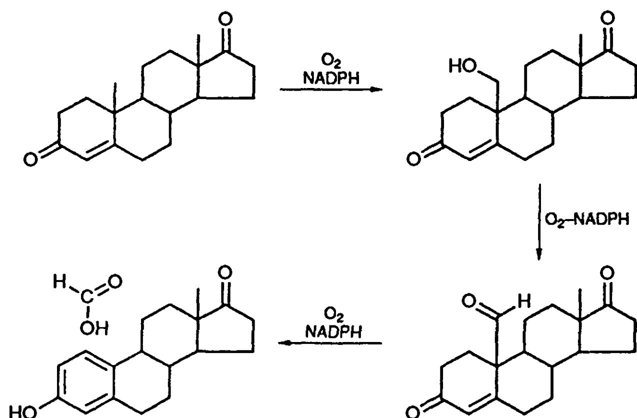
Novel 10β-Aziridinyl Steroids; Inhibitors of Aromatase

Vincent C. O. Njar,[a] Elam Safi,[a] J. V. Silverton[b] and Cecil H. Robinson[a,]*
[a] *Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore MD 21205, USA*
[b] *Laboratory of Chemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA*

The novel 10β-aziridinylestr-4-ene-3,17-diones **17** and **18** and the corresponding 10β-aziridinyl-17β-hydroxyestr-4-en-3-ones **19** and **20** have been synthesized from the 19-oximino-19-methyl intermediate **12b**. The key reaction was the conversion of the 19-oxime **12b** into the diastereoisomeric 10β-aziridines **13** and **14** by lithium aluminium hydride (LAH). Compounds **17–20** are powerful and stereoselective inhibitors of human placental microsomal aromatase. The most potent compound was **17** (*K*i = 3.4 nmol dm^{-3}). The 19*R*-isomers **17** and **19** are more effective than the corresponding 19*S*-isomers **18** and **20**, respectively. Unlike the corresponding 10β-oxiranes and -thiiranes which are classical competitive inhibitors, the (19*R*)-aziridines **17** and **19** appear to be slow-binding inhibitors. Spectroscopic studies with microsomal aromatase preparations indicate that the inhibition process involves binding of aziridine nitrogen to the heme-iron of the enzyme.

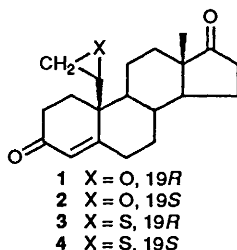
Human placental aromatase is a cytochrome P-450 enzyme complex which catalyses the conversion of androgens into estrogens. The process is illustrated by the conversion of androst-4-ene-3,17-dione into estrone *via* three steps each of which requires 1 mol of O_2 and 1 mol of NADPH (Scheme 1).[1]



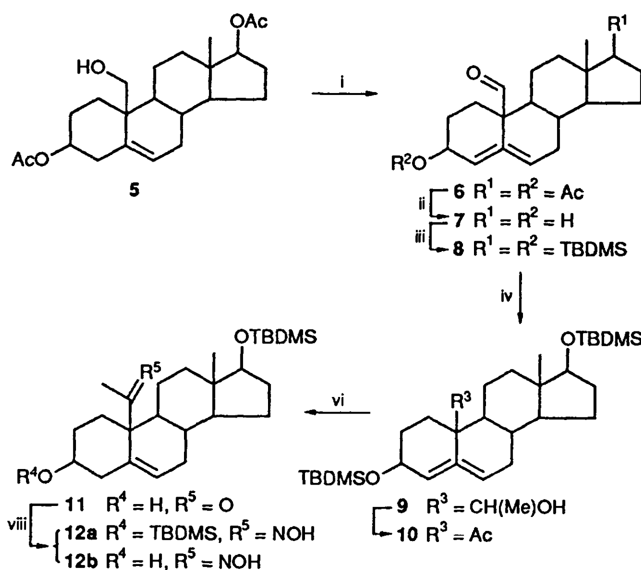
Scheme 1 Pathway for the biosynthesis of estrone from androstenedione

The potential therapeutic value of aromatase inhibitors in the treatment of estrogen-dependent diseases (*e.g.* breast cancer) has led to much recent interest in this area.[2]

Our laboratory has previously described[3] the synthesis of (19*R*)- and (19*S*)-10β-oxiranylestr-4-ene-3,17-dione **1**, **2** and (19*R*)- and (19*S*)-10β-thiiranylestr-4-ene-3,17-dione **3**, **4**. Each



of these compounds is a powerful inhibitor of human placental aromatase. The stereochemistry at C-19 plays an important



Scheme 2 *Reagents and conditions:* i, TPAP/NMO, CH_2Cl_2, room temp.; ii, 10% methanolic KOH; iii, TBDMS-Cl, DMF, Ar; iv, CH_3L, Et_2O, Ar; v, same as i; vi, TBAF, THF; vii, $H_2NOH{\cdot}HCl$, PY, Ar, reflux.

role, as the 19*R*-isomers **1** and **3** are more effective than the corresponding 19*S*-isomers **2** and **4**, respectively. Furthermore, the 19*R*-compounds **1** and **3** show[4] spectroscopic evidence for coordination of the epoxide or thiirane heteroatoms with the enzyme's heme iron.

Consequently, we embarked on the synthesis of the hitherto undescribed aziridinyl steroids **17**, **18**, **19** and **20** (Scheme 2 and 3) thinking that they might show strong coordination of the aziridine nitrogen to the heme iron of aromatase. We also considered the possibility that, because of the reactivity of the aziridine ring,[5] these aziridinyl steroids might react covalently at the active site of the enzyme, resulting in irreversible enzyme inhibition. These compounds are also especially interesting because attempts to prepare steroidal 19-primary amino-4-en-3-ones have been unsuccessful, although 19-amido[6–8] and 19-tertiary amino steroids[6] have been described. This paper describes the synthesis of novel aziridinyl steroids, and their evaluation as inhibitors of microsomal aromatase.[9]

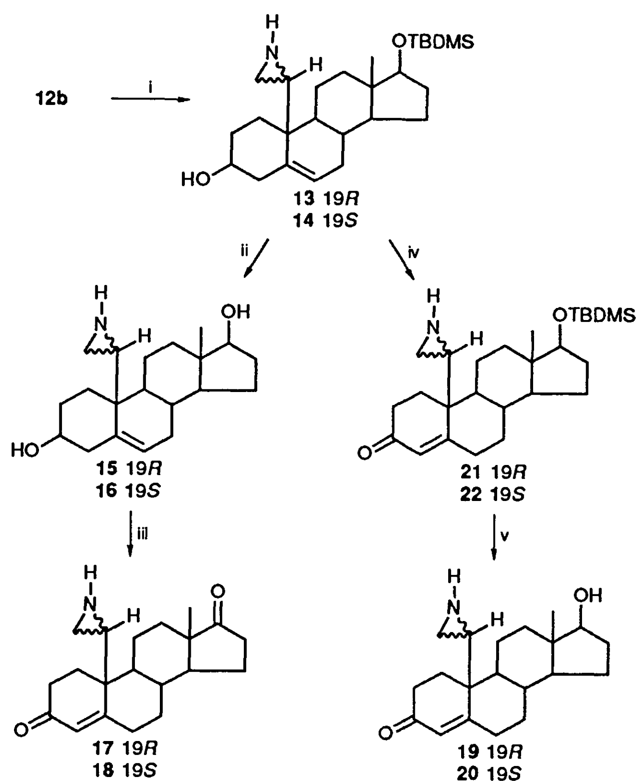
Results and Discussion

Synthesis.—Although a number of other steroidal aziridines have been reported,[10,11] we expected difficulties in the present case because of the sterically hindered 19-position. Attempts to transform 10β-oxiranylandrost-5-ene-3β,17β-diol into the corresponding 10β-aziridinyl steroid *via* a hydroxy azide failed. We eventually synthesized the desired 10β-aziridinyl steroids (Scheme 3) by the methodology of Tzikas *et al.*[12] which involved lithium aluminium hydride (LAH) reduction of the key intermediate 19-oximino-19-methylandrost-5-en-3β-ol 17β-*tert*-butyldimethylsilyl (TBDMS) ether **12b**. The preparation of **12b** is outlined in Scheme 2. The readily available 19-hydroxyandrost-5-ene-3β,17β-diyl diacetate **5** was oxidized with tetrapropylammonium perruthenate (TPAP) in CH_2Cl_2 in the presence of *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant, to give 19-oxoandrost-5-ene-3β,17β-diyl diacetate **6** in 90% yield. The diacetate **6** was cleaved (10% methanolic KOH under argon) to give the 3β,17β-diol **7** which was converted[13] into the 19-oxo-TBDMS ether **8**. Treatment of **8** with methyllithium[14] gave the expected 19-hydroxy-19-methyl TBDMS ether **9** in quantitative yield. Oxidation of **9** with TPAP–NMO gave the 19-oxo-19-methyl TBDMS ether **10**. Attempts to convert **10** (hydroxylamine hydrochloride, pyridine, Ar, reflux) into the corresponding 19-oximino-19-methyl TBDMS ether **12a** were unsuccessful. Instead, we obtained the 19-oximino compound **12b** in *ca.* 15% yield (note the cleavage of the 3β-TBDSM group) along with recovered starting material. The loss of the 3β-TBDMS group in the **12b** which was isolated suggested that it might be interfering sterically with the 19-position. Indeed, when the 3β-TBDMS group in **10** was *first* selectively cleaved to give the 3β-hydroxy-19-oxo-17β-silyl-19-methyl compound **11**, followed by the oximination reaction, the 19-oximino-19-methyl compound **12b** was obtained in 75% yield.

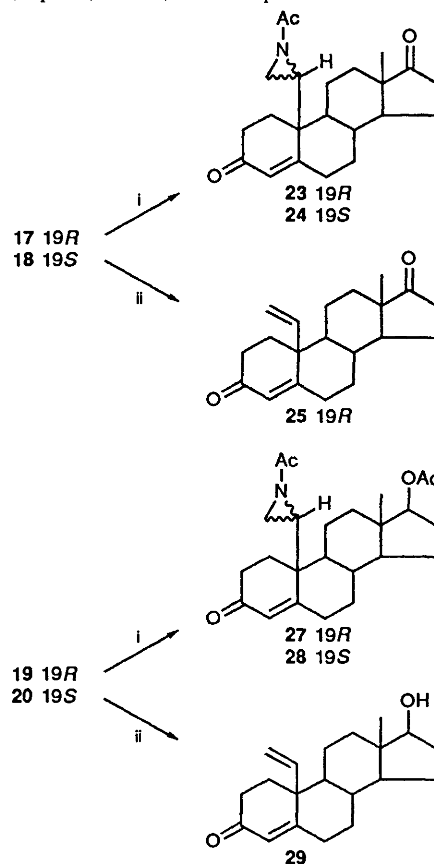
The key intermediate **12b** was then converted into the novel (19*R*)- and (19*S*)-10β-aziridinylestr-4-ene-3,17-diones **17** and **18** and the 10β-aziridinyl-17β-hydroxyestr-4-ene-3-ones **19** and **20**, as outlined in Scheme 3. Lithium aluminium hydride (LAH) reduction of **12b** in dry tetrahydrofuran (THF) under argon (Ar) at reflux for 17 h, gave the 19*R*- and 19*S*-isomers, **13** and **14** respectively, of 10β-aziridinylestr-5-en-3β-ol 17β-TBDMS ether in the ratio 1:1.5 (determined by ^1H NMR) in 88% yield. The aziridines **13** and **14** were separated (silica gel, TLC), and each was converted separately into the desired (19*R*)- and (19*S*)-10β-aziridinylestr-4-ene-3,17-dione **17** and **18** by desilylation at C-17 (aqueous HF in EtOH), to give the (19*R*)- and (19*S*)-aziridinyl diols **15** and **16**, respectively, followed by Oppenauer oxidation.

Rigorous establishment of the C-19 configuration required X-ray crystallographic analysis. We had tentatively assigned the C-19 configurations by comparison of the chromatographic mobilities and aromatase-inhibitory characteristics of **17** and **18**, with those of the corresponding pairs of oxiranes **1**, **2** and thiiranes **3**, **4** of established structure.[3] The (19*R*)- and (19*S*)-10β-aziridinylestr-4-ene-3,17-diones **17** and **18** were further characterized as the corresponding *N*-acetyl derivatives **23** and **24** respectively (acetic anhydride–pyridine, room temp., 16 h) (Scheme 4). As a final proof of structure, the aziridines **17** and **18** were each smoothly deaminated by treatment at 25 °C with aqueous sodium nitrite[12] in acetic acid to the known[6] 10β-vinylestr-4-ene-3,17-dione **25**.

The corresponding (19*R*)- and (19*S*)-10β-aziridinyl-17β-hydroxyestr-4-en-3-one **19** and **20** were prepared from compounds **13** and **14**, respectively, by a modified Oppenauer oxidation[15] to give the (19*R*)- and (19*S*)-aziridinyl-4-en-3-ones **21** and **22**, respectively, followed by cleavage (5% aq. HF in MeCN, room temp., 1 h) of the 17β-TBDMS ether groups (Scheme 3). These testosterone analogues **19** and **20** were each characterized as the *N*,*O*-diacetyl derivative **27** and **28**, respectively, and they were also each deaminated to give 17β-hydroxy-10β-vinylestr-4-en-3-one **29** (Scheme 4).



Scheme 3 *Reagents and conditions:* i, LAH, THF, Ar, reflux; ii, aq. HF, EtOH, room temp.; iii, Oppenauer oxidation; iv, modified Oppenauer oxidation; v, aq. HF, MeCN, room temp.



Scheme 4 *Reagents and conditions:* i, Ac_2O/PY, room temp., 17 h.

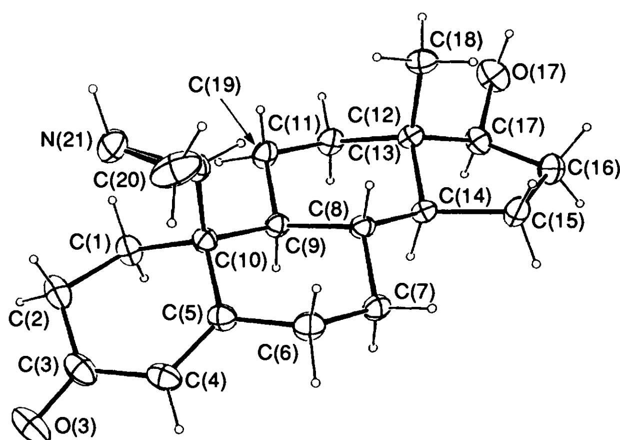


Fig. 1

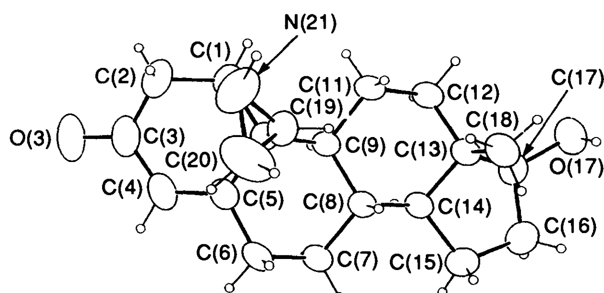


Fig. 2

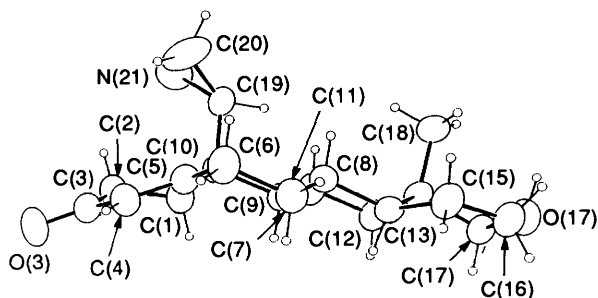


Fig. 3

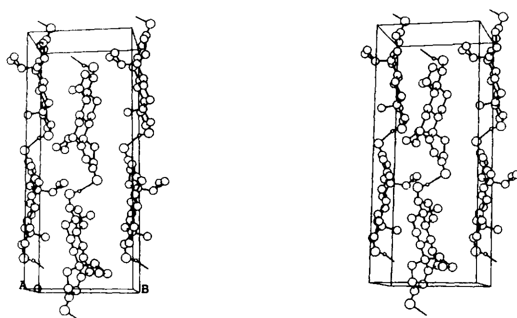


Fig. 4

Rigorous establishment of the C-19 configuration in the aziridinyl steroids was secured by the X-ray crystallographic analysis of a 10β-aziridinyltestosterone **19** which proved to be the 19*R*-isomer. The configurations at C-19 of the other aziridinyl steroids, **17**, **18** and **20**, followed from the above correlation. The molecular conformation is shown in Fig. 1, an ORTEP drawing produced by MolEN.[30] It will be noted that absolute configuration has been chosen to be the same as that of testosterone[16] itself and thus the aziridine substituent is also β. The pseudo-torsion or chiral angles[17] are −54.4, −69.7 and −71.5° respectively at C-17, C-13 and C-10. The aziridine ring rather neatly eclipses the A ring as shown in Figs. 2 and 3 (ORTEP drawings). As noted in the Experimental section, the choice of atomic labels for the aziridyl atoms seems quite conclusive. Assignment of the nitrogen H-atom position from the electron density was difficult but the position other than that chosen would lead to unlikely contacts. The dimensions and conformation of the steroid moiety are very similar to those reported for testosterone. The bond lengths, angles, torsion angles, atomic positions and thermal parameters are available as supplemental material from the Cambridge Crystallographic Database.*

A packing diagram, Fig. 4 (PLUTO drawing produced by MolEN), shows that the most important influence on the packing is a hydrogen bond producing infinite strings of molecules along the *c* axis. The dimensions involved in the bond are O(17)–H 0.94(3), H ··· O(3) 2.01(3), O(17) ··· O(3) 2.869(3) Å and O(17)–H ··· O(3) 149(2)°.

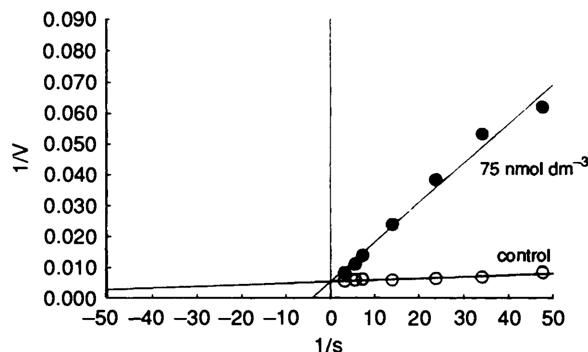


Fig. 5 Lineweaver–Burk analysis (1/v *vs.* 1/s) of (19*R*)-aziridine **17** at 75 mol dm^{-3}. The inhibition experiments with the other aziridines **18**–**20** gave plots that were essentially the same as shown above.

Table 1 Inhibitory potency of compounds **17**–**20** towards human placental microsomal aromatase

Compound	K_i/nmol dm^{-3} [a,b]
17	3.4
18	56.0
18	30.6
20	693.0

[a] Average of three experiments. [b] K_m for androstenedione = 13.5 nmol dm^{-3}.

Enzyme Studies.—The inhibitory potencies of the aziridines were determined as described in the Experimental section. Following the initial screening assays, all four aziridines **17**–**20** were evaluated further to determine the apparent K_i values (from Lineweaver–Burk plots; Fig. 5). The apparent K_i values are presented in Table 1 and show that all four aziridines are excellent competitive inhibitors of human placental microsomal aromatase. Differences in inhibitory potency due to C-19 stereochemistry, which were seen for the epoxides and thiiranes,[3] are observed in the aziridine series. The inhibitory potencies of the (*R*)- and (*S*)-10β-aziridinyl 3,17-diones **17** and **18** which have K_i values of 3.4 and 56 nmol dm^{-3}, respectively, are comparable with those of the corresponding (*R*)- and (*S*)-thiiranes **3** and **4** which had K_i values[3] of 1 and 75 nmol dm^{-3},

* For full details of the CCDC deposition scheme see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 1*, 1993, Issue 1.

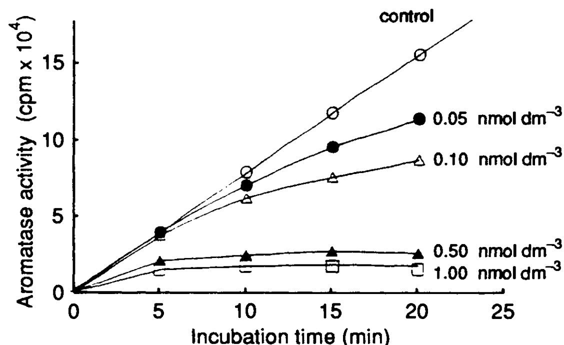


Fig. 6 Progress curves for the inhibition of placental microsomes by (19*R*)-aziridine **17** at different concentrations

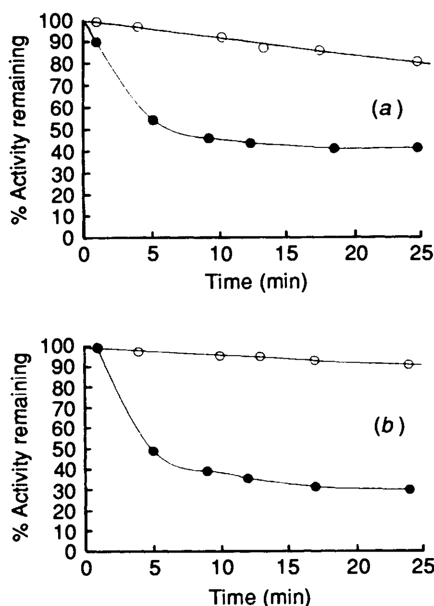


Fig. 7 Time-dependent, biphasic inactivation of aromatase by (19*R*)-aziridine **19**: (a) in the presence of NADPH; (b) in the absence of NADPH; ○ no inhibitor; ● 50 nmol dm^{-3} of **19**.

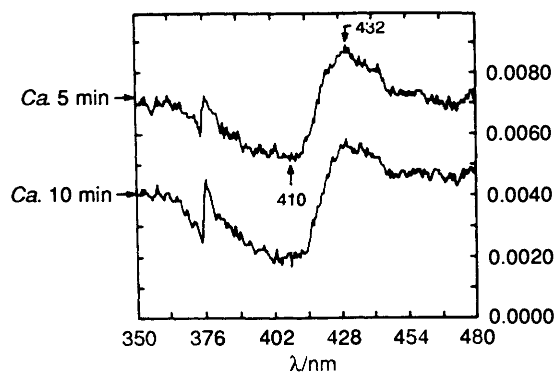


Fig. 8 Difference absorption spectra produced using placental microsomes. Sample contained 1 mg of protein/cm^{-3} and 0.1 μmol dm^{-3} androstenedione. Curves show the effect of addition of 0.5 μmol dm^{-3} (19*R*)-aziridine **17**, after *ca.* 5 min and after *ca.* 10 min.

respectively. The (19*R*)-aziridine **17** is one of the most potent aromatase inhibitors yet described. The differences in potency between the aziridinyl 3,17-diones **17** and **18** and the aziridinyl-17β-hydroxy 3-ones **19** and **20** are consistent with the known differences of K_m for aromatase between 17-oxo and 17β-hydroxy compounds.

Assays designed to examine possible time-dependent loss of enzyme activity at 37 °C (Fig. 6) suggest that the (19*R*)-aziridines, **17** and **19** may be slow-binding[18] inhibitors. Both

Table 2 Comparison of inhibition of aromatase by (19*R*)- and (19*S*)-aziridines and their corresponding *N*-acetyl derivatives

	% Inhibition of aromatase[a]			
Compd.	1 μmol dm^{-3} [b]	5 μmol dm^{-3} [b]	10 μmol dm^{-3} [b]	20 μmol dm^{-3} [b]
17	89	—[c]	—[c]	—[c]
18	13	40	61	73
23	0	—[c]	42	—[c]
24	—[c]	0	—[c]	0

[a] Substrate concentration = 1 μmol dm^{-3}. [b] Inhibitor concentration. [c] Not determined.

compounds exhibited a time-dependent, biphasic loss of enzymatic activity of 25 min (Fig. 7a). The loss in activity did not require NADPH (Fig. 7b). The mechanism underlying this biphasic loss of activity is unknown at this time. One possibility is that *N*-protonated aziridinyl steroid is first bound at the active site, followed by deprotonation by an active site grouping, thus permitting co-ordination of aziridine nitrogen with heme iron.

As this work neared completion, it was reported[19] that 19-azidoandrostenedione is a slow-binding inhibitor of aromatase. It was also established[19] that this compound exhibits a type II spectrum when incubated with aromatase, and it was hypothesized that the α-nitrogen of the azide group is likely to be involved in co-ordination with enzyme's heme iron.

To further investigate the mechanism of aromatase inhibition, the properties of the complexes formed between the 10β-aziridinyl-3,17-diones **17** and **18** and microsomal aromatase P-450 were studied using UV–VIS difference spectroscopy.[4] We observed that the 19*R*-isomer **17** shifted the Soret maximum of the enzyme-substrate complex from 390 to 432 nm (Fig. 8) while the 19*S*-isomer was ineffective under the experimental conditions. Thus, the 19*R*-isomer showed a type II spectrum, indicating coordination of the steroidal nitrogen to the heme iron of the cytochrome P-450, with formation of low spin iron. Although data are available for the binding of nitrogen ligands to cytochrome P-450 systems, resulting in complexes with a Soret maximum at 421–430 nm,[20] we are not aware of data for such complexes with the nitrogen of aziridine groupings.

The inhibitory potency of (19*R*)-10β-oxiranyl- and thiiranyl steroids stems in part from the additional stabilization due to coordination of the heteroatom of their three-membered ring to the heme iron of aromatase.[4] The spectroscopic data described above suggest that this may also be the case for the (19*R*)-10β-aziridinyl steroids of this work.

Presumably, the orientation as well as the availability of the lone pair of electrons on nitrogen would be important for effective co-ordination. It appeared worthwhile to examine the effects of *N*-acylation on the inhibitory effects of the aziridinyl steroids. Specifically, we examined the aromatase inhibitory effects of the *N*-acetyl derivatives, **27** and **28** respectively, of the aziridines **17** and **18**. It was found (Table 2) that *N*-acetylation of (19*R*)- and (19*S*)-aziridinyl steroids **17** and **18** resulted in significant losses of inhibitory power in each case. Although the process of *N*-acylation should result in some delocalization of the p-electrons of nitrogen, the results are not unambiguous because of the possible steric effects of the newly introduced acetyl group.

In conclusion, we have developed a method for the introduction of an aziridine group at the 10-carbon of a 4-en-3-one steroid. This enabled us to synthesize the 19-aziridinyl steroids **17**, **18**, **19** and **20** which proved to be powerful inhibitors of human placental aromatase. In addition, we have shown that the nitrogen atom of the most potent aziridine **17** coordinates to the enzyme's heme iron. We have also shown that the

orientation of the lone pair on the nitrogen heteroatom in the (19*S*)-aziridine **18** may be significant in its binding to the enzyme active site. As in the case of the analogous epoxides and thiiranes, these aziridines show significant differences in inhibitory potency related to the configuration at C-19. Unlike the thiiranes, which show normal competitive reversible kinetics, the (19*R*)-aziridines **17** and **19** show apparent slow binding behaviour. Additional experiments are needed to characterize fully the mechanism of aromatase inhibition by these novel aziridinyl steroids.

Experimental

General.—Most of the chemicals used were purchased from Aldrich Chemical Company or Sigma Chemical Company. Dehydroisoandrosterone (3β-hydroxyandrost-5-en-17-one) was purchased from G. D. Searle and converted into 19-hydroxyandrost-5-ene-3β,17β-diyl diacetate as described by Kalvoda *et al.*[21] [1β,^{3}H]Androst-4-ene-3,17-dione was purchased from New England Nuclear Corp. Tetrapropylammonium perruthenate (TPAP) was purchased from the Aldrich Chemical Co., and was used following the general procedure of W. P. Griffith and S. V. Ley [*Aldrichimica Acta*, 1990, **23**, 13].

M.p.s were determined on a Kofler micro hot-stage and are uncorrected. IR spectra were recorded on a Perkin-Elmer 710B Spectrometer as KBr discs or in $CHCl_3$. UV spectra were obtained in methanol or ethanol on a Perkin-Elmer Lambda 3 instrument. ^{1}H NMR spectra were recorded in $CDCl_3$ or [$^2H_4$]methanol with either IBM-80 MHz, Bruker 300 MHz, or Varian XL-400 MHz spectrometers; *J* values are recorded in Hz. Mass spectra were obtained on a VG 70-S spectrometer. HPLC separations were performed on a Waters Associates Model 6000 instrument, using a Whatman Magnum 10 Partisil semipreparative column. Column chromatography was performed on silica gel (Baker). Analytical and preparative TLS were carried out using pre-coated plates [Polygram SIL G/UV254, Macherey-Nagel, Germany and silica gel 60 (HF 254 + 366), Analtech Inc. Newark, DE, USA].

19-Oxoandrost-5-ene-3β,17β-diyl Diacetate **6**.—19-Hydroxyandrost-5-ene-3,17-diyl diacetate **5** (2.5 g, 6.41 mmol) was dissolved in dry CH_2Cl_2 (50 cm^3) containing both 4 Å molecular sieves and *N*-methylmorpholine *N*-oxide (NMO, 12.127 g, 9.615 mmol). The mixture was stirred for 10 min after which tetrapropylammonium perruthenate (TPAP; 0.112 g, 0.32 mmol) was added, and the reaction followed by TLC until complete (1–0.5 h). After dilution with CH_2Cl_2 (200 cm^3) the reaction mixture was washed with saturated aqueous sodium sulfite (50 cm^3), brine (50 cm^3) and saturated aqueous copper(II) sulfate (50 cm^3), and then dried (Na_2SO_4) and concentrated to give a green solid (2.6 g,). This was purified by flash chromatography (silica gel, 9:1 chloroform–ethyl acetate) to give the title compound **6** (2.25 g, 90%), m.p. 120–122 °C; δ_H(80 MHz, $CDCl_3$) 0.75 (3 H, s, 18-Me), 1.99 (3 H, s, 3β-OAc_3), 2.02 (3 H, s, 17β-OAc_3), 4.59 (2 H, m, 3α-H and 17α-H), 5.87 (1 H, m, 6-H) and 9.66 (1 H, s, 19-CHO).

19-Oxoandrost-5-ene-3β,17β-diol **7**.—A stirred solution of 19-oxoandrost-5-ene-3β,17β-diyl diacetate **6** (1.2 g, 3.1 mmol) in methanol (10 cm^3) under argon was treated with 10% methanolic KOH (40 cm^3). After being stirred at room temperature for 2 h, the reaction mixture was concentrated to *ca.* 15 cm^3, diluted with water (200 cm^3) and extracted with 5% methanol in $CHCl_3$ (50 cm^3 × 3). The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated to yield a white solid **7** (0.924 g, 98%), m.p. 183–185 °C; δ_H(80 MHz, $CDCl_3$) 0.69 (3 H, s, 18-Me), 3.55 (2 H, m, 3α-H and 17α-H), 5.82 (1 H, m, 6-H) and 9.65 (1 H, s, 19-CHO).

*19-Oxoandrost-5-ene-3β,17β-diyl Bis(*tert*-butyldimethylsilyl ether)* **8**.—A mixture of the diol **7** (0.91 g, 2.96 mmol), imidazole (1.222 g, 17.95 mmol) and *tert*-butylchlorodimethylsilane (1.804 g, 11.97 mmol) in dry DMF (20 cm^3) was stirred at room temperature under argon for 18 h. The mixture was then diluted with water (40 cm^3) and extracted with ether. The ether extract was washed with water and brine, dried (Na_2SO_4) and concentrated to give a dirty white solid (1.8 g). Crystallization of this from methanol gave the pure title compound **8** (1.48 g, 93%), m.p. 112–115 °C; δ_H(80 MHz, $CDCl_3$), 0.00 (6 H, s, one set of $SiMe_2$), 0.05 (6 H, s, one set of $SiMe_2$), 0.64 (3 H, s, 18-Me), 0.87 (18 H, s, $SiCMe_3$ × 2), 3.50 (2 H, m, 3α-H and 17α-H), 5.76 (1 H, m, 6-H) and 9.63 (1 H, s, 19-CHO).

*19-Hydroxy-19-methylandrost-5-ene-3β,17β-diyl Bis(*tert*-butyldimethylsilyl ether)* **9**.—To a cooled (ice-salt bath, −5 °C) solution of the 19-oxo compound **8** (1.375 g, 2.58 mmol) in dry ether (20 cm^3) under argon, a 1.4 mol dm^{-3} solution of methyllithium (6 cm^3, 3.5 equiv.) was added dropwise. The mixture was refluxed for 45 min and stirred at room temperature for 15 h. The excess of reagent was then decomposed with water (cooling) and the mixture diluted with ethyl acetate (20 cm^3). The organic layer was separated, washed with brine (25 cm^3 × 2), dried (Na_2SO_4) and concentrated to yield the title compound **9** as a white solid (1.41 g, 98%), m.p. 53–65 °C; δ_H(80 MHz, $CDCl_3$) 0.00 (6 H, s, one set of $SiMe_2$), 0.05 (6 H, s, one set of $SiMe_2$), 0.75 (3 H, s, 18-Me), 0.86 (21 H, s, $SiCMe_3$ × 2 and 20-Me), 3.55 (2 H, m, 3α-H and 17α-H), 4.25 (1 H, m, 19-H) and 5.60 (1 H, m, 6-H).

*19-Methyl-19-oxoandrost-5-ene-3β,17β-diyl Bis(*tert*-butyldimethylsilyl ether)* **10**.—Oxidation of **9** (1.36 g, 2.48 mmol) with TPAP/NMO as described above for **6** afforded **10** (1.22 g, 90%), m.p. 35–37 °C; δ_H(400 MHz, $CDCl_3$) 0.00 (6 H, s, one set of $SiMe_2$), 0.04 (6 H, s, one set of $SiMe_2$), 0.67 (3 H, s, 18-Me), 0.87 (18 H, s, $SiCMe_3$ × 2), 2.16 (3 H, s, 20-Me_3), 3.53 (2 H, m, 3α-H and 17α-H) and 5.72 (1 H, m, 6-H); *m/z* 546 (M^+, 2%) 489 (58), 371 (80) and 239 (31) [relative to 75 (100)].

*19-Methyl-19-oxoandrost-5-en-3β-yl 17β-*tert*-Butyldimethylsilyl Ether* **11**.—A solution of the disilyl ether **10** (1.2 g, 2.2 mmol) in dry THF (20 cm^3) was treated with a 1 mol dm^{-3} solution of tetrabutylammonium fluoride in THF (5 cm^3). The mixture was stirred at room temperature for 2 h and then poured into cold water (50 cm^3). The product was extracted with CH_2Cl_2 and the extract worked up to afford crude material (0.95 g) which was then purified by column chromatography (silica gel, 3:2, hexane–EtOAc) to give the title compound **11** (0.75 g, 79%), m.p. 140–142 °C; δ_H(400 MHz, $CDCl_3$) 0.00 (6 H, s, $SiMe_2$), 0.67 (3 H, s, 18-Me), 0.87 (9 H, s, $SiCMe_3$), 2.15 (3 H, s, 20-Me), 3.56 (2 H, m, 3α-H and 17α-H) and 5.65 (1 H, m, 6-H).

3β-Hydroxy-19-methyl-19-oximinoandrost-5-en-17β-yl tert-*Butyldimethylsilyl Ether* **12b**.—A mixture of the ether **11** (0.7 g, 1.62 mmol) and hydroxylamine hydrochloride (1.57 g, 22.7 mmol) in pyridine (20 cm^3) was refluxed under argon for 17 h. Pyridine was removed under reduced pressure and the residue was treated with a mixture of EtOAc (10 cm^3) and water (5 cm^3). The organic layer was washed with water and brine, dried (Na_2SO_4) and evaporated to give a yellow solid (0.71 g). This was purified by column chromatography (silica gel, 2:1 $CHCl_3$–EtOAc) to give the 19-oxime **12b** (0.52 g, 72%), m.p. 174–175 °C; δ_H(400 MHz; $CDCl_3$) 0.00 (6 H, s, $SiMe_2$), 0.66 (3 H, s, 18-Me, 0.87 (9 H, s, $SiCMe_3$), 1.88 (3 H, s, 19-Me), 3.60 (2 H, m, 3α-H and 17α-H) and 5.60 (1 H, m, 6-H); *m/z* 447 (M^+, 21%) 430 (34), 390 (36), 372 (31) and 331 (18) [relative to 167 (100)].

LAH Reduction of 3β-*Hydroxy*-19-*methyl*-19-*oximinoandrost*-5-*en*-17β-*yl* tert-*Butyldimethylsilyl Ether* **12b**: (19R)-10β-*Aziridin*-2-*yl*-3β-*hydroxyestr*-5-*en*-17β-*yl* tert-*Butyldimethylsilyl Ether* **13** *and* (19S)-10β-*Aziridin*-2-*yl*-3β-*hydroxyestr*-5-*en*-17β-*yl* tert-*Butyldimethylsilyl Ether* **14**.—A solution of the oxime **12b** (215 mg, 0.48 mmol) in dry THF (3 cm^3) was added dropwise to a stirred suspension of LAH (104 mg, 2.74 mmol) in dry THF (8 cm^3) which was then refluxed under an argon atmosphere. Stirring and heating were continued for 17 h after which excess of LAH was decomposed with water (10 cm^3). Insoluble inorganic material was filtered off and washed with 5% methanol in EtOAc (50 cm^3). The combined filtrate and washings were washed with water (10 cm^3 × 2) and brine (10 cm^3 × 2), dried and evaporated to give a dirty white solid (210 mg). Purification of this by preparative TLC (silica gel, $CHCl_3$–EtOH, 9:1) gave the (19*R*)-aziridine **13** (73 mg, 35%) and the (19*S*)-isomer **14** (110 mg, 53%). Compound **13** had m.p. 159–161 °C; δ_H(400 MHz; $CDCl_3$) 0.01 [3 H, s, 17β-OSi(*Me*)Me] 0.02 [3 H, s, 17β-OSi(*Me*)Me], 0.73 (3 H, s, 18-Me), 0.88 (9 H, s, $SiMe_3$) 2.21 (1 H, m), 2.31 (1 H, m) and 2.57 (1 H, m) (aziridine H), 3.55 (2 H, m, 3α-H and 17α-H) and 5.52 (1 H, m, 6-H) [Found: M^+, 431.3219 (10%). $C_{25}H_{45}NO_2Si$ requires *M*, 431.3220]. Compound **14** had m.p. 178–181 °C; δ_H(400 MHz, $CDCl_3$) 0.00 [3 H, 3, 17β-OSi(*Me*)Me], 0.02 [3 H, s, 17β-OSi(*Me*)Me], 0.78 (3 H, s, 18-Me), 0.88 (9 H, s, $SiMe_3$), 2.03 (1 H, m), 2.34 (1 H, m) and 2.4 (1 H, m) [aziridine H], 3.57 (2 H, m, 3α-H and 17α-H) and 5.59 (1 H, m, 6-H) [Found: M^+, 431.3224 (35%). $C_{26}H_{45}NO_2Si$ requires *M*, 431.3220].

(19R)-10β-*Aziridin*-2-*ylestr*-5-*ene*-3β,17β-*diol* **15**.—The ether **13** (200 mg, 0.464 mmol) was treated with 30% ethanolic HF (10 cm^3) [prepared by mixing 48% aq. HF (30 cm^3) and absolute ethanol (70 cm^3)]. The mixture was stirred at room temperature for 2.5 h and was then diluted with cold water (30 cm^3) and made alkaline with 50% aq. NaOH; it was then extracted with 9% butanol in EtOAc (20 cm^3 × 4). The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated to give the pure diol **15** (140 mg, 95%), m.p. 203–205 °C, ν_{max}(KBr)/cm^{-1} 3375; δ_H(300 MHz, $CDCl_3$ + CD_3OD, 2:1) 0.74 (3 H, s, 18-Me), 3.44 (1 H, m, 3α-H), 3.59 (1 H, t, *J* 8.4, 17α-H) and 5.51 (1 H, s, 6-H) [Found: M^+, 317.2351 (7%). $C_{20}H_{31}NO_2$ requires *M*, 317.2355].

(19S)-10β-*Aziridin*-2-*ylestr*-5-*ene*-3β,17β-*diol* **16**.—Treatment of compound **14** (250 mg, 0.58 mmol) with 30% ethanolic HF (25 cm^3) as described above for compound **15** afforded the title compound **16** (179 mg, 97%), m.p. 152–155 °C; ν_{max}(KBr)/cm^{-1} 3380; δ_H(300 MHz, $CDCl_3$ + CD_3OD) 0.73 (3 H, s, 18-Me), 3.52 (1 H, m, 3α-H), 3.60 (1 H, t, *J* 8.4, 17α-H) and 5.57 (1 H, s, 6-H) [Found: M^+, 317.2351 (25%). $C_{20}H_{31}NO_2$ requires *M*, 317.2355].

(19R)-10β-*Aziridin*-2-*ylestr*-4-*ene*-3,17-*dione* **17**.—A solution of the diol **15** (120 mg, 0.3785 mmol) in dry toluene (25 cm^3) and cyclohexanone (0.75 cm^3, 7.572 mmol) was refluxed under a Dean-Stark trap until *ca.* 3 cm^3 of distillate had collected. Dry aluminium isopropoxide (278 mg, 1.36 mmol) was added to the stirred reaction mixture which was then heated under reflux for 4 h before being cooled, diluted with EtOAc and washed successively with 5% aq. $NaHCO_3$ and brine, and then dried (Na_2SO_4). Evaporation of the organic phase under reduced pressure gave a yellow oil (210 mg) which was subjected to column chromatography (silica gel, 1:1 $CHCl_3$–EtOAc, then 1:1 EtOAc–EtOH) to give the title compound **17** (47 mg, 39%), m.p. 185–186 °C; ν_{max}(KBr)/cm^{-1} 3300, 1730, 1665 and 750; δ_H(400 MHz, $CDCl_3$) 0.95 (3 H, s, 18-Me), 2.42–2.56 (6 H, br m, aziridine H) and 5.85 (1 H, s, 4-H); λ_{max}(EtOH)/nm 239 (ε 12 700) [Found: M^+, 313.2041 (21%). $C_{20}H_{27}NO_2$ requires *M*, 313.2042].

(19Z)-10β-*Aziridin*-2-*ylestr*-4-*ene*-3,17-*dione* **18**.—The diol **16** (200 mg, 0.631 mmol) was subjected to Oppenauer oxidation as described above for **17** to give **18** (981 mg, 41%), m.p. 155–157 °C; ν_{max}(KBr)/cm^{-1} 3280, 1735, 1665 and 750; δ_H(400 MHz, $CDCl_3$) 0.95 (3 H, s, 18-Me) 2.48 (1 H, dd, *J* 19.4, 8.8) 2.6 (1 H, m) and 2.71 (1 H, t, *J* 14) (aziridine H) and 5.86 (1 H, s, 4-H); λ_{max}(EtOH)/nm 242 (ε 10 200) [Found: M^+, 313.2044 (43%). $C_{20}H_{27}NO_2$ requires *M*, 313.2042].

(19R)-10β-*Aziridin*-2-*yl*-3-*oxoestr*-4-*en*-17β-*yl* tert-*Butyldimethylsilyl Ether* **21**.—A solution of the ether **13** (250 mg, 0.58 mmol) in dry toluene (30 cm^3) and 4-methylpiperidone (0.86 cm^3) was refluxed under a Dean–Stark trap until *ca.* 5 cm^3 of distillate had collected. Dry aluminium isopropoxide (178 mg, 0.87 mmol) was added to the stirred reaction mixture which after being heated under reflux for 4 h was cooled, diluted with EtOAc and washed successively with 5% aq. $NaHCO_3$ and brine and dried (Na_2SO_4). Evaporation of the solvent gave a yellow oil which was purified by column chromatography (silica gel, hexane–EtOAc, 1:2) to yield the title compound **21** (200 mg, 80%), m.p. 175–178 °C; δ_H(80 MHz, $CDCl_3$) 0.00 (6 H, s, $SiMe_2$), 0.77 (3 H, s, 18-Me), 0.87 (9 H, s, $SiMe_3$), 3.60 (1 H, t, *J* 8.4, 17α-H) and 5.85 (1 H, s, 4-H).

(19S)-10β-*Aziridin*-2-*yl*-3-*oxoestra*-4-*en*-17β-*yl* tert-*Butyldimethylsilyl Ether* **22**.—Compound **14** (300 mg, 0.696 mmol) was subjected to the modified Oppenauer oxidation as described above for compound **21** to give compound **22** (244 mg, 81%), m.p. 193–195 °C; δ_H(80 MHz, $CDCl_3$) 0.00 (6 H, s, $SiMe_2$), 0.78 (3 H, s, 18-Me), 0.86 (9 H, s, $SiCMe_3$), 3.65 (1 H, t, *J* 8.6, 17α-H) and 5.9 (1 H, s, 4-H).

(19R)-10β-*Aziridin*-2-*yl*-17β-*hydroxyestr*-4-*en*-3-*one* **19**.—Compound **21** (100 mg, 0.2231 mmol) in MeCN (10 cm^3) was treated with 48% aq. HF (1 cm^3). After the mixture had been stirred at room temperature for 2 h it was diluted with cold water (10 cm^3), neutralized (sat. aqueous $NaHCO_3$) and extracted with $CHCl_3$ (15 cm^3 × 3). The combined extracts were washed with brine and evaporated to give the pure title compound **19** (70 mg, 95.3%), m.p. 179.5–180.5 °C (Found: C, 76.1; H, 9.3; N, 4.4. $C_{20}H_{29}NO_2$ requires C, 76.15; H, 9.27; N, 4.44%); ν_{max}($CHCl_3$)/cm^{-1} 3600, 3310 and 1665; δ_H(400 MHz, $CDCl_3$) 0.82 (3 H, s, 18-Me), 2.37–2.49 (3 H, br m), 3.67 (1 H, t, *J* 9) and 5.82 (1 H, s, 4-H); λ_{max}(MeOH)/nm 240 (ε 9900) [Found: M^+, 315.2198 (24%). $C_{20}H_{29}NO_2$ requires *M*, 315.2198].

(19S)-10β-*Aziridin*-2-*yl*-17β-*hydroxyestr*-4-*en*-3-*one* **20**.—Treatment of compound **22** (120 mg, 0.2677 mmol) with HF as described above gave the title compound **20** (85 mg, 96%), m.p. 194–196 °C (Found: C, 76.2; H, 9.2; N, 4.4. $C_{20}H_{29}NO_2$ requires C, 76.15; H, 9.27; N, 4.44%); ν_{max}($CHCl_3$)/cm^{-1} 3600, 3310 and 1670; δ_H(400 MHz, $CDCl_3$) 0.84 (3 H, s, 18-Me), 2.39 (1 H, dd, *J* 5.6, 3.6), 2.55–2.73 (2 H, br m), 3.64 (1 H, t, *J* 9, 17α-H) and 5.85 (1 H, s, 4-H); λ_{max}(MeOH)/nm 242 (ε 9700) [Found: M^+, 315.2201 (39%). $C_{20}H_{29}NO_2$ requires *M*, 315.2198].

Derivatization of the Aziridines **17–20**.—Each of the aziridines was separately acetylated (Ac_2O–pyridine, room temp., 17 h) and, following work-up and purification (prep. TLC, silica gel, $CHCl_3$–EtOAc, 1:1), yielded the corresponding *N*-acetyl or *N*,*O*-diacetyl derivatives. Analytical and spectroscopic data for these derivatives were as follows.

N-*Acetyl derivative of compound* **17**. Compound **23**, m.p. 208–210 °C, ν_{max}($CHCl_3$)/cm^{-1} 1735, 1690 and 1660; δ_H(400 MHz, $CDCl_3$) 1.0 (3 H, s, 18-Me), 2.16 (3 H, s, *N*-Ac), 2.41–2.53 (5 H, br m), 2.91 (1 H, dd, *J* 6.4, 3.6) and 5.85 (1 H, s, 4-H); λ_{max}(EtOH)/nm 239 (ε 11 600) [Found: M^+, 355.2146 (11%). $C_{22}H_{29}NO_3$ requires *M*, 355.2147].

N-*Acetyl derivative of compound* **18**. Compound **24**, m.p. 162–164 °C; ν_{max}(KBr)/cm^{-1} 1735, 1695 and 1665; δ_H(400 MHz, $CDCl_3$) 1.07 (3 H, s, 18-Me), 2.13 (3 H, s, *N*-Ac), 2.71 (2 H, m), 2.85 (1 H, dd, *J* 6.0, 3.4), 4.6 (1 H, t, *J* 8.3) and 5.82 (1 H, s, 4-H); λ_{max}(EtOH)/nm 242 (ε 11 700) [Found: M^+, 355.2149 (30%). $C_{22}H_{29}NO_3$ requires *M*, 335.2147].

N,O-*Diacetyl derivative of compound* **19**. Compound **27**, m.p. 165–167 °C; ν_{max}($CHCl_3$)/cm^{-1} 1720, 1680 and 1660; δ_H(300 MHz, $CDCl_3$) 0.9 (3 H, s, 18-Me), 2.04 (3 H, s, 17β-OAc), 2.15 (3 H, s, *N*-Ac), 2.2–2.5 (5 H, br m), 2.88 (1 H, dd, *J* 6.0, 3.4), 4.6 (1 H, t, *J* 8.3, 17α-H), and 5.82 (1 H, s, 4-H); λ_{max}(EtOH)/nm 239 (ε 12 800) [Found: M^+, 399.2412 (6%). $C_{22}H_{33}NO_4$ requires *M*, 399.2410].

N,O-*Diacetyl derivative of compound* **20**. Compound **28**, m.p. 150–153 °C; ν_{max}($CHCl_3$)/cm^{-1} 1725, 1695 and 1665; δ_H(300 MHz, $CDCl_3$) 0.95 (3 H, s, 18-Me), 2.05 (3 H, s, 17β-OAc), 2.11 (3 H, s, *N*-Ac), 2.43 (1 H, m), 2.68 (1 H, t, *J* 14.2), 2.85 (2 H, dd, *J* 6.0, 3.2), 4.6 (1 H, t, *J* 7.7, 17α-H) and 5.9 (1 H, s, 4-H); λ_{max}(EtOH)/nm 242 (ε 11 900) [Found: M^+, 399.2409 (5%). $C_{22}H_{33}NO_4$ requires *M*, 399.2410].

Deamination of the (19R)- *and* (19S)-10β-*Aziridin-2-ylestr-4-ene-3,17-diones* **17** *and* **18** *and* (19R)- *and* (19S)-10β-*Aziridinyl-17β-hydroxyestr-4-ene-3-one* **19** *and* **20**.—(*a*) A solution of the dione **17** (4 mg, 0.0128 mmol) in glacial acetic acid (120 mm^3) was treated with 10% aqueous sodium nitrite (25 mm^3, 0.0362 mmol). After the mixture had been stirred at room temperature for 0.5 h, it was diluted with water and extracted with CH_2Cl_2 (3 × 1 cm^3). The combined extracts were washed with 5% aqueous $NaHCO_3$, water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by preparative TLC (silica gel, $CHCl_3$–EtOAc, 4:1) to give 10β-vinylestr-4-ene-3,17-dione **25** (2.4 mg, 62%) identified by its m.p., IR, UV and ^{1}H NMR spectra and TLC comparison with authentic material.

(*b*) Treatment of **18** (10 mg, 0.0319 mmol) in glacial acetic acid (150 mm^3) and 10% aqueous $NaNO_2$ (40 mm^3), as described above, also gave the 10β-vinyl compound **25**.

(*c*) *and* (*d*) Treatment of **19** and **20** (1 mg and 5 mg, respectively) as described above gave the 17β-hydroxy-10β-vinylestr-4-en-3-one **29**, m.p. 165–166 °C; λ_{max}($CHCl_3$)/cm^{-1} 3450, 1665 and 1620; δ_H(400 MHz, $CDCl_3$) 0.78 (3 H, s, 18-Me), 3.65 (1 H, t, *J* 9, 17α-H), 4.99 (1 H, d, *J* 17.6, 20-H), 5.32 (1 H, d, *J* 10.8, 20-H), 5.92 (1 H, s, 4-H) and 5.95 (1 H, dd, *J* 7.2, 10.4, 19-H); λ_{max}(EtOH)/nm 237 (ε 12 725) [Found: M^+, 300.2091 (100%). $C_{20}H_{28}O_2$ requires *M*, 300.2089].

Preparation of Human Placental Microsomes.—Microsomes were obtained from human placentas immediately after delivery and prepared as described by Ryan.[22] They were stored at −78 °C. Protein concentration was determined with a Bio-Rad protein assay kit, obtained from Bio-Rad Laboratories.

Screening assay procedure. The method of Thompson and Siiteri[23] as modified by Reed and Ohno[24] was used in our studies. This assay quantitates the production of [^{3}H]H_2O released from [1β-^{3}H]androst-4-ene-3,17-dione after aromatization. All enzymatic studies were performed in 0.1 mol dm^{-3} phosphate buffer, pH 7.4, at a final incubation volume of 2.0 cm^3. The incubation mixture contained 10 mmol dm^{-3} phosphate buffer, 100 mmol dm^{-3} KCl, 1 mmol dm^{-3} EDTA, 5 mmol dm^{-3} DTT, 125 μmol dm^{-3} NADPH, [1β-^{3}H]androst-4-ene-3,17-dione, 1 μmol dm^{-3} (6 μCi); inhibitor concentrations varied from 50 nmol dm^{-3} to 30 μmol dm^{-3}; human placental microsomes, 0.125 mg cm^{-3}. The assay mixture was pre-incubated for 10 min at 37 °C and the reaction initiated by addition of placental microsomes. The incubations were performed in unstoppered 10 cm^3 culture tubes in a shaking water-bath at 37 °C. Aliquots were removed during the course of incubation (usually 20 min) at 5 min intervals and quenched in $CHCl_3$ (5 cm^3) followed by vortexing for *ca.* 30 s. After centrifugation at 1500 g for 10 min, aliquots (200 mm^3) of each aqueous phase were removed and added to scintillation mixture (10 cm^3) for determination of ^3H_2O production. From the measurements of ^3H_2O produced, the amount of estrogen formed can be calculated.

K_i *Assay Procedure*.—This procedure is essentially similar to that employed in the screening assay, except that the substrate concentration was varied between 20 nmol dm^{-3} and 295 nmol dm^{-3} using only 0.003 mg of microsomal protein to ensure a constant initial velocity, even at the lowest substrate concentration. Control samples with no inhibitor were incubated simultaneously for 15 min. Each inhibitor was examined at three concentrations (for **17** at 25, 50 and 75 nmol dm^{-3}; for **18** at 0.25, 0.5 and 1 μmol dm^{-3}, for **19** at 15, 50 and 100 nmol dm^{-3} and for **20** at 5 and 15 μmol dm^{-3}). Data from the various assays were used to obtain Lineweaver–Burk plots (*e.g.* Fig. 1). From these plots, K_i values for the inhibitors **17**–**20** and the K_m for androstenedione (substrate) were determined (Table 4).

Time-dependent Inactivation Procedure.—Various concentrations of inhibitors were incubated with placental microsomes (0.37 mg protein), 5 mmol dm^{-3} DTT, 125 μmol dm^{-3} NADPH in 10 mmol dm^{-3} phosphate buffer, pH 7.4 in a total volume of 0.5 cm^3 at 37 °C in air. Aliquots (50 mm^3) were removed at various time intervals (1–24 min) and added to a solution of [1β-^{3}H]androst-4-ene-3,17-dione (1 μmol dm^{-3}, 1.5 μCi), 5 mmol dm^{-3} DTT, 125 mm^3 NADPH in 10 mmol dm^{-3} phosphate buffer, pH 7.4, total volume (0.5 cm^3), and the mixture was incubated at 37 °C for 10 min. Tritiated water released was determined as described above.

Spectroscopic Studies.—Absorption spectra were recorded at room temperature using a Cary model 170 Spectrophotometer interfaced to an On-Line Instrument Systems Computer System. Steroids were added from stock solutions in ethanol. Repeated spectra were recorded to ensure sample equilibration.

X-Ray Crystallographic Determination.—Colourless prismatic crystal; formula: $C_{20}H_{28}NO_2$; approximate dimensions: 0.40 × 0.20 × 0.20 mm; X-radiation: Cu-Kα (λ = 1.541 84 Å); diffractometer: Enraf–Nonius CAD4 with graphite incident beam monochromator. Cell constants and orientation matrix from least-squares refinement using the setting angles of 21 reflections in the range $20 < \theta < 27°$. Crystal system: orthorhombic; cell parameters: a = 7.413 (1), b = 9.705 (1), c = 24.250 (1) Å, V = 1744.5 Å^3. For Z = 4 and F.W. = 314.45 the calculated density is 1.20 g cm^{-3}. Systematic absences of: $h00{:}h = 2n$; $0k0{:}k = 2n$; $00l{:}l = 2n$ and least-squares refinement indicate space group $P2_12_12_1$ (# 19). Data collection: 23 ± 1 °C; ω–θ scan; counter aperture; 2.0 mm horizontal by 4.0 mm vertical; 2052 unique reflections; Lorentz and polarization corrections applied; absorption coefficient: 5.6 cm^{-1}; no absorption correction.

Structure solution and refinement. The structure was solved by direct methods. A total of 23 atoms were located from an E-map prepared from the phase set with probability statistics: absolute figure of merit = 1.15, residual = 20.03, and psi zero = 1.370. Hydrogen atoms were located and added to the structure factor calculations but their positions were not refined. The structure was refined in full-matrix least squares where the function minimized was $\Sigma[w(|F_o| - |F_c|)]$ and the weight w is defined as $4{*}F_o/\sigma(F_o)$ with σ from counting statistics. Scattering factors were taken from Cromer and Waber.[26] Anomalous dispersion effects were included in F_c;[27] the values for $\Delta f'$ and $\Delta f''$ were those of Cromer.[28] Only the 1708

reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. The final cycle of refinement included 209 variable parameters and converged (largest parameter shift was under 0.01σ) with unweighted and weighted agreement factors of: $R = \Sigma[||F_o| - |F_c||]/\Sigma[|F_o|] = 0.040$, $R_w = \sqrt{\Sigma[w(|F_o| - |F_c|)^2]/\Sigma[wF_o^2]} = 0.054$. The standard deviation of an observation of unit weight was 1.90. The atomic labelling of atoms 20 and 21 was tested by reversing their labels. The values of R, R_w and the esd of a unit weight observation: 0.050, 0.068 and 2.29 respectively, confirm the assignment which gave the previously quoted values. It should be noted that the temperature factors for the aziridyl atoms are much more nearly equal with the correct choice of labels.

At the end of the refinement, an extinction correction parameters was introduced whose final value was $1.6(2) \times 10^{-6}$. There were no significant positional changes produced by the correction and the final R factor would be 0.042 without it. The highest peak in the final difference Fourier had a height of 0.22 e $Å^{-3}$ with an estimated error[29] based on ΔF of 0.01; the minimum negative peak had a height of -0.24 e $Å^{-3}$ with an estimated error based on ΔF of 0.01. Plots of $\Sigma[w(|F_o| - |F_c|)^2]$ *vs.* $|F_o|$, reflection order in data collection, sin v/λ, and various classes of indices showed no unusual trends. All calculations were performed on a DEC VAX-Station 3520 using MolEN.[30]

Acknowledgements

We thank Dr. P. A. Cole and Dr. K. Jaworski for the 400 MHz NMR spectra, and Dr. J. L. Kachinski, Jr. for the mass spectra. Gratitude is expressed to J. C. Jensen for preparation of the manuscript. This work was supported by National Institutes of Health Grant HD-11840.

References

1 E. A. Thompson and P. K. Siiteri, *J. Biol. Chem.*, 1974, **249**, 5364; P. K. Siiteri and E. A. Thompson, *J. Steroid Biochem.*, 1975, **6**, 317; M. Akhtar, M. R. Calder, D. L. Corina and J. N. Wright, *Biochem. J.*, 1982, **201**, 569.

2 P. A. Cole and C. H. Robinson, *J. Med. Chem.*, 1990, **33**, 2933.

3 M.-J. Shih, M. H. Carrell, H. L. Carrell, C. L. Wright, J. O. Johnston and C. H. Robinson, *J. Chem. Soc., Chem. Commun.*, 1987, 213; W. E. Childers and C. H. Robinson, *J. Chem. Soc., Chem. Commun.*, 1987, 320; W. E. Childers, P. S. Furth, M.-J. Shih and C. H. Robinson, *J. Org. Chem.*, 1988, **53**, 5947.

4 J. T. Kellis, Jr., W. E. Childers, C. H. Robinson and L. E. Vickery, *J. Biol. Chem.*, 1987, **262**, 4421.

5 J. A. Deyrup, *Heterocyclic Compounds*, ed. A. Hassner, Wiley, New York, 1983, vol. 42, part 1, ch. 1, p. 116.

6 O. Halpern, I. Delfin, L. Magana and A. Bowers, *J. Org. Chem.*, 1966, **31**, 693.

7 J. A. Lovett, M. V. Darby and R. E. Counsel, *J. Med. Chem.*, 1984, **27**, 734.

8 J. N. Wright, P. T. van Leersum, S. G. Chamberlin and M. Akhtar, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1647.

9 Part of this work has been presented by V. C. O. Njar, E. Safi and C. H. Robinson, at the 199th National Meeting of the American Chemical Society, Division of Organic Chemistry, Boston, MA, Spring 1990; Abstract 44.

10 J. A. Deyrup, ref. 5, p. 11.

11 G. J. Matthews and A. Hassner, *Organic Reactions in Steroid Chemistry*, vol. 2, ed. J. Fried and J. A. Edwards, Reinhold, New York, 1972, p. 22.

12 A. Tzikas, C. Tamm, A. Boller and A. Furst, *Helv. Chim. Acta*, 1976, **59**, 1850.

13 E. Corey and A. Venkatesurarlu, *J. Am. Chem. Soc.*, 1972, **94**, 6190.

14 E. Caspi and J. Wicha, *J. Chem. Soc., Chem. Commun.*, 1966, 209.

15 M. L. Raggio and D. S. Watt, *J. Org. Chem.*, 1976, **41**, 1873.

16 P. J. Roberts, R. C. Petterson, G. M. Sheldrick, N. W. Isaacs and O. Kennard, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1978.

17 S. Ito, M. Kasai, H. Ziffer and J. V. Silverton, *Can. J. Chem.*, 1987, **65**, 574.

18 J. F. Morrison and C. T. Walsh, *Adv. Enzymol.*, 1988, **61**, 201.

19 J. N. Wright, G. Slatcher and M. Akhtar, *Biochem. J.*, 1991, **273**, 533.

20 J. H. Dawson, L. A. Anderson and M. Sono, *J. Biol. Chem.*, 1982, **257**, 3606.

21 J. Kalvoda, K. Heusler, H. Ueberwasser, G. Anner and A. Wettstein, *Helv. Chim. Acta*, 1963, **46**, 1361.

22 K. J. Ryan, *J. Biol. Chem.*, 1959, **234**, 268.

23 E. A. Thompson, Jr. and P. K. Siiteri, *J. Biol. Chem.*, 1974, **249**, 5373.

24 K. C. Reed and S. Ohno, *J. Biol. Chem.*, 1976, **251**, 1625.

25 CAD4 Operations Manual, 1977, Enraf-Nonius, Delft, The Netherlands.

26 D. T. Gomer and J. T. Waber, *International Tables for X-ray Crystallography*, vol. 4, The Kynoch Press, Birmingham, England, Table 2.2B, 1974.

27 J. A. Ibers and W. C. Hamilton, *Acta Crystallogr.*, 1964, **17**, 781.

28 D. T. Gomer, *International Tables for X-ray Crystallography*, vol. 4, The Kynoch Press, Birmingham, England, Table 2.31, 1974.

29 D. W. T. Cruickshank, *Acta Crystallogr.*, 1949, **2**, 154.

30 *MolEN. An Interactive Structure Solution Procedure,* Enraf-Nonius, Delft, The Netherlands, 1990.

Paper 2/06563K
Received 10*th December* 1992
Accepted 4*th February* 1993