The 16,17-Double Bond Is Needed for Irreversible Inhibition of Human Cytochrome P450$_{17\alpha}$ by Abiraterone (17-(3-Pyridyl)androsta-5,16-dien-3β-ol) and Related Steroidal Inhibitors

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Abiraterone (17-(3-pyridyl)androsta-5,16-dien-3β-ol, **1**) is a potent inhibitor (IC_{50} 4 nM for hydroxylase) of human cytochrome P450$_{17\alpha}$. To assist in studies of the role of the 16,17-double bond in its mechanism of action, the novel 17α-(4-pyridyl)androst-5-en-3β-ol (**5**) and 17β-(3-pyridyl)-16,17α-epoxy-5α-androst-3β-ol (**6**) were synthesized. 3β-Acetoxyetienic acid was converted in three steps into **5** via photolysis of the thiohydroxamic ester **8**. Oxidation of an appropriate 16,17-unsaturated precursor (**21**) with CrO_3−pyridine afforded the acetate (**23**) of **6**. Inhibition of the enzyme by **1**, the similarly potent 5,6-reduced analogue **19** (IC_{50} 5 nM), and the 4,16-dien-3-one **26** (IC_{50} 3 nM) and by the less potent (IC_{50} 13 nM) 3,5,16-triene **25** is slow to occur but is enhanced by preincubation of the inhibitor with the enzyme. Inhibition following preincubation with these compounds is not lessened by dialysis for 24 h, implying irreversible binding to the enzyme. In contrast under these conditions the still potent (IC_{50} 27 nM) 17α-(4-pyridyl)androst-5-en-3β-ol (**5**) showed partial reversal after 5 h of dialysis and complete reversal of inhibition after 24 h. This behavior was also shown by the less potent 16,17-reduced 3-pyridyl compounds **3** and **24**. Further, in contrast to the compounds (**1**, **19**, **25**, **26**) with the 16,17-double bond, the inhibition of the enzymic reaction was not enhanced by preincubation either with **5** or with the 17β-pyridyl analogues **3**, **4**, and **24** which also lack this structural feature. The results show that the 16,17-double bond is necessary for irreversible binding of these pyridyl steroids to cytochrome P450$_{17\alpha}$. However oxidation to an epoxide is probably not involved since epoxide **6** was only a moderately potent inhibitor (IC_{50} 260 nM).

We have recently described potent steroidal inhibitors of cytochrome P450$_{17\alpha}$, a potential target enzyme in the treatment of hormone-dependent prostatic carcinoma.[1] One of these compounds, abiraterone (17-(3-pyridyl)-androsta-5,16-dien-3β-ol, **1**), has been selected for clinical evaluation based in part on its marked reduction of circulating testosterone levels in the male rat and mouse and of androgen-dependent organ weights in the mouse.[2]

We have also described potent nonsteroidal inhibitors of the target enzyme, which were esters containing a pyridyl residue.[3,4] Examples of each series having high inhibitory potency for the target enzyme coupled with metabolic stability toward esterases were selected for study in vivo. Unlike **1**, they did not lower circulating testosterone levels nor the weights of androgen-sensitive organs in the WHT mouse.[5] Two possible contributory factors were considered to explain this difference. First the inhibitory potencies of the nonsteroidal compounds toward the murine enzyme appear insufficient, in relation to the K_m values for the enzyme substrate, to inhibit the murine enzyme in vivo. Second, the binding of the nonsteroidal inhibitors to the target enzyme is reversible, whereas that of the steroidal inhibitor **1** is not. The present study extends investigations carried out on the murine cytochrome P450$_{17\alpha}$ to the human testicular enzyme and probes the structural features required for the irreversible binding by the steroidal inhibitors. It explores the hypothesis that the 16,17-double bond is the essential structural feature conferring this binding, either directly or through a potential for further metabolism to a reactive epoxide. In this context, Angelastro and co-workers have designed mechanism-based steroidal inhibitors of cytochrome P450$_{17\alpha}$ with cyclopropylamino[6] or cyclopropyloxy[7] substituents at C-17 proposed to be activated by enzymatic oxidation to covalently reactive species. In these studies, the stronger inhibition of the compounds observed following preincubation with the enzyme was evidence supporting the hypothesis of mechanism-based inhibition.

Analogues with the 16,17-double bond reduced have been synthesized previously, by reduction of **1** and its 4-pyridyl analogue **2**.[1] The 17β-3-pyridyl derivative **3** was 1 order of magnitude less potent than its precursor **1** (Table 1). The 17β-4-pyridyl derivative **4** was less potent still, though more potent than its precursor **2**, the pyridyl nitrogen of which was expected[1] to interact poorly with the target heme residue. In a molecular modeling study, the observed[4] relative inhibitory potencies of enantiomeric pairs of 1-(4-pyridyl)ethyl 1-adamantanecarboxylates and their 3-pyridyl-substituted counterparts were rationalized[8] in terms of overlays of the inhibitor molecules onto pregnenolone such that C-1 of the pyridylethyl residue corresponds to C-17 of the steroid. The proposed overlays would predict that the

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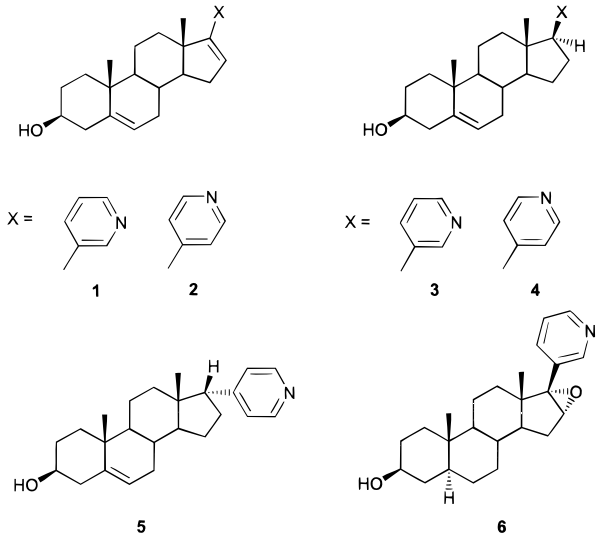
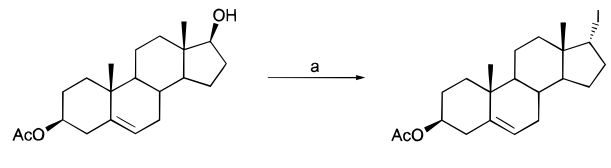
17α-4-pyridyl analogue **5** might be better orientated for interaction of the pyridyl nitrogen with the heme residue in the enzyme binding site than either **3** or **4** and could be a more potent inhibitor. Compound **5** might then provide a better comparison with **1** for assessing the putative role of the double bond in irreversible binding. The present study also describes the synthesis and evaluation of **5** and the epoxide **6**, a potential reactive metabolite of a 3-pyridyl steroid containing the 16,17-double bond.



Results and Discussion

Synthesis. The synthesis of a 17-substituted steroid with the pyridyl residue in the α configuration presented a synthetic challenge. According to literature precedents, electrophilic, radical, or nucleophilic attack at the C-17 position should occur preferentially at the less sterically crowded α face, owing to hindrance by the 18-methyl substituent, to give a product with the 17*β* configuration. Indeed this was seen in the reduction of **1** and **2** to the 17*β*-pyridyl derivatives **3** and **4**.[1] A synthetic strategy in which the bond between C-17 and the pyridyl residue was formed last was therefore envisaged. Unsuccessful approaches (results not shown) involved attempted inversion of configuration at C-17. Using Mitsonobu's conditions[9] 3*β*-acetoxy-17*β*-hydroxyandrost-5-ene was treated with ethyl cyanoacetate[10] or 4-pyridylzinc chloride[11] as the source of carbon nucleophile. Alternatively the 17*β*-mesylate was treated with diethyl malonate or ethyl cyanoacetate in the presence of CsF.[12] Another approach proceeded via the 17α-iodide **7** as a potential source of a free radical which, being planar, might trap a suitable reactive species on the less hindered α face to give a product of the required stereochemistry. The synthesis of **7** (Scheme 1) made use of the convenient reaction of secondary alcohols with iodine to give iodides, with inversion of configuration.[13,14] The alkylation at C-2 and C-4 of pyridines using alkyl iodides as sources of free radicals has been described,[15] but when **7** was subjected to the various reaction conditions used, no pyridine derivatives were isolated. Analogous attempts to use etienic acid as the free radical source also failed. However this acid was used in an eventually successful approach (Scheme 2) using methodology described by Barton et al.[16,17]

Scheme 1[a]



3β-acetoxy-17β-hydroxyandrost-5-ene 7

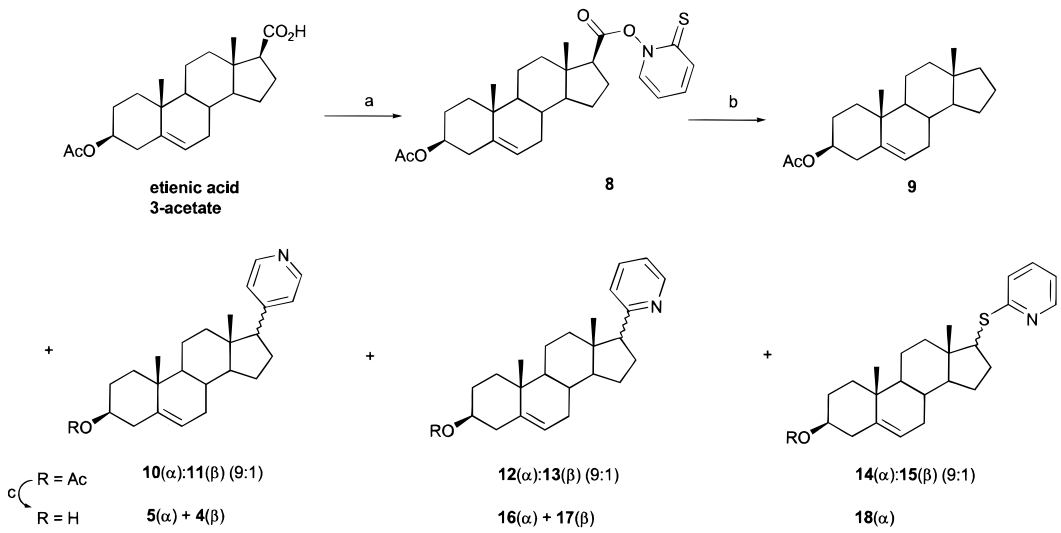
[a] (a) I_2, PPh_3, imidazole, PhMe.

Conversion of 3*β*-acetoxyetienic acid into the thiohydroxamic ester **8** followed by irradiation in pyridine/CH_2Cl_2 in the presence of camphorsulfonic acid gave in addition to the reduction product **9** (17% yield; also formed in the above-mentioned reaction with **7**) low yields of the isomeric 4-pyridyl derivatives (11%; **10**(α): **11**(*β*), 9:1) and the 2-pyridyl analogues (11.5%; **12**(α)**: 13**(*β*), 9:1). The major product (30%) comprised a 20:1 ratio of the chromatographically separable α (**14**) and *β* (**15**) epimers of the (2-pyridyl)thio derivative. Although (2-pyridyl)thio derivatives have been described as products of this radical approach,[18] their formation does not previously appear to have been a problem in the context of decarboxylative addition onto protonated pyridines.[16,17] It seems that such reactions at the C-17 position of steroids present particular difficulties, and attempts to improve the yield of the desired product by varying the solvent were unsuccessful. The target 4-pyridyl derivative **5** was readily separable by chromatography from the unwanted epimer (**4**) following deacetylation of the mixture of **10** and **11**. Alcohols **16** + **17** and **18** were similarly obtained by deacetylation of the mixture of 2-pyridyl derivatives **12** and **13** and the separated (2-pyridyl)thio derivative **14**, respectively.

Stereochemistry at C-17 for the 16,17-reduced derivatives was reflected in their ^{1}H NMR spectra. Thus for the 17*β* isomers the signal for H-17 appeared as an apparent triplet (J = 9.6 Hz), at higher field than for H-17 in the 17α epimers which appeared as a double doublet (J = 8.3 and ∼1.0 Hz). Additionally, Me-18 in 17*β* isomers appeared at δ ∼0.50, whereas in the 17α analogues it was more deshielded, appearing at δ ∼1.00.

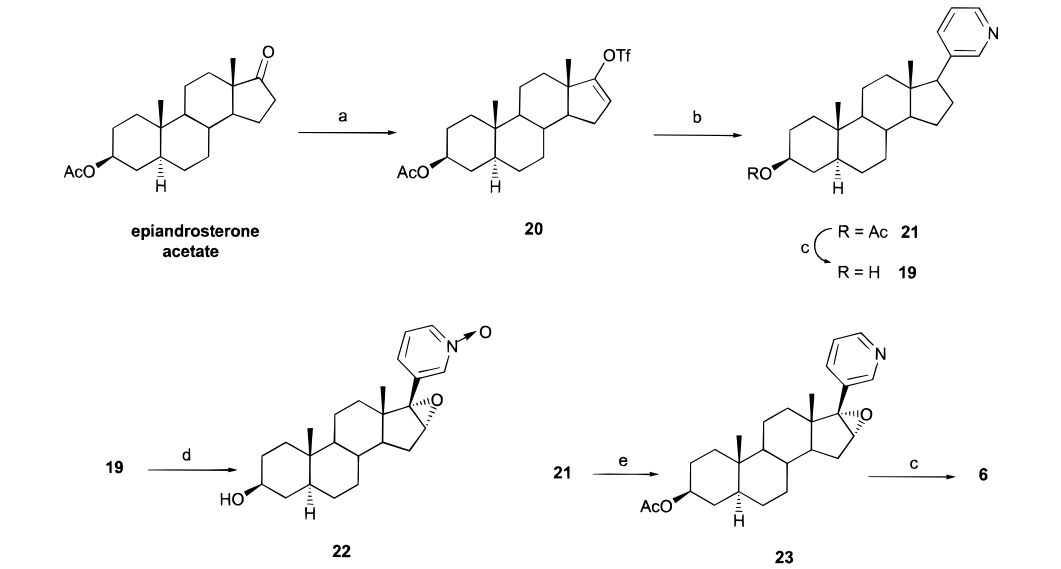
The synthesis of a 16,17-epoxide from the acetate of **1** proved intractable owing to the presence of the 5,6-double bond. Hence a precursor **(19)**, lacking the 5,6-double bond but otherwise analogous to **1,** was synthesized (Scheme 3) via the enol triflate **20** of epiandrosterone acetate using previously described methodology.[1] The synthesis (Scheme 3) of the target epoxide (**6)** from **19** was still a problem. The use of 1 equiv of MMPP (monoperoxyphthalic acid magnesium salt) as oxidant gave a mixture of **6**, the *N*-oxide of **19**, and the epoxide *N*-oxide **22**. Since these products were difficult to separate, the reaction was driven to completion using 4 equiv of MMPP with a view to subsequent selective reduction of the *N*-oxide residue in **22**. However, the use of borohydride ion-exchange resin combined with $CuSO_4$, recently reported[19] to reduce selectively amine *N*-oxides in the presence of epoxides, regenerated **19**. Finally acetate **21** was selectively epoxidized (Scheme 3) using Sarett's reagent.[20] Hydrolysis of the intermediate **23** gave the target compound **6** in 46% overall yield. The stereochemistry for **6** (and for **22** and **23**) was assigned based on a literature precedent.[21] For epoxides

Scheme 2[a]



[a] (a) (i) $(CO)_2Cl_2$, CH_2Cl_2, THF, (ii) *N*-hydroxypyridine-2-thione Na salt, CH_2Cl_2, dark; (b) PyH, camphorsulfonic acid, *hν*; (c) $LiAlH_4$, THF.

Scheme 3[a]



[a] (a) Tf_2O, base; (b) 3-PyBEt_2, $Pd(PPh_3)_2Cl_2$, THF, H_2O, Na_2CO_3; (c) NaOH, H_2O, MeOH; (d) MMPP, 4 equiv; (e) CrO_3, PyH, CH_2Cl_2.

within a five-membered ring, a trans orientation of the oxygen produced a small shielding of adjacent methyl protons while a cis relationship between epoxide oxygen and methyl had virtually no effect relative to the influence of a double bond. The signal for Me-18 appeared between δ 0.74 and 0.79 for epoxides **6**, **22**, and **23** but at δ 1.00−1.02 for the 16,17-unsaturated precursors **19** and **21**. This likewise suggested a trans orientation between the epoxide and the angular methyl group. Also, as mentioned above, a β orientation of a pyridyl ring in 16,17-reduced pyridyl steroids produced a ∼0.5 ppm shielding of the Me-18 signal relative to a 17α-pyridyl substituent.

Finally, for comparison with compounds **3**−**5**, the 17β-3-pyridyl derivative **24** was made by reduction of **19**.

Inhibition of Human Testicular 17α-Hydroxylase. A. Structure−Activity Relationships. The 17α-4-pyridyl derivative **5** proved, as predicted, a potent inhibitor of the target enzyme, with an IC_{50} for the hydroxylase of 27 nM, only 6−7-fold less potent than **1** (Table 1). Compounds **16** + **17** and **18**, byproducts of the synthesis of **5,** gave no inhibition at 1 μM (results not shown). To minimize ambiguity in the interpretation of the binding data (see below) which might arise by comparing compounds with markedly differing inhibitory potencies, a compound more similar in potency to that of **5**, but retaining the 16,17-double bond present in **1**, was selected to include in this study. The 3,5-diene

Table 1. Enzyme Inhibition Data

compd	progesterone 17α-hydroxylase IC_{50} (nM)	type of inhibition
1	4[a]	irreversible
3	47[a]	reversible
4	160[a]	
5	27	reversible
6	260	
19	5	irreversible
24	74	reversible
25	13[a]	irreversible
26	3[a]	irreversible

[a] Values taken from ref 1.

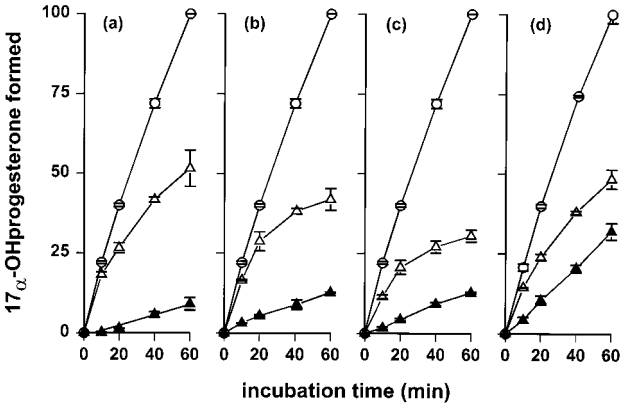
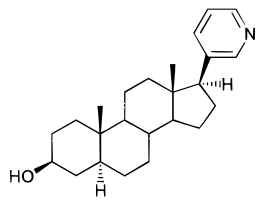


Figure 1. Time course for human 17α-hydroxylase activity after (▲) or without (△) preincubation of the enzyme with (a) **1** (3 nM), (b) **19** (5 nM), (c) **25** (30 nM), or (d) **26** (3 nM); control (○).

25, previously shown[1] to be ca. 3-fold less potent than **1**, was selected for this purpose. It was only twice as potent as **5**, making it unlikely that any marked difference in binding characteristics observed between **5** and **25** could be ascribed to differences in potency. Also included in the binding study as a further example of an inhibitor with the 16,17-double bond was the only other compound in this series which had previously been evaluated in vivo,[2] namely, the 3-en-4-one analogue **26**.[1]



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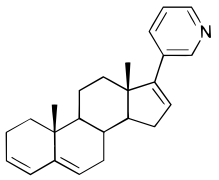

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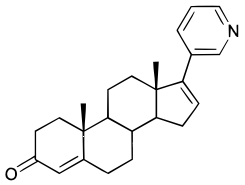

26

Turning to the potential role of epoxide formation, the parent steroid **19** of the epoxide **6** was of similar potency to that of **1**, in line with our previous observation[1] on its 3α epimer. The epoxide was however markedly less potent (IC_{50} 260 nM), and the inhibitory activity was not enhanced by preincubation with the enzyme (results not shown). Hence the present study does not suggest that such an epoxide is the ultimate inhibitory species.

B. Binding Characteristics. Inhibition of the human enzyme by steroidal compounds with the 16,17-ene structure (**1**, **19**, **25**, **26**) was slow to occur and appeared enhanced by preincubation with the enzyme (Figure 1). Once inhibited, there was no recovery of activity after 24 h of dialysis (Figure 2). This parallels findings with the testicular enzyme from WHT mice[5] and suggests irreversible inhibition of the enzyme by these compounds. Following preincubation of **1** with the human testicular cytochrome $P450_{17\alpha}$, the inhibition was unaffected by the substrate concentration (Figure 3), with an IC_{50} value of 1.2 nM. The hydroxylase reaction in the presence of steroidal compounds lacking the 16,17-double bond (**3**−**5**, **24**) showed essentially linear progress curves (Figure 4). There was no increase in the inhibitory effect after preincubation of the enzyme with the compounds. The inhibition by **3**, **5**, **24** was substantially reversed by dialysis (Figure 2) for 5 h and completely reversed after 24 h. The inhibition by **5** was competitive with respect to pregnenolone with a K_i of 0.35 ± 0.11 nM (Figure 5) compared to the K_m of 23 ± 1.7 nM.

Concluding Remarks

Previous studies showing tight irreversible binding for **1** to murine cytochrome $P450_{17\alpha}$ in contrast to reversibility for potent nonsteroidal esters containing the pyridyl residue could be interpreted in terms of a requirement for the steroidal skeleton for such binding. However the present study provides compelling evidence that the double bond adjacent to the pyridyl residue is the structural feature necessary for the apparently irreversible binding to human cytochrome $P450_{17\alpha}$ shown by **1** and its congeners. Thus three such compounds of varying potency have shown it. In contrast four steroidal compounds with this double bond reduced showed no evidence of irreversible binding. It remains to be seen if, by prolonging inhibition of enzyme activity compared with a reversible inhibitor, this structural feature of **1** confers therapeutic advantage in the treatment of carcinoma of the prostate.

Experimental Section

Chemical Methods. ^{1}H NMR spectra (250 MHz) (internal $Me_4Si = \delta$ 0) were determined in $CDCl_3$ (unless otherwise indicated) using a Bruker AC 250 spectrometer. Infrared spectra were determined with a Perkin-Elmer 1720X spectrometer. Fast atom bombardment mass spectra were determined with a VG ZAB-SE spectrometer, accurate masses being determined with *m*-nitrobenzyl alcohol + NaCl as the matrix. Melting points were determined with a Reichert micro-hot-stage apparatus and are uncorrected. Chromatography refers to column chromatography on silica gel (Merck Art. 15111) with solvent indicated applied under positive pressure. Light petroleum refers to the fraction with bp 60−80 °C. Elemental analyses were determined by CHN Analysis Ltd., South Wigston, Leicester, England.

3β-Acetoxy-17α-iodoandrost-5-ene (7). To a mixture of 3β-acetoxy-17β-hydroxyandrost-5-ene (1.000 g, 3.00 mmol), triphenylphosphine (3.156 g, 12.03 mmol), and imidazole (0.819 g, 12.03 mmol), in toluene (100 mL), under argon, was added iodine (2.290 g, 9.02 mmol) in two portions. The reaction mixture was stirred at 80 °C for 1 h and allowed to cool to room temperature. A saturated aqueous Na_2SO_3 solution (40 mL) was then added and the resulting mixture stirred until all the solids had dissolved. Ethyl acetate (100 mL) was added, and the organic phase was washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. Chromatography, on elution with Et_2O−light petroleum (1:25), afforded iodide **7** (1.18 g, 90%): mp 164−165 °C; ν_{max} 2942, 2903, 2890, 1732, 1440, 1376, 1250, 1200, 1036 cm^{-1}; ^{1}H NMR δ 0.86 (s, 3, H-19), 1.04 (s, 3, H-18), 2.05 (s, 3, CH_3CO), 4.38 (dd, 1, $J_{16,17}$ = 7.0 Hz, $J_{16',17}$ =1.0 Hz, H-17β), 4.63 (m, 1, H-3α), 5.40 (m, 1, H-6); HRMS calcd for $C_{21}H_{31}O_2INa$ 465.1267 (M + Na)$^+$, found 465.1260. Anal. ($C_{21}H_{31}O_2I$) H, N; C: calcd, 57.02; found, 56.59.

3β-Acetoxy-17-(4-pyridyl)androst-5-ene (10(α) + 11(β)), 3β-Acetoxy-17-(2-pyridyl)androst-5-ene (12(α) + 13(β)), and 3β-Acetoxy-17-(2-thiopyridyl)androst-5-ene (14(α) +

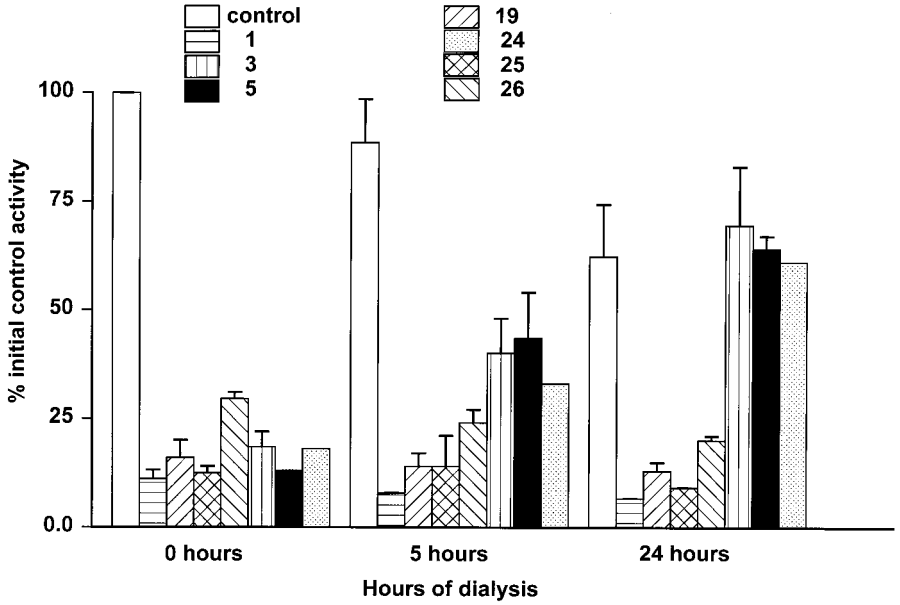

Figure 2. Effect of dialysis on the inhibition of human 17α-hydroxylase by compounds **1** (3 nM), **3** (150 nM), **5** (150 nM), **19** (5 nM), **24** (300 nM), **25** (30 nM), and **26** (3 nM).

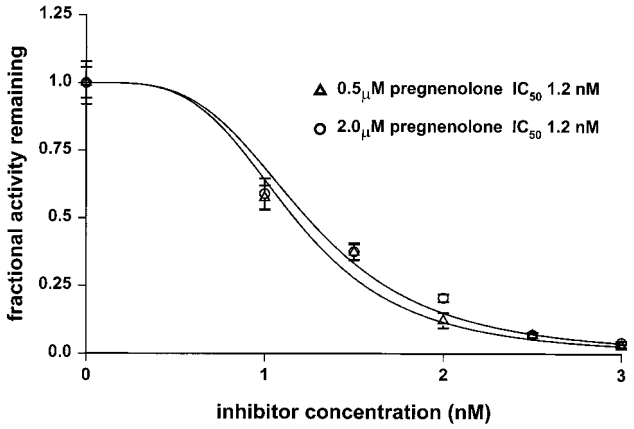

Figure 3. Human 17α-hydroxylase activity after preincubation with varying concentrations of **1** measured with two concentrations of pregnenolone.

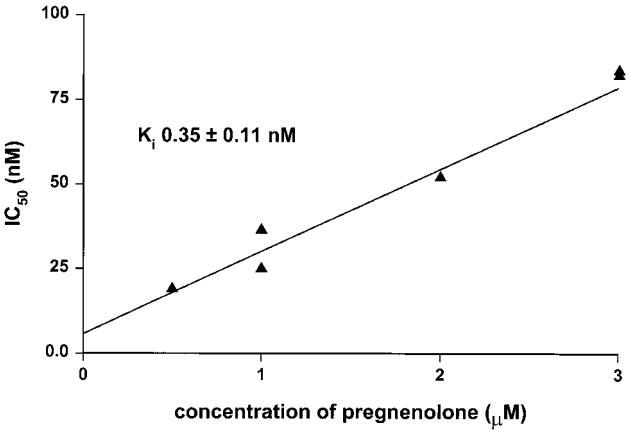

Figure 5. Plot of IC_{50} values for **5** vs pregnenolone concentration for human 17α-hydroxylase.

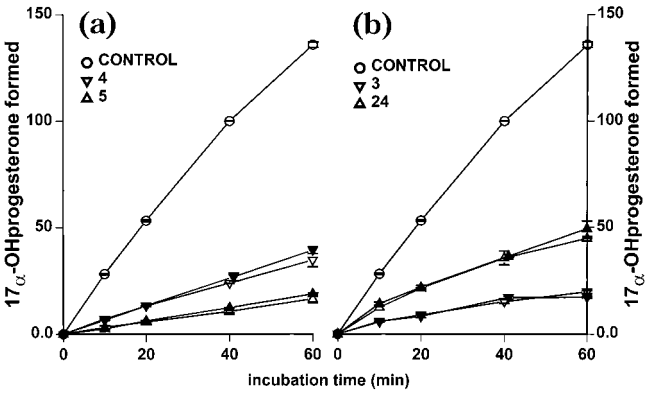

Figure 4. Time course for human 17α-hydroxylase after (solid symbols) or without (open symbols) preincubation of the enzyme with (a) **4** (400 nM) or **5** (150 nM) and (b) **3** (150 nM) or **24** (150 nM).

15(*β*)). 3*β*-Acetoxyetienic acid (200 mg, 0.555 mmol) was dissolved in dry CH_2Cl_2 (4 mL) and treated with oxalyl chloride (1.665 mL, 2 M solution in CH_2Cl_2) and 1 drop of DMF. The mixture was stirred at room temperature for 2 h and then concentrated, the residue dissolved in toluene, and the solution concentrated again. The crude acid chloride (0.555 mmol) was taken up in CH_2Cl_2 (5 mL), and *N*-hydroxypyridine-2-thione sodium salt (99.3 mg, 0.666 mmol) was added. The reaction mixture was shielded from light and stirred for 2 h at room temperature. To this mixture was added a solution of pyridine (263.4 mg, 270 μL, 3.33 mmol) and camphorsulfonic acid (777.6 mg, 3.33 mmol) in CH_2Cl_2 (5 mL). The resulting solution was irradiated (250 W, visible lamp) at ambient temperature for 1 h. Then CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added, and the organic phase was dried (Na_2SO_4) and evaporated. Chromatography, on elution with Et_2O−light petroleum (1:7), yielded first **9** (30 mg, 17%) followed by a mixture (20:1) of **14** and **15** (70 mg, 30%), separated by flash chromatography to give pure **14** (66 mg): ^{1}H NMR for **14** δ 0.97 (s, 3, H-19), 1.03 (s, 3, H-18), 2.04 (s, 3, CH_3CO), 4.10 (dd, 1, $J_{16,17} = 8.3$ Hz, $J_{16',17} = 1.5$ Hz, H-17*β*), 4.62 (m, 1, H-3α), 5.39 (m, 1, H-6), 6.95 (dd, 1, $J_{4,5} = 7.7$ Hz, $J_{5,6} = 5.0$ Hz, pyridyl H-5), 7.17 (d, 1, $J_{3,4} = 7.7$ Hz, pyridyl H-3), 7.45 (td, 1, $J_{4,6} = 1.8$ Hz, pyridyl H-4), 8.40 (dd, 1, pyridyl H-6); HRMS calcd for $C_{26}H_{36}NO_2S$ 426.2467 $(M + H)^+$, found 426.2460; ^{1}H NMR for **15** δ 0.84 (s, 3, H-18), 1.04 (s, 3H, H-19), 3.78 (t, $J = 9.5$ Hz, H-17α). Further elution with Et_2O−light petroleum (1:5) afforded a mixture (9:1) of **12** and **13** (25 mg, 11.5%): *m*/*z* 394 $(M + H)^+$; ^{1}H NMR for **12** δ 1.01 (s, 3, H-19), 1.02 (s, 3, H-18), 2.03 (s, 3, CH_3CO), 3.10 (dd, 1, $J_{16,17} = 8.3$ Hz, $J_{16'17} = 3.1$ Hz, H-17*β*), 4.57 (m, 1, H-3α), 5.39 (m, 1, H-6), 7.09 (m, 2, pyridyl H-3, H-5), 7.56 (td, 1, $J_{3,4} = J_{4,5} = 7.6$ Hz, $J_{4,6} = 1.8$ Hz, pyridyl H-4), 8.56 (dd, 1, $J_{5,6} = 4.7$ Hz, pyridyl H-6); ^{1}H NMR for **13** δ

0.50 (s, 3, H-18), 2.87 (t, 1, $J = 9.6$ Hz, H-17α). Further elution with Et_2O−light petroleum (2:1) gave a mixture (9:1) of **10** and **11** (25 mg, 11.5%): *m/z* 394 $(M + H)^+$; ^1H NMR for **10** δ 1.00 (s, 3, H-19), 1.01 (s, 3, H-18), 2.02 (s, 3, CH_3CO), 2.91 (dd, 1, $J_{16,17} = 8.3$ Hz, $J_{16',17} = 0.8$ Hz, H-17β), 4.57 (m, 1, H-3α), 5.39 (m, 1, H-6), 7.01 (AA′MM′, 2, pyridyl H-3, H-5), 8.48 (AA′MM′, 2, pyridyl H-2, H-6); ^1H NMR for **11** δ 0.49 (s, 3, H-18), 2.67 (t, 1, $J = 9.7$ Hz, H-17α), 7.13 (AA′MM′, 2, pyridyl H-3, H-5).

17α-(4-Pyridyl)androst-5-en-3β-ol (5). A solution of the above mixture of acetates **10** and **11** (25 mg, 0.064 mmol) in THF (2 mL) was treated with $LiAlH_4$ (63.5 μL, 1 M solution in THF) and then, after 5 min, with saturated aqueous Na_2SO_4 solution (19 μL). Solids were filtered off and washed with CH_2Cl_2, and the organic phase was concentrated. Chromatography, on elution with CH_2Cl_2−Et_2O (1:2), yielded pure **5** (18 mg, 81%): mp 210−212 °C; ν_{max} 3349, 2928, 2853, 1601, 1417, 1062, 732 cm^{-1}; ^1H NMR δ 1.00 (s, 6, H-18, H-19), 2.91 (d, 1, $J = 8.1$ Hz, H-17β), 3.49 (m, 1, H-3α), 5.36 (m, 1, H-6), 7.02 (AA′MM′, 2, pyridyl H-3, H-5), 8.48 (AA′MM′, 2, pyridyl H-2, H-6). Anal. ($C_{24}H_{33}NO$) C, H, N.

17-(2-Pyridyl)androst-5-en-3β-ol (16(α) + 17(β)). The foregoing method was followed, using the mixture of acetates **12** and **13** (50 mg, 0.13 mmol). Chromatography, on elution with Et_2O−light petroleum (1:1), yielded a mixture (9:1) of **16** and **17** (40 mg, 87.5%): ^1H NMR for **16** δ 1.00 (s, 6, H-18, H-19), 3.10 (dd, 1, $J_{16,17} = 8.5$ Hz, $J_{16'17} = 2.9$ Hz, H-17β), 3.48 (m, 1, H-3α), 5.36 (m, 1, H-6), 7.08 (m, 2, pyridyl H-3, H-5), 7.56 (td, 1, $J_{3,4} = J_{4,5} = 7.7$ Hz, $J_{4,6} = 1.9$ Hz, pyridyl H-4), 8.55 (dd, 1, $J_{5,6} = 4.0$ Hz, pyridyl H-6); HRMS calcd for $C_{24}H_{33}NO$ 352.2640 $(M + H)^+$, found 352.2640; ^1H NMR for **17** δ 0.49 (s, 3, H-18), 2.83 (t, 1, $J = 9.6$ Hz, H-17α).

17α-(2-Thiopyridyl)androst-5-en-3β-ol (18). The foregoing method was followed, using acetate **14** (90 mg, 0.211 mmol). Chromatography, on elution with Et_2O−light petroleum (1:3), afforded **18** (80 mg, 99%): mp 145−146 °C; ^1H NMR δ 0.97 (s, 3, H-19), 1.02 (s, 3, H-18), 3.54 (m, 1, H-3α), 4.09 (dd, 1, $J_{16,17} = 8.4$ Hz, $J_{16'17} = 1.7$ Hz, H-17β), 5.37 (m, 1, H-6), 6.96 (ddd, 1, $J_{4,5} = 7.8$ Hz, $J_{5,6} = 4.9$ Hz, $J_{3,5} = 0.9$ Hz, pyridyl H-5), 7.17 (dd, 1, $J_{3,4} = 7.8$ Hz, pyridyl H-3), 7.46 (td, 1, $J_{4,6} = 1.9$ Hz, pyridyl H-4), 8.41 (dd, 1, pyridyl H-6); HRMS calcd for $C_{24}H_{34}NOS$ 384.2361 $(M + H)^+$, found 384.2371. Anal. ($C_{24}H_{33}NOS$) H, N, S; C: calcd, 75.15; found, 74.51.

3β-Acetoxy-17-(3-pyridyl)-5α-androst-16-ene (21). Diethyl(3-pyridyl)borane (411.5 mg, 2.799 mmol) was added to a stirred solution of enol triflate **20**[22] (1 g, 2.153 mmol) in THF (10 mL) containing bis(triphenylphosphine)palladium(II) chloride (15.1 mg, 0.022 mmol). An aqueous solution of Na_2CO_3 (2 M, 4 mL) was then added, and the stirred mixture was heated at 80 °C for 1 h and then partitioned between Et_2O and H_2O. The organic phase was dried (Na_2CO_3), filtered through a short column of silica gel, and concentrated. Chromatography, on elution with Et_2O−light petroleum (2:1), afforded **21** (762 mg, 90%): mp 152−153 °C; ν_{max} 2937, 1729, 1599, 1562, 1370, 1250, 1150, 1132, 1080, 827 cm^{-1}; ^1H NMR δ 0.87 (s, 3, H-19), 1.00 (s, 3, H-18), 2.03 (s, 3, CH_3CO), 4.68 (m, 1, H-3α), 5.99 (m, 1, H-16), 7.24 (dd, 1, $J_{4,5} = 7.8$ Hz, $J_{5,6} = 5.0$ Hz, pyridyl H-5), 7.67 (d, 1, pyridyl H-4), 8.46 (d, 1, pyridyl H-6), 8.62 (s, 1, pyridyl H-2). Anal. ($C_{26}H_{35}NO_2$) C, H, N.

17-(3-Pyridyl)-5α-androst-16-en-3β-ol (19). To a solution of acetate **21** (400 mg, 1.016 mmol) in methanol (5 mL) was added an aqueous solution of NaOH (10% w/v, 1 mL). The mixture was stirred at 80 °C for 5 min and then allowed to cool, poured into water, and extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were dried (Na_2CO_3) and concentrated. Chromatography, on elution with Et_2O−CH_2Cl_2 (1:1), afforded **19** (300 mg, 84%): mp 215−216 °C; ν_{max} 3347, 2930, 2853, 1449, 1042, 799 cm^{-1}; ^1H NMR δ 0.90 (s, 3, H-19), 1.02 (s, 3, H-18), 3.62 (m, 1, H-3α), 5.99 (dd, 1, $J_{15,16} = 3.2$ Hz, $J_{15'16} = 1.8$ Hz, H-16), 7.23 (dd, 1, $J_{4,5} = 8.0$ Hz, $J_{5,6} = 4.7$ Hz, pyridyl H-5), 7.57 (dd, 1, $J_{2,4} = 1.9$ Hz, $J_{4,6} = 1.6$ Hz, pyridyl H-4), 8.46 (dd, 1, pyridyl H-6), 8.62 (d, 1, pyridyl H-2). Anal. ($C_{24}H_{33}NO$) C, H, N.

17β-(*N*-Oxo-3-pyridyl)-16,17α-epoxy-5α-androst-3β-ol (22). To a solution of alcohol **19** (100 mg, 0.284 mmol) in CH_2Cl_2 (10 mL) and methanol (3 mL) was added magnesium monoperoxyphthalate hexahydrate in one portion (703.6 mg, 1.138 mmol), and the resulting mixture was stirred at room temperature for 24 h. Water (50 mL) and CH_2Cl_2 (100 mL) were added, and the organic phase was separated, dried (Na_2SO_4), and concentrated. Chromatography, on elution with Et_2O−CH_2Cl_2−MeOH (1:4:0.2), afforded **22** (95 mg, 87%): mp 260−262 °C; ν_{max} 3369, 2962, 2850, 1429, 1042, 733, 682 cm^{-1}; ^1H NMR δ 0.79 (s, 3, H-18), 0.83 (s, 3, H-19), 3.60 (m, 1, H-16), 3.61 (m, 1, H-3α), 7.27 (m, 2, pyridyl H-4, H-5), 8.18 (m, 1, pyridyl H-6), 8.24 (m, 1, pyridyl H-2); HRMS calcd for $C_{24}H_{34}NO_3$ 384.2539 $(M + H)^+$, found 384.2530. Anal. ($C_{24}H_{33}NO_3$) C, H, N.

3β-Acetoxy-17β-(3-pyridyl)-16,17α-epoxy-5α-androstane (23). Dry CrO_3 (508 mg, 5.082 mmol) was added to a stirred solution of dry pyridine (804 mg, 822 μL, 10.164 mmol) in dry CH_2Cl_2 (15 mL) at 5 °C under argon. After the mixture was stirred at 5 °C for 1 h, acetate **21** (200 mg, 0.508 mmol) was added and stirring was continued at room temperature for 3 days; then the mixture was filtered and the solid washed with CH_2Cl_2. The filtrates were concentrated, and the residue was extracted several times with ether. The combined ether extracts were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated, and traces of pyridine removed by codistillation with toluene. Chromatography, on elution with Et_2O−light petroleum (2:1), afforded acetate **23** (115 mg, 55%): mp 166−170 °C; ν_{max} 2924, 2853, 1731, 1250, 1029, 718 cm^{-1}; ^1H NMR δ 0.74 (s, 3, H-18), 0.83 (s, 3, H-19), 2.02 (s, 3, CH_3CO), 3.62 (s, 1, H-16), 4.69 (m, 1, H-3α), 7.27 (dd, 1, $J_{4,5} = 7.8$ Hz, $J_{5,6} = 5.0$ Hz, pyridyl H-5), 7.70 (dt, 1, $J_{2,4} = J_{4,6} = 1.9$ Hz, pyridyl H-4), 8.55 (dd, 1, pyridyl H-6), 8.60 (d, 1, pyridyl H-2); HRMS calcd for $C_{26}H_{36}NO_3$ 410.2695 $(M + H)^+$, found 410.2690. Anal. ($C_{26}H_{35}NO_3$) H, N; C: calcd, 76.25; found, 75.53.

17β-(3-Pyridyl)-16,17α-epoxy-5α-androst-3β-ol (6). To a solution of acetate **23** (100 mg, 0.244 mmol) in methanol (2 mL) was added an aqueous solution of NaOH (10% w/v, 0.4 mL). The mixture was stirred at 80 °C for 5 min and then allowed to cool, poured into water, and extracted with CH_2Cl_2 (3 × 25 mL). The organic extracts were dried (Na_2CO_3) and concentrated. Chromatography, on elution with Et_2O−CH_2Cl_2 (3:1), afforded **6** (76 mg, 84%): mp 236−238 °C; ν_{max} 3338, 2978, 2930, 2856, 1451, 1057, 716 cm^{-1}; ^1H NMR δ 0.76 (s, 3, H-18), 0.83 (s, 3, H-19), 3.63 (m, 2, H-3α, H-16), 7.27 (m, 1, pyridyl H-5), 7.68 (m, 1, pyridyl H-4), 8.56 (m, 1, pyridyl H-6), 8.62 (m, 1, pyridyl H-2); HRMS calcd for $C_{24}H_{34}NO_2$ 368.2590 $(M + H)^+$, found 368.2595. Anal. ($C_{24}H_{33}NO_2$) C, H, N.

17β-(3-Pyridyl)-5α-androstan-3β-ol (24). To a solution of **19** (351 mg, 1 mmol) in ethanol (60 mL) were added hydrazine hydrate (1.6 mL, 5 mmol) and AcOH (1.0 mL). The mixture was heated at 80 °C for 24 h while a stream of air was passed through the solution. After cooling, the mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$, and the organic phase was dried (Na_2CO_3) and concentrated. Chromatography, on elution with MeOH−CH_2Cl_2 (1:50), afforded **24** (300 mg, 85%): mp 226−228 °C; ν_{max} 3361, 2926, 2845, 1449, 1378, 1081, 1045, 716 cm^{-1}; ^1H NMR δ 0.47 (s, 3, H-18), 0.81 (s, 3, H-19), 2.67 (t, 1, $J = 9.6$ Hz, H-17α), 3.62 (m, 1, H-3α), 7.22 (dd, 1, $J_{4,5} = 7.7$ Hz, $J_{5,6} = 4.8$ Hz, pyridyl H-5), 7.50 (d, 1, pyridyl H-4), 8.39 (m, 2, pyridyl H-2, H-6). Anal. ($C_{24}H_{35}NO$) C, H, N.

Enzyme Preparation and Assay Procedures for the 17α-Hydroxylase Activity of Human Cytochrome $P450_{17\alpha}$. The microsomal fraction from human testes was prepared as described elsewhere.[1] The standard assay with progesterone as substrate was also as described therein. To test the reversibility of the inhibition, the compounds were incubated with the enzyme for 30 min at 37 °C before being dialyzed against 250 volumes of 50 mM sodium phosphate buffer (pH 7.4) containing 0.137 mM dithiothreitol, 0.274 mM EDTA, 1.37% DMSO, and 3.6% glycerol. After 5 and 24 h, samples were removed and warmed at 37 °C for 5−10 min,

before the assay was started by the addition of [^{3}H]progesterone, NADPH, and its regenerating system. The final assay mixture was the same as in the standard assays.

For the studies on the type of inhibition shown by **1** and **5**, the assays used the substrate pregnenolone at concentrations up to 3 μM with specific activity of 3−5 mCi/μmol. The rest of the assay mixture was the same as in the standard assays. The cold mix added to stop the reaction contained pregnenolone, 17α-hydroxypregnenolone, and dehydroepiandrosterone. The HPLC separation used a 15-cm 5-μm Apex C1 column with an Uptight guard column packed with 75−125 μm of LC-Porosil silica (Waters Assoc.). The mobile phase was 52% methanol at a flow rate of 1.4 mL/min. The effluent was monitored at 214 nm before being mixed with Ecoscint A (National Diagnostics Ltd.) containing 25% acetonitrile flowing at 1.1 mL/min and monitored for ^{3}H using a Berthold LB506C detector. Activity was measured as the production of 17α-hydroxypregnenolone. No dehydroepiandrosterone nor androst-5-ene-3β,17β-diol were formed, but two unidentified peaks of long retention time were eluted.

The K_m for pregnenolone was calculated by fitting by nonlinear regression the initial rates at varying substrate concentrations to the Michaelis−Menton equation and was 23 ± 1.7 nM (mean ± SE from four determinations). IC_{50} values were calculated as before.[1]

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