Microbiological Hydroxylation. Part XX.[1] Hydroxylation of Dioxygenated 5α-Androstanes with the Fungi *Absidia regnieri* and *Syncephelastrum racemosum*

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Dioxygenated androstanes are readily hydroxylated by the title fungi. Although complex mixtures are generally formed, 3β-hydroxy-5α-androstan-7-one is converted efficiently into its 12α-hydroxy-derivative (51% yield) by *S. racemosum*. The poor steroid recoveries of incubations involving 3,17-dioxygenated substrates and *A. regnieri* are improved by using a medium containing cobalt(II) sulphate: under such conditions 3β-hydroxy-5α-androstan-17-one gives 3β,9α-dihydroxy-5α-androstan-17-one in 49% yield.

ONLY progesterone and its derivatives appear to have been used as substrates in previous work[2] on steroid hydroxylation with the fungi *Absidia regnieri* and *Syncephelastrum racemosum*. Progesterone was transformed into a range of products by both fungi, *viz.* 14α- and 15β-mono-, 6β,14α- and 7β,14α-di-, and 7β,-14α,15β-tri-hydroxylated derivatives with *A. regnieri*,[3] and 15β-mono- and 6β,11α-, 6β,15β-, 7β,14α-, and 7β,15β-di-hydroxylated derivatives with *S. racemosum*.[4] Incubations[5,6] of 17α-hydroxyprogesterone and cortexolone

[1] Part XIX, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, J. Pragnell, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 1552.

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

[3] M. Shirasaka, M. Ozaki, and S. Sugawara, *J. Gen. Appl. Microbiol.* (*Tokyo*), 1961, **7**, 341.

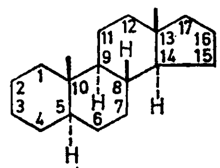
[4] Y. Sato, T. Tanaka, and K. Tsuda, *Chem. and Pharm. Bull.* (*Japan*), 1963, **11**, 1579; K. Tsuda, T. Asai, Y. Sato, T. Tanaka, and M. Kato, *J. Gen. Appl. Microbiol.* (*Tokyo*), 1959, **5**, 1.

[5] M. Shirasaka, *Chem. and Pharm. Bull* (*Japan*), 1961, **9**, 59.

[6] K. Tsuda, T. Asai, E. Ohki, A. Tanaka, and M. Hattori, *Chem. and Pharm. Bull.* (*Japan*), 1958, **6**, 387.

TABLE 1

Hydroxylations with *Absidia regnieri* (Ar) and *Syncephelastrum racemosum* (Sr)



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-17-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 incubated for the times (usually 4 days) specified in the Experimental section. The yields are calculated after making allowance for recovered starting material.

Substrate	Fungus	Substrate recovered	Main hydroxylation product(s)		Other products	%
3-CO	*Ar*	55%	**9α**, **15β**-(OH)$_2$	8%	**9α**-OH	4
3,7-(CO)$_2$	*Ar*	0	3β, **11α**-(OH)$_2$	6	Δ[1]-**17**-CO	3
					11α-OH	1
	Sr	3	3β, **12α**-(OH)$_2$	13	3β, **12β**-(OH)$_2$	6
					7α,**9α**-(OH)$_2$	5
					1β-OH	3
					11α-OH	2
					12α-OH	2
					7α,**9α**,**11α**-(OH)$_3$	2
9α-OH–3-CO	*Ar*	82	None isolated			
	Sr	10	3β, **12α**-(OH)$_2$	12		
3,11-(CO)$_2$	*Ar*	0	**Δ^{1,4}**-**17**β-OH	6	**Δ^{1,4}**-**16**β-OH	4
					Δ^{1,4}-**17**-CO	2
					16-CO	2
	Sr		**Δ^{1,4}**-**16**β-OH	18	**Δ^{1,4}**-**17**β-OH	9
					Δ^{1,4}-**17**-CO	9
11**α**-OH–3-CO	*Ar*	49	None isolated			
	Sr	25	3β, **7β**-(OH)$_2$	27	**7β**-OH	17
					3β-OH	4
12α-OH–C-CO	*Ar*	53			3β-OH	10
	Sr	65			3β-OH	6
3,17-(CO)$_2$	*Ar*	0	**9α**-OH	14	**7α**-OH	10
			7α,**9α**-(OH)$_2$	13	3β, **9α**-(OH)$_2$	5
	Ar *	0	**Δ^{1,4}**-**11α**-OH	16	3β, **11α**-(OH)$_2$	11
			11α-OH	14	**Δ^4**-**11α**-OH	9
					9α-OH	8
					3β, **9α**-(OH)$_2$	7
	Sr	0	3β, **7α**-(OH)$_2$	7	3β-OH	7
					3β, **7α**, 17β-(OH)$_3$	3
					3β, **9α**-(OH)$_2$	2

TABLE 1 (*Continued*)

Substrate	Fungus	Substrate recovered	Main hydroxylation product(s)		Other products	%
17β-OH-3-CO	*Ar*	5	9α-OH	14	7α,9α-$(OH)_2$-17-CO	7
					9α,14α-$(OH)_2$	2
	Ar *	13	9α-OH	30	3β,9α-$(OH)_2$	8
					9α-OH-17-CO	2
	Sr	0	3β,7α-$(OH)_2$	27	7β-OH	9
					9α-OH	7
					3β,7α-$(OH)_2$-17-CO	4
17β-OH-6-CO	*Sr*	19	11α-OH-17-CO	23		
3β-OH-7-CO	*Ar*	5	9α-OH	23	14α-OH	9
					11α-OH	5
	Sr	0	12α-OH	51	9α-OH	9
					12β-OH	8
3β-OAc-7-CO-Δ^5	*Ar*	0	3β,9α-$(OH)_2$	21		
			3β,14α-$(OH)_2$	19		
	Sr	0	3β,12α-$(OH)_2$	14		
			3β,12β-$(OH)_2$	14		
7,17-$(CO)_2$	*Ar*	33	5α-OH	6		
	Sr	4	1α,17β-$(OH)_2$	23	11α,17β-$(OH)_2$	11
					12β,17β-$(OH)_2$	7
					5α,17β-$(OH)_2$	4
					11α-OH	3
17β-OH-7-CO	*Ar*	18	3β-OH	24	3β-OH-17-CO	6
	Sr	0	3β,12β-$(OH)_2$	23	3β-OH	17
					12β-OH	11
					1β-OH	7
11,17-$(CO)_2$	*Ar*	34			17β-OH	28
	Sr	37	7α,17β-$(OH)_2$	27	5α,17β-$(OH)_2$	9
					7α-OH	5
17β-OH-11-CO	*Ar*	63			17-CO	25
	Sr	22			17-CO	15
3β-OH-17-CO	*Ar*	4	9α-OH	19	7β-OH	12
					7α,14α-$(OH)_2$	4
					9α,14α-$(OH)_2$	2
	Ar *	0	9α-OH	49	7β-OH	9
	Sr	0	7β-OH	27	7-CO	10
			7β,17β-$(OH)_2$	24		4
3β-OH-17,17$\left.{}^{-O}_{-O}\right]$	*Ar*	0	9α-OH-17-CO	14	7α,9α-$(OH)_2$-17-CO	4
					12,14α-$(OH)_2$-17-CO	4
3β-OH-17-CO-Δ^5	*Ar*	0	7α,11α-$(OH)_2$	39		
			7α-OH	21		
	Sr	0	7β-OH	17	7α-OH	11
					7-CO	5
3β-OAc-7,17-$(CO)_2$-Δ^5	*Ar*	16	3β,9α-$(OH)_2$	37	3β,14α-$(OH)_2$	10
	Sr	0	Not investigated			

* Incubation carried out in the presence of cobalt(II) sulphate.

(17α,21-dihydroxypregn-4-ene-3,20-dione) also gave mixtures, but with these substrates 11α-hydroxylation was the main microbiological process. In continuing our work, which is concerned mainly with the hydroxylation of androstane derivatives, both fungi were screened for activity towards mono- and di-oxygenated androstanes. Although hydroxylation of the mono-oxygenated substrates was inconveniently slow, the dioxygenated androstanes were metabolised readily, and a selection of them was used in the detailed study reported here.

A summary of the microbiological results is given in Table 1. The n.m.r. spectra of the steroids, substrates and products, involved here for which spectroscopic data have not appeared in earlier publications are listed in Table 2: the arabic serial number sequence of steroids dicussed earlier [7] is used in this Table, which contains steroids nos. **874—917**. A combination of spectrometric and chemical methods was used in establishing the structures of new compounds; their n.m.r. signals appear in Table 2 and the other information required for their characterisation is given in Table 3. Since the work involves only routine procedures fully described in earlier Parts the experimental details have beendeposited as Supplementary Publication No. SUP 21462 (14 pp., 1 microfiche).†

In general *A. regnieri* and *S. racemosum* give complex mixtures and are therefore less useful than the fungi investigated previously [8] for preparing oxygenated androstanes of the less common types. [The one notable exception is the efficient 12α-hydroxylation (51% yield) of 3β-hydroxy-5α-androstan-7-one with *S. racemosum.*] However, although most of the products were obtained in rather low yield they include compounds, *e.g.* 1α,17β-dihydroxy-5α-androstan-7-one (22%), which would be difficult to obtain by purely chemical methods. Both micro-organisms appear to be short-range hydroxylators, but it is difficult to discern any clear relationship between the positions of the substrates' oxygenated groups and the patterns of hydroxylation.

† For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1974, Index issue.

[7] 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.

[8] A. M. Bell, V. E. M. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, W. E. Müller, and J. Pragnell, *J.C.S. Perkin I*, 1974, 312.

TABLE 2
N.m.r. signals

The results, presented in the used earlier,[a] were obtained by examining solutions in $CDCl_3$ at 100 MHz

No.	Compound		τ_2	τ_2(calc.)	>CH–OR and other signals		
874	Androsta-3,5,8(9)-triene-7,17-dione	19	8.64	8.65	H-3, H-4 }	3.78	m (5)
		18	9.14	9.14	H-6	4.02	s
875	5α-Androst-1-ene-3,7,17-trione	19	8.70	8.69	H-1	2.89	d (10)
		18	9.09	9.02	H-2	4.05	d (10)
876	5α-Androstane-3,7,12,17-tetraone	19	8.62	8.59			
		18	8.76	8.74			
877	12β-Hydroxy-5α-androstan-3-one	19	8.98	8.96	H-12	6.62	*4* (11, 5)
		18	9.27	9.25			
878	1β-Hydroxy-5α-androstane-3,7-dione	19	8.68	8.65	H-1	6.17	*4* (10, 6)
		18	9.28	9.26			
879	5-Hydroxy-5α-androstane-7,17-dione	19	8.83	8.83			
		18	9.14	9.14			
880	9α-Hydroxy-5α-androstane-3,7-dione	19	8.60	8.57			
		18	9.26	9.23			
881	9α-Hydroxy-5α-androstane-3,15-dione	19	8.86	8.84			
		18	9.15	9.16			
882	11α-Hydroxy-5α-androstane-6,17-dione	19	9.13	9.13	H-11	5.95	*7* (10, 10, 5, 5)
		18	9.10	9.09			
883	12α-Hydroxy-5α-androstane-3,7-dione	19	8.72	8.69	H-12	6.15	m (5)
		18	9.24	9.21			
884	14α-Hydroxy-5α-androstane-3,7-dione	19	8.72	8.70			
		18	9.12	9.14			
885	1α,17β-Dihydroxy-5α-androstan-7-one	19	8.91	8.93	H-1	6.60	m (18)
		18	9.28	9.27	H-17	6.37	t (8)
886	1β,17β-Dihydroxy-5α-androstan-7-one	19	8.92	8.89	H-1	6.36	*4* (12, 5)
		18	9.27	9.27	H-17	6.36	*t* (8)
887	3β,7α-Diacetoxy-5α-androstan-17-one	19	9.15	9.13	H-3	5.26	*7* (10, 10, 5, 5)
		18	9.12	9.10	H-7	4.93	m (7)
388	3β,7α-Dihydroxyandrost-5-en-17-one	19	8.97	8.94	H-3	6.41	m (23)
					H-6	4.34	d (s)
		18	9.11	9.10	H-7	6.01	m (11)
889	3β,7β-Dihydroxyandrost-5-en-17-one	19	8.92	8.95	H-3	6.40	m (24)
					H-6	4.65	s
		18	9.10	9.12	H-7	6.02	m (16)
890	3β,9α-Dihydroxy-5α-androstan-7-one	19	8.80	8.78	H-3	6.38	m (25)
		18	9.29	9.26			
891	9α-Hydroxy-7-oxo-5α-androstan-3β-yl acetate	19	8.77	8.78	H-3	5.52	*7* (10, 10, 5, 5)
		18	9.28	9.25			
892	3β,9α-Dihydroxyandrost-5-en-7-one	19	8.66	8.65	H-3	6.34	m (18)
		18	9.28	9.28	H-6	4.25	s
893	9α-Hydroxy-17-oxo-5α-androstan-3β-yl acetate	19	9.02	9.02	H-3	5.32	m (22)
		18	9.12	9.09			
894	3β,12α-Dihydroxy-5α-androstan-7-one	19	8.93	8.92	H-3	6.41	m (23)
		18	9.26	9.24	H-12	6.22	m (7)
895	3β,12α-Dihydroxyandrost-5-en-7-one	19	8.80	8.77	H-3	6.34	m (17)
					H-6	4.28	s
		18	9.24	9.26	H-12	6.16	m (6)
896	3β,14α-Dihydroxy-5α-androstan-7-one	19	8.92	8.91	H-3	6.39	*7* (10, 10, 5, 5)
		18	9.20	9.17			
897	3β,14α-Dihydroxyandrost-5-en-7-one	19	8.78	8.78	H-3	6.33	m (17)
		18	9.17	9.19	H-6	4.27	s
898	5,17β-Dihydroxy-5α-androstan-7-one	19	8.85	8.85	H-17	6.33	t (7)
		18	9.27	9.29			
899	5,17β-Dihydroxy-5α-androstan-11-one	19	8.82	8.82	H-17	6.15	t (8)
		18	9.31	9.31			
900	7α,9α-Dihydroxy-5α-androstan-3-one	19	8.91	8.88	H-7	5.89	m (7)
		18	9.29	9.29			
901	7β,11α-Dihydroxy-5α-androstan-3-one	19	8.82	8.83	H-7	6.56	*7* (10, 10, 5, 5)
		18	9.21	9.21	H-11	5.98	*7* (10, 10, 5, 5)
902	7β,17β-Diacetoxy-5α-androstan-3-one	19	8.93	8.91	H-7, H-17 }	5.4	m (24)
		18	9.15	9.16			
903	9α,15β-Dihydroxy-5α-androstan-3-one	19	8.83	8.83	H-15	5.73	t (7)
		18	8.95	8.96			
904	12β,17β-Dihydroxy-5α-androstan-7-one	19	8.93	8.90	H-12	6.57	*4* (10, 5)
		18	9.20	9.17	H-17	6.12	t (7)
905	3β,9α-Dihydroxyandrost-5-ene-7,17-dione	19	8.63	8.63	H-3	6.31	m (20)
		18	9.11	9.11	H-6	4.28	s
906	3β,14α-Dihydroxyandrost-5-ene-7,17-dione	19	8.76	8.76	H-3	6.30	m (17)
		18	9.01	9.00	H-6	4.29	s
907	7α,9α-Dihydroxy-5α-androstane-3,17-dione	19	8.86	8.86	H-7	5.96	m (6)
		18	9.11	9.12			
908	5α-Androstane-3β,7β,11α-triol	19	9.04	9.02	H-3	6.5	m (22)
					H-7	6.35	m (20)
		18	9.24	9.26	H-11	6.06	m (20)
909	5α-Androstane-3β,9α,12α-triol	19	9.08	9.05	H-3	6.34	m (25)
		18	9.24	9.22	H-12	6.08	m (6)
910	7α,9α-Dihydroxy-17-oxo-5α-androstan-3β-yl acetate	19	9.05	9.05	H-3	5.32	*7* (10, 10, 5, 5)
		18	9.15	9.14	H-7	6.05	m (8)
911	3β,7α,11α-Trihydroxyandrost-5-en-17-one *	19	8.94		H-3	6.56	m (20)
					H-6	4.47	d (6)
		18	9.20		H-7	6.20	m (12)
					H-11	6.08	m (24)
912	3β,7α-Diacetoxy-14α-hydroxy-5α-androstan-17-one	19	9.16	9.13	H-3	5.08	*7* (11, 11, 5, 5)
		18	8.97	8.98	H-7	4.84	m (7)
913	9α,14α-Dihydroxy-17-oxo-5α-androstan-3β-yl-acetate	19	9.03	9.02	H-3	5.34	*7* (10, 10, 5, 5)
		18	9.00	8.97			
914	3β,12β,14α-Trihydroxy-5α-androstan-17-one	19	9.13	9.16	H-3	6.44	*7* (11, 11, 5, 5)
		18	8.96	8.93	H-12	5.92	*4* (11, 6)
915	3β,12β,17β-Trihydroxy-5α-androstan-7-one	19	8.90	8.89	H-3	6.40	m (25)
					H-12	6.66	*4* (11, 5)
		18	9.22	9.24	H-17	6.13	t (8)
916	7α,9α,11α-Trihydroxy-5α-androstan-3-one *	19	8.96		H-7	5.88	m (8)
		18	9.04		H-11	5.13	m (17)
917	9α,14α-Dihydroxy-3-oxo-5α-androstan-17β-yl acetate	19	8.86	8.83	H-17	4.73	m (11)
		18	9.06	9.04			

[a] Ref. 7. * Solution in $(CD_3)_2SO$.

TABLE 3
Characterisation of new compounds

Compound	M.p. (°C) *	[α]D (°) † (c)	Analytical figures (%)	C	H
Androsta-3,5,8(9)-triene-7,17-dione	193—195	−190	Found	80.5	7.9
		(0.2)	$C_{19}H_{22}O_2$ req.	80.8	7.85
5α-Androst-1-ene-3,7,17-trione	223—224	+56	Found	75.9	8.1
		(0.2)	$C_{19}H_{24}O_3$ req.	76.0	8.05
5α-Androstane-3,7,12,17-tetraone	282—285	+133	Found	72.3	7.7
		(0.1)	$C_{19}H_{24}O_4$ req.	72.1	7.65
1β-Hydroxy-5α-androstane-3,7-dione	181—182	−30	Found	75.2	8.9
		(0.25)	$C_{19}H_{28}O_3$ req.	75.0	9.3
5-Hydroxy-5α-androstane-7,17-dione	221—222	+42	Found	75.0	9.2
		(0.25)	$C_{19}H_{28}O_3$ req.	75.0	9.3
9α-Hydroxy-5α-androstane-3,7-dione	263—265	−68	Found	75.3	9.3
		(0.2)	$C_{19}H_{28}O_3$ req.	75.0	9.3
9α-Hydroxy-5α-androstane-3,15-dione	181—183	+36	Found	74.9	9.2
		(1.0)	$C_{19}H_{28}O_3$ req.	75.0	9.3
11α-Hydroxy-5α-androstane-6,17-dione	180—182	+45	Found	74.8	9.3
		(0.7)	$C_{19}H_{28}O_3$ req.	75.0	9.3
12α-Hydroxy-5α-androstane-3,7-dione	240—243	−19	Found	74.7	9.2
		(0.35)	$C_{19}H_{28}O_3$ req.	75.0	9.3
14α-Hydroxy-5α-androstane-3,7-dione	180—182	+29	Found	74.8	9.0
		(0.2)	$C_{19}H_{28}O_3$ req.	75.0	9.3
1α,17β-Dihydroxy-5α-androstan-7-one	197—199	−74	Found	74.6	9.7
		(1.0)	$C_{19}H_{30}O_3$ req.	74.5	9.9
1β,17β-Dihydroxy-5α-androstan-7-one	200—202	−82	Found	74.5	9.9
		(0.1)	$C_{19}H_{30}O_3$ req.	74-5	9.9
3β,7α-Diacetoxy-5α-androstan-17-one	183—185	+33	Found	70.65	8.9
		(0.5)	$C_{23}H_{34}O_5$ req.	70.75	8.8
3β,9α-Dihydroxy-5α-androstan-7-one	226—227	−80	Found	74.7	9.7
		(0.85)	$C_{19}H_{30}O_3$ req.	74.5	9.9
9α-Hydroxy-7-oxo-5α-androstan-3β-yl acetate	169—170	−75	Found	72.3	9.1
		(0.3)	$C_{21}H_{32}I_4$ req.	72.4	9.25
3β,9α-Dihydroxyandrost-5-en-7-one	201—203	−115	Found	74.6	9.3
		(1.05)	$C_{19}H_{28}O_3$ req.	75.0	9.3
9α-Hydroxy-17-oxo-5α-androstan-3β-yl acetate	177—179	+53	Found	72.4	9.1
		(0.5)	$C_{21}H_{32}O_4$ req.	72.4	9.25
3β,12α-Dihydroxy-5α-androstan-7-one	230—232	−32	Found	74.2	9.7
		(1.0)	$C_{19}H_{30}O_3$ req.	74.5	9.9
3β,12α-Dihydroxyandrost-5-en-7-one	206—209	−107	Found	74.9	9.15
		(0.2)	$C_{19}H_{28}O_3$ req.	75.0	9.3
3β,14α-Dihydroxy-5α-androstan-7-one	204—205	+32	Found	74.65	9.8
		(0.1)	$C_{19}H_{30}O_3$ req.	74.5	9.9
3β,14α-Dihydroxyandrost-5-en-7-one	218—219	−108	Found	74.7	9.2
		(0.7)	$C_{19}H_{28}O_3$ req.	75.0	9.3
5,17β-Dihydroxy-5α-androstan-7-one	155—156	−33	Found	74.6	10.0
		(0.25)	$C_{19}H_{30}O_3$ req.	74.5	9.9
5,17β-Dihydroxy-5α-androstan-11-one	211—213	−7	Found	74.5	9.7
		(0.4)	$C_{19}H_{30}O_3$ req.	74.5	9.9
7α,9α-Dihydroxy-5α-androstan-3-one	243—245	−68	Found	74.5	9.9
		(0.1)	$C_{19}H_{30}O_3$ req.	74.5	9.9
7β,11α-Dihydroxy-5α androstan-3-one	221—223	+16	Found	74.6	9.8
		(0.15)	$C_{19}H_{30}O_3$ req.	74.5	9.9
7β,17β-Diacetoxy-5α-androstan-3-one	180—182	+53	Found	70.95	8.8
		(0.2)	$C_{23}H_{34}O_5$ req.	70.75	8.8
9α,15β-Dihydroxy-5α-androstan-3-one	179—180	−21	Found	74.8	9.8
		(0.6)	$C_{19}H_{30}O_3$ req.	74.5	9.9
12β,17β-Dihydroxy-5α-androstan-7-one	122—123	−67	Found	74.5	10.1
		(0.2)	$C_{19}H_{30}O_3$ req.	74.5	9.9
3β,9α-Dihydroxyandrost-5-ene-7,17-dione	254—256	−71	Found	71.5	8.05
		(0.5)	$C_{19}H_{26}O_4$ req.	71.7	8.2
3β,14α-Dihydroxyandrost-5-ene-7,17-dione	246—249	−69	Found	71.5	8.4
		(0.2)	$C_{19}H_{26}O_4$ req.	71.7	8.2
7α,9α-Dihydroxy-5α-androstane-3,17-dione	267—268	+58	Found	71.2	8.6
		(0.7)	$C_{19}H_{28}O_3$ req.	71.2	8.8
5α-Androstane-3β,7β,11α-triol	186—188 ‡	+9 §	Found	74.1	10.6
		(1.0)	$C_{19}H_{32}O_3$ req.	74.0	10.5
5α-Androstane-3β,9α,12α-triol	283—286 ‡	−20 §	Found	73.9	10.3
		(0.15)	$C_{19}H_{32}O_3$ req.	74.0	10.5
7α,9α-Dihydroxy-17-oxo-5α-androstan-3β-yl acetate	213—215	+31	Found	69.25	8.7
		(0.35)	$C_{21}H_{32}O_5$ req.	69.2	8.85
3β,7α,11α-Trihydroxy-androst-5-ene-17-one	236—238	−58	Found	71.0	8.6
		(1.0)	$C_{19}H_{28}O_4$ req.	71.2	8.8
3β,7α-Diacetoxy-14α-hydroxy-5α-androstan-17-one	226—229	+53	Found	68.2	8.2
		(0.3)	$C_{23}H_{34}O_6$ req.	67.95	8.4
9α,14α-Dihydroxy-17-oxo-5α-androstan-3β-yl acetate	228—230	+25	Found	69.25	8.8
		(0.35)	$C_{21}H_{32}O_5$ req.	69.2	8.85
3β,12β,14α-Trihydroxy-5α-androstan-17-one	108—111	+41	Found	70.4	9.2
		(0.7)	$C_{19}H_{30}O_4$ req.	70.8	9.4
3β,12β,17β-Trihydroxy-5α-androstan-7-one	214—216	−57	Found	70.6	9.8
		(0.25)	$C_{19}H_{30}O_4$ req.	70.8	9.4
7α,9α,11α-Trihydroxy-5α-androstan-3-one	227—230	−70	Found	70.7	9.6
		(0.15)	$C_{19}H_{30}O_4$ req.	70.8	9.4
9α,14α-Dihydroxy-3-oxo-5α-androstan-17β-yl acetate	209—212	+12	Found	69.0	8.7
		(0.5)	$C_{21}H_{32}O_6$ req.	69.2	8.85

* From Me_2CO–hexane unless otherwise indicated † $CHCl_3$ as solvent unless otherwise indicated. ‡ From aqueous MeOH. § MeOH as solvent.

It seemed possible that the poor steroid recovery (substrate plus products) in some of the incubations might stem from initial 9α-hydroxylation, since this process is known [9] to be important in the microbiological

[9] C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *J. Biol. Chem.*, 1966, **241**, 140; D. T. Gibson, K. C. Wang, C. J. Sih, and H. Whitlock, *ibid.*, p. 551; K. Schubert, K. Bohme, and C. Horhold, *Steroids*, 1964, **4**, 581.

degradation of steroids. Several incubations were therefore repeated using a medium containing cobalt(II) sulphate, a reagent which has been reported [10] to inhibit hydroxylation at tertiary positions, but only those involving *A. regnieri* and 3,17-dioxygenated androstanes were appreciably affected. In the three such cases (Table 1) the total recoveries were improved, but other, unexpected, changes also occurred: while 17β-hydroxy-5α-androstan-3-one and the 3β-hydroxy-17-ketone gave much greater amounts of the 9α-hydroxy-derivatives, the opposite behaviour was found with 5α-androstane-3,17-dione, which underwent 11α-hydroxylation and ring A dehydrogenation as the main processes. (Some analogy for the dehydrogenation is found in the conversion of androst-4-ene-3,17-dione into the $\Delta^{1,4}$-diketone by *Mycobacterium smegmatis* in the presence of cobalt sulphate.[10])

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[10] G. Wix, K. G. Buki, E. Tomorkeny, and G. Ambrus, *Steroids*, 1968, **11**, 401.