Microbiological Hydroxylation of Steroids. Part V.[1] The Pattern of Hydroxylation of Dioxygenated 5α-Androstanes with Cultures of the Fungus *Calonectria decora*

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Dioxygenated 5α-androstanes are more readily hydroxylated with *Calonectria decora* than the mono-oxygenated substrates studied previously. Oxygen functions in rings A and D act as primary directing groups and, when a blocking effect by middle ring substituents is allowed for, the pattern of hydroxylation is predictable in most cases.

1β-Hydroxy-compounds are obtained in moderate yields from the readily accessible 5α-androstane-6,17- and 7,17-diones.

Two hydroxy-groups are introduced, on carbon atoms about 4 Å apart, into monoketones of the 5α-androstane and 5α-estrane series by the fungus *Calonectria decora*. The positions of the hydroxy-groups, which are equatorially oriented, are profoundly affected by the strong directing influence of the original keto-group,[1] as illustrated by the examples in Figure 1.

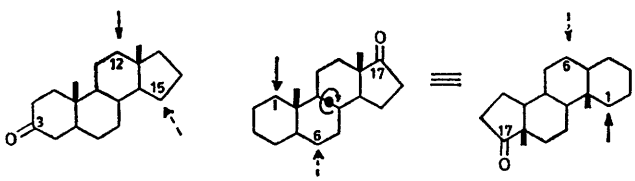
The present extension of the work is concerned mainly with the use of diketones and keto-alcohols as substrates. It was hoped that these would show how the basic behaviour is modified by competitive directing influences, and by additional polar groups which are less powerful than keto-groups in determining the course of hydroxylation. Further, it seemed likely that the presence of a second oxygen substituent would facilitate hydroxylation by increasing the solubility of the substrate and its ability to penetrate the cell walls of the micro-organism. (Substrates containing two or more oxygen substituents have been used in most previous steroid hydroxylations.[2]) This, and the possibility of limiting the fungus to monohydroxylation by a blocking effect of the second substituent on a position that would otherwise be attacked, suggested that dioxygenated substrates might be advantageous in preparative work.

[1] Part IV, 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.

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

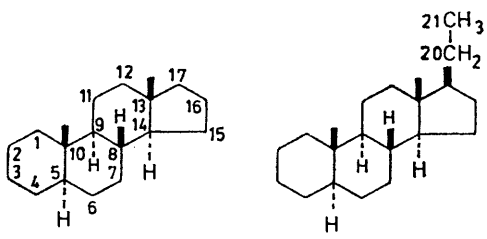
The composition of this paper follows the general plan already explained in detail.[1] Thus, assignments of structures to new compounds are based on the results of spectrometric examination, the conversion of selected

FIGURE 1 Hydroxylation of:
5α-androstan-3-one 5α-androstan-17-one



products into simpler, known steroids, and/or chemical interconversions (see Experimental section). Steroids nos. **483**—**509** (whose n.m.r. signals are listed in Table 4) and some of the new compounds with numbers below **375** in our steroid sequence are described in the present paper. The main microbiological results are summarised in Tables 1—3, whose form corresponds to that used earlier.[1] Of the substrates examined the potentially useful ones are those leading mainly to monohydroxylation (Table 1), or to dihydroxylation (Table 2). [Only 'new' hydroxy-groups are counted in this division. For example, although a 3,15-dihydroxy-compound is obtained from the 3,11-diketone (first case in Table 1), the 3-hydroxy-group is produced by reduction of a group already present rather than by hydroxylation.] With the other substrates (Table 3), the conversions were less satisfactory.

Hydroxylations by *Calonectria decora*



5α-Androstane 5α-Pregnane

In the products column those oxygen functions introduced during the incubation are in bold type. The entries under 'Conditions' refer to the use of ethanol (E) and dimethyl sulphoxide (D) as solvents for the substrate, and to the time of the incubation (in days). All but one of the substrates are derivatives of 5α-androstane and are represented by abbreviated names; the exception, the last substrate in Table 3, is derived from 5α-pregnane.

TABLE 1

Substantial monohydroxylations

Substrate	Conditions	Substrate recovered	Main product(s)	
3,11-$(CO)_2$	D2	27%	**15α**-OH	18%
			3β,15α-$(OH)_2$	23
3,12-$(CO)_2$	E4	10	**15α**-OH	11
			3β,15α-$(OH)_2$	26
3,17-$(CO)_2$-Δ^4	E2	4	**15α**-OH	36
17β-OH—3-CO-Δ^4	E4	25	**15α**-OH	27
			3,**6**-$(CO)_2$	11
3,7-$(CO)_2$	D2	26	**3β,12β**-$(OH)_2$	19
6,17-$(CO)_2$	E2	27	**1β**-OH	**64**
7,17-$(CO)_2$	D6	25	**1β**-OH	55
11,17-$(CO)_2$	E2	4	**6α**-OH	32
17β-OH—2-CO	D4	4	**6α**-OH	31

TABLE 2

Substantial dihydroxylations

Substrate	Conditions	Substrate recovered	Product(s)	
3β-OH—17-CO	D4	6%	**12β,15α**-$(OH)_2$—3-CO	32%
			3β,12β,15α-$(OH)_3$	21
3α-OH—17-CO	D4	0	**1β,3α,6α**-$(OH)_3$	37
			1β,3α, **15α**-$(OH)_3$	25
3β-OMe—17-CO	E4	12	**12β,15α**-$(OH)_2$	30
3α-OMe—17-CO	D4	3	**1β**, **6α**-$(OH)_2$	60
2,16-$(CO)_2$	D4	0	**6**-CO—**12β**-OH	27
			6α, **12β**-$(OH)_2$	12

TABLE 3

Modest conversions

Substrate	Conditions	Substrate recovered	Product(s)	
2,17-$(CO)_2$	D4	15%	**6α**-OH	13%
3,6-$(CO)_2$	D4	69	**12β,15α**-$(OH)_2$	11
			15α-OH	4
3,17-$(CO)_2$	E2	23	**12β,15α**-$(OH)_2$	5
14β-12β-OH—15-CO	D4	0	**7β**-OH	21
			7β, **14β**-$(OH)_2$	9
15α-OH—12-CO	D4	0	**6α**-OH	13
17β-OMe—3-CO	E4	16	**3β,12β,15α**-$(OH)_3$	8
3β-OH-pregnan-20-CO	D6	13	**12β,15α**-$(OH)_2$	24
			7β,12β,15α-$(OH)_3$—3-CO	8

Comparison with the study of androstane monoketones [1] shows that the mere presence of a second polar group in the substrate considerably increases the rate of its utilisation. Thus the recovery of the dioxygenated starting materials was usually low after incubation times of 4, or even 2, days (*cf.* the 6-day period used for monoketones). This was already adumbrated by our inability to interrupt the hydroxylation of monoketones at the monohydroxy-ketone stage, which indicated that the second hydroxylation must be fast.[1] The enzyme system(s) responsible for the formation of products at the trioxygenated level may be present initially in the fungus, or induced rapidly when suitable steroid substrates are added.

As with the simpler substrates, polar groups in the terminal rings exert a dominant directing influence, and the new hydroxy-groups of the major products are equatorial. In the first five examples in Table 1 the common ring A carbonyl group directs hydroxylation into rings C and D; in the remaining instances the presence of a 17-keto- or -hydroxy-group leads to substitution at positions 1 and 6 in rings A and B. Substituents in rings B and C appear to have only weak directing effects. The predominance of monohydroxylation with these substrates suggests that the substituents act by blocking their own positions and by impeding hydroxylation at adjacent sites (*e.g.*, 7-CO at position 6, 11-CO at 12, and possibly *vice versa*) and at positions which are spatially close (*e.g.*, 7-CO at 15, 11-CO at 1, 17-CO or –OH at 12). Consideration of the three sites involved in the monohydroxylations (*i.e.*, the positions of the substrates' two oxygen substituents and the carbon atoms which are attacked) reveals the rough geometric relationship shown in Figure 2: the hydroxy-groups are introduced at nuclear positions which are 3·5—4·5 Å from the nearer oxygen-bearing carbons and at 6·3—8·0 Å from either position 3 (substrates with ring A substituents) or position 17 (those substituted in ring D). This pattern is similar to, but less precise than, that found in the dihydroxylation of androstane and estrane monoketones.[1]

The dihydroxylations (Table 2) involve substitution at the usual positions (1β, 6α, 12β, and 15α): it is likely that some monohydroxylation could have been achieved by using shorter incubation times with ethanol rather than dimethyl sulphoxide as solvent. [The effect of the latter in promoting polysubstitution is shown with 3β-hydroxy-5α-pregnan-20-one. Of the present substrates only this one has been previously studied with *C. decora*, and found, with acetone as solvent, to give the 3β,12β,15α-triol in 40% yield.[3] Under the more vigorous conditions (Table 3) the triol, in smaller amount, is accompanied by the 7β,12β,15α-trihydroxy-3,20-diketone.]

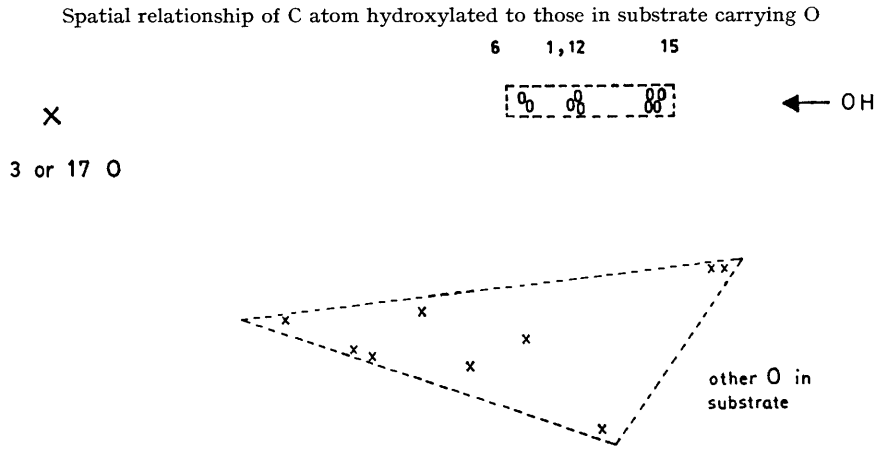


FIGURE 2 Monohydroxylation of dioxygenated substrates by *Calonectria decora*

Comparison of the products' structures with the results obtained with mono-substituted androstanes [1] leads to the following relationships between the directing strengths of some substituents: 3-CO(Δ^4 and, less clearly, 5α-H) and 3β-OR (R = H and Me) > 17-CO > 3α-OR (R = H and Me); 3-CO-Δ^4 > 17β-OH. Thus the nature of the oxygen function is less important than its spatial position relative to the steroid nucleus as a whole. It may be that the directing effect of an oxygen group arises from bonding with active hydrogen in one of the enzyme's hydrophilic sites: such a localised interaction would be consistent with the marked changes in the directing power and binding efficiency of a particular oxygen group as its position in the steroid nucleus is varied.

For preparative purposes the most interesting result is that 1β-hydroxy-compounds are obtained in satisfactory yield from the readily accessible 6,17- and 7,17-diketones.

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. 1. Where compounds with serial numbers below 483 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 results are reported in the abbreviated form used earlier.[1] I.r. spectra indicated by $\nu_{max.}$ (high resolution) refer to 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°.

5α-Androstane-2,16-dione (no. 33).*—(a) *Incubation.* 480 mg in Me_2SO (72 ml), 12 flasks, medium B, 4 d, extraction I ⟶ 675 mg combined extracts. P.l.c. [2 large plates, 8 × petrol–Me_2CO (7 : 3)] gave two main bands. The band of higher R_F afforded *12β-hydroxy-5α-androstane-2,6,16-trione* (no. 215) * (134 mg), m.p. 250—253° (decomp.) (from Me_2CO–hexane), $[\alpha]_D$ −152° (*c* 0·4) (Found: C, 71·3; H, 8·3. $C_{19}H_{26}O_4$ requires C, 71·7; H, 8·2%), $\nu_{max.}$ (high resolution) 3624, 1749, and 1717 cm^{-1}. The second band gave *6α,12β-dihydroxy-5α-androstane-2,16-dione* (no. 314)* (58 mg), m.p. 136—138° and 221·5—222·5° (from Me_2CO–hexane), $[\alpha]_D$ −99° (*c* 0·3) (Found: C, 67·3; H, 8·9. $C_{19}H_{28}O_4,H_2O$ requires C, 67·4; H, 8·9%), $\nu_{max.}$ ($CHCl_3$) 3590, 1739, and 1709 cm^{-1}.

(b) *Transformations.* Huang-Minlon reduction of 12β-hydroxy-5α-androstane-2,6,16-trione (no. 215) gave 5α-androstan-12β-ol (no. 130); * m.p. (from MeOH–H_2O) 115—117° (lit.,[4] 115—116°), identified by conversion into the 12β-acetate (no. 140), m.p. and [5] mixed m.p. 62—64°. Huang-Minlon reduction of 6α,12β-dihydroxy-5α-androstane-2,16-dione (no. 314) afforded 5α-androstane-6α, 12β-diol (no. 222),* m.p. (from Me_2CO–hexane) and [1] mixed m.p. 197·5—198·5°.

5α-Androstane-2,17-dione (no. 34).*—(a) *Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction I ⟶ 1·09 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 60 g). Petrol–Et_2O (1 : 1) gave s.m. (153 mg). Further elution with the same solvent mixture gave material which, after p.l.c. [1 large plate, 3 × petrol–Me_2CO (4 : 1)], afforded *6α-hydroxy-5α-androstane-2,17-dione* (no. 195)* (115 mg), m.p. 93·5—95·5° (from EtOAc–hexane), $[\alpha]_D$ +124° (*c* 1·0) (Found: C, 72·15; H, 9·2. $C_{19}H_{28}O_3$,-0·5EtOAc requires C, 72·4; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3590, 1732, and 1706 cm^{-1}.

(b) *Transformation.* Oxidation of 6α-hydroxy-5α-androstane-2,17-dione (no. 195) with 8N-H_2CrO_4 gave *5α-androstane-2,6,17-trione* (no. 483), m.p. 198·5—200·5° (from

[3] A. Schubert and R. Siebert, *Chem. Ber.*, 1958, **91**, 1856.
[4] C. Djerassi and L. Tökes, *J. Amer. Chem. Soc.*, 1966, **88**, 536.
[5] 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.

TABLE 4

N.m.r. signals

Solutions were examined at 100 MHz. Subscript arabic numerals of τ values refer to the solvent [1, CCl_4; 2, $CDCl_3$; 3, C_6H_6]. $\Delta_1^3 = \tau(C_6H_6) - \tau(CCl_4)$. 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_2(calc.)	τ_3	Δ_1^3
(483)	5α-Androstane-2,6,17-trione	19	9·26	9·25	9·28	9·60	+0·34
		18	9·15	9·11	9·11	9·58	+0·43
(484)	Androst-4-ene-3,6,17-trione	19	8·81	8·80	8·83	9·46	+0·65
		18	9·11	9·07	9·04	9·54	+0·43
(485)	5α-Androstane-3,7,12-trione	19		8·62	8·60	9·53	+0·93
		18		8·91	8·88	9·34	+0·46
(486)	Androst-4-ene-3,15,17-trione	19	8·78	8·77	8·76	9·41	+0·63
		18	8·97	8·96	8·98	9·43	+0·46
(487)	5α-Androstane-1,6,11,17-tetraone	19		8·66	8·65		
		18		9·19	9·14		

			τ_2	τ_2(calc.)	>CH-OR (in $CDCl_3$)		
(488)	12β-Hydroxy-5α,14β-androstan-15-one	19	9·24		H-12	6·64	*4*(11, 4)
		18	8·87				
(489)	15α-Hydroxy-5α-androstan-12-one	19	9·10	9·11	H-15	5·73	*6*(8, 8, 4)
		18	8·94	8·90			
(490)	3β-Hydroxy-5α-pregnan-20-one	19	9·19	9·20	H-3	6·38	m(22)
		18	9·39	9·39			
(491)	3α-Methoxy-5α-androstane-1,6-dione	19	8·88	8·87	H-3	6·13	m(9)
		18	9·29	9·26			
(492)	6α-Hydroxy-5α-androstane-11,17-dione	19	8·95	8·95			
		18	9·17	9·16			
(493)	11,17-Dioxo-5α-androstan-6α-yl acetate	19	8·89	8·95			
		18	9·16	9·16			
(494)	6β-Hydroxyandrost-4-ene-3,17-dione	19	8·60	8·59	H-6	5·62	m(7)
		18	9·06	9·02			
(495)	15α-Hydroxy-5α-androstane-3,6-dione	19	9·02	9·02	H-15	5·91	*6*(8, 8, 4)
		18	9·22	9·23			
(496)	15α-Hydroxy-5α-androstane-3,12-dione	19	8·88	8·87	H-15	5·70	*6*(8·5, 8·5, 5)
		18	8·86	8·86			
(497)	3β,12β-Dihydroxy-5α-androstan-7-one	19	8·90	8·90	H-3	6·38	m(17)
		18	9·29	9·24	H-12	6·62	*4*(10, 5)
(498)	3β,15α-Dihydroxy-5α-androstan-6-one	19	9·23	9·23	H-3	6·42	m(20)
		18	9·25	9·25	H-15	5·94	*6*(8, 8, 3)
(499)	3β,15α-Dihydroxy-5α-androstan-12-one	19	9·08	9·08	H-3	6·35	m(24)
		18	8·93	8·89	H-15	5·71	*6*(9, 9, 5)
(500)	1α,6α-Dihydroxy-5α-androstane-11,17-dione	19	8·91	8·94			
		18	9·17	9·16			
(501)	1β,6α-Dihydroxy-5α-androstane-11,17-dione	19	8·89	8·90			
		18	9·19	9·16			
(502)	1β,3α,6α-Triacetoxy-5α-androstan-17-one	19	8·97	9·01	H-1	5·03	*4*(12, 5)
		18	9·14	9·12	H-3	4·85	m(20)
					H-6	5·17	*6*(10, 10, 5)
(503)	1β,3α,6α-Trihydroxy-5α-androstan-17-one *	19	9·23		H-1	6·43	*4*(11,6)
		18	9·27		H-3	6·10	m(7)
					H-6	6·66	*6*(10, 10, 5)
(504)	1β,3α,15α-Triacetoxy-5α-androstan-17-one	19	9·02	9·04	H-1	5·04	*4*(11,5)
		18	9·05	9·05	H-3	4·92	m(7)
					H-15	4·75	m(12)
(505)	1β,3α,15α-Trihydroxy-5α-androstan-17-one	19	9·18	9·15	H-1	6·13	*4*(12,5)
		18	9·05	9·10	H-3	5·86	m(20)
					H-15	5·41	m(20)
(506)	3β,12β,15α-Trihydroxy-5α-pregnan-20-one	19	9·17	9·18			
		18	9·19				
(507)	3β,12β,15α-Triacetoxy-5α-pregnan-20-one	19	9·16	9·16	H-3	5·30	*7*(10, 10, 5, 5)
		18	9·10	9·10 †	H-12	5·16	*4*(12,5)
					H-15	4·97	*6*(8, 8, 4)
(508)	17β-Methoxy-5α-androstane-3β,12β,15α-triol	19	9·17	9·17	H-3	6·40	m(30)
		18	9·22	9·17	H-12	6·45	*4*(10, 5)
					H-15	5·80	*6*(9, 9, 4)
					H-17	6·35	t(8)
(509)	7β,12β,15α-Triacetoxy-5α-pregnane-3,20-dione	19	8·92	8·92	H-7	5·40	*6*(10, 10, 5)
		18	9·01	9·06 †	H-12	5·17	*4*(10, 5)
					H-15	4·94	*6*(8, 8, 3)

* In $(CD_3)_2SO$. † Calculated with $\Delta\tau_2$(H-18) = −0·19 for the 12β,15α-$(OAc)_2$ − 17β-COMe unit, as will be described later.

[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.

Me_2CO–hexane), $[\alpha]_D$ +70° (*c* 1·0) (Found: C, 75·6; H, 8·5. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ (high resolution) 1745 and 1717 cm^{-1}.

17β-*Hydroxy*-5α-*androstan*-2-*one* (no. 180).*—(a) *Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction I ⟶ 1·82 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (1 : 1) gave s.m. (44 mg), m.p. (from MeOH) and mixed m.p. 178—180°. Further elution with petrol–Et_2O (1 : 1) gave material which, after p.l.c. [1 large plate, 4 × petrol–Me_2CO (4 : 1)], afforded 6α,17β-*dihydroxy*-5α-*androstan*-2-*one* (no. 280)* (316 mg), m.p. 236—239° (from Me_2CO–hexane), $[\alpha]_D$ +61° (*c* 0·6) (Found: C, 74·4; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1706 cm^{-1}.

(b) *Transformation.* Oxidation of the dihydroxy-ketone (no. 280) with 8N-H_2CrO_4 gave 5α-androstane-2,6,17-trione (no. 483), m.p. (from MeOH) and mixed m.p. 198—201°.

5α-*Androstane*-3,6-*dione* (no. 35).*—*Incubation.* 840 mg in Me_2SO (126 ml), 21 flasks, medium B, 2 d, extraction II ⟶ 836 mg mycelial extract and 200 mg broth extract. Chromat. of the mycelial extract on Al_2O_3 (10% deactivated; 40 g). C_6H_6 gave s.m. (580 mg). P.l.c. [1 large plate, 1 × petrol–Me_2CO (4 : 1)] of the broth extract gave 15α-*hydroxy*-5α-*androstane*-3,6-*dione* (no. 495) (higher R_F) (12 mg), m.p. 182—185° (from Me_2CO–hexane), $[\alpha]_D$ −51° (*c* 0·2) (Found: C, 74·9; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3600 and 1715 cm^{-1}, and 3β,15α-*dihydroxy*-5α-*androstan*-6-*one* (no. 498) (lower R_F) (30 mg), m.p. 215—216° (from Me_2CO–hexane), $[\alpha]_D$ −15·5° (*c* 0·4) (Found: C, 74·7; H, 10·2. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3600 and 1715 cm^{-1}.

5α-*Androstane*-3,7-*dione* (no. 36).—(a) *Incubation.* 250 mg in Me_2SO (42 ml), 7 flasks, medium B, 2 d, extraction II ⟶ 340 mg combined extracts. Chromat. Al_2O_3 (10% deactivated; 30 g). Petrol–Et_2O (3 : 1) gave s.m. (66 mg). Petrol–Et_2O (1 : 1) gave 3α-*hydroxy*-5α-*androstan*-7-*one* (no. 145)* (17 mg), m.p. 177·5—178·5° (from EtOAc), $[\alpha]_D$ −74° (*c* 0·3) (Found: C, 78·7; H, 10·7. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·35%), $\nu_{max.}$ 3620 and 1712 cm^{-1}. Et_2O gave 3β,12β-*dihydroxy*-5α-*androstan*-7-*one* (no. 497) (37 mg), m.p. 185—188° (from MeOH–H_2O), $[\alpha]_D$ −43° (*c* 0·4) (Found: C, 75·1; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·8%), $\nu_{max.}$ 3610 and 1715 cm^{-1}.

(b) *Transformations.* Oxidation of 3α-hydroxy-5α-androstan-7-one (no. 145) with 8N-H_2CrO_4 gave 5α-androstan-3 7-dione (no. 36), m.p. (from Me_2CO–hexane) and [6] mixed m.p. 148—149°. Oxidation of 3β,12β-dihydroxy-5α-androstan-7-one (no. 497) gave 5α-*androstane*-3,7,12-*trione* (no. 485), m.p. 263—265° (from Me_2CO–petrol), $[\alpha]_D$ −7° (*c* 0·6) (Found: C, 75·15; H, 8·5. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1715 cm^{-1}.

5α-*Androstane*-3,11-*dione* (no. 37).*.—(*a*) *Incubation.* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 2 d, extraction II ⟶ 300 mg mycelial extract and 400 mg broth extract. Chromat. of the mycelial extract on Al_2O_3 (5 g). C_6H_6 gave s.m. (170 mg). Chromat. of the broth extract on Al_2O_3 (10 g). Petrol–$CHCl_3$ (10 : 1) gave further s.m. (100 mg). Petrol–$CHCl_3$ (3 : 1) gave 15α-hydroxy-5α-androstane-3,11-dione (no. 372)* (140 mg), m.p. (from Me_2CO–hexane) and [7] mixed m.p. 167—170°, $\nu_{max.}$ (high resolution) 3629, 3610w, and 1715 cm^{-1}. Petrol–$CHCl_3$ (1 : 1) gave 3β,15α-dihydroxy-5α-androstan-11-one (no. 375) (180 mg), m.p. (from Me_2CO–hexane) and [7] mixed m.p. 228—230°.

(b) *Transformation.* Huang-Minlon reduction of 15α-hydroxy-5α-androstane-3,11-dione (no. 372) (30 mg) under vigorous conditions [1] gave 5α-androstan-15α-ol (no. 131)* (20 mg), m.p. (from MeOH) and [7] mixed m.p. 159—162°.

5α-*Androstane*-3,12-*dione* (no. 379).*—(a) *Incubation.*

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320 mg in EtOH (16 ml), 8 flasks, medium A, 4 d, extraction II ⟶ 120 mg mycelial extract and 360 mg broth extract. Chromat. of the mycelial extract on Al_2O_3 (deactivated; 4 g). C_6H_6 gave s.m. (31 mg). P.l.c. [1 large plate, 3 × Et_2O] of the broth extract gave 15α-*hydroxy*-5α-*androstane*-3,12-*dione* (no. 496) (higher R_F) (30 mg), m.p. 217—219° (from Me_2CO–H_2O), $[\alpha]_D$ +122° (*c* 0·1) (Found: C, 74·8; H, 9·0. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3630 and 1717 cm^{-1}, and 3β,15α-*dihydroxy*-5α-*androstan*-12-*one* (no. 499) (lower R_F) (72 mg), m.p. 236—238° (from MeOH), $[\alpha]_D$ +79° (*c* 0·3) (Found: C, 74·1; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3630 and 1717 cm^{-1}.

(b) *Transformations*. Oxidation of both metabolites (nos. 496 and 499) with 8N-H_2CrO_4 gave 5α-androstane-3,12,15-trione (no. 86),* m.p. (from EtOH) and [1] mixed m.p. 203—205°.

5α-*Androstane*-3,17-*dione* (no. 42).*—(a) *Incubation*. 4·4 g in EtOH (440 ml), 88 flasks, medium A, 2 d, extraction III ⟶ 4·0 g total extract. Chromat. Al_2O_3 (10% deactivated; 200 g). Petrol–Et_2O (1 : 1) gave s.m. (1·0 g). Et_2O–MeOH (10 : 1) gave a mixture (2·4 g) containing at least six compounds. P.l.c. of the mixture [6 large plates, 6 × Et_2O–EtOAc (19 : 1)] gave 12β,15α-*dihydroxy*-5α-*androstane*-3,17-*dione* (no. 315)* (200 mg), m.p. 220—222° (from EtOAc), $[\alpha]_D$ +104° (*c* 0·9) (Found: C, 70·9; H, 8·7. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%). The other metabolites could not be purified.

(b) *Transformation*. Huang-Minlon reduction of the preceding dihydroxy-dione (no. 315) (50 mg) gave 5α-androstane-12β,15α-diol (no. 229)* (20 mg), m.p. (from Me_2CO–hexane) and [1] mixed m.p. 139—140° and 170—171°.

Androst-4-*ene*-3,17-*dione* (no. 43).*—(a) *Incubation*. 7·0 g in EtOH (770 ml), 140 flasks, medium A, 2 d, extraction III ⟶ 6·5 g total extract. Chromat. Al_2O_3 (10% deactivated; 200 g). Et_2O gave s.m. (2·8 g), m.p. and mixed m.p. 171—173°. Et_2O–MeOH (20 : 1) gave a mixture (3·4 g) which was separated into three bands by p.l.c. [7 large plates, 6 × Et_2O–EtOAc(19 : 1)]. The band of highest R_F afforded 15α-hydroxyandrost-4-ene-3,17-dione (no. 205)* (2·6 g), m.p. 200—202° (from EtOAc), $[\alpha]_D$ +218° (*c* 0·9) (lit.,[8] m.p. 194—196°, $[\alpha]_D$ +206°). The second band gave 6β-hydroxyandrost-4-ene-3,17-dione (no. 494) (100 mg), m.p. 192—193° (from EtOAc), $[\alpha]_D$ +115° (*c* 0·7 (lit.,[9] m.p. 192—193°, $[\alpha]_D$ +114°). The band of lowest R_F gave 12β,15α-*dihydroxyandrost*-4-*ene*-3,17-*dione* (no. 374)* (80 mg), m.p. 238—239° (from EtOAc), $[\alpha]_D$ +175° (*c* 1·1) (Found: C, 71·6; H, 8·2. $C_{19}H_{26}O_4$ requires C, 71·7; H, 8·2%).

(b) *Transformations*. Oxidation of 15α-hydroxyandrost-4-ene-3,17-dione (no. 205) (50 mg) with 8N-H_2CrO_4 gave androst-4-ene-3,15,17-trione (no. 486) (30 mg), m.p. 172—173° (from EtOH), $[\alpha]_D$ +123° (*c* 0·5) (lit.,[8] m.p. 172—173°, $[\alpha]_D$ +124°). Oxidation of 6β-hydroxyandrost-4-ene-3,17-dione (no. 494) (50 mg) gave androst-4-ene-3,6,16-trione (no. 484) (42 mg), m.p. 227—229° (from EtOH), $[\alpha]_D$ +41° (*c* 0·6) (lit.,[9] m.p. 223—225°), $[\alpha]_D$ +43°.

17β-*Hydroxyandrost*-4-*en*-3-*one* (no. 182).*—*Incubation*. 4·0 g in EtOH (200 ml), 80 flasks, medium A, 4 d, extraction I ⟶ 4·5 g combined extract. Chromat. Al_2O_3 (10% deactivated; 700 g). Et_2O gave androst-4-ene-3,17-dione (no. 43)* (320 mg), m.p. (from MeOAc) and [6] mixed m.p. 171—173°. Further elution with Et_2O gave s.m. (1·0 g). $CHCl_3$–MeOH (50 : 1) gave a mixture which was separated into two components by p.l.c. [1 large plate, 10 × petrol–Me_2CO (5 : 1)]. The band of higher R_F gave 17β-hydroxy-5α-androstane-3,6-dione (no. 208)* (340 mg), m.p. 233—236° (from MeOAc), $[\alpha]_D$ −13° (*c* 0·9) (lit.,[10] m.p. 232—236°, $[\alpha]_D$ −10°). The second band afforded 15α,17β-dihydroxyandrost-4-en-3-one (no. 309)* (850 mg), m.p. 203—205° (from EtOAc), $[\alpha]_D$ +134° (*c* 1·1) (lit.,[11] m.p. 102—110° and 203—206°, [α] +136°).

3β-*Hydroxy*-5α-*androstan*-17-*one* (no. 151).*—(a) *Incubation*. 2·8 g in EtOH (350 ml), 70 flasks, medium A, 2 d, extraction III ⟶ 1·8 g total extract. P.l.c. [3 large plates, 6 × petrol–Me_2CO (5 : 1)] gave two bands. That of higher R_F afforded 12β,15α-dihydroxy-5α-androstane-3,17-dione (no. 315)* (160 mg), m.p. (from EtOAc) and mixed m.p. 219—222°. The second band gave 3β,12β,15α-*trihydroxy*-5α-*androstan*-17-*one* (no. 335)* (74 mg), m.p. 219—221° (from EtOAc), $[\alpha]_D$ +8° (*c* 0·9) (Found: C, 70·5; H, 9·1. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%).

A repetition of the foregoing incubation in Me_2SO for 4 d gave 12β,15α-dihydroxy-5α-androstane-3,17-dione (no. 315) (32%) and 3β,12β,15α-trihydroxy-5α-androstan-17-one (no. 335) (21%).

(b) *Transformations*. Huang-Minlon reduction of 12β,15α-dihydroxy-5α-androstane-3,17-dione (no. 315) (30 mg) gave 5α-androstane-12β,15α-diol (no. 229)* (8 mg), m.p. (from MeOH) and [1] mixed m.p. 138—139°; similarly 3β,12β,15α-trihydroxy-5α-androstan-17-one (no. 335) (35 mg) gave 5α-androstane-3β,12β,15α-triol (no. 461)* (11 mg), m.p. (from MeOH) and [1] mixed m.p. 245—248°.

3α-*Hydroxy*-5α-*androstan*-17-*one* (no. 146).—(a) *Incubation*. 600 mg in Me_2SO (90 ml), 15 flasks, medium B, 4 d, extraction II ⟶ 230 mg mycelial extract and 600 mg broth extract. Mycelial extract contained no s.m. and was discarded. Crystallisation of the broth extract from $CHCl_3$ gave 1β,3α,6α-*trihydroxy*-5α-*androstan*-17-*one* (no. 503) (190 mg), m.p. 293—295° (from MeOH), $[\alpha]_D$ +100° (*c* 0·5) (Found: C, 70·7; H, 9·4. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%), $\nu_{max.}$ (Nujol) 3460 and 1735 cm^{-1}. P.l.c. [1 large plate, 2 × petrol–Me_2CO (3 : 1)] of the material in the $CHCl_3$ filtrate gave more 1β,3α,6α-trihydroxy-5α-androstan-17-one (29 mg), and 1β,3α,15α-*trihydroxy*-5α-*androstan*-17-*one* (no. 505) (145 mg), m.p. 238—240° (from MeOH), $[\alpha]_D$ +113° (*c* 0·3) (Found: C, 70·5; H, 9·45. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%), $\nu_{max.}$ (Nujol) 3640, 3470, and 1735 cm^{-1}.

(b) *Transformations*. Acetylation [Ac_2O–C_5H_5N (3 : 1) for 2 d] of 1β,3α,6α-trihydroxy-5α-androstan-17-one (no. 503) gave the *triacetate* (no. 502), m.p. 203—205° (from Me_2CO–hexane), $[\alpha]_D$ +62° (*c* 0·8) (Found: C, 66·75; H, 8·1. $C_{25}H_{36}O_7$ requires C, 66·8; H, 8·1%), $\nu_{max.}$ 1742br cm^{-1}. Similar acetylation of 1β,3α,15α-trihydroxy-5α-androstan-17-one (no. 505) gave the corresponding triacetate (no. 504) as an oil, *m/e* 448 (M^+), $\nu_{max.}$ 1740br cm^{-1}.

3β-*Methoxy*-5α-*androstan*-17-*one* (no. 155).*—(a) *Incubation*. 3·1 g in EtOH (400 ml), 80 flasks, medium A, 4 d, extraction III ⟶ 3·5 g total extract. P.l.c. [6 large plates, 8 × petrol–Me_2CO (5 : 1)] gave 4 bands. That of highest R_F gave s.m. (370 mg). The second band afforded 12β-*hydroxy*-3β-*methoxy*-5α-*androstan*-17-*one* (no.

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259)* (27 mg), m.p. 136—136·5° (from EtOAc), $[\alpha]_D$ +52° (*c* 0·7) (Found: C, 74·6; H, 9·8. $C_{20}H_{32}O_3$ requires C, 74·9; H, 10·1%), $\nu_{max.}$ (high resolution) 3567 and 1736 cm^{-1}. The third band (37 mg) consisted mainly of a compound presumed to be 15α-hydroxy-3β-methoxy-5α-androstan-17-one (no. 260),* $\nu_{max.}$ 3612 and 1745 cm^{-1}. The fourth band afforded 12β,15α-*dihydroxy*-3β-*methoxy*-5α-*androstan*-17-*one* (no. 336)* (920 mg), m.p. 193—194° (from EtOAc), $[\alpha]_D$ −32° (*c* 1·2) (Found: C, 71·5; H, 9·7. $C_{20}H_{32}O_4$ requires C, 71·4; H, 9·6%).

(b) *Transformations*. Huang-Minlon reduction of 12β,15α-dihydroxy-3β-methoxy-5α-androstan-17-one (no. 336) (50 mg) and oxidation of the product with 8N-H_2CrO_4 gave 3β-*methoxy*-5α,14β-*androstane*-12,15-*dione* (no. 92)* (45 mg), m.p. 122—125° (from EtOAc), $[\alpha]_D$ +66° (*c* 0·9) (Found: C, 75·5; H, 9·4. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%), $\nu_{max.}$ 1747 and 1715 cm^{-1}.

3α-*Methoxy*-5α-*androstan*-17-*one* (no. 154).*—(a) *Incubation*. 1·56 g in Me_2SO (300 ml), 50 flasks, medium B, 4 d, extraction I ⟶ 649 mg mycelial extract and 1·5 g broth extract. P.l.c. [1 large plate, 3 × petrol–Me_2CO (9 : 1)] of the mycelial extract gave s.m. (49 mg). The broth extract was dissolved in EtOAc and refluxed with activated charcoal for 15 min. The solution was filtered through Celite and evaporated to give 1β,6α-*dihydroxy*-3α-*methoxy*-5α-*androstan*-17-*one* (no. 332),* m.p. 190—192° (from EtOAc) (1·02 g), $[\alpha]_D$ +94° (*c* 0·4) (Found: C, 71·2; H, 9·5. $C_{20}H_{32}O_4$ requires C, 71·4; H, 9·6%), $\nu_{max.}$ 3622 and 1742 cm^{-1}.

(b) *Transformations*. Oxidation of 1β,6α-dihydroxy-3α-methoxy-5α-androstan-17-one (no. 332) (150 mg) with 8N-H_2CrO_4 gave 3α-*methoxy*-5α-*androstane*-1,6,17-*trione* (no. 214)* (97 mg), m.p. 201—203° (from Me_2CO–hexane), $[\alpha]_D$ +132° (*c* 1·1) (Found: C, 72·2; H, 8·4. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ (high resolution) 1746, 1717sh, and 1713 cm^{-1}. Huang-Minlon reduction of the metabolite (no. 332) (300 mg) gave 3α-*methoxy*-5α-*androstane*-1β,6α-*diol* (no. 322)* (250 mg), m.p. 201—203° (from Me_2CO–hexane), $[\alpha]_D$ +15° (*c* 0·8) (Found: C, 74·4; H, 10·5. $C_{20}H_{34}O_3$ requires C, 74·5; H, 10·6%), $\nu_{max.}$ 3622 cm^{-1}. Oxidation of the methoxy-diol (no. 322) (150 mg) with 8N-H_2CrO_4 gave 3α-*methoxy*-5α-*androstane*-1,6-*dione* (no. 491) (84 mg), m.p. 114—116° (from Me_2CO–hexane), $[\alpha]_D$ +59° (*c* 0·7) (Found: C, 75·2; H, 9·7. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%), $\nu_{max.}$ 1715 cm^{-1}.

17β-*Methoxy*-5α-*androstan*-3-*one* (no. 183).*—*Incubation*. 800 mg in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 900 mg mycelial extract and 850 mg broth extract. P.l.c. [2 large plates, 1 × Et_2O] of the mycelial extract gave s.m. (130 mg). P.l.c. [2 large plates, 6 × Et_2O] of the broth extract gave 17β-*methoxy*-5α-*androstane*-3β,12β,15α-*triol* (no. 508) (62 mg), m.g. 239—243° (from Me_2CO), $[\alpha]_D$ +75° (*c* 1·1) (Found: C, 70·8; H, 9·8. $C_{20}H_{34}O_4$ requires C, 71·0; H, 10·1%), $\nu_{max.}$ 3622 cm^{-1}.

3β-*Hydroxy*-5α-*pregnan*-20-*one* [6] (no. 490).—(a) *Incubation*. 4·0 g in Me_2SO (570 ml), 100 flasks, medium B, 6 d, extraction III ⟶ 2·9 g total extract. Chromat. Al_2O_3 (10% deactivated; 250 g). Petrol–$CHCl_3$ (2 : 1) gave s.m. (500 mg). $CHCl_3$–MeOH (49 : 1) gave 3β,12β,15α-*trihydroxy*-5α-*pregnan*-20-*one* (no. 506), m.p. 230° (decomp.) (from EtOH) (910 mg), $[\alpha]_D$ +76° (*c* 0·5) (Found: C, 71·6; H, 9·7. $C_{21}H_{34}O_4$ requires C, 72·0; H, 9·8%), $\nu_{max.}$ (Nujol) 3330 and 1705 cm^{-1}. The residues from the foregoing crystallisation were acetylated [Ac_2O–C_5H_5N (3 : 1) for 2 d] and the product was separated into 2 bands by p.l.c. [1 large plate, 3 × petrol–Me_2CO (6 : 1)]. The band of higher R_F gave 3β,12β,15α-*triacetoxy*-5α-*pregnan*-20-*one* (no. 507) (150 mg), m.p. 156—158° (from Me_2CO–hexane), $[\alpha]_D$ +57° (*c* 0·9) (Found: C, 67·9; H, 8·3. $C_{27}H_{40}O_7$ requires C, 68·0; H, 8·5%), $\nu_{max.}$ 1736, 1713, and 1238 cm^{-1}. The second band afforded 7β,12β,15α-*triacetoxy*-5α-*pregnane*-3,20-*dione* (no. 509) (430 mg), m.p. 221—227° (from Me_2CO–hexane), $[\alpha]_D$ +124° (*c* 0·9) (Found: C, 66·1; H, 7·7. $C_{27}H_{38}O_8$ requires C, 66·1; H, 7·8%), $\nu_{max.}$ 1735, 1717, and 1235 cm^{-1}.

(b) *Transformation*. Oxidation of 3β,12β,15α-trihydroxy-5α-pregnan-20-one (no. 506) (400 mg) with 8N-H_2CrO_4 gave 5α-pregnane-3,12,15,20-tetraone (no. 364),* m.p. 206—212° (from Me_2CO–hexane) (290 mg), $[\alpha]_D$ +210° (*c* 1·0) (lit.,[3] m.p. 203—212°, $[\alpha]_D$ +147°).

5α-*Androstane*-6,17-*dione* (no. 48).*—(a) *Incubation*. 6·8 g in EtOH (700 ml), 140 flasks, medium A, 2 d, extraction III ⟶ 8·12 g total extract. Chromat. Al_2O_3 (10% deactivated; 250 g). Petrol–Et_2O (4 : 1) gave s.m. (1·84 g). Petrol–Et_2O (3 : 2) eluted material which was purified by p.l.c. [1 large plate, 4 × hexane–EtOAc (3 : 1)] to give 17β-*hydroxy*-5α-*androstan*-6-*one* (no. 84)* (151 mg), m.p. 150—153° (from MeOH), $[\alpha]_D$ −16° (*c* 0·5) (Found: C, 78·4; H, 10·3. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ (high resolution) 3626 and 1713 cm^{-1}. Petrol–Et_2O (2 : 3) eluted material which was rechromatographed on Al_2O_3 (10% deactivated; 200 g). Petrol–Et_2O (1 : 1) eluted 1β-*hydroxy*-5α-*androstane*-6,17-*dione* (no. 189)* (3·38 g), m.p. 200—203° (from MeOH), $[\alpha]_D$ +51° (*c* 0·4) (Found: C, 75·4; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ (high resolution) 3653w, 3616, 1742, and 1713 cm^{-1}.

(b) *Transformations*. Oxidation of 1β-hydroxy-5α-androstane-6,17-dione (no. 189) (150 mg) with 8N-H_2CrO_4 gave 5α-androstane-1,6,17-trione (no. 64) (120 mg), m.p. (from MeOH) and [1] mixed m.p. 203—205°. Huang-Minlon reduction of 1β-hydroxy-5α-androstane-6,17-dione (no. 189) (350 mg) gave, after p.l.c. [1 large plate, 1 × hexane–EtOAc (9 : 1)], 5α-androstan-1β-ol (no. 102)* (152 mg), m.p. 120—122° (from hexane), $[\alpha]_D$ −17° (*c* 0·4) (lit.,[12] m.p. 123—125°, $[\alpha]_D$ −19°). Oxidation of 5α-androstan-1β-ol (no. 102) (150 mg) with 8N-H_2CrO_4 gave 5α-androstan-1-one (no. 1)* (66 mg, m.p. 70—71° (from MeOH–H_2O), $[\alpha]_D$ +118° (lit.,[12] m.p. 68—70°, $[\alpha]_D$ +125°).

A solution of 1β-hydroxy-5α-androstane-6,17-dione (no. 189) (500 mg) in MeOH (25 ml)–KOH (1·2 g) was heated under reflux for 24 h. The product, separated by p.l.c. [2 large plates, 5 × PhMe–EtOAc (6 : 1)], gave s.m. (265 mg) and 1β-*hydroxy*-5β-*androstane*-6,17-*dione* (no. 190)* (138 mg), m.p. 257—258° (from MeOH), $[\alpha]_D$ −8° (*c* 0·1) (Found: C, 74·8; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3610, 1745, and 1710 cm^{-1}. Oxidation of the foregoing hydroxy-diketone (no. 190) (15 mg) gave 5β-androstane-1,6,17-trione (no. 356) (7 mg), m.p. (from MeOH) and [1] mixed m.p. 240—243°.

Huang-Minlon reduction of 1β-hydroxy-5β-androstane-6,17-dione (no. 190) (200 mg) gave, after purification by p.l.c. [1 large plate, 3 × petrol–C_6H_6 (1 : 1)], 5β-*androstan*-1β-*ol* (no. 104)* (95 mg) as an oil, *m/e* 276·2453 (M^+) ($C_{19}H_{32}O$ requires *M*, 276·2461). Oxidation of this alcohol (no. 104) (100 mg) with 8N-H_2CrO_4 gave 5β-*androstan*-1-*one* (no. 2)* (82 mg), m.p. 92—94° (from MeOH), $[\alpha]_D$ −122° (*c* 0·9) (Found: C, 83·3; H, 10·9. $C_{19}H_{30}O$ requires C, 83·2; H, 11·0%).

[12] G. von Mutzenbecher and A. D. Cross, *Steroids*, 1965, **5**, 429.

5α-*Androstane*-7,17-*dione* (no. 51).*—(a) *Incubation.* 1 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction II ⟶ 300 mg mycelial extract and 1·5 g broth extract. Chromat. of mycelial extract on Al_2O_3 (10 g). Petrol–Et_2O (4 : 1) gave s.m. (236 mg). Et_2O–MeOH (9 : 1) gave a mixture which was combined with the broth extract and chromat. on Al_2O_3 (10% deactivated; 50 g). Petrol–Et_2O (4 : 1) gave more s.m. (16 mg). Et_2O gave 1β-*hydroxy*-5α-*androstane*-7,17-*dione* (no. 191)* (431 mg), m.p. 180—182° (from MeOH), $[\alpha]_D$ −17° (*c* 0·3) (Found: C, 74·9; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3610, 1750, and 1718 cm^{-1}. Et_2O–MeOH (49 : 1) gave a mixture of polar compounds (114 mg).

(b) *Transformations.* Oxidation of the hydroxy-diketone (no. 191) (40 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-1,7,17-*trione* (no. 67)* (34 mg), m.p. 235—237° (from MeOH), $[\alpha]_D$ +106° (*c* 0·6) (Found: C, 75·65; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Huang-Minlon reduction of the hydroxy-diketone (no. 191) (160 mg) gave 5α-androstan-1β-ol (no. 102)* (95 mg), m.p. (from hexane) and mixed m.p. 120—121°, which was oxidised with 8N-H_2CrO_4 to 5α-androstan-1-one (no. 1)* (76 mg), m.p. (from MeOH–H_2O) and mixed m.p. 69—71°.

5α-*Androstane*-11,17-*dione* (no. 54).*—(a) *Incubation.* 3·52 g in EtOH (350 ml), 176 flasks, medium A, 2 d extraction III ⟶ 3·8 g total extract. Chromat. Al_2O_3 (10% deactivated; 270 g). C_6H_6 gave s.m. (150 mg). C_6H_6–Et_2O (1 : 1) gave 6α-*hydroxy*-5α-*androstan*-11,17-*dione* (no. 492), m.p. 155—156° (from C_6H_6–hexane) (1·31 g), $[\alpha]_D$ +155° (*c* 1·0) (Found: C, 75·1; H, 9·5. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3600, 1742, and 1710 cm^{-1}. Et_2O afforded 1β,6α-*dihydroxy*-5α-*androstane*-11,17-*dione* (no. 501), m.p. 282—284° (from $CHCl_3$–hexane) (43 mg), $[\alpha]_D$ +137° (*c* 0·7) (Found: C, 71·1; H, 8·9. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 3580, 3450, 1749, and 1695 cm^{-1}. Et_2O–MeOH (4 : 1) gave 1α,6α-*dihydroxy*-5α-*androstane*-11,17-*dione* (no. 500), m.p. 216—218° (from $CHCl_3$–hexane) (126 mg), $[\alpha]_D$ +120° (*c* 0·2) (Found: C, 70·7; H, 8·6. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 3580, 1745, and 1705 cm^{-1}.

(b) *Transformations.* Oxidation of the dihydroxy-ketones (nos. 500 and 501) with 8N-H_2CrO_4 gave 5α-*androstane*-1,6,11,17-*tetraone* (no. 487), m.p. 270—272° (from $CHCl_3$–hexane), $[\alpha]$ +152° (*c* 0·3) (Found: C, 72·4; H, 7·6. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%), $\nu_{max.}$ (high resolution) 1746 and 1719 cm^{-1}. Oxidation of 6α-hydroxy-5α-androstane-11,17-dione (no. 492) with 8N-H_2CrO_4 gave 5α-androstane-6,11,17-trione (no. 96),* m.p. (from C_6H_6–hexane) and [1] mixed m.p. 218—220°.

Acetylation of 6α-hydroxy-5α-androstane-11,17-dione (no. 492) [Ac_2O–C_5H_5N (3 : 1) for 2 d] gave 11,17-*dioxo*-5α-*androstan*-6α-*yl acetate* (no. 493), m.p. 139—140° (from MeOH–H_2O), $[\alpha]_D$ +177° (*c* 0·4) (Found: C, 72·5; H, 8·6. $C_{21}H_{30}O_4$ requires C, 72·8; H, 8·7%), $\nu_{max.}$ 1742, 1728, and 1718 cm^{-1}.

15α-*Hydroxy*-5α-*androstan*-12-*one* (no. 489).—*Incubation.* 800 mg in Me_2SO (120 ml), 20 flasks, medium B, 4 d, extraction II ⟶ 1·23 g total extract. P.l.c. [3 large plates, 1 × petrol–Me_2CO (3 : 1)] gave a major band (275 mg) which was acetylated [Ac_2O–C_5H_5N (3 : 1) for 2 d] and purified by p.l.c. [3 small plates, 3 × petrol–Et_2O (2 : 1)] to give 6α,15α-diacetoxy-5α-androstan-12-one (no. 442)* (111 mg), m.p. (from Me_2CO) and [1] mixed m.p. 222—224°.

12β-*Hydroxy*-5α-*androstan*-15-*one* (no. 488).—*Incubation.* 80 mg in Me_2SO (12 ml), 2 flasks, medium B, 4 d, extraction II ⟶ 181 mg total extract. P.l.c. [1 small plate, 1 × petrol–Me_2CO (2 : 1)] gave 7β,12β-dihydroxy-5α,14β-androstan-15-one (no. 445)* (higher R_F) (18 mg), m.p. (from Me_2CO–hexane) and [1] mixed m.p. 167—169°, and 7β,12β,14-trihydroxy-5α,14β-androstan-15-one (no. 473)* (lower R_F) (7 mg), m.p. (from Me_2CO–hexane) and [1] mixed m.p. 146—148°.

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