Steroid hormones share a very similar structure, but they behave distinctly. We present structures of human estrogentic 17β-hydroxysteroid dehydrogenase (17β-HSD1) complexes with dehydroepiandrosterone (DHEA) and dihydrotestosterone (DHT), providing the first pictures to date of DHEA and DHT bound to a protein. Comparisons of these structures with that of the enzyme complexed with the most potent estrogen, estradiol, revealed the structural basis and general model for sex hormone recognition and discrimination. Although the binding cavity is almost entirely composed of hydrophobic residues that can make only nonspecific interactions, the arrangement of residues is highly complementary to that of the estrogenic substrate. Relatively small changes in the shape of the steroid hormone can significantly affect the binding affinity and specificity. The \( K_m \) of estrone is more than 1000-fold lower than that of DHEA and the \( K_m \) of estradiol is about 10 times lower than that of DHT. The structures suggest that Leu-149 is the primary contributor to the discrimination of C-19 steroids and estrogens by 17β-HSD1. The critical role of Leu-149 has been well confirmed by site-directed mutagenesis experiments, as the Leu-149 \( \rightarrow \) Val variant showed a significantly decreased \( K_m \) for C-19 steroids while losing discrimination between estrogens and C-19 steroids. The electron density of DHEA also revealed a distortion of its 17-ketone toward a \( \beta \)-oriented form, which approaches the transition-state conformation for DHEA reduction.

Steroid hormones are involved in a wide variety of physiological functions, including metabolism, reproduction, aging, and cancer. Steroid hormones follow a common pathway: they diffuse into cells and bind to their receptors, which in turn bind hormone response elements to the DNA and interact with the transcriptional machinery to activate or repress transcription of target genes (1, 2). One important mechanism by which cells can modify hormone action is the modulation of the steroid structure itself (3). Steroid hormones share a very similar structure that has as its core the cyclo-pentenophenanthrene ring. The addition of an 18th carbon atom results in the estrogen ring (C-18), the addition of a 19th carbon atom results in the C-19 ring (including androgens such as DHT\(^1\) and testosterone), and the addition of a two-carbon lateral chain produces the pregnane ring (C-21). Despite their very similar structures, steroid hormones have significantly different physiological activities. DHT, the most potent androgen, estradiol (E\(_2\)), the most potent estrogen, and DHEA, the most abundant hormone precursor in humans and other mammals, exert numerous and diverse actions on their target tissues (Fig. 1) (4–6). The detailed mechanism of steroid discrimination has not been well elucidated up to now.

The enzymes of the 17β-hydroxysteroid dehydrogenase (17β-HSD) family are responsible for the last step in the formation of all androgens and estrogens. The reduction of the 17-ketone to a 17β-hydroxyl increases the affinity of the steroids to their cognate receptors (7). The 17β-reduction activity of these enzymes is thus required for the synthesis of all active androgens and estrogens. Molecular cloning has revealed the presence of seven isozymes of 17β-HSD, six of which are members of the short chain dehydrogenase/reductase superfamily (8, 9). 17β-HSD1 primarily catalyzes the interconversion of estrone (E\(_1\)) and estradiol, but it also has some catalytic activity for the interconversion between DHEA and 5-androstan-3,17-diol, 4-androene-3,17-dione and testosterone, as well as between A-dione and DHT (9, 10), thus constituting a good model to study C-18/C-19 steroid or estrogen/androgen discrimination. We have previously proved that 17β-HSD1 is a dimer consisting of two identical subunits (11). Immunochemical analyses have confirmed the presence of this enzyme in human placenta, breast, and ovary granulosa cells (12, 13). Since it is well known that estradiol stimulates the proliferation of mammary tumor cells, this enzyme is an important target for breast cancer therapy (14, 15).

The structure of 17β-HSD1 from human placenta was first determined at 2.2 Å resolution (16). More recently, the structure of the complex of 17β-HSD1 with E\(_2\), as well as the enzyme structure in the presence of E\(_2\) and NADP, have been reported (17, 18). Nevertheless, it is still unclear why a small structural modification, like the addition of a C-19 methyl group, can result in a very different catalytic activity, and how steroids are recognized by different isozymes and receptors. To answer these questions, we have determined and compared the structures of 17β-HSD1 in complex with different steroids. Here we report the crystal structures of 17β-HSD1 complexed with ei-

\( ^1 \) The abbreviations used are: DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; E\(_1\), estrone; E\(_2\), estradiol; 17β-HSD, 17β-hydroxysteroid dehydrogenase.
ther DHEA or DHT. The comparison of the structures of DHEA, DHT, and E\textsubscript{2} complexes with 17\textbeta-HSD1 gives an informative picture of important steroid hormone recognition and discrimination by the enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—DHEA, DHT, 17\textbeta-estradiol, and estrone were purchased from Aldrich. NAD, NADH, \( \beta \)-octylglucoside, dithiothreitol, polyethylene glycol 4000, and glycerol were from Sigma. We packed the Q-Sepharose ion exchange and the Blue-Sepharose CL-6B columns ourselves with media and columns from Amersham Pharmacia Biotech (Montreal, Canada). Phenyl-Sepharose HR 10/10 was purchased directly from the same company. All reagents were of the best grade available. *Spodoptera frugiperda* cells (Sf9 cells), Bac-N-Blue\textsuperscript{TM} transfection kit, and transfer vector pBlueBac4.5 were purchased from Invitrogen Corp.; Bac-N-Blue\textsuperscript{TM} transfection kit (Stratagene) following the manufacturer’s instructions. The mutation to obtain the variant L149V, was constructed by using the QuickChange site-directed mutagenesis kit (Stratagene) following the manufacturer’s instructions. The mutation used two primers: 5'-CGTCATTGAAAGGCACCCCCATCAATCCTCC-3' and 5'-GAGGGATGATGGGCGTGCTTTTCAATGACG-3'. Both structures were refined with good geometry (Table I). The electron densities for DHEA and DHT are well defined, and they have been modeled and refined with full occupancies. The electron densities for the 43 C-terminal residues and for the flexible segment between residues 192 and 207 are stronger than those found in the first enzyme structure and in the estradiol complex (17), but they are still not clear enough to define the atomic location of those residues (15% of the protein by molecular mass). These missing portions of the model may contribute to the somewhat high free \( R \) factor for the structure of the DHT complex.

**Steady-state Kinetic Study**—Kinetic data for the wild type 17\textbeta-HSD1 with DHEA and DHT and for the mutant 17\textbeta-HSD1 with E\textsubscript{1}, E\textsubscript{2}, DHEA, and DHT were obtained by spectrophotometric measurement of NAD(P)(H) reduction and/or oxidation at 37 °C by measuring the absorbance change at 340 nm. The reaction mixture contained 50 mM phosphate buffer, pH 7.5, 100 \( \mu \)M NAD(P)H, and 10–60 \( \mu \)M steroid (with smaller upper concentrations for estrogens: 10–30 \( \mu \)M for estradiol and 1–10 \( \mu \)M for estrone). The cell subfractions obtained from the wild type, and from the variants, were sonicated and centrifuged at 100,000 \( \times \)g for 0.5 h to separate the mitochondrial and microsomal fractions resulting in samples ready for assaying the enzyme activity for various steroids. Lineweaver-Burk plots were used to determine the \( K_{M} \) and \( V_{max} \) values. A blank value lacking steroids was obtained under the same condition and subtracted. Protein concentrations were determined by the method of Bradford (26). Protein content was rapidly assayed using a Coomassie Blue dye-binding procedure combined with scanning densitometry (27). The enzymatic activity was calculated as described previously (11).

**Site-directed Mutagenesis**—The human 17\textbeta-HSD1 cDNA coding region was subcloned from the vector pVL17\textbeta-HSD (28) by polymerase chain reaction to reconstruct the vector pBlueBac4.5/17\textbeta-HSD1 for site-directed mutagenesis. The mutation to obtain the variant L149V, was constructed by using the QuickChange site-directed mutagenesis kit (Stratagene) following the manufacturer’s instructions. The mutation used two primers: 5'-GGAGGATTGATGGGCGTGCTTTTCAATGACG-3' and 5'-CTGCTTTGAAAGGCACCCCCATCAATCCTCC-3'. After verifying the mutation by dideoxynucleotide sequencing, the mutagenized transfer vector was cotransfected in Sf9 cells with linearized pBlueBac DNA following the protocol described by the manufacturer (35).

**RESULTS AND DISCUSSION**

**Binding Pocket**—In these complexes, the core of the protein maintains the same structure as that of the enzyme (16) and as

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**TABLE I**

|                           | 17\textbeta-HSD-DHEA | 17\textbeta-HSD-DHT |
|---------------------------|-----------------------|----------------------|
| Resolution (\( \AA \))    | 2.3                   | 2.24                 |
| Total reflections         | 25,462                | 39,772               |
| Unique reflections (all data) | 14,316              | 14,692               |
| Redundancy                | 1.8                   | 2.7                  |
| Completeness (%)          | 95                    | 97                   |
| \( R_{sym} \) (%)         | 9                     | 3.8                  |
| Resolution (\( \AA \))    | 8.2–3.3               | 8.2–2.24             |
| \( R_{free} \) (%)        | 0.184/0.249           | 0.189/0.278          |
| Atoms (protein/water)     | 2179/40               | 2179/44              |

**Bond distance (\( \AA \))**

| Bond distance (\( \AA \)) | 0.008                  | 0.010                |

**Bond angle (\( \AA \))**

| Bond angle (\( \AA \)) | 1.38                   | 1.46                  |

**Estimated average coordinate error (\( \AA \); see Ref. 33)**

| Estimated average coordinate error (\( \AA \)) | 0.25                   | 0.27                  |

**Average B factor (\( \AA ^2 \))**

| Average B factor (\( \AA ^2 \)) | 29.3                   | 28.6                  |
|----------------------------------|------------------------|-----------------------|
| Main chain                       | 32.4                   | 31.8                  |
| All atom                         | 57.3                   | 56.1                  |
| Steroid                          | 36.2                   | 37.4                  |
| Solvent                          | 42.2                   | 43.2                  |

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2 Q. Han and S.-X. Lin, unpublished results.
that of the 17β-HSD1-E2 complex (17). The quality of the electron density map for both DHEA and DHT enabled the unambiguous localization of their positions. Both DHEA and DHT, like E2, are deeply engulfed in the hydrophobic cleft (Fig. 2) that is located at the interface of helices αG (209–227), αH (274–284), and αH′ (251–271). This cavity is composed of valines 143 and 225, leucine 149, phenylalanines 226 and 259, glycine 186, methionines 193 and 279, glutamic acid 282, tyrosine 218, serines 142 and 222, proline 187, and histidine 221. The binding pocket is approximately 7.5 Å by 9 Å in cross-section and 16 Å deep. Hydrophobic atoms constitute 79% of the total 335 Å² surface of the pocket. It also contains the Tyr-X-X-Lys sequence that is conserved in all members of the short chain dehydrogenase/reductase superfamily and essential for the enzymatic activity.

DHEA and DHT Recognition and Sex Hormone Discrimination—The O-17 atom of DHEA (and that of DHT) forms two hydrogen bonds with the protein. These hydrogen bonds involve Tyr-155 and Ser-142 in the active site, and form a triangular-shaped network similar to that observed between the steroid and the catalytic residues in the 17β-HSD-E2 complex. The O-3 atom in DHEA forms two hydrogen bonds with Nε2 of His-221 and Oε2 of Glu-282. The hydrogen bond between Glu-282 and O-3 is weak, as shown by the flexibility of the side chain of Glu-282, which has a high B-factor and a weak electron density. The hydrogen bond formed with His-221 is strong and important for orientating the bound substrate. The latter hydrogen bond is also found in the 17β-HSD1-DHT complex, and a similar hydrogen bond is found in the complex of rat liver 3α-hydroxysteroid dehydrogenase with testosterone (19).

When the structures of the three substrate complexes of 17β-HSD1 are superimposed, it is found that the core of DHEA rotates by nearly 20° about an axis along the length of the steroid binding pocket, and shifts 1.5 Å at the O-3 end and 0.6 Å at the O-17 end as compared with the position of E2 in the E2 complex (Figs. 3 and 4). DHT shifts 1.4 Å at the O-3 end and 0.6 Å at the O-17 end, but the core of DHT rotates only slightly. These results are surprising, since it was thought that the binding pocket fitted the steroid tightly (16). In all structures of DHEA, DHT, and E2, the C-10 is in a precise alignment with the fork-like side chain of Leu-149. In the E2 structure, the distance between E2 and the Cδ of Leu-149 is 4.6 Å. Because there is a β-methyl C-19 attached to C-10 of DHEA and DHT, these steroids obviously cannot remain at the same position due to steric hindrance. The position of Leu-149 is stabilized by two main chain hydrogen bonds with Pro-150 O and Gly-148 O. The electron density of Leu-149 is well defined, and the B-factors of its Cδ1 and Cδ2 are half the average B-factor for the whole protein. The interaction of Leu-149 with both DHEA and DHT is sufficiently strong to shift Cδ1 and Cδ2 of Leu-149 by 0.4 Å from their original position in the presence of E2. This rigidity of part of the active site forces the shift in position of DHEA and DHT. Compared with E2 binding, the different binding locations of DHT and DHEA result in reduced interactions of these steroids with several important non-polar residues in the binding pocket, although they can make new interactions with the more polar Ser-222 and Pro-187. The E2 complex structure and our modeling study have revealed that Phe-259 plays an important part in E2 binding. Its side chain forms five van der Waals contacts with the A-ring of E2. Because the interactions with DHEA and DHT are decreased, we observed that the side chain of Phe-259 in the DHEA and DHT complexes has shifted a little from its binding position in the E2 complex and that its B-factor has increased. Due to the hydrophobic nature of steroids, van der Waals contacts between 17β-HSD1 and the non-polar atoms of the steroid provide major contributions to the binding energy. A loss of favorable interactions would be expected to decrease the affinity of binding. The reason for the difference between the binding positions of DHEA and of DHT may be explained by the differences in their shapes. The A- and B-rings of DHT are in the same plane...
while the double bond between C-5 and C-6 in DHEA forces the C-3 and 3-hydroxyl group toward the \( \alpha \) surface. DHT makes stronger interactions with Val-283 and Tyr-218 than DHEA, while DHEA makes stronger interactions with Glu-282 and a stronger hydrogen bond with N-\( \epsilon_2 \) of His-221. The positions of most of the active site residues are unchanged, but the helix-turn-helix motif (\( \alpha G^\prime-\alpha G^\prime \)) (16) has conformational mobility that allows different positions for His-221 and Phe-226 in the presence of various ligands (Fig. 3). The flexibility of helix \( \alpha G^\prime \), which contains several residues in the binding pocket, may be important for steroid binding.

Evidence for the relative binding affinities of various substrates can be obtained from the steroid electron density in the different complex structures. The position of \( E_2 \) in the binding pocket was proven by its high level of electron density. In contrast, the electron densities of DHEA and DHT are weaker than that of \( E_2 \) in the cognate complex, although they were crystallized in the presence of higher concentrations of DHEA and DHT (about 1 mM) than the one that was used in the eocrystallization of \( E_2 \) (500 \( \mu \)M) (17). The slightly higher B factor values of the DHEA and DHT complexes also show that their positions are less well defined than was \( E_2 \) in the binding pocket.

Steady-state kinetic studies of these steroids with DHEA were carried out in parallel, to compare with the structural information from various complexes. These kinetic studies show that the \( K_m \) for DHEA is more than 1000-fold greater than that for estrone, and the \( K_m \) value for DHT is about 10-fold greater than that for estradiol, in agreement with the structural information (Table II).

The catalytic mechanism of 17\( \beta \)-HSD1 for estrone reduction is thought to involve the direct transfer of a hydride ion from the C-4 position of the nicotinamide nucleotide to the acceptor carbonyl C-17 of estrone to produce a 17\( \beta \)-hydroxyl. The NADP\(^+\) molecule binds in an extended conformation, with the nicotinamide moiety in a \( \text{syn} \) conformation pointing toward the substrate binding site of 17\( \beta \)-HSD1 (17, 20). The shift of DHT and DHEA could increase the distance between C-17 of the steroid and C-4 of the nicotinamide. A modeling study using the model from Azzi \textit{et al.} (17) and the NADP\(^+\) model from Breton \textit{et al.} (18) indicated that the distance is 3.6 Å for \( E_2 \), 4.0 Å for DHT, and 3.9 Å for DHEA. If these longer distances are maintained when 17\( \beta \)-HSD1 binds both the cofactor and these substrates, then we would expect a lower rate of hydride transfer for the C-19 steroids. Thus, we propose that the weak affinity of DHEA and DHT for 17\( \beta \)-HSD1 is consistent with their increased \( K_m \) values and that the longer distance between C-17 and C-4 of NADP\(^+\) is in accord with their lower \( k_{cat} \) values when compared with \( E_2 \). As a result of the \( K_m \) and \( k_{cat} \) modification, the specificity difference between the estrogens and C-19 steroids could be between 250- and 50,000-fold. The discrimination between these steroids is thus guaranteed.
Three-dimensional Structure and Specificity of 17β-HSD1

Table II

Steady-state kinetic parameters of 17β-HSD1 with different substrates

| Ligands  | Wild type 17β-HSD1 | 149V variant |
|----------|---------------------|--------------|
|          | $K_m$ (μM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ | $K_m$ (μM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ |
| Estrone  | 0.03 | 5.8 | 194 | 0.3 | 0.26 | 0.87 |
| Estradiol | 4.6 | 4.4 | 0.98 | 10 | 0.24 | 0.024 |
| DHEA     | 33 | 0.33 | 0.01 | 4.3 | 0.34 | 0.079 |
| DHT      | 45 | 0.16 | 0.0035 | 17.2 | 0.12 | 0.007 |

Of all the steroid hormones, only the estrogens E$_1$ and E$_2$ lack the C-19 methyl group. 17β-HSD1 is well designed for its high specificity toward estrogen hormones. Although the binding cavity is almost entirely composed of hydrophobic residues having nonspecific interactions, the arrangement of residues yields highly specific interactions allowing only one orientation for the steroid, corresponding to the strongest binding. Thus, relatively small changes in the shape of the hormones can significantly affect the binding position and affinity resulting in critical hormone recognition and substrate selectivity. The interactions between C-19 and the hydrophobic side chains around Leu-149 appear to be the most important for discriminating C-19 steroids and in preventing them from binding in an ideal position. Therefore, the shape of the binding pocket plays a critical role in steroid hormone discrimination.

In parallel with the crystallographic study, site-directed mutagenesis was carried out to produce the variant by replacing the Leu-149 by the very similar but smaller valine. As expected from our knowledge of the structure, the catalytic efficiency and substrate discrimination were significantly modified in the variant, which, unlike the native enzyme, demonstrates similar affinities for E$_2$ and DHEA. The $K_m$ of variant enzyme for DHEA has decreased from 33 μM to 4.3 μM, while the $K_m$ for E$_1$ has increased from 0.03 μM to 0.3 μM (Table II). Similarly, the variant enzyme showed a lower $K_m$ value for DHT as compared with the wild type, though the effect is not so significant as in the case of DHEA (Table II). These changes appear to be due to some loss of favorable interactions with estrogens and to the removal of unfavorable interactions with C-19 steroids and thus are in good agreement with the above structural analysis.

In Table II, we can see that the specificity values of the Leu-149 → Val variant for estrogens may decrease, while these values significantly increased for C-19 steroids, resulting in a loss of C-18/C-19 steroid discrimination. In fact, the specificity value for C-19 steroids and estrogens became similar (Table II). These results confirm the crucial role of the Leu-149 in the substrate recognition of 17β-HSD1.

Enzyme-induced Strain and the Mechanism of the Reduction of 17-Ketone—The structure of 17β-HSD1 complexed with DHEA gives important information about how the enzyme catalyzes the conversion of the 17-ketone to the 17β-hydroxyl. The omit difference electron density map shows that the C-17 ketone oxygen is oriented toward the β-position. The refinement was started with an energy-minimized DHEA model created with Insight II (Biosym, Inc.), in which the d-ring conformation position and O-17 are oriented toward the α-face of the steroid. This conformation is likely favored by the presence of the C-18 methyl group. However, the d-ring and O-17 did not fit into the electron density and O-17 was far from a position in which it could form hydrogen bonds with Tyr-155 (4.8 Å) and Ser-142 (4.4 Å). Small molecule structures of DHEA demonstrate a greater degree of flexibility of the d-ring compared with the rest of the steroid core (21). Therefore, the conformation of the d-ring was adjusted to fit the density (Fig. 5). O-17, C-17, and C-16 were moved 1.2, 0.6, and 0.7 Å, respectively, toward the β-surface. The O-17–C-17 bond was shifted by 24° compared with its orientation in the energy-minimized and small molecular models. In this new conformation, the 17-ketone oxygen can form hydrogen bonds with Tyr-155 (3.6 Å) and Ser-142 (3.3 Å). This suggests that the hydroxyl groups of Tyr-155 and Ser-142 can induce the new position of the 17-ketone oxygen of DHEA, thereby allowing Tyr-155 to donate its proton and to form a hydrogen bond. These hydrogen bonds could stabilize this high energy state, thus facilitating the proton transfer from Tyr-155 to O-17, and the hydride transfer from nicotinamide ring to C-17 of the steroid. The presence of the positively charged side chain of Lys-159, in proximity to the Tyr-155 hydroxyl oxygen, could also facilitate the stabilization of the β-oriented ketone and proton transfer. By comparison, in the structure of the E$_2$ complex, the 17-hydroxyl and d-ring of estradiol are β-oriented, forming strong hydrogen bonds with Tyr-155 and Ser-142. Thus, the β-oriented ketone shows the characteristics of the intermediate of 17β-HSD reduction, and this is evidence for enzyme-induced strain of the substrate. The stabilization of a β-oriented ketone is an obligatory step in the formation of the 17β-hydroxyl, because it decreases the energy barrier for the conversion of 17-ketone to 17β-hydroxyl. Therefore, the present results may indicate a new catalytic mechanism that includes a conformational modification during the reduction of DHEA, and this may also apply to the reduction of other 17-keto-steroid hormones (Fig. 6).

The present structure and kinetic studies of inhibitors have determined that a 17β-hydroxyl or 17-ketone in the ligand is essential for tight binding with 17β-HSD1 (24). This is consistent with the formation of strong hydrogen bonds with Tyr-155 and Ser-142 as seen in the 17β-HSD1-E$_2$ complex. Even though the orientations of the core of DHEA and DHT have been changed in the binding pocket, the hydrogen bonds to Tyr-155 have remained. Hydrogen bonds are highly directional and therefore are important in the determination of the binding orientation, while the non-directional van der Waals forces are important for the binding affinity of steroids.

General Characteristics of the Steroid-binding Site—The three 17β-HSD1 binary complex structures shed light upon the general features of steroid hormone recognition by the 17β-HSD family. The steroid binding pocket appears to have three

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4 A. Gangloff and S. X. Lin, unpublished results.
regions. The first region recognizes the steroid phenolic A-ring. It contains the conserved His-221 and Glu-282 residues that could form hydrogen bonds with O-3. The second region binds to the central hydrophobic core of the steroid. It contributes to the main thermodynamic force favoring the binding of substrates. The catalytic region surrounds the D-ring, and contains the absolutely conserved catalytic residue Tyr-155 that is located on the β-face of the steroid and forms a hydrogen bond with O-17. The α-face of the steroid is accessible to the nicotinamide to facilitate hydride transfer. Because both estrogens and androgens (with C-19) may have 3- and 17-hydroxyl or ketone groups, the critical residues that are expected to determine specificity for the estrogenic and androgenic members of the 17β-HSD family must lie within the hydrophobic binding region. In estrogen-specific enzymes, some residues will sterically hinder the C-19-methyl group of the androgens, resulting in an unfavorable binding position or no binding at all. In androgen-specific enzymes, such as 17β-HSD3, there may be a hydrophobic cavity that can strongly interact with the C-19. We predict that the hydrophobic region of 17β-HSD2 will be more tolerant than that of 17β-HSD1, as it shows similar activity with both C-18 and C-19 hormones.

We propose that other C-19 steroids (androstenedione, androsterone, androst-5-ene-3β,17β-diol, and testosterone) must have similar interactions with 17β-HSD1 as do DHEA and DHT. This prediction is in agreement with our kinetic data, which show that these C-19 steroids have similar $K_m$ and $K_{cat}$ values for 17β-HSD1. The combination of hydrogen bonding and hydrophobic interactions in the construction of the specific steroid binding site in 17β-HSD1 is expected to be a common feature in other protein-steroid interactions. Hydrogen bonds alone are insufficient for the requisite ligand-binding affinity; van der Waals contacts contribute significantly to binding energy. Similar mechanisms were recently shown in the interactions between the estrogen receptor and 17β-estradiol.

**Inhibitor Implication**—Forty percent of all cancer cases are sex steroid-sensitive, including breast, prostate, ovarian, and uterine cancers. Steroid-metabolizing enzymes are thus prime candidates for approaches based on control of intracrine activity. Because the 17β-HSD isozymes have much greater molecular specificity than the receptor, this family of enzymes is an attractive target for the design of potent and selective drugs to combat steroid-related disorders (23, 24). The 17β-HSD1 complex structures suggest several ways to design potent inhibitors. The narrow hydrophobic binding region contributes the main thermodynamic force for binding. For the best access to the restricted pocket, a planar hydrophobic ring structure like that of E$_2$ should be chosen as the core of an inhibitor. A β-oriented electron withdrawing group should be present to form hydrogen bonds with Tyr-155 and Ser-142. In addition, an α-oriented hydrophobic group at C-17 or C-16 would help to prevent the nicotinamide nucleotide cofactor from binding. Despite the narrowness of the hydrophobic cleft, there appears to be sufficient space to accommodate substituents at the Ta-position of the steroid. 17β-Estradiol derivatives with Ta-alkyl substituents have been synthesized and shown to inhibit 17β-HSD1 (25). The selectivity of an inhibitor toward androgenic or estrogenic HSDs would be controlled by the shape of its hydrophobic core. A group in the C-19 position of the androgen would greatly decrease its affinity toward 17β-HSD1 while increasing the affinity toward 17β-HSD3.

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Three-dimensional Structure and Specificity of 17β-HSD1
Dehydroepiandrosterone and Dihydrotestosterone Recognition by Human Estrogenic 17 β-Hydroxysteroid Dehydrogenase: C-18/C-19 STEROID DISCRIMINATION AND ENZYME-INDUCED STRAIN
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