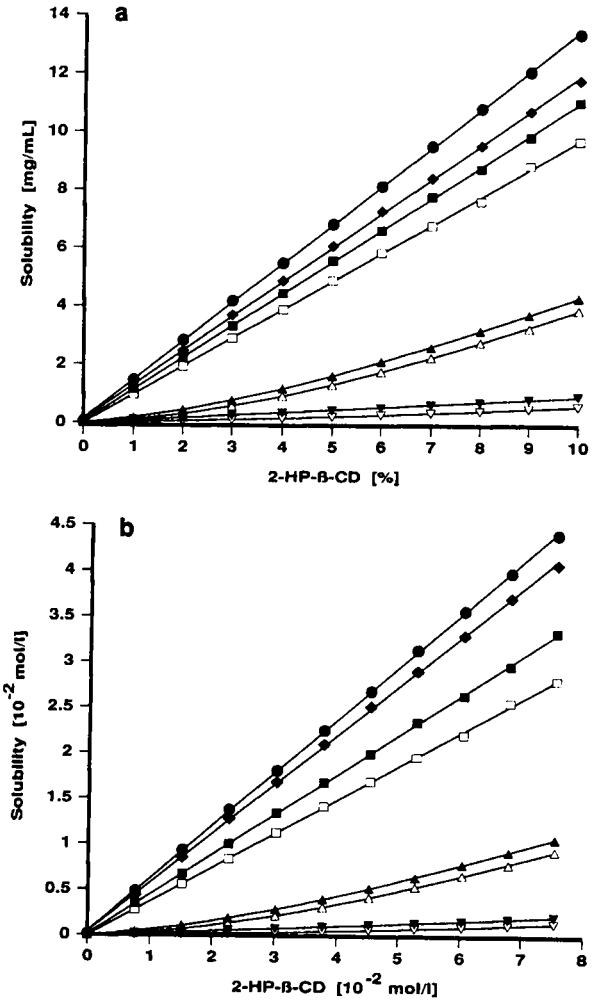

Figure 1—Phase solubility diagram of methyltestosterone (●), testosterone (◆), testosterone 17-acetate (■), testosterone 17-propionate (□), testosterone 17-enanthate (▲), testosterone 17-cypionate (△), cyproterone acetate (▼), and testosterone 17-benzoate (▽) with 2-HP-β-CD in pH 7.4 phosphate buffer at 25 °C (**a**) 2-HP-β-CD expressed in percent; (**b**) 2-HP-β-CD expressed in molar concentration.

isotherms of the other esters changed to the exponential Ap type with increasing chain length. This transition from the linear to the exponential type of isotherm took place between the propionate and the enanthate for the testosterone esters, whereas for the estradiol esters, it was apparent even with the acetate. Esterification of the secondary alcohol group generally had a considerably greater adverse effect on the solubilization of estradiol than on that of testosterone. In Figure 1b, the solubilization of androgens is also given in molar quantities. The results are strictly comparable with those shown in Figure 1a because there are only slight differences in the molecular weights of the steroid esters. Bs isotherms were found with β-cyclodextrin, except for Cyp (Figures 3a and 3b). This type of isotherm passes through a plateau, indicating the formation of insoluble complexes. For long-chain esters, only insignificant differences were apparent in the solubilization behavior with β-CD. The order of the isotherms for β-CD was the same as for 2-HP-β-CD, which formed only soluble complexes. Our previous work in this area demonstrated that the maximum solubility of the complex is actually given by the solubility isotherm and no enhancement of the solubilization capacity of 2-HP-β-CD can be achieved by any conceivable, suitable combination with further solution mediators.[28]

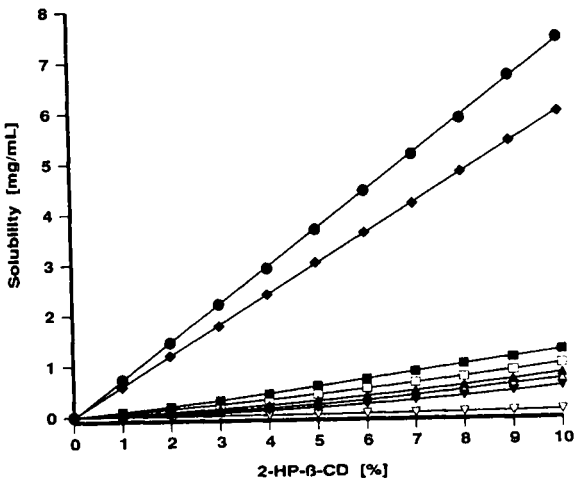

Figure 2—Phase solubility diagram of estradiol 3-methyl ether (●), estradiol (◆), estradiol 17-acetate (■), estradiol 17-propionate (□), estradiol 17-valerate (▲), estradiol 3-benzoate (△), estradiol 17-enanthate (▼), and estradiol 17-undecylate (▽) with 2-HP-β-CD in pH 7.4 phosphate buffer at 25 °C.

Curve fittings of the general form $y = a + bx$ for the linear isotherms and $y = a \cdot x^c$ for the exponential isotherms were carried out with the experimental data from the solubility isotherms. In its actual definition, the exponential function $y = a^x$ was not valid to characterize solubility isotherms, because the saturation solubility is not allowed to deviate from 1 according to $a^0 = 1$. However, the term "exponential" was retained with respect to the equation given above. The calculated curve parameters are shown in Table II. The gradient of the linear isotherms is represented by "b." The minimum cyclodextrin concentration required to solubilize any given quantity of steroid (e.g., to produce a certain test dose) can be calculated from these parameters. The isotherms can be assigned to a given type on the basis of the correlation coefficient for the particular fitting. Both linear and exponential descriptions are possible for the isotherms of MeT, T, Tac, Tpr, E3me, and E. However, if we consider the values of c to be ~1 for these steroids, the equation $y = a \cdot x^c$ transforms to the linear form $y = ax$. For the isotherms of the other steroid esters, the correlation coefficients for the linear type decrease with increasing chain length, and only exponential representation is possible in these cases. Regarding the precision of curve fittings, the best results could be achieved by polynomial regression analysis of the general from $y = a_0 + a_1x + a_2x^2 + \ldots a_nx^0$. With increasing polynomial degree n, the correlation coefficient (r) approximates the value 1; however, the significance of the model decreases. In the case of Eva, for example, n increases to 8, with $r^2 \approx 1$. Consequently, polynomial regression should not be applied for the evaluation of curve parameters in this context.

The exponential solubility isotherms of long-chain steroid esters indicate that these steroids have a greater tendency to form higher order complexes that deviate from 1:1 stoichiometry. Isotherms that could be misinterpreted as exponential can also result if the buffer capacity is exceeded. However, effects of this nature can be ruled out, because checks on the pH of the filtrates showed no changes from the initial value. To summarize, the solubilization of steroid hormones with 2-HP-β-CD decreases in the following orders: for androgens, MeT > T > Tac > Tpr > Ten > Tcy > Cyp > Tbe; for estrogens, E3me > E > Eac > Epr > Eva > E3be > Een > Eun.

The order in complexation determined for steroid hormone derivatives was confirmed by ^{1}H NMR investigations with 0.1 mol · L^{-1} solutions of 2-HP-β-CD in D_2O that was saturated with the steroids. The relative ratios of the intensities and

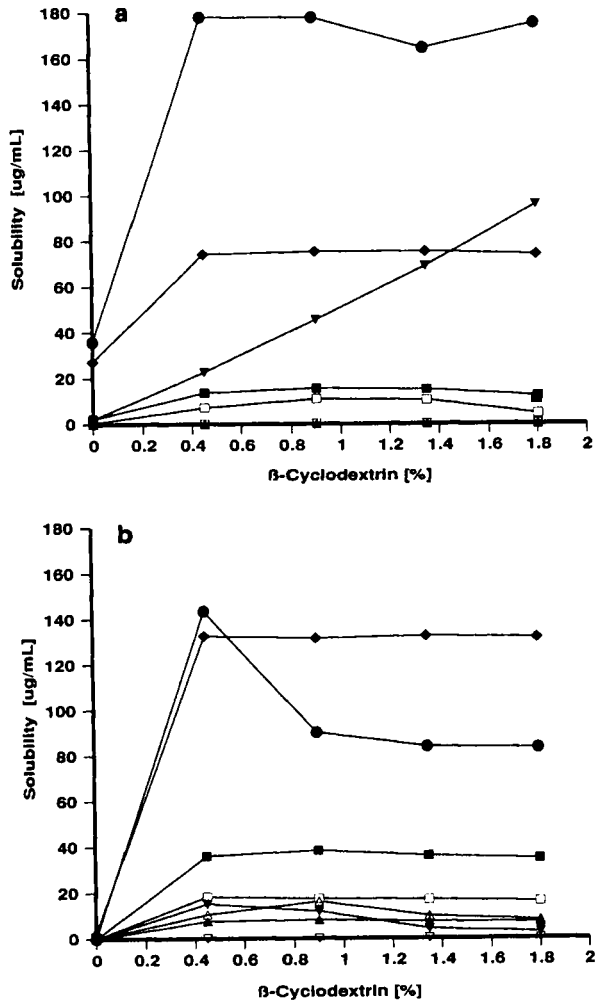

Figure 3—(a) Phase solubility diagram of methyltestosterone (●), testosterone (◆), cyproterone acetate (▼), testosterone 17-acetate (■), testosterone 17-propionate (□), testosterone 17-enanthate (▲), and testosterone 17-benzoate (▽) with β-CD in pH 7.4 phosphate buffer at 25 °C. (b) Phase solubility diagram of estradiol 3-methyl ether (●), estradiol (◆), estradiol 17-acetate (■), estradiol 17-propionate (□), estradiol 3-benzoate (△), estradiol 17-valerate (▲), estradiol 17-enanthate (▼), and estradiol 17-undecylate (▽) with β-CD in pH 7.4 phosphate buffer at 25 °C.

integrals of 2-HP-β-CD:steroid ^{1}H resonances increased within the order given above.

For a more detailed investigation of the relationship between the solubilization of steroid esters and the ester chain length, the molar solubilities of the steroids, in moles of dissolved drug per mole of 2-HP-β-CD, were plotted against the number of carbon atoms in the ester side chain (Figure 4). The linear relationship for testosterone esters also includes T and MeT if the abscissa values 0 and −1, respectively, are assigned to these compounds. Estradiol, however, does not fall on the straight line found for the estradiol esters. The transition from E to Eac is abrupt, and the gradient of the line is smaller. This relationship is shown by the following regression equations: for testosterone derivatives, $y = 0.539 - 0.054x$ ($r^2 = 0.996$); for estradiol derivatives, $y = 0.058 - 0.005x$ ($r^2 = 0.967$). Within a homologous series of guest molecules, a correlation between structure or substituent parameters (e.g., the number of carbon atoms in the ester side chain) and interaction parameters (e.g., complex solubility) can be established. The solubilization of esters that were not investigated, such as the formate, the butyrate, and the caproate, can be deduced on the basis of this direct dependence on the chain length of the esters. Thus, the y intercept of testosterone formate was interpolated with C = 1 (where C is

Table II—Regression Parameters of the Fitting Functions $y = a + bx$ for Linear Solubility Isotherms (l) and $y = a \cdot x^c$ for Nonlinear (nl) Exponential Solubility Isotherms of Testosterone and Estradiol Derivatives with 2-HP-β-CD

Drug	Model	Regression Parameter			r^2
		a	b	c	
MeT	l	0.132	1.332	—[a]	0.999
	nl	1.448	—	0.965	1.000
T	l	0.109	1.180	—	0.999
	nl	1.258	—	0.975	1.000
Tac	l	0.033	1.097	—	0.999
	nl	1.130	—	0.987	0.999
Tpr	l	−0.014	0.972	—	0.999
	nl	0.954	—	1.008	0.999
Ten	l	−0.388	0.440	—	0.981
	nl	0.159	—	1.436	1.000
Tcy	l	−0.436	0.396	—	0.969
	nl	0.092	—	1.632	0.999
Cyp	l	−0.018	0.090	—	0.998
	nl	0.077	—	1.059	0.999
Tbe	l	−0.050	0.056	—	0.973
	nl	0.022	—	1.378	0.998
E3me	l	−0.024	0.738	—	0.999
	nl	0.721	—	1.009	0.999
E	l	−0.004	0.596	—	1.000
	nl	0.587	—	1.008	0.999
Eac	l	−0.049	0.126	—	0.997
	nl	0.088	—	1.150	0.999
Epr	l	−0.051	0.097	—	0.992
	nl	0.061	—	1.187	0.999
Eva	l	−0.072	0.079	—	0.975
	nl	0.030	—	1.399	0.999
Ebe	l	−0.081	0.068	—	0.957
	nl	0.015	—	1.623	0.999
Een	l	−0.071	0.052	—	0.942
	nl	0.009	—	1.745	0.999
Eun	l	−0.009	0.005	—	0.897
	nl	0.000	—	2.688	0.996

[a] —, Not applicable.

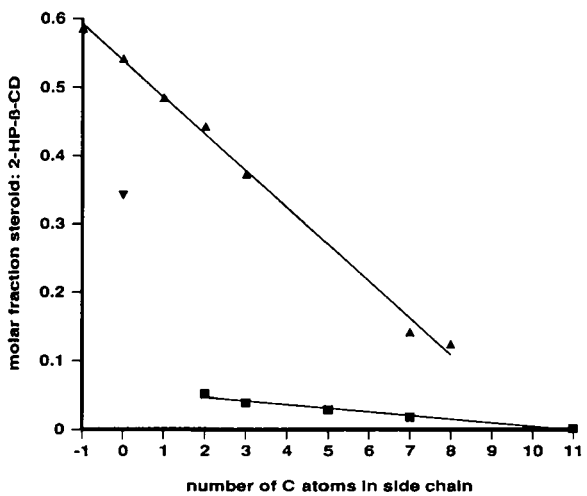

Figure 4—Dependence of the solubilization of steroid hormone esters with 2-HP-β-CD on the number of C atoms in the ester side chain for various testosterone esters (▲), estradiol esters (■), and estradiol (▼).

the number of carbon atoms in the side chain) between T and Tac. According to this interpolation, 0.485 mol of testosterone formate can be solubilized by 1 mol of 2-HP-β-CD. In comparison with their demethyl derivatives T and E, the more hydrophobic guest molecules MeT and E3me were preferentially complexed by the hydrophobic cyclodextrin cavity. In accordance with these results, recent investigations with 17α-ethynylestradiol[11] revealed a predominant complexation with 2-HP-β-CD compared with the unsubstituted guest molecule. The reduction of the interactions by acylation can be explained in the case of Tac by the introduction of the polar carbonyl group. If the hydrophobicity of the guest molecule was the only factor governing the interactions with cyclodextrins, the curve in Figure 4 should rise again as it proceeds from Tac to the higher fatty acid esters. However, the steric features of the guest molecule appear to be of crucial importance so that the complexibility of the steroid hormones is blocked by esterification with longer chain fatty acids, even though the latter are highly lipophilic. The linear relationship between the solubilization of the steroids and the length of the ester side chain is also valid for alicyclic structures, as shown by the behavior of a cyclic ester, the 3-cyclopentanepropionate. This so-called cypionate behaved as an aliphatic caprylic acid ester. The solubilization properties of the benzoic acid esters of the steroids were very poor and depended on the position of the aromatic structure. Position 3 on the A ring of estradiol was more favorable than position 17 on the D ring of testosterone. This difference can be attributed to conformation effects. The benzoyloxy group in position 3 of the planar aromatic A ring of estradiol rotates freely in space, whereas substitution in position 17 of testosterone increases the probability of steric proximity to the steroid skeleton and allows the formation of a bulky partial structure. On the basis of the results for the benzoates, a comparable low solubilization for the nicotinates is concluded. Interactions with cyclodextrins were also massively reduced by the introduction of bulky or polar groups into the steroid structure, as exemplified in Cyp by the methylene bridge in the A ring and the chlorine substitution on ring B. The molar ratio of drug to solubilizer for MeT and 2-HP-β-CD was calculated to be ≈0.6. In addition to our previous findings,[28] this value greatly exceeded the molar ratios found for conventional solubilizers (e.g., deoxycholic acid sodium salt, 0.03; nicotinamide, 0.002–0.01; and 1,2-propylene glycol, $1–3 \cdot 10^{-4}$). These results demonstrate that inclusion complexation with 2-HP-β-CD is the most effective solubilizing principle for steroid hormones. However, in the latter case (i.e., testosterone esters), the strong dependency of the complexation on structural requirements of the guest molecules has to be considered.

Information on relationships between structure and interaction is obtainable not only from the solubilization behavior but also indirectly from stability studies on complexed guest molecules.[29] Investigation of the photostability of the light-sensitive steroid hormones would have been of pharmaceutical interest, but a photochemical kinetic study seemed pointless, because the photoreactivity of these substances is unusually complex[30] and also solvent dependent. On the other hand, the alkaline hydrolysis of the steroid hormone esters has a uniform kinetic basis.[31,32] The OH^--catalyzed decomposition of 21-hydroxy corticosteroids, such as hydrocortisone 17-butyrate or betamethasone 17-valerate, however, involves intramolecular transesterification steps.[33,34] It was possible to follow the alkaline hydrolysis of the steroid hormone esters by HPLC. The only hydrolysis products detected were T and E, which were separated from the undecomposed esters. A qualitative indication of the degree of hydrolysis was provided by the decrease in the area of the ester peak accompanied by the progressive increase in the area of the T or E peak. Investigations on the decomposition kinetics of the steroid esters listed in Table III were carried out at pH 9 in methanolic solutions and in solutions containing 2-HP-β-CD with various cyclodextrin concentrations and at various temperatures. The alkaline hydrolyses of the acetates and propionates of T and E and the effect of 2-HP-β-CD are shown in Figures 5 and 6, respectively, on a semilogarithmic plot, as an example.

Linear relationships between log c and t (where c is

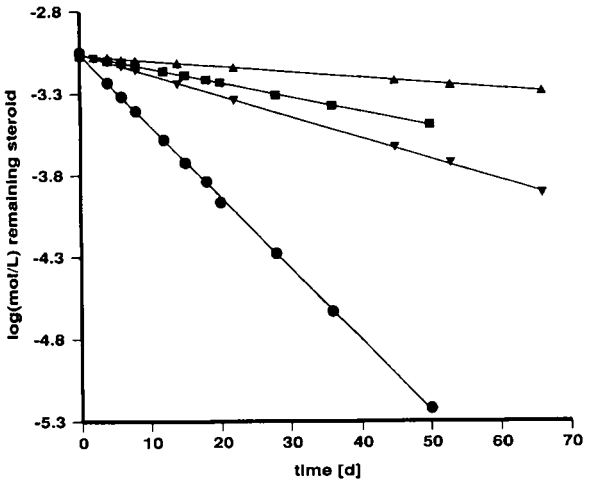

Figure 5—Semilogarithmic representation of the decomposition kinetics of testosterone acetate in methanolic solution (●) and in 10% aqueous 2-HP-β-CD solution (■) and of testosterone propionate in methanolic solution (▼) and in 10% aqueous 2-HP-β-CD solution (▲) at 40 °C and pH 9 (d, days).

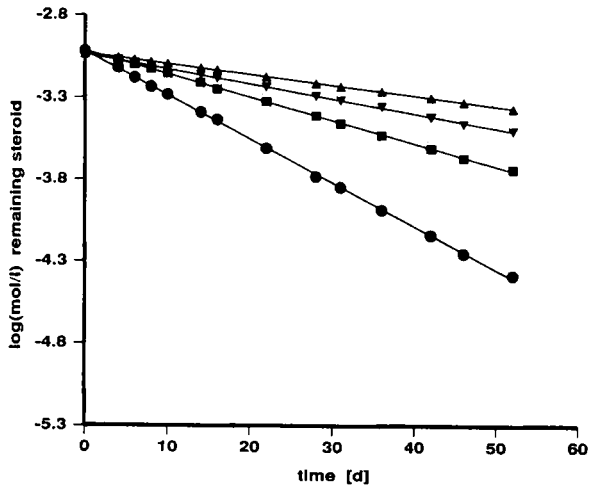

Figure 6—Semilogarithmic representation of the decomposition kinetics of estradiol acetate in methanolic solution (●) and in 10% aqueous 2-HP-β-CD solution (■) and of estradiol propionate in methanolic solution (▼) and in 10% aqueous 2-HP-β-CD solution (▲), at 40 °C and pH 9 (d, days).

concentration, and t is time) were found for all the steroid esters investigated ($r > 0.999$). The straight lines shown in Figures 5 and 6 are typical. The pseudo-first-order kinetic behavior deduced from these results is also valid for the systems with 2-HP-β-CD. To compare the extent of the stabilizing effects of 2-HP-β-CD on the steroid esters, instantaneous rate constants were calculated and statistically analyzed (Table III). The rates of hydrolysis of the steroid esters ($^1K_{obs}$) depended on the ester structure, as shown by the decrease in $^1K_{obs}$ values and the increasing half-life ($t_{1/2}$) values in methanolic solution. The T esters were less stable than the E esters. As a general rule, the stability within a series increased with increasing chain length. The observed rate constants were significantly reduced by 2-HP-β-CD (unpaired, two-sided t test). No significant difference was found for Een. Investigations with higher cyclodextrin concentrations led to only an insignificant further increase in the stabilizing effect. The differences in the rate constants (Δ^1K_{obs}) show that the stabilizing effect of 2-HP-β-CD is greatest for the T esters. The preferential stabilization of the T esters is likewise evident from the distances between the regression lines of free and complexed steroid esters (Figures 5 and 6) for the acetates and propionates. Within a given series, stabilization was greatest for the acetate and decreased with increasing ester chain length. The results of the stability tests with steroid esters show that the stabilizing effect of 2-HP-β-CD correlates with the strength of the solubilizing effect. A possible explanation for the protective effect of 2-HP-β-CD is that the ester function is at least partly enclosed and hence shielded against nucleophilic attack by OH^-.

The temperature dependence of the stabilizing effect of 2-HP-β-CD was investigated for Tac. Kinetic stress tests in the absence and presence of 2-HP-β-CD at four temperatures (Figure 7) indicated a linear relationship between the natural logarithm of $^1K_{obs}$ and the reciprocal absolute temperature. Consequently, there is no change in the reaction mechanism within the temperature range investigated, and the Arrhenius equation is valid. The stabilizing effect of 2-HP-β-CD is evident in the Arrhenius diagram from the displacement of the regression line. The energy of activation and frequency factor of the degradation were calculated from the gradients of the regression lines and intercepts of each plot, respectively. The results, including kinetic characteristics at 40 °C, are summarized in Table IV. Complexation with 2-HP-β-CD led to an appreciable increase in activation energy and resulted in a 4.2-fold improvement in stability. With higher 2-HP-β-CD concentration, steeper Arrhenius lines were obtained, and the energy of activation rose to 103.2 kJ · mol^{-1} for a 2-HP-β-CD concentration of 10%, representing the

Table III—$^1K_{obs}$ and $t_{1/2}$ Values of the Alkaline Hydrolysis of Various Steroid Esters[a]

Ester	Solution[a]	$^1K_{obs} \cdot 10^{-2}$, day^{-1}	SD · 10^{-2}[b]	$CI_{95\%} \cdot 10^{-2}$[c]	$t_{1/2}$, day	$\Delta^1K_{obs} \cdot 10^{-2}$[d]	p
Tac	ME	10.247	0.181	0.130	6.8		
	CD	1.955	0.072	0.052	35.5	8.292	<0.0001
Trp	ME	2.913	0.083	0.064	23.8		
	CD	0.734	0.120	0.092	94.4	2.178	<0.0001
Tcy	ME	1.140	0.032	0.027	60.8		
	CD	0.312	0.141	0.118	22.0	0.827	<0.0001
Tbe	ME	0.275	0.063	0.067	252.2		
	CD	0.095	0.067	0.107	732.8	0.180	0.0025
Eac	ME	6.115	0.130	0.079	11.3		
	CD	3.154	0.117	0.071	22.0	2.961	<0.0001
Epr	ME	1.994	0.109	0.065	34.8		
	CD	1.390	0.094	0.057	49.9	0.605	<0.0001
Een	ME	0.671	0.049	0.033	103.3		
	CD	0.552	0.372	0.250	125.6	0.119	0.3074

[a] Methanolic aqueous solution (ME) or 10% 2-HP-β-CD solution (CD) at 40 °C and pH 9. [b] Standard deviation. [c] Confidence interval. [d] Difference between $^1K_{obs}$ values in ME and CD solutions.

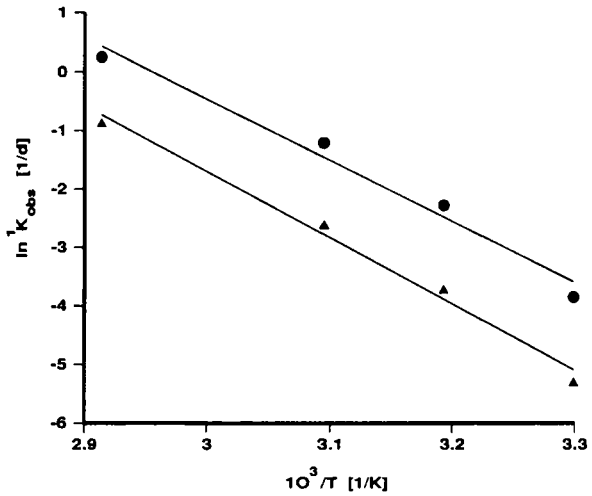

Figure 7—Arrhenius diagram for the decomposition of testosterone acetate at pH 9 in methanolic aqueous solution (●) and in 2% aqueous 2-HP-β-CD (▲) (d, days; T, temperature).

Table IV—Arrhenius and Kinetic Parameters for the Alkaline Hydrolysis of Testosterone Acetate[a]

Solution[a]	$^1K_{obs} \cdot 10^{-2}$, day^{-1}	$t_{1/2}$, day	$t_{90\%}$, day[b]	E_a, KJ · mol^{-1}[c]	log A, day^{-1}[d]
ME	10.25	6.77	1.03	87.00	13.42
CD	2.44	28.46	4.32	94.28	14.02

[a] In methanolic aqueous solution (ME) or 2% 2-HP-β-CD solution (CD) at 40 °C and pH 9. [b] Time for an extent of degradation of 10%. [c] Energy of activation. [d] Frequency factor.

maximal stability enhancement that could be achieved by complexation in aqueous solution.

Conclusions

2-HP-β-CD is a suitable solubilizing agent for steroid hormones and their derivatives, especially for androgens. The results reflect a strong dependency of the complexing ability of 2-HP-β-CD on substituent parameters (e.g., length and structure of the ester side chain). The crucial structural requirements of steroid hormones with respect to strong interactions with cyclodextrins are methyl or methoxy functions directly attached to the A or D ring. Because of the correlation of solubilizing and stabilizing effects of 2-HP-β-CD on steroid hormones, the gradient of the solubility isotherm indicates the magnitude of the obtainable stabilizing effect. The modified degradation kinetics has to be taken into account, and steroid esters must be applied on the basis of their prodrug abilities. The stabilizing effects of 2-HP-β-CD on steroid esters with long ester side chains are comparatively low. However, the improved chemical stability in aqueous solution should allow sufficient enzymatic reactivity to enable the delivery of the active compounds from the prodrugs. Thus, complexes with cyclodextrins are recommended for official steroid prodrugs (e.g., testosterone enanthate or estradiol valerate injections of the USP XXII; Table I).

It would be of considerable importance to extend the current investigations to the enhancing effects of cyclodextrin derivatives on the permeation and absorption behavior of steroid esters.

Whereas the relationships between the structure of the guest molecule and the interaction with 2-HP-β-CD are consistent within a homologous series of active substances, the same relationships are not necessarily valid for other groups of active substances. Benzoylation of the steroids led to a considerable reduction in the interactions with cyclodextrins. On the other hand, the aromatic substitution of dihydropyridine derivatives was a necessary structural requirement to obtain therapeutically useful solubilized systems.[28,35] The determination of structural elements that increase the guest–host interactions and the establishment of general rules for structure–interaction relationship will facilitate predictions of inclusion behavior with new, homologous, practically insoluble drug molecules, which is of particular interest when only small quantities of investigational drugs are available.

References and Notes

1. Müller, B. W.; Brauns, U. *Int. J. Pharm.* 1985, *26*, 77–88.
2. Crabbe, P.; Diczfalusy, E.; Djerassi, C. *Science* 1980, *209*, 992–994.
3. Chien, Y. W. *Drug Dev. Ind. Pharm.* 1987, *13*, 589–651.
4. Uekama, K.; Fujinaga, T.; Hirayama, F.; Otagiri, M.; Yamasaki, M. *Int. J. Pharm.* 1982, *10*, 1–15.
5. Pitha, J.; Anaissie, E. J.; Uekama, K. *J. Pharm. Sci.* 1987, *76*, 788–790.
6. Kralova, K.; Mitterhauszerova, L. *Pharmazie* 1989, *44*, 623–625.
7. Habon, I.; Stadler-Sz ke, A.; Szejtli, J. *Acta Biochim. Biophys. Acad. Sci. Hung.* 1984, *19*, 86.
8. Fujinaga, T.; Uemura, Y.; Hirayama, F.; Otagiri, M.; Uekama, K. *J. Pharmacobio-Dyn.* 1982, *5*, s-11.
9. Pitha, J.; Pitha, J. *J. Pharm. Sci.* 1985, *74*, 987–990.
10. Jensen, A. B., Diplomarbeit; Christian Albrecht University, Kiel, Germany, and University of Oslo, Norway, 1986.
11. Loftsson, T.; Bodor, N. *Acta Pharm. Nord.* 1989, *4*, 185–194.
12. Loftsson, T.; Olafsdottir, B. J.; Bodor, N. *Eur. J. Pharm. Biopharm.* 1991, *37*, 30–33.
13. Hermens, W. A. J. J.; Deurloo, M. J. M.; Romeyn, S. G.; Verhoef, J. C.; Merkus, F. W. H. M. *Pharm. Res.* 1990, *7*, 500–503.
14. Schipper, N. G. M.; Hermens, W. A. J. J.; Romeyn, S. G.; Verhoef, J.; Merkus, F. W. H. M. *Int. J. Pharm.* 1990, *64*, 61–66.
15. Miura, T.; Takagi, H.; Adachi, K.; Kimura, M. *Chem. Pharm. Bull.* 1982, *30*, 3154–3159.
16. Salole, E. G. In *Analytical Profiles of Drug Substances*; Florey, K., Ed.; Academic: New York, 1986; Vol. 15, pp 283–318.
17. Higuchi, T.; Connors, K. A. *Advances in Analytical Chemistry and Instrumentation*; Interscience: New York, 1965; Vol. 4, pp 117–212.
18. Higuchi, T.; Lach, J. L. *J. Am. Pharm. Assoc., Sci. Ed.* 1954, *43*, 349–354.
19. Carignan, G.; Lodge, B. A.; Skakum, W. *J. Pharm. Sci.* 1980, *69*, 1214–1216.
20. Karunanithy, R.; Becket, G.; Leung, S. L.; Ng, S. E. *J. Pharm. Biomed. Anal.* 1985, *3*, 375–379.
21. Matlin, S. A.; Chan, L.; Prazeres, M. A.; Mehani, S.; Cass, Q. B. *J. High Resolut. Chromatogr. Chromatogr. Commun.* 1987, *10*, 186–190.
22. Scott, J. C.; Soltero, R. A. *J. Chromatogr. Sci.* 1987, *25*, 415–417.
23. Satyaswaroop, P. G.; de la Osa, E. L.; Gurpide, E. *Steroids* 1977, *30*, 139–145.
24. Capitano, G.; Tscherne, R. *J. Pharm. Sci.* 1979, *68*, 311–314.
25. Roos, R. W. *J. Assoc. Off. Anal. Chem.* 1980, *63*, 80–87.
26. Grimm, W.; Schepky, G. *Stabilitätsprüfung in der Pharmazie*; Editio Cantor: Aulendorf, 1980; p 140.
27. Sachs, L. *Angewandte Statistik*; Springer: Berlin, 1983; p 89.
28. Müller, B. W.; Albers, E. *J. Pharm. Sci.* 1991, *80*, 599–604.
29. Zhang, D.-D.; Huang, N.-J.; Xue, L.; Huang, Y.-M. *J. Incl. Phenom.* 1987, *5*, 443–447.
30. Bellus, D.; Kearns, D. R.; Schaffner, K. *Helv. Chim. Acta* 1969, *52*, 971–1009.
31. Schenck, M.; Junkmann, K. *Arch. Exp. Pathol. Pharmakol.* 1955, *227*, 210–211.
32. Vesely, Z.; Pospisek, J.; Trojanek, J. *Collect. Czech. Chem. Commun.* 1969, *34*, 685–689.
33. Po, A. L. W.; Irwin, W. J.; Yip, Y. W. *J. Chromatogr.* 1979, *176*, 399–405.
34. Andersen, F. M.; Bundgaard, H. *Int. J. Pharm.* 1984, *20*, 155–162.
35. Müller, B. W.; Albers, E. *Int. J. Pharm.* 1992, *79*, 273–288.

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