Microbiological Hydroxylation. Part XXI.† Hydroxylations of 3-Halogeno-17-oxo-, 3-Halogeno-7-oxo-, and 17-Halogeno-3-oxo-androstanes by the Fungi *Calonectria decora*, *Rhizopus nigricans*, and *Aspergillus ochraceus*

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The hydroxylations set out in the title have been studied and the results compared with those (obtained in earlier work) of hydroxylating the parent 3-, 7-, and 17-ketones. The microbiological effect of a halogeno-substituent depends on its nature, position, and configuration in the steroid nucleus. The 3α-fluoro-, 3α-chloro-, and 3α-bromo-5α-androstan-17-ones undergo 1β,6α-dihydroxylation with *C.decora*; while the 3β-chloro- and 3β-bromo-analogues are more reactive (and are also hydroxylated initially in this way), the 3β-fluoro-17-ketone is recovered essentially unchanged. Loss of halogen generally occurs during incubations of 3-halogeno-17-ketones with *R.nigricans*, the products being 3-oxo- and 3β-hydroxy-compounds. *A.ochraceus* converts the fluoro-ketones (but not the chloro-ketones) into their 11α-hydroxy- or 7β,11α-dihydroxy-derivatives; the former process has been utilised in the preparation of 3α- and 3β-fluoro-5α-androstane-11,17-dione.

So far, most of our microbiological work has been concerned with the hydroxylation of hydroxy- and oxo-steroids. The main general conclusions are that, with certain fungi, oxygenated groups of the substrates exert characteristic directing effects which determine the outcome of all the hydroxylation processes; with other fungi, the site-specific type, there is a marked tendency for attack at a certain steroidal position almost irrespective of the substrates' structures. In the present study the plan was to compare some previously investigated hydroxylations of 5α-androstane monoketones with those of related substrates having halogeno-substituents at positions remote from the ketone groups. Using fungi whose modes of hydroxylation are susceptible to structural variations in the substrate, this comparison would reveal any possible directing effects of halogen atoms, since such effects would be in competition with those of the ketone groups; with the site-specific fungi, interest would lie in the possibility of using microbiological methods for preparing poly-oxygenated halogeno-steroids not conveniently accessible by standard chemical routes. In view of the extensive earlier work, *C. decora* [1,2,3] and *R. nigricans* [4,5] (fungi influenced by directing effects) and *A. ochraceus* [6,7] (a site-specific fungus) were deemed the most suitable micro-organisms for the comparisons.

Table 1 shows the fifteen halogenoandrostanones used as substrates, and the results of incubating them with the three fungi. Although routes to most of the substrates, apart from the 3-halogeno-7-ketones, have been described elsewhere (references are given in the Experimental section ‡), the present requirement for bulk quantities of the substrates necessitated, in certain cases, the development of the modified procedures recorded in the Experimental section. As usual the structures of the new compounds follow from chemical relationships and spectrometric examination; the chemical transformations which may be of interest are depicted in the Scheme (which also portrays some of the microbiological conversions). Table 2 lists the n.m.r. signals of the steroids, substrates and products, for which spectrometric data have not appeared in earlier publications; the arabic serial number sequence discussed earlier is used in this Table, which contains steroids nos. 918 to 976. The n.m.r. signals of new compounds appear in Table 2, and the other information required for their characterisation is given in Table 3.

In Table 1 the incubations are divided between two (almost equal) categories according to whether the substrate is hydroxylated to a useful extent (section A) or largely unchanged (section B). The position of the 3-halogeno-7-ketones (in the second group) indicates that the presence of a halogeno-substituent does not confer appreciably greater activity on a 7-oxo-substrate. (It may be noted that 5α-androstan-7-one is among the least reactive monoketones.[1,4,6]) However, inspection of the results for the other substrates with *C. decora* reveals marked effects which depend on the nature, position, and configuration of the halogen atoms. Comparison of the 3α- and 3β-fluoro-17-ketones illustrates the configurational dependence: the 3α-isomer's be-

† Part XX, A. M. Bell, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 2040.

‡ The Experimental section of the present work is available as Supplementary Publication No. SUP 21537 (18 pp., 1 microfiche) (for details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, Index issue, 1974).

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

[2] 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.

[3] V. E. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, J. T. Pinhey, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 1359.

[4] 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.

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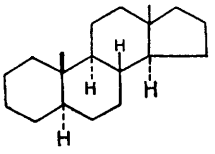
[6] A. M. Bell, J. W. Browne, W. A. Denny, Sir Ewart R. H. Jones, A. Kasal, and G. D. Meakins, *J.C.S. Perkin I*, 1972, 2930.

[7] A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *J.C.S. Perkin I*, 1973, 2137.

TABLE 1

Hydroxylations of 3-halogeno-17-oxo-, 3-halogeno-7-oxo-, and 17-halogeno-3-oxo-androstanes by *Calonectria decora* (Cd), *Rhizopus nigricans* (Rn), and *Aspergillus ochraceus* (Ao)



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; n.i. indicates that no product was isolated (or that a small amount of a mixture of products was obtained). The entries under conditions refer to the use of ethanol (E) as solvent for the substrate and to the time of incubation (in days). The yields are calculated after making allowance for recovered starting material.

Substrate	Fungus	Conditions	Substrate recovered (%)	Main product(s)			Other product(s)		
Section A									
3α-F–17-CO	Cd	E4	12	**1β,6α-**	$(OH)_2$	41%	**6α,11α-**	$(OH)_2$	7%
							11α,15α-	$(OH)_2$	5
							1β, **15α-**	$(OH)_2$	4
							12β,15α-	$(OH)_2$	3
	Rn	E6	32	**3**-CO–**11α-**	OH	12	3β,**7β-**	$(OH)_2$	9
				3β, 11α-	$(OH)_2$	11	3-CO–**6α-**	OH	5
	Ao	E2	44	**11α-**	OH	49	**7β,11α-**	$(OH)_2$	17
		E4	18	**7β,11α-**	$(OH)_2$	41	**11α-**	OH	18
		E4 *	66	**11α-**	OH	73	**7β,11α-**	$(OH)_2$	16
3α-Cl–17-CO	Cd	E4	28	**1β,6α-**	$(OH)_2$	70	**1β, 15α,17β-**	$(OH)_3$	4
							6α,11α-	$(OH)_2$	4
	Rn	E6	30	**3**-CO–**11α-**	OH	13	**3**-CO–**6α-**	OH	6
				3β,7β-	$(OH)_2$	12	**3β,6α-**	$(OH)_2$	5
				3β, 11α-	$(OH)_2$	11			
3α-Br–17-CO	Cd	E4	33	**1β,6α-**	$(OH)_2$	65			
	Rn	E6	38	**3**-CO–**11α-**	OH	13	**3β,6α-**	$(OH)_2$	7
				3β,7β-	$(OH)_2$	12	**3**-CO–**6α**	OH	4
				3β, 11α-	$(OH)_2$	11			
3β-F–17-CO	Rn	E6	28	**6α,11α-**	$(OH)_2$	24	**3β,7β-**	$(OH)_2$	8.5
				3-CO–**11α-**	OH	12	**11α-**	OH	5
							3β, 11α-	$(OH)_2$	3.5
	Ao	E2	45	**11α-**	OH	49	**7β,11α-**	$(OH)_2$	17
		E4	18	**7β,11α-**	$(OH)_2$	40	**11α-**	OH	18
		E4 *	66	**11α-**	OH	66	**7β,11α-**	$(OH)_2$	16
3β-Cl–17-CO	Cd	E4	22	**Δ²–1**-CO–**6α-**	OH	20	**1β,6α-**	$(OH)_2$	4
				Δ²–1-CO–**6α-**	**19**-$(OH)_2$	12	**1β, 15α-**	$(OH)_2$	4
	Rn	E6	29	**3**-CO–**11α-**	OH	11	**3β,7β-**	$(OH)_2$	8
				3β, 11α-	$(OH)_2$	10	**3**-CO–**6α**	OH	5
3β-Br–17-CO	Cd	E4	26	**Δ²–1**-CO–**6α-**	OH	25	**Δ²–1**-CO–**6α,**	**19**-$(OH)_2$	10
	Rn	E6	34	**3**-CO–**11α-**	OH	14	**3β,7β-**	$(OH)_2$	7
				3β, 11α-	$(OH)_2$	10	**3**-CO–**6α-**	OH	6
3β-F–17-CO–Δ^5	Cd	E4	9	**7β,11α-**	$(OH)_2$	41	**1β,7β-**	$(OH)_2$	10
							7β,12β-	$(OH)_2$	4
	Ao	E4	7	**7,β11α-**	$(OH)_2$	41	**7α,11α-**	$(OH)_2$	10
3β-Cl–17-CO–Δ^5	Cd	E4	40	**7β,12β-**	$(OH)_2$	20	**1β,7β-**	$(OH)_2$	7
							7β,11α-	$(OH)_2$	6
17α-F–3-CO	Cd	E4	42	**12β,15α-**	$(OH)_2$	17			
	Ao	E4	34	**11α-**	OH	46			
3α-F–11,17-$(CO)_2$	Rn	E4	23	**3**-CO– **17β-**	OH	37			
Section B									
3α-Cl–17-CO	Ao	E4	96	n.i.					
3α-Br–17-CO	Ao	E4	95	n.i.					
3β-F–17-CO	Cd	E4	81	n.i.					
3β-Cl–17-CO	Ao	E4	95	n.i.					
3β-Br–17-CO	Ao	E4	92	n.i.					
3β-F–17-CO–Δ^5	Rn	E4	80	**7β-**	OH	23			
3β-Cl–17-CO–Δ^5	Rn	E4	77	**7β-**	OH	13			
	Ao	E4	68	**7β,11α-**	$(OH)_2$	30	**7α,11α-**	$(OH)_2$	12
3α-F–7-CO	Cd	E4	60	**1β, 15α-**	$(OH)_2$	14			
	Rn	E6	55	**3**-CO– **16β-**	OH	11	**3α, 16β-**	$(OH)_2$	8
				3β, 16β-	$(OH)_2$	10			
	Ao	E4	92	n.i.					
3α-Cl–7-CO	Cd	E4	60	n.i.					
	Rn	E6	51	**3**-CO– **16β-**	OH	10	**3α, 16β-**	$(OH)_2$	6
				3β, 16β-	$(OH)_2$	10			
	Ao	E4	92	n.i.					

TABLE 1 (*Continued*)

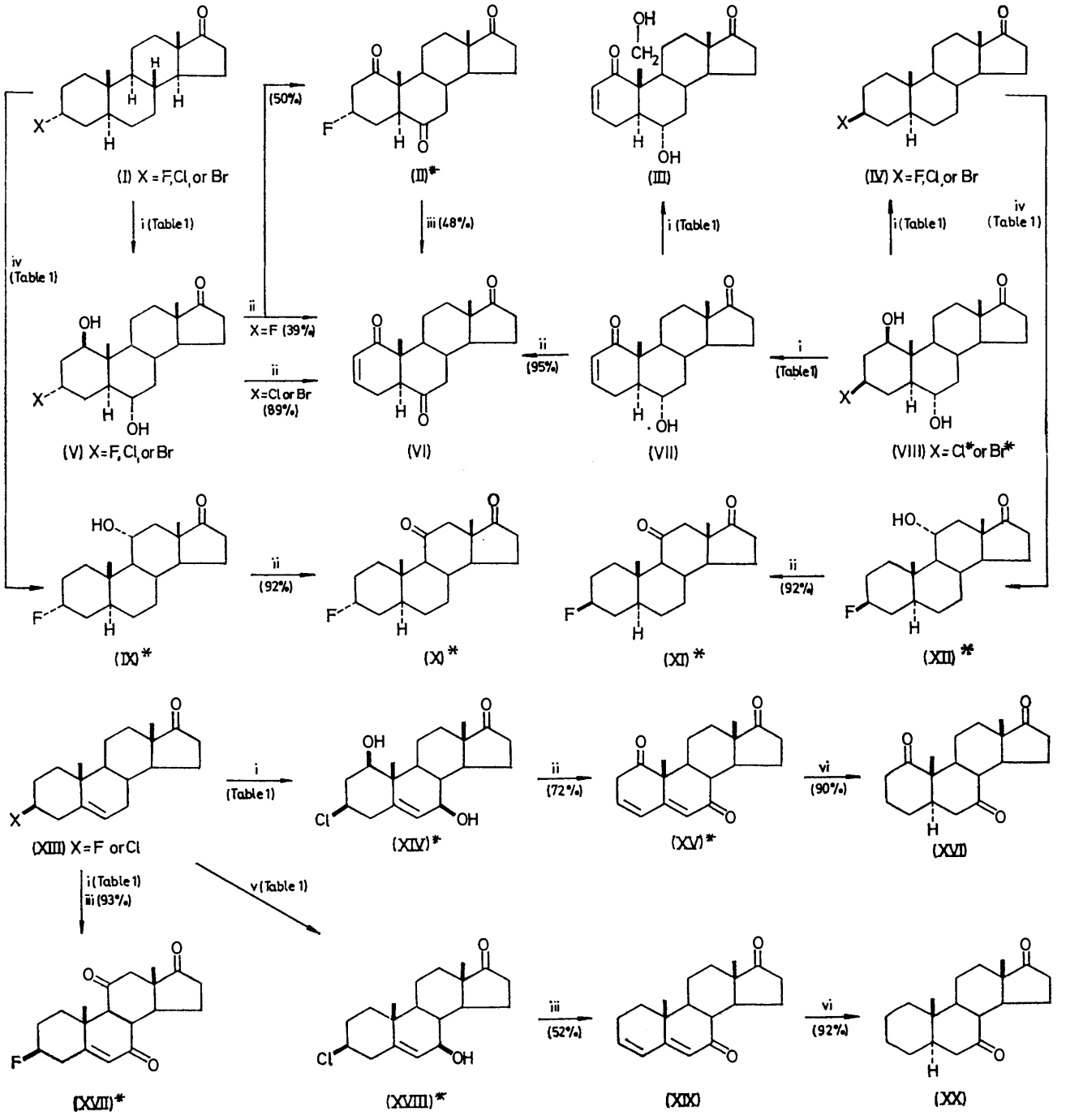
Substrate	Fungus	Conditions	Substrate recovered (%)	Main product(s)
3β-F-7-CO	Cd	E4	70	n.i.
	Rn	E6	60	**3**-CO- **16**β- OH 10
	Ao	E4	96	n.i.
17α-F-3-CO	Rn	E4	95	n.i.
17α-Cl-3-CO	Cd	E4	92	n.i.
	Rn	E4	95	n.i.
	Ao	E4	94	n.i.
3β-F-11,**17**-$(CO)_2$	Rn	E4	53	n.i.

* 10 g incubation using Biotech fermentor.

SCHEME

Some chemical and microbiological transformations

References to known compounds are given in the Experimental section, new compounds are marked with an asterisk



Reagents: i, *C. decora*; ii, H_2CrO_4–Me_2CO; iii, $AgNO_3$–Me_2SO, reflux; iv, *A. ochraceus*; v, *R. nigricans*; vi, H_2–Pd.

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 τ_2(calc) values are based, where possible, on earlier work. [b] Calculated increments for the halogeno-substituents appear at the foot of the Table

No.	Compound		τ_2	τ_2 (calc.) *		>CHX † *etc.*
918	3β-Fluoroandrost-5-ene	19	8.96	8.97	H-3	5.40 **7** (10, 10, 5, 5) 5.88 **7** (10, 10, 5, 5)
		18	9.28	9.28	H-6	4.57 d (6)
919	17α-Chloro-5α-androstan-3-one	19	8.98	8.98	H-17	5.95 d (6)
		18	9.18	9.18		
920	17α-Fluoro-5α-androstan-3-one	19	8.97	8.98	H-17	5.24 d (5) 5.78 d (5)
		18	9.32	9.32		
921	5α-Androst-2-en-7-one	19	9.27	9.28		
		18	8.95	8.95		
922	2α,3α-Epoxy-5α-androstan-17-one	19	9.22	9.22	H-2	6.86 m (3)
		18	9.16	9.16	H-3	
923	3α-Chloro-5α-androstan-7-one	19	8.92	8.92	H-3	5.50 m (7)
		18	9.30	9.30		
924	3α-Fluoro-5α-androstan-7-one	19	8.91	8.92	H-3	5.00 m (7) 5.47 m (7)
		18	9.29	9.30		
925	3β-Fluoro-5α-androstan-7-one	19	8.89	8.97	H-3	5.33 **7** (10, 10, 5, 5) 5.82 **7** (10, 10, 5, 5)
		18	9.31	9.30		
926	3β-Fluoroandrost-5-en-7-one	19	8.72	8.75	H-3	5.26 **7** (10, 10, 5, 5) 5.75 **7** (10, 10, 5, 5)
		18	9.30	9.30		
927	3α-Bromo-5α-androstan-17-one	19	9.19	9.19	H-3	5.28 m (7)
		18	9.15	9.14		
928	3α-Chloro-5α-androstan-17-one	19	9.18	9.19	H-3	5.50 m (7)
		18	9.14	9.14		
929	3α-Fluoro-5α-androstan-17-one	19	9.19	9.19	H-3	4.99 m (7) 5.48 m (7)
		18	9.14	9.14		
930	3β-Bromo-5α-androstan-17-one	19	9.15	9.14	H-3	6.02 **7** (10, 10, 5, 5)
		18	9.15	9.14		
931	3β-Bromoandrost-5-en-17-one	19	8.94	8.95	H-3	6.13 **7** (10, 10, 5, 5)
		18	9.10	9.11	H-6	4.22 d (5)
932	3β-Chloro-5α-androstan-17-one	19	9.14	9.14	H-3	6.27 **7** (10, 10, 5, 5)
		18	9.14	9.14		
933	3β-Chloroandrost-5-en-17-one	19	8.95	8.95	H-3	6.29 **7** (10, 10, 5, 5)
		18	9.12	9.11	H-6	4.66 d (6)
934	3β-Fluoro-5α-androstan-17-one	19	9.14	9.14	H-3	5.32 **7** (10, 10, 5, 5) 5.80 **7** (10, 10, 5, 5)
		18	9.14	9.14		
935	3β-Fluoroandrost-5-en-17-one	19	8.94	8.95	H-3	5.40 **7** (10, 10, 5, 5) 5.88 **7** (10, 10, 5, 5)
		18	9.11	9.11	H-6	4.58 d (6)
936	Androsta-3,5-diene-7,17-dione	19	8.85	8.83	H-3	3.75 s
					H-4	
		18	9.07	9.10	H-6	4.20 s
937	3α-Fluoro-5α-androstane-11,17-dione	19	8.96	8.97	H-3	4.98 m (7) 5.50 m (7)
		18	9.16	9.17		
938	3β-Fluoro-5α-androstane-11,17-dione	19	8.93	8.92	H-3	5.32 **7** (10, 10, 5, 5) 5.81 **7** (10, 10, 5, 5)
		18	9.16	9.17		
939	3α-Fluoro-5α-androstane-1,6,17-trione	19	8.85	8.86	H-3	4.53 m (7) 5.01 m (7)
		18	9.12	9.10		
940	Androsta-3,5-diene-1,7,17-trione	19	8.44	8.45	H-3	3.78 s
					H-4	
		18	9.08	9.08	H-6	4.22 s
941	3β-Fluoro-5α-androstane-6,11,17-trione	19	8.99	8.97	H-3	5.22 **7** (10, 10, 5, 5) 5.80 **7** (10, 10, 5, 5)
		18	9.14	9.15		
942	3β-Fluoroandrost-5-ene-7,11,17-trione	19	8.55	8.51	H-3	5.27 **7** (10, 10, 5, 5) 5.76 **7** (10, 10, 5, 5)
		18	9.15	9.18		
943	3β-Chloro-7β-hydroxyandrost-5-en-17-one	19	8.89	8.92	H-3	6.12 m (22)
					H-6	4.68 s
		18	9.10	9.08	H-7	6.16 d (7)
944	3β-Fluoro-7β-hydroxyandrost-5-en-17-one	19	8.90	8.92	H-3	5.38 m (22) 5.88 m (22)
		18	9.10	9.08	H-6	4.68 s
					H-7	6.12 d (7)
945	17α-Fluoro-11α-hydroxy-5α-androstan-3-one	19	8.87	8.86	H-11	6.02 **6** (10, 10, 5)
		18	9.28	9.29	H-17	5.25 d (6) 5.77 d (6)
946	3α-Fluoro-11α-hydroxy-5α-androstan-17-one	19	9.05	9.07	H-3	4.97 m (7) 5.48 m (7)
		18	9.12	9.10	H-11	6.03 **6** (10, 10, 5)
947	3β-Fluoro-11α-hydroxy-5α-androstan-17-one	19	9.00	9.02	H-3	5.29 **7** (10, 10, 5, 5) 5.79 **7** (10, 10, 5, 5)
		18	9.11	9.11	H-11	6.05 **6** (10, 10, 5)
948	17β-Hydroxy-5α-androstane-3,11-dione	19	8.76	8.76	H-17	6.15 t (8)
		18	9.28	9.26		
949	3α-Bromo-1β,6α-dihydroxy-5α-androstan-17-one ‡	19	9.13	9.14		
		18	9.13	9.14		
950	1β,6α-Diacetoxy-3α-bromo-5α-androstan-17-one	19	8.96	8.99	H-1	4.89 **4** (10, 5)
					H-3	5.39 m (7)
		18	9.15	9.13	H-6	5.15 **6** (10, 10, 5)
951	3α-Chloro-1β-6α-dihydroxy-5α-androstan-17-one ‡	19	9.14	9.11		
		18	9.14	9.14		
952	1β,6α-Diacetoxy-3α-chloro-5α-androstan-17-one	19	8.97	8.99	H-1	4.90 **4** (10, 5)
					H-3	5.53 m (7)
		18	9.15	9.13	H-6	5.17 **6** (10, 10, 5)
953	1β,6α-Diacetoxy-3β-chloro-5α-androstan-17-one	19	8.93	8.94	H-1	5.29 **4** (10, 5)
					H-3	6.18 **7** (10, 10, 5, 5)
		18	9.15	9.13	H-6	5.17 **6** (10, 10, 5)

TABLE 2 (*Continued*)

No.	Compound		τ_2	τ_2 (calc.) *		>CHX † *etc.*
954	3α-Fluoro-1β,6α-dihydroxy-5α-androstan-17-one	19	9.15	9.12	H-1	6.20 **4** (10, 5)
		18	9.15	9.14	H-3	4.82 m (7) 5.32 m (7)
					H-6	6.47 **6** (10, 10, 5)
955	3β-Chloro-1β,7β-dihydroxyandrost-5-en-17-one	19	8.90	8.90	H-1	6.63 **4** (10, 5)
		18	9.10	9.10	H-3	6.25 m (24)
					H-7	6.15 d (6)
956	3β-Fluoro-1β,7β-dihydroxyandrost-5-en-17-one	19	8.89	8.87	H-1	6.61 **4** (10, 4)
		18	9.10	9.08	H-3	5.25 m (22) 5.78 m (22)
					H-7	6.08 d (7)
957	3α-Fluoro-1β,15α-dihydroxy-5α-androstan-7-one	19	8.86	8.88	H-1	6.23 **4** (10, 5)
		18	9.30	9.27	H-3	4.98 m (7)
					H-15	5.45 m (7) 5.94 **6** (9, 9, 4)
958	3α-Fluoro-1β,15α-dihydroxy-5α-androstan-17-one	19	9.14	9.13	H-1	6.23 **4** (10, 5)
		18	9.10	9.11	H-3	4.93 m (7) 5.43 m (7)
					H-15	5.67 q (8)
959	1β,15α-Diacetoxy-3β-chloro-5α-androstan-17-one	19	9.01	9.01	H-1	5.07 **4** (10, 5)
		18	9.06	9.06	H-3	6.21 **7** (10, 10, 5, 5)
					H-15	5.32 **6** (9, 9, 4)
960	3α-Chloro-6α,11α-dihydroxy-5α-androstan-17-one	19	9.03	9.04	H-3	5.43 m (7)
		18	9.13	9.11	H-6	6.65 m (22)
					H-11	6.05 **6** (10, 10, 5)
961	6α,11α-Diacetoxy-3α-chloro-5α-androstan-17-one	19	9.01	9.05	H-3	5.50 m (7)
		18	9.08	9.07	H-6	5.32 **6** (10, 10, 5)
					H-11	4.84 **6** (10, 10, 5)
962	3α-Fluoro-6α,11α-dihydroxy-5α-androstan-17-one	19	9.03	9.04	H-3	4.92 m (7) 5.40 m (7)
		18	9.13	9.11	H-6	6.65 m (22)
					H-11	6.07 **6** (10, 10, 5)
963	3β-Fluoro-6α,11α-dihydroxy-5α-androstan-17-one	19	8.99	8.99	H-3	5.30 **7** (10, 10, 5, 5) 5.81 **7** (10, 10, 5, 5)
		18	9.12	9.11	H-6	6.54 **6** (10, 10, 5)
					H-11	6.04 **6** (10, 10, 5)
964	3β-Chloro-7α,11α-dihydroxyandrost-5-en-17-one	19	8.80	8.83	H-3	6.25 m (22)
					H-7	
		18	9.10	9.07	H-11	6.00 m (25)
965	3β-Fluoro-7α,11α-dihydroxyandrost-5-en-17-one	19	8.80	8.83	H-3	5.30 m (23) 5.81 m (23)
		18	9.09	9.07	H-7	
					H-11	5.97 m (28)
966	3α-Fluoro-7β,11α-dihydroxy-5α-androstan-17-one	19	9.03	9.04	H-3	4.97 m (7) 5.46 m (7)
		18	9.10	9.11	H-7	6.45 m (20)
					H-11	6.02 **6** (10, 10, 5)
967	3β-Fluoro-7β,11α-dihydroxy-5α-androstan-17one	19	8.98	8.99	H-3	5.28 **7** (10, 10, 5, 5) 5.89 **7** (10, 10, 5, 5)
		18	9.09	9.11	H-7	6.42 m (20)
					H-11	6.03 **6** (10,10,5)
968	3β-Chloro-7β,11α-dihydroxyandrost-5-en-17-one	19	8.80	8.80	H-3	6.23 m (23)
		18	9.08	9.05	H-7	
					H-11	5.93 m (28)
969	3β-Fluoro-7β,11α-dihydroxyandrost-5-en-17-one	19	8.78	8.80	H-3	5.33 m (23) 5.83 m (23)
		18	9.07	9.05	H-7	
					H-11	5.98 m (28)
970	3β-Chloro-7β,12β-dihydroxyandrost-5-en-17-one	19	8.88	8.88	H-3	6.23 m (28)
					H-7	6.15 d (7)
		18	9.02	9.00	H-12	6.26 **4** (10, 5)
971	3β-Fluoro-7β,12β-dihydroxyandrost-5-en-17-one	19	8.89	8.91	H-3	5.26 m (23) 5.78 m (23)
		18	9.02	9.00	H-7	6.10 d (7)
					H-12	6.24 **4** (10, 5)
972	3α-Fluoro-11α,15α-dihydroxy-5α-androstan-17-one	19	9.04	9.06	H-3	4.95 m (7) 5.45 m (7)
		18	9.09	9.08	H-11	6.08 **6** (10, 10, 5)
					H-15	5.71 q (8)
973	17α-Fluoro-12β,15α-dihydroxy-5α-androstan-3-one	19	8.96	8.98	H-12	6.07 **4** (11, 5)
					H-15	5.87 m (19)
		18	9.27	9.24	H-17	5.07 d (6) 5.61 d (6)
974	3α-Fluoro-12β,15α-dihydroxy-5α-androstan-17-one	19	9.16	9.17	H-3	4.94 m (7) 5.44 m (7)
		18	9.03	9.03	H-12	6.17 **4** (10, 5)
					H-15	5.51 m (18)
975	3α-Chloro-5α-androstane-1β,15α,17β-triol	19	9.15	9.15	H-1	6.11 **4** (10, 5)
					H-3	5.56 m (8)
		18	9.25	9.25	H-15	5.97 **6** (8, 8, 4)
					H-17	6.15 t (9)
976	1β,15α,17β-Triacetoxy-3α-chloro-5α-androstane	19	9.05	9.05	H-1	4.94 **4** (10, 5)
					H-3	5.58 m (8)
		18	9.17	9.16	H-15	5.09 **6** (9,9, 4)
					H-17	5.30 t (9)

[a] Ref. 2. [b] 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; A. M. Bell, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, W. E. Muller, and E. E. Richards, *J.C.S. Perkin I*, 1973, 2131.

* Calculated increments (C-19, C-18) for halogeno-substituents (X = Br, Cl, or F): 3α-X (−0.01, 0.00), 3β-X (−0.06, 0.00), 3β-X–Δ^5 (−0.25, −0.03): 17α-Cl (0.00, −0.09), 17α-F (0.00, +0.05). † X = OH or halogen. ‡ Dilute solution; only methyl group signals recorded.

haviour, 1β,6α-dihydroxylation, is similar to that of the parent ketone [2] (*i.e.* the halogen has little effect), but the 3β-compound is poorly utilised. The contrast between these results and those of the corresponding

TABLE 3

Characterisation of new compounds

Compound	M.p. (°C) *	$[\alpha]_D$ † (°) (c)	Analyticail figures (%)	C	H
3β-Fluoroandrost-5-ene	98—100	−99 (0.6)	Found $C_{19}H_{29}F$ req.	82.6 82.6	10.6 10.5
17α-Fluoro-5α-androstan-3-one	194—197	+29 (1.0)	Found $C_{19}H_{29}FO$ req.	78.3 78.1	9-5 9.9
5α-Androstan-2-en-7-one	72—75	−27 (0.7)	Found $C_{19}H_{28}O$ req.	83.7 83.8	10.2 10.3
3α-Chloro-5α-androstan-7-one	157—160	−70 (0.45)	Found $C_{19}H_{29}ClO$ req.	73.9 73.9	9.3 9.4
3α-Fluoro-5α-androstan-7-one	100—103	−63 (0.95)	Found $C_{19}H_{29}FO$ req.	78.2 78.1	10.1 9.9
3β-Fluoro-5α-androstan-7-one	135—137	−64 (1.0)	Found $C_{19}H_{29}FO$ req.	77.9 78.1	9.8 9.9
3β-Fluoroandrost-5-en-7-one	155—158	−174 (0.65)	Found $C_{19}H_{27}FO$ req.	78.8 78.6	9.5 9.3
3α-Fluoro-5α-androstane-11,17-dione	108—109	+114 (1.1)	Found $C_{19}H_{27}FO_2$ req.	74.2 74.5	8.7 8.8
3β-Fluoro-5α-androstane-11,17-dione	147—149	+105 (0.6)	Found $C_{19}H_{27}FO_2$ req.	74.6 74.5	8.9 8.8
3α-Fluoro-5α-androstane-1,6,17-trione	209—210	+148 (0.85)	Found $C_{19}H_{25}FO_3$ req.	71.4 71.25	7.95 7.8
Androsta-3,5-diene-1,7,17-trione	184—186	−305 (0.2)	Found $C_{19}H_{22}O_3$ req.	76.2 76.5	7.25 7.4
3β-Fluoro-5α-androstane-6,11,17-trione	212—214	+85 (0.6)	Found $C_{19}H_{25}FO_3$ req.	71.0 71.25	7.9 7.8
3β-Fluoroandrost-5-en-7,11,17-trione	209—210	−66 (0.5)	Found $C_{19}H_{23}FO_3$ req.	71.8 71.65	7.2 7.3
17α-Fluoro-11α-hydroxy-5α-androstan-3-one	149—152	+20 (0.95)	Found $C_{19}H_{29}FO_2$ req.	73.9 74.0	9.4 9.4
3β-Chloro-7β-hydroxyandrost-5-en-17-one	165—167	+38 (1.2)	Found $C_{19}H_{27}ClO_2$ req.	70.7 70.7	8.2 8.4
3β-Fluoro-7β-hydroxyandrost-5-en-17-one	184—185	+10 (0.4)	Found $C_{19}H_{27}FO_2$ req.	74.8 74.5	8.9 8.8
3α-Fluoro-11α-hydroxy-5α-androstan-17-one	173—176	+54.5 (0.95)	Found $C_{19}H_{29}FO_2$ req.	74.1 74.0	9.65 9.4
3β-Fluoro-11α-hydroxy-5α-androstan-17-one	191—194	+45 (1.0)	Found $C_{19}H_{29}FO_2$ req.	74.0 74.0	9.2 9.4
1β-6α-Diacetoxy-3α-bromo-5α-androstan-17-one	181—184	+60 (0.2)	Found $C_{23}H_{33}BrO_5$ req.	59.0 58.85	7.2 7.0
1β-6α-Diacetoxy-3α-chloro-5α-androstan-17-one	178—180	+63 (0.3)	Found $C_{23}H_{33}ClO_5$ req.	65.2 65.0	7.6 7.8
1β,6α-Diacetoxy-3β-chloro-5α-androstan-17-one	182—185	+68 (1.55)	Found $C_{23}H_{33}ClO_5$ req.	65.3 65.0	7.6 7.8
3α-Fluoro-1β,6α-dihydroxy-5α-androstan-17-one	209—211	+92 (1.0)	Found $C_{19}H_{29}FO_3$ req.	70.1 70.35	8.8 9.0
3β-Chloro-1β,7β-dihydroxy-androst-5-en-17-one	191—193	+21 (0.6)	Found $C_{19}H_{27}ClO_3$ req.	67.2 67.35	8.1 8.0
3β-Fluoro-1β,7β-dihydroxy-androst-5-en-17-one	199—201	+8 (0.4)	Found $C_{19}H_{27}FO_3$ req.	70.6 70.8	8.5 8.4
3α-Fluoro-1β,15α-dihydroxy-5α-androstan-7-one	222—224	−63 (0.3)	Found $C_{19}H_{29}FO_3$ req.	70.15 70.35	9.2 9.0
3α-Fluoro-1β,15α-dihydroxy-5α-androstan-17-one	189—190	+89 (1.6)	Found $C_{19}H_{29}FO_3$ req.	70.5 70.35	8.9 9.0
1β,15α-Diacetoxy-3β-chloro-5α-androstan-17-one	212—215	+75 (1.05)	Found $C_{23}H_{33}ClO_5$ req.	64.8 65.0	7.9 7.8
6α,11α-Diacetoxy-3α-chloro-5α-androstan-17-one	172—174	+67 (0.65)	Found $C_{23}H_{33}ClO_5$ req.	65.1 65.0	7.6 7.8
3α-Fluoro-6α,11α-dihydroxy-5α-androstan-17-one	187—189	+69 (1.3)	Found $C_{19}H_{29}FO_3$ req.	70.2 70.35	9.3 9.0
3β-Fluoro-6α,11α-dihydroxy-5α-androstan-17-one	207—209	+80 (0.5)	Found $C_{19}H_{29}FO_3$ req.	70.3 70.35	9.15 9.0
3β-Chloro-7α,11α-dihydroxy-androst-5-en-17-one	189—190	−24 (0.5)	Found $C_{19}H_{27}ClO_3$ req.	67.5 67.35	8.2 8.0
3β-Fluoro-7α,11α-dihydroxy-androst-5-en-17-one	212—213	−22 (0.3)	Found $C_{19}H_{27}FO_3$ req.	70.6 70.8	8.1 8.4
3α-Fluoro-7β,11α-dihydroxy-5α-androstan-17-one	196—199	+63 (1.1)	Found $C_{19}H_{29}FO_3$ req.	70.1 70.35	9.1 9.0
3β-Fluoro-7β,11α-dihydroxy-5α-androstan-17-one	212—214	+77 (1.5)	Found $C_{19}H_{29}FO_3$ req.	70.4 70.35	8.9 9.0
3β-Chloro-7β,11α-dihydroxy-androst-5-en-17-one	171—173	+15 (0.5)	Found $C_{19}H_{27}ClO_3$ req.	67.2 67.35	8.1 8.0
3β-Fluoro-7β,11α-dihydroxy-androst-5-en-17-one	184—186	+22 (0.25)	Found $C_{19}H_{27}FO_3$ req.	70.6 70.8	8.25 8.4
3β-Chloro-7β,12β-dihydroxy-androst-5-en-17-one	175—178	+51 (0.7)	Found $C_{19}H_{27}ClO_3$ req.	67.1 67.35	8.0 8.0
3β-Fluoro-7β,12β-dihydroxy-androst-5-en-17-one	202—205	+26 (0.65)	Found $C_{19}H_{27}FO_3$ req.	70.9 70.8	8.2 8.4
3α-Fluoro-11α,15α-dihydroxy-5α-androstan-17-one	213—214	+84 (0.4)	Found $C_{19}H_{29}FO_3$ req.	70.5 70.35	9.15 9.0
17α-Fluoro-12β,15α-dihydroxy-5α-androstan-3-one	186—189	+52 (0.75)	Found $C_{19}H_{29}FO_3$ req.	70.4 70.35	9.1 9.0
3α-Fluoro-12β,15α-dihydroxy-5α-androstan-3-one	220—222	+63 (0.45)	Found $C_{19}H_{29}FO_3$ req.	70.2 70.35	8.9 9.0
1β,15α,17β-Triacetoxy-3α-chloro-5α-androstane	Oil	+45 (0.25)	Found $C_{25}H_{37}ClO_6$ req.	64.2 64.0	8.1 7.9

* From Me_2CO–hexane. † $CHCl_3$ as solvent.

chloro- and bromo-ketones exemplifies the influence of the halogens' nature. While the hydroxylation of the 3α-chloro- and 3α-bromo-ketones resembles that of the fluorine analogue, the 3β-chloro- and 3β-bromo-compounds appear to be more reactive than 5α-androstan-17-one. (The hydroxylation sequence is shown in the Scheme, and is reminiscent of that found with the parent ketone under the more vigorous microbiological procedure of incubation in the presence of dimethyl sulphoxide.[3]) A different order of halogenated substrate activity is manifest in the 17α-halogeno-3-ketones; here the chloro-compound is unreactive whereas the fluoro-compound undergoes 12β,15α-dihydroxylation (as does 5α-androstan-3-one [2]).

Although the 17-halogeno-3-ketones are not metabolised by *R. nigricans*, the isomeric 3-halogeno-17-ketones are converted into 3-oxo- or 3β-hydroxy-derivatives. Removal of the halogeno-substituents most probably involves hydroxylation at position 3 to form unstable halogenohydrins; subsequent microbial reduction of the resulting 3-oxo-derivatives would then give the 3β-hydroxy-derivatives.[5] (The parent 17-ketone is attacked only slowly by *R. nigricans*, but 3-hydroxylation [4] is the dominant microbiological process.) The attack appears to be faster with the 3α-halogeno-substrates than with the 3β-isomers (as indicated by the isolation of some 3β-fluoro-11α-hydroxy-5α-androstan-17-one from the 3β-fluoro-17-ketone); this accords with the preference [4,5] of *R. nigricans* for equatorial rather than axial hydroxylation. When a double bond is near to the halogen function (in the 3-halogeno-Δ^5-17-ketones) hydroxylation and consequential loss of halogen is not observed.

A clear, and useful, difference between fluoro-ketones and other halogeno-compounds is found with *A. ochraceus*. The chloro- and bromo-derivatives of 3- and 17-ketones are not metabolised (section B), thus parallelling the behaviour of the parent ketones.[6] In contrast, the corresponding fluoro-ketones are efficiently hydroxylated at position 11, behaviour found previously with dioxygenated androstanes. The material in the centre of the Scheme illustrates the application of these processes for preparing 3β- and 3α-fluoro-5α-androstane-11,17-diones (both new compounds); the microbiological stages were carried out on a 10 g scale by the technique described earlier.[7]

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