Microbiological Hydroxylation of Steroids. Part IX.[1] Hydroxylation of Diketones and Keto-alcohols Derived from 5α-Androstane with the Fungi *Rhizopus arrhizus* and *Rhizopus circinnans*. Steroidal 18- and 19-Proton Magnetic Resonance Signals[2]

By Alan M. Bell, Ian M. Clark, William A. Denny, Sir Ewart R. H. Jones, G. Denis Meakins,* Wilhelm E. Müller, and (Mrs.) Eva E. Richards, Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY

The hydroxylations of seven diketones and keto-alcohols derived from 5α-androstane with *Rhizopus arrhizus* and *Rhizopus circinnans* are similar to, but not identical with, those observed previously using *Rhizopus nigricans*. *R. circinnans* is useful for introducing a 4α-hydroxy-group into 5α-androstane-11,17-dione.

N.m.r. shift values are given for the influence on steroidal 18- and 19-H signals of various single substituents, and of systems containing two or more substituents whose effects are not additive.

PREVIOUSLY we investigated the hydroxylation of a range of dioxygenated 5α-androstanes with *Calonectria decora*,[3] *Aspergillus ochraceus*,[4] and *Rhizopus nigricans*.[1] These fungi, of different genera, lead to characteristic patterns of hydroxylation which differ fundamentally from each other. We have now examined other fungi, from the same genus as one of those already studied, using a selection of the substrates employed earlier. The literature [5] suggests that such related fungi should show similar, but not identical, behaviour in steroid hydroxylation. However, as already emphasised,[6] the uniformity of the substrates employed (in particular, the concentration on 3-oxo-Δ^4-compounds) may well have masked significant differences between certain fungi. The species selected, for comparison with *Rhizopus nigricans* (*Rn*), were *Rhizopus arrhizus* (*Ra*) and *Rhizopus circinnans* (*Rc*). Of these, the former is known [5] to cause monohydroxylation (at the 11α- and, much less frequently, at the 6β- and 7β-positions) and 7β,11α-dihydroxylation of certain 5α-steroids (androstane and pregnane derivatives), and to hydroxylate a few 5β-substrates (cardenolides) at the 1β- and 5β-positions; *Rhizopus circinnans* does not appear to have been used previously for steroid hydroxylation.

Table 1 summarises the results. [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.[6] Compounds nos. 634—650 (whose n.m.r. signals are listed in Table 2) are described here.]

Comparison of the present hydroxylations with those reported earlier [1] shows that there is a greater similarity between *Rn* and *Ra* than between either of these and *Rc*: the extent to which *Rc* differs from the other two varies with the nature of the substrate, as illustrated in the Scheme. The model proposed to explain the *Rn* results [1] (the presence of three dual-purpose binding and hydroxylating sites on the enzyme surface) works well with *Ra*; the *Rc* hydroxylations are more complicated, the difference arising from the greater tendency of this fungus to substitute the middle rings of the steroid nucleus. In cases where *Rn* and *Ra* show a clear preference for hydroxylating a particular carbon atom in a middle ring *Rc* behaves similarly [substrates (I) and (II)], and where the first two attack two middle ring positions *Rc* differs only slightly [substrate (III)]. However with androstanes which are hydroxylated at the same terminal positions by *Rn* and *Ra* the differences between these fungi and *Rc* become more marked [substrates (IV), (V), (VI), and (less clearly) (VII)]. With so little detailed knowledge of the hydroxylation processes, it does not seem profitable to speculate about the reasons for these differences.

An advantage of *Rc* over the other two fungi for preparative work lies in its greater ability to introduce a 4-hydroxy-group, as shown by the conversion of 5α-androstane-11,17-dione (VII) into 4α,17β-dihydroxy-5α-androstan-11-one in 44% yield.

Our earlier survey [7] of the effects of substituents on steroidal angular methyl groups' signals was based on the results in the literature and those from the 344 compounds already encountered in the microbiological work. The subsequent study of a further 306 steroids has given shift values (Table 3) for systems not covered by the previous compilation. As before,[7] the material is divided into four sections corresponding to the different configurations at positions 5 and 14. Within a section the order is determined by the steroidal number of the substituent, and, for compounds with further groups, by the number of the second substituent. In ordering

[1] Part VIII, V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. Pragnell, *J.C.S. Perkin I*, 1973, 1500.

[2] Details of the experimental work (which is recorded only briefly in the Experimental section), and the considerations leading to the shift values in Table 3 are given by I. M. Clark, D.Phil. Thesis, Oxford, 1972.

[3] 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.

[4] 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.

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

[6] 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.

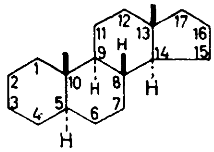
[7] J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc.* (*C*), 1970, 250.

substituents with the same number, priority is given in the sequence CO > acetal > thioacetal > OH > OAc > OMe > epoxy (denoted by, *e.g.* 2β,3β-O) > olefin, for epimeric pairs α > β, and a single substituent is listed before systems containing that substituent and an extra substituent [*e.g.* 12β-OH > 12β-OH–17-CO > 12β,17β-(OH)$_2$].

Most of the entries refer to two substituents whose combined effect is appreciably different from that predicted on the basis of independent action by the separate groups, *i.e.* cases where the individual shifts are not additive. Although interaction would be expected in many of the systems, there are several instances, *e.g.* 12α,17β-(OH)$_2$, in which it would have appeared reasonable to apply normal shift values. A few of the earlier figures[7] have been revised. The largest change is for the 15-CO in 5α,14β-steroids where the earlier value was deduced incorrectly, as already

TABLE 1

Hydroxylations with *Rhizopus arrhizus* (*Ra*) and *Rhizopus circinnans* (*Rc*)



5α-Androstane

The substrates, all derivatives of 5α-androstane, are indicated by abbreviated names, *e.g.* 3β-OH–17-CO represents 3β-hydroxy-5α-androstan-3-one. In the ' products ' columns those oxygen functions introduced during the incubation are in bold type. The substrates were introduced as solutions in ethanol, and the incubations were carried out for 2 or 4 days (see Experimental section). The yields are calculated after making allowance for recovered starting material.

Substrate	Fungus	Substrate recovered	Main monohydroxylation product(s)		Other products	
3β-OH–17-CO	*Ra*	0	**7**β- OH	57%	**7**- CO	1%
			7α- OH	20	**6**α- OH	8
3β-OH–17-CO	*Rc*	7%	**7**β- OH	43		
			6α- OH	25		
6α-OH–17-CO	*Ra*	4	**11**α- OH	72	**11**α,17β- (OH)$_2$	5
					3β- OH	5
6α-OH–17-CO	*Rc*	37	**11**α- OH	56	**3**β- OH	5
17β-OH–3-CO	*Ra*	21	**11**α- OH	23	3β,**7**β- (OH)$_2$	5
			6α- OH	12	3β,**6**α- (OH)$_2$	4
					3β, **11**α- (OH)$_2$	4
					5α- OH	4
17β-OH–3-CO	*Rc*	19	**6**α- OH	33		
			11α- OH	26		
3α-OH–17-CO	*Ra*	0	**11**α- OH	30	**7**β- OH	8
3,7-(CO)$_2$	*Ra*	3	3β, **16**β-(OH)$_2$	31	**16**α- (OH)	3
			16β- OH	15	3β-OH– **16**- CO	2
			3α, **16**β-(OH)$_2$	10	**17**α- OH	2
					11α,**16**β- OH)$_2$	3
3,7-(CO)$_2$	*Rc*	7	3β, **11**α- (OH)$_2$	22	3β, **16**β- (OH)$_2$	6
			16β- OH	10	**11**α,**16**β- (OH)$_2$	5
					11α- OH	4
					17α- OH	4
					16α- OH	3
3,11-(CO)$_2$	*Ra*	16	3β, **16**β-(OH)$_2$	22	**16**β- OH	7
			9α,**16**β-(OH)$_2$	23	**9**α,**16**α- (OH)$_2$	7
3,11-(CO)$_2$	*Rc*	17	**6**α- OH	20	3β,7β- (OH)$_2$	3
			16β- OH	15	**9**α,**16**β- (OH)$_2$	2
			7β- OH	10		
7,17-(CO)$_2$	*Ra*	11	**3**α- OH	31	**3**β- OH	6
					3β, **17**β- (OH)$_2$	6
					3α, **17**β- (OH)$_2$	2
					3α, **11**α- (OH)$_2$ *	6
					3β, **11**α- (OH)$_2$ *	2
					11α- OH	3
					1α- OH	4
7,17-(CO)$_2$	*Rc*	38	**11**α- OH	18	**11**α,**17**β- (OH)$_2$	8
			3β- OH	15	**4**α- OH	6
					3β, **17**β- (OH)$_2$	5
					3α- OH	5
					3β, **11**α- (OH)$_2$ *	3
					2β- OH	2
11,17-(CO)$_2$	*Ra*	11	**4**α, 17β-(OH)$_2$	27	**7**β, **17**β- (OH)$_2$	8
			3α, 17β-(OH)$_2$	20		
11,17-(CO)$_2$	*Rc*	19	**4**α, 17β-(OH)$_2$	54		
			7β, 17β-(OH)$_2$	18		

* Isolated as the corresponding diacetate.

explained.[8] Values given under 5α,14α-steroids for substituents in ring A and at positions 6 and 11 may be used with reasonable confidence for the same substituents in 5α,14β-steroids; similarly those for substituents in rings C and D and at position 7 can be applied to 5β,14α-steroids.

EXPERIMENTAL

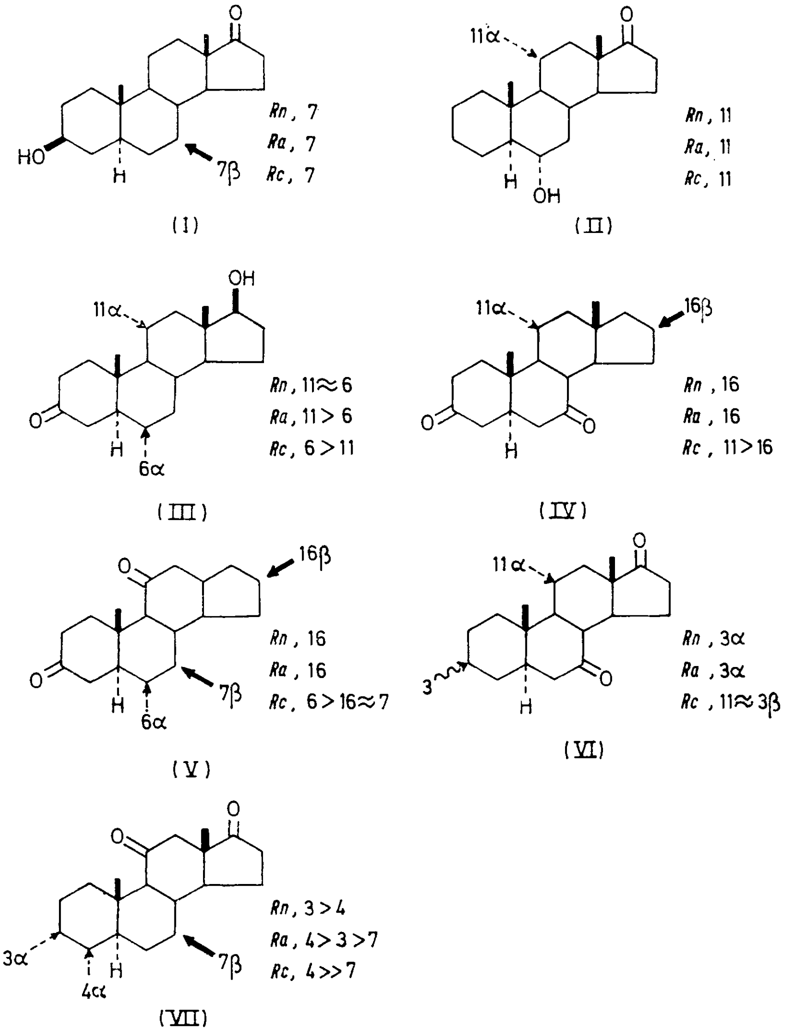
For general directions see ref. 6. Where compounds with serial numbers below 634 are stated to have been identified by mixed m.p., the original preparations are contained in, or can be found from, the papers cited. The microbiological procedures and the abbreviations used in reporting the results are given fully in ref. 9. Components of mixtures isolated by p.l.c. are reported in order of decreasing R_F value. Petrol refers to light petroleum, b.p. 60—80°, *Ra* to *Rhizopus arrhizus*, and *Rc* to *Rhizopus circinnans*.

3β-*Hydroxy*-5α-*androstan*-17-*one* (no. 151). (a) *Incubation with* Ra: 2·0 g in EtOH (100 ml), 50 flasks, medium B, 2 d, extraction II ⟶ 3·8 g combined extracts. Chromat. Al_2O_3 (2% deactivated; 150 g). Et_2O eluted a mixture which was separated by p.l.c. [2 small plates, 1 × petrol–Me_2CO (4 : 1)] to give 3β-hydroxy-5α-androstane-7,17-dione (no. 558) (20 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 200—203°, and 11α-hydroxy-5α-androstane-3,17-dione (no. 519) (10 mg), m.p. (from Me_2CO–hexane) and mixed [4] m.p. 193—195°. Et_2O–MeOH (49 : 1) eluted 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (1·2 g), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 240—241°, and 3β,7α-dihydroxy-5α-androstan-17-one (no. 249) (420 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 194—195°. Et_2O–MeOH (9 : 1) eluted 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (170 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 222—223°.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks,

SCHEME

Comparison of main monohydroxylation products obtained with *Rhizopus* species *Rn*(*nigricans*), *Ra*(*arrhizus*), and *Rc*(*circinnans*)



[8] 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.

[9] 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.

[10] 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*, 1973, 1493.

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.

No.	Compound		τ_2	τ_2(calc.)*	$>CH$–OR		
634	1α-Hydroxy-5α-androstane-7,17-dione †	19	8·94	8·91	H-1	6·23	m(7)
		18	9·14	9·13			
635	2β-Hydroxy-5α-androstane-7,17-dione	19	8·67	8·66	H-2	5·82	m(10)
		18	9·13	9·12			
636	3β-Hydroxy-5α-androstane-7,16-dione	19	8·88	8·87	H-3	6·36	m(22)
		18	9·13	9·12			
637	4α-Hydroxy-5α-androstane-7,17-dione †	19	8·91	8·90	H-4	6·38	6(11,11,5)
		18	9·13	9·13			
638	6α-Hydroxy-5α-androstane-3,11-dione	19	8·77	8·73	H-6	6·52	m(22)
		18	9·31	9·30			
639	7β-Hydroxy-5α-androstane-3,11-dione	19	8·74	8·73	H-7	6·46	m(20)
		18	9·28	9·27			
640	11α-Hydroxy-5α-androstane-7,17-dione	19	8·80	8·80	H-11	5·86	6(10,10,5)
		18	9·14	9·10			
641	16α-Hydroxy-5α-androstane-3,7-dione	19	8·73	8·71	H-16	5·56	m(18)
		18	9·27	9·25			
642	17α-Hydroxy-5α-androstane-3,7-dione	19	8·72	8·72	H-17	6·28	m(10)
		18	9·33	9·33			
643	9α-Hydroxy-5α-androstane-3,11,16-trione	19	8·69	8·70			
		18	9·12	9·12			
644	3β,7β-Dihydroxy-5α-androstan-11-one †	19	8·94	8·94	H-3 / H-7	6·52	m(20)
		18	9·30	9·30			
645	7β,17β-Dihydroxy-5α-androstan-11-one	19	8·95	8·97	H-7	6·48	m(20)
		18	9·29	9·28	H-17	6·17	t(8)
646	11α,17β-Dihydroxy-5α-androstan-7-one	19	8·81	8·82	H-11	5·90	6(11,11,5)
		18	9·27	9·24	H-17	6·30	m(15)
647	3β,11α-Diacetoxy-5α-androstane-7,17-dione †	19	8·77	8·79	H-3	5·34	7(10,10,5,5)
		18	9·08	9·04	H-11	4·72	6(10,10,6)
648	9α,16α-Dihydroxy-5α-androstane-3,11-dione	19	8·74	8·75	H-16	5·45	m(18)
		18	9·28	9·28			
649	9α,16β-Dihydroxy-5α-androstane-3,11-dione	19	8·72	8·72	H-16	5·49	m(17)
		18	9·04	9·04			
650	11α,16β-Dihydroxy-5α-androstane-3,7-dione	19	8·60	8·56	H-11	5·86	6(10,10,5)
		18	9·01	8·98	H-16	5·48	m(15)

* Calculated using the shift values in ref. 6 and Table 3 of this paper. † Not fully characterised; no analytical figures available. The proposed structures are based on the results of spectrometric examination, and of conversions [by oxidation, or, in the case of the diacetate (no. 647), by hydrolysis and oxidation] into known ketones.

[a] Ref. 6.

medium B, 2 d, extraction II ⟶ 1·45 g combined extracts. P.l.c. [3 large plates, 1 × petrol–Me_2CO (3 : 1)] gave s.m. (67 mg), 3β,7β-dihydroxy-5α-androstan-17-one (no. 250) (421 mg), m.p. and mixed m.p. 241—243°, and 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (244 mg), m.p. and mixed m.p. 222—223°.

6α-Hydroxy-5α-androstan-17-one (no. 552).[11] (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract yielded s.m. (41 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 50 g). Petrol–$CHCl_3$ (1 : 3) eluted 6α,11α-dihydroxy-5α-androstan-17-one (no. 529) (730 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 183—185°. $CHCl_3$–MeOH (95 : 5) gave a mixture, which was purified by p.l.c. [2 small plates, 2 × EtOAc] to give 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (53 mg), m.p. and mixed m.p. 225—228°, and 5α-androstane-6α,11α,17β-triol (no. 462) (50 mg), m.p. (from Me_2CO–hexane) and mixed [6] m.p. 228—230°.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), medium B, 25 flasks, 4 d, extraction II ⟶ mycelial and broth extracts. Purification of the mycelial extract gave s.m. (371 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 50 g). Petrol–$CHCl_3$ (1 : 5) eluted 6α,11α-dihydroxy-5α-androstan-17-one (no. 529) (369 mg), m.p. and mixed m.p. 182—185°. $CHCl_3$–MeOH (19 : 1) eluted material which was purified by p.l.c. [1 small plate, 2 × EtOAc] to give 3β,6α-dihydroxy-5α-androstan-17-one (no. 246) (31 mg), m.p. and mixed m.p. 225—228°.

17β-Hydroxy-5α-androstan-3-one (no. 411). (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract gave s.m. (207 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 60 g). Petrol–$CHCl_3$ (1 : 4) eluted material which was further purified by p.l.c. [1 small plate, 2 × EtOAc] to give 5,17β-dihydroxy-5α-androstan-3-one (no. 585) (32 mg), m.p.

TABLE 3

The effect of substituents ($\Delta\tau_2$, $CDCl_3$) on 19-H and 18-H signals

A positive $\Delta\tau_2$ indicates a shift to higher field. For substituents marked * the present values are preferable to those given earlier.[a] Values marked † are based on one or two examples only.

Substituent 5α,14α-Steroids	19-H	18-H
1,3-$(CO)_2$	−0·46	−0·04
1,6-$(CO)_2$–Δ^2	−0·29	−0·07
1β-OH–3-CO	−0·10 †	+0·01 †
1β-OH–Δ^2*	−0·01	−0·02
1β-OAc–3-CO	−0·33 †	−0·01 †
1α,2α-O–3-CO	−0·06	0·00
1β,11α-O	−0·06	−0·03
Δ^1	−0·08	−0·04
2-CO–3β-OH	+0·01	+0·04
2-CO–3β-OAc	+0·01	0·00
2,7-$(CO)_2$–$\Delta^{3,5}$	−0·50	−0·06
2,2-(-O—, -O—)	−0·12	−0·01
2α-OAc–3-CO	−0·35	−0·03
2β,3β-O	−0·07	+0·01
Δ^2	+0·01	−0·02
Δ^2–3-OAc	−0·03	0·00
Δ^2–3-OMe	+0·04	−0·01
3-CO–4α,5α-O	−0·27	−0·04
3-CO–9α,11α-O	−0·49	−0·06
3-CO–$\Delta^{4,6}$	−0·35	−0·12
3,3-(-S—, -S—)	−0·02	+0·01
3-OMe–$\Delta^{3,5}$	−0·16	−0·03
3α,4α-O	+0·01	+0·04
Δ^3	+0·04	−0·01
$\Delta^{3,5,8}$–7-CO	−0·55 †	0·00 †
Δ^4	−0·21	−0·02
Δ^4–6β-OAc	−0·30	−0·06
5α-OH–6-CO	0·00	0·00
5α,6β-$(OH)_2$	−0·37	−0·02
5α-OH–7-CO	−0·37	0·00
5α,6α-O	−0·12	+0·04
6α-OAc*	−0·06	0·00
7α,9α-$(OH)_2$	−0·10	+0·02
7β-OAc *	−0·05	−0·03
7β-OAc–17-CO	−0·07	−0·15
7,7-(-O—, -O—)	−0·03	−0·01
Δ^7–15-CO	+0·01	−0·21
9α-OH–11-CO	−0·24	+0·02
$\Delta^{9(11)}$	−0·14	+0·04
$\Delta^{9(11)}$–12-CO	−0·27	−0·23
11α,12β-$(OH)_2$	−0·11	−0·03
11α,12β-$(OAc)_2$	−0·12	−0·14
11β-OAc	−0·10	−0·13
11α,12α-O	−0·07	−0·12
12-CO *	−0·09	−0·32
12,17-$(CO)_2$	−0·11	−0·52
12α,17β-$(OH)_2$	+0·03 †	+0·01 †
12β-OH *	−0·02	−0·02
12β-OH–17-CO	−0·03	−0·25
12β,17β-$(OH)_2$	−0·04	−0·13
12α-OAc *	0·00	−0·09
14α-OH	0·00	−0·12
14α,15α-O	−0·03 †	−0·23 †
Δ^{14}	−0·03	−0·32
Δ^{14}–16-CO	−0·13 †	−0·55 †
Δ^{14}–17-CO	−0·06 †	−0·42 †
Δ^{14}–17-OH	−0·05 †	−0·40 †
Δ^{14}–17,17-(-O—, -O—)	−0·03 †	−0·38 †
$\Delta^{14,16}$	−0·11 †	−0·32 †
15-CO–Δ^{16}	−0·07	−0·29
15,15-(-S—, -S—)	−0·05 †	−0·20 †
15β-OH–Δ^{16}	−0·09	−0·40
15β-OAc	−0·06	−0·26
15β-OMe	−0·04 †	−0·22 †
15β-OMe–Δ^{16}	−0·07 †	−0·36 †
15β,16β-O–17-CO	−0·06 †	−0·46 †
Δ^{15}–17,17-(-O—, -O—)	−0·03 †	−0·22 †
Δ^{15}–17β-OH	−0·03 †	−0·12 †
16-CO–17β-OAc	−0·04	−0·13
16,16-(-O—, -O—)	0·00	−0·21
16,16-$(OMe)_2$	−0·01	−0·09
16α-OH–17-CO	−0·03	−0·26
16α,17α-$(OH)_2$	0·00	0·00
16α,17β-$(OH)_2$	−0·01	−0·06
16α-OAc–17-CO	−0·03	−0·26
16α-OAc–17β-OH	−0·03	−0·10
16α,17β-$(OAc)_2$	−0·02	−0·09
16β-OAc–17-CO	−0·04	−0·27
16β-OAc–17α-OH	−0·04	−0·09
16β-OAc–17β-OH	−0·03	−0·12
16α-OEt	0·00	−0·01
16β-OMe	0·00	−0·18
16α,17α-O	0·00	−0·03
16β,17β-O	0·00	−0·12
Δ^{16}	−0·04	−0·09
Δ^{16}–17-Ac	−0·02	−0·18
Δ^{16}–16-Me–17-Ac	−0·03	−0·27
Δ^{16}–17-OAc	−0·03	−0·20
17,17-(-S—, -S—)	−0·04 †	−0·20 †
17α-Ac	−0·01	−0·20
17α-OAc	−0·01	−0·04
5β,14α-Steroids		
2β-OAc	−0·07	0·00
3-CO–4β,5β-O	−0·22	−0·04
3,3-$(OMe)_2$	−0·02	0·00
3α-OH	−0·01	0·00
3β-OH	−0·06	−0·01
3β,4β-O	+0·04	−0·01
Δ^3–5β-OH	−0·06	−0·01
5β-OH	+0·02 †	0·00 †
5β-OH–6-CO	+0·22 †	−0·01 †
5β,6β-O	−0·03	+0·02
6β-OAc	−0·09	−0·05
5α,13α-Steroids		
15-CO	−0·05	−0·06
15α-OH	−0·01	−0·25
15α-OH–Δ^{16}	+0·02	−0·29
15α,16α-O–17-CO	+0·05 †	−0·28 †
Δ^{15}–17-CO	+0·02 †	−0·22 †
16α,17α-O	0·00	−0·26
16β,17β-O	0·00	+0·02
Δ^{16}–17-OAc	+0·02	−0·14
17-CO	+0·06	−0·11
5α,14β-Steroids		
7-CO	−0·19	0·00
7β-OH	−0·03	−0·02
12-CO	−0·09	−0·22
12,15-$(CO)_2$	−0·04	−0·38
12α-OH	−0·01	−0·02
12β-OH	−0·03	+0·02
14β-OH–15-CO	+0·02	−0·06
14β,15α-$(OH)_2$	+0·02	−0·01
14β,15β-$(OH)_2$	−0·05	−0·23
14β,15β-O	−0·03 †	−0·05 †
15-CO *	+0·03	−0·18
15-CO–Δ^{16}	+0·01 †	−0·22 †
15,15-(-S—, -S—)	+0·04 †	−0·13 †
15α-OH	+0·03	−0·03
15β-OH	0·00	−0·04

[a] Ref. 7.

(from Me_2CO–hexane) and mixed [1] m.p. 237—239°. Petrol–$CHCl_3$ (1 : 8) gave 6α,17β-dihydroxy-5α-androstan-3-one (no. 590) (103 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 207—209°. $CHCl_3$ and $CHCl_3$–EtOAc (10 : 1) eluted material which was purified by p.l.c. [3 small plates,

[11] The preparation of this compound will be described in a later paper.

3 × $CHCl_3$–MeOH (10 : 1)] to give 11α,17β-dihydroxy-5α-androstan-3-one (no. 296) (192 mg), m.p. (from Me_2CO–hexane) and mixed [3] m.p. 203—204°, and 5α-androstane-3β,7β,17β-triol (no. 617) (42 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 218—220°. $CHCl_3$–MeOH (4 : 1) gave material which was purified by p.l.c. [1 small plate, 3 × $CHCl_3$–MeOH (10 : 1)] to give 5α-androstane-3β,6α,17β-triol (no. 611) (30 mg), m.p. (from Me_2CO) and mixed [1] m.p. 238—240°, and 5α-androstane-3β,11α,17β-triol (no. 521) (30 mg), m.p. (from Me_2CO) and mixed [4] m.p. 245—248°.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 2 d, extraction II ⟶ 1·2 g combined extracts. P.l.c. [3 large plates, 1 × petrol–Me_2CO (3 : 1)] gave s.m. (193 mg), 6α,17β-dihydroxy-5α-androstan-3-one (no. 590) (279 mg), m.p. and mixed m.p. 206—209°, and 11α,17β-dihydroxy-5α-androstan-3-one (no. 296) (222 mg), m.p. and mixed m.p. 198—201°.

3α-Hydroxy-5α-androstan-17-one (no. 146). *Incubation with* Ra: 800 mg in EtOH (40 ml), 20 flasks, medium B, 2 d, extraction II ⟶ 1·28 g combined extracts. Several crystallisations from Me_2CO gave 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) (190 mg), m.p. (from Me_2CO–hexane) and mixed [9] m.p. 191—193°. P.l.c. [2 large plates, 2 × EtOAc] of the material from the mother liquors yielded more 3α,11α-dihydroxy-5α-androstan-17-one (no. 242) (60 mg), and 3α,7β-dihydroxy-5α-androstan-17-one (no. 241) (71 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 197—200°.

5α-Androstane-3,7-dione (no. 36). (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. Purification of mycelial extract gave s.m. (32 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 70 g) and the various fractions were further purified by p.l.c. (development with Et_2O) to give 16β-hydroxy-5α-androstane-3,7-dione (no. 207) (158 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 185—186°; *17α-hydroxy-5α-androstane-3,7-dione* (no. 642) (25 mg), m.p. 203—205° (from Me_2CO–hexane), $[\alpha]_D$ −59° (*c* 0·5) (Found: C, 75·1; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 1715 cm^{-1}; *3β-hydroxy-5α-androstane-7,16-dione* (no. 636) (25 mg), m.p. 189—191° (from Me_2CO–hexane), $[\alpha]_D$ −239° (*c* 0·5) (Found: C, 75·0; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 1745 and 1712 cm^{-1}; *16α-hydroxy-5α-androstane-3,7-dione* (no. 641) (30 mg), m.p. 172—173° (from Me_2CO–hexane), $[\alpha]_D$ −52° (*c* 0·5) (Found: C, 74·9; H, 9·4. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 1716 cm^{-1}; 3β,16β-dihydroxy-5α-androstan-7-one (no. 263) (315 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 234—236°; 3α,16β-dihydroxy-5α-androstan-7-one (no. 243) (103 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 266—267°; and *11α,16β-dihydroxy-5α-androstane-3,7-dione* (no. 650) (38 mg), m.p. 255—257° (from Me_2CO–hexane), $[\alpha]_D$ −63° (*c* 0·5) (Found: C, 71·35; H, 8·7. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 1713 cm^{-1}.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract gave s.m. (72 mg). The broth extract was filtered through Al_2O_3 (5% deactivated; 20 g) to give two fractions which were purified by p.l.c. The less polar fraction [250 mg, on 1 large plate, 3 × Et_2O] gave 11α-hydroxy-5α-androstane-3,7-dione (no. 203) (42 mg), m.p. (from Me_2CO–hexane) and mixed [4] m.p. 185—186°; 16β-hydroxy-5α-androstane-3,7-dione (no. 207) (95 mg), m.p. and mixed m.p. 185—186°; 17α-hydroxy-5α-androstane-3,7-dione (no. 642) (43 mg), m.p. and mixed m.p. 203—205°; and 16α-hydroxy-5α-androstane-3,7-dione (no. 641) (27 mg), m.p. and mixed m.p. 172—173°. The more polar fraction [420 mg, on 1 large plate, 4 × petrol–Me_2CO (2 : 1)] gave 3β,11α-dihydroxy-5α-androstan-7-one (no. 254) (211 mg), m.p. (from Me_2CO–hexane) and mixed [4] m.p. 205—208°; 3β,16β-dihydroxy-5α-androstan-7-one (no. 263) (60 mg), m.p. and mixed m.p. 234—236°; and 11α,16β-dihydroxy-5α-androstane-3,7-dione (no. 650) (54 mg), m.p. and mixed m.p. 255—257°.

(c) *Transformations:* Oxidation of 16α-hydroxy-5α-androstane-3,7-dione (no. 641) and of 3β-hydroxy-5α-androstane-7,16-dione (no. 636) with 8N-H_2CrO_4 gave 5α-androstane-3,7,16-trione (no. 82), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 240—242°. Oxidation of 17α-hydroxy-5α-androstane-3,7-dione (no. 642) and of 11α,16β-dihydroxy-5α-androstane-3,7-dione (no. 650) gave, respectively, 5α-androstane-3,7,17-trione (no. 84), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 239—241°, and 5α-androstane-3,7,11,16-tetraone (no. 546), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 260—262°.

5α-Androstane-3,11-dione (no. 37). (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. Purification of the mycelial extract gave s.m. (161 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 60 g). Elution with petrol–$CHCl_3$ (1 : 1) gave 16β-hydroxy-5α-androstane-3,11-dione (no. 564) (65 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 174—176·5°. $CHCl_3$ eluted 3β,16β-dihydroxy-5α-androstan-11-one (no. 264) (180 mg), m.p. (from Me_2CO–hexane) and mixed [10] m.p. 232—234°. $CHCl_3$–MeOH (9 : 1) eluted a mixture which was separated by p.l.c. [1 large plate, 2 × EtOAc] into *9α,16β-dihydroxy-5α-androstane-3,11-dione* (no. 649) (218 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%), $\nu_{max.}$ 1710 cm^{-1}, and *9α,16α-dihydroxy-5α-androstane-3,11-dione* (no. 648) (62 mg), m.p. 247—248·5° (from Me_2CO–hexane), $[\alpha]_D$ +96° (*c* 0·5) (Found: C, 71·1; H, 8·9. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 1710 cm^{-1}.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract gave s.m. (173 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 50 g). Petrol–$CHCl_3$ (1 : 2) eluted material which was separated by p.l.c. [1 large plate, 1 × EtOAc] into *7β-hydroxy-5α-androstane-3,11-dione* (no. 639) (82 mg), m.p. 207—210° (from Me_2CO–hexane), $[\alpha]_D$ +82° (*c* 0·9) (Found: C, 74·8; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3610 and 1712 cm^{-1}, and 16β-hydroxy-5α-androstane-3,11-dione (no. 564) (126 mg), m.p. and mixed m.p. 173—176°. Petrol–$CHCl_3$ (1 : 3) eluted *6α-hydroxy-5α-androstane-3,11-dione* (no. 638) (177 mg), m.p. 168—170° (from Me_2CO–hexane), $[\alpha]_D$ +86° (*c* 0·8) (Found: C, 75·2; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3620 and 1710 cm^{-1}. $CHCl_3$ and $CHCl_3$–MeOH (99 : 1) gave a mixture which was separated by p.l.c. [2 small plates, 1 × EtOAc, followed by 1 small plate, 5 × Et_2O] into 9α,16β-dihydroxy-5α-androstane-3,11-dione (no. 649) (15 mg), m.p. and mixed m.p. 219—221° and material [22 mg, $\nu_{max.}$ ($CHCl_3$) 3565 and 1703 cm^{-1}] presumed to be 3β,7β-dihydroxy-5α-androstan-11-one (no. 644).

(c) *Transformations*: On oxidation with 8N-H_2CrO_4, 9α,-16β-dihydroxy-5α-androstane-3,11-dione (no. 649) and the 16α-epimer (no. 648) gave 9α-*hydroxy*-5α-*androstane*-3,11,16-*trione* (no. 643), 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%), $\nu_{max.}$ 3600, 1746, and 1712 cm^{-1}; 7β-hydroxy-5α-androstane-3,11-dione (no. 639) and 3β,7β-dihydroxy-5α-androstan-11-one (no. 644) gave 5α-androstane-3,7,11-trione (no. 80), m.p. (from Me_2CO–hexane) and mixed [4] m.p. 176—177°; and 6α-hydroxy-5α-androstane-3,11-dione (no. 638) gave 5α-androstane-3,6,11-trione (no. 72), m.p. (from Me_2CO–hexane) and mixed [3] m.p. 191—192°.

5α-*Androstane*-7,17-*dione* (no. 51). (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract gave s.m. (110 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 60 g). Petrol–$CHCl_3$ (1 : 2) eluted a mixture (70 mg), indicated by n.m.r. examination to consist of 1α-hydroxy-5α-androstane-7,17-dione (no. 634) (40 mg) and 11α-hydroxy-5α-androstane-7,17-dione (no. 640) (30 mg). [Repeated p.l.c. failed to separate this mixture; the constants of the 11α-hydroxy-dione (no. 640) are given in the following incubation.] Petrol–$CHCl_3$ (1 : 3) eluted a mixture which, on crystallisation from Me_2CO–hexane, gave 3α-hydroxy-5α-androstane-7,17-dione (no. 556) (290 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 220—223°. The material from the mother liquors was purified by p.l.c. [3 × Et_2O] to give 3β-hydroxy-5α-androstane-7,17-dione (no. 558) (54 mg), m.p. and mixed m.p. 200—202°. $CHCl_3$ eluted 3β,17β-dihydroxy-5α-androstan-7-one (no. 266) (62 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 195—198°. $CHCl_3$–EtOAc (1 : 1) eluted material which was purified by p.l.c. [1 small plate, 2 × petrol–Me_2CO (1 : 1)] to give 3α,17β-dihydroxy-5α-androstan-7-one (no. 245) (20 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 195—198°. EtOAc–MeOH (9 : 1) eluted polar material which was separated by p.l.c. [1 small plate, 4 × petrol–Me_2CO (1 : 1)] into two fractions. Acetylation of one fraction gave material (18 mg; $\nu_{max.}$ 1742, 1735, and 1712 cm^{-1}) formulated as 3β,11α-diacetoxy-5α-androstane-7,17-dione (no. 647); acetylation of the other gave 3α,11α-diacetoxy-5α-androstane-7,17-dione (no. 602) (70 mg), m.p. (from Et_2O-hexane) and mixed [1] m.p. 148—150°.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract gave s.m. (380 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 60 g). Petrol–$CHCl_3$ (1 : 2) eluted a mixture which was separated by p.l.c. [2 small plates, 1 × petrol–Me_2CO (1 : 1)] into 11α-*hydroxy*-5α-*androstane*-7,17-*dione* (no. 640) (118 mg), m.p. 168—170° (from Me_2CO–hexane), $[\alpha]_D$ −26° (*c* 0·7) (Found: C, 74·7; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3605, 1742, and 1711 cm^{-1}, and 2β-*hydroxy*-5α-*androstane*-7,17-*dione* (no. 635) (14 mg), m.p. 204—206° (from Me_2CO–hexane), $[\alpha]_D$ +4° (*c* 0·5) (Found: C, 75·1; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3620, 1742, and 1713 cm^{-1}. Petrol–$CHCl_3$ (1 : 3) eluted material which was purified by p.l.c. [2 small plates, 3 × Et_2O] to give 3β-hydroxy-5α-androstane-7,17-dione (no. 558) (96 mg), m.p. and mixed m.p. 201—203°, and a mixture (72 mg) indicated by n.m.r. examination to consist of 4α-hydroxy-5α-androstane-7,17-dione (no. 637) (40 mg) and 3α-hydroxy-5α-androstane-7,17-dione (no. 556) (32 mg). (Repeated p.l.c. failed to separate this mixture.) EtOAc and EtOAc–MeOH (9 : 1) eluted material which was separated by p.l.c. [1 small plate, 3 × petrol–Me_2CO (1 : 1)] into 11α,17β-*dihydroxy*-5α-*androstan*-7-*one* (no. 646) (54 mg), m.p. 196—198° (from EtOAc), $[\alpha]_D$ −88° (*c* 0·42) (Found: C, 74·2; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1710 cm^{-1}; 3β,17β-dihydroxy-5α-androstan-7-one (no. 266) (30 mg), m.p. and mixed m.p. 199—202°; and a fraction which, after acetylation, afforded material (25 mg) identical with that (see earlier) formulated as 3β,11α-diacetoxy-5α-androstane-7,17-dione (no. 647).

(c) *Transformations:* The mixture of 1α- and 11α-hydroxy-5α-androstane-7,17-diones (nos. 634 and 640) was oxidised with 8N-H_2CrO_4. P.l.c. [1 × EtOAc] of the product gave 5α-androstane-7,11,17-trione (no. 97), m.p. (from Me_2CO–hexane) and mixed [4] m.p. 171—173°, and 5α-androstane-1,7,17-trione (no. 67), m.p. (from Me_2CO–hexane) and mixed [3] m.p. 235—237°. Similarly, the mixture of 3α- and 4α-hydroxy-5α-androstane-7,17-diones (nos. 556 and 637) afforded 5α-androstane-3,7,17-trione (no. 84), m.p. and mixed m.p. 241—243°, and 5α-androstane-4,7,17-trione (no. 542), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 210—212°. Oxidation of both 11α-hydroxy-5α-androstane-7,17-dione (no. 640) and 11α,17β-dihydroxy-5α-androstan-7-one (no. 646) gave 5α-androstane-7,11,17-trione (no. 97), m.p. and mixed m.p. 171—173°.

A solution of 3β,11α-diacetoxy-5α-androstane-7,17-dione (no. 647) (25 mg) in MeOH (10 ml)–2N-NaOH (1 ml) was kept at 40 °C for 20 h. The product was oxidised with 8N-H_2CrO_4 to give 5α-androstane-3,7,11,17-tetraone (no. 547), m.p. (from Me_2CO-hexane) and mixed [1] m.p. 242—246°.

5α-*Androstane*-11,17-*dione* (no. 54). (a) *Incubation with* Ra: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. Purification of the mycelial extract gave s.m. (108 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 60 g). Petrol–$CHCl_3$ (1 : 4 and 1 : 8) eluted material which was purified by p.l.c. [1 large plate, 2 × EtOAc] to give 4α,17β-dihydroxy-5α-androstan-11-one (no. 584) (253 mg), m.p. (from Me_2CO) and mixed [1] m.p. 212—214°, and 7β,17β-*dihydroxy*-5α-*androstan*-11-*one* (no. 645) (73 mg), m.p. 210—212° (from Me_2CO–hexane), $[\alpha]_D$ +85° (*c* 0·8) (Found: C, 74·2; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3550 and 1705 cm^{-1}. $CHCl_3$ eluted 3α,17β-dihydroxy-5α-androstan-11-one (no. 574) (189 mg), m.p. (from Me_2CO–hexane) and mixed [1] m.p. 232—234°.

(b) *Incubation with* Rc: 1·0 g in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ mycelial and broth extracts. The mycelial extract yielded s.m. (191 mg). The broth extract was chromatographed on Al_2O_3 (5% deactivated; 50 g). Petrol–$CHCl_3$ (1 : 2) gave 4α,17β-dihydroxy-5α-androstan-11-one (no. 584) (455 mg), m.p. and mixed m.p. 210—214°. Petrol–$CHCl_3$ (1 : 3) eluted material which was further purified by p.l.c. [2 small plates, 3 × petrol–Me_2CO (2 : 1)] to give 7β,17β-dihydroxy-5α-androstan-11-one (no. 645) (151 mg), m.p. and mixed m.p. 210—212°.

We thank the Rhodes Trust for a scholarship (to I. M. C.), I.C.I. Ltd. for a Postdoctoral Fellowship (to W. A. D.), and Glaxo Laboratories Ltd. for grants and gifts of chemicals.

[3/601 *Received*, 22*nd March*, 1973]