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IN VITRO STUDIES ON THE INHIBITION OF PIG LIVER STEROID Δ^4-5β-REDUCTASE ACTIVITY BY NATURALLY OCCURRING AND SYNTHETIC ESTROGENS

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SUMMARY

1. The effects of various estrogens on the *in vitro* 5β-reduction by a partially purified pig liver enzyme preparation of the Δ^4-3-ketosteroids particularly testosterone, have been investigated to demonstrate the possibility of their being involved in the *in vivo* regulation of steroid hormone metabolism, and the possible side effects of oral contraceptives and drugs used for the treatment of androgen-dependent disorders.

2. Of the naturally occurring estrogens studied, estradiol(17β), 2-hydroxyestradiol, and estradiol-3-sulfate were found to inhibit testosterone 5β-reduction by 17%, 35% and 37%, respectively.

3. Ethinylestradiol and diethylstilbestrol, commonly used in the treatment of prostatic carcinoma, inhibited testosterone 5β-reduction by 56% and 87%, respectively.

4. The antiandrogen, cyproterone acetate, also inhibited testosterone 5β-reduction (51%).

5. The 5β-reduction of testosterone was inhibited by members of the equine series of estrogens.

6. The 5β-reduction of cortisol and aldosterone was inhibited by ethinylestradiol to the same extent as that of testosterone.

Abbreviations (trivial names used in this communication): testosterone, 17β-hydroxy-4-androsten-3-one; cortisol, 11β,17α,21-trihydroxy-4-pregnene-3,20-dione; aldosterone, 18,11-hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al; estrone, 3-hydroxy-1,3,5(10)-estratrien-17-one; estradiol(17β), 1,3,5(10)-estratriene-3,17β-diol; estradiol(17α), 1,3,5(10)-estratriene-3,17α-diol; estradiol(16α), 1,3,5(10)-estratriene-3,16α-diol; estriol, 1,3,5(10)-estratriene-3,16α,17β-triol; equilin = 1,3,5(10),7-estratetraene-3,17β-diol; equilenin, 1,3,5(10),6.8(9)-estrapentaene-3,17β-diol; diethylstilbestrol, trans-3,4-bis(*p*-hydroxyphenyl)-hex-3-ene; cyproterone acetate, 1,2α-methylene-6-chloro-4,6-pregnadiene-17α-ol-3,20-dione-17α-acetate; chlorotrianisene, tri-*p*-anisylchloroethylene; 6-ketoestradiol, 3,17β-dihydroxy-1,3,5(10)-estratrien-6-one; 2-hydroxyestradiol, 1,3,5(10)-estratriene-2,3,17β-triol; 15α-hydroxyestradiol, 1,3,5(10)-estratriene-3,15α-17β-triol; 4-bromoestradiol, 3,17β-dihydroxy-1,3,5(10)-estratriene-4-bromide; 15α-hy-droxyestrone,3,15α-dihydroxy-1,3,5(10)-estratrien-17-one; mestranol, 17α-ethinylestradiol-3-methyl ether.

7. The inhibition by diethylstilbestrol was found to be non-competitive, and the apparent K_i for diethylstilbestrol was calculated to be $1.0 \cdot 10^{-6}$ M.

INTRODUCTION

Factors which control the rate of steroid hormone metabolism can affect the rate of secretion of the hormone and the amount of hormone available for exertion of biological activity. Quantitatively, the liver is the principal site of steroid metabolism[1,2]. Estrogenic compounds have a number of known effects on steroid metabolism.

An important step in the hepatic metabolism of steroids containing the Δ^4-3-ketone configuration in ring A is the irreversible reduction of the double bond between carbon atoms 4 and 5. This reduction occurs in both the microsomal (principally 5α) and the soluble fraction (principally 5β). Estrogen administration to male rats increases the levels of microsomal Δ^4-reductase (ref. 3 and Patterson, D., Clark, A. F. and Bird, C. E., unpublished). In female rats, the administration of estrogens causes the levels to remain the same[3], or decrease[4]. These contradictory results could be due to the different doses of estrogen or the different dosage schedules[5]. In *in vitro* experiments, estrogens inhibit the microsomal reduction of cortisone and testosterone[6] and cortisol[7,8].

In vivo alterations in the rate of Δ^4-reduction of steroids by the liver appear to occur primarily in the microsomes. However, a decrease in the rate of testosterone 5β-reduction was observed in patients with cholestasis, which can be produced by liver cirrhosis or estrogen treatment[9]. Also, the inhibition by estrone of the conversion of cortisol to tetrahydrocortisol by human liver slices has been reported[10].

Our laboratory has previously reported that estrogen administration to men causes a decrease in the metabolic clearance rate of testosterone[11,12]. This is at least in part due to (1) the decreased plasma testosterone levels[13], which result from decreases in the secretion of gonadotropins by the pituitary and (2) increased levels of the specific sex hormone binding globulin in plasma[14].

In this paper, we report on the *in vitro* inhibitory effect of some estrogens on a partially purified pig liver steroid Δ^4-5β-reductase.

MATERIALS AND METHODS

Reagents

$NADP^+$ and glucose 6-phosphate were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Glucose-6-phosphate dehydrogenase (yeast) was purchased from Sigma and P.L. Biochemicals (Milwaukee, Wisc., U.S.A.).

The substrates and estrogens used in this study were products of Sigma, Schwartz/Mann (Orangeburg, N.Y., U.S.A.), Ikapharm (Ramat-Gan, P.O. Box 31, Israel), and Steraloids (Pawling, N.Y., U.S.A.).

15α-Hydroxyestrone and 15α-hydroxyestradiol were kindly supplied by Dr S. Solomon and Dr B. Bhavnani, Department of Endocrinology, Royal Victoria Hospital, Montreal, P.Q., Canada.

Materials for Sephadex G-100 column chromatography were purchased from Pharmacia (Montreal, P.Q., Canada).

Enzyme grade $(NH_4)_2SO_4$ was a product of Schwartz/Mann.

Methods

Partial purification of the enzyme. The soluble fraction of the female pig liver was obtained as described by Nduaguba and Clark[15], except that the homogenate was prepared in 3 vol. (w/v) 0.05 M Tris–HCl buffer (pH 8.0) rather than sucrose solution. The 105 000 × *g* supernatant fraction was brought to 40% $(NH_4)_2SO_4$ saturation by addition of the solid salt and centrifuged for 15 min at 10 000 × *g*. The precipitate was discarded and the supernatant was brought to 70% $(NH_4)_2SO_4$ saturation and again centrifuged for 15 min at 10 000 × *g*. The supernatant solution was discarded and the precipitate was dissolved in a minimal amount of 0.05 M Tris–HCl buffer, pH 8.0. This fraction was either subjected to further treatment or stored at 0–4 °C.

The 40–70% $(NH_4)_2SO_4$ saturation precipitate, dissolved in 0.05 M Tris–HCl buffer (pH 8.0), was applied to a Sephadex G-100 column (2.5 cm × 145 cm) which was prepared as described by Whitaker[16]. Equilibration of the column and elution of the enzyme in an ascending fashion was achieved with 0.05 M Tris–HCl buffer (pH 8.0) at a flow rate of 15 ml/h. Fractions of 50 drops (≈ 3.6 ml) were collected and the fractions with enzyme activity greater than 0.010 absorbance units/30 min were pooled. These pooled fractions were used for subsequent studies.

Enzyme and protein assays. The standard steroid Δ^4-5β-reductase assay mixture contained 100 μmoles Tris–maleate buffer (pH 6.4), 5.0 μmoles $MgCl_2$, 3.0 μmoles $NADP^+$, 15.0 μmoles glucose 6-phosphate, 2 units glucose 6-phosphate dehydrogenase activity, and 75 nmoles testosterone dissolved in 0.05 ml methanol, in a total volume of 3.0 ml. In the estrogen inhibition studies, 75 nmoles of each estrogen was also added to the incubation mixture. The assay mixture was incubated at 37 °C for 10 min. The reaction was started by the addition of enzyme, and a 1.0-ml sample was immediately removed and extracted with 5.0 ml methylene chloride. The remainder of the reaction mixture was incubated for 20 min (unless otherwise indicated) at 37 °C at which time another 1.0-ml sample was removed and extracted with 4.0 ml methylene chloride. The two extracted solutions were centrifuged at 1500 rev./min for 10 min. The aqueous and protein layers were removed by suction and the absorbance of the methylene chloride layers was determined at 240 nm. The difference in absorbance of the two methylene chloride extracts is proportional to the amount of substrate reduced under the chosen experimental conditions. Specific activity is expressed as nmoles of testosterone reduced per min per mg protein.

Protein concentrations were determined by the Miller[17] modification of the Lowry method[18].

Inhibition kinetics of testosterone 5β-reductase activity were determined by varying the substrate concentration (2.5–7.5 μM) in the absence and presence of 1.25 μM and 2.50 μM concentrations of diethylstilbestrol.

The effect of ethinylestradiol on testosterone, cortisol, and aldosterone 5β-reductase activity was determined with 75 nmoles of each substrate in the absence and presence of 75 nmoles of ethinylestradiol in the assay mixture.

RESULTS

The pig liver soluble steroid Δ^4-5β-reductase preparation used in these studies was partially purified by the procedures summarized in Table I. As indicated, the enzyme preparation was purified 8.3-fold with respect to the soluble fraction. The enzyme activity was not purified further because of its instability following further chromatographic procedures.

TABLE I

PURIFICATION OF STEROID Δ^4-5β-REDUCTASE FROM 400 g OF FEMALE PIG LIVER

Purification step	*Specific activity**	*Purification factor (-fold)*	*Total activity***	*Yield (%)*
Homogenate	0.331	—	20 000	—
Soluble fraction	0.393	1.00	8 350	100
40–70% $(NH_4)_2SO_4$ satn ppt.	0.435	1.11	5 110	61
Sephadex G-100 pooled eluates	3.26	8.30	3 710	44

* nmoles substrate reduced/min/mg protein.
** Specific activity × mg total protein.

Inhibition studies

The results obtained for the inhibition of testosterone 5β-reduction by the variety of naturally occurring and synthetic estrogens are summarized in Table II. Of the naturally occurring estrogens studied, 2-hydroxyestradiol and estradiol-3-sulfate were the most potent inhibitors of testosterone 5β-reduction (35.2% and 37.0%, respectively). Inhibition of 5β-reduction observed with some other naturally occurring estrogens are as follows: 17β-estradiol, 17.6%; 17α-estradiol, 17.2%; 6-keto-17β-estradiel, 0%; 15α-hydroxy-17β-estradiol, 4.2%; 17β-estradiol-17-(β-D-glucuronide), 5.9%; estrone, 18.0%; 15α-hydroxyestrone, 18.5%; estriol-16-(β-D-glucuronide), 8.4%. Considerable inhibition was observed with synthetic estrogens such as 16α-estradiol (54.1%), ethinylestrrdiol 55.6%), and diethyl- (86.8%). Less inhibition was observed with 17β-estradiol-3-phosphate (27.4%), 17β-estradiol-3-wethyl ether (20.2%), estriol-3-methyl ether (9.3%), 17β-estradiol-3-acetate (19.9%), 17β-estradiol-17-acetate (5.5%), 17β-estradiol-3,17-diacetate (2.7%), 17β-estradiol-3-hemisuccinate (13.7%), 17β-estradiol-17-hemisuccinate (34.0%), 17β-estradiol-3, 17-dihemisuccinate (21.9%), 17β-estradiol-17-valerate (19.9%), 4-bromo-17β-estradiol (33.6%), mestranol (11.1%), and chlorotrianisene (1.8%).

Equilin and equilenin, members of the equine group of steroids, exhibited 56.3% and 16.8% inhibition of testosterone 5β-reduction, respectively. Equilin-3-acetate inhibited testosterone 5β-reduction by 36.3%, while equilin-3-benzoate had no inhibitory activity.

A non-estrogenic antiandrogen, cyproterone acetate, inhibited the enzyme to the extent of 50.9%.

Several of the estrogen compounds studied exhibit a very wide range of inhibition (see Table II). This may be due to the fact that these are the pooled results obtained with different enzyme preparations. These enzyme preparations differed slightly in their degree of purity.

TABLE II

INHIBITION STUDIES OF TESTOSTERONE 5β-REDUCTION BY ESTROGEN COMPOUNDS

Estrogen	*Mean inhibition (%)*	*Range*	*No. of assays*
17β-Estradiol	17.6	11.3 – 23.3	9
17α-Estradiol	17.2	7.8 – 25.7	7
16α-Estradiol	54.1	53.4 – 54.8	2
6-Keto-17β-estradiol	0	0	2
2-Hydroxy-17β-estradiol	35.2	18.4 – 54.0	7
15α-Hydroxy-17β-estradiol	4.2	4.2	2
17β-Estradiol-3-sulfate	37.0	29.4 – 44.5	2
17β-Estradiol-3-phosphate	27.4	23.3 – 31.5	2
17β-Estradiol-3-methyl ether	20.2	16.4 – 24.4	4
17β-Estradiol-3-acetate	19.9	19.2 – 20.5	2
17β-Estradiol-17-acetate	5.5	2.7 – 8.2	2
17β-Estradiol-3,17-diacetate	2.7	(– 1.4)– 6.8	2
17β-Estradiol-3-hemisuccinate	13.7	12.3 – 15.1	2
17β-Estradiol-17-hemisuccinate	34.0	27.4 – 46.2	4
17β-Estradiol-3,17-dihemisuccinate	21.9	20.5 – 23.3	2
17β-Estradiol-17-valerate	19.9	12.3 – 27.4	2
17β-Estradiol-17-(β-D-glucuronide)	5.9	4.2 – 7.6	2
4-Bromo-17β-estradiol	33.6	26.0 – 41.1	2
17α-Ethinyl-17β-estradiol	55.6	37.9 – 69.9	9
Mestranol	11.1	0.7 – 20.4	7
Estrone	18.0	8.0 – 25.5	7
15α-Hydroxyestrone	18.5	16.4 – 20.5	2
Estriol	(–3.3)	(–15.2)– 4.4	7
Estriol-3-methyl ether	9.3	4.2 – 14.3	2
Estriol-16-(β-D-glucuronide)	8.4	7.6 – 9.2	2
Equilin	56.3	54.6 – 58.0	2
Equilin-3-acetate	36.3	34.2 – 38.4	2
Equilin-3-benzoate	(–4.1)	(– 8.2)– 0	2
Equilenin	16.8	14.3 – 19.3	2
Cyproterone Acetate	50.9	30.9 – 77.0	7
Chlorotrianisene	1.8	(– 8.2)– 13.3	7
Diethylstilbestrol	86.8	75.2 –100.0	7

Inhibition kinetics

Conventional Lineweaver–Burke plots, with the Lee and Wilson[19] modification for reactions exceeding 50% conversion of substrate, were obtained by varying the substrate concentration in the presence and absence of two different concentrations of diethylstilbestrol. The data obtained is illustrated in Fig. 1, and indicates that diethylstilbestrol is a non-competitive inhibitor of testosterone 5β-reduction. The apparent K_i for diethylstilbestrol was calculated to be $1.0 \cdot 10^{-6}$ M. The K_m for testosterone Δ^4-5β-reductase was found to be $6.4 \cdot 10^{-6}$ M.

Inhibition of 5β-reduction of some steroids by ethinylestradiol

Substitution of either cortisol or aldosterone for testosterone as substrate in the enzyme assay procedure, in the absence and presence of ethinylestradiol as an inhibitor, indicated that the 5β-reduction of these substrates was inhibited to the same extent. The results obtained are summarized in Table III.

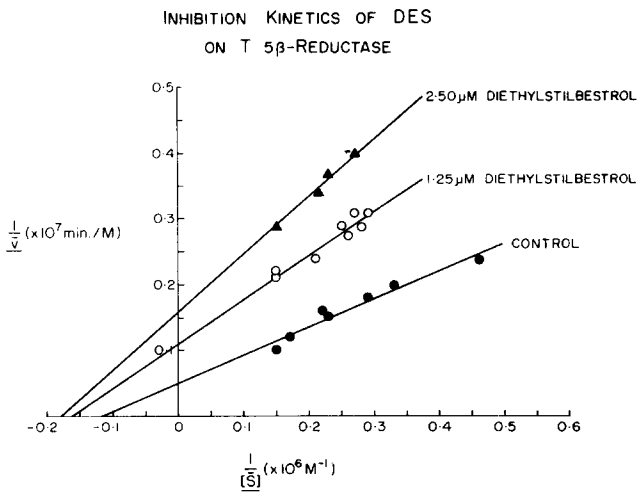

Fig. 1. Reciprocal plot of rate of reduction of testosterone in absence and presence of diethylstilbestrol. For experimental details see text.

TABLE II

INHIBITION OF TESTOSTERONE, CORTISOL AND ALDOSTERONE 5β-REDUCTASE ACTIVITY BY ETHINYL ESTRADIOL

Substrate	*Inhibitor*	*Average* $\Delta A_{240\ nm}$	*% inhibition*
Testosterone	—	0.0410	—
Testosterone	ethinyl estradiol	0.0175	56.3
Cortisol	—	0.0375	—
Cortisol	ethinyl estradiol	0.0165	56.0
Aldosterone	—	0.0805	—
Aldosterone	ethinyl estradiol	0.0275	65.8

DISCUSSION

The partially purified soluble pig liver Δ^4-5β-reductase utilized in these studies reduces a large number of steroids, both C_{19} and C_{21} (unpublished results), and it may or may not contain more than one enzyme. However, since our laboratory is primarily concerned with studies on the control of androgen metabolism, we used testosterone as the substrate for most of our experiments. The concentration of testosterone ($2.5 \cdot 10^{-5}$ M) was chosen since V conditions are observed at this concentration, and continue to exist until a concentration of $1.0 \cdot 10^{-5}$ M is approached, *i.e.* V conditions persist throughout the course of the experiment. The concentration of our steroid inhibitors were arbitrarily chosen to make up an equimolar solution of substrate and inhibitor. At this inhibitor concentration, it is probable that maximal inhibition is observed, since we demonstrated that the inhibition is non-competitive, at least for diethylstilbestrol.

Under the conditions of our assay system, 17β-estradiol inhibited the rate of testosterone 5β-reduction to the extent of 17.6%. The state of the oxygen function

at C-17 is not important as both estrone (17-ketone) and 17α-estradiol inhibited 5β-reduction to the same extent as 17β-estradiol. When the hydroxyl group in ring D was moved to position 16 (16α-estradiol) the extent of inhibition was greatly increased (54.1%). The presence of both 17β- and 16α-hydroxyl groups (estriol) eliminated the inhibitory effects of the estrogen. This was also the case when both 17β- and 15α-hydroxyl groups were present. However, the presence of a 17-ketone and a 15α-hydroxy group (15α-hydroxyestrone) did not eliminate the inhibitory effect of the estrogen. Therefore, it would appear as if a single hydroxyl group on ring D of the estrogen molecule results in the inhibition of steroid Δ^4-5β-reductase activity, while two hydroxyl groups on ring D eliminates the inhibition exhibited by the estrogen. The elimination of inhibitory activity by estrogens with two hydroxyl groups on ring D is probably due to some interaction between them which prevents appropriate binding of the steroid to the enzyme.

2-Hydroxyestradiol inhibited testosterone Δ^4-5β-reductase activity to the extent of 35.2% while 6-ketoestradiol exhibited no inhibitory ability. Thus, it would appear as if oxygen functions on ring A result in increased inhibitory activity for the estrogen, whereas an oxygen function on ring B results in a complete loss of inhibitory activity for the steroid. 4-Bromoestradiol also exhibited an increased inhibition of 5β-reduction of testosterone (33.6%). Therefore, we may extend our previous conclusion of the effects of ring A substituents. Not only do hydroxyl groups on ring A, other than at C-3, increase the inhibitory ability of the steroid, but nucleophilic species in general may increase the inhibitory ability of the estrogen.

The naturally occurring conjugates of the estrogens, the glucuronides and sulfates, have varied effects on the inhibition of steroid Δ^4-5β-reductase activity. The 17-(β-D-glucuronides) of estradiol and estriol exhibited little inhibitory activity. This is probably due to the lack of an unhindered oxygen function on ring D of the estrogen molecule. The 3-sulfate conjugate of estradiol shows a markedly different effect in that it inhibits testosterone 5β-reduction to a greater extent than estradiol itself. This latter observation may be due to the ionic nature of the sulfate group, which also appears to be the reason for the increased inhibitory activity exhibited by estradiol-3-phosphate. Estrone sulfate, being the most abundant estrogen in human plasma[20], may have some physiological importance in the regulation of steroid Δ^4-5β-reductase activity, as a result of our findings.

A study of the inhibitory effects of some estrogen esters produced a very complex set of results, complex in that no definite pattern has emerged. The 3-acetate and 3-hemisuccinate esters of estradiol exhibit inhibitory activity similar to that of estradiol itself. However, equilin-3-acetate, compared to equilin, exhibits less inhibition of testosterone 5β-reduction. Estradiol-3-hemisuccinate is unstable in a methanol solution, resulting in estradiol formation. This would explain the similarity in the results observed with estradiol-3-hemisuccinate and estradiol itself. This may also be the reason estradiol-3-acetate showed similar inhibition, as the estradiol-3-acetate might be hydrolyzed in the assay procedure. Estradiol-17-acetate was observed to have little inhibitory activity, whereas estradiol-17-valerate had an effect similar to that of estradiol itself. The latter observation might possibly, again, be due to the hydrolysis of the estrogen ester in the assay procedure. Estradiol-17-hemisuccinate was a better inhibitor of testosterone 5β-reduction than estradiol, which is probably due to the ionic nature of the substituent group; however, the glucuronides

of estradiol and estriol, also ionic compounds had no inhibitory activity. The contrast in the latter results may be due to the size of the substituent group and its flexibility on the estrogen molecule. Estradiol-3,17-diacetate exhibits little, if any, inhibitory activity, while estradiol-3,17-dihemisuccinate exhibits inhibitory activity similar to that observed for estradiol. From these observations it would seem quite apparent that the stability of the estrogen esters in our assay system is questionable. A thorough investigation of the behaviour of these estrogens in our assay system must be made before we can conclusively ascertain their roles in the inhibition of testosterone 5β-reduction.

Kupfer and Peets[7], who studied the effects of estrogens on rat liver Δ^4-5α-reductase activity for cortisol, observed a decreased inhibitory activity for estrone-3-methyl ether as compared to estrone, indicating that the lack of a free phenolic group decreases the inhibitory activity. Such also appears to be the case in our study with respect to ethinylestradiol and mestranol, its 3-methyl ether, but not with estradiol and its 3-methyl ether. This seems to indicate that the 3-methoxy group alone does not determine the decrease in inhibitory activity. Some interaction may exist between the 3-methoxy group and other parts of the steroid molecule, such as the 17α-ethinyl group of ethinylestradiol, which may act in a cooperative or antagonistic manner to result in a decrease in the inhibition of steroid Δ^4-5β-reductase activity by the estrogen. Another explanation for the lack of a decrease in the inhibition of 5β-reduction of testosterone by estradiol-3-methyl ether may be the existence of a soluble demethylase enzyme for the naturally occurring estrogens. However, it has been reported that a demethylase enzyme is present in rat liver microsomes for the conversion of mestranol to ethinylestradiol[21], and the fact that chlorotrianisene, a compound with 3-methoxy groups which is a very active estrogen *in vivo*, did not inhibit testosterone 5β-reduction in our system would seem to rule out the possibility of such a hypothesis.

Equilin and equilenin inhibit steroid Δ^4-5β-reductase activity by 56.3% and 16.8%, respectively, but the significance of these results is not known since these compounds have as yet not been found to be naturally occurring in the pig. The conversion of equilin to equilenin has been demonstrated in porcine adrenal tissue[22].

Since prostatic carcinoma was shown to be an androgen-dependent tumor[23], the administration of estrogens and non-estrogenic antiandrogens have been used to treat this disease. The administration of diethylstilbestrol, chlorotrianisene, and ethinylestradiol to men with prostatic carcinoma has been reported to cause a decrease in the metabolic clearance rate of testosterone[11,12]. This decrease in the metabolic clearance rate of testosterone may be attributed in part to decreased plasma testosterone levels[13] and increases in the plasma levels of the specific sex hormone binding globulin[14]. From the results obtained in this study with diethylstilbestrol (86.8% inhibition) and ethinylestradiol (55.6% inhibition), a third possibility arises. Estrogen administration may result in a direct inhibition of steroid metabolic pathways, thus decreasing the metabolic clearance rate of the steroids. Although chlorotrianisene is a potent estrogen *in vivo*, it did not exhibit any significant inhibitory activity in our *in vitro* study. This may be due to the fact that there are no free phenolic groups in chlorotrianisene. Cyproterone acetate, also used in the treatment of prostatic carcinoma but having a different mode of action[24], also inhibited steroid Δ^4-5β-reductase activity in our system by 50.9%.

If one or several steroid Δ^4-5β-reductase enzymes exist for the steroids with the Δ^4-3-keto groups as an integral part of their molecular structure, the results in Table III suggest that 5β-reduction of all these steroids would be inhibited by ethinylestradiol, and a decrease in the metabolic clearance rate of these steroids is possible when administering ethinylestradiol in sufficient quantities. Further investigation is required before a firm conclusion can be reached.

From this study of steroid Δ^4-5β-reductase inhibition by estrogens, it would seem as if a phenolic A ring and an intact oxygen function on ring D are necessary structural requirements for the steroid to exhibit inhibitory activity. Blocking the availability of the 17-oxy function and introduction of hydroxyl groups at C-15 and C-16 of estradiol decreases the inhibitory activity of the steroid. The presence of ionic groups, such as the sulfate at C-3, increases the inhibitory activity of the estrogens. Nucleophilic groups on ring A increase estrogen inhibition of steroid Δ^4-5β-reductase activity.

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