Microbiological Hydroxylation of Steroids. Part VII.[1] The Pattern of Dihydroxylation of Mono-oxo-5α-androstanes and -5α-estranes with the Fungus *Rhizopus nigricans*

By **John W. Browne, William A. Denny, Sir Ewart R. H. Jones, G. Denis Meakins,* Yasu Morisawa, Anthony Pendlebury,** and **John Pragnell,** Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY

Although steroidal monoketones with the carbonyl group in ring B or C are relatively unreactive towards *Rhizopus nigricans*, 2-, 3-, 16-, and 17-ketones give modest yields of dihydroxy-derivatives. The position of the carbonyl group influences the direction of the hydroxylation process: comparison of the 11,16-dihydroxylation of 3-ketones with the 3,7-dihydroxylation of 17-ketones suggests that a reversal effect is operating.

16-Hydroxylation, not previously recorded with this fungus, occurs commonly with the present androstane and estrane derivatives, *i.e.* steroids lacking side-chains at position 17. Estr-4-en-3-one gives three 16-oxygenated products (total yield 68%), the main one being the 10β,16β-dihydroxy-Δ⁴-3-ketone.

THE pioneering work of Peterson and Murray [2] showed that the fungus *Rhizopus nigricans* efficiently hydroxylates certain steroids. Since this fungus has been used mainly for the preparation of steroid hormones, most of the substrates which have been studied contain the 3-oxo-Δ⁴-system; with these 11α-hydroxylation generally predominates,[3] although attack does sometimes occur (notably with 5α-pregnane derivatives [4]) at the 6β- or 7β-position. There is little information about the hydroxylation of other steroidal types.

In continuing our investigation of the relationship between substrate structure and microbiological hydroxylation pattern we have studied the effect of *R. nigricans* on a range of simple monohydroxy- and mono-oxo-steroids (mostly derived from 5α-androstane). Since the alcohols (*e.g.* 1α- 3β-, 6α-, 11α-, and 17β-hydroxy-5α-androstane) were not hydroxylated to an appreciable extent (75—95% recovery of starting materials) details are not recorded in the Experimental section. In this respect *R. nigricans* differs from *Calonectria decora*,[5] the fungus used in our first survey of

[1] Part VI, A. M. Bell, J. W. Browne, W. A. Denny, Sir Ewart R. H. Jones, A. Kasal, and G. D. Meakins, *J.C.S. Perkin I*, 1972, 2930.

[2] D. H. Peterson and H. C. Murray, *J. Amer. Chem. Soc.*, 1952, **74**, 1871.

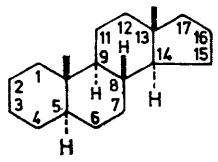
[3] W. Charney and H. L. Herzog, 'Microbiological Transformations of Steroids,' Academic Press, New York, 1967.

[4] B. Görlich and J. Walter, *Annalen*, 1971, **753**, 106, 116.

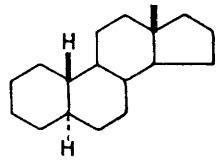
[5] A. M. Bell, P. C. Cherry, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and P. D. Woodgate, *J.C.S. Perkin I*, 1972, 2081.

TABLE 1

Hydroxylation of monoketones by *Rhizopus nigricans*



5α-Androstane



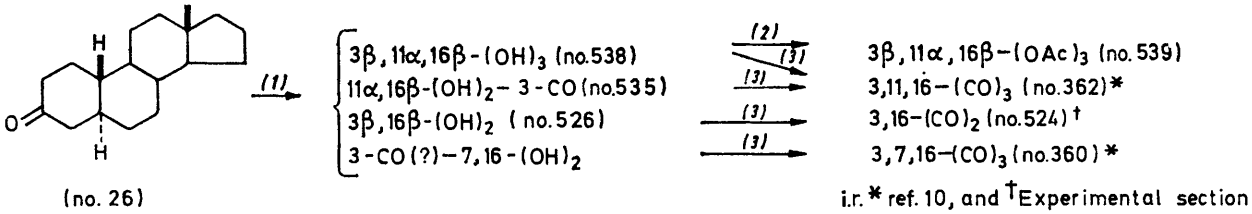
5α-Estrane

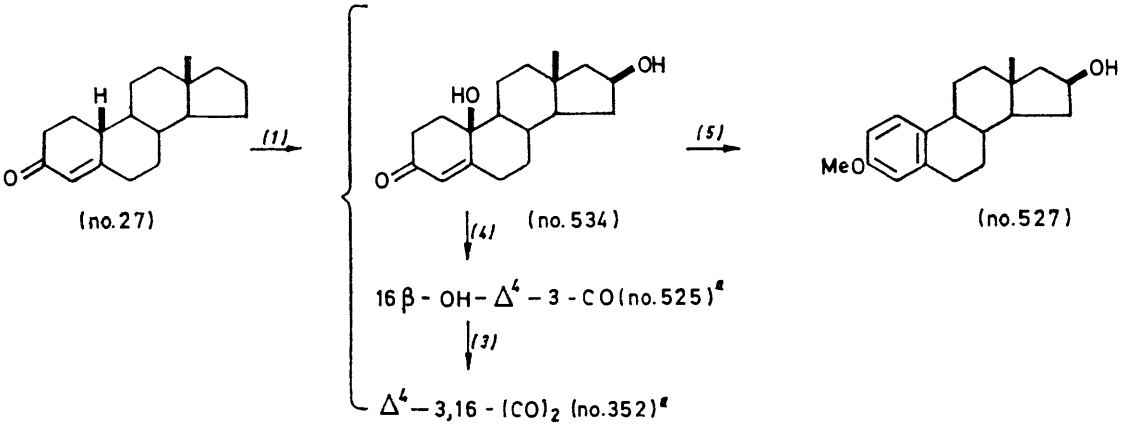
Substrates derived from 5α-androstane are indicated by abbreviated names, *e.g.* 2-CO represents 5α-androstan-2-one. Those derived from 5α-estrane are named fully. In the Products column those oxygen functions introduced during the incubation are in bold type. The Conditions refer to the use of dimethylsulphoxide (D) or ethanol (E) 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 steroid material after incubation and removal of the substrate.

Substrate	Conditions	Substrate recovered	Main product(s)		Other product(s)	
2-CO	D6	51%	**6α, 16α**-$(OH)_2$	10%	2α,**6α, 16α**-$(OH)_3$	2·5%
3-CO	D6	46	**11α,16β**-$(OH)_2$	33	**6β,11α**-$(OH)_2$	4
					11α-OH	1
Estran-3-one	D6	59	3β, **11α,16β**-$(OH)_3$	38	3β, **16β**-$(OH)_2$	6
			11α,16β-$(OH)_2$	23	**7**, **16**-$(OH)_2$	2 *
3-CO-Δ^1	D6	13	1,2-H_2-**11α,16β**-$(OH)_2$	13·5		
			1,2-H_2-3β, **11α,16β**-$(OH)_3$	12		
3-CO-Δ^4	E6	20	**11α,16β**-$(OH)_2$	31	4,5α-H_2-**11α,16β**-$(OH)_2$	9
Estr-4-en-3-one	D6	27	**10β,16β**-$(OH)_2$	58	**16β**-OH	23
					16-CO	11
4-CO	D6	40	**11α,16β**-$(OH)_2$	9 †	4β, **11α,16β**-$(OH)_3$	7 †
6-CO	E6	90	None isolated			
7-CO	D6	90	**3α, 16β**-$(OH)_2$	34	**4β, 16β**-$(OH)_2$	16
			4α, 16β-$(OH)_2$	33		
11-CO	D6	62	**4α, 16β**-$(OH)_2$	7 †	**3α, 16β**-$(OH)_2$	4·5 †
					3β, 16β-$(OH)_2$	3 †
15-CO	D4	59	None isolated			
16-CO	E6	34	**3β,7α**-$(OH)_2$	46		
17-CO	D6	59	**3α, 11α**-$(OH)_2$	10	**7β,11α**-$(OH)_2$	2·5
			3β,7β-$(OH)_2$	9		
			6α,11α-$(OH)_2$	9		
Estran-17-one	D6	60	**3β,7β**-$(OH)_2$	29	**3α,6α**-$(OH)_2$	8

* Isolated as the 3,7,16-triketone formed by oxidation. † Isolated as the product formed by acetylation.

SCHEME Products from 5α-estran-3-one and estr-4-en-3-one (The abbreviated names indicate the positions of substituents)





Reagents: (1), *R. nigricans*; (2), Ac_2O–C_5H_5N; (3), H_2CrO_4–Me_2CO; (4), Zn–AcOH; (5), HCl–MeOH.

[a] Ref. 14.

steroidal hydroxylation. Table 1 and the Scheme summarise the results obtained with the monoketones. [The use of the (arabic) serial number sequence of steroids throughout this work, and considerations about the structural elucidation and the reporting of new compounds have been explained earlier.[5] Compounds nos. 524—539 (whose n.m.r. signals are listed in Table 2) and some of the new steroids with numbers below 375 are described here.]

An interesting feature is the propensity, hitherto undetected, of *R. nigricans* for attack at the 16-position. There are various instances in which microbiological hydroxylation at a certain position has been shown to be inhibited by the presence of neighbouring groups;[6] the 17β-oxygenated side-chains of the substrates studied previously with *R. nigricans* must have acted in this way in preventing substitution of the 16-methylene group.

The tendency of most of the monoketone substrates to give dihydroxy-ketones parallels their behaviour with *Calonectria decora*,[5] and it seems likely that the two

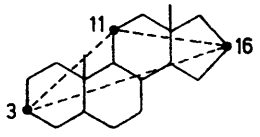


FIGURE 1 Positions involved in normal mode of hydroxylation

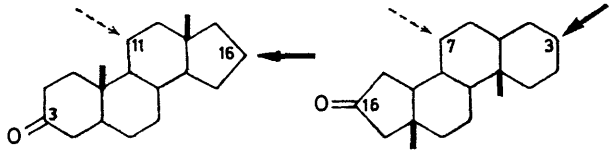


FIGURE 2 Reversal effect

hydroxy-groups are generally introduced in a concerted manner (*i.e.* in a single enzyme–substrate complex) by both micro-organisms. (In contrast, few of the monoketones react with *Aspergillus ochraceus*; those which do are dihydroxylated by a sequential process.[1]) With *C. decora*[5] the two hydroxy-groups were invariably equatorial; with *R. nigricans* there is less stereoselectivity, both equatorial and axial (*e.g.* 3α and 7α) substitution occurring. Further, while the carbon atoms attacked by *C. decora* are about 4 Å apart, there is less uniformity with *R. nigricans* and the most common C–C distances involved (11—16 and 3—7) are about 5 Å. Despite these differences a broad pattern can be discerned by interpreting the results in the way used previously with *C. decora*.[5] This assumes a triangular arrangement on the enzyme surface of three sites, each of which has both binding and hydroxylating capabilities.* The substrate's keto-group is bound at one of these and the others hydroxylate such C–H bonds of the steroid as come into their vicinity. The idea of three dual-purpose sites is not essential in explaining the hydroxylation of the simple ketones, but it has the merits of simplicity, and of satisfactorily accommodating the more extensive results obtained with dioxygenated substrates (see following paper). With *R. nigricans* the geometric requirements appear to be best satisfied by the steroidal 3,11,16- or 3,7,16-positions (Figures 1 and 2). The reversal effect of terminal ring ketones (observed earlier with *C. decora*[5,7]) is seen clearly with the 3- and 16-ketones, and is interpreted, on the basis of the three-site arrangement, as shown in Figure 2. Thus, the 3-ketone is attached, and hydroxylated, in the 'normal' mode; to satisfy the same geometric requirements the 16-ketone must be rotated into the 'reverse' mode. [This incorporates the idea of symmetry effects operating in steroidal microbiological hydroxylation which, in certain circumstances, lead to approximate equivalence of two positions, *e.g.* 11 and 6 (or 7).[8]]

While the behaviour of 5α-estran-3-one is similar to that of 5α-androstan-3-one, the hydroxylation of estr-4-en-3-one (which is the most efficient of those studied here) leads to a 10β,16β-dihydroxy-product. It is reasonable to suppose that the site responsible for 11-hydroxylation of 10-methyl substrates is suitably disposed for 10-hydroxylation of estrane derivatives. With 5α-estran-3-one attack at the 11-position is still preferred, but with estr-4-en-3-one removal of the (now allylic) 10β-hydrogen atom appears to be facilitated;† the isolation of substantial amounts of products resulting solely from 16-substitution of the latter substrate is surprising. The occurrence of 6,16- rather than 11,16-substitution with the 2-ketone was unexpected. The rather high recovery of starting material indicates that neither mode of binding gives a situation in which the steroid is readily attacked; the observed substitution in ring B suggests that the reverse mode is the less unfavourable in this respect. Binding in the normal and reverse modes accounts for the 3,11- and 3,7-dihydroxylation of 5α-androstan-17-one. The third product, the 6α,11α-dihydroxy-17-ketone, is probably formed by a (rare) sequential process, since 6α-hydroxy-17-ketones are hydroxylated at the 11α-position (following paper). The poor utilisation of the 7- and 11-ketones, and the lack of reaction with the 6-ketone suggest that, as with *C. decora*, binding is less efficient with middle ring ketones.

The reactions in the Scheme illustrate the transformations used in establishing the products' structures. (Many more are recorded in the Experimental section.) In detecting 16-hydroxylation the standard n.m.r.

* As is general,[5] there is an alternative explanation: the micro-organism may prefer to attack certain positions [here 3 (or 4), 6 (or 7), 11, and 16], with the keto-groups exerting only a modifying effect on this basic tendency. However, the lack of reactivity of 3β- and 17β-acetyl-5α-androstane towards *R. nigricans*, *C. decora*, and *A. ochraceus* suggests a positive role for a carbonyl group attached directly to the steroid nucleus.

† This hydroxylation does not appear to be merely due to autoxidation because the substrate is not affected by shaking in air with the aqueous medium used, and is hydroxylated at *other* positions by different micro-organisms in the same medium.[3,5]

[6] L. Tan and P. Falardeau, *J. Steroid Biochem.*, 1970, **1**, 221.
[7] 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.
[8] D. R. Brannon, F. W. Parrish, B. J. Wiley, and L. Long, jun., *J. Org. Chem.*, 1967, **32**, 1521.

approach [9] is usefully supplemented by i.r. study, since 16-ketones are characterised by the positions and intensities of their perturbed methylene scissoring absorptions.[10] The presence of a hydroxy-group at the 10- rather than the 9-position in the main product (no. 534) from estr-4-en-3-one was indicated by u.v. and n.m.r. examination, and confirmed by the aromatisation under acidic (but not under alkaline) conditions. Assignment of the proposed 10β-configuration is based only on the general observation of stereochemical retention in microbiological hydroxylation.

TABLE 2

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 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
524	5α-Estrane-3,16-dione	18	9·09	9·09	9·10	9·42	+0·33
			τ_2	τ_2(calc.)	>CH–OR (in $CDCl_3$)		
525	16β-Hydroxyestr-4-en-3-one	18	8·97	8·98	H-16	5·58	7(8,5,5,4)
526	5α-Estrane-3β,16β-diol	18	9·05	9·02	H-3	6·44	m(25)
					H-16	5·60	m(20)
527	3-Methoxyestr-1,3,5(10)-trien-16β-ol	18	9·01		H-16	5·45	m(20)
528	3α,16β-Diacetoxy-5α-androstan-11-one	19	8·97	8·96	H-3	4·98	m(8)
		18	9·14	9·17	H-16	4·75	m(20)
529	6α,11α-Dihydroxy-5α-androstan-17-one	19	9·04	9·05	H-6	6·56	m(24)
		18	9·15	9·11	H-11	6·04	6(11,11,6)
530	11α,16β-Dihydroxy-5α-androstan-4-one	19	9·12	9·12	H-11	6·07	6(10,10,5)
		18	9·03	9·04	H-16	5·56	m(16)
531	11α,16β-Diacetoxy-5α-androstan-4-one	19	9·14	9·16	H-11, H-16	4·83	m(20)
		18	9·04	9·09			
532	3α,6α-Dihydroxy-5α-estran-17-one	18	9·13	9·13	H-3	5·98	m(8)
					H-6	6·4	m(22)
533	3β,7β-Dihydroxy-5α-estran-17-one	18	9·11	9·10	H-3, H-7	6·4	m(25)
534	10β,16β-Dihydroxyestr-4-en-3-one *	18	8·97	8·97	H-16	5·58	7(7,5,5,4)
535	11α,16β-Dihydroxy-5α-estran-3-one	18	8·99	8·99	H-11	6·16	m(25)
					H-16	5·55	m(20)
536	5α-Androstane-4β,11α,16β-triol	19	8·82	8·84	H-4	6·17	m(7)
		18	9·04	9·03	H-11	6·02	6(10,10,5)
					H-16	5·56	m(17)
537	4β,11α,16β-Triacetoxy-5α-androstane	19	8·88	8·89	H-4	5·03	m(7)
		18	9·07	9·08	H-11, H-16	4·81	m(15)
538	5α-Estrane-3β,11α,16β-triol	18	9·05	9·02			
539	3β,11α,16β-Triacetoxy-5α-5α-estrane	18	9·05	9·06	H-3	5·35	m(25)
					H-11	5·04	6(10,10,5)
					H-16	4·86	m(20)

* H-4, 4·21, d(2); *cf.* 10β-H analogues with H-4 signal as t(2).

[a] Ref. 9. [b] Ref. 10. [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. 5. Where compounds with serial numbers below 523 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. 11. 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°. The abbreviation s.m. indicates starting material.

5α-Androstan-2-one (no. 4).* (a) *Incubation*: 1·88 g in Me_2SO (282 ml), 47 flasks, medium B, 6 d, extraction I ⟶ 2·22 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (19 : 1) eluted s.m. (950 mg). Et_2O–MeOH (49 : 1 to 7 : 1) eluted a mixture which was separated by p.l.c. [2 large plates, 6 × petrol–Me_2CO (7 : 3)]. The band of higher R_F yielded 6α,16α-*dihydroxy-5α-androstan-2-one* (no. 275) * (106 mg), as an oil (Found: C, 74·4; H, 9·8%; *M*, 306. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%; *M*, 306), $\nu_{max.}$ ($CHCl_3$) 3600 and 1704 cm^{-1}. The band of lower R_F gave *5α-androstane-2α,6α,16α-triol* (no. 323) * (25 mg), m.p. 219—222° (from Me_2CO–hexane), $[\alpha]_D$ −13° (*c* 0·05) (Found: C, 74·2; H, 10·2. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ 3610 cm^{-1}.

(b) *Transformations*: Acetylation (Ac_2O–C_5H_5N; 2 : 1, for 2 d) of 5α-androstane-2α,6α,16α-triol (no. 323) gave *2α,6α,16α-triacetoxy-5α-androstane* (no. 324),* m.p. 151·5—153·5° (from MeOH), $[\alpha]_D$ +15° (*c* 0·3) (Found: C, 69·0; H, 8·7. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%), $\nu_{max.}$ 1728 and 1244 cm^{-1}. Similarly 6α,16α-dihydroxy-5α-androstan-2-one (no. 275) gave 6α,16α-diacetoxy-5α-androstan-2-one (no. 276) * as an oil, *m/e* 390 (M^+), $\nu_{max.}$ 1728, 1714, and 1250 cm^{-1}.

Oxidation of the dihydroxy-ketone (no. 275) (50 mg) with 8N-H_2CrO_4 gave *5α-androstane-2,6,16-trione* (no. 68) * (38 mg), m.p. 251—253° (from Me_2CO–hexane) (Found: C, 75·2; H, 8·9. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Huang-Minlon reduction of the dihydroxy-ketone (no. 275) (30 mg) gave *5α-androstane-6α,16α-diol* (no. 223) * (20 mg), m.p. 162—164 and 174·5—176·5° (from Me_2CO–hexane) (Found: C, 78·0; H, 10·7. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ (high resolution) 3630, 3622, and 3608 cm^{-1}.

5α-Androstan-3-one (no. 5).* (a) *Incubation*: 3·0 g in Me_2SO (450 ml), 75 flasks, medium B, 6 d, extraction II ⟶ 1·8 g mycelial extract + 2·0 g broth extract. Chromat. of mycelial extract on Al_2O_3 (10% deactivated; 50 g) and elution with C_6H_6 gave s.m. (1·38 g). P.l.c. [3 large plates, 1 × C_6H_6–EtOAc (2 : 1)] of the broth extract gave 11α-hydroxy-5α-androstan-3-one (no. 163) * (highest R_F) (20 mg), m.p. (from MeOH–H_2O) and mixed [1] m.p. 123—125°; 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) * (intermediate R_F) (595 mg), m.p. (from Me_2CO) and mixed [1] m.p. 206—207°; and 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) * (lowest R_F) (70 mg), m.p. (from Me_2CO) and mixed [1] m.p. 193—194°.

(b) *Transformations*: Acetylation (Ac_2O–C_5H_5N; 3 : 1, for 3 d) of 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) gave *11α,16β-diacetoxy-5α-androstan-3-one* (no. 293),* m.p. 131—133° (from hexane), $[\alpha]_D$ −8° (*c* 0·3) (Found: C, 70·75; H, 8·8. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1754 and 1714 cm^{-1}. A solution of this diacetoxy-ketone (38 mg) in tetrahydrofuran (1 ml) was treated with $LiAlH(OBu^t)_3$ (200 mg) at 20 °C for 30 min. Work-up gave *11α,16β-diacetoxy-5α-androstan-3β-ol* (no. 327) * (30 mg), m.p. 152—155° (from Et_2O–hexane), $[\alpha]_D$ −49° (*c* 0·3) (Found: C, 70·5; H, 9·0. $C_{23}H_{36}O_5$ requires C, 70·4; H, 9·2%), $\nu_{max.}$ 3620, 1735, and 1230 cm^{-1}.

Oxidation of 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (60 mg), with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85) * (50 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 174—176°. A solution of this triketone (no. 85) (62 mg) in tetrahydrofuran (3 ml) was treated with $LiAlH(OBu^t)_3$ (300 mg) at 0 °C for 1·5 h to give 3β,16β-*dihydroxy-5α-androstan-11-one* (no. 264) * (40 mg), m.p.

[9] 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.

[10] 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.

[11] 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.

232—234° (from Me_2CO–hexane), $[\alpha]_D$ +50° (*c* 0·3) (Found: C, 74·9; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3620 and 1708 cm^{-1}. Acetylation of this dihydroxy-ketone (no. 264) with Ac_2O–C_5H_5N gave 3β,16β-*diacetoxy*-5α-*androstan*-11-*one* (no. 265),* m.p. 149—151° (from hexane), $[\alpha]_D$ +27° (*c* 0·7) (Found: C, 70·8; H, 8·7. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1736, 1710, and 1237 cm^{-1}.

Huang-Minlon reduction of 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (130 mg) afforded 5α-*androstane*-11α,16β-*diol* (no. 226),* m.p. 164—166° (from MeOH–H_2O) (40 mg), $[\alpha]_D$ −28° (*c* 0·3) (Found: C, 77·9; H, 11·0. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3610 cm^{-1}. Oxidation of this diol (no. 226) (40 mg) with 8N-H_2CrO_4 gave 5α-androstane-11,16-dione (no. 53) * (34 mg), m.p. (from MeOH) and mixed [12] m.p. 131—133°.

Reduction of this diketone (no. 53) (150 mg) in tetrahydrofuran (4 ml) with $LiAlH(OBu^t)_3$ (400 mg) at 0 °C for 2 h gave 16β-*hydroxy*-5α-*androstan*-11-*one* (no. 177) * (109 mg), m.p. 157—158° (from Me_2CO–hexane), $[\alpha]_D$ +59° (*c* 0·3) (Found: C, 78·5; H, 10·3. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ (high resolution) 3625 and 1706 cm^{-1}. This hydroxy-ketone (no. 177) (90 mg) in Et_2O (4 ml) was reduced with $LiAlH_4$ (20 mg) at 20 °C for 20 h to give 5α-*androstane*-11β,16β-*diol* (no. 227) * (85 mg), m.p. 162—164° (from Me_2CO–hexane), $[\alpha]_D$ +5° (*c* 0·3) (Found: C, 77·45; H, 10·8. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%). Acetylation (Ac_2O–C_5H_5N; 10 : 1, at 0 °C for 2 d) of this diol (no. 227) (80 mg) gave 11β-*hydroxy*-5α-*androstan*-16β-*yl acetate* (no. 228) * (83 mg), m.p. 146—147° (from MeOH), $[\alpha]_D$ +13° (*c* 0·2) (Found: C, 75·5; H, 10·1. $C_{21}H_{34}O_3$ requires C, 75·4; H, 10·25%). Oxidation of this hydroxy-acetate (no. 228) (70 mg) with 8N-H_2CrO_4 gave 11-*oxo*-5α-*androstan*-16β-*yl acetate* (no. 178) * (60 mg), m.p. 156—157° (from MeOH), $[\alpha]_D$ +51° (*c* 0·2) (Found: C, 72·5; H, 9·9. $C_{21}H_{32}O_3$,MeOH requires C, 72·5; H, 9·95%). Vigorous Huang-Minlon reduction [11] of this acetoxy-ketone (no. 178) (40 mg) gave an oil (35 mg) (estimated by n.m.r. examination to be a 2 : 3 mixture of 5α-androstan-16β-ol and 5α-androstan-16α-ol) which was oxidised with 8N-H_2CrO_4 to 5α-androstan-16-one (no. 19) * (31 mg), m.p. (from MeOH) and mixed [13] m.p. 106—107°.

5α-*Estran*-3-*one* (no. 26). (a) *Incubation*: 3·92 g in Me_2SO (598 ml), 40 flasks, medium B, 6 d, extraction I ⟶ 5·0 g combined extracts. Chromat. SiO_2 (10% deactivated; 100 g). C_6H_6 eluted s.m. (2·3 g). EtOAc–MeOH (9 : 1) eluted a mixture which was separated by p.l.c. [5 large plates, 16 × petrol–Me_2CO (6 : 1)]. The band of highest R_F gave, after further p.l.c. purification, 5α-*estrane*-3β,16β-*diol* (no. 526) (110 mg), m.p. 166—167·5° (from Me_2CO–hexane), $[\alpha]_D$ +12° (*c* 1·0) (Found: C, 73·2; H, 10·6. $C_{18}H_{30}O_2$,$2C_3H_6O$ requires C, 73·1; H, 10·7%), $\nu_{max.}$ 3600 cm^{-1}. The material in the second band was dissolved in $CHCl_3$, boiled with activated charcoal, and then crystallised from Me_2CO to give 11α,16β-*dihydroxy*-5α-*estran*-3-*one* (no. 535) (410 mg), m.p. 182·5—184·5°, $[\alpha]_D$ −97° (*c* 1·0) (Found: C, 73·8; H, 9·5. $C_{18}H_{23}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ 3600 and 1710 cm^{-1}. The third band gave 5α-*estrane*-3β,11α,16β-*triol* (no. 538) (680 mg), m.p. 211—213° (from EtOH–Me_2CO), $[\alpha]_D$ (in EtOH) −33° (*c* 0·5) (Found: C, 73·3; H, 10·4. $C_{18}H_{30}O_3$ requires C, 73·4; H, 10·3%), $\nu_{max.}$ (Nujol) 3400 cm^{-1}.

The residues from the crystallisation of 11α,16β-dihydroxy-5α-estran-3-one (no. 535) were oxidised with 8N-H_2CrO_4 to a mixture which was separated by p.l.c. [2 small plates, 6 × petrol–Et_2O (100 : 1)] to give the 3,11,16-triketone (no. 362) (lower R_F) (10 mg) (see later) and 5α-*estrane*-3,7,16-*trione* (no. 360) * (higher R_F) (45 mg), m.p. 195—200° (from Me_2CO–hexane), $[\alpha]_D$ +30° (*c* 1·0) (Found: C, 75·2; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%).

(b) *Transformations*: Acetylation (Ac_2O–C_5H_5N; 2 : 1, for 1 d) of 5α-estrane-3β,11α,16β-triol (no. 538) (80 mg) gave 3β,11α,16β-*triacetoxy*-5α-*estrane* (no. 539) (113 mg), m.p. 116—118° (from MeOH), $[\alpha]_D$ −31° (*c* 1·0) (Found: C, 68·3; H, 8·9. $C_{24}H_{36}O_6$ requires C, 68·5; H, 8·6%), $\nu_{max.}$ 1735 and 1243 cm^{-1}.

On oxidation with 8N-H_2CrO_4 5α-estrane-3β,11α,16β-triol (no. 538) (150 mg) and 11α,16β-dihydroxy-5α-estran-3-one (no. 535) (150 mg) gave 5α-*estrane*-3,11,16-*trione* (no. 362) * (140 and 130 mg, respectively), m.p. 217—220° (from Me_2CO–hexane), $[\alpha]_D$ −7° (*c* 0·9) (Found: C, 74·9; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); 5α-estrane-3β,16β-diol (no. 526) (120 mg) gave, after p.l.c. [2 small plates, 2 × petrol–Me_2CO (5 : 1)], 5α-*estrane*-3,16-*dione* (no. 524) (80 mg), m.p. 170—173° (from Me_2CO–hexane), $[\alpha]_D$ −128° (*c* 0·3) (Found: C, 78·7; H, 9·5. $C_{18}H_{26}O_2$ requires C, 78·7; H, 9·6%), $\nu_{max.}$ (high resolution) 1748, 1722, and 1410 (ε 110) cm^{-1}.

5α-*Androst*-1-*en*-3-*one* (no. 6).* (a) *Incubation*: 1·92 g in Me_2SO (288 ml), 48 flasks, medium A, 6 d, extraction III ⟶ 1·62 g total extract. Chromat. Al_2O_3 (5% deactivated; 80 g). C_6H_6 eluted s.m. (255 mg). C_6H_6–EtOAc (1 : 1) eluted 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (250 mg), m.p. (from EtOAc) and mixed [1] m.p. 206—208°. EtOAc eluted 5α-*androstane*-3β,11α,16β-*triol* (no. 325) * (225 mg), m.p. 250·5—251·5° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) −15° (*c* 1·0) (Found: C, 73·8; H, 10·5. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ (Nujol) 3600 cm^{-1}.

(b) *Transformations*: Acetylation of the triol (no. 325) gave 3β,11α,16β-*triacetoxy*-5α-*androstane* (no. 326),* m.p. 170—171° (from Me_2CO–hexane), $[\alpha]_D$ −23° (*c* 0·9) (Found: C, 68·7; H, 8·9. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%), $\nu_{max.}$ 1745, 1735, and 1235 cm^{-1}. Oxidation of the triol (no. 325) (50 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85) (44 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 170—174°.

Solutions of 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (60 mg) in AcOH (1 ml) and of CrO_3 (16 mg) in H_2O (0·1 ml) were mixed and kept at 20 °C for 45 min. P.l.c. [1 medium plate, 1 × EtOAc] gave 5α-androstane-3,11,16-trione (no. 85) (highest R_F) (32 mg), 11α-hydroxy-5α-androstane-3,16-dione (no. 204) * (intermediate R_F) (9 mg), m.p. and mixed [1] m.p. 259—261°, and s.m. (20 mg).

Androstan-4-*en*-3-*one* (no. 7).* (a) *Incubation*: 2·0 g in EtOH (100 ml), 50 flasks, medium B, 6 d, extraction II ⟶ 4·0 g mycelial extract + 2·0 g broth extract. Mycelial extract chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (49 : 1) eluted s.m. (390 mg). Et_2O–MeOH (19 : 1) eluted material (160 mg) which was combined with the broth extract and separated by p.l.c. [4 large plates, 2 × Et_2O] to give 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (higher R_F) (160 mg) and 11α,16β-*dihydroxyandrost*-4-*en*-3-*one* (no. 294) * (lower R_F) (550 mg), m.p. 205—207°

[12] A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, V. Kumar, G. D. Meakins, and V. E. M. Thomas, *J. Chem. Soc.* (*C*), 1972, 492.

[13] 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.

(from MeOH), $[\alpha]_D$ +59° (*c* 0·4) (Found: C, 74·9; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\lambda_{max.}$ 241 nm (ε 11,500), $\nu_{max.}$ 3610 and 1677 cm^{-1}.

(b) *Transformations*: A solution of the dihydroxy-ketone (no. 294) (50 mg) in dioxan (3 ml)–Et_2O (3 ml) was added over 10 min to a stirred solution of Li (40 mg) in liquid NH_3 (50 ml). After 45 min NH_4Cl was added and the NH_3 allowed to evaporate. Isolation with $CHCl_3$ gave 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (46 mg).

Estr-4-en-3-one (no. 27).* (a) *Incubation*: 3·4 g in Me_2SO (510 ml), 34 flasks, medium B, 6 d, extraction I ⟶ 5·0 g combined extracts. Chromat. SiO_2 (10% deactivated; 100 g). C_6H_6 eluted s.m. (917 mg). EtOAc–MeOH (9 : 1) eluted a mixture of 3 compounds which was separated by p.l.c. [5 large plates, 15 × petrol–Me_2CO (6 : 1)] to give estr-4-ene-3,16-dione (no. 352) * (300 mg), m.p. 139—141° (from Me_2CO–hexane), $[\alpha]_D$ −154° (*c* 1·0) (lit.,[14] m.p. 138·5—139·5°, $[\alpha]_D$ −147°); 16β-hydroxyestr-4-en-3-one (no. 525) (600 mg), m.p. 151—153·5° (from Me_2CO–hexane), $[\alpha]_D$ +34° (*c* 1·0) (lit.,[14] m.p. 149—150·5°, $[\alpha]_D$ +23°), and 10β,16β-*dihydroxyestr-4-en-3-one* (no. 534) (1·6 g), m.p. 181—182·5° (from Me_2CO–hexane), $[\alpha]_D$ +45° (*c* 1·0) (Found: C, 74·4; H, 8·8. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%), $\lambda_{max.}$ 236 nm (ε 9300), $\nu_{max.}$ ($CHCl_3$) 3600 and 1667 cm^{-1}.

(b) *Transformations*: Oxidation of 16β-hydroxyestr-4-en-3-one (no. 525) (170 mg) with 8N-H_2CrO_4 gave estr-4-ene-3,16-dione (no. 352) (135 mg).

A solution of 10β,16β-dihydroxyestr-4-en-3-one (no. 534) (300 mg) in MeOH (40 ml) and 2N-HCl (0·1 ml) was heated under reflux for 5 min, and the product was purified by p.l.c. [4 small plates, 4 × $CHCl_3$] to give 3-*methoxyestra*-1,3,5(10)-*trien*-16β-*ol* (no. 527) (150 mg), m.p. 97—104° (after repeated crystallisation from hexane), $[\alpha]_D$ +71° (*c* 0·9) (Found: C, 79·1; H, 8·85%; *M*, 286. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·15%; *M*, 286), $\lambda_{max.}$ 222 (ε 7700), 278 (1950), and 287 nm (1850), $\nu_{max.}$ 3600 cm^{-1}. A solution of 10β,16β-dihydroxyestr-4-en-3-one (no. 534) (45 mg) in AcOH (5 ml) was heated under reflux with Zn dust (145 mg) for 20 min. Work-up, and p.l.c. [1 small plate, 3 × petrol–Me_2CO (6 : 1)] gave 16β-hydroxyestr-4-en-3-one (no. 525) (lower R_F) (25 mg) and an oil (higher R_F) (15 mg) (thought to be 3-oxoestr-4-en-16β-yl acetate). The u.v. absorption of a solution of the dihydroxy-ketone (no. 534) (4 mg) in EtOH (25 ml) was unchanged after the solution had been boiled under reflux for 30 min with 5% KOH aq. (0·25 ml).

5α-*Androstan-4-one* (no. 11).* (a) *Incubation*: 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction II ⟶ 578 mg mycelial extract and 678 mg broth extract. Mycelial extract chromat. Al_2O_3 (10 g). C_6H_6 eluted s.m. (400 mg). P.l.c. [2 large plates, 2 × petrol–Et_2O (4 : 1)] of the broth extract gave s.m. (higher R_F) (4 mg) and a mixture (lower R_F) which was acetylated [Ac_2O–C_5H_5N; 3 : 1, for 2 d]. P.l.c. [2 small plates, 4 × petrol–Et_2O (4 : 1)] gave 11α,16β-*diacetoxy*-5α-*androstan*-4-*one* (no. 531) (78 mg), m.p. 124—128° (from hexane), $[\alpha]_D$ −33° (*c* 0·5) (Found: C, 70·6; H, 9·0. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1741 and 1718 cm^{-1}, and 4β,11α,16β-triacetoxy-5α-androstane (no. 537) (63 mg), $\nu_{max.}$ 1735 and 1230 cm^{-1}. The other products could not be fully purified.

(b) *Transformations*: Hydrolysis of the diacetate (no. 531) (52 mg) with 5% methanolic KOH gave 11α,16β-*dihydroxy*-5α-*androstan*-4-*one* (no. 530) (43 mg), m.p. 183—185° (from Me_2CO), $[\alpha]_D$ −22° (*c* 0·5) (Found: C, 74·7; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·8%), $\nu_{max.}$ 3627 and 1715 cm^{-1}.

Huang-Minlon reduction of the dihydroxy-ketone (no. 530) (100 mg) gave, after purification by p.l.c., 5α-androstane-11α,16β-diol (no. 226) * (55 mg), m.p. and mixed m.p. 164—167°.

Hydrolysis of the triacetate (no. 537) (63 mg) as above gave 5α-*androstane*-4β,11α,16β-*triol* (no. 536) (38 mg), m.p. 200—203° (from Me_2CO), $[\alpha]_D$ −20° (*c* 0·5) (Found: C, 74·0; H, 10·4. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ 3610 cm^{-1}.

Oxidation with 8N-H_2CrO_4 of the dihydroxy-ketone (no. 530) and triol (no. 536) gave 5α-androstane-4,11,16-trione (no. 92),* m.p. (from EtOH) and mixed [1] m.p. 194—197°.

5α-*Androstan-7-one* (no. 15).* (a) *Incubation*: 2·0 g in Me_2SO (300 ml), 50 flasks, medium B, 6 d, extraction II ⟶ 3·0 g mycelial extract + 84 mg broth extract. Mycelial extract chromat. SiO_2 (10% deactivated; 30 g). C_6H_6 eluted s.m. (1·8 g). P.l.c. [2 large plates, 5 × C_6H_6–EtOAc (2 : 1)] of the broth extract gave 3α,16β-*dihydroxy*-5α-*androstan*-7-*one* (no. 243) * (lower R_F) (73 mg), m.p. 266—267° (from $CHCl_3$–Et_2O), $[\alpha]_D$ −71° (*c* 0·6) (Found: C, 74·3; H, 9·5. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1710 cm^{-1}. Further p.l.c. purification of the less polar material gave 4β,16β-*dihydroxy*-5α-*androstan*-7-*one* (no. 269) * (higher R_F) (36 mg), m.p. 197—198° (from Me_2CO–hexane), $[\alpha]_D$ −49° (*c* 0·2) (Found: C, 74·1; H, 9·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1710 cm^{-1}, and 4α,16β-*dihydroxy*-5α-*androstan*-7-*one* (no. 267) * (lower R_F) (74 mg), m.p. 200—202° (from Me_2CO–hexane), $[\alpha]_D$ −94° (*c* 0·6) (Found: C, 74·5; H, 9·95. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1704 cm^{-1}.

(b) *Transformations*: Acetylation of 3α,16β-dihydroxy-5α-androstan-7-one (no. 243) gave 3α,16β-*diacetoxy*-5α-*androstan*-7-*one* (no. 244),* m.p. 214—215° (from Et_2O–hexane), $[\alpha]_D$ −55° (*c* 0·4) (Found: C, 70·6; H, 8·6. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1740 and 1710 cm^{-1}. Oxidation of the dihydroxy-ketone (no. 243) with 8N-H_2CrO_4 gave 5α-*androstane*-3,7,16-*trione* (no. 82),* m.p. 241—242° (from Et_2O), $[\alpha]_D$ −210° (*c* 0·6) (Found: C, 75·1; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1747, 1717, and 1409 cm^{-1}.

Oxidation of both 4α,16β-dihydroxy-5α-androstan-7-one (no. 267) and its 4β-epimer (no. 269) with 8N-H_2CrO_4 gave 5α-*androstane*-4,7,16-*trione* (no. 90),* m.p. 231—233° (from hexane) (Found: C, 75·3; H, 8·9. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1750 and 1716 cm^{-1}.

5α-*Androstan*-11-*one* (no. 16).* (a) *Incubation*: 2·0 g in Me_2SO (300 ml), 50 flasks, medium A, 6 d, extraction II ⟶ 1·75 g mycelial extract and 450 mg broth extract. Mycelial extract chromat. Al_2O_3 (20 g). C_6H_6 eluted s.m. (1·24 g). EtOAc gave a mixture (201 mg) which was combined with the broth extract. P.l.c. [2 large plates, 6 × petrol–Et_2O (4 : 1)] gave the steroidal material in one broad band. Acetylation [Ac_2O–C_5H_5N; 2 : 1, for 1 d] followed by p.l.c. [1 large plate, 5 × C_6H_6–$CHCl_3$ (7 : 1)] gave 4α,16β-*diacetoxy*-5α-*androstan*-11-*one* (no. 268) * (highest R_F) (72 mg), m.p. 179—182° (from Me_2CO–hexane), $[\alpha]_D$ +36° (*c* 0·8) (Found: C, 70·7; H, 8·6. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1738, 1712, and 1238 cm^{-1}; 3α,16β-*diacetoxy*-5α-*androstan*-11-*one* (no. 528) (intermediate R_F) (48 mg), m.p. 187—189° (from Me_2CO–hexane), $[\alpha]_D$ +45° (*c* 0·9) (Found: C, 70·9; H, 8·8. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1736 and 1712 cm^{-1}; and 3β,16β-

[14] K. L. Sax, R. H. Blank, R. H. Evans, jun., L. I. Feldman, and C. E. Holmlund, *J. Org. Chem.*, 1964, **29**, 2351.

diacetoxy-5α-androstan-11-one (no. 265) (lowest R_F) (36 mg).

(b) *Transformations*: Hydrolysis of 3β,16β-diacetoxy-5α-androstan-11-one (no. 265) (29 mg) with KOH (0·5 g)–MeOH (10 ml) for 12 h at 20 °C gave 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (23 mg). Similar hydrolysis of 3α,16β-diacetoxy-5α-androstan-11-one (no. 528) (37 mg) followed by oxidation with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85) (26 mg). Similarly, 4α,16β-diacetoxy-5α-androstan-11-one (no. 268) (36 mg) gave 5α-androstane-4,11,16-trione (no. 92) * (29 mg), m.p. (from EtOH) and mixed m.p. 195—197°.

5α-*Androstan*-16-*one* (no. 19). (a) *Incubation*: 1·45 g in EtOH (72 ml), 36 flasks, medium B, 6 d, extraction II ⟶ 600 mg mycelial extract + 1·34 g broth extract. Mycelial extract chromat. Al_2O_3 (10 g). $CHCl_3$ eluted s.m. (490 mg). The broth extract was crystallised from MeOH to give 3β,7α-*dihydroxy*-5α-*androstan*-16-*one* (no. 247) * (490 mg), m.p. 257—259°, $[\alpha]_D$ −166° (*c* 0·2) (Found: C, 74·0; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1740 cm^{-1}.

(b) *Transformation*: Oxidation of the dihydroxy-ketone (no. 247) (30 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,7,16-trione (no. 82) (24 mg).

5α-*Androstan*-17-*one* (no. 20).* (a) *Incubation*: 2·0 g in Me_2SO (300 ml), 50 flasks, medium B, 6 d, extraction I ⟶ 2·26 g combined extracts. Chromat. Al_2O_3 (5% deactivated; 80 g). Petrol–Et_2O (9 : 1) eluted s.m. (1·18 g). Et_2O–MeOH (9 : 1) gave a mixture which was separated by p.l.c. [2 large plates, 3 × C_6H_6–EtOAc (1 : 1)] into the following compounds, listed in order of descending R_F: 7β,11α-dihydroxy-5α-androstan-17-one (no. 287) (22 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 190—191°; 6α,11α-*dihydroxy*-5α-*androstan*-17-*one* (no. 529) (79 mg), m.p. 183—185° (from Me_2CO–hexane), $[\alpha]_D$ +75° (*c* 1·0) (Found: C, 74·7; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1730 cm^{-1}; 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) * (89 mg), m.p. (from Me_2CO) and mixed [11] m.p. 192—194°; and 3β,7β-*dihydroxy*-5α-*androstan*-17-*one* (no. 250) * (80 mg), m.p. 241—243° (from Et_2O–Me_2CO), $[\alpha]_D$ +130° (*c* 0·6) (Found: C, 74·5; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1735 cm^{-1}.

(b) *Transformations*: Acetylation of 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) gave 3β,7β-*diacetoxy*-5α-*androstan*-17-*one* (no. 251),* m.p. 142—145° (from MeOH) (Found: C, 70·9; H, 8·5. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ (Nujol) 1745, 1740, 1735, and 1250 cm^{-1}.

On oxidation with 8N-H_2CrO_4, 6α,11α-dihydroxy-5α-androstan-17-one (no. 529) (50 mg) gave 5α-androstane-6,11,17-trione (no. 96) (42 mg), m.p. (from Me_2CO–hexane) and mixed [5] m.p. 214—215°; and 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (150 mg) gave 5α-androstane-3,7,17-trione (no. 84) (110 mg), m.p. 239—241° (from Me_2CO–hexane), $[\alpha]_D$ (MeOH) +20° (*c* 0·2) {lit.,[15] m.p. 237—239°, $[\alpha]_D$ (dioxan) +22·5°}.

Huang-Minlon reduction of 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) (88 mg) gave 5α-androstane-3α,11α-diol (no. 218) (46 mg), m.p. (from Et_2O) and mixed [11] m.p. 162—163°.

5α-*Estran*-17-*one* (no. 29).* (a) *Incubation*: 3·0 g in Me_2SO (540 ml), 36 flasks, medium B, 6 d, extraction I ⟶ 4·0 g combined extracts. Chromat. SiO_2 (10% deactivated; 100 g). C_6H_6 eluted s.m. (1·8 g). EtOAc–MeOH (9 : 1) eluted a mixture which was separated by p.l.c. [3 large plates, 15 × petrol–Me_2CO (5 : 1), and then 2 large plates, 6 × $CHCl_3$–MeOH (99 : 1)] to give 3α,6α-*dihydroxy*-5α-*estran*-17-*one* (no. 532) (higher R_F) (105 mg), m.p. 255—257° (from Me_2CO–hexane), $[\alpha]_D$ +133° (*c* 0·5) (Found: C, 73·7; H, 9·5. $C_{18}H_{28}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ (Nujol) 3350 and 1735 cm^{-1}; and 3β,7β-*dihydroxy*-5α-*estran*-17-*one* (no. 533) (lower R_F) (380 mg), m.p. 208—210° (from Me_2CO–hexane), $[\alpha]_D$ +143° (*c* 0·7) (Found: C, 73·8; H, 9·7. $C_{18}H_{28}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ 3617 and 1739 cm^{-1}.

(b) *Transformations*: On oxidation with 8N-H_2CrO_4, 3β,7β-dihydroxy-5α-estran-17-one (no. 533) (100 mg) gave 5α-*estrane*-3,7,17-*trione* (no. 361) * (70 mg), m.p. 174—177° (from Me_2CO–hexane), $[\alpha]_D$ +30° (*c* 1·0) (Found: C, 75·2; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); and 3α,6α-dihydroxy-5α-estran-17-one (no. 532) (70 mg) gave 5α-*estrane*-3,6,17-*trione* (no. 359) * (65 mg), m.p. 148—150° (from Me_2CO–hexane), $[\alpha]_D$ +79° (*c* 0·3) (Found: C, 74·7; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%).

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[15] H. B. Kagan and J. Jacques, *Bull. Soc. chim. France*, 1960, 1551.