Microbiological Hydroxylation of Steroids. Part VIII.[1] The Pattern of Monohydroxylation of Diketones and Keto-alcohols derived from 5α-Androstane with Cultures of the Fungus, *Rhizopus nigricans*

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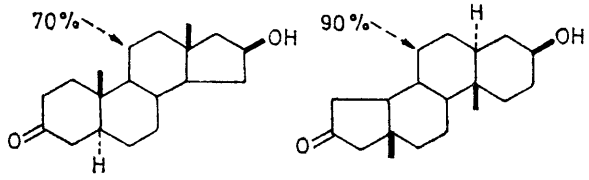
Diketones and keto-alcohols derived from 5α-androstane are readily converted into monohydroxy-derivatives by *Rhizopus nigricans*. Varying the positions and oxidation levels of the oxygen functions leads to hydroxylation at different positions. Substrates with one oxygen substituent in each of the terminal rings are attacked at position 11 or 7; those with one group in rings B or C are hydroxylated at position 16 if the second group is in ring A, and at position 3 if the second group is in ring D.

The results are rationalised by assuming the presence of three dual-purpose sites on the enzyme surface capable of binding to the steroidal oxygen groups and of hydroxylating those positions of the steroid nucleus which come into their vicinity.

In the preceding paper[1] the hydroxylation of mono-oxygenated 5α-androstanes and 5α-estranes with *Rhizopus nigricans* was reported. The present work is concerned with dioxygenated substrates (diketones and keto-alcohols) in which the positions of the polar groups around the steroid nucleus have been varied systematically. Table 1 summarises the main microbiological results obtained by incubating **36** such substrates with *R. nigricans*. [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.[2] Compounds nos. **540**—**633** (whose n.m.r. signals are listed in Table 2) and some of the new steroids with numbers below **375** are described here.]

Monoketones are hydroxylated slowly by *R. nigricans*, and even after 6 days most of them give rather low yields of dihydroxylated products.[1] The much higher reactivity of dioxygenated substrates (Table 1) may arise from their ability to permeate the cell walls more easily (an effect caused partly by their increased solubility in water), or to bind more efficiently to the appropriate enzyme sites (see later). Shorter incubation times are needed, comparatively little starting material is recovered, and in most cases the monohydroxylated products are isolated without difficulty.

The broad pattern of these monohydroxylations can be interpreted in terms of the idea[1] of three dual-purpose (binding or hydroxylating) sites on the enzyme surface, located in positions corresponding to those of carbon atoms **3**, **11**, and **16** (or **3**, **7**, and **16**) of the steroid nucleus. Their role in a particular instance depends upon whether those portions of the steroid molecule which become adjacent to them in the enzyme–substrate complex already bear oxygen atoms. The dioxygenated substrate orients itself so as to maximise hydrophilic binding between its two substituents and two of the sites; the remaining site then becomes involved in the hydroxylation of the nearest steroid carbon atom. The symmetrical disposition of these sites allows a suitable disubstituted steroid to take up more than one orientation; two such orientations have been termed the normal and the reverse mode.[1] For substrates with an oxygen function in each terminal ring, the normal mode [*e.g.* (I)] leads to hydroxylation at position **11**, and the reverse mode [*e.g.* (II)] to hydroxylation in ring B (position **7** or **6**). In general, when the substrate's oxygen groups are at two of the **3**-, **11**- (**7**- or **6**-), or **16**-positions then substantial monohydroxylation occurs at the third position. Even when adjacent positions are involved, *e.g.* **2**,**16**-$(CO)_2$, **7**,**17**-$(CO)_2$, and **11**-CO–**17**β-OH, there is reasonable conformity with this rule.



(I) Example of normal mode (II) Example of reverse mode

Two types of substrates, diketones and keto-alcohols, containing oxygen in rings A and D, have been examined. Of the four androstanediones, three (**2**,**16**-, **2**,**17**-, and **3**,**16**-) appear to hydroxylate only in the reverse mode. Models show that for the first two binding in the normal mode does not bring a steroid position close to the third enzyme site; hydroxylation in this mode is therefore unlikely. The geometric requirements are satisfied by both modes with the third diketone (5α-androstane-**3**,**16**-dione), and the observation of ring-B hydroxylation suggests that a 'directing influence' effect[2] (16-CO > 3-CO) is operating. Hydroxylation of 5α-androstane-**3**,**17**-dione, the fourth example, to about the same extent in both modes indicates that there is not much difference between the directing strengths of these carbonyl groups. (It may be that the 17-CO is slightly the stronger, as the **3**-monoketone undergoes only normal mode hydroxylation whereas the **17**-monoketone gives small amounts of products of both types.)[1]

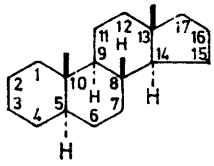
The most striking results are the **11**α-hydroxylation

[1] Part VII, J. W. Browne, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, Y. Morisawa, A. Pendlebury, and J. Pragnell, *J.C.S. Perkin I*, preceding paper.

[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.

TABLE 1

Hydroxylation of dioxygenated 5α-androstanes by *Rhizopus nigricans*



5α-Androstane

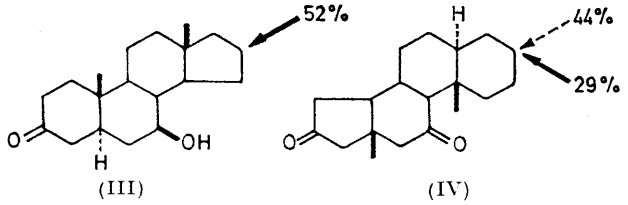
In the ' Products ' columns those oxygen functions introduced during the incubation are in bold type. The entries under conditions refer to the use of ethanol (E), acetone (A), and dimethyl sulphoxide (D) as solvents for the substrate and to the time of incubation (in days). The yields are calculated after making allowance for recovered starting material.

Substrate	Conditions	Substrate recovered	Main product(s)		Other product(s)	
2,16-$(CO)_2$	D6	0%	**2α,7α-** $(OH)_2$	55%	**7α-** OH	7%
					2α,6α- $(OH)_2$	5
2,17-$(CO)_2$	D6	0	**2α,7α-** $(OH)_2$	20	**2α,7β-** $(OH)_2$	6
					2α,6α- $(OH)_2$	2
3,16-$(CO)_2$	D2	0	**3β,7α-** $(OH)_2$	50		
3,17-$(CO)_2$	E4	32	**11α-** OH	23	**3β,7β-** $(OH)_2$	4
			6α- OH	15	**3β,6α-** $(OH)_2$	3
16β-OH-3-CO	D2	0	**11α-** OH	71		
			3β, **11α-** $(OH)_2$	27		
17β-OH-2-CO	D6	17	**6α-** OH	15		
17β-OH-3-CO	E2	8	**11α-** OH	34	**3β,7β-** $(OH)_2$	7
			6α- OH	30	**5α-** OH	6
					3β,6α- $(OH)_2$	5
					3β, **11α-** $(OH)_2$	3
17β-OH-estr-4-en-3-one	D6	35	**6β-** OH	18	**16β-** OH	3
			10β- OH	11		
			11α- OH	9		
3β-OH-16-CO	D2	0	**7α-** OH	90		
3α-OH-17-CO	A2	7	**11α-** OH	23	**6α-** OH	5
			7β- OH	24		
3β-OH-17-CO	E2	0	**7β-** OH	48	**7α-** OH	15
					6α- OH	12
3,6-$(CO)_2$	E2	6	3β, **16β-** $(OH)_2$	26	**16β-** OH	9
			3β, **16α-** $(OH)_2$	23		
			16α- OH	23		
6α-OH-3-CO	E2	0	**16α-** OH	30	**17α-** OH	7
			16β- OH	27	**11α-16β-** $(OH)_2$	4
			11α- OH	14		
6β-OH-3-CO	E2	0	**16β-** OH	39	3β, **16β-** $(OH)_2$	2
			11α,16β- $(OH)_2$	30		
			11α- OH	14		
3β-OH-6-CO	D5	0	**16α-** OH	43		
			16β- OH	18		
3β,6α-$(OH)_2$	E4	6	**16α-** OH	37	**17α-** OH	10
			16β- OH	17		
3β,6β-$(OH)_2$	E4	17	**16β-** OH	38		
			15α- OH	17		
3,6-$(CO)_2$-Δ^4	E4	20	3β, **16α-** $(OH)_2$	32	3β, **16β-** $(OH)_2$	7
					4,5α-H_2-3β,16α- $(OH)_2$	3
6β-OH-3-CO-Δ^4	D5	4	**16β-** OH	61		
			11α,16β- $(OH)_2$	22		
3,7-$(CO)_2$	D3	4	**16β-** OH	32	3β, **16β-** $(OH)_2$	9
7α-OH-3-CO	D6	0	**16β-** OH	33		
			3β, **16β-** $(OH)_2$	24		
7β-OH-3-CO	D6	31	**16β-** OH	52		
3β-OH-7-CO	E4	0	**16β-** OH	55		
			16β- OH	22		
3,11-$(CO)_2$	E2	4	**16β-** OH	55	**9α,16β-** $(OH)_2$	6
			3β, **16β-** $(OH)_2$	20	**9α,16α-** $(OH)_2$	5
					3β, **16α-** $(OH)_2$	4
					16α- OH	2
3β-OH-11-CO	E4	0	**16β-** OH	34	**7β,16β-** $(OH)_2$	8
			16α- OH	11	**7β-OH-16-CO**	7
			6α- OH	9	**16-CO**	6
					5α,16β- $(OH)_2$	5
					7β,17α- $(OH)_2$	4
3-CO-11α-OH	E3	5	**16β-** OH	45	**16α-** OH	14
					3β, **16β-** $(OH)_2$	11
					16-CO	4

TABLE 1 (*Continued*)

Substrate	Conditions	Substrate recovered	Main product(s)		Other product(s)	
3-CO–11β-OH	E3	27	11-CO-**9α,16β**-$(OH)_2$	13	3β, **16β**-$(OH)_2$	4
			16β- OH	11	3β,**6α**- 11-CO-$(OH)_2$	2
			11-CO-**16β**- OH	9	3β-11-CO-**16α**-$(OH)_2$	1
			3β-11-CO-**16β**-$(OH)_2$	9		
17β-OH–6-CO	E4	0	**3β**- OH	23	**11α**- OH	11
			3α- OH	21	**5α,11α**- $(OH)_2$	7
					3α, 11α- $(OH)_2$	7
7,17-$(CO)_2$	E4	0	**3α**- OH	37	**3β**- OH	7
					3α- 17β-$(OH)_2$	6
					3α, 11α- $(OH)_2$	5
					3β, 17β-$(OH)_2$	4
6α-OH–17-CO	E4	16	**11α**- OH	58	**3β**- OH	10
17β-OH–7-CO	E4	0	**3α**- OH	52	**3β**- OH	12
					4α- OH	3
7α-OH–17-CO	E4	10	**3β**- OH	77		
7β-OH–17-CO	E4	25	**3β**- OH	57		
11,16-$(CO)_2$	E2	22	**3α**- OH	44		
			3β- OH	29		
11,16-$(CO)_2$	E6	23	**3β,7β**- $(OH)_2$	24	**3α**- OH	10
					3β- OH	5
11,17-$(CO)_2$	E4	21	**3α,** 17β-$(OH)_2$	36	**4α,** 17β-$(OH)_2$	15
					3α- OH	14
17β-OH–11-CO	E4	26	**3α**- OH	47		
			4α- OH	25		

(98%) of the 16β-hydroxy-3-ketone and the 7α-hydroxylation (90%) of the 3β-hydroxy-16-ketone [see (I) and (II)]. The mono-alcohols are unaffected by *R. nigricans*,[1] but in conjunction with 3- and 16-carbonyl groups respectively the 16β- and 3β-hydroxy-groups not only facilitate hydroxylation but also enhance the directing effect of the carbonyl groups, presumably by leading to precise modes of binding at the active sites. In association with a 3β-hydroxy-group the 17-CO shows a marked preference for hydroxylation in one (the reverse) mode; the 3α-hydroxy-group is not so effective, however, 3α-hydroxy-5α-androstan-17-one giving products from both modes. The presence of a 17β-hydroxy-group in the 17β-OH–3-CO substrate leads to easier hydroxylation but not to selective processes; this group's asymmetric location, as compared with that of a 16β-hydroxy-group, may impede binding in the normal manner and hence encourage attack in the reverse mode.



Hydroxylations of all but one (the 6α-OH–17-CO) of the 25 substrates with one oxygen function in ring B or C and the second in ring A or D are readily interpreted on the basis of three dual-purpose sites. Thus, those with the second substituent in ring A are hydroxylated in ring D [*e.g.* (III)]; conversely, a ring D substituent leads to ring A hydroxylation [*e.g.* (IV)].

With both diketones and keto-alcohols the hydroxylations are highly specific in a positional sense. For example, hydroxylation in ring D occurs almost entirely at position 16; minor products, where present, arise from 17-hydroxylation. Similarly ring A hydroxylation gives 3-hydroxy-products, sometimes accompanied by small amounts of 4-hydroxy-isomers. However, as already observed with monoketones,[1] hydroxylations with *R. nigricans* are subject to considerable variation in a stereochemical sense. Thus, although substitution at position 11 leads exclusively to 11α-products, hydroxylation at the other common positions (3, 7, and 16) gives α- or β-products, or mixtures of both. Changing the configuration of a hydroxy-group in the substrate influences the stereochemical outcome in some cases (*e.g.* 6β-OH–3-CO ⟶ 16β-OH, but 6α-OH–3-CO ⟶ 16α- and 16β-OH) but not in others (*e.g.* both 7α- and 7β-OH–17-CO ⟶ 3β-OH).*

R. nigricans has been used frequently for the 11-monohydroxylation of 3,17-disubstituted steroids.[4] The present work shows that by selecting the substrates judiciously, hydroxylation can be directed to other positions. For this purpose the oxidation level of the substrates' substituents may be as important as their positions. Particularly striking is the comparison of 17β-OH–3-CO ⟶ mainly 11-OH (34%) with 3β-OH–17-CO ⟶ 7-OH (63%). The ability to switch substitution in this way has been exploited in large-scale preparative work which will be described later.[5]

* The yields shown in Table 1 differ from those recorded in the preliminary account,[3] which referred to initial small-scale incubations. Further, the main reaction with 11β-hydroxy-5α-androstan-3-one is 16β-hydroxylation (Table 1); previously[3] the major product was formulated incorrectly as 11β,16α-dihydroxy-5α-androstan-3-one.

[3] J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, and J. Wicha, *Chem. Comm.*, 1969, 1491.

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

[5] Part XI, in preparation.

TABLE 2

N.m.r. signals

The results, presented in the form used earlier,[a] were obtained by examining solutions in $CDCl_3$ at 100 MHz. The weak signals recorded with saturated solutions of some relatively insoluble triols are not given: each of these triols is followed by an entry for the corresponding triacetate.

No.			τ_2	τ_2 (calc.)	>CH−OR		
540	Androst-4-ene-3,6-dione	19	8·83	8·85			
		18	9·22	9·21			
541	5α-Androstane-3,6,15-trione *	19	9·03	9·02			
		18	9·16	9·17			
542	5α-Androstane-4,7,17-trione	19	8·97	8·96			
		18	9·13	9·14			
543	5α-Androstane-4,11,17-trione	19	9·02	9·02			
		18	9·17	9·18			
544	5α-Androstane-3,6,11,16-tetraone	19	8·79	8·77			
		18	9·12	9·11			
545	5α-Androstane-3,6,11,17-tetraone	19	8·81	8·79			
		18	9·12	9·11			
546	5α-Androstane-3,7,11,16-tetraone	19	8·48	8·44			
		18	9·13	9·12			
547	5α-Androstane-3,7,11,17-tetraone	19	8·51	8·46			
		18	9·13	9·12			
548	3β-Hydroxy-5α-androstan-11-one	19	8·97	8·97	H-3	6·21	7(10,10,5,5)
		18	9·32	9·33			
549	5-Hydroxy-5α-androstan-6-one †	19	9·22	9·22			
		18	9·31	9·31			
550	5-Hydroxy-5β-androstan-6-one	19	9·30				
		18	9·30				
551	6α-Hydroxy-5α-androstan-3-one	19	8·97	8·95	H-6	6·50	m(13)
		18	9·27	9·27			
552	6α-Hydroxy-5α-androstan-17-one	19	9·18	9·17	H-6	6·57	6(10,10,5)
		18	9·14	9·14			
553	6β-Hydroxy-5α-androstan-3-one	19	8·77	8·75	H-6	6·24	m(10)
		18	9·23	9·23			
554	7α-Hydroxy-5α-androstan-17-one	19	9·18	9·20	H-7	6·02	m(6)
		18	9·13	9·13			
555	17β-Hydroxy-5α-androstan-11-one	19	8·97	9·00	H-17	6·16	t(8)
		18	9·31	9·31			
556	3α-Hydroxy-5α-androstane-7,17-dione	19	8·93	8·92	H-3	5·91	m(8)
		18	9·14	9·12			
557	3α-Hydroxy-5α-androstane-11,17-dione	19	8·98	8·98	H-3	5·95	m(8)
		18	9·19	9·16			
558	3β-Hydroxy-5α-androstane-7,17-dione	19	8·88	8·89	H-3	6·35	7(10,10,5,5)
		18	9·13	9·12			
559	3β-Hydroxy-5α-androstane-11,16-dione	19	8·95	8·93	H-3	6·41	7(10,10,5,5)
		18	9·18	9·16			
560	6α-Hydroxy-5α-androstane-3,17-dione	19	8·94	8·93	H-6	6·45	6(10,10,5)
		18	9·10	9·10			
561	16α-Hydroxy-5α-androstane-3,6-diene	19	9·04	9·04	H-16	5·51	4(12,6)
		18	9·24	9·24			
562	16α-Hydroxy-5α-androstane-3,11-dione	19	8·79	8·77	H-16	5·48	m(16)
		18	9·30	9·29			
563	16β-Hydroxy-5α-androstane-3,6-dione	19	9·03	9·01	H-16	5·60	m(14)
		18	9·01	9·00			
564	16β-Hydroxy-5α-androstane-3,11-dione	19	8·77	8·74	H-16	5·50	m(16)
		18	9·06	9·05			
565	5-Hydroxy-5α-androstane-3,11,16-trione	19	8·61	8·54			
		18	9·14	9·13			
566	5-Hydroxy-5α-androstane-6,11,17-trione †	19	8·98	8·98			
		18	9·18	9·17			
567	9α-Hydroxy-5α-androstane-3,11,16-trione †	19	8·69	8·70			
		18	9·12	9·12			
568	5α-Androstane-3β,6α-diol	19	9·17	9·16	H-3	6·47	m(29)
		18	9·30	9·30	H-6		
569	2α,6α-Dihydroxy-5α-androstan-16-one ‡	19	9·12	9·13	H-2	6·16	6(10,10,5)
		18	9·15	9·14	H-6	6·56	6(10,10,5)
570	2α,7α-Dihydroxy-5α-androstan-17-one §	19	9·18	9·18	H-2	6·19	6(11,11,5)
		18	9·14	9·13	H-7	6·02	m(7)
571	2α,7β-Dihydroxy-5α-androstan-17-one §	19	9·14	9·15	H-2	6·19	6(11,11,5)
		18	9·11	9·11	H-7	6·60	m(18)
572	3α,17β-Dihydroxy-5α-androstan-6-one	19	9·25	9·27	H-3	5·84	m(7)
		18	9·25	9·25	H-17	6·32	4(15,7)
573	3α,17β-Diacetoxy-5α-androstan-7-one	19	8·93	8·90	H-3	4·96	m(18)
		18	9·22	9·21	H-17	5·36	t(8)
574	3α,17β-Dihydroxy-5α-androstan-11-one	19	8·98	9·00	H-3	5·96	m(7)
		18	9·30	9·30	H-17	6·15	t(8)
575	3β,6α-Dihydroxy-5α-androstan-11-one	19	8·94	8·92	H-3	6·45	m(28)
		18	9·31	9·33	H-6		
576	3β,6α-Diacetoxy-5α-androstan-17-one	19	9·08	9·11	H-3	5·35	m(20)
		18	9·14	9·11	H-6		
577	3β,16α-Diacetoxy-5α-androstan-6-one	19	9·25	9·21	H-3	5·32	6(10,5,5)
		18	9·22	9·20	H-16	4·78	4(13,6)
578	3β,16α-Dihydroxyandrost-4-en-6-one	18	8·99	8·99	H-3	5·72	m(15)
		18	9·25	9·23	H-16	5·48	m(15)
579	3β,16α-Dihydroxy-5α-androstan-7-one	19	8·92	8·92	H-3	6·37	m(22)
		18	9·30	9·28	H-16	5·55	4(13,6)
580	3β,16α-Dihydroxy-5α-androstan-11-one	19	8·99	8·98	H-3	6·43	m(25)
		18	9·34	9·32	H-16	5·50	m(14)
581	3β,16β-Dihydroxyandrost-4-en-6-one ‡	19	8·97	8·96	H-3	5·67	m(27)
		18	9·00	8·99	H-16		
582	3β,17β-Dihydroxy-5α-androstan-6-one	19	9·23	9·24	H-3	6·36	m(23)
		18	9·25	9·25	H-17		
583	4α,17β-Dihydroxy-5α-androstan-7-one	19	8·92	8·92	H-4	6·45	6(10,10,4)
		18	9·26	9·27	H-17	6·32	t(8)
584	4α,17β-Dihydroxy-5α-androstan-11-one	19	8·96	8·98	H-4	6·62	6(11,11,5)
		18	9·30	9·31	H-17	6·14	t(8)
585	5,17-Dihydroxy-5α-androstan-3-one	19	8·81	8·80	H-17	6·36	t(8)
		18	9·24	9·24			
586	6α,11α-Dihydroxy-5α-androstan-3-one	19	8·84	8·83	H-6	6·56	6(10,10,5)
		18	9·25	9·24	H-11	6·02	6(10,10,5)
587	6α,16α-Dihydroxy-5α-androstan-3-one	19	8·98	8·96	H-6	6·53	m(20)
		18	9·26	9·26	H-16	5·53	m(15)
588	6α,16β-Dihydroxy-5α-androstan-3-one	19	8·96	8·93	H-6	6·55	6(8,8,4)
		18	9·02	9·02	H·16	5·60	m(15)

TABLE 2 (*Continued*)

No.			τ_2	τ_2 (calc.)	>CH−OR		
589	6α,17α-Dihydroxy-5α-androstan-3-one	19	8·96	8·97	H-6	6·55	m(24)
		18	9·31	9·33	H-17	6·25	d(6)
590	6α,17β-Dihydroxy-5α-androstan-3-one	19	8·97	8·95	H-6	6·57	6(10,10,5)
		18	9·24	9·24	H-17	6·36	t(8)
591	6β,16β-Dihydroxy-5α-androstan-3-one	19	8·77	8·75	H-6	6·23	4(3,3,1·5)
		18	8·98	8·98	H-16	5·58	m(15)
592	7α,16β-Dihydroxy-5α-androstan-3-one	19	8·97	8·96	H-7	6·13	d(2)
		18	9·02	9·01	H-16	5·57	m(17)
593	7β,16β-Dihydroxy-5α-androstan-3-one †	19	8·94	8·94	H-7	6·65	m(20)
		18	9·00	8·99	H-16	5·60	m(19)
594	11α,16α-Dihydroxy-5α-androstan-3-one	19	8·87	8·87	H-11	6·02	6(10,10,5)
		18	9·24	9·23	H-16	5·55	m(18)
595	11α,17β-Dihydroxy-5α-androstan-6-one	19	9·12	9·15	H-11	6·02	6(10,10,5)
		18	9·26	9·23	H-17	6·32	t(7)
596	11β,16β-Dihydroxy-5α-androstan-3-one	19	8·71	8·73	H-11	5·66	m(14)
		18	8·78	8·77	H-16	5·60	
597	10β-Hydroxy-3-oxoestr-4-en-17β-yl acetate	18	9·15	9·14	H-17	5·39	4(9,7)
598	6β,17β-Diacetoxyestr-4-en-3-one	18	9·11	9·09	H-6	4·55	t(7)
					H-17	5·37	4(9,7)
599	11α,17β-Diacetoxyestr-4-en-3-one	18	9·08	9·08	H-11	4·96	6(10,10,5)
					H-17	5·34	4(9,7)
600	16β,17β-Diacetoxyestr-4-en-3-one	18	9·03		H-16	4·73	6(8,8,5)
					H-17	5·43	d(8)
601	3β,7β-Dihydroxy-5α-androstane-11,16-dione	19	8·90	8·90	H-3	6·43	m(25)
		18	9·16	9·13	H-7		
602	3α,11α-Diacetoxy-5α-androstane-7,17-dione	19	8·80	8·82	H-3	5·00	m(7)
		18	9·08	9·05	H-11	4·70	6(10,10,6)
603	9α,16α-Dihydroxy-5α-androstane-3,11-dione †	19	8·75	8·75	H-16	5·50	m(17)
		18	9·28	9·28			
604	9α,16β-Dihydroxy-5α-androstane-3,11-dione †	19	8·72	8·72	H-16	5·55	m(15)
		18	9·04	9·04			
[605	5α-Androstane-3β,6α,16α-triol]						
606	4β,6α,16α-Triacetoxy-5α-androstane	19	9·11	9·13	H-3	5·32	m(23)
		18	9·26	9·22	H-6		
					H-16	4·78	m(12)
[607	5α-Androstane-3β,6α,16β-triol]						
608	3β,6α,16β-Triacetoxy-5α-androstane ‡	19	9·10	9·12	H-3	5·32	m(24)
		18	9·12	9·13	H-6		
					H-16	4·88	m(16)
[609	5α-Androstane-3β,6α,17α-triol]						
610	3β,6α,17α-Triacetoxy-5α-androstane ‡	19	9·10	9·15	H-3	5·33	m
		18	9·27	9·26	H-6		6(8,8,4)
					H-17	5·20	d(6·5)
[611	5α-Androstane-3β,6α,17β-triol]						
612	3β,6α,17β-Triacetoxy-5α-androstane ‡	19	9·11	9·12	H-3	5·33	m(22)
		18	9·22	9·21	H-6		
					H-17		
613	3β,6β,15α-Triacetoxy-5α-androstane †	19	8·98	9·01	H-3	5·16	
		18	9·18	9·16	H-15		
					H-6	5·02	
[614	5α-Androstane-3β,6β,16β-triol]						
615	3β,6β,16β-Triacetoxy-5α-androstane ‡	19	8·97	9·99			
		18	9·07	9·07			
616	5α-Androstane-3β,7α,16β-triol	19	9·18	9·18	H-3	6·37	m(20)
		18	9·05	9·04	H-7	6·15	m(7)
					H-16	5·57	m(18)
[617	5α-Androstane-3β,7β,17β-triol]						
618	3β,7β,17β-Triacetoxy-5α-androstane †	19	9·12	9·10	H-3	5·38	m(22)
		18	9·19	9·18	H-7		
					H-17		
619	3β,11β,17β-Triacetoxy-5α-androstane	19	9·06	9·07	H-3	5·33	m
		18	9·17	9·14	H-17	5·36	t(8)
					H-11	4·86	6(10,10,5)
620	5α-Androstane-3β,11β,16β-triol	19	8·94	8·94	H-3	6·40	7(10,10,5,5)
		18	8·81	8·80	H-11	5·66	m(20)
					H-16	5·60	
[621	3α,11α,17β-Trihydroxy-5α-androstan-6-one]						
622	3α,11α,17β-Triacetoxy-5α-androstan-6-one	19	9·15	9·15	H-3	4·90	m(8)
		18	9·12	9·13	H-11	4·82	6(10,10,5)
					H-17	5·32	t(8)
623	3β,1β6-Diacetoxy-5-hydroxy-5α-androstan-11-one	19	8·80	8·75	H-3	4·80	m(25)
		18	9·15	9·16	H-16		
624	3β,5,16β-Trihydroxy-5α-androstan-11-one	19	8·81	8·77	H-3	6·46	m(20)
		18	9·09	9·08	H-16	5·49	q(7)
625	3β,7β,16β-Trihydroxy-5α-androstan-11-one	19	8·92	8·92	H-3	6·56	m(24)
		18	9·08	9·05	H-7		
					H-16	5·52	q(7)
[626	3β,7β,17α-Trihydroxy-5α-androstan-11-one]						
627	3β,7β,17α-Triacetoxy-5α-androstan-11-one †	19	8·89	8·91	H-3	5·28	m
		18	9·26	9·26	H-7		
					H-17	5·28	d(6)
628	5,11α,17β-Trihydroxy-5α-androstan-6-one †	19	9·08	9·10			
		18	9·26	9·25			
629	11α,17β-Diacetoxy-5-hydroxy-5α-androstan-6-one	19	9·11	9·12	H-11	4·94	6(10,10,5)
		18	9·18	9·16	H-17	5·35	t(8)
630	6α,11α,16β-Trihydroxy-5α-androstan-3-one	19	8·83	8·81			
		18	8·99	8·99			
631	6α,11α,16β-Triacetoxy-5α-androstan-3-one	19	8·81	8·85	H-6	5·29	6(10,10,5)
		18	9·02	9·04	H-11	4·82	m(30)
					H-16		
632	6β,11α,16β-Trihydroxy-5α-5α-androstan-3-one	19	8·66	8·61			
		18	8·97	8·95			
633	6β,11α,16β-Triacetoxy-5α-androstan-3-one	19	8·70	8·72	H-6	5·12	q(3)
		18	8·98	8·98	H-11	4·78	m(30)
					H-16		

* Δ_1^3 +0·56 (H-19) and +0·44 (H-18). † τ_2(calc.) figures from substituent values which will appear in Part IX. ‡ Not fully characterised. § Obtained as a mixture of 7-epimers.

[a] Ref. 2.

EXPERIMENTAL

For general directions, references to standard procedures, and the meanings of abbreviations see the preceding paper.[1] Where compounds with serial numbers below 543 are stated to have been identified by mixed m.p., the original preparations are contained in, or can be found from, the papers cited.

5α-*Androstane*-2,16-*dione* (no. 33).[6] (a) *Incubation*: 480 mg in Me_2SO (72 ml), 12 flasks, medium B, 6 d, extraction I ⟶ 1·27 g combined extracts. P.l.c. [1 large plate, 3 × petrol–Me_2CO (7 : 3)] gave, in order of decreasing R_F, 2α,6α-dihydroxy-5α-androstan-16-one (no. 569) (25 mg), as an oil, *m/e* 306 (M^+), $\nu_{max.}$ ($CHCl_3$) 3588 and 1734 cm^{-1}; 2α,7α-*dihydroxy*-5α-*androstan*-16-*one* (no. 239)* (277 mg), m.p. 209—212° (from Me_2CO–hexane), $[\alpha]_D$ −175° (*c* 1·0) (Found: C, 74·4; H, 9·75. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1739 cm^{-1}; and 7α-*hydroxy*-5α-*androstane*-2,16-*dione* (no. 196)* (36 mg), m.p. 188—191° (from Me_2CO–hexane), $[\alpha]_D$ −146° (*c* 0·45) (Found: C, 74·8; H, 8·9. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3617, 1739, and 1703 cm^{-1}.

(b) *Transformations*: Oxidation of 2α,6α-dihydroxy-5α-androstan-16-one (no. 569) (50 mg) with 8N-H_2CrO_4 gave 5α-androstane-2,6,16-trione (no. 68) (38 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 251—252°. Oxidation of 2α,7α-dihydroxy-5α-androstan-16-one (no. 239) (25 mg) gave 5α-*androstane*-2,7,16-*trione* (no. 69) * (19 mg), m.p. 270—272° (decomp.) (from Me_2CO–hexane), $[\alpha]_D$ −225° (*c* 0·5) (Found: C, 75·5; H, 8·95. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ ($CHCl_3$) 1740 and 1707 cm^{-1}. Oxidation of 7α-hydroxy-5α-androstane-2,16-dione (no. 196) (50 mg) gave the 2,7,16-trione (no. 69) (39 mg).

5α-*Androstane*-2,17-*dione* (no. 34).[6] (a) *Incubation*: 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction I ⟶ 1·94 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (49 : 1) eluted a mixture (277 mg) [2α,7α-dihydroxy-5α-androstan-17-one (no. 570) and 2α,7β-dihydroxy-5α-androstan-17-one (no. 571) in a 3 : 1 ratio], double m.p. 211—214 and 221—224° (from Me_2CO–hexane) (Found: C, 74·6; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1732 cm^{-1}, which could not be separated by repeated p.l.c. Et_2O–MeOH (99 : 1) gave 2α,6α-*dihydroxy*-5α-*androstan*-17-*one* (no. 238) * (18 mg), m.p. 193—195° (from Me_2CO–hexane), $[\alpha]_D$ +90° (*c* 0·3) (Found: C, 74·2; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3601 and 1737 cm^{-1}.

(b) *Transformations*: Oxidation of the mixture of 2,7-dihydroxy-17-ketones (nos. 570 and 571) (100 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-2,7,17-*trione* (no. 70) * (85 mg), m.p. 216—218° (from Me_2CO–hexane), $[\alpha]_D$ +11° (*c* 0·5) (Found: C, 75·2; H, 8·65. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1743 and 1717 cm^{-1}. Oxidation of 2α,6α-dihydroxy-5α-androstan-17-one (no. 238) gave 5α-androstane-2,6,17-trione (no. 483), m.p. (from Me_2CO–hexane) and mixed [7] m.p. 196—198°.

17β-*Hydroxy*-5α-*androstan*-2-*one* (no. 180).[6] (a) *Incubation*: 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction I ⟶ 2·1 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 100 g). Petrol–Et_2O (1 : 1) gave s.m. (170 mg), m.p. and mixed m.p. 179—180°. Petrol–Et_2O (1 : 9) gave a gum (300 mg) which was purified by p.l.c. [1 large plate, 4 × petrol–Me_2CO (7 : 3)] to give 6α,17β-dihydroxy-5α-androstan-2-one (no. 280) (130 mg), m.p. (from Me_2CO–hexane) and mixed [7] m.p. 235—238°.

5α-*Androstane*-3,6-*dione* (no. 35).[8] (a) *Incubation*: 670 mg in EtOH (34 ml), 17 flasks, medium B, 2 d, extraction (II) ⟶ 800 mg mycelial extract and 1·4 g broth extract. Filtration of the mycelial extract in Et_2O through Al_2O_3 (5% deactivated; 20 g) gave s.m. (40 mg). The broth extract, in Et_2O–MeOH (1 : 1), was filtered through Al_2O_3 (10% deactivated: 20 g) and then separated by p.l.c. [2 large plates, 2 × Et_2O–MeOH (49 : 1)] to give, in order of decreasing R_F, 16β-*hydroxy*-5α-*androstane*-3,6-*dione* (no. 563) (62 mg), m.p. 164—166° (from Me_2CO), $[\alpha]_D$ −20° (*c* 1·0), *m/e* 304·205 ($C_{19}H_{28}O_3$ requires M^+, 304·204), $\nu_{max.}$ ($CHCl_3$) 3610 and 1710 cm^{-1}; 16α-*hydroxy*-5α-*androstane*-3,6-*dione* (no. 561) (156 mg), m.p. 198—200° (from Me_2CO–hexane), $[\alpha]_D$ −19° (*c* 0·3) (Found: C, 75·2; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1710 cm^{-1}; 3β,16β-*dihydroxy*-5α-*androstan*-6-*one* (no. 262) * (175 mg), m.p. 186—187° (from Me_2CO), $[\alpha]_D$ −34° (*c* 0·4) (Found: C, 74·2; H, 10·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1712 cm^{-1}; and 3β,16α-*dihydroxy*-5α-*androstan*-6-*one* (no. 261) * (147 mg), m.p. 201—203° (from Me_2CO), $[\alpha]_D$ −32° (*c* 0·3) (Found: C, 74·6; H, 10·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}.

(b) *Transformations*: Acetylation (Ac_2O–C_5H_5N; 2 : 1, for 2 d) of 3β,16α-dihydroxy-5α-androstan-6-one (no. 261) gave 3β,16α-*diacetoxy*-5α-*androstan*-6-*one* (no. 577), m.p. 136—138° (from Me_2CO–hexane), $[\alpha]_D$ −41° (*c* 0·6) (Found: C, 70·9; H, 8·7. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1740 and 1723 cm^{-1}.

A portion (150 mg) of a crude broth extract in Me_2CO was oxidised with 8N-H_2CrO_4 and the product (120 mg) was purified by p.l.c. to give 5α-*androstane*-3,6,16-*trione* (no. 76) * (80 mg), m.p. 224—226° (from Me_2CO–hexane), $[\alpha]_D$ −158° (*c* 0·5) (Found: C, 75·7; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1749 and 1719 cm^{-1}.

6α-*Hydroxy*-5α-*androstan*-3-*one* (no. 551).[9] (a) *Incubation*: 600 mg in EtOH (30 ml), 15 flasks, medium B, 2 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract contained no s.m. and was discarded. Filtration of the broth extract in Et_2O–MeOH (9 : 1) through Al_2O_3 (10% deactivated; 20 g) followed by p.l.c. [2 large plates, 2 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] gave, in order of decreasing R_F, 6α,11α-*dihydroxy*-5α-*androstan*-3-*one* (no. 586) (88 mg), m.p. 137—138 and 180—182° (from Me_2CO–hexane), $[\alpha]_D$ 0° (*c* 0·9) (Found: C, 74·1; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3590 and 1703 cm^{-1}; 6α,17α-*dihydroxy*-5α-*androstan*-3-*one* (no. 589) (45 mg), m.p. 176—178° (from Me_2CO–hexane), $[\alpha]_D$ +22° (*c* 0·2) (Found: C, 74·3; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; 6α,16β-*dihydroxy*-5α-*androstan*-3-*one* (no. 588) (172 mg), m.p. 171·5—173·5° (from Me_2CO–hexane), $[\alpha]_D$ +26° (*c* 1·0) (Found: C, 74·2; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3605 and 1705 cm^{-1}; 6α,16α-*dihydroxy*-5α-*androstan*-3-*one* (no. 587) (190 mg), m.p. 227—228° (from Me_2CO–hexane), $[\alpha]_D$ (EtOH) +37° (*c* 0·8) (Found: C, 74·55; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; and 6α,11α,16β-*trihydroxy*-

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[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] 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.

[9] Unpublished work.

5α-*androstan*-3-*one* (no. 630) (25 mg), m.p. 255—257° (from MeOH–Me_2CO), $[\alpha]_D$ (EtOH) −10° (*c* 0·2) (Found: C, 70·7; H, 9·4. $C_{10}H_{30}O_4$ requires C, 70·8; H, 9·4%), $\nu_{max.}$ ($CHCl_3$) 3600 cm^{-1}.

(b) *Transformations*: On oxidation with 8N-H_2CrO_4 6α,11α-dihydroxy-5α-androstan-3-one (no. 586) gave 5α-androstane-3,6,11-trione (no. 72), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 188—190°; 6α,17α-dihydroxy-5α-androstan-3-one (no. 589) gave 5α-androstane-3,6,17-trione (no. 78), m.p. (from Me_2CO–hexane) and mixed [2] m.p. 194—196°; 6α,16β- (no. 588) and 6α,16α-dihydroxy-5α-androstan-3-one (no. 587) gave 5α-androstane-3,6,16-trione (no. 76), m.p. and mixed m.p. 223—225°; and 6α,11α,16β-trihydroxy-5α-androstan-3-one (no. 630) gave 5α-*androstane*-3,6,11,16-*tetraone* (no. 544), m.p. 279—281° (from Me_2CO–hexane), $[\alpha]_D$ −116° (*c* 0·1) (Found: C, 71·7; H, 7·9. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%), $\nu_{max.}$ ($CHCl_3$) 1743 and 1710 cm^{-1}. Acetylation of the trihydroxy-ketone (no. 630) gave 6α,11α,16β-*triacetoxy*-5α-*androstan*-3-*one* (no. 631), m.p. 199—201·5° (from Me_2CO–hexane), $[\alpha]_D$ −4° (*c* 0·2) (Found: C, 67·2; H, 8·3. $C_{25}H_{36}O_7$ requires C, 66·9; H, 8·1%) $\nu_{max.}$ 1733 and 1712 cm^{-1}.

6β-*Hydroxy*-5α-*androstan*-3-*one* (no. 533).[9] (a) *Incubation*: 600 mg in EtOH (30 ml), 15 flasks, medium B, 2 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract contained no steroidal material and was discarded. The broth extract, in $CHCl_3$–MeOH (9 : 1), was filtered through Al_2O_3 (10% deactivated; 20 g). P.l.c. [2 large plates, 2 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] gave, in order of decreasing R_F, 6β,11α-dihydroxy-5α-androstan-3-one (no. 281) (91 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 194—195°; 6β,16β-*dihydroxy*-5α-*androstan*-3-*one* (no. 591) (250 mg), m.p. 214·5—215·5° (from Me_2CO–hexane), $[\alpha]_D$ −11: (*c* 0·9) (Found: C, 74·7; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1703 cm^{-1}; 6β,11α,16β-*trihydroxy*-5α-*androstan*-3-*one* (no. 632) (202 mg), m.p. 259—260° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) −31° (*c* 0·4) (Found: C, 70·8; H, 9·3. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%); and 5α-*androstane*-3β,6β,16β-*triol* (no. 614) (14 mg), m.p. 263—266° (from Me_2CO–MeOH), $[\alpha]_D$ (MeOH) −20·5° (*c* 0·4) (Found: C, 73·7; H, 10·35. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%).

(b) *Transformations*: Acetylation of the trihydroxy-ketone (no. 632) gave 6β,11α,16β-triacetoxy-5α-androstan-3-one (no. 633), m.p. 166—169° (from Me_2CO–hexane), *m/e* 448 (M^+), $\nu_{max.}$ 1733 and 1712 cm^{-1}; the triol (no. 614) gave 3β,6β,16β-triacetoxy-5α-androstane (no. 615), an oil, *m/e* 434 (M^+). Oxidation of the trihydroxy-ketone (no. 632) gave 5α-androstane-3,6,11,16-tetraone (no. 544), m.p. and mixed m.p. 279—281°. Oxidation of the 3β,6β,16β-triol (no. 614) and of the 6β,16β-dihydroxy-3-ketone (no. 591) gave 5α-androstane-3,6,16-trione (no. 76), m.p. and mixed m.p. 222—224°.

3β-*Hydroxy*-5α-*androstan*-6-*one* (no. 147).[9] (a) *Incubation*: 100 mg in Me_2SO (12 ml), 2 flasks, medium B, 5 d, extraction II ⟶ 200 mg combined extracts. P.l.c. [1 medium plate, 1 × C_6H_6–EtOAc (1 : 1)] gave 3β,16β-dihydroxy-5α-androstan-6-one (no. 262) (higher R_F) (19 mg), m.p. and mixed m.p. 185—187°, and 3β,16α-dihydroxy-5α-androstan-6-one (no. 261) (lower R_F) (45 mg), m.p. and mixed m.p. 197—199°.

5α-*Androstane*-3β,6α-*diol* (no. 568).[9] (a) *Incubation*: 600 mg in EtOH (30 ml), 15 flasks, medium B, 4 d, extraction II ⟶ 400 mg mycelial extract and 703 mg broth extract. P.l.c. [1 large plate, 1 × Et_2O–MeOH (49 : 1)] of the mycelial extract gave s.m. (33 mg). The broth extract was separated by p.l.c. [3 large plates, 3 × CH_2Cl_2–Me_2CO–EtOH (8 : 4 : 1)] to give, in order of decreasing R_F, 5α-*androstane*-3β,6α,17α-*triol* (no. 609) (58 mg), m.p. 280—281° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) +19° (*c* 0·6) (Found: C, 74·1; H, 10·6. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%); 5α-*androstane*-3β,6α,16β-*triol* (no. 607) (100 mg), m.p. 231—232° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) +14° (*c* 0·5) (Found: C, 74·1; H, 10·3. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%); and 5α-*androstane*-3β,6α,16α-*triol* (no. 605) (220 mg), m.p. 237—238° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) +37° (*c* 0·1) (Found: C, 74·2; H, 10·5. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%).

(b) *Transformations*: Acetylation of the 3β,6α,17α-triol (no. 609) and of the 3β,6α,16β-triol (no. 607) gave 3β,6α,17α-triacetoxy-5α-androstane (no. 610) and 3β,6α,16β-triacetoxy-5α-androstane (no. 608), respectively, as oils, *m/e* 314 (M^+ − 2AcOH), $\nu_{max.}$ 1732 cm^{-1}. Acetylation of the 3β,6α,16α-triol (no. 605) gave 3β,6α,16α-*triacetoxy*-5α-*androstane* (no. 606), m.p. 70—75° (from petrol), $[\alpha]_D$ +14° (*c* 1·1) (Found: C, 68·9; H, 8·9. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%), $\nu_{max.}$ 1732 cm^{-1}. Oxidation of the 3β,6α,17α-triol (no. 609) (10 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,6,17-trione (no. 78) (9 mg), m.p. and mixed m.p. 193—195°. Oxidation of the 3β,6α,16β- and 3β,6α-16α-triols (nos. 607 and 605) (30 mg) gave 5α-androstane-3,6,16-trione (no. 76) (25 mg), m.p. and mixed m.p. 223—225°.

5α-*Androstane*-3β,6β-*diol* (no. 220).[9] (a) *Incubation*: 120 mg in EtOH (6 ml), 3 flasks, medium B, 4 d, extraction II ⟶ 237 mg mycelial extract and 99 mg broth extract. P.l.c. [1 large plate, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 5 : 1)] of the mycelial extract gave s.m. (20 mg). Separation of the broth extract by p.l.c. [2 small plates, 2 × CH_2Cl_2–Me_2CO–EtOH (12 : 8 : 1)] afforded 5α-androstane-3β,6β,16β-triol (no. 614) (higher R_F) (40 mg), m.p. and mixed m.p. 263—266°, and material (18 mg) which was acetylated (Ac_2O–C_5H_5N; 2 : 1, for 2 d) to give 3β,6β,15α-triacetoxy-5α-androstane (no. 613) as an oil, *m/e* 434 (M^+), $\nu_{max.}$ 1732 cm^{-1}.

(b) *Transformations*: Oxidation of the 3β,6β,16β-triol (no. 614) (8 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,6,16-trione (no. 76) (8 mg). The 3β,6β,15α-triacetate (no. 613) (30 mg) was boiled under reflux with NaOH (40 mg) in H_2O (1 ml)–MeOH (6 ml). The resulting 3β,6β,15α-triol was oxidised with 8N-H_2CrO_4 to 5α-*androstane*-3,6,15-*trione* (no. 541) (10 mg), m.p. 218—219° (from Me_2CO–hexane), $[\alpha]_D$ +22° (*c* 0·3) (Found: C, 75·3; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ ($CHCl_3$) 1738 and 1710 cm^{-1}.

Androst-4-*ene*-3,6-*dione* (no. 540).[11] (a) *Incubation*: 880 mg in EtOH (44 ml), 22 flasks, medium B, 4 d, extraction II ⟶ 438 mg mycelial extract and 898 mg broth extract. P.l.c. [1 large plate, 1 × Et_2O] of the mycelial extract gave s.m. (173 mg). P.l.c. [3 large plates, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] of the broth extract gave several unidentified products (total 64 mg) and, in order of decreasing R_F, 3β,16β-dihydroxyandrost-4-en-6-one (no. 581) (53 mg) as an oil, *m/e* 304 (M^+); 3β,16α-*dihydroxyandrost*-4-*en*-6-*one* (no. 578) (240 mg), m.p. 198—200° (from Me_2CO–hexane), $[\alpha]_D$ −34° (*c* 0·9) (Found: C, 74·7; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600, 1683, and

[10] 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.

[11] R. Sciaky and A. Consonni, *Gazzetta*, 1962, **92**, 730.

1630 cm^{-1}; and 3β,16α-dihydroxy-5α-androstan-6-one (no. 261) (20 mg), m.p. and mixed m.p. 199—201°.

(b) *Transformations*: On oxidation with 8N-H_2CrO_4, 3β,16β- and 3β,16α-dihydroxy-5α-androst-4-en-6-one (nos. 581 and 578) (30 mg) gave *androst-4-ene-3,6,16-trione* (no. 77) * (25 mg), m.p. 181—182° (from Me_2CO–hexane), $[\alpha]_D$ −131° (*c* 0·6) (Found: C, 75·9; H, 8·0. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·05%), $\lambda_{max.}$ 250 nm (ε 9000), $\nu_{max.}$ 1750 and 1691 cm^{-1}.

6β-*Hydroxyandrost-4-en-3-one* (no. 160).[8] (a) *Incubation*: 50 mg in Me_2SO (6 ml), 1 flask, medium B, 5 d, extraction II ⟶ 100 mg combined extracts. P.l.c. [1 medium plate, 2 × C_6H_6–EtOAc (2 : 1)] gave, in order of decreasing R_F, s.m. (2 mg); 6β,16β-*dihydroxyandrost-4-en-3-one* (no. 285) * (31 mg), m.p. 188—190° (from Me_2CO–hexane), $[\alpha]_D$ −2·5° (*c* 0·4) (Found: C, 74·6; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3600, 1679, and 1620 cm^{-1}; and 6β,11α,16β-trihydroxyandrost-4-en-3-one (11 mg), m.p. 226—228° (from Me_2CO–Et_2O), $\nu_{max.}$ ($CHCl_3$) 1680 cm^{-1}.

(b) *Transformation*: Oxidation of 6β,16β-dihydroxyandrost-4-en-3-one (no. 285) gave androst-4-ene-3,6,16-trione (no. 77), m.p. and mixed m.p. 178—180°.

5α-*Androstane-3,7-dione* (no. 36).[8] (a) *Incubation*: 160 mg in Me_2SO (24 ml), 4 flasks, medium B, 3 d, extraction II ⟶ 300 mg combined extracts. P.l.c. [1 large plate, 1 × petrol–Me_2CO (2 : 1)] gave, in order of decreasing R_F, s.m. (7 mg); 16β-*hydroxy-5α-androstane-3,7-dione* (no. 207) * (51 mg), m.p. 185—186° (from Me_2CO–hexane), $[\alpha]_D$ −48° (*c* 1·0) (Found: C, 75·2; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 1710 cm^{-1}; and 3β,16β-*dihydroxy-5α-androstan-7-one* (no. 263) * (14 mg), m.p. 235—237° (from Me_2CO), $[\alpha]_D$ −65° (*c* 1·0) (Found: C, 74·5; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1715 cm^{-1}.

(b) *Transformations*: Oxidation of 16β-hydroxy-5α-androstane-3,7-dione (no. 207) and of 3β,16β-dihydroxy-5α-androstan-7-one (no. 263) gave 5α-androstane-3,7,16-trione (no. 82), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 240—242°.

7α-*Hydroxy-5α-androstan-3-one* (no. 161).[8] (a) *Incubation*: 160 mg in Me_2SO (24 ml), 4 flasks, medium B, 6 d, extraction III ⟶ total extract, which was purified by p.l.c. [1 medium plate, 1 × petrol–EtOAc (4 : 1)] to give 7α,16β-*dihydroxy-5α-androstan-3-one* (no. 592) (higher R_F) (55 mg), m.p. 203—204° (from Me_2CO–hexane), $[\alpha]_D$ −9° (*c* 0·6) (Found: C, 74·7; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1710 cm^{-1}, and 5α-*androstane-3β,7α,16β-triol* (no. 616) (lower R_F) (40 mg), m.p. 170—172° (from Me_2CO–hexane), $[\alpha]_D$ −14° (*c* 1·0) (Found: C, 73·8; H, 10·3. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ 3610 cm^{-1}.

(b) *Transformations*: Oxidation with 8N-H_2CrO_4 of both products gave 5α-androstane-3,7,16-trione (no. 82), m.p. and mixed m.p. 240—242°.

7β-*Hydroxy-5α-androstan-3-one* (no. 383).[8] (a) *Incubation*: 160 mg in Me_2SO (24 ml), 4 flasks, medium B, 6 d, extraction III ⟶ total extract which was separated by p.l.c. [1 medium plate, 1 × petrol–EtOAc (4 : 1)] to give s.m. (50 mg) (higher R_F), and 7β,16β-dihydroxy-5α-androstan-3-one (no. 593) (lower R_F) (60 mg), as an oil, $\nu_{max.}$ 3610 and 1715 cm^{-1}.

[12] K. Heusler and A. Wettstein, *Helv. Chim. Acta*, 1952, **35**, 284.

[13] D. H. Williams, N. S. Bhacca, and C. Djerassi, *J. Amer. Chem. Soc.*, 1963, **85**, 2810.

3β-*Hydroxy-5α-androstan-7-one* (no. 148).* *Preparation*: A mixture of 1·85M-$(Bu^tO)_2CrO_2$ (prepared as in ref. 12; 200 ml), AcOH (65 ml), and Ac_2O (25 ml) was added during 1 h to a vigorously stirred solution of androst-5-en-3β-yl acetate (21·7 g) in CCl_4 (145 ml) at 20 °C. The stirred mixture was boiled under reflux for 14 h, then cooled to 0 °C. Oxalic acid (43 g) in H_2O (433 ml) was added during 40 min. After a further 15 min, more oxalic acid (30 g) was added, and the mixture was stirred until effervescence ceased. The material isolated with CCl_4 was dissolved in $CHCl_3$ and filtered through Al_2O_3 (10% deactivated; 20 g) to give 7-oxoandrost-5-en-3β-yl acetate (no. 149) (18·6 g), m.p. 178—180° (from EtOH–H_2O) (lit.,[13] 179—180°). [Adding the $(Bu^tO)_2CrO_2$ to a boiling solution of androst-5-en-3β-yl acetate [12] results in a lower yield.]

Hydrogenation of 7-oxoandrost-5-en-3β-yl acetate (no. 149) (6 g) in EtOH (180 ml)–EtOAc (20 ml) for 1 h over 10% Pd–C (450 mg), followed by hydrolysis with KOH (6 g)–MeOH (100 ml) under N_2 for 20 h at 20 °C gave 3β-hydroxy-5α-androstan-7-one (3·7 g), m.p. 144—146° (from Me_2CO–hexane) (lit.,[13] 128—129·5°).

(a) *Incubation*: 600 mg in EtOH (30 ml), 15 flasks, medium B, 4 d, extraction II ⟶ 460 mg mycelial extract and 800 mg broth extract. The mycelial extract contained no s.m. and was discarded. The broth extract was separated by p.l.c. [2 large plates, 3 × Et_2O–MeOH (25 : 1)] to give 3β,16β- dihydroxy-5α-androstan-7-one (no. 263) (higher R_F) (350 mg), m.p. and mixed m.p. 235—236°, and 3β,16α-*dihydroxy-5α-androstan-7-one* (no. 579) (lower R_F) (140 mg), m.p. 203—204° (from Me_2CO), $[\alpha]_D$ −65° (*c* 0·7) (Found: C, 74·6; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}.

(b) *Transformation*: Oxidation of the 3β,16α-dihydroxy-7-ketone (no. 579) with 8N-H_2CrO_4 gave 5α-androstane-3,7,16-trione (no. 82), m.p. and mixed m.p. 240—242°.

5α-*Androstane-3,11-dione* (no. 37).[14] (a) *Incubation*: 1 g in EtOH (50 ml), 25 flasks, medium B, 2 d, extraction II ⟶ 400 mg mycelial extract and 1·4 g broth extract. P.l.c. [1 large plate, 1 × Et_2O] of the mycelial extract gave s.m. (42 mg). P.l.c. [4 large plates, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 5 : 1)] of the broth extract gave, in order of decreasing R_F, 16β-*hydroxy-5α-androstane-3,11-dione* (no. 564) (555 mg), m.p. 174—175° (from Me_2CO–hexane), $[\alpha]_D$ +58° (*c* 0·7) (Found: C, 75·3; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}; 16α-*hydroxy-5α-androstane-3,11-dione* (no. 562) (21 mg), m.p. 177—180° (from Me_2CO–hexane), $[\alpha]_D$ +72° (*c* 0·2) (Found: C, 74·8; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; 9α,16β-*dihydroxy-5α-androstane-3,11-dione* (no. 604) (64 mg), m.p. 219—221° (from Me_2CO–hexane), $[\alpha]_D$ +91° (*c* 1·0) (Found: C, 71·35; H, 8·8. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (204 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 232—234°; and a mixture, separated by further p.l.c. [2 small plates, 3 × Et_2O–MeOH (97 : 3)] into 9α,16α-*dihydroxy-5α-androstane-3,11-dione* (no. 603) (higher R_F) (51 mg), m.p. 247—248·5° (from Me_2CO–hexane), $[\alpha]_D$ +100° (*c* 0·4) (Found: C, 71·1; H, 8·9. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1710 cm^{-1}, and 3β,16α-*dihydroxy-5α-androstan-11-one* (no. 580) (lower R_F) (38 mg), m.p. 193—195° (from Me_2CO–hexane), $[\alpha]_D$ +17°

[14] 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.

(*c* 1·0) (Found: C, 74·65; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1703 cm^{-1}.

(b) *Transformations*: Oxidation of 16β-hydroxy-5α-androstane-3,11-dione (no. 564), 16α-hydroxy-5α-androstane-3,11-dione (no. 562), and 3β,16α-hydroxy-5α-androstan-11-one (no. 580) with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 172—174°. Huang-Minlon reduction of the 16β-hydroxy-diketone (no. 564) (20 mg) gave 5α-androstan-16β-ol (no. 134) (18 mg), m.p. (from MeOH) and mixed [15] m.p. 108—110°. Oxidation of the dihydroxy-diketones (nos. 603 and 604) gave 9α-*hydroxy*-5α-*androstane*-3,11,16-*trione* (no. 567), m.p. 215—216° (from Me_2CO–hexane), $[\alpha]_D$ −72° (*c* 0·45) (Found: C, 72·0; H, 8·1. $C_{19}H_{26}O_4$ requires C, 71·7; H, 8·2%), $\nu_{max.}$ 1745 and 1710 cm^{-1}. This compound, whose properties are different from those of 5-hydroxy-5α-androstane-3,11,16-trione (no. 565) and 14α-hydroxy-5α-androstane-3,11,16-trione,[9] was unchanged by treatment with 2N-NaOH at 20 °C for 5 h; similar treatment of the 5α-hydroxy- and 14α-hydroxy-triketones gave solutions showing strong absorption at 240 nm.

3β-*Hydroxy*-5α-*androstan*-11-*one* (no. 548).[16] (a) *Incubation*: 1·4 g in EtOH (70 ml), 35 flasks, medium B, 4 d, extraction II ⟶ mycelial extract (which contained no s.m. and was discarded) and broth extract (1·6 g). The broth extract, in $CHCl_3$–MeOH (9 : 1), was filtered through Al_2O_3 (10% deactivated; 10 g) and separated by p.l.c. [5 large plates, 2 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] to give, in order of decreasing R_F, 3β-*hydroxy*-5α-*androstane*-11,16-*dione* (no. 559) (90 mg), m.p. 232—234° (from Me_2CO–hexane), $[\alpha]_D$ −119° (*c* 0·9) (Found: C, 75·2; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600, 1745, and 1708 cm^{-1}; 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (500 mg), m.p. (from Me_2CO–hexane) and mixed m.p. 232—234°; a mixture, separated by further p.l.c. [3 small plates, 2 × Et_2O–MeOH (97 : 3)] into 3β,6α-*dihydroxy*-5α-*androstan*-11-*one* (no. 575) (138 mg), m.p. 176—177° (from Me_2CO–hexane), $[\alpha]_D$ +82° (*c* 0·2) (Found: C, 74·8; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}, and 3β,16α-dihydroxy-5α-androstan-11-one (no. 580) (164 mg), m.p. (from Me_2CO–hexane) and mixed m.p. 193—195°; 3β,7β,16β-*trihydroxy*-5α-*androstan*-11-*one* (no. 625) (117 mg), m.p. 240—241° (from Me_2CO–hexane), $[\alpha]_D$ +81° (*c* 1·1) (Found: C, 70·5; H, 9·0. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1703 cm^{-1}; 3β,7β-*dihydroxy*-5α-*androstane*-11,16-*dione* (no. 601) (102 mg), m.p. 203—205° (from Me_2CO–hexane), $[\alpha]_D$ −84° (*c* 0·2), *m/e* 320·1983 ($C_{19}H_{28}O_4$ requires M^+, 320·1987), $\nu_{max.}$ ($CHCl_3$) 3610, 1740, and 1710 cm^{-1}; 3β,5,16β-*trihydroxy*-5α-*androstan*-11-*one* (no. 624) (81 mg), m.p. 225—226° (from Me_2CO), $[\alpha]_D$ (EtOH) +42° (*c* 0·5) (Found: C, 70·5; H, 9·4. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%); and 3β,7β,17α-*trihydroxy*-5α-*androstan*-11-*one* (no. 626) (69 mg), m.p. 255—256·5° (from MeOH–Me_2CO), $[\alpha]_D$ (EtOH) +65° (*c* 0·3) (Found: C, 71·0; H, 9·45. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%).

(b) *Transformations*: On oxidation with 8N-H_2CrO_4, 3β-hydroxy-5α-androstane-11,16-dione (no. 559) gave 5α-androstane-3,11,16-trione (no. 85), m.p. and mixed m.p. 174—176°; 3β,6α-dihydroxy-5α-androstan-11-one (no. 575) gave 5α-androstane-3,6,11-trione (no. 72), m.p. and mixed m.p. 188—190°; the 3β,7β,16β-trihydroxy-11-ketone (no. 625) and the 3β,7β-dihydroxy-11,16-diketone (no. 601) gave 5α-*androstane*-3,7,11,16-*tetraone* (no. 546), m.p. 260—263° (from Me_2CO–hexane), $[\alpha]_D$ −155° (*c* 0·1) (Found: C, 72·2; H, 7·5. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%), $\nu_{max.}$ ($CHCl_3$) 1750 and 1715 cm^{-1}; 3β,5,16β-trihydroxy-5α-androstan-11-one (no. 624) gave 5-*hydroxy*-5α-*androstane*-3,11,16-*trione* (no. 565), m.p. 233—236° (from Me_2CO–hexane), *m/e* 318·1828 ($C_{19}H_{26}O_4$ requires M^+, 318·1831), $\nu_{max.}$ ($CHCl_3$) 3610, 1743, and 1710 cm^{-1}; and 3β,7β,17α-trihydroxy-5α-androstan-11-one (no. 626) gave 5α-*androstane*-3,7,11,17-*tetraone* (no. 547), m.p. 239—241° (from Me_2CO–hexane), $[\alpha]_D$ +63° (*c* 0·2) (Found: C, 72·2; H, 7·7. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%), $\nu_{max.}$ ($CHCl_3$) 1742 and 1710 cm^{-1}. Acetylation of the 3β,5α,16β-trihydroxy-11-ketone (no. 624) and of the 3β,7β,17α-trihydroxy-11-ketone (no. 626) gave, respectively, 3β,16β-*diacetoxy*-5-*hydroxy*-5α-*androstan*-11-*one* (no. 623), m.p. 193—195° (from Me_2CO–hexane), $[\alpha]_D$ +2° (*c* 0·1) (Found: C, 67·6; H, 8·5. $C_{23}H_{34}O_6$ requires C, 68·0; H, 8·4%), $\nu_{max.}$ 3600, 1740, and 1710 cm^{-1}, and 3β,7β,17α-*triacetoxy*-5α-*androstan*-11-*one* (no. 627), m.p. 145—151° (from Me_2CO), $[\alpha]_D$ +27° (*c* 0·1) (Found: C, 64·45; H, 8·2. $C_{25}H_{36}O_7,H_2O$ requires C, 64·4; H, 8·2%), *m/e* 448 (M^+, $C_{25}H_{36}O_7$), $\nu_{max.}$ 1740, 1716, and 1705 cm^{-1}.

11α-*Hydroxy*-5α-*androstan*-3-*one* (no. 163).[10] (a) *Incubation*: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 3 d, extraction II ⟶ 350 mg mycelial extract and 1·2 g broth extract. Chromat. Al_2O_3 (5% deactivated; 25 g) of mycelial extract gave s.m. (50 mg). The broth extract was separated by p.l.c. [3 large plates, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] to give, in order of decreasing R_F, 11α-hydroxy-5α-androstane-3,16-dione (no. 204) (39 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 256—258°; 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (451 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 200—204°; 11α,16α-*dihydroxy*-5α-*androstan*-3-*one* (no. 594) (136 mg), m.p. 220—223° (from Me_2CO), $[\alpha]_D$ −22° (*c* 0·4) (Found: C, 74·75; H, 10·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1707 cm^{-1}; and 5α-androstane-3β,11α,16β-triol (no. 325) (111 mg), m.p. (from Me_2CO–MeOH) and mixed [1] m.p. 250·5—251·5°.

(b) *Transformation*: Oxidation of 11α,16α-dihydroxy-5α-androstan-3-one (no. 594) with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85).

11β-*Hydroxy*-5α-*androstan*-3-*one* (no. 166).[5] (a) *Incubation*: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 3 d, extraction II ⟶ 1·28 g mycelial extract and 870 mg broth extract. P.l.c. [3 large plates, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 2 : 1)] of the mycelial extract gave s.m. (275 mg). P.l.c. [2 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] of the broth extract gave, in order of decreasing R_F, 16β-hydroxy-5α-androstane-3,11-dione (no. 564) (66 mg), m.p. and mixed m.p. 174—175°; 11β,16β-*dihydroxy*-5α-*androstan*-3-*one* (no. 596) (83 mg), m.p. 214—215° (from Me_2CO–hexane), $[\alpha]_D$ +28° (*c* 0·4) (Found: C, 74·2; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3605 and 1708 cm^{-1}; 9α,16β-dihydroxy-5α-androstane-3,11-dione (no. 604) (105 mg), m.p. and mixed m.p. 216—217°; 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (68 mg), m.p. and mixed m.p. 232—233°; and a mixture (91 mg), separated by further p.l.c. [2 small plates, 2 × Et_2O–MeOH (49 : 1)] into 5α-*androstane*-3β,11β,16β,*triol* (no. 620) (30 mg), m.p. 185—186° (from Me_2CO–hexane), $[\alpha]_D$ +13° (*c* 0·6) (Found: C,

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[16] S. Binns, J. S. G. Cox, Sir Ewart R. H. Jones, and B. G. Ketcheson, *J. Chem. Soc.*, 1964, 1161.

73·8; H, 10·6. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ ($CHCl_3$) 3608 cm^{-1}, and material (24 mg) shown by n.m.r. to be a 3 : 1 mixture of 3β,6α-dihydroxy-5α-androstan-11-one (no. 575) and 3β-16α-dihydroxy-5α-androstan-11-one (no. 580).

(b) *Transformations*: Oxidation of 11β,16β-dihydroxy-5α-androstan-3-one (no. 596) and of 5α-androstane-3β,11β,16β-triol (no. 620) with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85). Reduction of 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (50 mg) with $LiAlH_4$ (10 mg) in Et_2O (5 ml) gave 5α-androstane-3β,11β,16β-triol (no. 620) (45 mg).

5α-Androstane-3,16-dione (no. 40).[6]. (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, 1 × C_6H_6–EtOAc (2 : 1)] gave 3β,7α-dihydroxy-5α-androstan-16-one (no. 247) (42 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 255—258°.

16β-Hydroxy-5α-androstan-3-one (no. 176).[6] (a) *Incubation*: 100 mg in Me_2SO (18 ml), 3 flasks, medium B, 2 d, extraction II ⟶ 140 mg combined extracts. P.l.c. [1 medium plate, 2 × C_6H_6–EtOAc (2 : 1)] gave 11α,16β-dihydroxy-5α-androstan-3-one (no. 292) (higher R_F) (75 mg), m.p. and mixed m.p. 207—208°, and 5α-androstane-3β,11α,16β-triol (no. 325) (lower R_F) (29 mg), m.p. and mixed m.p. 247—248°.

3β-Hydroxy-5α-androstan-16-one (no. 150).[6] (a) *Incubation*: 80 mg in Me_2SO (12 ml), 2 flasks, medium B, 2 d, extraction II ⟶ 100 mg combined extracts. Crystallisation from Me_2CO–hexane gave 3β,7α-dihydroxy-5α-androstan-16-one (no. 247) (76 mg), m.p. and mixed m.p. 257—259°.

(b) *Transformation*: Oxidation of the dihydroxy-ketone (no. 247) with 8N-H_2CrO_4 gave 5α-androstane-3,7,16-trione (no. 82), m.p. and mixed m.p. 240—242°.

5α-Androstane-3,17-dione (no. 42). (a) *Incubation*: 960 mg in EtOH (48 ml), 24 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. Filtration of the mycelial extract, in Et_2O, through Al_2O_3 (5% deactivated; 20 g) gave s.m. (310 mg). The broth extract (700 mg), in EtOAc–MeOH (9 : 1), was filtered through Al_2O_3 (10% deactivated; 40 g). P.l.c. [2 large plates, 2 × petrol–Me_2CO (3 : 1)] gave, in order of decreasing R_F, 11α-hydroxy-5α-androstane-3,17-dione (no. 519) (160 mg), m.p. (from Me_2CO–hexane) and mixed [17] m.p. 190—193°; *6α-hydroxy-5α-androstane-3,17-dione* (no. 560) (100 mg), m.p. 206—207° (from Me_2CO–hexane), $[\alpha]_D$ +119° (*c* 0·9) (Found: C, 75·1; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 35,80 1735, and 1710 cm^{-1}; 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (25 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 243—244°; and *3β,6α-dihydroxy-5α-androstan-17-one* (no. 246) * (20 mg), m.p. 222—224° (from Me_2CO), $[\alpha]_D$ +111° (*c* 0·5) (Found: C, 74·7; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3610 and 1747 cm^{-1}.

(b) *Transformations*: The material (50 mg) from the mother liquor of the crystallisation of 6α-hydroxy-5α-androstan-3,17-dione (no. 560) was dissolved in $CHCl_3$, filtered through Al_2O_3 (5% deactivated; 5 g), and oxidised with 8N-H_2CrO_4 to give material (40 mg) shown by n.m.r. to be a 1 : 1 mixture of 5α-androstane-3,7,17-trione (no. 84) (presumably formed from 7β-hydroxy-5α-androstane-3,17-dione) and 5α-androstane-3,6,17-trione (no. 78).

3α-Hydroxy-5α-androstan-17-one (no. 146). (a) *Incubation*: 1 g in Me_2CO (150 ml), 25 flasks, medium A, 2 d, extraction III ⟶ 2·0 g total extract. Chromat. alumina (5% deactivated; 100 g). Petrol gave non-steroidal material. EtOAc afforded a mixture (1·23 g), separated by p.l.c. [2 large plates, 2 × EtOAc] to give, in order of decreasing R_F, s.m. (70 mg); 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) (227 mg), m.p. (from Me_2CO) and mixed [14] m.p. 191—193°; *3α,7β-dihydroxy-5α-androstan-17-one* (no. 241) * (238 mg), m.p. 201—202° (from Me_2CO–hexane), $[\alpha]_D$ +129° (*c* 0·6) (Found: C, 74·8; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1735 cm^{-1}; and *3α,6α-dihydroxy-5α-androstan-17-one* (no. 240) * (51 mg), m.p. 222—224° (from Me_2CO), $[\alpha]_D$ +110° (*c* 0·3) (Found: C, 74·75; H, 9·75. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3600 and 1740 cm^{-1}.

(b) *Transformations*: Oxidation of 3α,7β-dihydroxy-5α-androstan-17-one (no. 241) (50 mg) with 8N-H_2CrO_4 gave 5α-androstane-3,7,17-trione (no. 84) (43 mg), m.p. and mixed m.p. 238—240° (lit.,[18] 236—238°); 3α,6α-dihydroxy-5α-androstan-17-one (no. 240) (25 mg) gave 5α-androstane-3,6,17-trione (no. 78) (18 mg), m.p. and mixed m.p. 195—197°.

3β-Hydroxy-5α-androstan-17-one (no. 151). (a) *Incubation*: 2·0 g in EtOH (100 ml), 50 flasks, medium B, 2 d, extraction II ⟶ mycelial extract (no s.m., discarded) and broth extract, chromat. Al_2O_3 (2% deactivated, 100 g). Petrol–$CHCl_3$ (1 : 1) eluted *3β-hydroxy-5α-androstane-7,17-dione* (no. 558) (10 mg), m.p. 202—204° (from Me_2CO), $[\alpha]_D$ −2° (*c* 0·4) (Found: C, 75·3; H, 9·15. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3610, 1740, and 1710 cm^{-1}. $CHCl_3$ eluted 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (1·0 g), m.p. and mixed [1] m.p. 240—242°, and then *3β,7α-dihydroxy-5α-androstan-17-one* (no. 249) * (310 mg), m.p. 194—195° (from Me_2CO–hexane), $[\alpha]_D$ +58° (*c* 0·6) (Found: C, 74·4; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3625 and 1743 cm^{-1}. $CHCl_3$–MeOH (9 : 1) eluted 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (250 mg), m.p. and mixed m.p. 222—224°.

(b) *Transformations*: Oxidation of 3β,7α-dihydroxy-5α-androstan-17-one (no. 249) with 8N-H_2CrO_4 gave 5α-androstane-3,7,17-trione (no. 84), m.p. and mixed m.p. 238—240°. Acetylation of 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) gave *3β,6α-diacetoxy-5α-androstan-17-one* (no. 576), m.p. 158—160° (from Me_2CO–hexane), $[\alpha]_D$ +95·5° (*c* 0·9) (Found: C, 70·75; H, 8·8. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1745 cm^{-1}.

17β-Hydroxy-5α-androstan-3-one (no. 411). (a) *Incubation*: 2·0 g in EtOH (100 ml), 50 flasks, medium B, 2 d, extraction II ⟶ mycelial and broth extracts. P.l.c. of the mycelial extract gave s.m. (164 mg). The broth extract, in $CHCl_3$–MeOH (9 : 1), was filtered through Al_2O_3 (10% deactivated; 20 g) and separated by p.l.c. [5 large plates, 1 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] to give, in order of decreasing R_F, 5,17β-*dihydroxy-5α-androstan-3-one* (no. 585) (111 mg), m.p. 237—239° (from Me_2CO), $[\alpha]_D$ +21° (*c* 1·0) (Found: C, 74·2; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; 6α,17β-*dihydroxy-5α-androstan-3-one* (no. 590) (587 mg), m.p. 207—209° (from Me_2CO–hexane), $[\alpha]_D$ +42° (*c* 1·0) (Found: C, 74·3; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3600 and 1707 cm^{-1}; 11α,17β-dihydroxy-5α-androstan-3-one (no. 296) * (667 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 204—205°; *5α-androstane-3β,7β,17β-triol* (no.

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

617) (131 mg), m.p. 218—220° (from Me_2CO–hexane), $[\alpha]_D$ +41° (*c* 1·0) (Found: C, 74·2; H, 10·45. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ ($CHCl_3$) 3600 cm^{-1}; *5α-androstane-3β,6α,17β-triol* (no. 611) (90 mg), m.p. 238—240° (from Me_2CO), $[\alpha]_D$ (EtOH) +28° (*c* 0·3) (Found: C, 73·7; H, 10·6. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ ($CHCl_3$) 3600 cm^{-1}; and 5α-androstane-3β,11α,17β-triol (no. 523) (60 mg), m.p. (from Me_2CO) and mixed [10] m.p. 245—248°.

(b) *Transformations*: A solution of 5,17β-dihydroxy-5α-androstan-3-one (no. 585) (15 mg) in MeOH (1 ml) was stirred with 2N-NaOH (0·1 ml) at 20 °C for 5 h. Work-up gave 17β-hydroxyandrost-4-en-3-one (no. 182) (8 mg), m.p. (from Me_2CO–hexane) and mixed m.p. 153—156°. On oxidation with 8N-H_2CrO_4, 6α,17β-dihydroxy-5α-androstan-3-one (no. 590) and 5α-androstane-3β,6α,17β-triol (no. 611) gave 5α-androstane-3,6,17-trione (no. 78); and 5α-androstane-3β,7β,17β-triol (no. 617) gave 5α-androstane-3,7,17-trione (no. 84). Acetylation of the 3β,7β,17β-triol (no. 617) gave 3β,7β,17β-triacetoxy-5α-androstane (no. 618), m.p. 174—175° (from hexane), $[\alpha]_D$ +34° (*c* 0·7) (lit.,[12] m.p. 172—173°, $[\alpha]_D$ +39°); the 3β,6α,17β-triol (no. 611) gave 3β,6α,17β-triacetoxy-5α-androstane (no. 612) as an oil, *m/e* 434 (M^+), $\nu_{max.}$ 1735 cm^{-1}; and the 3β,11α,17β-triol (no. 523) gave 3β,11α,17β-*triacetoxy-5α-androstane* (no. 619), m.p. 138—140° (from hexane), $[\alpha]_D$ −39° (*c* 0·9) (Found: C, 69·2; H, 9·0. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%), $\nu_{max.}$ 1735 cm^{-1}. A solution of 6α,17β-dihydroxy-5α-androstan-3-one (no. 590) (40 mg) in THF (5 ml) was refluxed with $LiAlH_4$ (10 mg) for 30 min. Work-up gave 5α-androstane-3β,6α,17β-triol (no. 611) (30 mg), m.p. and mixed m.p. 238—240°.

17β-*Hydroxyestr-4-en-3-one* (no. 187). (a) *Incubation*: 3·98 g in Me_2SO (588 ml), 100 flasks, medium B, 6 d, extraction III ⟶ total extract, chromat. Al_2O_3 (10% deactivated; 250 g). Petrol–$CHCl_3$ (1 : 1) gave s.m. (1·4 g), and then a mixture (100 mg) which was discarded. $CHCl_3$–MeOH (19 : 1) gave a mixture which was acetylated and separated by p.l.c. [5 large plates, 2 × petrol–Me_2CO (5 : 1)]. The material with highest R_F was separated by further p.l.c. [1 large plate, 5 × petrol–Me_2CO (5 : 1)] to give 16β,17β-diacetoxyestr-4-en-3-one (no. 600) [19a] (122 mg), m.p. 182—184°, $\nu_{max.}$ 1745 and 1678 cm^{-1}, and 10β-hydroxy-3-oxoestr-4-en-17β-yl acetate (no. 597) (333 mg), m.p. 178—179·5° (from Me_2CO–hexane), $[\alpha]_D$ +48° (*c* 1·0) (lit.,[20] m.p. 184—185°). The other two bands from the original p.l.c. afforded 6β,17β-diacetoxyestr-4-en-3-one (no. 598) (medium R_F) (635 mg), m.p. 134·5—135·5° (from Me_2CO–hexane), $[\alpha]_D$ −31° (*c* 0·9) (lit.,[19b] m.p. 132—133°, $[\alpha]_D$ −34°), and 11α,17β-diacetoxyestr-4-en-3-one (no. 599) (lowest R_F) (332 mg), m.p. 189—190·5° (from Me_2CO–hexane), $[\alpha]_D$ −42° (*c* 1·0) (lit.,[19c] m.p. 189—192°, $[\alpha]_D$ −39°).

6α-*Hydroxy-5α-androstan-17-one* (no. 552).[9] (a) *Incubation*: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. Mycelial extract chromat. Al_2O_3 (5% deactivated; 40 g). Petrol–$CHCl_3$ (2 : 1) eluted s.m. (157 mg). Broth extract chromat. Al_2O_3 (5% deactivated; 70 g). $CHCl_3$–petrol (3 : 1) eluted 6α,11α-dihydroxy-5α-androstan-17-one (no. 529) (517 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 183—185°. $CHCl_3$–MeOH (97 : 3) eluted 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (86 mg), m.p. and mixed m.p. 223—226°.

17β-*Hydroxy-5α-androstan-6-one* (no. 184).[9] (a) *Incubation*: 1 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial extract (very little s.m., discarded) and broth extract (1·3 g), which was separated by p.l.c. [4 large plates, 4 × CH_2Cl_2–Me_2CO–EtOH (16 : 4 : 1)] to give, in order of decreasing R_F, an unidentified dihydroxy-ketone (55 mg), m.p. 187—191° (decomp.) (from Me_2CO–hexane), $[\alpha]_D$ +25° (*c* 0·1) (Found: C, 74·2; H, 9·85. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3600 and 1709 cm^{-1}; *3α,17β-dihydroxy-5α-androstan-6-one* (no. 572) (220 mg), m.p. 223—224° (from Me_2CO–hexane), $[\alpha]_D$ −13·5° (*c* 0·3) (Found: C, 74·7; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}; *3β,17β-dihydroxy-5α-androstan-6-one* (no. 582) (243 mg), m.p. 217—218° (from Me_2CO), $[\alpha]_D$ −25° (*c* 0·6) (Found: C, 74·1; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1708 cm^{-1}; *11α,17β-dihydroxy-5α-androstan-6-one* (no. 595) (118 mg), m.p. 179—181° (from Me_2CO), $[\alpha]_D$ (EtOH) −42° (*c* 0·7) (Found: C, 74·5; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1706 cm^{-1}; a mixture (255 mg), see later; and *3α,11α,17β-trihydroxy-5α-androstan-6-one* (no. 621) (77 mg), m.p. 228—231° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) −33° (*c* 0·4) (Found: C, 70·5; H, 9·5. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%). Further p.l.c. of the mixture (255 mg) gave an unidentified trihydroxy-ketone (105 mg), m.p. 229—234° (from Me_2CO–MeOH), $[\alpha]_D$ (EtOH) −17° (*c* 0·8) (Found: C, 70·9; H, 9·6. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%), and 5,11α,17β-trihydroxy-5α-androstan-6-one (no. 628) (58 mg) as a gel, *m/e* 322 (M^+, $C_{19}H_{30}O_4$).

(b) *Transformations*: Oxidation of 3α- and 3β,17β-dihydroxy-5α-androstan-6-one (nos. 572 and 582) with 8N-H_2CrO_4 gave 5α-androstane-3,6,17-trione (no. 78); 11α,17β-dihydroxy-5α-androstan-6-one (no. 595) gave 5α-androstane-6,11,17-trione (no. 96), m.p. (from Me_2CO–hexane) and mixed [2] m.p. 215—218°; and 5,11α,17β-trihydroxy-5α-androstan-6-one (no. 628) (12 mg) gave *5-hydroxy-5α-androstane-6,11,17-trione* (no. 566) (11 mg), m.p. 281—282° (decomp.) (from Me_2CO–hexane), $[\alpha]_D$ +74·5° (*c* 0·1), *m/e* 318·1831 ($C_{19}H_{26}O_4$ requires M^+, 318·1831), $\nu_{max.}$ (high resolution) 3603, 1750, and 1722 cm^{-1}, whose structure was confirmed by spectrometric comparison with the model 5-hydroxy-6-ketones described later. Oxidation of 3α,11α-17β-trihydroxy-5α-androstane-6-one (no. 621) (25 mg) gave *5α-androstane-3,6,11,17-tetraone* (no. 545) (18 mg), m.p. 248—249° (from Me_2CO–hexane), $[\alpha]_D$ +134° (*c* 0·2) (Found: C, 71·85; H, 7·6. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%), $\nu_{max.}$ ($CHCl_3$) 1745 and 1715 cm^{-1}. Acetylation of the trihydroxy-ketone (no. 621) gave 3α,11α,17β-*triacetoxy-5α-androstan-6-one* (no. 622), m.p. 195—202° (from Me_2CO–hexane), $[\alpha]_D$ −62° (*c* 0·4) (Found: C, 67·1; H, 8·0. $C_{25}H_{36}O_7$ requires C, 66·9; H, 8·1%), $\nu_{max.}$ 1730 cm^{-1}; the trihydroxy-ketone (no. 628) gave 11α,17β-*diacetoxy-5-hydroxy-5α-androstan-6-one* (no. 629), m.p. 265—266° (from Me_2CO), $[\alpha]_D$ −114° (*c* 0·2) (Found: C, 68·15; H, 8·3. $C_{23}H_{34}O_6$ requires C, 67·95; H, 8·4%), $\nu_{max.}$ (high resolution) 3604, 1735, and 1720 cm^{-1}.

5-Hydroxy-5α-androstan-6-one (no. 549) *and 5-Hydroxy-5β-androstan-6-one* (no. 550).—Solutions of *m*-chloroperoxybenzoic acid (2 g) in $CHCl_3$ (30 ml) and of androst-5-ene (2 g) in $CHCl_3$ (10 ml) were mixed and stirred at 20 °C for 5 h. Work-up gave material (2·2 g) which was dissolved in dioxan (60 ml) and stirred with 2N-H_2SO_4 (10 ml) at 20 °C

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for 20 h. After work-up and chromatography on Al_2O_3 (2% deactivated; 200 g) the material (1·65 g, presumed to be 5α-androstane-5,6β-diol) eluted with Et_2O–petrol (3 : 1) was oxidised with 8N-H_2CrO_4 to give *5-hydroxy-5α-androstan-6-one* (no. 549) (1·4 g), m.p. 183—184° (from Me_2CO), $[\alpha]_D$ −85° (*c* 1·0) (Found: C, 78·85; H, 10·3. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ (high resolution) 3605 and 1719 cm^{-1}. A solution of this hydroxy-ketone (310 mg) and KOH (8 g) in MeOH (80 ml) was boiled under reflux for 9 h to give *5-hydroxy-5β-androstan-6-one* (no. 550), m.p. 115—116° (from MeOH) (252 mg), $[\alpha]_D$ −52° (*c* 1·0) (Found: C, 78·7; H, 10·3%), $\nu_{max.}$ 3480 and 1705 cm^{-1}.

5α-Androstane-7,17-dione (no. 51).[8] (a) *Incubation*: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial extract (containing no s.m.) and broth extract, chromat. Al_2O_3 (5% deactivated; 70 g). Petrol–$CHCl_3$ and $CHCl_3$–MeOH eluted material separated by p.l.c. [1 × petrol–Me_2CO (1 : 1)] to give, in order of decreasing R_F, 3β-hydroxy-5α-androstane-7,17-dione (no. 558) (76 mg), m.p. and mixed m.p. 202—204°; 3α-hydroxy-5α-androstane-7,17-dione (no. 556) (393 mg), m.p. 222—223·5° (from Me_2CO–hexane), $[\alpha]_D$ −6·5° (*c* 1·0) (lit.,[20] m.p. 220—221°, $[\alpha]_D$ −10·5°); 3β,17β-dihydroxy-5α-androstan-7-one (no. 266) * (43 mg), m.p. 200—202° (from Me_2CO–hexane), $[\alpha]_D$ −58° (*c* 0·2) (lit.,[12] m.p. 198·5—199·5°, $[\alpha]_D$ −53°); and 3α,17β-dihydroxy-5α-androstan-7-one (no. 245) * (66 mg), m.p. 198—199° (from Me_2CO–hexane), $[\alpha]_D$ −51·5° (*c* 0·5) (lit.,[21] m.p. 190—192°, $[\alpha]_D$ −80°). $CHCl_3$–MeOH (9 : 1) eluted material which was acetylated to give *3α,11α-diacetoxy-5α-androstane-7,17-dione* (no. 602) (70 mg), m.p. 148·5—150° (from Et_2O–hexane), $[\alpha]_D$ −19° (*c* 0·6) (Found: C, 68·5; H, 8·0. $C_{23}H_{32}O_6$ requires C, 68·3; H, 8·0%), $\nu_{max.}$ 1735 cm^{-1}.

(b) *Transformations*: Oxidation of 3α- and 3β-hydroxy-5α-androstane-7,17-diones (nos. 556 and 558) with 8N-H_2CrO_4 gave 5α-androstane-3,7,17-trione (no. 84). Acetylation of 3α,17β-dihydroxy-5α-androstan-7-one (no. 245) gave *3α,17β-diacetoxy-5α-androstan-7-one* (no. 573), m.p. 186—188° (from MeOH), $[\alpha]_D$ −36·5° (*c* 0·8) (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}.

17β-Hydroxy-5α-androstan-7-one (no. 388).[8] (a) *Incubation*: 600 mg in EtOH (30 ml), 15 flasks, medium B, 4 d, extraction II ⟶ combined extract, chromat. Al_2O_3 (5% deactivated; 70 g). $CHCl_3$ eluted 3β,17β-dihydroxy-5α-androstan-7-one (no. 266) (78 mg), m.p. and mixed m.p. 198—201°, and a mixture, which was separated by p.l.c. [1 large plate, 2 × EtOAc] into *4α,17β-dihydroxy-5α-androstan-7-one* (no. 583) (lower R_F) (22 mg), m.p. 207—208° (from Me_2CO–hexane), $[\alpha]_D$ −60° (*c* 0·1) (Found: C, 74·7; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3620 and 1710 cm^{-1}, and 3α,17β-dihydroxy-5α-androstan-7-one (no. 245) (higher R_F) (326 mg), m.p. and mixed m.p. 197—199°.

(b) *Transformation*: Oxidation of 4α,17β-dihydroxy-5α-androstan-7-one (no. 583) with 8N-H_2CrO_4 gave *5α-androstane-4,7,17-trione* (no. 542), m.p. 210—212° (from Me_2CO–hexane), $[\alpha]_D$ +20° (*c* 0·2), *m/e* 302·1926 ($C_{19}H_{26}O_3$ requires M^+, 302·1882), $\nu_{max.}$ 1748 and 1720 cm^{-1}.

7α-Hydroxy-5α-androstan-17-one (no. 554).[22] (a) *Incubation*: 80 mg in EtOH (4 ml), 2 flasks, medium B, 4 d, extraction II ⟶ 110 mg combined extracts. P.l.c. [2 small plates, 1 × EtOAc] gave s.m. (8 mg) and 3β,7α-dihydroxy-5α-androstan-17-one (no. 249) (57 mg), m.p. and mixed m.p. 193—195°.

7β-Hydroxy-5α-androstan-17-one (no. 369).[22] (a) *Incubation*: 93 mg in EtOH (6 ml), 3 flasks, medium B, 4 d, extraction II ⟶ 125 mg combined extracts. P.l.c. [2 small plates, 1 × EtOAc] gave s.m. (23 mg) and 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (42 mg), m.p. and mixed m.p. 242—244°.

5α-Androstane-11,16-dione (no. 53).[8] (a) *Incubation*: 160 mg in EtOH (8 ml), 4 flasks, medium B, 2 d, extraction III ⟶ total extract. Chromat. Al_2O_3 (5% deactivated; 6 g). Petrol–Et_2O (4 : 1) eluted s.m. (36 mg). Petrol–Et_2O (1 : 4) eluted material (95 mg) shown by n.m.r. to be a 3 : 2 mixture of 3α-hydroxy-5α-androstane-11,16-dione and 3β-hydroxy-5α-androstane-11,16-dione (no. 559). Fractional crystallisation afforded 3β-hydroxy-5α-androstan-11,16-dione (no. 559), m.p. and mixed m.p. 232—234°; the 3α-hydroxy-isomer was not isolated.

Incubation of the diketone (no. 53) (488 mg) for 6 days gave s.m. (113 mg), a mixture of 3α- and 3β-hydroxy-5α-androstane-11,16-diones (64 mg), and 3β,7β-dihydroxy-5α-androstane-11,16-dione (no. 601) (101 mg), m.p. and mixed m.p. 203—205°.

(b) *Transformation*: Oxidation of the mixture (50 mg) of 3α- and 3β-hydroxy-11,16-diones with 8N-H_2CrO_4 gave 5α-androstane-3,11,16-trione (no. 85) (44 mg).

5α-Androstane-11,17-dione (no. 54).[10] (a) *Incubation*: 1·4 g in EtOH (70 ml), 35 flasks, medium B, 4 d, extraction II ⟶ 3·8 g mycelial extract and 2·4 g broth extract. Chromat. of the mycelial extract on Al_2O_3 (5% deactivated; 40 g) and elution with petrol–Et_2O (4 : 1) gave s.m. (295 mg). The broth extract, in Et_2O–MeOH (9 : 1), was filtered through Al_2O_3 (5% deactivated; 50 g). P.l.c. [2 large plates, 1 × petrol–Me_2CO (1 : 1)] gave, in order of decreasing R_F, 3α-hydroxy-5α-androstane-11,17-dione (no. 557) (168 mg), m.p. 150—152° (from Me_2CO–hexane), $[\alpha]_D$ +129° (*c* 0·5) (lit.,[23] m.p. 150—151°, $[\alpha]_D$ +124°); *4α,17β-dihydroxy-5α-androstan-11-one* (no. 584) (180 mg), m.p. 212—214° (from Me_2CO–hexane), $[\alpha]_D$ +67° (*c* 0·9) (Found: C, 74·3; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1705 cm^{-1}; and *3α,17β-dihydroxy-5α-androstan-11-one* (no. 574) (417 mg), m.p. 233—234° (from Me_2CO–hexane), $[\alpha]_D$ +55° (*c* 0·8) (Found: C, 74·6; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1705 cm^{-1}.

(b) *Transformations*: On oxidation with 8N-H_2CrO_4, 3α-hydroxy-5α-androstane-11,17-dione (no. 557) and 3α,17β-dihydroxy-5α-androstan-11-one (no. 574) gave 5α-androstane-3,11,17-trione (no. 358), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 176—178°; 4α,17β-dihydroxy-5α-androstan-11-one (no. 584) gave *5α-androstane-4,11,17-trione* (no. 543), m.p. 220—224° (from Me_2CO–hexane), $[\alpha]_D$ +133° (*c* 0·7) (Found: C, 75·3; H, 8·5. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1740 and 1710 cm^{-1}.

17β-Hydroxy-5α-androstan-11-one (no 555).[24] (a) *Incubation*: 400 mg in EtOH (20 ml), 10 flasks, medium B, 4 d, extraction I ⟶ mycelial and broth extracts. Chromat. of the mycelial extract on Al_2O_3 (5% deactivated; 40 g) and elution with petrol–Et_2O (4 : 1) gave s.m. (98 mg). Broth extract chromat. Al_2O_3 (5% deactivated; 50 g). Petrol–Et_2O (4 : 1) gave s.m. (5 mg). Et_2O–MeOH (99 : 1)

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eluted material which, on purification by p.l.c. [1 small plate, 2 × petrol–Me_2CO (1 : 1)], gave 4α,17β-dihydroxy-5α-androstan-11-one (no. 584) (80 mg), m.p. and mixed m.p. 212—214°. Et_2O–MeOH (48 : 1) eluted 3α,17β-dihydroxy-5α-androstan-11-one (no. 574) (150 mg), m.p. and mixed m.p. 232—234°.

We thank Mr. J. W. Keeping for assistance in the microbiological work. We also thank the S.R.C. for a studentship (to V. E. M. C.), a fellowship (to J. M. E.), and a Research Grant, I.C.I. Ltd., for a Postdoctoral Fellowship (to W. A. D.), The Salters' Company for a Scholarship (to J. H. P.), the Czechoslovak Academy of Science whose Agreement on Co-operation with the Royal Society allowed A. K. to participate, and Glaxo Laboratories Ltd. for a grant and gifts of chemicals.

[3/290 *Received*, 8*th February*, 1973]