Esters of 3-Pyridylacetic Acid That Combine Potent Inhibition of 17α-Hydroxylase/$C_{17,20}$-Lyase (Cytochrome P45017α) with Resistance to Esterase Hydrolysis

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Esters of 3- and 4-pyridylacetic acid have been prepared and tested for inhibitory activity toward the human testicular 17α-hydroxylase/$C_{17,20}$-lyase and human placental aromatase enzymes. The structural features required for optimal inhibition of the hydroxylase/lyase enzyme were a 3-pyridine ring, methyl substitution α to the carbonyl group, and a bulky alkoxycarbonyl substituent. The compounds with the greatest selectivity were isopinocampheyl 2-methyl-2-(3-pyridyl)propanoate, **9**, 1-adamantyl 2-methyl-2-(3-pyridyl)propanoate, **12**, and 2-methyl-2-adamantyl 2-methyl-2-(3-pyridyl)propanoate, **14**, which, while inhibiting the aromatase activity with IC_{50} values of 30, 35, and 40 μM, respectively, exhibited IC_{50} values toward hydroxylase/lyase of between 13 and 90 nM. For comparison, ketoconazole gave an IC_{50} value of 15 μM against aromatase and values of 65 and 26 nM for inhibition of the hydroxylase and lyase activities, respectively. Some of the structural features required for enzyme inhibition also conferred resistance to esterase hydrolysis, *in vitro* using rat liver microsomes as a source of the esterase activity. Therefore these esters are lead compounds in the development of inhibitors of androgen biosynthesis for the treatment of hormone-dependent prostatic cancer.

Carcinoma of the prostate is one of the most prevalent causes of cancer death in Western males. A high proportion of these tumors are dependent upon androgens for growth and respond to ablative or therapeutic measures designed to deplete circulating androgens. Most of the circulating androgens are derived from the testes and can be eliminated by treatments such as orchiectomy or gonadotrophin-releasing hormone agonists. However, adrenal androgens may continue to stimulate tumor growth, and therefore a more effective therapeutic result might be produced by abolishing adrenal as well as testicular androgen synthesis. The 17α-hydroxylase/$C_{17,20}$-lyase enzyme catalyzes the conversion of the C_{21} steroids pregnenolone and progesterone into the C_{19} steroids dehydroepiandrosterone and androstenedione, respectively; the latter is then converted into testosterone, the major circulating androgen. This cytochrome P450 enzyme (cytochrome P45017α, the product of the *CYP17* gene) is located in both testicular and adrenal tissue.[1] A single microsomal enzyme catalyzes the two consecutive oxidation reactions, namely, the 17α-hydroxylation of the C_{21} steroids and the cleavage of the C_{17-20} bond of the 17α-hydroxylated intermediates to produce the C_{19} steroids. Some inhibitors of this enzyme have been identified.[4-6,21,22]

The imidazole-based drug ketoconazole was originally developed as an antifungal agent, but in high doses the drug was observed to cause a decrease in androgen biosynthesis due to blockade of the hydroxylase/lyase enzyme.[7-9] This led to its clinical application in treating advanced prostate cancer; however, it has now been withdrawn from this use due to undesirable side effects, which are thought to be due to its poor selectivity.[10,11] In the treatment of prostate cancer it is desirable that a compound should be a strong inhibitor of only the 17α-hydroxylase/$C_{17,20}$-lyase enzyme and not of other cytochrome P450-dependent steroidogenic enzymes, such as 11β-hydroxylase, 18-hydroxylase, cholesterol side chain cleavage, or 21-hydroxylase. Inhibition of these enzymes would block the production of glucocorticosteroids and mineralocorticoids. Although the conversion of androgens into estrogens by the aromatase enzyme represents a minor pathway in males, aromatization does play a major role in regulation of negative feedback between the gonad–hypothalamic–pituitary axis. The blockade of androgen synthesis leads to the reduction of estrogen levels, which in turn produces an elevation in the gonadotrophins that drives androgen production from the gonads.[12]

As part of a program to develop new agents to treat prostate cancer, we have previously reported that esters of 4-pyridylacetic acid displayed inhibitory activity toward the hydroxylase/lyase enzyme.[13] However, the need to increase both the potency and selectivity of the hydroxylase/lyase inhibitors remained. Therefore we have undertaken two synthetic programs. First, building on the information in the earlier study,[13] we synthesized derivatives of the 4-pyridyl ester of isopinocampheol with alkyl substitutions at the position α to the ester carbonyl group. Second, our previous finding that the 3-pyridyl analogue of rogletimide [3-ethyl-3-(4-pyridyl)piperidine-2,6-dione] was not an inhibitor of aromatase[14] prompted the investigation of the esters of 3-pyridylacetic acid in the hope that they might share this property and provide some interesting lead compounds. This present study identifies a number of 3-pyridylacetic acid esters as potent inhibitors of human 17α-hydroxylase/$C_{17,20}$-lyase with significantly diminished activity toward aromatase. The structures

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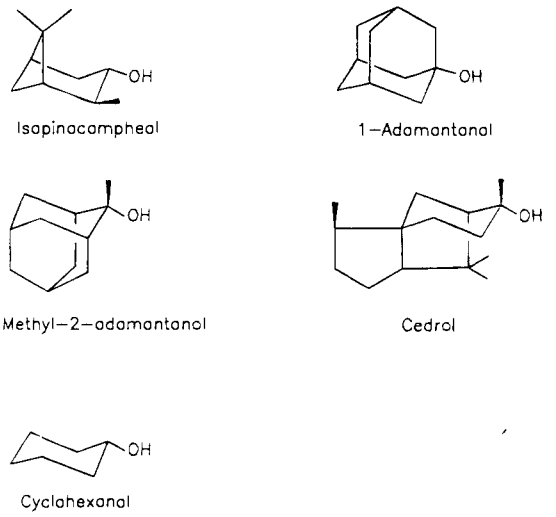


Figure 1. Structures of carbocyclic alcohols used in this study.

of all the carbocyclic alcohols from which the esters referred to in this study were prepared are shown in Figure 1.

Results and Discussion

Synthesis. The general procedure for the synthesis of the new α-unsubstituted esters of 4-pyridylacetic acid (**6**) and 3-pyridylacetic acid (**7**, **10**, **13**, **15**) was that previously reported for esters of 4-pyridylacetic acid,[13] namely, transesterification between methyl 3-pyridylacetate and the appropriate alcohol, by the method of Meth-Cohn.[15] This methodology, involving an *n*-butyllithium-mediated ester exchange, was originally between a methyl ester and the appropriate alcohol. As in our earlier synthesis of 1-adamantyl 4-pyridylacetate in 11% yield by this method, the yields of present products (**6**, **10**, **13**, **15**) from tertiary alcohols were modest, ranging from 24% to 38%. Esters of 2-(3-pyridyl)propanoic acid (**8**, **11**) were prepared by careful monoalkylation of corresponding the α-unsubstituted esters **7** and **10**, respectively, as was 2-(4-pyridyl)-butanoate ester **4** from **1**. The α-methylated counterpart of **4**, isopinocampheyl 2-(4-pyridyl)propanoate (**2**), had been previously prepared by a procedure which avoids dialkylated products, namely, a Meth-Cohn type transesterification of the 5,5-disubstituted Meldrum's acid derivative 2,2,5-trimethyl-5-(4-pyridyl)-4,6-dioxo-1,3-dioxane with isopinocampheol.[16] Alkylation of **7**, **10**, and **15** with an excess of methyl iodide afforded the 2-methyl-2-(3-pyridyl)propanoate esters **9**, **12**, and **16** and of **1** with ethyl iodide the 2-ethyl-2-(4-pyridyl)-butanoate ester **5**.

Comparison of Inhibitory Activity toward Human and Rat Enzymes. The hydroxylase assay quantifies the conversion of tritiated progesterone into 17α-hydroxyprogesterone, while the subsequent lyase step is assayed by the determination of the production of radiolabeled testosterone and androstenedione. Previously, the compounds were tested using the microsomal fraction from rat testes as a source of the enzyme.[13] In the present study microsomal fraction from human testes obtained from previously untreated patients undergoing orchiectomy for prostate cancer was used. A comparison was made between the inhibitory activity, expressed as IC_{50}, displayed by three of the pyridyl esters and ketoconazole toward the enzyme located in rat and human testicular microsomes. The results are shown in Table 1. Ketoconazole is a relatively weak inhibitor of the rat hydroxylase and lyase activities (IC_{50} values of 6 and 11 μM, respectively) compared to all three esters assayed, which ranged in potency from 47 to 520 nM. With the human enzyme, ketoconazole has IC_{50} values of 65 and 26 nM, and the pyridyl esters **1** and **9** were of equal or greater potency but **7** was less active. Owing to the difference in the structure–activity pattern and the relevance to the clinical situation, all further assays were carried out using the human enzyme.

Selectivity toward Hydroxylase/Lyase Compared with Aromatase. In addition to the hydroxylase/lyase assays, all the esters were tested for inhibition toward the aromatase enzyme located in the microsomes from human placenta. The results of both assays on the previously synthesized isopinocampheyl esters **1** and **2** and the 4-pyridyl esters synthesized in this study are shown in Table 2. The isopinocamphenyl 4-pyridyl ester **1** has IC_{50} values of 14 nM for the hydroxylase step and 5 nM for the lyase; despite this potency, it lacks selectivity, having an IC_{50} of 120 nM for aromatase. Substitution of a methyl group onto the position α to the carbonyl group of **1** to give **2** caused a 10-fold decrease in aromatase inhibitory activity but comparable inhibition of the hydroxylase/lyase enzyme. The inclusion of a further methyl group to give the α,α-dimethylated ester **3** resulted in a decrease in activity toward both enzymes.

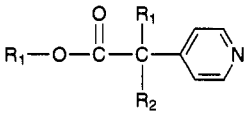
For inhibition of the hydroxylase/lyase enzyme, these results are similar to those previously found with the cyclohexyl 4-pyridyl ester series and the rat enzyme.[13] That the α,α-dimethylated ester showed a decrease in activity when compared with the corresponding racemic mix of α-monomethylated esters suggests that one of the two methyl groups must be so orientated as to disfavor binding to the enzyme, from which it follows that only one of the two enantiomers of the α-monomethylated esters should favorably overlay the steroid substrate molecule. In the molecular modeling studies[17] (Figure 2), the cyclohexyl group of the ester overlays the B ring of the natural substrate and the 4-pyridyl

Table 1. Comparison of Inhibitory Activities of Esters and Ketoconazole against Rat and Human Testicular 17α-Hydroxylase/$C_{17,20}$-Lyase[a]

	rat IC_{50} (nM)		human IC_{50} (nM)	
compd	17α-hydroxylase	$C_{17,20}$-lyase	17α-hydroxylase	$C_{17,20}$-lyase
1	260	280	14	5
7	460	520	260	88
9	47	52	29	14
ketoconazole	6 μM	11 μM	65	26

[a] The standard errors were usually <10% of the IC_{50} values. The substrate concentration for both the hydroxylase and lyase assays was 3 μM.

Table 2. Inhibition of Human Placental Aromatase and Human Testicular 17α-Hydroxylase/$C_{17,20}$-Lyase by 4-Pyridyl Esters[a]



compd	R_1	R_2	R_3	IC_{50} values: 17α-hydroxylase (nM)	$C_{17,20}$-lyase (nM)	aromatase (μM)
1	isopinocampheyl	H	H	14	5	0.12
2	isopinocampheyl	Me	H	19	6	1.32
3	isopinocampheyl	Me	Me	26	10	3.85
4	isopinocampheyl	Et	H	34	9	3.96
5	isopinocampheyl	Et	Et	140	35	55.00
6	cedryl	H	H	270	52	0.44

[a] The standard errors were <8%, with an average of 5.6% of the IC_{50} values. A substrate concentration of 3 μM was used for the hydroxylase and lyase assays and 0.38 μM for the aromatase assays.

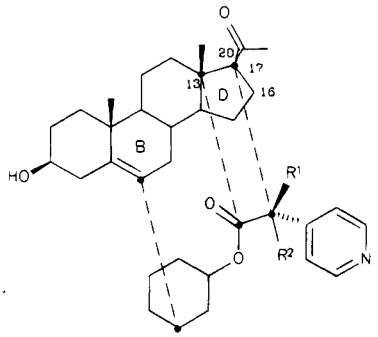


Figure 2. Overlay of cyclohexyl 4-pyridylacetate onto pregnenolone.

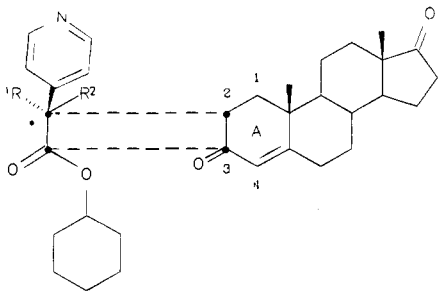
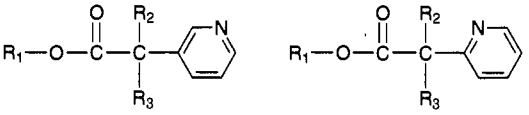


Figure 3. Overlay of cyclohexyl 4-pyridylacetate onto androstenedione.

group extends from the 17α-position, below the plane of the steroid. This mode of overlap places the carbonyl groups of the steroid and inhibitor almost parallel to each other, and both could interact with a hydrogen bond donor grouping in the active site and the α-methyl group to overlay either the C(16)- or C(20)-position of the natural steroid substrate. Synthesis of the individual enantiomers would clarify which position is favored. For aromatase inhibition, the esters would overlay the natural substrate (androstenedione) so that the ester carbonyl group maps onto the steroid C(3) carbonyl function (Figure 3). The ring system would occupy a hydrophobic pocket hypothesized as extending from the C(4)-position. In this mode of overlap, the 4-pyridyl group is positioned above the β-face of the steroid A ring in the vicinity of C(19). With the α-monomethylated esters, the chiral α-carbon atom adjacent to the carbonyl group occupies a spatial position mapping onto that of the C(2) atom of androstenedione. In one enantiomer, the methyl group maps onto C(1), whereas in the other it extends outward from C(2) into a region which is thought to be intolerant to steric bulk, and this could explain the decrease in aromatase inhibitory activity observed for the racemic α-methylated esters. The importance of the steric constraints at the C(2)-position and the hydrophobic pocket at the C(4)-position of androstenedione has been recognized by analysis of inhibitors[18,19] and site-directed mutagenesis.[20] Substitution of an ethyl group for the α-methyl to give compound **4** produced similar inhibitory results to the dimethylated ester with both enzymes. The α,α-diethylated analogue **5** showed a further decrease in potency and was the poorest inhibitor of aromatase in this series. This provides further evidence that, on the basis of the proposed binding mode for aromatase inhibition, the position occupied by the C(2) of the steroid substrate is intolerant to steric bulk.

The results in Table 3 refer to the 3-pyridyl ester series. The isopinocampheyl esters of 3-pyridylacetic acid (compounds **7**–**9**) were synthesized, and it was discovered that activity against aromatase was significantly diminished compared with the 4-pyridyl series. A further reduction in aromatase inhibitory potency could be achieved by introduction of greater bulk into the alkoxy moiety as demonstrated by the substitution with adamantyl (**10**–**12**) and 2-methyl-2-adamantyl (**13**, **14**). However, this was not an inevitable consequence of increased steric bulk since the cedryl analogue **15** possesses increased inhibition of aromatase activity. Interestingly, in contrast to the 4-pyridyl ester series, the α,α-dimethylated esters in the 3-pyridyl series were generally even better inhibitors of hydroxylase/lyase than their corresponding racemic α-monomethylated counterparts and much better than the unmethylated compounds. This is illustrated by comparison of the IC_{50} values for the isopinocampheyl compounds **7**–**9**. A similar feature is observed for the adamantyl (**11**, **12**) and 2-methyl-2-adamantyl (**13**, **14**) analogues. These results suggest that the binding mode of the 3-pyridyl esters differs from that of the corresponding 4-pyridyl esters. From the structure–activity results, three analogues combine the requirements of potent inhibition of the target enzyme and selectivity compared with aromatase. These are the 3-pyridyl α,α-dimethylated esters of isopinocampheol (**9**), adamantanol (**12**), and 2-methyl-2-adamantanol (**14**). All have IC_{50} values toward hydroxylase/lyase between 13 and 90 nM, while for aromatase the IC_{50} values range from 30 to 40 μM. An interesting exception to this general trend of increased activity for the α,α-dimethylated derivatives is the marked reduction in inhibitory potency toward hydroxylase/lyase of the α-disubstituted cedryl ester **16**

Table 3. Inhibition of Human Placental Aromatase and Human Testicular 17α-Hydroxylase/$C_{17,20}$-Lyase by 3-Pyridyl Esters **7**–**16** and the 2-Pyridyl Ester **17**[a]



7-16 **17**

compd	R_1	R_2	R_3	IC_{50} values: 17α-hydroxylase (nM)	IC_{50} values: $C_{17,20}$-lyase (nM)	IC_{50} values: aromatase (μM)
7	isopinocampheyl	H	H	260	88	5.6
8	isopinocampheyl	Me	H	82	14	8.9
9	isopinocampheyl	Me	Me	29	15	30.0
10	1-adamantyl	H	H	930	130	8.9
11	1-adamantyl	Me	H	220	35	9.1
12	1-adamantyl	Me	Me	90	13	35.0
13	2-methyl-2-adamantyl	H	H	1900	320	10.8
14	2-methyl-2-adamantyl	Me	Me	75	13	40.0
15	cedryl	H	H	230	38	1.7
16	cedryl	Me	Me	4000	670	80.5
17	isopinocampheyl	H	H	>1000	>1000	>20.0

[a] The standard errors were <13%, with an average of 7.5% of the IC_{50} value. A substrate concentration of 3 μM was used for the hydroxylase and lyase assays and 0.38 μM for the aromatase assays.

Table 4. Comparative Hydrolysis Rates of Pyridyl Esters by Rat Liver Microsomal Esterase

compd	hydrolysis rate $t_{1/2}$ (min)
1	17.5
2	100
3	nd[a]
4	88
5	nd
7	16
8	50
9	nd
13	nd
14	nd
15	nd

[a] nd = none detected, which means no hydrolysis was observed after 60 min of incubation.

compared with the α-unsubstituted compound **15**. It is likely that a steric clash between the two methyl substituents in the cedryl residue and those in the 2-methyl-2-(3-pyridyl)propanoate moiety prevents the molecule from adopting a conformation favorable for binding.

Finally, the improved selectivity and retained activity, in transferring from the 4-pyridyl to the 3-pyridyl series, prompt the question of whether 2-pyridyl derivatives might also be active. Only one such compound was made, isopinocampheyl 2-pyridylacetate (**17**), and this was inactive (Table 3).

Resistance to Esterase Activity. All the compounds described are esters and therefore expected to be susceptible to hydrolysis by the esterase enzymes which are found in many tissues. This would reduce the effectiveness of the compounds *in vivo*. Previously, we demonstrated that methylation on the carbon atom adjacent to the ester bond gave some protection to the 4-pyridylcyclohexyl esters from esterase attack.[13] Therefore a rat liver microsomal preparation was utilized as a source of esterase activity in order to assess the lability of some of the compounds synthesized in this present study. The hydrolysis rates, expressed as half-lives, are shown in Table 4. The unsubstituted 4-pyridyl isopinocampheyl ester **1** is rapidly hydrolyzed by rat liver esterase. The introduction of a methyl group α to the ester bond improves the resistance to hydrolysis over 5-fold, and disubstitution to give **3** has an even greater effect. Substitution with an ethyl group, **4**, produces a similar hydrolysis rate to that of the monomethylated compound. Therefore slightly increasing the size of the alkyl group does not give increased resistance. Relocation of the pyridyl nitrogen had little effect, so hydrolysis rates for 3-pyridyl derivatives were similar to those of their 4-pyridyl counterparts. Thus in the 3-pyridyl isopinocampheyl series, namely, compounds **7**–**9**, the effect of increased α-substitution on the half-life of hydrolysis is similar to that in the 4-pyridyl series. The 3-pyridyl derivatives **13**–**15** were tested in order to assess the influence of bulky alcoholic moieties, e.g., 2-methyl-2-adamantanol and cedryl, on the esterase liability. For all three compounds, no hydrolysis was observed after 60 min of incubation. Therefore, in conclusion, the structural features that confer resistance to hydrolysis by the esterase in rat liver microsomes are alkyl substitution α to the carbonyl group and the presence of bulky alkoxy moieties with an angular methyl group adjacent to the ester oxygen atom.

Conclusions

We have described a number of 3- and 4-pyridylacetic acid esters that are potent inhibitors of human testicular 17α-hydroxylase/$C_{17,20}$-lyase *in vitro* with minimal activity toward human placental aromatase. The lead compounds, namely, **9**, **12**, and **14**, are of a similar order of potency against the hydroxylase/lyase enzyme as ketoconazole. Other reported inhibitors include 4-pregnen-3-one-20β-carboxaldehyde oxime, which is comparable in potency to ketoconazole but also exhibits inhibition of the testosterone 5α-reductase enzyme (K_i = 16 nM)[6] and 17β-(cyclopropylamino)androst-5-en-3β-ol with a K_i of 90 nM toward the hydroxylase/lyase enzyme.[5] Potent activity toward the rat enzyme was displayed by 17β-ureido-substituted steroids.[21] Our group has identified 17-(3 pyridyl)androsta-5,16-dien-3β-ol as a particularly potent inhibitor of the human hydroxylase/lyase enzyme in testicular microsomes with K_i < 1 nM.[22] This compound markedly suppressed testosterone levels in the mouse despite the reflex rise in luteinizing hormone which would have stimulated

androgen biosynthesis. In addition, adrenal weights were unchanged indicating no inhibition of corticosterone production.[23] Future experiments on the lead compounds identified in this present study will include determination of their activity toward androgen levels and adrenal weights in the mouse as a measure of *in vivo* potency and selectivity toward other cytochrome P450-dependent enzymes.

The structural features of the pyridylacetic acid esters responsible for optimal inhibition of the hydroxylase/lyase complex compared with the aromatase enzyme were identified as a 3-pyridine ring, methyl substitution α to the carbonyl group, and a bulky alkoxy substituent. In addition some of these features conferred resistance to *in vitro* esterase hydrolysis, using a rat liver microsomal preparation. Inhibitors of the hydroxylase/lyase complex may be useful in the treatment of androgen-dependent prostate cancer. We have identified the 3-pyridylacetic acid esters **9**, **12**, and **14** as potent inhibitors of this enzyme and worthy of further investigation.

Experimental Section

Chemical Methods. Standard Procedures. ^{1}H NMR spectra (250 MHz, $CDCL_3$) were recorded on a Bruker AC250 spectrometer. Mass spectra (electron impact, 70 eV) were obtained by direct insertion with a VG 7070H spectrometer and VG 2235 data system. Chromatography refers to column chromatography on silica gel (Merck Art. 15111) with the eluent indicated applied at a positive pressure of 0.5 atm. Melting points were determined with a Reichert hot stage and are uncorrected. Light petroleum refers to the fraction of bp 60–80 °C. The preparation of esters of 4-pyridylacetic acid, **1** and **2**, were as previously described.[13]

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-Methyl-2-(4-pyridyl)propanoate (3). A solution of **1**[13] (797 mg, 2.92 mmol) in THF (4 mL) was added to a stirred suspension of KH (35% by weight dispersion in oil, 736 mg, 6.42 mmol) in THF (10 mL) under Ar at 0 °C. After 10 min, MeI (0.37 mL, 5.84 mmol) was added, and after 1 h at 20 °C, the mixture was partitioned between Et_2O and H_2O. The organic extracts were dried ($MgSO_4$) and concentrated under vacuum to give the crude product which after chromatography with 50:50:1 light petroleum–Et_2O–Et_3N afforded **3** (669 mg, 76%) as an oil: ^{1}H NMR δ_H 0.05–2.57 (m, 7, isopinocampheyl ring H), 0.95 and 1.20 (2s, 6, isopinocampheyl CMe_2), 1.06 (d, 3, J = 7.4 Hz, isopinocampheyl $CHCH_3$), 1.57 (s, 6, $COCMe_2$), 5.05 (m, 1, isopinocampheyl OCH), 7.25 (d, 2, J = 6.3 Hz, pyridyl H-3, H-5), 8.56 (d, 2, pyridyl H-2, H-6). Anal. ($C_{19}H_{27}NO_2$) C, H, N.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-(4-Pyridyl)butanoate (4). The foregoing procedure was carried out with **1** (912 mg, 3.34 mmol) in THF (4 mL), KH (35% by weight dispersion in oil, 383 mg, 3.34 mmol) in THF (10 mL), and EtI (0.21 mL, 2.68 mmol) to give **4** (770 mg, 77%) as an oil: ^{1}H NMR δ_H 0.77 (t, 3, J = 7.3 Hz, CH_2CH_3), 0.94–2.62 (m, 7, isopinocampheyl CH), 0.94 and 1.21 (2s, 6, isopinocampheyl CMe_2), 1.08 (d, 3, J = 7.4 Hz, isopinocampheyl OCH), 7.26 (d, 2, J = 6.0 Hz, pyridyl H-3, H-5), 8.50 (d, 2, J = 5.7 Hz, pyridyl H-2, H-6). Anal. ($C_{19}H_{27}NO_2$) C, H, N.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-Ethyl-2-(4-pyridyl)butanoate (5). The method essentially followed that described for **4**, using isopinocampheyl 4-pyridylacetate (797 mg, 2.92 mmol) in THF (4 mL), KH (35% weight dispersion in oil, 736 mg, 6.42 mmol) in THF (10 mL), and EtI (0.47 mL, 5.84 mmol). Chromatography on elution with 75:50:1 light petroleum–Et_2O–Et_3N gave **5** (769 mg, 80%) as an oil: ^{1}H NMR δ_H 0.77 (t, 6, J = 7.4 Hz, 2 CH_2CH_3), 0.95 and 1.20 (2s, 6, isopinocampheyl CMe_2), 0.96–2.60 (m, 7, isopinocampheyl CH), 1.03 (d, 3, J = 7.4 Hz, isopinocampheyl $CHCH_3$), 2.06 (q, 4, J = 7.4 Hz, 2 CH_2CH_3), 5.04 (m, 1, isopinocampheyl OCH), 7.20 (d, 2, J = 6.3 Hz, pyridyl H-3, H-5), 8.55 (d, 2, J = 6.3 Hz, pyridyl H-2, H-6). Anal. ($C_{21}H_{31}NO_2$) C, H, N.

(1*S*,2*R*,5*S*,8*R*)-Cedryl 4-Pyridylacetate (6). To a stirred solution of (+)-cedrol (489 mg, 2.2 mmol) in dry THF (7.5 mL) at −15 °C were added dropwise *n*-BuLi (2.5 M, 0.88 mL, 2.2 mmol) in hexane and then methyl 4-pyridylacetate (302 mg, 2.0 mmol) in THF (5 mL). After allowing the reaction mixture to attain room temperature, stirring was maintained for an additional 24 h; then the mixture was partitioned between Et_2O and H_2O, and the organic phase was concentrated. Chromatography, eluting with 250:50:1 light petroleum–Et_2O–Et_3N, gave **6** together with unreacted cedrol. On passing HCl gas through a solution of the crude product in Et_2O, the hydrochloride of **6** precipitated as white crystals (100 mg, 13%) from which **6** was reliberated as an oil: ^{1}H NMR δ_H 0.83 (d, 3, J = 7.2 Hz, cedryl CH*Me*), 0.96 and 1.09 (2s, 6, cedryl CMe_2), 1.52 (s, 3, cedryl OCMe), 3.53 (s, 2, $COCH_2$), 7.21 (d, 2, J = 5.7 Hz, pyridyl H-3, H-5), 8.54 (d, 2, pyridyl H-2, H-6); MS *m/z* 341 (M^+). Anal. ($C_{22}H_{31}NO_2 \cdot 0.5H_2O$) H, N.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 3-Pyridylacetate (7). A stirred solution of (+)-isopinocampheol (3.086 g, 20 mmol) in dry THF (20 mL) under N_2 was cooled with an ice–salt bath. A solution of *n*-BuLi (1.6 M, 12.5 mL, 20 mmol) in hexane was added followed, after 5 min, by a solution of ethyl 3-pyridylacetate (2.746 g, 16.7 mmol) in THF (5 mL) and the clear yellow solution allowed to attain room temperature. After 4 h, the mixture was partitioned between Et_2O and H_2O, and the organic extracts were concentrated. Chromatography of the residue gave on elution with 50:50:1 light petroleum–Et_2O–Et_3N **7** (3.70 g, 76%) as an oil. On passing HCl gas through a solution of **7** in Et_2O, the hydrochloride was obtained. This was recrystallized from dioxane–Et_2O, 1:1:mp 158–160 °C. Anal. ($C_{17}H_{21}NO_2Cl$) C, H, N. Cl.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-(3-Pyridyl)propanoate (8). A solution of **7** (free base) (912 mg, 3.34 mmol) in dry THF (3 mL) was added to a stirred suspension of KH (35% by weight dispersion in oil, 383 mg, 3.34 mmol) in THF (10 mL) under N_2 at 0 °C. After 10 min, MeI (0.17 mL, 380 mg, 2.68 mmol) was added, and after 1 h at 20 °C, the mixture was treated as for **7** above. Chromatography with 5:4 Et_2O–light petroleum gave **8** (345 mg, 36%) as an oil: ^{1}H NMR δ_H 0.97, 1.20 (2s, 6, Me_2C), 1.53 (d, 3, J = 6.8 Hz, $COCHCH_3$), 3.75 (q, 3, $COCHCH_3$), 5.10 (m, 1, OCH), 7.30 (dd, 1, J = 4.8, 7.95 Hz, pyridyl H-5), 7.80 (m, 1, pyridyl H-4), 8.60 (m, 2, pyridyl H-2, H-6). Anal. ($C_{18}H_{24}NO_2$) C, H, N.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-Methyl-2-(3-pyridyl)propanoate (9). A solution of **7** (706 mg, 2.58 mmol) in dry THF (8 mL) was added to a stirred suspension of KH (35% by weight dispersion in oil, 650 mg, 5.68 mmol) in THF (6 mL) under Ar at 0 °C. After 10 min, MeI (733 mg, 5.16 mmol) was added in two equal portions, each in THF (2 mL). On addition of 1 equiv, the mixture became cloudy and H_2 evolved. On adding the second equivalent, the yellow solution turned colorless. After 20 min, the reaction was quenched by addition of *i*-PrOH (0.5 mL). Extraction as for **7** above with chromatography in the same eluent gave **9** (539 mg, 69%) as a colorless oil which similarly gave a crystalline hydrochloride: mp 144–146 °C. Anal. ($C_{19}H_{27}NO_2Cl$) C, H, N, Cl.

1-Adamantyl 3-Pyridylacetate (10). The method essentially followed that described for **7**, using 1-adamantanol (3.35 g, 22 mmol) in dry THF (20 mL), *n*-BuLi (1.6 M, 12.5 mL, 20 mmol) in hexane, and methyl 3-pyridylacetate (3.02 g, 20 mmol) in THF (8 mL). After allowing the reaction mixture to attain room temperature, it was heated under reflux for 18 h. The product obtained following extraction and chromatography, as described for **4** above, contained unreacted 1-adamantanol. It was further purified by conversion to the hydrochloride, from which pure **10** (1.30 g, 24%) was reliberated and crystallized from hexane: mp 71–72 °C; ^{1}H NMR δ_H 1.70 and 2.08 (2s, 12, adamantyl CH_2), 2.14 (s, 3, adamantyl CH), 3.54 (s, 2, $COCH_2$), 7.27 (dd, 1, pyridyl H-5), 7.66 (m, 1, pyridyl H-4), 8.50 (m, 2, pyridyl H-2, H-6). Anal. ($C_{17}H_{21}NO_2$) C, H, N.

1-Adamantyl 2-(3-Pyridyl)propanoate (11). The method essentially followed that described for **8**, using 1-adamantyl 3-pyridylacetate (542 mg, 2.0 mmol) in dry THF (2 mL), KH (35% by weight dispersion in oil, 229 mg, 2.0 mmol) in THF (6 mL), and MeI (0.10 mL, 1.6 mmol). Chromatography, upon

elution with 50:50:1 light petroleum–Et_2O–Et_3N, gave **11** (143 g, 25%) as an oil: ^1H NMR δ_H 1.47 (d, 3, J = 7.2 Hz, CH*Me*), 7.26 (m, 1, pyridyl H-5), 7.66 (m, 1, pyridyl H-4), 8.52 (m, 2, pyridyl H-2, H-6). Anal. ($C_{18}H_{23}NO_2$) C, H, N.

1-Adamantyl 2-Methyl-2-(3-pyridyl)propanoate (12). The method essential followed that described for **9**, using 1-adamantyl 3-pyridylacetate (542 mg, 2.0 mmol) in dry THF (2 mL), KH (35% by weight dispersion in oil, 504 mg, 4.4 mmol) in THF (6 mL), and methyl iodide (0.25 mL, 4.0 mmol). Chromatography upon elution with 50:50:1 light petroleum–Et_2O–Et_3N afforded **12** (262 mg, 39%) as an oil: ^1H NMR δ_H 1.56 (s, 6, CMe_2), 1.63 and 2.03 (2s, 12, adamantyl CH_2), 2.13 (s, 3, adamantyl CH), 7.26 (m, 1, pyridyl H-5), 7.65 (m, 1, pyridyl H-4), 8.47 (m, 1, pyridyl H-2 or H-6), 8.62 (m, 1, pyridyl H-2 or H-6). Anal. ($C_{19}H_{26}NO_2$) C, H, N.

2-Methyl-2-adamantyl 3-Pyridylacetate (13). The method essentially followed that described for **7**, but using 2-methyl-2-adamantanol (3.66 g, 22 mmol) in dry THF (30 mL), *n*-BuLi (2.5 M, 8.8 mL, 22 mmol) in hexane, and methyl 3-pyridylacetate (3.02 g, 20 mmol) in THF (10 mL). After allowing the reaction mixture to attain room temperature, stirring was maintained for an additional 96 h. Following workup and chromatography, eluting with 250:50:1 light petroleum–Et_2O–Et_3N, the product obtained contained some unreacted 2-methyl-2-adamantanol. It was further purified by forming the hydrochloride and reliberating **13** (1.77 g, 31%) as an oil: ^1H NMR δ_H 1.59 (s, 3, adamantyl OCMe), 3.60 (s, 2, $COCH_2$), 7.26 (dd, 1, pyridyl H-5), 7.65 (ddd, 1, pyridyl H-4), 8.52 (m, 2, pyridyl H-2, H-6); FAB-MS *m/z* 286 (M + 1).

2-Methyl-2-adamantyl 2-Methyl-2-(3-pyridyl)propanoate (14). The method essentially followed that described for **12**, using 2-methyl-2-adamantyl 3-pyridylacetate (571 mg, 2.0 mmol) in dry THF (2 mL), KH (35% by weight dispersion in oil, 504 mg, 4.4 mmol) in THF (6 mL), and MeI (0.25 mL, 4.0 mmol). Chromatography upon elution with 3:1 light petroleum–Et_2O afforded **14** (494 mg, 83%) as an oil: ^1H NMR δ_H 1.53 (s, 3, adamantyl OCMe), 1.62 (s, 6, CMe_2), 7.25 (dd, 1, pyridyl H-5), 7.70 (ddd, 1, pyridyl H-4), 8.48 (dd, 2, pyridyl H-6), 8.67 (d, 1, pyridyl H-2); FAB-MS *m/z* 314 (M + 1). Anal. ($C_{20}H_{27}NO_2$) C, H, N.

(1*S*,2*R*,5*S*,8*R*)-Cedryl 3-Pyridylacetate (15). The method essentially followed that described in **6**, using (+)-cedrol (2.45 g, 11 mmol), *n*-BuLi (2.5 M, 4.4 mL, 11 mmol) in hexane, and methyl 3-pyridylacetate (1.51 g, 10 mmol). The product was purified via the hydrochloride, which was reconverted to **15** (1.30 g, 38%), an oil: ^1H NMR δ_H (cedryl signals as for **6**), 7.26 (dd, 1, pyridyl H-5), 7.63 (ddd, 1, pyridyl H-4), 8.51 (m, 2, pyridyl H-2, H-6). Anal. ($C_{22}H_{31}NO_2$) C, H, N.

(1*S*,2*R*,5*S*,8*R*)-Cedryl 2-Methyl-2-(3-pyridyl)propanoate (16). The method followed essentially that described for **9**, using **15** (683 mg, 2 mmol) in THF (5 mL), KH (35% by weight dispersion in oil, 504 mg, 4.4 mmol) in THF (5 mL), and MeI (568 mg, 4.0 mmol). Chromatography with stepwise change from 4:1 to 2:1 light petroleum–Et_2O gave **16** (625 mg, 85%) as an oil which solidified to a white crystalline solid on standing: mp 59–60 °C; ^1H NMR δ_H 0.81 (d, 3, J = 7.0 Hz, cedryl CH*Me*), 0.85 and 0.90 (2s, 6, cedryl CMe_2), 1.42 (s, 3, cedryl OCMe), 1.57 and 1.60 (2s, 6, $COCMe_2$), 7.24 (dd, 1, pyridyl H-5), 7.69 (ddd, 1, pyridyl H-4), 8.47 (dd, 1, pyridyl H-6), 8.65 (d, 1, pyridyl H-2); MS *m/z* 369 (M^+). Anal. ($C_{24}H_{35}NO_2$) C, H, N.

(1*S*,2*S*,3*S*,5*R*)-Isopinocampheyl 2-Pyridylacetate (17). The method followed essentially that of **7** above using (+)-isopinocampheol (1.70 g, 11 mmol) in THF (15 mL), *n*-BuLi (2.5 M, 4.4 mL, 11 mmol), and methyl 2-pyridylacetate (1.51 g, 10 mmol) in THF (5 mL). Chromatography with 50:10:1 light petroleum–Et_2O–Et_3N gave **17** (2.24 g, 82%) as an oil: ^1H NMR δ_H 0.94 and 1.21 (2s, 6, CMe_2), 1.08 (d, 3, J = 7.5 Hz, CHMe), 3.86 (s, 2, $COCH_2$), 5.10 (ddd, 1, OCH), 7.18 (dd, 1, pyridyl H-5), 7.32 (d, 1, pyridyl H-3), 7.65 (ddd, 1, pyridyl H-4), 8.56 (dd, 1, pyridyl H-6); this was converted into the hydrochloride which sublimed at 120 °C and 4 × 10^{-2} mbar; MS *m/z* 274 ($[M + H]^+$). Anal. ($C_{17}H_{24}ClNO_2$) C, H, Cl, N.

Aromatase and 17α-Hydroxylase/$C_{17,20}$-Lyase Assays. The reagents and conditions for the assay of the human placental aromatase and rat testicular hydroxylase/lyase enzymes were those previously described.[13] In the aromatase assay, the K_m for androstenedione was 38 ± 5 nM, so a final substrate concentration of 0.38 μM was used. Under these conditions, the rate of reaction was proportional to protein concentration and time of incubation. For the human hydroxylase/lyase assay, a microsomal fraction was prepared from testes removed at orchiectomy from previously untreated prostatic cancer patients.[24] Microsomes were resuspended in 50 mM sodium phosphate buffer (pH 7.4):glycerol (3:1) at the equivalence of 1 mL/g of fresh tissue and stored in liquid nitrogen. The assay contained 3 μM [^{3}H]substrate (1–3 μCi/nmol), 250 μM NADPH, 10 mM D-glucose-6-phosphate, 1 mM $MgCl_2$, 2 units/mL D-glucose-6-phosphate dehydrogenase, 0.1 mM dithiothreitol, 0.2 mM EDTA, 1% ethanol, 1% DMSO, 3% glycerol, and 95% 50 mM sodium phosphate buffer, pH 7.4. The test compounds were prepared in 50% DMSO, the controls receiving just 50% DMSO. The reaction, at 37 °C, was started by the addition of the microsomal preparation and stopped by adding 2 vol of MeOH–MeCN (2:1) containing unlabeled steroids. The samples were stored at −20 °C until analyzed by HPLC. The reaction was linear with time, and the rate was proportional to the protein concentration under the conditions used (data not shown). Details of the HPLC analysis are as described previously.[22] Although in human testes the pregnenolone pathway predominates, the assays used progesterone and 17α-hydroxyprogesterone as substrates as it was not possible to obtain tritiated 17α-hydroxypregnenolone commercially.

Each compound was tested at at least four different concentrations in triplicate, and the data were fitted by nonlinear regression to the median effect equation of Chou.[25] The correlation coefficients were all greater than 0.97. This method of analysis was chosen as it is valid for calculating IC_{50} values whatever the IC_{50}/[enzyme] ratio. In contrast, methods based on the Michaelis–Menten equation become invalid for values of IC_{50} < 100[enzyme].[26] The estimates of the enzyme concentration were obtained by fitting some of the data to the equation derived by Henderson for tight binding inhibitors.[27] For the assays of the human hydroxylase/lyase, the enzyme concentration was estimated to be 4–5 nM except for the hydroxylase assays of **1**, **7**, and **9** when it was ~2 nM and for the assays of ketoconazole when it was 10 nM. For a tight binding inhibitor: $IC_{50} = K_{i_{app}} + 0.5$[enzyme].

The K_m for progesterone was 0.2 ± 0.05 μM, and that for 17α-hydroxyprogesterone was 2.2 ± 0.15 μM.

Microsomal Hydrolysis of Esters. Esters (0.5 mg) were incubated with a rat liver microsomal preparation (17 mg of protein) in 0.1 M potassium phosphate buffer, pH 7.4 (5 mL), at 37 °C.[28] Aliquots (1 mL) were removed at various time points, and unhydrolyzed ester was extracted with ethyl acetate (3 × 5 mL) in the presence of sodium chloride (0.2 g). An internal standard (100 μg of another ester) was added to the sample at the time of extraction. Extracts were reduced to dryness and reconstituted in 0.5 mL of HPLC mobile phase (10 mM sodium phosphate buffer, pH 6.8:acetonitrile, 65:35). Aliquots (50 μL) were analyzed by HPLC using a 15 cm Apex column containing 5 μM C18 stationary phase, and esters were detected by UV absorption at 254 and 229 nm. No hydrolysis was observed when the esters were incubated in the presence of the esterase inhibitor phenylmethanesulfonyl fluoride (0.1 mM). Half-lives were derived from the graphs plotted of percent ester remaining against time.

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