Synthesis and Evaluation of 7α-Iodo-5α-dihydrotestosterone as a Potential Radioligand for Androgen Receptor

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7α-Iodo-17β-hydroxy-5α-androstan-3-one (7α-iodo-5α-dihydrotestosterone, 7α-IDHT) has been synthesized as a potential radioligand for the detection and measurement of androgen receptor and for imaging of androgen-receptor-containing tissues when labeled with the γ-emitting radionuclides ^{125}I and ^{123}I, respectively. *In vitro* binding studies show that 7α-IDHT binds with high affinity to the rat and human androgen receptor (RBA = 74) compared to R1881 (RBA = 100). Further, this compound showed high specificity for the androgen receptor. 7α-IDHT showed only a marginal affinity for the progestin receptor and even less affinity for the estrogen receptor. No binding was detected to the glucocorticoid receptor. These characteristics make 7α-IDHT a potentially ideal agent for imaging and evaluation of androgen-receptor-containing tissues.

Introduction

The synthesis of γ-emitting steroids that are capable of binding to steroid hormone receptors with high affinity and specificity has been a goal of many investigators.[1] When compared to the more common β-emitting steroids (labeled with ^{3}H or ^{14}C), the higher specific activities attainable with γ-emitting isotopes and their more penetrating radiation offer important advantages of higher sensitivity in receptor analyses and ease of external imaging *in vivo*. A number of useful steroid-derived γ-emitting ligands have been prepared as probes for the estrogen[2] and progesterone[3] receptors. However, the design of such a steroid ligand for the androgen receptor has proved to be much more challenging because this receptor is highly specific and can discriminate among androgens labeled with bulky isotopes. Consequently, none of the early attempts to make γ-emitting androgens produced useful ligands.[1c,4] These attempts involved syntheses of radiohalogenated derivatives of the natural androgens testosterone and 5α-dihydrotestosterone (5α-DHT) and focused primarily on placing the halogen atom (or a short carbon chain containing halogen) in either the steroid A-ring using the C-3 carbonyl as a synthetic handle[1c,4a] or the D-ring of the steroid using the 17-hydroxyl as a handle.[4b,c] Some B- and C-ring-iodinated derivatives of testosterone have also been reported.[4d] These early studies produced compounds which were found to be either chemically or metabolically unstable or had low affinity for the androgen receptor. More recently, potential radiolabeled androgens of other structural types have been investigated with some promising results. These include derivatives of 7α-methyl-19-nortestosterone, mibolerone, and methyltrienolone (R1881).[5]

In cases of low receptor affinity of testosterone and 5α-DHT derivatives, especially that of the A- and D-ring substitutions, it is generally believed that the bulky iodine atom (or the group containing it) interferes with the donor–acceptor interactions between the receptor and the C-3 carbonyl or between the receptor and the 17β-hydroxyl group. To avoid these problems, we have focused our attention on the C-7α-iodinated derivative of 5α-dihydrotestosterone in which the absence of unsaturation and the greater distance between the iodine and the above functional groups should promote greater stability and minimize the interactions which are disruptive to receptor binding. Herein, we report the synthesis of 7α-iodo-17β-hydroxy-5α-androstan-3-one (7α-iodo-5α-dihydrotestosterone, 7α-IDHT) and present an evaluation of its binding affinity for both the rat and human androgen receptor and its cross-reactivity with other steroid receptors.

Results

Chemistry. The chemical synthesis of 7α-iodo-5α-dihydrotestosterone (7) was accomplished as shown in Scheme 1. 17β-Acetoxy-3,3-(ethylenedioxy)-5-androstene (1) was prepared by established methods[6] and oxidized to the Δ^5-7-ketone 2 with CrO_3–3,5-dimethylpyrazole.[7] Catalytic hydrogenation of this material gave the 5α-7-ketone 3 in accordance with the stereochemical course of similar hydrogenations in several steroid systems.[8] The 5α stereochemical assignment is further supported by the mass spectral fragmentation data for compounds **5a,b**, the products of reduction of 3 and deprotection of the C-3 carbonyl group. Specifically, it has been demonstrated[9] that A/B *trans* (5α) fused androstanes oxygenated at C-3, C-7, and C-17 show prominent ions at *m/z* 99 (D-ring fragment) and 176 (A-ring + B-ring + C-11). The *m/z* 99 ion is almost absent in similar A/B *cis* (5β) fused androstanes, and the *m/z* 176 ion is produced at much lower abundance. The mass spectra of compounds **5a,b** show both of these ions prominently.

The reduction of 3 was carried out with lithium aluminum hydride–*tert*-butyl alcohol (1:1.1) in THF–ethyl ether at −78 °C to give the 7α-hydroxy compound **4a** as the major product. High- field NMR analysis of this material shows it to contain 20% of the 7β-hydroxy epimer **4b**. This contrasts with earlier reductions of 7-keto steroids[10] with other hydride-reducing agents in which, in

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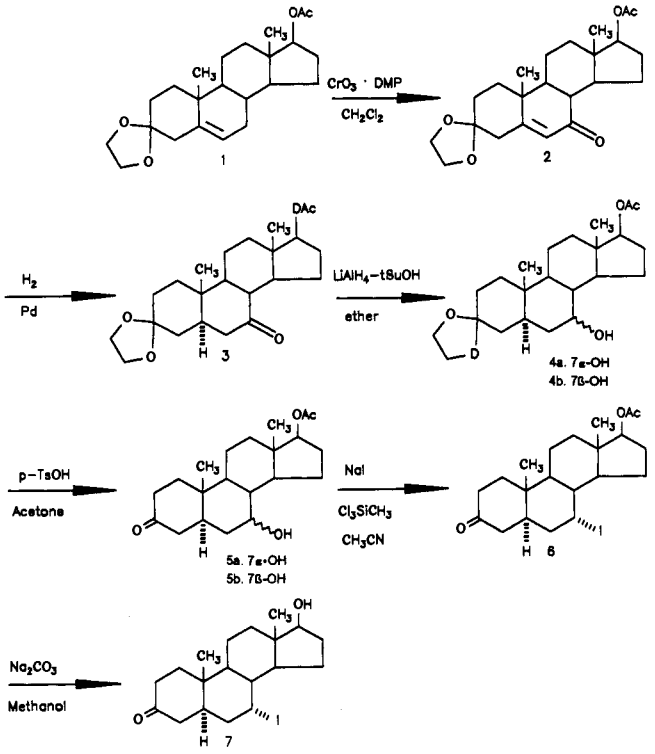
Scheme 1



most cases, it is reported that the major product is the 7β-hydroxy epimer. Our assignment of stereochemistry in this reduction is based on NMR chemical shift data, signal widths, and component coupling constants for the H-7 resonances of compounds **4a,b** and the resulting products of deprotection of the C-3 carbonyl, **5a,b**. The H-7β (equatorial) resonances of compounds **4a** and **5a** are found at 3.83 and 3.88 ppm, respectively, which are consistent with chemical shift data published for other 7α-hydroxy steroids.[11] The 7β-hydroxy epimers **4b** and **5b** show the H-7α (axial) resonance further upfield at 3.40 ppm which is consistent with data for other 7β-hydroxylated steroids.[8b,11] In addition, the H-7 resonances of **4a** and **5a** have signal half-widths of 6.9 and 6.1 Hz which are reflective of the small coupling constants expected[12] for an equatorial proton coupled through 60° dihedral angles to three equatorial and axial neighboring protons. The C-7 axial protons of the 7β-hydroxy epimers **4b** and **5b** have broader signal half-widths of 26.9 and 26.4 Hz, respectively, which are due to component axial–axial couplings.[8b,12]

While earlier studies indicate generally that hydride reductions of 7-keto steroids produce mostly the 7β-hydroxy epimer, it has been observed that the ratio of the epimeric products is strongly influenced by reaction conditions such as the nature of the solvent and the specific reducing agent employed.[10a,d] The reagent employed here (lithium aluminum hydride–*tert*-butyl alcohol, 1:1.1) is presumably lithium mono-*tert*-butoxyaluminohydride ($LiAlH_3Ot$-Bu) by analogy with the selective mono-ethoxy reagent described by Lythgoe.[13] It is interesting that the 17β-acetoxy group was not cleaved by this reagent under the conditions used. Additionally, while the effect of temperature was not investigated, we note that in all of the prior cases the reductions were carried out at 0 °C or higher temperatures, whereas the reduction reported here was conducted at −78 °C. Since 7β-hydroxy steroids are believed to be more stable than the 7α-hydroxy epimers,[10e,14] the preponderance of the 7α-epimer at −78 °C may reflect its more rapid formation under the conditions employed in this investigation.

To obtain the target compound **7**, the carbonyl deprotection product, which was a mixture of 90% **5a** and 10% **5b**, was iodinated by the method of Olah[15] to give **6** followed by saponification of the 17β-acetoxy group. This method of iodination, which employs trichloromethylsilane and sodium iodide in acetonitrile, was chosen because it allows direct conversion of the alcohol to the iodide without the necessity of an intermediate compound such as a sulfonate ester. While the reaction produced **7** in low yield, the only observed iodinated product was 7α-IDHT. No 7β-iodo product was detected. Further, when the separated 7α- and 7β-hydroxy epimers **5a,b** were iodinated by this method, they each gave 17β-acetoxy-7α-iodo-5α-androstan-3-one (**6**) as the only iodinated product. While the mechanism of this alcohol to iodide transformation has not been fully elucidated, these results are consistent with the intermediacy of a carbocation as proposed by Olah.[15] The 7α-iodo product is formed in preference to the 7β-iodo product, probably as a result of strong repulsion between the large 7β-iodo substituent and the C-15 methylene group.

Biological Evaluation. Competition assays were conducted to compare the binding of 7α-IDHT with established androgen receptor ligands to rat and human androgen, estrogen, and progestin and rat glucocorticoid receptors. 7α-IDHT efficiently displaced the binding of the synthetic androgen receptor ligand [^{3}H]R1881 to rat prostate cytosol even more than testosterone (relative binding affinity (RBA) = 74% vs 46%; Figure 1A, Table 1). Higher RBA values were obtained with DHT and R1881. Consistent with our earlier findings, E-MIVNT displaced binding with an RBA of about 10% relative to R1881.[5a] Progesterone also displaced [^{3}H]R1881 binding but only at much higher concentrations.

The ability of 7α-IDHT to displace [^{3}H]R1881 binding from the human androgen receptor was approximately 3- and 6-fold higher than that of testosterone and E-MIVNT, respectively (Figure 1B, Table 1). Although the affinity relative to R1881 was nearly identical to that measured in the rat, 7α-IDHT exhibited an RBA value for the human androgen receptor essentially equivalent to that of DHT.

Consistent with previous reports,[5a,16] the androgen receptor ligand R1881 displaced [^{3}H]R5020 binding from the progestin receptor as efficiently as progesterone (Figure 1C,D, Table 1). The remaining androgen receptor ligands tested displaced [^{3}H]R5020 binding only weakly. Of all the substances examined, 7α-IDHT exhibited the lowest RBA for the rat progestin receptor and was among the lowest for the human.

None of the androgen receptor ligands bound appreciably to either the rat or human estrogen receptor. Both 7α-IDHT and E-MIVNT competed very weakly for [^{3}H]estradiol binding (RBA < 0.1, relative to estradiol; Table 1). DHT, R1881, and testosterone exhibited essentially no affinity for the estrogen receptor.

7α-IDHT and DHT exhibited no binding affinity for the rat glucocorticoid receptor at concentrations approaching 10^{-5} M. Of the remaining androgens tested, R1881 exhibited the highest relative binding affinity (1.59, relative to unlabeled DEX, Table 1) followed by E-MIVNT (0.159). Testosterone displaced [^{3}H]DEX binding only slightly at concentrations above 10^{-6} M.

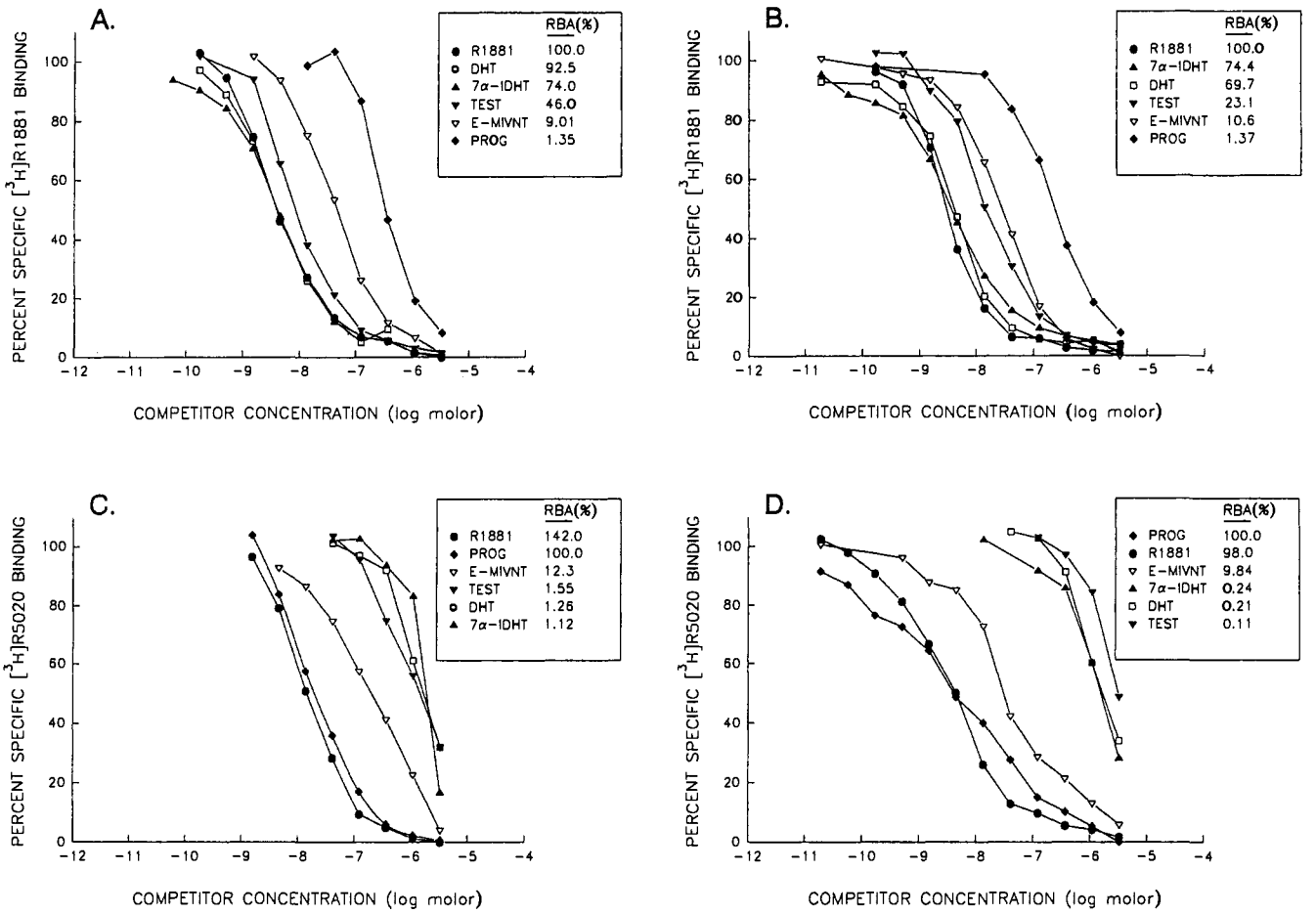


Figure 1. Competition binding isotherms for [^{3}H]R1881 binding to rat prostate (A) and human breast cancer cell (B) cytosol and [^{3}H]R5020 binding to rat uterine (C) and human breast cancer cell (D) cytosol. All incubations were conducted at 4 °C for 20 h. DHT = dihydrotestosterone, PROG = progesterone, E-MIVNT = 7α-methyl-17α-[(*E*)-2′-iodovinyl]-19-nortestosterone, TEST = testosterone, and 7α-IDHT = 7α-iodo-5α-dihydrotestosterone. The curves were analyzed by a least-squares nonlinear curve-fitting method with the use of the computer program ALLFIT.[12] RBA values were calculated on the basis of the concentration of competitor required to displace saturable binding by 50%. For further details, refer to Table 1.

Table 1. Comparison of Relative Binding Affinities (% ± SE) of 7α-IDHT with Other Natural and Synthetic Androgen Receptor Ligands for Steroid Hormone Receptor Binding in Rat Tissue and Human MCF-7 Cell Cytosol Extracts[a]

	androgen receptor		progestin receptor		estrogen receptor		glucocorticoid
competitor	rat[b]	human[c]	rat[d]	human[e]	rat[f]	human[g]	receptor rat[h]
R1881	100	100	142.0 ± 17.8	98.0 ± 15.2	<0.005	<0.005	1.59 ± 0.23
DHT	92.5 ± 14.3	69.7 ± 5.46	1.26 ± 0.157	0.205 ± 0.032	<0.005	0.022 ± 0.012	<0.001
7α-IDHT	74.0 ± 11.1	74.4 ± 7.13	1.12 ± 0.126	0.239 ± 0.038	0.038 ± 0.008	0.006 ± 0.003	<0.001
testosterone	46.0 ± 6.69	23.1 ± 2.01	1.55 ± 0.209	0.112 ± 0.018	<0.005	<0.005	<0.001
E-MIVNT	9.01 ± 1.39	10.6 ± 0.87	12.3 ± 1.74	9.84 ± 1.550	0.060 ± 0.007	0.030 ± 0.003	0.14 ± 0.14
progesterone	1.35 ± 0.25	1.37 ± 0.14	100	100			
estradiol					100	100	
corticosterone							22.9 ± 2.31
DEX							100

[a] RBA values are based on the concentration of competitor required to displace saturable binding by 50% using a dose–response curve-fitting program, ALLFIT.[20] [b] Rat prostate crystal extract incubated for 20 h at 4 °C with 3.8 nM [^{3}H]R1881 and 5 mM TA in the presence or absence of a range of competitor concentrations. RBA values are relative to R1881. [c] MCF-7 cell cytosol extract incubated for 20 h at 4 °C with 3.8 nM [^{3}H]R1881 and 5 mM TA in the presence or absence of a range of competitor concentrations. RBA values are relative to R1881. [d] Rat uterine cytosol extract incubated for 20 h at 4 °C with 1.0 nM [^{3}H]R5020 in the presence or absence of a range of competitor concentrations. RBA values are relative to progesterone. [e] MCF-7 cell cytosol extract incubated for 20 h at 4 °C with 1.0 nM [^{3}H]R5020 in the presence of a range of competitor concentrations. RBA values are relative to progesterone. [f] Rat uterine cytosol extract incubated for 20 h at 4 °C with 2.0 nM [^{3}H]estradiol in the presence of a range of competitor concentrations. RBA values are relative to estradiol. [g] MCF-7 cell cytosol extract incubated for 20 h at 4 °C with 2.0 nM [^{3}H]estradiol in the presence or absence of a range of competitor concentrations. RBA values are relative to estradiol. [h] Rat liver cytosol extract incubated for 20 h at 4 °C with 4.0 nM [^{3}H]dexamethasone in the presence or absence of a range of competitor concentrations. RBA values are relative to DEX.

Discussion

In vitro displacement assays indicate that iodine substitution at the 7α position of DHT does not impact on the affinity of DHT for the androgen receptor. In both rat and human preparations, 7α-IDHT displaced [^{3}H]-R1881 binding more efficiently than testosterone. In fact, 7α-IDHT displaced [^{3}H]R1881 binding to the human androgen receptor in MCF-7 cytosol essentially equivalent to DHT. Although R1881 has a slightly greater affinity for the androgen receptor, it also binds to the progestin receptor nearly as avidly as progesterone. A difficult problem with many androgen receptor ligands is their

excellent affinity for the progestin receptor. However, 7α-IDHT exhibited only very weak affinity for the progestin or estrogen receptor and virtually no affinity for the glucocorticoid receptor (Table 1).

The RBA of E-MIVNT observed in this study agrees with that previously obtained in binding displacement assays.[5a] However, we found a markedly higher apparent affinity of E-MIVNT for the androgen receptor with saturation binding analysis using ^{125}I-labeled E-MIVNT (RBA = 50% vs 10%) to verify the actual steroid concentration in each incubate.[5a] The disparity in the results of the methods can be attributed to increased adsorption of the iodinated steroid relative to the non-iodinated steroids such as R1881 and DHT. If 7α-IDHT also adsorbs strongly to surfaces, then the RBA value reported in this study may be an underestimate of its true affinity for the androgen receptor.

In all, 7α-IDHT exhibits excellent characteristics for a steroid hormone receptor ligand: high affinity for its receptor as well as specificity, i.e., very low affinity for other steroid receptors. Thus, it is highly likely that 7α-IDHT, labeled with radioiodine, should be an excellent probe for the detection and quantification of the androgen receptor in various tissues and tumors.

Experimental Section

Melting points were obtained in a Koffler hot stage or in a Mel-temp apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide disks on a Beckman Acculab 4 spectrophotometer or a Perkin-Elmer Model 1600 FT-IR instrument. NMR spectra were obtained at 100 or 600 MHz with Bruker WP100SY or AMX 600 FT instruments. Mass spectra were obtained using a Hewlett-Packard Model 5985A spectrometer at 70 eV with a direct-insertion probe. FAB mass spectra were obtained with a Kratos MS890 spectrometer. High-performance liquid chromatography was performed on a Beckman Model 334 gradient system equipped with Model 421 controller, Altex CR-1A integrator–recorder, and Hitachi Model 100–10 variable-wavelength detector. Elemental analyses were performed by Schwarzkopf Micro Analytical Laboratory, Woodside, NY.

[17α-Methyl-^{3}H]methyltrienolone (R1881; specific activity = 83.2 Ci/mmol), [17α-methyl-^{3}H]promegestone (R5020; specific activity = 84.7 Ci/mmol), [2,4,6,7-^{3}H]estradiol (specific activity = 103 Ci/mmol), [6,7-^{3}H]dexamethasone (DEX; specific activity = 44.7 Ci/mmol), and unlabeled R1881 were purchased from DuPont Canada (Mississauga, Ontario, Canada). 7α-Methyl-17-[(*E*)-2′-iodovinyl]-19-nortestosterone (E-MIVNT) was synthesized as reported previously.[5a] 17β-Acetoxy-3,3-(ethylenedioxy)-5-androstene was prepared by previously described methods.[6] All other steroids with the exception of 7α-IDHT were purchased from Sigma Chemical Co. (St. Louis, MO).

17β-Acetoxy-3,3-(ethylenedioxy)-5-androsten-7-one (2). Methylene chloride (180 mL) was placed in a 500-mL flask which was charged with nitrogen and cooled to −23 °C in a dry ice–carbon tetrachloride bath. Anhydrous chromium trioxide (36 g, 0.356 mol) was added, and the mixture was stirred mechanically for 2 min. 3,5-Dimethylpyrazole (DMP) (34.7 g, 0.361 mol) was then added, and the mixture was stirred for an additional 15 min following which 17β-acetoxy-3,3-(ethylenedioxy)-5-androstene (1) (6.0 g, 0.016 mol) was added. After stirring for 2.5 h, the mixture was brought to 0 °C and 157 mL of 5 N NaOH was slowly added. After stirring for 1 h at this temperature, the mixture was transferred to a separatory funnel, diluted with 250 mL of water, and extracted with 250 mL of ether. After thorough agitation, the organic layer was separated from the aqueous and emulsion layers and washed with water and 0.1 N HCl. The acid washes were done quickly to avoid ketal hydrolysis and were followed immediately by washing with 5% NaOH, water, and saturated sodium chloride. The ether solution was then dried over sodium sulfate, filtered, and evaporated to give a brown residue. Emulsions which formed at various stages of the workup of the organic layer were combined and filtered through a bed of Celite. The resulting filtrate was extracted with methylene chloride and the organic extract washed with water and saturated sodium chloride. After drying and evaporation of solvent, a brown residue remained which was composed primarily of additional ketone. The residues from the organic layer and the treatment of the emulsions were combined and recrystallized from methanol–water containing a trace of pyridine to give 3.5 g of ketone **2** (56.2% yield): mp 253–256 °C; IR (KBr) 1720, 1659 cm^{-1}; NMR ($CDCl_3$) δ 5.66 (s, 1, H-6 vinyl), 4.59 (dd, 1, H-17α), 3.94 (s, 4, 3-ketal), 2.02 (s, 3, 17β-OAc), 1.19 (s, 3, H-19), 0.79 (s, 3, H-18). Anal. ($C_{23}H_{32}O_5$) C: calcd, 71.09; found, 70.47. H: calcd, 8.32; found, 8.02.

17β-Acetoxy-3,3-(ethylenedioxy)-5α-androstan-7-one (3). A mixture of compound **2** (4.7 g, 12.1 mmol), 228 mL of methanol, and 2.28 g of 10% palladium on charcoal was hydrogenated under 35 psi H_2 using a Parr low-pressure hydrogenation apparatus for 3 h. The mixture was filtered to remove the catalyst, and the solvent was evaporated. The weight of this residue was 4.0 g (84.3%): mp 169–172 °C; IR (KBr) 1720, 1700 cm^{-1}; NMR ($CDCl_3$) δ 4.62 (dd, H-17α), 3.89 (s, 4, 3-ketal), 2.01 (s, 3, 17β-OAc), 1.06 (s, 3, H-19), 0.79 (s, 3, H-18). Anal. ($C_{23}H_{34}O_5$) C: calcd, 70.72; found, 69.81. H: calcd, 8.80; found, 8.60.

17β-Acetoxy-3,3-(ethylenedioxy)-5α-androstan-7α-ol (4a) and 17β-Acetoxy-3,3-(ethylenedioxy)-5α-androstan-7β-ol (4b). A mixture of lithium aluminum hydride (562 mg, 14.8 mmol) and 140 mL of anhydrous ether in a 1-L flask was cooled to −78 °C (dry ice–acetone) in an atmosphere of nitrogen. *tert*-Butyl alcohol (1.53 mL, 16.2 mmol) was added followed by a solution of 1.95 g (4.97 mmol) of compound **3** in 280 mL of tetrahydrofuran. This was allowed to stir for 5 min and then the bath was removed. The mixture was allowed to warm to room temperature and then 280 mL of saturated ammonium chloride was added with stirring. The mixture was transferred to a separatory funnel, and the organic phase was washed with water and saturated sodium chloride and then dried over sodium sulfate. Filtration and evaporation gave a white crystalline residue weighing 1.4 g (72% yield). This material was a mixture of 80% **4a**, 7α-hydroxy, and 20% **4b**, 7β-hydroxy compounds, as determined by integration of the H-7β and H-7α resonances in the 600-MHz NMR spectrum: mp 189–191 °C; IR (KBr): 3560 cm^{-1}, 1715 cm^{-1}; NMR ($CDCl_3$) δ signals assigned to **4a** 4.64 (dd, H-17α), 3.93 (s, 4, 3-ketal), 3.83 (m, 1, half-width 6.9 Hz, H-7β), 2.04 (s, 3, 17-OAc), 0.83 (s, 3, H-19), 0.79 (s, 3, H-18); signals assigned to **4b** 4.59 (dd, H-17α), 3.94 (s, 4, 3-ketal), 3.40 (m, 1, half-width 26.9 Hz, H-7α), 2.04 (s, 3, 17-OAc), 0.85 (s, 3, H-19), 0.82 (s, 3, H-18). Anal. ($C_{23}H_{36}O_5$) C: calcd, 70.35; found, 69.97. H: calcd, 9.27; found, 9.10.

17β-Acetoxy-7α-hydroxy-5α-androstan-3-one (5a) and 17β-Acetoxy-7β-hydroxy-5α-androstan-3-one (5b). A solution of 58 mg (0.31 mmol) of *p*-toluenensulfonic acid in 58 mL of acetone was added to 962 mg (2.44 mmol) of the above mixture of **4a,b** in a screw-capped Erlenmeyer flask. The flask was closed and heated at 60 °C overnight. After cooling, the solution was evaporated to half its original volume, diluted with 130 mL of water, and cooled at 4 °C for 6 h. Crystalline product formed which was collected by filtration and weighed 562 mg. The filtrate was extracted with ether, and the extract was washed with water and saturated sodium chloride and then dried over sodium sulfate. Filtration and evaporation gave a residue of additional product weighing 291 mg. The total yield was 853 mg (99%). Recrystallization of the crystalline material above with aqueous methanol and then methylene chloride–petroleum ether gave **5** which was a mixture consisting of 90% 7α-hydroxy and 10% 7β-hydroxy compounds, as determined by integration of the H-7β and -7α resonances in the 600-MHz NMR spectrum: mp 223–226 °C. Anal. ($C_{21}H_{32}O_4$) C: calcd, 72.34; found, 71.88. H: calcd, 9.28; found, 9.16. A portion of this material was converted to **6** as described below. Another portion (220 mg) was separated into the pure epimers **5a,b** by flash chromatography on silica gel-H. Elution with 50% benzene–ethyl acetate gave 177 mg of **5a**, 12.5 mg of **5b**, and 15 mg of a mixture of the two. The purified epimers were recrystallized with methylene chloride–petroleum ether.

5a: mp 227–228 °C; IR (KBr) 3520, 1718 cm^{-1}; NMR ($CDCl_3$) δ 4.64 (dd, H-17α), 3.88 (m, 1, half-width 6.1 Hz, H-7β), 2.05 (s, 3, 17β-OAc), 1.02 (s, 3, H-19), 0.82 (s, 3, H-18); MS *m/z* 348 (0.3, parent, M), 330 (1.3, M − H_2O), 288 (0.8, M − HOAc), 270 (7.5,

M – H_2O – HOAc), 255 (6.5, M – H_2O – HOAc – CH_3), 176 (10.6, $C_{12}H_{16}O^+$, A- + B-rings and C-11), 99 (61.2, $C_6H_{11}O^+$, D-ring cleavage). Anal. ($C_{21}H_{32}O_4$) C: calcd, 72.34; found, 72.66. H: calcd, 9.28; found, 9.48.

5b: mp 172–173 °C; IR (KBr) 3520, 1718 cm^{-1}; NMR ($CDCl_3$) δ 4.59 (dd, H-17α), 3.40 (m, 1, half-width 26.4 Hz, H-7α), 2.05 (s, 3, 17β-OAc), 1.06 (s, 3, H-19), 0.85 (s, 3, H-18); MS m/z 348 (2.5, parent, M), 330 (3.1, M – H_2O), 288 (5.8, M – HOAc), 270 (47.5, M – H_2O – HOAc), 255 (28.1, M – H_2O – HOAc– CH_3), 176 (24.8, $C_{12}H_{16}O^+$, A- + B-rings and C-11), 99 (18.9, $C_6H_{11}O^+$, D-ring cleavage). Anal. ($C_{21}H_{32}O_4$) C: calcd, 72.34; found, 72.12. H: calcd, 9.28; found, 9.52.

17β-Acetoxy-7α-iodo-5α-androstan-3-one (6). Compound **5**, a mixture consisting of 90% 7α- and 10% 7β-hydroxy epimers (280 mg, 0.805 mmol), was placed in a screw capped test tube followed by 0.4 mL of chloroform, 1.2 mL of 0.8 M sodium iodide in acetonitrile (0.97 mmol), and 113 μL (144 mg, 0.962 mmol) of trichloromethylsilane under nitrogen. The tube was closed and heated with magnetic stirring in an oil bath at 80–85 °C for 2 h. After cooling, the mixture was diluted with 100 mL of methylene chloride, and the solution was washed with 10% sodium thiosulfate and water. After drying over sodium sulfate, filtration, and evaporation, an oily residue remained. Flash chromatography on a 12-cm × 2-cm column of silica gel-H (Merck) using 6:1 benzene–ethyl acetate gave 75.1 mg of product **6** which was homogeneous by TLC (3:1 benzene–ethyl acetate, R_f = 0.51) and 52.0 mg of product containing a less polar impurity (38% yield). Rechromatography of the latter fraction on a 2.5-cm × 26-cm column of silica gel-H using 10:1 benzene–ethyl acetate and recrystallization with acetone–petroleum ether gave the analytical sample: mp 176–178 °C; IR(KBr) 1720, 1700 cm^{-1}; NMR ($CDCl_3$) δ 4.64 (dd, 1, H-17α), overlaps with 4.62 (ddd, 1, width 8.6 Hz, H-7β), 2.05 (s, 3, 17β-OAc), 1.07 (s, 3, H-19), 0.86 (s, 3H-18); MS (FAB) m/z 459 (M + 1), 399 (M – CH_3COOH + 1), 331 (M – HI + 1), 271 (M – CH_3COOH – HI + 1). Anal. ($C_{21}H_{31}O_3I$) C: calcd, 55.00; found, 55.32. H: calcd, 6.83; found, 7.01. I: calcd, 27.70; found, 27.98.

Impurities separated in the above chromatographic procedures were not characterized. NMR analysis suggests that these may be elimination side products. There was no evidence (NMR, TLC) of the presence of a 7β-iodo product.

In separate experiments, the purified 7-hydroxy epimers **5a,b** were iodinated by the procedure described above. Both epimers were converted to the 7α-iodo product with no evidence of the presence of a 7β-iodo product.

17β-Hydroxy-7α-iodo-5α-androstan-3-one (7). A 16.2-mg portion of **6** was stirred for 4 days with 0.24 mL of saturated Na_2CO_3 in 2.2 mL of methanol. The mixture was diluted with 30 mL of water and extracted with methylene chloride. The extracts were dried over sodium sulfate, filtered, and evaporated to give 14.3 mg of crude product. HPLC purification on a 25-cm × 1-cm silica gel column (1% isopropyl alcohol in methylene chloride, 5 mL/min, 280 nm) gave 9.6 mg of material (65% yield) which eluted as a symmetrical peak at 14.3 min and was recrystallized from acetone to give needles: mp 164–166 °C; TLC (3:1 benzene–ethyl acetate, developed twice) single spot, R_f = 0.38. Rechromatography of this material in a second HPLC system (25-cm × 4.6-mm Lichrosorb-Diol column, 50% isooctane–methylene chloride, 1 mL/min) gave a single peak migrating at 10 min: IR (KBr) 3400 (br, 17β-OH), 1700 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 4.64 (ddd, 1, width 9.1 Hz, H-7β, J = 2.7–3.0 Hz), 3.70 (dd, 1, H-17α), 1.08 (s, 3, H-19), 0.82 (s, 3, H-18); MS (FAB) m/z 417 (M + 1) (low-res EI), 289 (M – I), 271 (M – I – H_2O) (high res). Calcd for $C_{19}H_{29}O_2$ (M – I): 289.2167; found, 289.2166.

Steroid Receptor Analyses. Cytosol Preparation. Cytosols prepared from rat tissues and the human breast cancer cell line MCF-7 were used to compare the binding of 7α-IDHT with other androgens to different classes of steroid receptors. All rat cytosols were prepared from tissues removed from adult Sprague–Dawley CD-strain rats obtained from Charles River Breeding Laboratories (St. Constance, Quebec, Canada). For androgen-receptor-binding experiments, prostate glands obtained from male rats gonadectomized (GDX) 48 h before sacrifice were homogenized in buffer TEGTMo (10 mM Tris, 1.5 mM Na_2-EDTA, 10% (v/v) glycerol, 12 mM monothioglycerol, 25 mM sodium molybdate, pH 7.4, at 4 °C; 1.5 mL/prostate). For glucocorticoid-receptor-binding experiments, liver extirpated from a rat adrenalectomized 1 week before sacrifice was homogenized in 5 volumes of buffer TEGTMo. For estrogen- and progestin-receptor-binding experiments, uteri from rats GDX 6 months before sacrifice were homogenized in buffer TEGT (TEGTMo without sodium molybdate; 2 mL/uterus). Uteri taken for progestin receptor binding were from animals treated for 6 months with a subcutaneously placed 1.0 cm Silastic (Dow Corning, Midland, MI) capsule containing crystalline estradiol diluted to 10% with cholesterol.[17]

Cytosols prepared from the human breast cancer cell line MCF-7 were used as a source of human androgen, estrogen, and progestin receptors. Cells were grown in RPMI 1640 medium (without phenol red) supplemented with 5% fetal calf serum. Four days before harvest, the medium was changed to RPMI 1640 containing 5% charcoal-stripped fetal calf serum[18] with (for progestin receptors) or without (for estrogen and androgen receptors) 10 nM estradiol. Cells were grown in T-75 tissue culture flasks and harvested with a cell scraper when confluent. Cells combined from six flasks were washed twice with RPMI 1640 medium, suspended in 2 mL of buffer TEGTMo, and homogenized with a Teflon pestle.

All homogenates were centrifuged at 105000g for 45 min at 0–4 °C. The supernatants (cytosol) were decanted, frozen on dry ice, and stored at –80 °C until assay (<1 month). At the time of assay, cytosols were allowed to thaw on ice in a 4 °C room and diluted with assay buffer before use. Protein concentration was adjusted to 6–10 mg/mL for prostate cytosol, 0.8–1.2 mg/mL for uterine cytosol, and 1.0–1.5 mg/mL for MCF-7 cytosols.

Specificity of 7α-IDHT Binding. Binding affinities of 7α-IDHT relative to reference compounds were determined for rat and human androgen, estrogen, and progestin receptors and for rat glucocorticoid receptors. For androgen receptor binding, 75-mL aliquots of cytosol extracted from GDX rat prostates or from MCF-7 cells were incubated at 4 °C for 20 h with 50 mL of buffer TEGTMo containing 3.8 nM [^{3}H]R1881 (final concentration) in the presence or absence of a range of different concentrations of unlabeled R1881, 7α-IDHT, testosterone, DHT, E-MIVNT, or progesterone. To suppress any possible binding to progestin or glucocorticoid receptors, all incubates contained 1.0 mM triamcinolone acetonide.[16a] For progestin receptor binding, 75-mL aliquots of cytosol extracted from uteri of estrogen-treated GDX rats or from estrogen-treated MCF-7 cells were incubated at 4 °C for 20 h with 50 mL of buffer TEGT (rat) or TEGTMo (human) containing 1.0 nM [^{3}H]R5020 (final concentration) in the presence or absence of a range of different concentrations of unlabeled R1881, 7α-IDHT, testosterone, DHT, E-MIVNT, or progesterone. For estrogen receptor binding, 75-mL aliquots of cytosol extracted from GDX rat uteri or from MCF-7 cells were incubated at 4 °C for 20 h with 50 mL of buffer TEGT (rat) or TEGTMo (human) containing 2.0 nM [^{3}H]estradiol (final concentration) in the presence or absence of a range of different concentrations of unlabeled R1881, 7α-IDHT, testosterone, DHT, E-MIVNT, or estradiol. For glucocorticoid receptor binding, 75-mL aliquots of cytosol extracted from ADX rat liver were incubated at 4 °C for 20 h with 50 mL of buffer TEGT (rat) or TEGTMo (human) containing 4.0 nM [^{3}H]DEX (final concentration) in the presence or absence of a range of different concentrations of unlabeled R1881, 7α-IDHT, testosterone, DHT, E-MIVNT, corticosterone, or DEX.

Macromolecular bound ^{3}H ligand was separated from free by gel filtration on 7-mm × 35-mm Sephadex LH-20 columns at 4 °C.[19] Aliquots of incubate (100 mL) were loaded onto the columns and washed into the column bed with 100 mL of assay buffer. Thirty minutes after sample application, the macromolecular bound fraction was eluted with 400 mL of assay buffer into liquid scintillation counting vials. Radioactivity was determined at 55% efficiency using an ICN Micromedic Taurus liquid scintillation counter after overnight extraction of radioligand into 5 mL of BetaMax (ICN) scintillation counting fluid. Displacement curves were analyzed by a least-squares curve-fitting method with the use of the computer program ALLFIT.[20] Relative binding affinities (RBAs) were calculated on the basis of the concentration of competitor required to displace saturable binding by 50%.

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