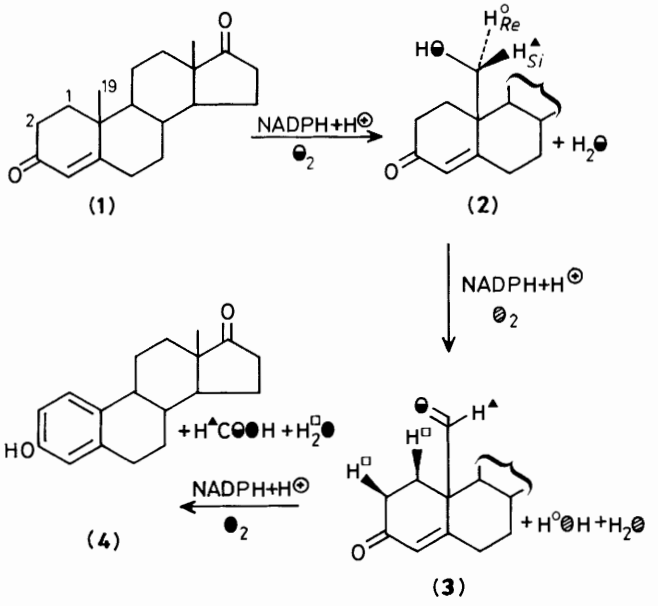
Inhibition of Aromatase by Steroids Substituted at C-19 with Halogen, Sulphur, and Nitrogen

J. Neville Wright, Phillip T. van Leersum, (in part) Stephen G. Chamberlin, and Muhammad Akhtar
Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU

The known displacement of 19-methanesulphonates in 3-acetoxy-5-ene steroids was extended to the bis(ethylene acetal) compounds (**12**) to provide a convenient route to 19-halogenoandost-4-ene-3,17-diones (**10 m,n,o**). It was discovered that iodine in the 19 position in compounds (**7**) and (**13**) can be smoothly displaced by reactive nucleophiles (CN^-, $MeSO_2S^-$, N_3^-) without rearrangement. This enabled us to synthesize a series of steroid derivatives, containing sulphur and nitrogen at C-19, which were tested as aromatase inhibitors. Of potential interest were the 19-thioalkyl compounds (**10 f—j**) which showed increasing competitive inhibition as the size of the alky substituent decreased. The most potent was the 19-thiomethylandrost-4-ene-3,17-dione (**10f**) ($K_i = 1 \times 10^{-9}$M). The most potent of the 19-aza steroids was 19-azidoandrost-4-ene-3,17-dione (**10d**) ($K_i = 5 \times 10^{-9}$M). It is proposed that both these inhibitors act by providing a sixth ligand to the haem iron of cytochrome P-450 (arom).

The conversion of androgens into oestrogens, *e.g.* androstenedione (**1**) to oestrone (**4**) occurs through the participation of three sequential reactions at position 19, each requiring 1 mol of NADPH and 1 mol of O_2.[1] This process culminates in the release of the target C-19 atom as formic acid and the aromatization of ring A.[2] The overall transformation is catalysed by a P-450* dependent enzyme system, designated as aromatase, in conjunction with a reductase (NADPH–cytochrome–P-450 reductase).[3] The function of the reductase is to transfer, in stepwise fashion, two electrons from NADPH to the haem-iron of the cytochrome. The mechanisms of the reactions catalysed by aromatase have been studied through the use of stereo and regiospecifically labelled substrates and the fate of each atom undergoing chemical change during the conversion is shown in Scheme 1.[4]

There is much recent medical interest in aromatase since inhibitors of the enzyme may be of value as contraceptives and also for treating oestrogen-dependent diseases, in particular breast cancer.[5] In more than 30% of breast cancer patients, the tumour is oestrogen-dependent and the current treatment of this type of breast cancer involves the use of anti-oestrogens with or without surgery. The most commonly used anti-oestrogens are compounds of the triphenylethylene type (*e.g.* tamoxifen) which are directed to the oestrogen receptor.[6] Recent work on animal models has, however, shown that tamoxifen possesses some oestrogenic activity and hence, instead of inhibiting, could promote tumour growth.[7] Another form of therapy being investigated is the use of aromatase inhibitors. An example of this is aminoglutethimide which inhibits oestrogen biosynthesis and indeed promotes tumour regression.[8] This compound is however a general inhibitor of P-450 enzymes and suffers from the disadvantage of affecting other steroidal transformations.[9] There is, therefore, need to design specific and potent inhibitors of aromatase and indeed



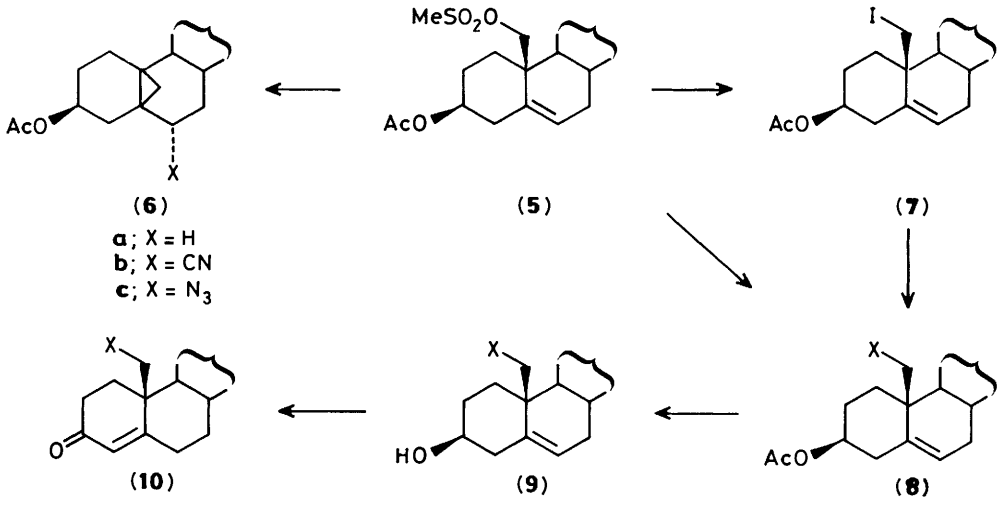
Scheme 1. Pathway for the biosynthesis of oestrone (**4**) from androstenedione (**1**) showing the origin and fate of the oxygen and hydrogen atoms involved in the transformation[4]

nearly a decade ago we initiated such a programme of work aimed at the synthesis of steroid compounds containing various heteroatoms at C-19. This paper is concerned with the synthesis and testing of the derivatives of androstenedione substituted with sulphur, nitrogen, and halogen atoms at C-19, the position that is the key target for aromatase. A preliminary account of part of this work has already been published.[10]

Results and Discussion

Synthesis of 19-Halogeno Derivatives of Androstenedione.—Methods for the introduction of a hydroxy group at position 19 of 3β-acetoxy-5-ene steroids have been available since the sixties.[11] It is also known that the *p*-tolylsulphonyl and

* P-450 type cytochromes contain haem b as a prosthetic group and derive their name from the difference spectrum obtained by the addition of CO to the reduced form when a peak at 450 nm is produced. The cytochromes of this class catalyse a variety of oxidative transformations but are particularly involved in hydroxylation reactions.



a; X = CN
b; X = N_3
c; X = $NHCOCH_2Cl$
d; X = $NHCO_2Bu^t$
e; X = NH
f; X = SMe
g; X = SEt
h; X = SPr
i; X = $SCH_2CH{=}CH_2$
j; X = $SCH_2C{\equiv}CH$
k; X = SOMe
l; X = SO_2Me

a; X = CN
b; X = N_3
c; X = NH_2
d; X = $NHCOCH_2Cl$
e; X = $NHCO_2Bu^t$
f; X = SMe
g; X = SEt
h; X = SPr
i; X = $SCH_2CH{=}CH_2$
j; X = $SCH_2C{\equiv}CH$

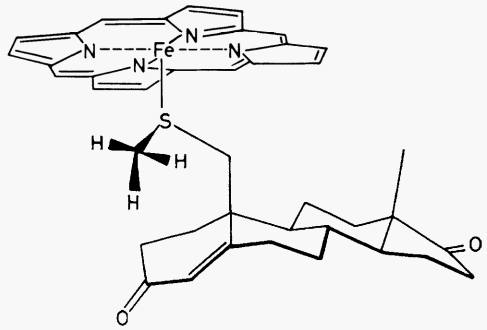
a; X = Br
b; X = Cl
c; X = CN
d; X = N_3
e; X = SSO_2Me
f; X = SH
g; X = OH

Scheme 2.

methylsulphonyl derivatives (**5**) of these 19-hydroxy compounds undergo smooth displacement with halides to produce the corresponding 19-halogenated steroids of the type (**7**) and (**8a,b**) (Scheme 2).[12] For the biological work described in this paper, we required 19-functionalized steroids in the 3-oxo-4-ene series which, in principle, could be obtained by an oxidation–isomerization reaction sequence from the 3β-hydroxy-5-ene compounds (**9**) and indeed such a protocol for the synthesis of 19-chloroandrost-4-ene-3,17-dione (**10m**) has already been described.[12] The more convenient sequence in our hands however, was the one in which 19-hydroxyandrostenedione following mesylation was converted into the bis(ethylene acetal) (**12**) and the latter then treated with LiCl, LiBr, or NaI in refluxing isopropylalcohol (Scheme 3). When carried out for 2 h, instead of giving the expected 19-halogenated compounds in the bis-ethylene acetal series, this reaction gave the deprotected compounds (**10m,n,o**) directly. Presumably, the displaced mesylate catalyses the deprotection. Since it is extremely difficult to displace the 19-methylsulphonyl group in steroids containing the 3-oxo-4-ene system,[13] the displacement must have occurred before deprotection. This was confirmed by the fact that reducing the reaction time from 2 h to 2 min enabled us to obtain the 19-iodide bis(ethylene acetal) (**13**) in high yield.

Displacement at C-19 *with* CN^- *and* N_3^-.—In contrast to the uncomplicated displacement of 19-methylsulphonyl or *p*-tolylsulphonyl groups of 5-ene stereoids with halides, the earlier attempts at achieving equivalent displacements with other nucleophiles such as H^-, CN^-, N_3^- had only produced rearranged compounds of the type (**6a,b,c**).[13] We then discovered that clean displacement at C-19, with a variety of nucleophiles might be achieved by using the 19-iodo compound (**7**) rather than the toluene-*p*-sulphonates or methanesulphonates. Treatment of the 19-iodo compound with NaCN or NaN_3 in hexamethylphosphoramide (HMPA) gave the corresponding substituted compounds (**8c**) and (**8d**) in high yields. The 19-azide (**9b**) was smoothly reduced, with H_2 in the presence of Lindlar catalyst[14] to the 19-amine (**9c**). The latter was converted into the *N*-chloroacetyl and *N*-t-butoxycarbonyl derivatives (**9d**) and (**9e**) by conventional methods. The 19-cyanide and the 19-azide (**9a**) and (**9b**) were converted into the biologically more important 4-en-3-one compounds (**10a,b**) [Marcotte and Robinson have published an alternative synthesis of compound (**10a**)[26]] by treatment with methanolic KOH followed by Jones oxidation[15] and isomerization with oxalic acid in refluxing ethanol. The 19-*N*-chloroacetyl compound (**9d**) was also converted into the corresponding conjugated ketone (**10c**) by oxidation with pyridinium chlorochromate[16] followed by acid catalysed isomerization. Because of the need to avoid acid conditions, the 19-*N*-t-butoxycarbonyl derivative (**9e**) was oxidized to the corresponding conjugated ketone (**10d**) using Oppenauer oxidation.[17] We had hoped to obtain 19-aminoandrost-4-ene-3,17-dione (**10e**) as its hydrochloride, from the hydrolysis of (**10d**) with HCl, however this expectation has not yet been satisfactorily realized. The difficulty of synthesizing this compound has been highlighted by the work of Lovett and co-workers who have tried several other routes but without success.[18]

Introduction of Sulphur at C-19.—We have recently described a convenient method for the preparation of ammonium methanethiosulphonate and have used the reagent for the introduction of sulphur into organic compounds.[19] Displacement of the 19-iodo substituent of (**7**) and (**13**) with ammonium methanethiosulphonate in HMPA was effected to give the corresponding 19-methylsulphonyl sulphide derivatives (**8e**) and (**14**) in good yields. The latter were converted into the



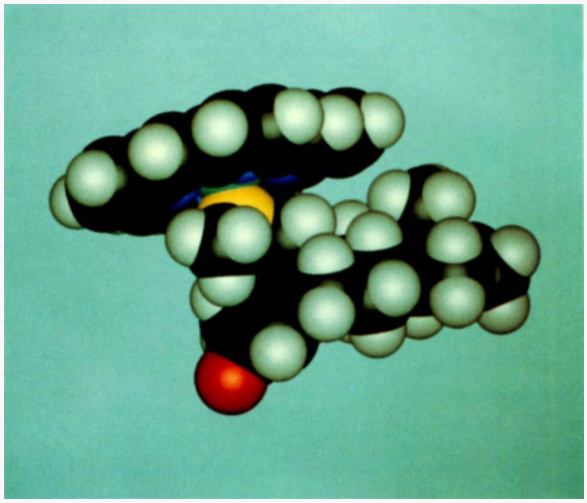


Figure. The illustration shows the restricted space available between the porphyrin ring of cytochrome P-450$_{(aromatase)}$ and the nucleus of the inhibitory steroid. Since in modelling studies the side chains of haem were not used these are also omitted in the illustration.

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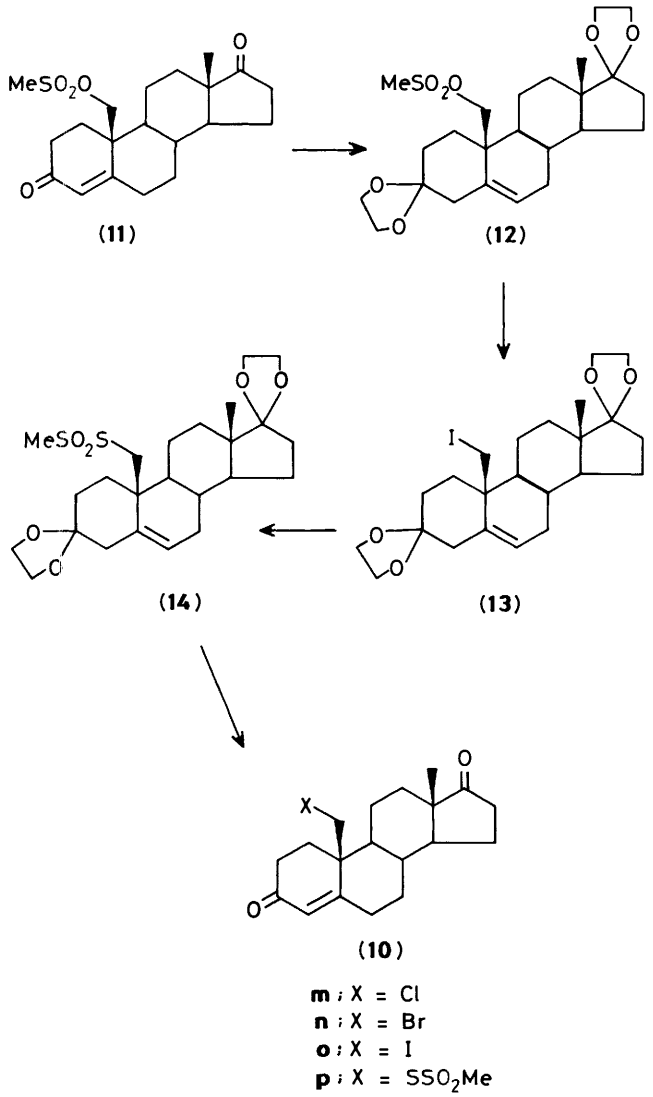

Scheme 3.

Table. The inhibition of aromatase by 19-substituted steroids with androstenedione used as the substrate (see Experimental section for details)

Compd.	[Substrate]/μM	[Inhibitor]/μM	% Inhibition
(10b)	0.1	0.1	84
(10c)	0.1	0.1	4
(10d)	0.1	0.1	17
(10f)	0.5	0.5	92
(10g)	0.5	2.5	75
(10h)	0.5	2.5	14
(10i)	0.5	2.5	38
(10j)	0.5	2.5	49
(10k)	0.5	2.5	14
(10l)	0.5	2.5	6
(10m)	0.5	0.5	35
(10n)	0.5	0.5	24
(10o)	0.5	0.5	7
(10p)	0.1	0.1	44

19-thiol derivatives by reduction with $LiAlH_4$ or more conveniently with dithiothreitol at pH 10 for 10 min at 25 °C. The latter procedure in one of the mildest methods available at present for the generation of mercapto groups from masked sulphides normally produced from nucleophilic displacement reactions. An alternative route to 19-thiols has been described which used potassium ethyl dithiocarbonate to displace 19-trifluoromethanesulphonate in the 4-en-3-one series.[19] The disadvantage of this method is that the reduction of the dithiocarbonate to thiol, using ethylenediamine gives a poor yield (36%). The mercapto group in 3β-acetoxy-19-mercapto-androst-5-en-17-one (**8f**) was treated with various alkyl halides to give the alkylthio derivatives (**9f—j**) which were transformed into the desired ring A conjugated ketones by Oppenauer oxidation.[17] The 19-methylthioandrost-4-ene-3,17-dione (**10f**) was also converted into either the sulphoxide (**10k**) or the sulphone (**10l**) using 1 and 2 mol equiv. respectively of *m*-choroperbenzoic acid.

The 19-methylsulphonyl sulphide in the 3,17-bis(ethylene acetal) series (**14**) was deprotected with toluene-*p*-sulphonic acid in acetone to give the corresponding 4-en-3-one compound (**10p**).

Enzymic Studies and Conclusions.—The source of enzyme used in the present study was a placental microsome preparation that contains aromatase as well as NADPH-cytochrome P-450 reductase. The enzyme reaction was monitored by determining the release of ^3H_2O from [1β,2β-^{3}H]-androst-4-ene-3,17-dione. Since it is known that in the aromatization process the 1β and 2β hydrogen atoms are the ones removed[4c,f] (Scheme 1), the release of ^3H_2O from the [1β,2β-^{3}H]substrate provides a convenient method for estimating the activity of aromatase. It should though be emphasized that in the calculation of the rate of product formation from the ^3H_2O released, no allowance is made for the possible operation of an isotopic effect. The results obtained by using the method are thus only valid for the comparative study of the type described here.

In order to estimate the inhibitory potency of the compounds synthesized in the present work, the tritiated substrate, a NADPH generating system and microsomes were incubated at 37 °C in air in the presence or absence of the inhibitor. The reaction was normally monitored for 20 min during which time the formation of oestrogen was linear. The Table records the percentage inhibition data and highlights that the 19-halogenated compounds (**10m,n,o**) were only moderate inhibitors. By contrast, some of the thioalkyl derivatives (**10f—j**) were strong inhibitors of the enzyme and the inhibitory potency progressively increased as the bulk of the alkyl substituent was decreased. The thiomethyl derivative (**10f**) at a substrate:inhibitor ratio of 1:1 completely abolished the activity of aromatase. A detailed kinetic study on the compound has revealed that the derivative combines with aromatase tightly in a slow-binding mode with an overall K_i value of 1×10^{-9}M (K_m of androstenedione 6.3×10^{-8}M). This compound is thus one of the most potent aromatase inhibitors described to date. We had originally shown that the thiomethyl compound (**10f**) interacts with aromatase through the co-ordination of the steroidal sulphur to the haem iron of aromatase[10] as shown in the Figure. It would now appear that co-ordination is most effective with the smallest alkyl substituent. This feature may be rationalized by assuming that, at the active site, the haem prosthetic group and the inhibitory steroid form a molecular sandwich in which the iron-sulphur bond is roughly central and orthogonal to the porphyrin and steroid nuclei. We envisage that the cavity produced in such a sandwich structure is both rather confined and insufficient to accommodate alkyl substituents larger than a methyl group. Our preliminary molecular modelling studies produced the computer-drawn picture of the Figure that adequately illustrates our intuitive view[10] of the binding mode of 19-methylthioandrost-4-ene-3,17-dione (**10f**) to the haem iron

of aromatase. If this view is extended to enzyme catalysis, then it can be concluded that in the Michaelis complex, the C-19 of the substrate is relatively tightly packed against the haem iron. This conclusion is further supported by the fact that the sulphoxide (**10k**) which though containing a potent oxygen ligand, had an impaired binding to aromatase because of the adverse steric repulsion caused by the bulk of an additional oxygen atom. In view of this consideration it was not surprising to find that the sulphone (**10l**) is a rather poor inhibitor of aromatase. The lack of inhibition by the sulphoxide (**10k**) is in contrast to the inhibition of the cholesterol side-chain cleavage enzyme (cyt-P450scc) by 22-thiacholesterol. In this case the inhibition by the sulphoxide derivatives was 10^3 times greater than that by the parent sulphide.[21]

The 19-methylsulphonyl sulphide (**10p**) is also a powerful aromatase inhibitor, causing time-dependent inhibition in the presence of NADPH and O_2. The mechanism of this is unknown and is at present the subject of further investigations.

Since our original discovery[10] that the C-19 sulphur analogues of steroids constitute a novel group of sixth ligand inhibitors for aromatase, another example of a similar phenomenon has been described by Kellis and Childers, and their colleagues.[22] This work has shown that the (19*R*)-10-thiiranylestr-4-ene-3,17-dione is a high affinity competitive inhibitor (K_i 2×10^{-9}M, K_m of androstenedione 6×10^{8}M) in which the sulphur atom of the 10-thiirane is bound to the haem iron. It was also shown that the 19*R*-isomer of this compound binds 80 fold more tightly than the 19*S*. This discrimination in favour of only one of the diastereosiomers of the thiirane reinforces the assertion that there is a rather restricted space around the C-19 binding region of aromatase.

The other powerful aromatase inhibitor was the 19-azide (**10b**) (K_i 5×10^{-9}M). We have obtained evidence to suggest that, in this case also, the inhibitor is locked in union with aromatase through the co-ordination of the azido ligand to the haem iron. It is interesting to note that our original demonstration[10] of the effectiveness of the 19-azido derivative (**10b**) as an inhibitor of aromatase has been confirmed in a perfused placental system[23] where it was the most potent of the inhibitors tested including 4-hydroxyandrostenedione which is an irreversible inactivating (suicide) inhibitor.[24] This indicates that powerful competitive inhibitors may have an advantage clinically where *in vivo* conditions are not necessarily ideal for suicide inactivation which requires low substrate and high O_2 and NADPH concentrations.

Experimental

Chemicals were generally obtained from B.D.H. Ltd, Poole, Dorset, or Aldrich Chemical Co Ltd, Gillingham, Dorset, except for the following: 3β-hydroxyandrost-5-en-17-one, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and dithiothreitol (DTT) (Sigma Chemical Co, Poole, Dorset); [1β,2β-^{3}H]androst-4-ene-3,17-dione (New England Nuclear, Stevenage).

M.p.s were measured on an Electrothermal melting-point apparatus and are uncorrected. I.r. spectra were recorded on a Pye-Unicam S.P. 1000 spectrometer in Nujol on NaCl plates. N.m.r. spectra were recorded on the following instruments: 60 MHz (Perkin-Elmer R12B), 100 MHz (Varian XL-100A), 270 MHz (JEOL GX-270), 360 MHz (Bruker AM-360). Mass spectra were recorded on an A.E.I. M.S.30 or a V.G. TS-250 instrument using electron impact (e.i.) at 70 eV or fast atom bombardment (f.a.b.) by a xenon beam at 30 μA, 10 kV with the sample suspended in glycerol. Mass spectra were recorded over the mass range *m/z* 100—520 and for e.i. were normalized to 100% for the most intense peak in each spectrum. Optical rotations, $[\alpha]_D$, were recorded on a Perkin-Elmer 141 polarimeter. Radioactivity was measured, in a solution of Labscint (Lablogic Ltd, Sheffield, UK), with a Phillips PW4700 Liquid Scintillation Counter, programmed for automatic quench correction.

Analytical and preparative t.l.c. plates were prepared from silica gel GF_{254} and PF_{254} respectively (Merck, Dormstadt, F.R.G.) according to the manufacturer's instructions.

Methods used to ensure Anhydrous Conditions.—Where moisture-sensitive reagents were used, full precautions were taken to ensure dryness. These included the flaming out of all apparatus, maintenance of a slight positive pressure of nitrogen, and the use of dry solvents. Tetrahydrofuran (THF) was freshly distilled under nitrogen, from sodium–benzophenone. Dimethyl sulphoxide (DMSO) was supplied dry, over molecular seive, by Fluka (Glossop, Derbyshire, UK).

3-*Acetoxy*-3β,19-*dihydroxyandrost*-5-*en*-17-*one* (**8g**).—This was prepared from 3β-acetoxy-5α-androst-5-en-17-one *via* 3β-acetoxy-5-bromo-6β,19-epoxy-5α-androstan-17-one[11a] which was reductively cleaved to give the desired alcohol (**8g**).[2]

19-*Hydroxyandrost*-4-*ene*-3,17-*dione* (**2a**).—This was prepared from 3β-acetoxy-5-bromo-6β,19-epoxy-5α-androstan-17-one (*vide supra*), using the method of Skinner and Akhtar[2] (based on the route developed by Syntex)[25] except that the reductive cleavage was performed using the method described for the synthesis of 19-[O^{18}]hydroxy-4-androstene-3,17-dione.[4a]

3β-*Acetoxy*-19-*methylsulphonylandrost*-5-*en*-17-*one* (**5**).—Compound (**8g**) (31.7 g) was dissolved in pyridine (dried over KOH; 100 ml) and methanesulphonyl chloride (10 ml) was added. After 1.5 h at room temperature the solution was diluted with water until the crystals of pyridinium chloride dissolved. The resulting solution was poured slowly into rapidly stirred dilute hydrochloric acid (7% w/v; 1 l). The colourless solid was filtered off at the pump and washed on the filter with dilute hydrochloric acid (500 ml) and water (500 ml), and then dried *in vacuo* over silica gel. The colourless solid (38.3 g, 98.4%), had m.p. 140—141.5 °C; $\nu_{max.}$(Nujol) 1 740, 1 260, 1 175, and 970 cm^{-1}; δ_H($CDCl_3$; 60 MHz), 0.90 (3 H, s, 18-Me), 1.96 (3 H, s, OAc), 2.95 (3 H, s, Me), 4.10 (1 H,d, J_{gem} 11 Hz, 19-H), 4.40 (1 H, d, J_{gem} 11 Hz, 19-H), and 5.64 (1 H, m, 6-H vinylic).

3β-*Acetoxy*-19-*iodoandrost*-5-*en*-17-*one* (**7**).—Compound (**5**) (23 g) was added to a hot saturated solution of sodium iodide (75 g) in butan-2-one (500 ml) that had been clarified by the addition of a small volume of water (*ca.* 15 ml). The solution immediately became cloudy and was heated at reflux for 3 h. The resultant was washed with water (3 × 250 ml) and dilute aqueous sodium thiosulphate (2 × 100 ml). Any reduction in the volume of organic solvent was counteracted by the addition of ethyl acetate. The organic solution was dried ($MgSO_4$), filtered, and evaporated to provide a yellow gum. This, upon tituration with diethyl ether, yielded pale yellow crystals (18.81 g, 76%), m.p. 144—145 °C; $[\alpha]_D^{20}$ −42.7° (*c* 1.0, $CHCl_3$); $\nu_{max.}$(Nujol) 1 735, 1 260, and 1 030 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.95 (3 H, s, 18-Me), 1.97 (3 H, s, OAc), 3.20 (1 H, d, J_{gem} 11 Hz, 19-H), 3.62 (1 H, d, J_{gem} 11 Hz, 19-H), 4.2—4.7 (1 H, br m, 3α-H), 5.65 (1 H, s, 6-H vinylic); *m/z* 396 (M − AcOH)$^+$ (57%), 328 (M − HI)$^+$ (4.5), and 269 (M − AcOH − I)$^+$ (100) (Found: C, 55.4; H, 6.6. $C_{21}H_{29}IO_3$ requires C, 55.70; H, 6.41%).

3β-*Acetoxy*-19-*methylsulphonylthioandrost*-5-*en*-17-*one* (**8e**).—Compound (**7**) (20 g) and ammonium methanethiosulphonate[19] (20 g) were stirred under argon in HMPA (200 ml) at 80 °C for 7 h. The brown solution was then poured slowly into a beaker of rapidly stirred water (1.5 l). The precipitate was

filtered off at the pump and washed on the filter with water before dessication. The crude, brown product (19.2 g) was purified by column chromatography on silica gel (15 g/g steroid) using chloroform as the eluant. This yielded a creamy coloured crystalline compound (14.1 g, 73%), m.p. 175—178 °C; $[\alpha]_D^{20}$ −84.7° (*c* 1.1, $CHCl_3$); δ_H($CDCl_3$; 60 MHz) 1.04 (3 H, s, 18-Me), 2.08 (3 H, s, OAc), 3.34 (1 H, d, J_{gem} 11 Hz, 19-H), 3.76 (1 H, d, J_{gem} 11 Hz, 19-H), 3.36 (3 H, s, $MeSO_2S$), 4.3—4.9 (1 H, br m, 3α-H), and 5.7 (1 H, m, 6-H vinylic); $\nu_{max.}$(Nujol) 1 735, 1 340, 1 250, and 1 125 cm^{-1}; *m/z* 361 ($M - SO_2Me$)$^+$ (10%), 301 ($M - SO_2Me - AcOH$)$^+$ (100), and 255 ($M - CH_2SSO_2Me - AcOH$)$^+$ (47) (Found: C, 60.0; H, 7.5. $C_{22}H_{32}O_5S_2$ requires C, 59.97; H, 7.32%).

3β-*Acetoxy*-19-*mercaptoandrost*-5-*en*-17-*one* (**8f**).—Purified compound (**8e**) (15.0 g) and dithiothreitol (5.9 g) were stirred in methanol (150 ml) containing aqueous tris(hydroxymethyl)-aminomethane (2.75 M; 15 ml) at room temperature until a clear solution was obtained. After a further 10 min, the volume was reduced (to *ca.* 15 ml) on a rotary evaporator and the concentrated solution poured into water (700 ml). The resultant suspension was extracted with diethyl ether (4 × 100 ml). The combined organic extracts were washed with water (4 × 150 ml), dried ($MgSO_4$), and evaporated to yield a clear colourless gum. This was normally used directly for further steps. An analytical sample was obtained by crystallization from aqueous methanol to yield white crystals, m.p. 119—121 °C; $\nu_{max.}$(Nujol) 1 740, 1 250, and 1 035 cm^{-1}; δ_H($CDCl_3$; 360 MHz) 0.98 (1 H, s, 18-Me), 2.04 (3 H, s, OAc), 3.18 (1 H, dd, *J* 11 Hz and *J* 4 Hz, 19-H), (signals from the other 19-CH are obscured by the steroid CH and CH_2 envelope), 4.65 (1 H, m, 3α-H), and 5.78 (1 H, m, 6-H vinylic); *m/z* 362 (M^+) (20%) 348 (10), 302 (75), and 255 (100) (M^+, 362.1913. $C_{21}H_{30}O_3S$ requires *M*, 362.1956).

3β-*Hydroxy*-19-*methylthioandrost*-5-*en*-17-*one* (**9f**).—The gum (**8f**) (*vide supra*) was dissolved in methanol (150 ml) and treated with aqueous potassium hydroxide (10M; 5 ml). This solution gives a strong positive reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) solution. Iodomethane (7.5 ml) was added and the disappearance of the thiol group was followed with DTNB solution. Aqueous potassium hydroxide solution (10M; 10 ml) was added and the solution left at room temperature for 2 h before the volume was reduced (to *ca.* 50 ml) on a rotary evaporator. The concentrated solution was poured into dilute hydrochloric acid (6% w/v; 700 ml) which was then extracted with ethyl acetate (4 × 100 ml). The combined organic layers were washed with water (4 × 150 ml), dried ($MgSO_4$), filtered, and evaporated to yield a pale yellow gum (9.33 g, 82%). Recrystallization of this gum from ethyl acetate–hexane yielded pale yellow crystals (7.94 g, 69.5%), m.p. 168—172 °C; $[\alpha]_D^{20}$ −67.6° (*c* 1.1, $CHCl_3$); $\nu_{max.}$(Nujol) 3 480 and 1 730 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.98 (3 H, s, 18-Me), 2.03 (3 H, s, SMe), 2.53 (1 H, d, J_{gem} 11 Hz, 19-H), 2.98 (1 H, d, *J* 11 Hz, 19-H), 3.1—3.8 (1 H, br m, 3α-H), and 5.54 (1 H, m, 6-H, vinylic); *m/z* 334 (M^+ 1%), 317 ($M - OH$)$^+$ (20), and 256 ($M - OH - MeSCH_2$)$^+$ (100) (M^+, 334.1985. $C_{20}H_{20}O_2S$ requires 334.1966).

19-*Methylthioandrost*-4-*ene*-3,17-*dione* (**10f**).—Compound (**9f**) (4.92 g) was refluxed in toluene (250 ml) containing cyclohexanone (20 ml) under a Dean and Stark separator until the distillate was no longer turbid. Aluminium t-butoxide (8.0 g) was added and the mixture refluxed for 30 min. The solution was then washed with dilute hydrochloric acid (6% w/v; 3 × 200 ml), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield an oil. This was heated at 100 °C at 10 mmHg for 20 min, and then poured in light petroleum (b.p. 60—80 °C; 50 ml) and the mixture cooled overnight. The solid produced was chromatographed on silica gel (75 g) using chloroform as the eluant. The desired ketone was isolated pure as colourless crystals (2.83 g, 57%), m.p. 159 °C; $[\alpha]_D^{20}$ +194° (*c* 0.9, $CHCl_3$); $\nu_{max.}$(Nujol) 1 735 and 1 665 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.93 (3 H, s, 18-Me), 2.05 (3 H, s, SMe), 2.70 (1 H, d, J_{gem} 11 Hz, 19-H), 3.03 (1 H, d, J_{gem} 11 Hz, 19-H), and 5.79 (1 H, br s, 4-H vinylic); *m/z* 332 (M^+) (75%), 271 (100), and 284 (50) (Found: C, 72.3; H, 8.5. $C_{20}H_{28}O_2S$ requires C, 72.25; H, 8.5).

19-*Alkylthio*-3-*hydroxy*-5-*ene Steroids* (**9g—j**).—These compounds were synthesized by treating the 19-thiol (**8f**) with a variety of alkyl halides using the method as described above for the synthesis of (**9f**). The reagents used were: iodoethane for (**9g**); 1-iodopropane for (**9h**); allyl iodide for (**9i**); prop-2-ynyl bromide for (**9j**). The data obtained are reported below.

19-*Ethylthio*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9g**) (64%) had m.p. 116—118 °C; $\nu_{max.}$(Nujol) 3 250 and 1 735 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.87 (3 H, s, 18-Me), 1.13 (3 H, t, *J* 7 Hz, CH_3CH_2S), 2.95 (1 H, d, J_{gem} 11 Hz, 19-H), 3.47 (1 H, br m, 3α-H), and 5.49 (1 H, br m, 6-CH-vinylic); *m/z* 330 ($M - H_2O$)$^+$ (10%), 288 ($M - MeCHS$)$^+$ (15), and 255 ($M - EtSCH_2 - H_2O$)$^+$ (100); *m/z* (f.a.b.-m.s.) 349 ($M + H$)$^+$ and 331 ($M + H - H_2O$)$^+$.

3β-*Hydroxy*-19-*propylthioandrost*-5-*en*-17-*one* (**9h**) had m.p. 97—101 °C; $\nu_{max.}$(Nujol) 3 310, 1 740, and 1 060 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.99 (3 H, s, 18-Me), 3.08 (1 H, d, J_{gem} 11 Hz, 19-H), 3.55 (1 H, br m, 3α-H), and 5.58 (1 H, br m, 6-CH-vinylic); *m/z* 344 ($M - H_2O$)$^+$ (10%) and 255 ($M - PrSCH_2$)$^+$ (60); *m/z* (f.a.b.-m.s.) 363 ($M + H$)$^+$ and 344 ($M - H_2O$)$^+$.

3β-*Hydroxy*-19-*allylthioandrost*-5-*en*-17-*one* (**9i**) had m.p. 113—115 °C; $\nu_{max.}$(Nujol) 3 320, 1 740, 1 640, 1 230, 1 055, and 910 cm^{-1}; δ_H($CDCl_3$; 270 MHz) 0.995 (3 H, s, 18-Me), 2.118 (1 H, d, J_{gem} 10 Hz, 19-H), 2.467 (1 H, d, J_{gem} 11 Hz, 19-H), 3.090 (1 H, d, *J* 7 Hz, =CHCH_2S) 3.170 (1 H, d, *J* 7 Hz, =CHCH_2S), 3.564 (1 H, m, 3α-H), 5.133 (2 H, m, H_2C=CH), 5.616 (1 H, br d, 6-H vinylic), and 5.780 (1 H, m, H_2C=C*H*CH_2); *m/z* 360 (M^+) (25%), 342 ($M - OH$)$^+$ (30), 320 ($M - CH_2$=CHCH)$^+$ (50), and 302 ($M - H_2O - CH_2$=CHCH)$^+$ (100) (M^+, 360.2106. $C_{22}H_{32}O_2S$ requires *M*, 360.2123.

3β-*Hydroxy*-19-(*prop*-2-*ynylthio*)*androst*-5-*en*-17-*one* (**9j**) had m.p. 111.5—113 °C; $\nu_{max.}$(Nujol) 1 740, 1 055, and 795 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.95 (3 H, s, 18-Me), 2.67 (1 H, d, J_{gem} 12 Hz, 19-H), 3.18 (1 H, d, J_{gem} 12 Hz, 19-H), 3.0—3.7 (3 H, m, 3α-H and ≡CCH_2S), and 5.57 (1 H, m, 6-H vinylic); *m/z* 358 (M^+) (25%), 319 ($M - HC{\equiv}CCH_2$)$^+$ (10), 310 ($M - HC{\equiv}CCH_2 - H_2O$)$^+$ (45), and 255 ($M - HC{\equiv}CCH_2SCH_2 - H_2O$)$^+$ (100) (M^+, 358.1967. $C_{22}H_{30}O_2S$ requires *M*, 358.1967).

19-*Alkylthio*-3-*oxo*-4-*ene Steroids* (**10g—j**).—These compounds were synthesized from the corresponding 3-hydroxy-5-ene compounds (**9g—j**) by Oppenauer oxidation [17] as described above for the synthesis of (**10f**). The data obtained are reported below.

19-*Ethylthioandrost*-4-*ene*-3,17-*dione* (**10g**) had m.p. 113.5—115 °C; $\nu_{max.}$ 1 745, 1 680, 1 630, 1 275, 1 045, and 870 cm^{-1}; δ_H($CDCl_3$; 360 MHz) 0.94 (3 H, s, 18-Me), 1.26 (3 H, t, *J* 7.4 Hz, CH_3CH_2S), 2.54 (2 H, q, *J* 7.4 Hz, CH_3CH_2S), 2.80 (1 H, d, J_{gem} 12 Hz, 19-H), 3.05 (1 H, d, J_{gem} 12 Hz, 19-H), and 5.88 (1 H, d, *J* 1.4 Hz, 4-H vinylic); *m/z* 346 (M^+) (100%), 317 ($M - Et$)$^+$ (55), 248 ($M - EtSH$)$^+$ (100), 271 ($M - EtSCH_2$)$^+$ (100), and 253 (50) (M^+, 346.1975. $C_{21}H_{30}O_2S$ requires *M*, 346.1967).

19-*Propylthioandrost*-4-*ene*-3,17-*dione* (**10h**) had m.p. 111—113 °C; $\nu_{max.}$(Nujol) 1 745, 1 675, 1 620, 1 040, and 865 cm^{-1}; δ_H($CDCl_3$; 360 MHz) 0.94 (3 H, s, 18-Me), 0.98 (3 H, t, *J* 7.3 Hz, $CH_3CH_2CH_2S$), 2.49 (2 H, q, *J* 7.1 Hz, $CH_3CH_2CH_2S$), 2.78 (1 H, d, J_{gem} 11.7 Hz, 19-H), 3.04 (1 H, d, J_{gem} 11.7 Hz, 19-H), and 5.88 (1 H, d, *J* 1.3 Hz, 4-H vinylic); *m/z* 360 (M^+) (100%), 317 ($M - Pr$)$^+$ (30), 284 ($M - PrSH$)$^+$ (100), 271 ($M - PrSCH_2$)$^+$ (100), and 253 ($M - PrSCH_2 - H_2O$)$^+$ (30) (M^+, 360.2159. $C_{22}H_{32}O_2S$ requires *M*, 360.2132).

19-*Allylthioandrost*-4-*ene*-3,17-*dione* (**10i**) had m.p. 142—144 °C; $[\alpha]_D^{20}$ +174.5 (*c* 0.9, $CHCl_3$); $\nu_{max.}$(Nujol) 1 738 and 1 672 cm^{-1}; δ_H($CDCl_3$; 270 MHz) 0.925 (3 H, s, 18-Me), 2.172 (1 H, d, J_{gem} 11.7 Hz, 19-H), 2.934 (1 H, d, J_{gem} 11.7 Hz, 19-H), signals for C^aH_2=C^bH-C^cH_2-S, 3.114 (2 H, d, J_{cb} 7.1 Hz, CH_2=CHCH_2S), 5.065—5.160 (2 H, m, CH_2=$CHCH_2$S), 5.760 (1 H, ddt, 12 peaks resolved, $J_{ba(cis)}$ 10.01 Hz, $J_{ba(trans)}$ 16.84 Hz, J_{bc} 7.08 Hz, CH_2=$CHCH_2$S), and 5.878 (1 H, s, 4-H vinylic); *m/z* 358 (M^+) (20%), 317 (M − CH_2=$CHCH_2$)$^+$ (90), 284 (M − CH_2=$CHCH_2SH$)$^+$ (15), 271 (M − CH_2=$CHCH_2SCH_2$)$^+$ (20), and 253 (M − CH_2=$CHCH_2SCH_2$ − H_2O)$^+$ (10) (M^+, 358.1929. $C_{22}H_{30}O_2S$ requires M, 358.1966).

19-(*Prop*-2-*ynylthio*)*androst*-4-*ene*-3,17-*dione* (**10j**) had m.p. 147—150.5 °C; $\nu_{max.}$(Nujol) 1 745, 1 680, 1 630, 1 245, 1 045, 885, and 795 cm^{-1}; δ_H($CDCl_3$; 360 MHz) 0.94 (3 H, s, 18-Me), 2.96 (1 H, d, J_{gem} 12 Hz, 19-H), 3.22 (1 H, d, J_{gem} 12 Hz, 19-H), 3.24 (2 H, d, *J* 3 Hz, CH≡CCH_2S), 5.88 (1 H, d, *J* 1.4 Hz, 4-H vinylic); *m/z* 356 (M^+) (25%), 317 (M − CH≡CCH_2)$^+$ (65), 271 (M − CH≡CH_2SCH_2)$^+$ (45), and 253 (M − CH≡CCH_2-SCH_2 − H_2O)$^+$ (100) (M^+, 356.1839. $C_{22}H_{28}O_2S$ requires M, 356.1810).

19-*Methylsulphinylandrost*-4-*ene*-3,17-*dione* (**10k**).—A solution of *m*-chloroperbenzoic acid (325 mg) in dichloromethane (10 ml) was added to a solution of compound (**10f**) (500 mg) in dichloromethane (10 ml). After 15 min at room temperature the mixture was washed with saturated aqueous sodium hydrogen carbonate (2 × 50 ml) and water (2 × 50 ml), dried ($MgSO_4$), filtered, and evaporated to give a colourless crystalline solid (477 mg, 91%), m.p. 195—200 °C (decomp); $\nu_{max.}$(Nujol) 1 743, 1 765, and 1 045 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.89 (3 H, s, 18-Me), 2.63 (3 H, s, MeSO), and 5.88 (1 H, br s, 4-H vinylic); *m/z* 331 (M − OH)$^+$ (40%), 316 (10), 284 (M − CH_2SOCH_2)$^+$ (40), and 270 (10); *m/z* (f.a.b.-m.s.) 349 (M + H^+), 285 (M + H − CH_2SOCH_2)$^+$, and 267.

19-*Methylsulphonylandrost*-4-*ene*-3,17-*dione* (**10l**).—A solution of *m*-chloroperbenzoic acid (650 mg) in dichloromethane (20 ml) was added to a solution of compound (**10f**) (500 mg) in dichloromethane (10 ml). After 15 min at room temperature the mixture was washed with saturated aqueous sodium hdyrogen carbonate (2 × 50 ml) and water (2 × 50 ml), dried ($MgSO_4$), filtered, and evaporated to give a colourless crystalline solid (509 mg, 93%), m.p. 140—141.5 °C; $\nu_{max.}$(Nujol) 1 729, 1 665, 1 308, and 1 140 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.90 (3 H, s, 18-Me), 2.94 (3 H, s, $MeSO_2$), 3.26 (1 H, d, J_{gem} 14 Hz, 19-H), 3.73 (1 H, d, J_{gem} 14 Hz, 19-H), 5.85 (1 H, br s, 4-H vinylic); *m/z* 364 (M^+) (40%), 284 (M − $MeSO_2H$)$^+$ (35), and 271 (M − SO_2CH_2)$^+$ (40) (M^+, 364.1714. $C_{20}H_{28}O_4S$ requires M, 364.1708).

3β-*Acetoxy*-19-*azidoandrost*-5-*en*-17-*one* (**8d**).—Compound (**7**) (1 g) was added to a suspension of finely divided sodium azide (70 mg) in HMPA (10 ml) and stirred under argon at 80 °C for 7 h. The reaction mixture was poured into cold water (200 ml) and extracted with diethyl ether (3 × 50 ml). The combined extracts were washed with water (100 ml), dried (Na_2SO_4), and evaporated under reduced pressure to give an oil which, on addition of ethyl acetate–light petroleum, yielded a white crystalline solid (0.5 g, 62%), m.p. 131—132 °C; $[\alpha]_D^{20}$ −93.0° (*c* 1.2, $CHCl_3$); $\nu_{max.}$(Nujol) 2 060, 1 740, and 1 260 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.89 (3 H, s, 18-Me), 1.97 (3 H, s, OAc), 3.23 (1 H, d, J_{gem} 12.6 Hz, 19-H), 3.73 (1 H, d, J_{gem} 12.6 Hz, 19-H), 4.6 (1 H, br m, 3α-H), and 5.7 (1 H, m, 6-CH-vinylic); *m/z* 343 (M − N_2)$^+$, (3%), 315 (M − CH_2N_2)$^+$ (3), 283 (M − N_2 − AcOH)$^+$ (20), 256 (94), 255 (M − CH_2N_2 − OAc)$^+$ (100), and 237 (12) (Found: C, 68.1; H, 8.1; N, 11.4. $C_{21}H_{29}N_3O_3$ requires C, 67.9; H, 7.87; N, 11.31%).

19-*Azido*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9b**).—Compound (**8d**) (0.5 g) was dissolved in a 5% solution of potassium hydroxide in methanol (10 ml). After 3 h at room temperature, the mixture was cooled in ice–water and neutralized with aqueous acetic acid (2 M) and the product was crystallized by dropwise addition of water. The white crystals were filtered off, washed with water, and dried *in vacuo* overnight to yield the product (0.4 g, 90%), m.p. 136—137 °C, $[\alpha]_D^{20}$ −108.7° (*c* 1.3, $CHCl_3$; $\nu_{max.}$(Nujol) 3 500, 3 250, 2 080, and 1 740 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.95 (3 H, s, 18-Me), 3.21 (1 H, d, J_{gem} 12.6 Hz, 19-H), and 3.72 (1 H, d, J_{gem} 12.6 Hz, 19-H) (the signals from 19-CH_2 were superimposed on the signal for 3α-H), and 5.65 (1 H, m, 6-CH-vinylic); *m/z* 301 (M − N_2)$^+$ (5%), 286 (M − N_3H)$^+$ (2), 283 (M − N_2 − H_2O)$^+$ (5), 273 (M − CH_2N_3)$^+$ (22), and 255 (M − CH_2N_3 − H_2O)$^+$ (100) (Found: C, 68.3; H, 8.7; N, 12.8. $C_{19}H_{27}N_3O_2$ requires C, 69.28; H, 8.26; N, 12.75%).

19-*Azidoandrost*-4-*ene*-3,17-*dione* (**10b**).—Compound (**9b**) (0.2 g) was dissolved in acetone (distilled from $KMnO_4$) (5 ml) and stirred under argon at 0 °C. Jones reagent[15] (0.25 ml) was added dropwise and the reaction continued for a further 5 min. Excess of Jones reagent was destroyed by the addition of a few drops of methanol and the product precipitated by the slow addition of ice-cold water. The crude product was filtered off and recrystallized from methanol to yield 19-azidoandrost-5-ene-3,17-dione (120 mg). This was dissolved in 95% aqueous ethanol (10 ml) containing oxalic acid dihydrate (126 mg) and refluxed for 75 min. After dilution with water the product was extracted into chloroform, washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated under reduced pressure. The resulting oil was crystallized from diethyl ether to yield white crystals (85 mg), m.p. 90—91 °C; $[\alpha]_D^{20}$ +273.6° (*c* 1.1, $CHCl_3$); $\nu_{max.}$(Nujol) 2 090, 1 740, and 1 675 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.88 (3 H, s, 18-Me), 3.51 (1 H, d, J_{gem} 12 Hz, 19-H), 3.77 (1 H, d, J_{gem} 12 Hz, 19-H), and 5.87 (1 H, s, 4-CH-vinylic); *m/z* 299 (M − N_2)$^+$ (23%), 279 (31), 272 (100), and 271 (M − CH_2N_3)$^+$ (100) (Found: C, 70.0; H, 7.6. $C_{19}H_{25}N_3O_2$ requires C, 69.7; H, 7.7%).

19-*Amino*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9c**).—Compound (**9b**) (0.2 g) was dissolved in ethanol (5 ml) and stirred with Lindlar catalyst,[14] (5% Pd/$CaCO_3$ poisoned with lead; 80 mg) under 1 atm of hydrogen at room temperature for 4.5 h. The catalyst was filtered off and the filtrate evaporated under reduced pressure. The resulting oil was crystallized from ethanol–light petroleum to yield white crystals (130 mg, 70%), m.p. 285 °C (decomp.); $\nu_{max.}$(Nujol) 3 390, 3 280, and 1 740 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.93 (3 H, s, 18-Me), 2.65 (1 H, d, J_{gem} 13.2 Hz, 19-H), 3.08 (1 H, d, J_{gem} 13.2 Hz, 19-H), 3.5 (1 H, m, 3α-H), 5.65 (1 H, m, 6-CH-vinylic); *m/z* 285 (M − NH_4)$^+$ (21%), 272 (M − CH_2NH_3)$^+$ (65), and 256 (100) (Found: C, 74.2; H, 9.4; N, 4.5. $C_{19}H_{24}NO_2$ requires C, 75.21; H, 9.62; N, 4.61). Analysis by t.l.c. on silica gel G plates developed in ethyl acetate–acetone (7:3) made basic with a few drops of 35% aqueous ammonia, showed the product as a ninhydrin positive spot R_F 0.32 compared to starting material (**9b**), R_F 0.59 (visualized with iodine vapour).

19-*t*-*Butoxycarbonylamino*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9e**).—Compound (**9c**) (0.3 g) was dissolved in methanol and the solution made basic with a few drops of aqueous K_2CO_3. Di-t-butyl dicarbonate (0.24 g) was added and the mixture stirred at room temperature for 1 h. The reaction mixture was maintained at a basic pH by further addition of aqueous K_2CO_3. After the mixture had been poured into water, the product was extracted into ethyl acetate, and the extract dried (Na_2SO_4), and evaporated under reduced pressure. Addition of diethyl ether–light petroleum to the residue yielded white crystals (300 mg, 75%), m.p. 203—204 °C; $\nu_{max.}$(Nujol) 3 500, 3 440, 1 750, 1 700,

and 1 530 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.85 (3 H, s, 18-Me), 1.37 (9 H, s, CO_2Bu^t), 3.35 (2 H, m, 19-CH_2) superimposed on 3.5 (1 H, m, 3α-H), 4.1 (1 H, br m, NH), and 5.6 (1 H, m, 5-CH vinylic); *m/z* 386 (M − OH)$^+$ (3%), 329 (M − HOCBu^t)$^+$ (45), 286 (11), 272 (M − CH_2NHBoc)$^+$ (24), 268 (100), and 255 (M − OH − CH_2NHBoc)$^+$ (100).

19-*t-Butoxycarbonylamino*-3β-*hydroxyandrost*-4-*ene*-3,17-*dione* (**10d**).—Compound (**9e**) (0.3 g) was subjected to Oppenauer oxidation[17] as described above for compound (**10f**), except that during work-up the reaction mixture was washed with aqueous potassium sodium tartrate instead of dilute HCl. The product was crystallized from ethyl acetate–light petroleum without the need for column chromatography to yield white crystals (0.2 g, 66%), m.p. 185—187 °C; $[\alpha]_D^{20}$ +121.3° (*c* 0.7, $CHCl_3$); $\nu_{max.}$(Nujol) 3 320, 1 755, 1 730, 1 670, 1 560, 1 265, and 1 180 cm^{-1}; δ_H($CDCl_3$; 100 MHz) 0.92 (3 H, s, 18-Me), 1.44 (9 H, s, CO_2Bu^t), 3.58 (2 H, m, 19-CH_2), 4.42 (1 H, br m, NH), and 5.98 (1 H, s, 4-H-vinylic); *m/z* 401 (M)$^+$ (2%), 345 (M − $CH_2C(CH_3)_2$ (6), 328 (11), and 272 (M − CH_2NHBoc)$^+$ (100) (M^+, 401.2572. $C_{24}H_{35}NO_4$ requires M, 401.2566).

19-*Chloroacetamido*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9d**).—Compound (**9c**) (100 mg) suspended in dry THF (5 ml) was stirred with a stoicheiometric amount (46 μl) of dry triethylamine. Chloroacetic anhydride (70 mg) was added to give immediate dissolution of the steroid. After 15 min at 25 °C, the mixture was poured into ice-cold water and extracted into chloroform; the extract was then dried (Na_2SO_4) and evaporated under reduced pressure. The resulting oil was crystallized from diethyl ether to yield a white crystalline solid (100 mg, 80%), m.p. 158—160 °C; $[\alpha]_D^{22}$ +26.8° (*c* 1.1, $CHCl_3$); $\nu_{max.}$(Nujol) 3 400, 1 725, 1 670, and 1 540; δ_H($CDCl_3$; 60 MHz) 0.8 (3 H, s, 18-Me), 3.5 (2 H, m, 19-CH_2N superimposed on 1 H, m, 3α-H), 3.95 (2 H, s, $COCH_2Cl$), 5.7 (1 H, m, 5-H vinylic), and 6.4 (1 H, br m, NH); *m/z* 381/379 (M^+) (0.6/1.7%), 363/361 (M − H_2O)$^+$ (1/3), 286 (47), and 255 (M − $CH_2NHCOCH_2$-Cl − H_2O)$^+$ (100).

19-*Chloroacetamidoandrost*-4-*ene*-3,17-*dione* (**10c**).—Compound (**9d**) (50 mg) dissolved in methylene dichloride was rapidly stirred with pyridinium chlorochromate (60 mg) and crushed molecular sieve type 3A (80 mg) for 25 min at 25 °C.[16] The reaction mixture was poured into diethyl ether (200 ml) and filtered through a small plug of silica gel. The clear filtrate was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to leave a pale yellow oil. This was dissolved in acetone (5 ml) containing toluene-*p*-sulphonic acid (4 mg) and the solution left at 25 °C overnight. The product was extracted into methylene dichloride, washed with aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated under reduced pessure. The resulting oil was purified on preparative t.l.c. plates (silica gel PF_{254}) developed in ethyl acetate–chloroform–methanol (49:49:2). The product was located under u.v. light as a dark band (R_F 0.26) which ran just ahead of a non-u.v. absorbing impurity (R_F 0.2) which stained strongly in iodine vapour. The product was eluted with ethyl acetate–methanol (99:1) and the solvent removed under reduced pressure to leave an oil which, on addition of diethyl ether, yielded colourless crystals (32 mg, 64%), m.p. 141—142 °C; $\nu_{max.}$(Nujol) 3 280, 1 740, 1 675, and 1 530 cm^{-1}; δ_H($CDCl_3$; 100 MHz) 0.94 (3 H, s, 18-Me), 3.73 (2 H, d, *J* 6 Hz, 19-CH_2NH), 4.02 (2 H, s, $COCH_2Cl$), 6.02 (1 H, s, 4-H vinylic), and 6.54 (1 H, br m, NH); *m/z* 379/377 (M^+) (2/6%) and 272 (M − $CH_2NHCOCH_2Cl$)$^+$ (100) (M^+, 377.1746. $C_{21}H_{28}ClNO_3$ requires M, 377.1758).

3β-*Acetoxy*-19-*cyanoandrost*-5-*en*-17-*one* (**8c**).—Powdered sodium cyanide (0.5 g) was suspended in HMPA (10 ml) and stirred under N_2 at 80 °C for 20 min. The 19-iodide (**7**) (1 g) was then added and the reaction continued for 5.5 h. The reaction mixture was poured into water (500 ml) containing a trace of sodium sulphite and extracted into diethyl ether (2 × 300 ml); the extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to leave an oil which, on addition of diethyl ether–light petroleum, yielded a white crystalline solid (0.7 g, 90%), m.p. 159—162 °C; $[\alpha]_D^{20}$ −28.5° (*c* 1.0, $CHCl_3$); $\nu_{max.}$(Nujol) 1 730 and 1 260 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.95 (3 H, s, 18-Me), 2.0 (3 H, s, OAc), 2.42 (1 H, d, J_{gem} 18 Hz, partially obscured by steroid CH envelope, 19-H), 2.68 (1 H, d, J_{gem} 18 Hz, 19-H), 4.55 (1 H, br m, 3α-H), 5.7 (1 H, m, 6-H vinylic); *m/z* 295 (M − HOAc)$^+$ (53%), 256 (13) and 254 (M − OAc − CH_2CN)$^+$ (100); [(M − HOAc)$^+$, 295.1895. $C_{20}H_{25}NO$ requires M 295.1936].

19-*Cyano*-3β-*hydroxyandrost*-5-*en*-17-*one* (**9a**).—Compound (**8c**) (0.6 g) was added to 5% methanolic potassium hydroxide (30 ml) and the mixture stirred at 25 °C for 3 h; it was then poured into dilute acetic acid (500 ml) and extracted into methylene dichloride; the extract was washed with water and aqueous sodium hydrogen carbonate and then evaporated under reduced pressure. The resulting oil was crystallized from ethyl acetate–light petroleum to yield white crystals (0.5 g, 94%), m.p. 205 °C; $[\alpha]_D^{20}$ −29.2° (*c* 1.0, $CHCl_3$); $\nu_{max.}$(Nujol) 3 480, 2 220, 1 730, and 1 065 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.93 (3 H, s, 18-Me), 2.39 (1 H, d, J_{gem} 18 Hz partially obscured by steroid CH envelope, 19-H), 2.65 (1 H, d, J_{gem} 18 Hz, 19-H), 3.45 (1 H, br m, 3α-H), 5.6 (1 H, m, 6-CH vinylic); *m/z* 313 (M^+) (23%), 295 (M − H_2O)$^+$ (21), 273 (18), 256 (23), and 254 (M − H_2O − MeCN)$^+$ (100) (M^+, 313.2030. $C_{20}H_{27}NO_2$ requires M, 313.2042).

19-*Cyanoandrost*-4-*ene*-3,17-*dione* (**10a**).—Compound (**9a**) (400 mg) was oxidized and isomerized under conditions identical to those used for the conversion of (**9b**) into (**10b**). Crude (**10a**) was recrystallized from ethanol to yield colourless crystals (250 mg, 62%), m.p. 178—180 °C (lit.,[26] m.p. 179—181 °C); $[\alpha]_D^{20}$ +174.5° (*c* 0.9, $CHCl_3$); $\nu_{max.}$(Nujol) 2 240w, 1 745, and 1 680 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.9 (3 H, s, 18-Me), 2.68 (2 H, s, 19-CH_2), and 5.85 (1 H, 4-CH vinylic); *m/z* 311 (M^+) (100%) and 148 (74) (M^+, 311.1876. $C_{20}H_{25}NO_2$ requires M, 311.1885).

19-*Methylsulphonylandrost*-4-*ene*-3,17-*dione* (**11**).—19-Hydroxyandrostenedione [compound of the type (**2**)] (1 g) was dissolved in dry pyridine (10 ml) and cooled to 0 °C. Methanesulphonyl chloride (0.5 ml) was added dropwise to the stirred solution under a blanket of nitrogen. The mixture was then stoppered and stirred at 25 °C for 1.5 h. Ice-cold water (100 ml) was added and the oil that separated, crystallized by scratching with a glass rod. The crude material was filtered off, redissolved in methylene dichloride, and the extract washed with dilute hydrochloric acid and aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated under reduced pressure. The resulting oil, on addition of ethyl ether, yielded white crystals (925 mg, 74%), m.p. 147—148 °C; $\nu_{max.}$(Nujol) 1 730, 1 670, 1 360, 1 175, and 970 cm^{-1}; δ_H($CDCl_3$; 60 MHz) 0.9 (3 H, s, 18-Me), 3.0 (3 H, s, SO_2Me), 4.41 (1 H, d, J_{gem} 10 Hz, 19-H), 4.55 (1 H, d, J_{gem} 10 Hz, 19-H), and 5.91 (1 H, s, 4-CH vinylic).

19-*Methylsulphonylandrost*-5-*ene*-3,17-*dione Bis*(*ethylene acetal*) (**12**).—Compound (**11**) (1.8 g) was dissolved in dry THF (32 ml) containing ethylene glycol (4.3 ml), trimethyl orthoformate (4.4 ml), and toluene-*p*-sulphonic acid (70 mg) and the reaction mixture was refluxed under nitrogen for 2 h. It

was then poured into saturated aqueous sodium hydrogen carbonate, extracted into methylene dichloride, and the extract washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to leave an oil. This on addition of methanol–diethyl ether gave white crystals (1.1 g, 50%), m.p. 86—88 °C; $\delta_H(CDCl_3$; 60 MHz) 0.87 (3 H, s, 18-Me), 2.92 (3 H, s, SO_2Me), 3.84 (8 H, m, 3 and 17 OCH_2CH_2O), 4.14 (1 H, d, J_{gem} 10 Hz, 19-H), 4.33 (1 H, d, J_{gem} 10 Hz, 19-H), and 5.57 (1 H, m, 6-CH vinylic).

19-*Iodoandrost*-5-*ene*-3,17-*dione Bis*(*ethylene acetal*) (**13**).—Compound (**12**) (0.2 g) was added to a rapidly stirred solution of sodium iodide (4 g) in refluxing isopropyl alcohol (20 ml). After 2 min, the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate containing a trace of sodium sulphite and the product extracted into methylene dichloride. The extract was washed with water (containing a trace of pyridine), dried (Na_2SO_4), and evaporated under reduced pressure to yield an oil. This on addition of diethyl ether, gave white crystals (139 mg, 65%), m.p. 104—106 °C; $\delta_H(CDCl_3$; 60 MHz) 0.92 (3 H, s, 18-Me), 3.24 (1 H, d, J_{gem} 11 Hz, 19-H), 3.5 (1 H, d, J_{gem} 11 Hz, 19-H), 3.83 (8 H, m, 3 and 17 OCH_2CH_2O), and 5.51 (1 H, m, 6-CH vinylic); *m/z* 373 $(M - I)^+$ (25%), 329 (100), and 311 (67).

19-*Iodoandrost*-4-*ene*-3,17-*dione* (**10o**).—This compound was synthesized from compound (**12**) by the method used to prepare compound (**13**) except that the reaction was allowed to continue for 2 h. Crystallization from chloroform–diethyl ether yielded white crystals, m.p. 143—147 °C; $[\alpha]_D^{20}$ +116.9° (*c* 1.0, $CHCl_3$); $\nu_{max.}$(Nujol) 1 735, 1 675, and 865 cm^{-1}; $\delta_H(CDCl_3$; 60 MHz) 0.88 (3 H, s, 18-Me), 3.37 (1 H, d, J_{gem} 11 Hz, 19-H), 3.63 (1 H, d, J_{gem} 11 Hz, 19-H), and 5.89 (1 H, s, 4-CH vinylic); *m/z* 285 $(M - I)^+$ (100%), 267 (23), and 243 (10) (Found: C, 55.3; H, 6.0. $C_{19}H_{25}IO_2$ requires C, 55.35; H, 6.11%).

19-*Bromoandrost*-4-*ene*-3,17-*dione* (**10n**).—Compound (**12**) (100 mg) was added to a refluxing solution of anhydrous lithium bromide in dry isopropyl alcohol (10 ml). After 3 h the mixture was poured into water and extracted into methylene dichloride, dried (Na_2SO_4), and evaporated under reduced pressure. The resulting oil, on addition of diethyl ether, yielded white crystals (35 mg, 45%), m.p. 165—169 °C; $[\alpha]_D^{20}$ +133.0° (*c* 0.9, $CHCl_3$); $\nu_{max.}$(Nujol) 1 735 and 1 675 cm^{-1}; $\delta_G(CDCl_3$; 60 MHz) 0.93 (3 H, s, 18-Me), 3.64 (1 H, d, J_{gem} 12 Hz, 19-H), 3.86 (1 H, d, J_{gem} 12 Hz, 19-H), 5.96 (1 H, s, 4-CH vinylic); *m/z* 366/364 (M^+) (93/100%), 320/318 (17/19), and 285 $(M - Br)^+$ (94) (M^+, 364.1029. $C_{19}H_{25}{}^{79}BrO_2$ requires *M*, 364.1038).

19-*Chloroandrost*-4-*ene*-3,17-*dione* (**10m**).—This compound was prepared from compound (**12**) by the method used to prepare compound (**10n**) except that anhydrous lithium chloride was used instead of anhydrous lithium bromide. The oil on addition of diethyl ether yielded white crystals, m.p. 167—171 °C (lit,[12c] 167—177 °C); $\delta_H(CDCl_3$; 60 MHz) 0.88 (3 H, s, 18-Me), 3.75 (1 H, d, J_{gem} 10.8 Hz, 19-H), 3.95 (1 H, d, J_{gem} 10.8 Hz, 19-H), and 5.91 (1 H, s, 4-CH vinylic); *m/z* 322/320 (M^+) (33/100%), 285 $(M - Cl)^+$ (13), 284 $(M - HCl)^+$ (20), 278 (10), 276 (16), 271 (30), 243 (20), and 229 (18) (M^+, 320.1559. $C_{19}H_{25}{}^{35}ClO_2$ requires *M*, 320.1543).

19-*Methylsulphonylthioandrost*-5-*ene*-3,17-*dione Bis*(*ethylene acetal*) (**14**).—Compound (**13**) (0.3 g) dissolved in HMPA (5 ml) with ammonium methanethiosulphonate[19] (0.5 g) was heated to 80 °C under nitrogen for 5 h. The reaction mixture was then poured into aqueous sodium hydrogen carbonate and extracted with diethyl ether. The extract was washed with water (containing a trace of pyridine), dried (Na_2SO_4), and evaporated under reduced pressure to leave a brown oil which on addition of diethyl ether–light petroleum gave a light brown crystalline solid (175 mg, 62%), m.p. 103—104 °C; $\nu_{max.}$(Nujol) 1 315, 1 130, and 1 300 cm^{-1}; $\delta_H(CDCl_3$; 60 MHz) 0.88 (3 H, s, 18-Me), 3.23 (1 H, d, J_{gem} 12 Hz, 19-H), 3.24 (3 H, s, SSO_2Me), 3.54 (1 H, d, J_{gem} 12 Hz, 19-H), 3.86 (8 H, m, 3 and 17 OCH_2CH_2O), and 5.52 (1 H, m, 6-CH vinylic); *m/z* 405 $(M - SO_2Me)^+$ (100%), 360 $(M - CHSSO_2Me)^+$ (92), 345 (23), and 316 (32).

19-*Methylsulphonylthioandrost*-4-*ene*-3,17-*dione* (**10p**).—Compound (**14**) (90 mg) dissolved in butan-2-one (20 ml) with toluene-*p*-sulphonic acid (8 mg) was heated under reflux for 1 h. The reaction mixture was then poured into aqueous sodium hydrogen carbonate and extracted with methylene dichloride. The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give an oil. This was crystallized from diethyl ether to yield white crystals (48 mg, 65%), m.p. 110—111 °C; $[\alpha]_D^{20}$ +180° (*c* 1.0, $CHCl_3$); $\nu_{max.}$(Nujol) 1 735, 1 680, 1 325, and 1 140 cm^{-1}; $\delta_H(CDCl_3$; 60 MHz) 0.92 (3 H, s, 18-Me), 3.3 (3 H, s, SSO_2Me), 3.42 (1 H, d, J_{gem} 12.7 Hz, 19-H) (partially obscured by signal from SSO_2Me), 3.66 (1 H, d, J_{gem} 12.7 Hz, 19-H), and 5.87 (1 H, s, 4-CH vinylic); *m/z* 317 $(M - SO_2Me)^+$ (100%), 271 (16), 261 (9), and 242 (10); *m/z* (f.a.b.-m.s.) 397 $(M + H)^+$ (Found: C, 60.1; H, 7.1 $C_{20}H_{28}O_4S_2$ requires C, 60.58; H, 7.11%).

Preparation of Human Placental Microsomes.—The microsomal fraction was prepared as described previously[27] except that the final buffer contained 20% glycerol. Immediately before use the microsomes were thawed and diluted 500-fold with 0.1M potassium phosphate buffer pH 7.4.

Screening Assay Procedure.—This assay is based on the method of Thompson and Siiteri.[1] [1β,2β-^{3}H]Androst-4-ene-3,17-dione substrate was mixed with inhibitor in a glass vial and solvent removed with a stream of argon. The mixture was redissolved in methanol (25 μl) and added to the assay buffer (0.1M potassium phosphate pH 7.4; 4 ml) containing NADP monosodium salt (4 mg), glucose-6-phosphate monosodium salt (9 mg) and glucose-6-phosphate dehydrogenase (5 units). The assay mixture was pre-incubated for 15 min and the reaction initiated by adding a placental microsome suspension (0.1 mg microsomal protein in 0.1M potassium phosphate buffer pH 7.4; 1 ml) which had also been pre-incubated at 37 °C for 15 min. The final concentration of the components in the incubations (final volume 5 ml) are as follows: [1β,2β-^{3}H]androst-4-ene-3,17-dione, 0.5 μM (0.1 μCi/ml); inhibitor, 0.5 or 2.5 μM; human placental microsomes, 0.02 mg/ml; NADP, mM; glucose-6-phosphate, 6.4 mM; glucose-6-phosphate dehydrogenase, 1 unit/ml; potassium phosphate buffer, pH 7.4, 0.1M. The incubations were performed in unstoppered 25 ml conical flasks in a shaking water-bath at 37 °C. Samples (0.5 ml) were taken immediately after addition of microsome suspension (to give a zero-time blank) and then every 5 min for 30 min. The samples were added to polypropylene microcentrifuge tubes (1.5 ml) containing methylene dichloride (0.5 ml) and immediately capped and shaken to stop the reaction and remove tritiated steroid from the aqueous layer. The aqueous and organic layers were separated by centrifugation in a microcentrifuge for 20 s and the organic layer removed with a drawn-out Pasteur pipette. Remaining tritiated steroid was removed from the aqueous layer by shaking with activated charcoal (18 mg) and leaving overnight at 4 °C. After centrifugation (in a microcentrifuge for 4 min) to pellet the charcoal, aliquots of the supernatant (0.45 ml) were taken, added to scintillation fluid (Labscint, 4 ml) and the radioactivity measured by liquid scintillation counting with automatic quench correction. From this measurement of ^3H_2O produced,

the amount of oestrogen formed can be calculated. Under these conditions, the control rates (no inhibitor added) were 90 ± 10 pmol min^{-1} mg^{-1}. The rate was linear over the first 20 min during which time an average of 9% of the substrate was converted to product. The blanks (zero-time samples) showed <0.01% conversion. The rates of product formation for incubations containing inhibitors was compared to that of control incubations run simultaneously and are reported as percent inhibition of control incubations (Table 1).

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