Microbiological Hydroxylation of Steroids. Part VI.[1] Hydroxylation of Simple Mono- and Di-oxygenated 5α-Androstanes and of 3-Oxoestranes with the Fungus *Aspergillus ochraceus*

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Of the eleven 5α-androstane monoketones, only two are hydroxylated by *Aspergillus ochraceus*. 5α-Androstan-3-one (and 5α-estran-3-one and the related Δ^4-3-ketones) give 11α-hydroxy- and then 6β,11α-dihydroxy-compounds: 5α-androstan-17-one gives a 7β,11α-dihydroxy-derivative.

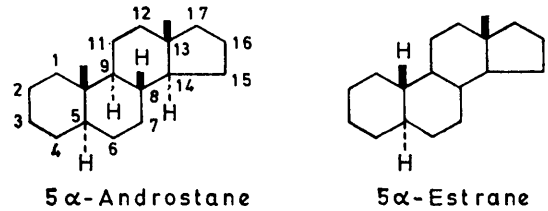
The predilection of *A. ochraceus* for 11α-hydroxylation is emphasized by the results with dioxygenated androstanes which, although representing a range of structural types, are all hydroxylated efficiently at the 11α-position.

PREVIOUS work in this series [1,2] was concerned with the hydroxylation of steroids (mostly mono- and di-oxygenated 5α-androstanes in which the positions of the substituents around the steroid nucleus were varied systematically) with the fungus *Calonectria decora*. The effect of a second micro-organism, *Aspergillus ochraceus*, on a selection of these substrates has now been studied. Our main object was, as before, to examine the possibility of there being spatial relationships between the positions of the substituents (corresponding to binding sites of the enzyme systems involved in the hydroxylations) and those of the new hydroxy-groups (corresponding to hydroxylating sites). The existence of such relationships would widen the potential use of *Aspergillus ochraceus* in steroid synthesis, possibly along the lines of the microbiological stages employed in preparing 15-oxygenated androstanes.[3,4]

Aspergillus ochraceus is well known as an efficient 11α-hydroxylator of steroids;[5] the 6β-position appears to be an additional, or occasionally an alternative, site for hydroxylation,[6] and we have reported briefly the unusual 1β,11α-dihydroxylation of some pregnane derivatives.[7] Recent work[8,9] with cell-free cultures has shown that two hydroxylase enzymes act independently in causing the (equatorial) 11α- and (axial) 6β-hydroxylations undergone by many of the usual steroid substrates. The two enzyme systems can be induced, also independently, by appropriate steroids. Thus progesterone itself induces only 11α-hydroxylase activity; the product, 11α-hydroxyprogesterone, is responsible for the generation of the 6β-hydroxylase and is converted by it into the 6β,11α-dihydroxy-compound.[8] In some cases the second stage, 6β-hydroxylation, can be selectively inhibited by zinc ions.[10]

Substrates derived from 5α-androstane are indicated by abbreviated names, *e.g.* 6β-OH-3-CO represents 6β-hydroxy-5α-androstan-3-one. Derivatives of estrane are named fully. In the Products column those oxygen functions introduced during the incubation are in bold type. The Conditions refer to the use of ethanol (E),

Hydroxylation of androstanes and estranes by *Aspergillus ochraceus*



5α-Androstane 5α-Estrane

dimethyl sulphoxide (D), and acetone (A) as solvents for the substrate and to the time of incubation (in days). The yields are calculated after making allowance for recovered starting material, *i.e.* they refer to the composition of the steroidal material after incubation and removal of the substrate.

TABLE 1

Monoketone substrates

Substrate	Conditions	Substrate recovered	Main product(s)	
3-CO	D4	56%	**6β,11α**-$(OH)_2$	51%
5α-Estran-3-one	D3	65	**11α**- OH	11
5α-Estran-3-one	D5	12	**6β,11α**-$(OH)_2$	50
3-CO-Δ^1	D6	67	**6β,11α**-$(OH)_2$	39
3-CO-Δ^4	E3	33	**6β,11α**-$(OH)_2$	68
			11α- OH	21
3-CO-Δ^4	E6	0	**6β,11α**-$(OH)_2$	75
Estr-4-en-3-one	D6	5	**6β,11α**-$(OH)_2$	53
			11α- OH	32
1-, 2-, 4-, 6-, 7-, 11-, 12-, 15-, and 16-CO	E6 and D6	75—92	None isolated	
17-CO	D4	65	**7β,11α**-$(OH)_2$	27
			11α- OH	5

Tables 1 and 2, and the Scheme summarise the results obtained here with vegetative cell cultures of *A. ochraceus*. [The use of the (arabic) serial number sequence of steroids

[1] Part V, A. M. Bell, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and W. E. Müller, *J.C.S. Perkin I*, 1972, 2759.

[2] A. M. Bell, P. C. Cherry, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and P. D. Woodgate, *J.C.S. Perkin I*, 1972, 2081.

[3] J. W. Blunt, I. M. Clark, J. M. Evans, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *J. Chem. Soc.* (*C*), 1971, 1136.

[4] I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, J. T. Pinhey, and A. Pendlebury, *J.C.S. Perkin I*, 1972, 2765.

[5] W. Charney and H. L. Herzog, 'Microbial Transformations of Steroids,' Academic Press, New York, 1967.

[6] T. Okumura, Y. Nozaki, and D. Satoh, *Chem. and Pharm. Bull.* (*Japan*), 1962, **12**, 1143; L. L. Smith, G. Greenspan, R. Rees, and T. Foell, *J. Amer. Chem. Soc.*, 1966, **88**, 3120.

[7] A. S. Clegg, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *Chem. Comm.*, 1970, 1029.

[8] M. Shibahara, J. A. Moody, and L. L. Smith, *Biochim. Biophys. Acta*, 1970, **202**, 172.

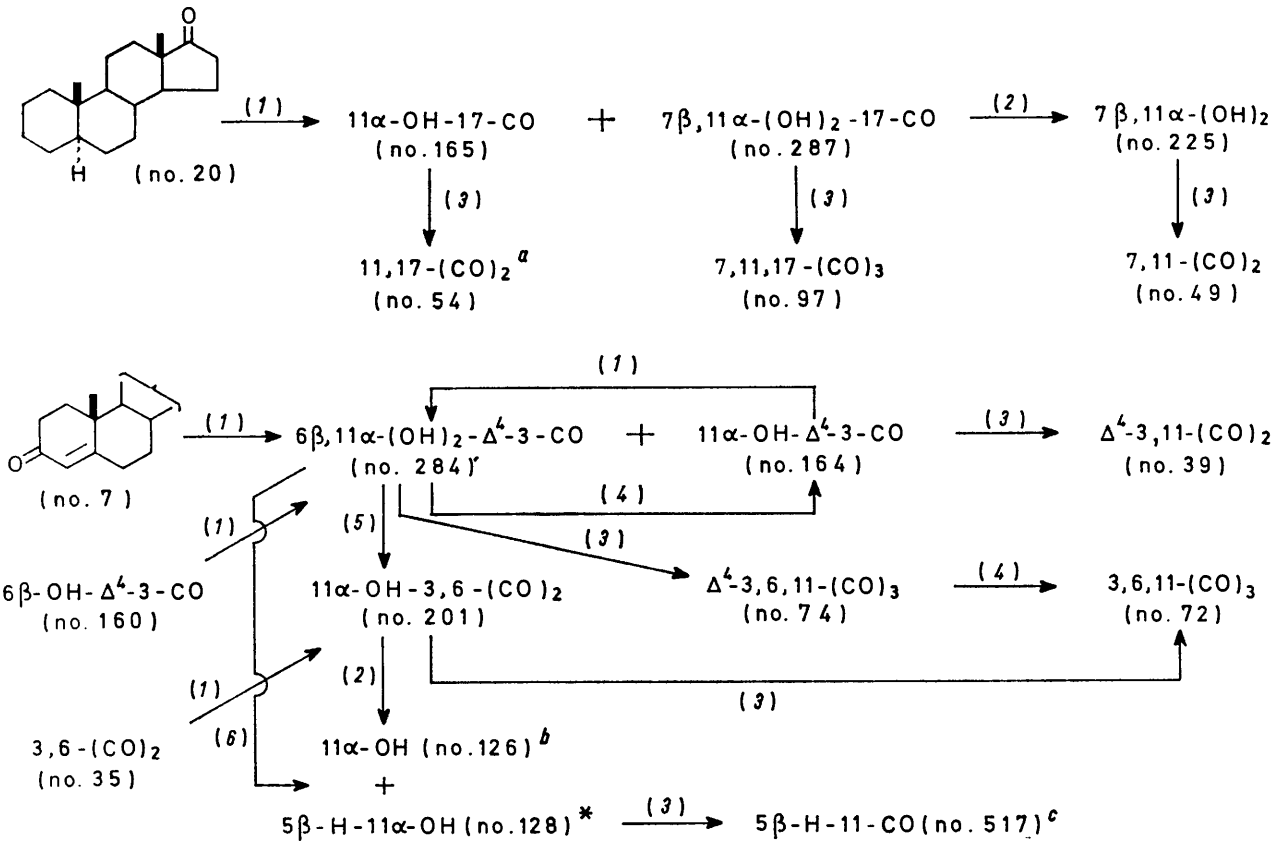
[9] L. Tan and P. Falardeau, *J. Steroid Biochem.*, 1970, **1**, 221.

[10] Ref. 5, p. 297.

throughout this work, and considerations about the structural elucidation and the reporting of new compounds have been explained earlier.[2] Compounds nos. 517—523 (whose n.m.r. signals are listed in Table 3) and some of the new steroids with numbers below 375 are described here.]

The results are in accord with the sequential nature of use of *C. decora*. Nine 5α-androstanones, exemplifying substrates with a keto-group in each of the steroid rings, were not hydroxylated to a significant extent even under forcing conditions. 3-Ketones in the androstane and estrane series gave 6β,11α-dihydroxy-products, the highest yield (75%) being obtained with androst-4-en-3-one. Apart from these only the 17-oxoandrostane was

SCHEME Some reactions involved in structural elucidation work (abbreviated names indicate the positions of substituents in the 5α-androstane nucleus)



Reagents: (1), *A. ochraceus*; (2), Huang-Minlon reduction; (3), H_2CrO_4–Me_2CO; (4), Zn–AcOH; (5), $Fe_2(SO_4)_3$–CH_2Cl_2, or HCl–EtOH; (6) KOH, 120°C, then KOH–N_2H_4, 210 °C

* Not fully characterised. [a] Ref. 20. [b] Ref. 18. [c] Ref. 19.

dihydroxylation by *A. ochraceus*. Even after incubation of some monoketones for 4—6 days, the products contained significant amounts of 11α-monohydroxylated materials but none arising from 6β-monohydroxylation. Study of the products from androst-4-en-3-one during the first 24 h of incubation showed that the 11α-hydroxy-compound is formed quickly, and is then gradually converted into 6β,11α-dihydroxyandrost-4-en-3-one. (The results, recorded in the Experimental section, are similar to those found earlier [11] with progesterone.) In this respect *A. ochraceus* differs sharply from *Calonectria decora*,[1,2] which appears to possess either a single dihydroxylating enzyme system or, as with *Curvularia lunata*,[12] two closely interdependent monohydroxylases.

The results with the monoketones (Table 1) were disappointing in comparison with those obtained by hydroxylated, giving mainly a product shown (see Scheme) to be the 7β,11α-dihydroxy-17-ketone.

As expected,[1] the presence of a second oxygen group (keto- or hydroxy-) in the substrate (Table 2) greatly facilitates hydroxylation. (The one substrate which did not react is discussed later.) An 11α-hydroxy-group is introduced into all the substrates which do not already possess an 11-oxygen substituent. Although the variation in conditions makes comparisons difficult, it is noticeable that those monoketones (Table 1) which do react are dihydroxylated, whereas only monohydroxylation occurs with the dioxygenated substrates. The difference could be interpreted in terms of enzyme sites;

[11] E. L. Dulaney, W. J. McAleer, M. Koslowski, E. O. Stapley, and J. Jaglom, *Appl. Microbiol.*, 1955, **3**, 336.
[12] M. H. J. Zuidweg, *Biochim. Biophys. Acta*, 1968, **152**, 144.

more simply it could be that the increased solubility of a trioxygenated androstane causes it to leave the enzyme surface at this oxidation level. Of the dioxygenated substrates only the two with 11α-hydroxy-groups undergo 6β-hydroxylation. Here the structural feature required to induce 6β-hydroxylase activity is already present, and such hydroxylation is necessary for the trioxygenated

TABLE 2

Dioxygenated substrates

Substrate	Conditions	Substrate recovered	Main product(s)	
2,16-$(CO)_2$	D6	4%	**11**α- OH	61%
2,17-$(CO)_2$	D6	9	**11**α- OH	57
17β-OH—2-CO	D6	10	**11**α- OH	32
3,6-$(CO)_2$	D3	7	**11**α- OH	60
			3β,**11**α-$(OH)_2$	25
6β-OH—3-CO	D3	0	**11**α- OH	76
6β-OH—3-CO-Δ⁴	E6	3	**11**α- OH	61
3,7-$(CO)_2$	D5	48	**11**α- OH	56
			3β,**11**α-$(OH)_2$	34
3,7-$(CO)_2$	E2	5	**11**α- OH	74
			3β,**11**α-$(OH)_2$	20
3,11-$(CO)_2$	D6	85	None isolated	
11α-OH—3-CO	D6	79	**6**β- OH	34
11α-OH—3-CO-Δ⁴	E6	37	**6**β- OH	76
3,16-$(CO)_2$	D2	0	**11**α- OH	55
			3β,**11**α-$(OH)_2$	14
16β-OH—3-CO	D2	0	**11**α- OH	41
3β-OH—16-CO	D2	0	**11**α- OH	71
3,17-$(CO)_2$	D4	0	**11**α- OH	52
17β-OH—3-CO	A3	0	**11**α- OH	79
17β-OH—3-CO-Δ⁴	E1	0	**11**α- OH	73
3α-OH—17-CO	E3	6	**11**α- OH	66
3α-OH—17-CO	A2	0	**11**α- OH	81
3β-OH—17-CO	E3	4	**11**α- OH	53
3β-OAc—17-CO	E2	3	3β,**11**α-$(OH)_2$	61
3β-OH—17-CO-Δ⁵	E2	48	**11**α- OH	87
3β-OMe—17-CO-Δ⁵	E2	27	**11**α- OH	41

state to be reached. 5α-Androstane-3,11-dione is exceptional in not reacting. Apparently the micro-organism cannot reduce the 11-oxo-group of this substrate; since an 11α-hydroxy-group can be neither generated nor introduced hydroxylation at position 6 is also prevented.

To explain the inhibiting effect of a 17-ethynyl group on the hydroxylation of testosterone by *A. ochraceus* it was assumed[9] that binding through *both* the 3- and 17-oxygen functions is essential for effective hydroxylation. (This followed a similar explanation of the results obtained by incubating 3,17-dioxygenated steroids with *Aspergillus tamarii*.[13]) Our results show that the 3-oxo-group alone is sufficient, and that in dioxygenated substrates the substituents need not be at positions 3 and 17. Whereas *Calonectria decora* was found to produce a variety of substitution patterns, *A. ochraceus* shows a monotonous predilection for 11α-hydroxylation; the latter micro-organism appears to be site-specific, and the effect of structural variation in the substrate is mainly on the rate of hydroxylation.

The structures of the products shown in the Scheme are based, as usual,[2] on spectrometric results. Since 7,11-dihydroxylation by *A. ochraceus* is unprecedented it was important to establish that the main product from 5α-androstan-17-one is not a 6,11-dihydroxy-compound. Huang-Minlon reduction followed by oxidation gave a diketone which is different from 5α-androstane-6,11-dione[2] but identical with the 5α-androstane-7,11-dione prepared by another route.[14]

The products from androst-4-en-3-one and 3,6-dioxygenated substrates were easily inter-related. Some of the early incubations of androst-4-en-3-one gave 11α-hydroxy-5α-androstane-3,6-dione rather than the 'correct' product, 6β,11α-dihydroxyandrost-4-en-3-one. This was traced to isomerisation (6β-hydroxy-4-en-3-one ⟶ 3,6-dione) caused by the rust present in some of the steel drums used in the isolation process. Such isomerisations have been observed under basic conditions;[15] it was found here that iron(III) salts and mineral acids are also effective catalysts. The base-induced rearrangement is involved in the one-stage conversion of 6β,11α-dihydroxyandrost-4-en-3-one into a mixture of the 5α- and 5β-androstan-11α-ols.

TABLE 3

Solutions were examined at 100 MHz. Subscripts to τ refer to the solvent [1, CCl_4; 2, $CDCl_3$; 3, C_6H_6]. $\Delta^3_1 = \tau(C_6H_6) - \tau(CCl_4)$. τ_2(calc.) values were obtained, where possible, from earlier work.[a,b] Signals are described in the form used previously.[c]

No.	Compound		τ_1	τ_2	τ_3	Δ^3_1
517	5β-Androstan-11-one	19	8·87	8·84	8·67	−0·20
		18	9·34	9·34	9·45	+0·11

No.	Compound		τ_2	τ_2 (calc.)	>C*H*-OR (in $CDCl_3$)		
518	3-βMethoxyandrost-5-en-17-one	19	8·96	8·95	H-3	6·95	7(10,10,5,5)
		18	9·11	9·10			
519	11α-Hydroxy-5α-androstane-3,17-dione	19	8·85	8·84	H-11	6·00	6(10,10,5)
		18	9·10	9·07			
520	3β,11α-Dihydroxyandrost-5-en-17-one	19	8·78	8·82	H-3	6·45	m(20)
		18	9·09	9·07	H-11	5·85	6(10,10,5)
521	11α-Hydroxy-3β-methoxyandrost-5-en-17-one	19	8·82	8·83	H-3	6·90	m(20)
		18	9·08	9·07	H-11	5·90	6(10,10,5)
522	11α,17β-Dihydroxyandrost-4-en-3-one	19	8·69	8·68	H-11	5·92	m(18)
		18	9·18	9·17	H-17	6·27	m(18)
523	5α-Androstane-3β,11α17β-triol	19	9·06	9·07			
		18	9·24	9·24			

[a] J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc.* (*C*), 1970, 250. [b] A. D. Boul, J. W. Blunt, J. W. Browne, V. Kumar, G. D. Meakins, J. T. Pinhey, and V. E. M. Thomas, *J. Chem. Soc.* (*C*), 1971, 1130. [c] M. G. Combe, W. A. Denny, G. D. Meakins, Y. Morisawa, and E. E. Richards, *J. Chem. Soc.* (*C*), 1971, 2300.

EXPERIMENTAL

For general directions and use of an asterisk to indicate that the n.m.r. signals, and possibly also the i.r. absorptions, of a compound have already been reported, see ref. 2. Where compounds with serial numbers below 517 are stated to have been identified by mixed m.p., the original preparations are contained in, or can be found from, the papers cited. The microbiological procedures and the abbreviations used in reporting the results are given fully in ref. 3. I.r. spectra indicated by $\nu_{max.}$ (high resolution) refer to

[13] D. R. Brannon, F. W. Parish, B. J. Wiley, and L. Long, *J. Org. Chem.*, 1967, **32**, 1521.

[14] Details will appear in Part XI of this series.

[15] C. Amendolla, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 1954, 1226.

dilute solutions in CCl_4 examined at a spectral slit-width of 1·5—2 cm^{-1}. Petrol refers to light petroleum, b.p. 60—80°. The abbreviation s.m. indicates starting material.

5α-Androstan-3-one (no. 5).*—(a) *Incubation.* 1·25 g in Me_2SO (375 ml), 25 flasks, medium A, 4 d, extraction I → 1·45 g combined extracts. Chromat. Al_2O_3 (5% deactivated; 60 g). Petrol gave s.m. (700 mg). Petrol–Et_2O (1 : 1) eluted *11α-hydroxy-5α-androstan-3-one* (no. 163)* (57 mg), m.p. 123—125° (from MeOH–H_2O), $[\alpha]_D$ −12° (*c* 0·3) (Found: C, 78·1; H, 10·25. $C_{19}H_{30}O_2$ requires C, 78·55; H, 10·4%), $\nu_{max.}$ (high resolution) 3655w, 3611, and 1715 cm^{-1}. Et_2O–MeOH (19 : 1) eluted *6β,11α-dihydroxy-5α-androstan-3-one* (no. 281)* (305 mg), m.p. 194—195° (from Me_2CO–hexane), $[\alpha]_D$ −39° (*c* 1·0) (Found: C, 74·4; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·8%), $\nu_{max.}$ 3631, 3613, and 1710 cm^{-1}.

(b) *Transformations.* Oxidation of 11α-hydroxy-5α-androstan-3-one (no. 163) (20 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,11-dione (no. 37)* (15 mg), m.p. (from hexane) and [3] mixed m.p. 120—121°.

Oxidation of 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) (30 mg) gave *5α-androstane-3,6,11-trione* (no. 72)* (24 mg), m.p. 188—190° (from EtOAc), $[\alpha]_D$ +57° (*c* 0·6) (Found: C, 75·6; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Huang-Minlon reduction of the dihydroxy-ketone (no. 281) (60 mg) gave *5α-androstane-6β,11α-diol* (no. 224)* (46 mg), m.p. 91—99° (unchanged after several crystallisations from Me_2CO–hexane) (Found: C, 78·0; H, 11·2. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%). Oxidation of this diol (20 mg) afforded 5α-androstane-6,11-dione (no. 46)* (16 mg), m.p. (from Me_2CO–hexane) and [2] mixed m.p. 170—173°.

5α-Estran-3-one (no. 26).*—(a) *Incubation.* 2·5 g in Me_2SO (975 ml), 65 flasks, medium A, 5 d, extraction I → 3·0 g combined extracts. P.l.c. [5 large plates, 10 × petrol–Me_2CO (5 : 1)]. The band of higher R_F was s.m. (300 mg). The band of lower R_F yielded *6β,11α-dihydroxy-5α-estran-3-one* (no. 310)* (1·2 g), m.p. 186—187°(from Me_2CO–hexane), $[\alpha]_D$ −34° (*c* 1·0) (Found: C, 73·8; H, 9·6. $C_{18}H_{28}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ 3600 and 1717 cm^{-1}.

A similar incubation carried out for 3 days gave s.m. (1·62 g) and a compound presumed to be 11α-hydroxy-5α-estran-3-one (no. 185)* (133 mg).

(b) *Transformations.* Huang-Minlon reduction of 11α-hydroxy-5α-estran-3-one (no. 185) (100 mg) followed by oxidation with 8N-H_2CrO_4 gave *5α-estran-11-one* (no. 28)* (84 mg), m.p. 86·5—88·5° (from MeOH–H_2O), $[\alpha]_D$ +169° (*c* 1·0) (Found: C, 82·8; H, 11·0. $C_{18}H_{28}O$ requires C, 83·0; H, 10·8%). Reduction of this ketone (no. 28) (80 mg) with $NaBH_4$ (4 mg) in MeOH (5 ml) gave *5α-estran-11β-ol* (no. 142)* (75 mg), m.p. 93—94° (from MeOH–H_2O), $[\alpha]_D$ +50° (*c* 0·1) (Found: C, 82·5; H, 11·7. $C_{18}H_{30}O$ requires C, 82·4; H, 11·5%), $\nu_{max.}$ 3610 cm^{-1}.

Oxidation of 6β,11α-dihydroxy-5α-estran-3-one (no. 310) (300 mg) gave *5α-estrane-3,6,11-trione* (no. 98)* (210 mg), m.p. 147—148° (from Me_2CO–hexane), $[\alpha]_D$ +143° (*c* 1·0) (Found: C, 74·8; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%), $\nu_{max.}$ 1715 cm^{-1}. Huang-Minlon reduction of 6β,11α-dihydroxy-5α-estran-3-one (no. 310) (500 mg) yielded *5α-estrane-6β,11α-diol* (no. 231)* (450 mg), m.p. 151·5—152·5° (from MeOAc), $[\alpha]_D$ −44° (*c* 1·0) (Found: C, 77·9; H, 10·8. $C_{18}H_{30}O_2$ requires C, 77·65; H, 10·9%), $\nu_{max.}$ 3600 cm^{-1}. Oxidation of this diol (no. 231) (200 mg) with 8N-H_2CrO_4 gave *5α-estrane-6,11-dione* (no. 58)* (187 mg), m.p. 121·5—122·5° (from Me_2CO–hexane), $[\alpha]_D$ +138° (*c* 1·0) (Found: C, 78·9; H, 9·6. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·6%).

5α-Androst-1-en-3-one (no. 6).*—(a) *Incubation.* 4·0 g in Me_2SO (1200 ml), 80 flasks, medium A, 6 d, extraction III → 5·9 g total extract. Chromat. SiO_2 (160 g). C_6H_6–Et_2O (19 : 1) gave s.m. (2·7 g), m.p. (from Me_2CO) and [16] mixed m.p. 104—105°. C_6H_6–EtOAc (1 : 1) gave *6β,11α-dihydroxy-5α-androst-1-en-3-one* (no. 282)* (560 mg), m.p. 212—215° (from Me_2CO–hexane), $[\alpha]_D$ −53° (*c* 0·3) (Found: C, 74·9; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·2%), $\lambda_{max.}$ 230 nm (ε 7850), $\nu_{max.}$ 3631, 3614, and 1679 cm^{-1}.

(b) *Transformations.* Acetylation (Ac_2O–C_5H_5N; 2 : 1, for 1 d) of the dihydroxy-ketone (no. 282) gave *6β,11α-diacetoxy-5α-androst-1-en-3-one* (no. 283),* m.p. 136—137° (from hexane), $[\alpha]_D$ −59° (*c* 1·1) (Found: C, 71·2; H, 8·3. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·25%), $\lambda_{max.}$ 237 nm (ε 7690), $\nu_{max.}$ 1740 and 1685 cm^{-1}. Oxidation of the dihydroxy-ketone (no. 282) with 8N-H_2CrO_4 afforded 5α-androst-1-ene-3,6,11-trione (no. 73), m.p. (from $CHCl_3$–hexane) and [2] mixed m.p. 171—175°. A solution of 6β,11α-dihydroxy-5α-androst-1-en-3-one (no. 282) (70 mg) in EtOH (10 ml) was hydrogenated over 10% Pd–C (10 mg) for 15 min at 20 °C to give 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) (56 mg), m.p. and mixed m.p. 194—195°.

Androst-4-en-3-one (no. 7).*—(a) *Incubation*: 4·0 g in EtOH (500 ml), 100 flasks, medium B, 3 d, extraction II → 7·6 g combined extracts. Chromat. Al_2O_3 (deactivated; 75 g). Petrol gave s.m. (1·3 g). Et_2O eluted *11α-hydroxyandrost-4-en-3-one* (no. 164) * (600 mg), m.p. 147—149° (from MeOH), $[\alpha]_D$ +77° (*c* 0·2) (Found: C, 79·0; H, 9·9. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%), $\lambda_{max.}$ 242 nm (ε 15400), $\nu_{max.}$ 3609 and 1678 cm^{-1}. Et_2O–MeOH (9 : 1) afforded *6β,11α-dihydroxyandrost-4-en-3-one* (no. 284)* (2·1 g), m.p. 252—255° (from Me_2CO–hexane), $[\alpha]_D$ +7° (*c* 0·8) (Found: C, 75·1; H, 9·4. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\lambda_{max.}$ 234 nm (ε 12,800), $\nu_{max.}$ 3615 and 1690 cm^{-1}. Incubation for 6 days under identical conditions gave 6β,11α-dihydroxyandrost-4-en-3-one as the only product isolated.

In a similar incubation pairs of flasks were removed after the times specified. Work-up and analysis of the steroidal material gave the following compositions (%):

Time (h)	3	6	9	12	15	18	24
Androst-4-en-3-one	98	88	62	37	26	21	16
11α-Hydroxyandrost-4-en-3-one	2	9	31	49	52	49	31
6β,11α-Dihydroxyandrost-4-en-3-one	0	3	7	14	22	30	43

Related Incubations.—(a) *11α-Hydroxyandrost-4-en-3-one* (no. 164): 80 mg in EtOH (4 ml), 2 flasks, medium B, 6 d, extraction III → 110 mg total extract. P.l.c. [1 medium plate, 1 × petrol–Me_2CO (3 : 2)] gave s.m. (higher R_F) (30 mg) and 6β,11α-dihydroxyandrost-4-en-3-one (no. 284) (lower R_F) (40 mg), m.p. and mixed m.p. 252—255°.

(b) *6β-Hydroxyandrost-4-en-3-one* (no. 160): * [17] 80 mg, as in the preceding experiment → s.m. (2 mg) and the dihydroxy-ketone (no. 284) (50 mg).

(c) *5α-Androstane-3,6-dione* (no. 35): * [17] 240 mg in Me_2SO (36 ml), 6 flasks, medium B, 3 d, extraction II → 310 mg combined extracts. P.l.c. [1 large plate, 6 × petrol–Me_2CO (5 : 1)] gave s.m. (highest R_F) (17 mg);

[16] I. M. Clark, A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and A. Pendlebury, *J.C.S. Perkin I*, 1972, 499.

[17] A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, V. Kumar, G. D. Meakins, and V. E. M. Thomas, *J.C.S. Perkin I*, 1972, 492.

11α-*hydroxy*-5α-*androstane*-3,6-*dione* (no. 201)* (intermediate R_F) (140 mg), m.p. 176—178° (from EtOAc), $[\alpha]_D$ −54° (*c* 0·7) (Found: C, 74·7; H, 9·0. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3615 and 1720 cm^{-1}; and 3β,11α-*dihydroxy*-5α-*androstan*-6-*one* (no. 253)* (lowest R_F) (60 mg), m.p. 198—199·5° (from Me_2CO), $[\alpha]_D$ −53° (*c* 0·4) (Found: C, 74·9; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1716 cm^{-1}.

Transformations (*lower part of Scheme*). A solution of 6β,11α-dihydroxyandrost-4-en-3-one (no. 284) (50 mg) in AcOH (3 ml) was boiled under reflux with Zn dust (150 mg) for 30 min to give 11α-hydroxyandrost-4-en-3-one (no. 164) (45 mg). This hydroxy-ketone (220 mg) was oxidised with 8N-H_2CrO_4 to *androst*-4-*ene*-3,11-*dione* (no. 39)* (170 mg), m.p. 119—120° (from MeOH), $[\alpha]_D$ +237° (*c* 1·0) (Found: C, 79·6; H, 9·1. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·2%), $\lambda_{max.}$ 240 nm (ε 18,000), $\nu_{max.}$ 1713 and 1680 cm^{-1}.

6β,11α-Dihydroxyandrost-4-en-3-one (no. 284) (250 mg) was oxidised with 8N-H_2CrO_4 to *androst*-4-*ene*-3,6,11-*trione* (no. 74) * (230 mg), m.p. 145—147° (from MeOH), $[\alpha]_D$ +106° (*c* 0·9) (Found: C, 76·1; H, 8·2. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·1%), $\lambda_{max.}$ 248 nm (ε 14,500), $\nu_{max.}$ 1713 and 1694 cm^{-1}. A solution of this triketone (375 mg) in AcOH (25 ml) was boiled under reflux with Zn dust (900 mg) for 2·5 h. P.l.c. [1 × petrol–Me_2CO (5 : 1)] of the product afforded 5α-androstane-3,6,11-trione (no. 72) (155 mg), m.p. and mixed m.p. 187—190°.

A solution of 6β,11α-dihydroxyandrost-4-en-3-one (no. 284) (237 mg) and iron(III) sulphate (50 mg) in CH_2Cl_2 (50 ml) was boiled under reflux for 2 h to give 11α-hydroxy-5α-androstane-3,6-dione (no. 201) (205 mg), m.p. and mixed m.p. 174—177°. This reaction was also carried out (82% yield) by boiling under reflux for 2 h a solution of the dihydroxy-ketone (no. 284) in 10N-HCl (0·1 ml)–EtOH (10 ml)–H_2O (0·5 ml). Oxidation of the hydroxy-diketone (no. 201) (127 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,6,11-trione (no. 72) (101 mg); similarly the dihydroxy-ketone (no. 253) (105 mg) gave the triketone (no. 72) (86 mg).

Huang-Minlon reduction of the hydroxy-diketone (no. 201) (600 mg) gave material (444 mg) which was purified by p.l.c. [3 large plates, 3 × petrol–Me_2CO (19 : 1)]. The band of lower R_F afforded 5α-androstan-11α-ol (no. 126) * (200 mg), m.p. 107—108° (from hexane) (lit.,[18] 108°), $[\alpha]_D$ −27° (*c* 0·9). The material of higher R_F [210 mg, $\nu_{max.}$ 3610 cm^{-1}, *m/e* 276 (M^+), formulated as 5β-androstan-11α-ol (no. 128) *] was oxidised with 8N-H_2CrO_4 to 5β-androstan-11-one (no. 517) (190 mg), m.p. 118—120° (from MeOH), $[\alpha]_D$ +55° (*c* 0·9) (lit.,[19] m.p. 120—121°, $[\alpha]_D$ +55°).

N_2 was bubbled for 1 h through a stirred solution of 6β,11α-dihydroxyandrost-4-en-3-one (no. 284) (2·2 g) in diethylene glycol (100 ml). KOH (1·7 g) was added, and the temperature was raised to 120 °C for 1 h. N_2H_4,H_2O (10 ml) was added, the N_2 stream was stopped, and the temperature was raised to 170 °C for 3 h. H_2O and the excess of N_2H_4 were allowed to distil while the solution was heated to 210 °C and kept at 210 °C for 4 h. After work-up the product was dissolved in petrol–Et_2O (19 : 1) and filtered through Al_2O_3 (10% deactivated; 30 g). P.l.c. [4 large plates, 3 × petrol–Me_2CO (19 : 1)] gave 5α-androstan-11α-ol (no. 126) (lower R_F) (320 mg) and material (higher R_F) (330 mg) identical (i.r., mixed t.l.c.) with the compound formulated as 5β-androstan-11α-ol (no. 128).

Estr-4-*en*-3-*one* (no. 27).*—(a) *Incubation*. 4·0 g in Me_2SO (1500 ml), 100 flasks, medium A, 6 d, extraction I ⟶ 5·0 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 300 g). Petrol–Et_2O (9 : 1) gave s.m. (200 mg). Petrol–Et_2O (1 : 1) afforded 11α-hydroxyestr-4-en-3-one (no. 186)* (1·3 g), m.p. 135—137° (from Me_2CO–hexane), $[\alpha]_D$ −69° (*c* 1·0) (Found: C, 78·9; H, 9·8. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·55%), $\lambda_{max.}$ 244 nm (ε, 13,700), $\nu_{max.}$ 3605 and 1678 cm^{-1}. Et_2O gave a mixture which was separated by p.l.c. [5 large plates, 12 × petrol–Me_2CO (6 : 1)] to give 11α-*hydroxy*-5α-*estrane*-3,6-*dione* (no. 210) * (higher R_F) (200 mg), m.p. 148—149·5° (from Me_2CO–hexane), $[\alpha]_D$ −66° (*c* 1·1) (Found: C, 74·5; H, 8·8. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%), $\nu_{max.}$ 3595 and 1715 cm^{-1}, and 6β,11α-dihydroxyestr-4-en-3-one (no. 311) * (lower R_F) (2·2 g), m.p. (from Me_2CO–hexane) and [2] mixed m.p. 160—162°.

(b) *Transformations*. Oxidation of 11α-hydroxyestr-4-en-3-one (no. 186) (400 mg) gave *estr*-4-*ene*-3,11-*dione* (no. 57) * (350 mg), m.p. 132—133° (from Me_2CO–hexane), $[\alpha]_D$ +240° (*c* 1·0) (Found: C, 79·3; H, 8·9. $C_{18}H_{24}O_2$ requires C, 79·4; H, 8·9%). Oxidation of 11α-hydroxy-5α-estrane-3,6-dione (no. 210) (100 mg) gave 5α-estrane-3,6,11-trione (no. 98) (80 mg), m.p. and mixed m.p. 144—146°.

Huang-Minlon reduction of 11α-hydroxy-5α-estrane-3,6-dione (no. 210) (600 mg) gave a mixture which was separated by p.l.c. [4 small plates, 8 × petrol–Et_2O (49 : 1)] to give 5β-*estran*-11α-*ol* (no. 143) * (higher R_F) (150 mg), m.p. 88·5—89·5° (from MeOH–H_2O), $[\alpha]_D$ −24° (*c* 1·0) (Found: C, 82·5; H, 11·6. $C_{18}H_{30}O$ requires C, 82·4; H, 11·5%), $\nu_{max.}$ 3604 cm^{-1}, and 5α-estran-11α-ol (no. 141) * (lower R_F) (250 mg) as an oil, $\nu_{max.}$ 3610 cm^{-1}, *m/e* 262 (M^+).

Oxidation of 5β-estran-11α-ol (no. 143) (100 mg) gave 5β-*estran*-11-*one* (no. 349) *, m.p. 103—105° (from MeOH–H_2O) (64 mg), $[\alpha]_D$ +37° (*c* 0·3) (Found: C, 82·8; H, 10·8. $C_{18}H_{28}O$ requires C, 83·0; H, 10·8%). Reduction of this ketone (35 mg) with $NaBH_4$ (3 mg) in MeOH (4 ml) gave 5β-estran-11β-ol (no. 144) * (28 mg) as an oil, $\nu_{max.}$ 3610 cm^{-1}, *m/e* 262 (M^+).

5α-*Androstan*-17-*one* (no. 20) *.—(a) *Incubation*. 1·9 g in Me_2SO (600 ml), 38 flasks, medium A, 4 d, extraction III ⟶ 2·3 g total extract. Chromat. Al_2O_3 (deactivated; 150 g). Petrol–Et_2O (9 : 1) gave s.m. (1·24 g). Et_2O eluted 11α-*hydroxy*-5α-*androstan*-17-*one* (no. 165) * (36 mg), m.p. 136—137° (from hexane), $[\alpha]_D$ +59° (*c* 0·4) (Found: C, 78·5; H, 10·4. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ 3611 and 1744 cm^{-1}. Et_2O–MeOH (9 : 1) eluted 7β,11α-*dihydroxy*-5α-*androstan*-17-*one* (no. 287) * (205 mg), m.p. 190·5—192·5° (from Me_2CO–hexane), $[\alpha]_D$ +99° (*c* 0·6) (Found: C, 74·4; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3614 and 1743 cm^{-1}.

(b) *Transformations*. Oxidation of 11α-hydroxy-5α-androstan-17-one (no. 165) (25 mg) gave 5α-androstane-11,17-dione (no. 54) * (20 mg), m.p. 126—127° (from hexane) (lit.,[20] 126—127·5°).

Oxidation of 7β,11α-dihydroxy-5α-androstan-17-one (no. 287) (30 mg) gave 5α-*androstane*-7,11,17-*trione* (no. 97) * (25 mg), m.p. 173—174° (from hexane), $[\alpha]_D$ +39° (*c* 0·9) (Found: C, 75·5; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Huang-Minlon reduction of 7β,11α-dihydroxy-5α-androstan-17-one (no. 287) (80 mg) gave 5α-*androstane*-7β,11α-*diol* (no. 225) * (70 mg), m.p. 203—205° (from EtOAc), $[\alpha]_D$ +7° (*c* 0·1) (Found: C, 77·9; H, 11·1. $C_{19}H_{32}O_2$

[18] A.-M. Giroud, A. Rassat, and T. Rull, *Bull. Soc. chim. France*, 1963, 2563.

[19] F. Sondheimer, E. Batres, and G. Rosenkranz, *J. Org. Chem.*, 1957, **22**, 1090.

[20] W. Klyne and S. Palmer, *J. Chem. Soc.*, 1958, 4545.

requires C, 78·0; H, 11·0%). Oxidation of this diol (30 mg) gave 5α-*androstane*-7,11-*dione* (no. 49) * (29 mg), m.p. 141—143° (from EtOH), $[\alpha]_D$ −11° (*c* 0·5) (Found: C, 79·0; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·2; H, 9·7%).

5α-*Androstane*-2,16-*dione* (no. 33) *.—(a) *Incubation.* 480 mg in Me_2SO (72 ml), 12 flasks, medium B, 6 d, extraction I ⟶ 1·21 g combined extracts. P.l.c. [1 large plate, 3 × petrol–Me_2CO (7 : 3)] afforded s.m. (20 mg) (higher R_F), and 11α-*hydroxy*-5α-*androstane*-2,16-*dione* (no. 199) * (lower R_F) (295 mg), m.p. 209—210° (from Me_2CO–hexane), $[\alpha]_D$ −164° (*c* 1·0) (Found: C, 74·9; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3588, 1740, and 1700 cm^{-1}.

(b) *Transformation.*—Huang-Minlon reduction of the hydroxy-diketone (no. 199) gave 5α-androstan-11α-ol (no. 126),* m.p. and mixed m.p. 107—108°.

5α-*Androstane*-2,17-*dione* (no. 34) *.—(a) *Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction I ⟶ 1·88 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (3 : 2) gave s.m. (90 mg). Petrol–Et_2O (2 : 3) gave 11α-*hydroxy*-5α-*androstane*-2,17-*dione* (no. 200) * (544 mg), m.p. 185—186° (from Me_2CO–hexane), $[\alpha]_D$ +70° (*c* 1·0) (Found: C, 74·8; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3611, 1744, and 1714 cm^{-1}. Et_2O–MeOH (1 : 1) gave 11α,17β-*dihydroxy*-5α-*androstan*-2-*one* (no. 295) * (30 mg), m.p. 244·5—246·5° (from Me_2CO–hexane), $[\alpha]_D$ +11° (*c* 0·6) (Found: C, 74·6; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3596 and 1699 cm^{-1}.

(b) *Transformations.* Huang-Minlon reduction of 11α-hydroxy-5α-androstane-2,17-dione (no. 200) (100 mg) gave 5α-androstan-11α-ol (no. 126) (71 mg).

Oxidation of both 11α-hydroxy-5α-androstane-2,17-dione (no. 200) and 11α,17β-dihydroxy-5α-androstan-2-one (no. 295) with 8N-H_2CrO_4 gave 5α-*androstane*-2,11,17-*trione* (no. 71) * (yields 81 and 83%, respectively), m.p. 210—211° (from Me_2CO–hexane), $[\alpha]_D$ +145° (*c* 1·0) (Found: C, 72·5; H, 8·55. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%).

17β-*Hydroxy*-5α-*androstan*-2-*one* (no. 180) *.—*Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction I ⟶ 1·7 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (49 : 1) gave s.m. (95 mg). Petrol–Et_2O (1 : 1) gave a mixture which was separated by p.l.c. [1 large plate, 2 × petrol–Me_2CO (7 : 3)] to give 11α-hydroxy-5α-androstane-2,17-dione (no. 200) (higher R_F) (20 mg) and 11α,17β-dihydroxy-5α-androstan-2-one (no. 295) (lower R_F) (304 mg).

6β-*Hydroxy*-5α-*androstan*-3-*one* (no. 159) *.[21]—*Incubation.* 20 mg in Me_2SO (3 ml), 1 flask, medium B, 3 d, extraction III ⟶ 30 mg total extract. P.l.c. [1 small plate, 2 × petrol–acetone (5 : 1)] gave 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) (16 mg), m.p. and mixed m.p. 193—195°.

5α-*Androstane*-3,7-*dione* (no. 36) *.—(*a*) *Incubation.* 1·0 g in EtOH (50 ml), 25 flasks, medium B, 2 d, extraction II ⟶ 1·6 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 50 g). C_6H_6 eluted s.m. (50 mg). $CHCl_3$ eluted 11α-*hydroxy*-5α-*androstane*-3,7-*dione* (no. 203) * (740 mg), m.p. 203—204° (from Me_2CO–hexane), $[\alpha]_D$ −74° (*c* 0·5) (Found: C, 75·2; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·2%), $\nu_{max.}$ 3600 and 1715 cm^{-1}, and then 3β,11α-*dihydroxy*-5α-*androstan*-7-*one* (no. 254) * (200 mg), m.p. 205—208° (from Me_2CO–hexane), $[\alpha]_D$ −88° (*c* 0·55) (Found: C, 74·4; H, 9·75. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·85%), $\nu_{max.}$ 3610 and 1710 cm^{-1}.

A similar incubation using Me_2SO for 5 d gave s.m. (480 mg), 11α-hydroxy-5α-androstane-3,7-dione (no. 203) (293 mg), and 3β,11α-dihydroxy-5α-androstan-7-one (no. 254) (178 mg).

(b) *Transformation.* Oxidation of 11α-hydroxy-5α-androstane-3,7-dione (no. 203) (50 mg) with 8N-H_2CrO_4 gave 5α-*androstaae*-3,7,11-*trione* (no. 80) * (40 mg), double m.p. 170—171° and 176—177° (from Me_2CO–hexane), $[\alpha]_D$ +23° (*c* 0·7) (Found: C, 75·8; H, 9·0. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1715 cm^{-1}.

Huang-Minlon reduction of 3β,11α-dihydroxy-5α-androstan-7-one (no. 254) (70 mg) gave 5α-*androstane*-3β,11α-*diol* (no. 221) * (50 mg), m.p. 187—189° (from Me_2CO–hexane), $[\alpha]_D$ −28° (*c* 1·05) (Found: C, 78·2; H, 11·1. $C_{19}H_{32}O_2$ requires C, 78·15; H, 11·0%), $\nu_{max.}$ 3615 cm^{-1}.

11α-*Hydroxy*-5α-*androstan*-3-*one* (no. 163) *.—*Incubation.* 33 mg in Me_2SO (4 ml), 1 flask, medium A, 6 d, extraction III ⟶ 40 mg total extract. P.l.c. [1 small plate, 1 × petrol–Me_2CO (3 : 2)] gave s.m. (26 mg) and 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) (2·5 mg).

5α-*Androstane*-3,16-*dione* (no. 40) *.[22]—(a) *Incubation.* 80 mg in Me_2SO (12 ml), 2 flasks, medium B, 2 d, extraction II ⟶ 100 mg combined extracts. P.l.c. [1 medium plate, 2 × petrol–Me_2CO (5 : 1)] gave 11α-*hydroxy*-5α-*androstane*-3,16-*dione* (no. 204) * (higher R_F) (46 mg), m.p. 259—262° (from Me_2CO–hexane), $[\alpha]_D$ −124° (*c* 1·2) (Found: C, 74·7; H, 9·0. $C_{19}H_{28}O_3$ requires C, 74·95; H, 9·3%), $\nu_{max.}$ 3600, 1740, and 1710 cm^{-1}, and 3β,11α-*dihydroxy*-5α-*androstan*-16-*one* (no. 255) * (lower R_F) (12 mg), m.p. 202—204° (from Me_2CO–hexane), $[\alpha]_D$ −170° (*c* 0·45) (Found: C, 74·5; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3595 and 1740 cm^{-1}.

(b) *Transformation.* Oxidation with 8N-H_2CrO_4 of a sample (50 mg) of the total extract from a similar incubation gave 5α-*androstane*-3,11,16-*trione* (no. 85) * (30 mg), m.p. 174—176° (from Me_2CO–hexane), $[\alpha]_D$ −96° (*c* 0·7) (Found: C, 75·3; H, 8·55. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·6%).

16β-*Hydroxy*-5α-*androstan*-3-*one* (no. 176) *.[22]—(a) *Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, 2 d, medium B, extraction II ⟶ 2·0 g combined extracts. P.l.c. [1 large plate, 1 × petrol–Me_2CO (2 : 1)] of a portion (200 mg) gave 11α,16β-*dihydroxy*-5α-*androstan*-3-*one* (no. 292) * (43 mg), m.p. 206—207° (from Me_2CO), $[\alpha]_D$ −20° (*c* 0·4) (Found: C, 74·4; H, 9·8. $C_{19}H_{30}O_2$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1710 cm^{-1}.

(b) *Transformation.* Oxidation of a portion (200 mg) of the extracts gave 5α-androstane-3,11,16-trione (no. 85) (40 mg), m.p. and mixed m.p. 173—176°.

3β-*Hydroxy*-5α-*androstan*-16-*one* (no. 150) *.[22]—*Incubation.* 80 mg in Me_2SO (30 ml), 2 flasks, medium B, 2 d, extraction II ⟶ 100 mg combined extracts. P.l.c. [1 large plate, 1 × petrol–Me_2CO (5 : 1)] gave 11α-hydroxy-5α-androstane-3,16-dione (no. 204) (6 mg) and 3β,11α-dihydroxy-5α-androstan-16-one (no. 255) (60 mg).

5α-*Androstane*-3,17-*dione* (no. 42) *.—(a) *Incubation.* 40 mg in Me_2SO (6 ml), 1 flask, medium B, 4 d, extraction I ⟶ 24 mg combined extracts. P.l.c. [1 small plate, 1 × C_6H_6–EtOAc (1 : 2)] gave 11α-hydroxy-5α-androstane-3,17-dione (no. 519) (22 mg), m.p. 192—194° (from Me_2CO–hexane), $[\alpha]_D$ +66° (*c* 0·1) (lit.,[23] m.p. 194—195°, $[\alpha]_D$ +66°).

(b) *Transformation.* Oxidation of the hydroxy-diketone

[21] The preparation of this compound will be described later.

[22] J. E. Bridgeman, C. E. Butchers, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and P. D. Woodgate, *J. Chem. Soc.* (*C*), 1970, 244.

[23] Ch. Meystre, J. Kalvoda, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, 1963, **46**, 2844.

(no. 519) (25 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,11,17-trione (no. 358) * (19 mg), m.p. 176—177° (from Me_2CO–hexane), $[\alpha]_D$ +156° (*c* 0·9) (lit.,[24] m.p. 174—176°, $[\alpha]_D$ +150°).

17β-*Hydroxy*-5α-*androstan*-3-*one* (no. 411) *.—(*a*) *Incubation.* 450 mg in Me_2CO (60 ml), 12 flasks, medium A, 3 d, extraction III ⟶ 410 mg total extract. Crystallisation from Me_2CO–hexane gave 11α,17β-*dihydroxy*-5α-*androstan*-3-*one* (no. 296) * (375 mg), m.p. 202—204°, $[\alpha]_D$ −1° (*c* 0·4) (Found: C, 74·5; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ (Nujol) 1700 cm^{-1}.

(b) *Transformation.* Oxidation of the dihydroxy-ketone (no. 296) (100 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,11,17-trione (no. 358) (80 mg).

17β-*Hydroxyandrost*-4-*en*-3-*one* (no. 182) *.—*Incubation.* 4 g in EtOH (396 ml), 66 flasks, medium A, 1 d, extraction I ⟶ 4·5 g combined extracts. Crystallisation from MeOH gave a solid (1·95 g). The material in the filtrate was purified by p.l.c. [3 large plates, 1 × petrol–Me_2CO (5 : 1)] to give a main fraction (1·1 g) shown by t.l.c. to be identical with the solid. The materials were combined (3·05 g) and recrystallised from Me_2CO–hexane to give 11α,17β-dihydroxyandrost-4-en-3-one (no. 522), m.p. 180·5—181·5°, $[\alpha]_D$ +95° (*c* 0·9) (lit.,[25] m.p. 181—181·5°, $[\alpha]_D$ +93°).

3α-*Hydroxy*-5α-*androstan*-17-*one* (no. 146) *.—*Incubation.* 1·0 g in Me_2CO (150 ml), 25 flasks, medium A, 2 d, extraction III ⟶ 1·1 g total extract. Crystallisation from Me_2CO gave 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) * (850 mg), m.p. and [3] mixed m.p. 191—193°.

A similar incubation using EtOH for 3 d gave s.m. (60 mg) and the dihydroxy-ketone (no. 242) (720 mg).

3β-*Hydroxy*-5α-*androstan*-17-*one* (no. 151) *.—(a) *Incubation.* 2·0 g in EtOH (300 ml), 50 flasks, medium A, 3 d, extraction II ⟶ 2·6 g combined extracts. Chromat. Al_2O_3 (5% deactivated; 200 g). C_6H_6 eluted s.m. (80 mg). $CHCl_3$ eluted 3β,11α-dihydroxy-5α-androstan-17-one (no. 256) * (1·10 g), m.p. 102—106° (from Me_2CO), $[\alpha]_D$ +49° (*c* 0·9) (lit.,[24] m.p. 103—106°, $[\alpha]_D$ +50°). $CHCl_3$–EtOAc (1 : 1) eluted 5α-*androstane*-3β,11α,17β-*triol* (no. 523) (84 mg), m.p. 247—249° (from Me_2CO), $[\alpha]_D$ −7·5° (*c* 0·7) (Found: C, 73·75; H, 10·3. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ (Nujol) 3580 cm^{-1}.

(b) *Transformation.* Oxidation of the triol (no. 521) (36 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,11,17-trione (no. 358) (30 mg).

17-*Oxo*-5α-*androstan*-3β-*yl Acetate* (no. 152) *.—*Incubation.* 5·0 g in EtOH (500 ml), 100 flasks, medium B, 2 d, extraction II ⟶ 6·0 g combined extracts. Chromat. Al_2O_3 (6% deactivated; 150 g). Prolonged elution with EtOAc gave s.m. (158 mg), then 11α-hydroxy-5α-androstane-3,17-dione (no. 519) (134 mg), and then 3β,11α-dihydroxy-5α-androstan-17-one (no. 256) (3·0 g).

3β-*Hydroxyandrost*-5-*en*-17-*one* (no. 153) *.—*Incubation.* 840 mg in EtOH (42 ml), 21 flasks, medium B, 2 d, extraction II ⟶ 500 mg mycelial extract and 950 mg broth extract. The mycelial extract in $CHCl_3$ was filtered through Al_2O_3 (deactivated; 30 g) to give s.m. (405 mg). P.l.c. of the broth extract [2 large plates, 1 × EtOAc] gave 3β,11α-*dihydroxyandrost*-5-*en*-17-*one* (no. 520) (400 mg), m.p. 211—213° (from Me_2CO–hexane), $[\alpha]_D$ −27° (*c* 0·6) (Found: C, 74·65; H, 9·1. $C_{19}H_{28}O_3$ requires C, 74·95; H, 9·3%), $\nu_{max.}$ 3595 and 1740 cm^{-1}.

3β-*Methoxyandrost*-5-*en*-17-*one* (no. 518).—Treatment of 3β-hydroxyandrost-5-en-17-one (no. 153) (2 g) with CH_2N_2–HBF_4 [16] gave 3β-methoxyandrost-5-en-17-one (1·7 g), m.p. 139—142·5° (from MeOH) (lit.,[26] 140—142°).

Incubation. 1·0 g in EtOH (50 ml), 25 flasks, medium B, 2 d, extraction II ⟶ 720 mg mycelial extract and 1·2 g broth extract. The mycelial extract in $CHCl_3$ was filtered through Al_2O_3 (deactivated; 30 g) to give s.m. (230 mg). Broth extract, chromat. Al_2O_3 (5% deactivated; 50 g). Petrol–Et_2O (3 : 2) eluted s.m. (40 mg). Petrol–Et_2O (1 : 4) eluted 11α-*hydroxy*-3β-*methoxy*-5α-*androst*-5-*en*-17-*one* (no. 521) (303 mg), m.p. 161—161·5° (from Et_2O), $[\alpha]_D$ −16° (*c* 0·9) (Found: C, 75·7; H, 9·5. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%), $\nu_{max.}$ 3600 and 1735 cm^{-1}.

Androsta-4,6-*dien*-3-*one* (no. 8) *.—Dry HCl was passed through a solution of androst-4-en-3-one (2 g) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1·8 g) in dioxan (100 ml) until the solution became cloudy. After 5 h the mixture was filtered, and the filtrate was worked up to give *androsta*-4,6-*dien*-3-*one* (1·2 g), m.p. 138—140° (from Me_2CO–hexane), $[\alpha]_D$ +53° (*c* 1·1) (Found: C, 84·4; H, 9·8. $C_{19}H_{26}O$ requires C, 84·4; H, 9·7%), $\lambda_{max.}$ 285 nm (ε 23,200), $\nu_{max.}$ 1670 and 1620 cm^{-1}.

Incubation in EtOH for 6 d gave s.m. (59%) and a complex mixture of products.

We thank the S.R.C. for a Studentship (to J. W. B.) and a Research Grant, I.C.I. Ltd. for a Postdoctoral Fellowship (to W. A. D.), and Glaxo Research, Ltd. for grants and gifts of chemicals. A. K. participated as a result of the Agreement on Co-operation between the Czechoslovak Academy of Science and the Royal Society.

[2/1607 *Received*, 7*th July*, 1972]

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