Microbiological Hydroxylation. Part 23.[1] Hydroxylations of Fluoro-5α-androstanones by the Fungi *Calonectria decora*, *Rhizopus nigricans*, and *Aspergillus ochraceus*

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A series of monofluoro- and gem-difluoro-5α-androstanones and the parent ketones were incubated, under standard conditions, with the fungi named in the title. The results may be rationalised by comparing the positions of the fluorine atoms in the substrates with those of the favoured hydroxylation sites in the parent ketones. With few exceptions hydroxylation does not occur at, or adjacent to, the carbon to which a fluorine substituent is attached even though one of these centres is a favoured site (in the parent ketone). In such cases hydroxylation is usually diverted to an alternative position. Where the favoured site is more distant from the fluorine substituent(s) the behaviour of a fluoro-ketone resembles that of its parent. Hydroxylation of several fluoro-ketones by *Aspergillus ochraceus* gives the 11α-hydroxy-derivatives cleanly and in yields which are satisfactory for preparative work.

In previous work [2] comparisons were made between the hydroxylations of 5α-androstane monoketones and those of related substrates having halogeno-substituents at positions (3 and 17) remote from the keto-groups; on the basis of their known activities the fungi selected for study were *Calonectria decora* [3] and *Rhizopus nigricans* [4] (which are influenced by directing effects) and *Aspergillus ochraceus* [5] (which is usually site-specific). The emergence of the fluoro-ketones as the most interesting substrates prompted the present work involving a range of monofluoro- and *gem*-difluoro-5α-androstanones prepared by the methods described recently.[6] Since the object was to elucidate the effect of the fluoro-substituents on the hydroxylation processes rather than to optimise the yield of any particular product it was essential to use standard conditions for the incubations. Preliminary experiments led to a general procedure in which most of the fluoro-ketones were converted into mono- or di-hydroxy-derivatives but did not undergo degradation to smaller water-soluble products. However, it transpired that 3-, 16-, and 17-oxo-5α-androstanes (the appropriate reference compounds) had not been incubated previously under precisely these conditions, and a re-examination of the parent ketones was therefore required.

RESULTS AND DISCUSSION

Table 1 summarises the results obtained in the hydroxylations of the fluoro-ketones and the reference ketones by *C. decora* (*Cd*), *R. nigricans* (*Rn*), and *A. ochraceus* (*Ao*). Table 2 lists the n.m.r. spectra of the steroids involved here for which spectrometric data have not appeared in previous publications; the arabic serial number sequence discussed earlier [3a] is used in this Table, which contains steroids nos. 1028—1080. The structures of new compounds follow, as usual,[3a,b] from a combination of spectroscopic and chemical methods. With three 16-oxygenated-17,17-difluoro-compounds (nos. 1043, 1067, and 1075) there is poor agreement between the observed and calculated positions of the 18-H signals. The proposed structures are supported, however, by the large coupling of the 16-H signal in the hydroxy-diketone (no. 1043) and by a comparison of the ^{19}F signals of the other compounds with those of 17,17-difluoro-androstanes lacking 16-substituents.[6] Jones oxidation of the 11α,16β-dihydroxy-3-ketone (no. 1067) gave the 16β-hydroxy-3,11-diketone (no. 1043); apparently the 17-fluoro-substituents markedly reduce the tendency of a neighbouring hydroxy-group to form a chromate ester. The properties, other than the n.m.r. characteristics, of the products which were fully characterised are collected in Table 3. Since the microbiological and chemical operations of the present work are routine applications of techniques described in earlier parts details are not given here.†

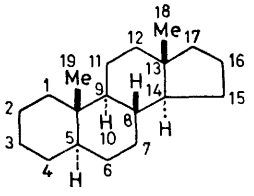
Inspection of Table 1 shows that the introduction of fluorine substituents has marked effects on the positions and rates at which a parent ketone is hydroxylated by *Cd*, *Rn*, and *Ao*. While no comprehensive explanation of these effects can be given, several trends emerge from an analysis based on a comparison of the positions of the fluorine atoms in the substrates with those of the favoured hydroxylation sites in the parent ketones. To simplify discussion the latter sites (whose number and positions in the steroid nucleus vary with the structure of the parent ketone and the nature of the micro-organism) [3-5] are termed the standard sites, and the fluoro-ketone substrates are represented as $F{-}C_x{-}C_y\cdots C_z\cdots$. The two basically different situations which arise may be considered as follows.

(*i*) The standard site (or one of the standard sites) corresponds to C_x or C_y. An overall inspection of the results shows that, with few exceptions, hydroxylation does not occur at C_x or C_y. Thus, the tendency of fluorine to inhibit substitution at, or adjacent to, the carbon to which it is attached outweighs the directing effect of the substrate's keto-group [3,4] or the site-specificity of the micro-organism.[5] There is one instance in which the

† The experimental work is recorded in Supplementary Publication No. SUP 22690 (12 pp.). For details of Supplementary Publications see Notice to Authors, No. 7 in *J.C.S. Perkin I*, 1979, Index issue.

TABLE 1

Hydroxylation of fluoro-5α-androstanones and the parent ketones 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.* 3-CO-16,16-F_2 represents 16,16-difluoro-5α-androstan-3-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 the complex mixture of products was not investigated. The substrates were introduced as solutions in ethanol, and the incubations were carried out for the times (in days) specified. The yields are calculated after making allowance for recovered starting material.

Substrate	Fungus (Time in days)	Substrate recovered	Main product(s)	Other product(s)
3-CO	Cd (4)	15%	3β,**12**β,**15**α-$(OH)_3$ 30%	**12**β,**15**α-$(OH)_2$ 17%
	Rn (4)	31	**11**α,**16**β-$(OH)_2$ 24	3β,**11**α,**16**β-$(OH)_3$ 11
	Ao (6)	90	**6**β,**11**α- $(OH)_2$ 84	
3-CO-7,7-F_2	Cd (4)	8	3β,**12**β,**15**α-$(OH)_3$ 35	**12**β,**15**α-$(OH)_2$ 17
	Rn (4)	0	n.i.	
3-CO-12,12-F_2	Cd (4)	23	3β,**7**β,**15**α-$(OH)_3$ 30	
	Rn (4)	21	**1**β,**16**β-$(OH)_2$ 21	4α,**16**β-$(OH)_2$ 5
3-CO-16,16-F_2	Cd (4)	30	**7**β,**12**β-$(OH)_2$ 16	3β,**7**β,**12**β-$(OH)_3$ 11
	Ao (6)	71	**11**α- OH 42	**6**β,**11**α-$(OH)_2$ 34
3-CO-17α-F *	Cd (4)	42	**12**β,**15**α-$(OH)_2$ 17	
	Rn (4)	95	n.i.	
	Ao (4)	34	**11**α- OH 46	
3-CO-Δ[16]-17-F	Cd (4)	0	3β,**12**β,**15**α-$(OH)_3$ 25	**12**β,**15**α-$(OH)_2$ 6
3-CO-17,17-F_2	Rn (4)	6	**11**α,**16**β-$(OH)_2$ 15	3β,**11**α,**16**β-$(OH)_3$ 8
16-CO	Cd (4)	90	n.i.	
	Rn (6)	38	**3**β,**7**α-$(OH)_2$ 48	
	Ao (6)	90	n.i.	
16-CO-3α-F	Cd (4)	33	**6**α,**11**α-$(OH)_2$ 59	**6**-CO-**11**α- OH 10
	Rn (4)	46	**3**β,**7**α-$(OH)_2$ 24	**3**-CO-**11**α- OH 16
			3-CO-**7**α-OH 21	**3**-CO-**6**α- OH 7
	Ao (4)	8	**7**β,**11**α-$(OH)_2$ 10	**6**β,**11**α-$(OH)_2$ 5
				11α- OH 5
17-CO	Cd (4)	14	1β,**6**α-$(OH)_2$ 33	**1**-CO-**6**α- OH 4
			1-CO-**6**α,**19**-$(OH)_2$ 8	**6**α,**11**α-$(OH)_2$ 3
	Rn (4)	41	**6**α,**11**α-$(OH)_2$ 11	**3**α,**11**α-$(OH)_2$ 8
				3β,**7**β-$(OH)_2$ 7
	Ao (6)	67	**7**β,**11**α-$(OH)_2$ 34	**11**α- OH 4
17-CO-3α-F *	Cd (4)	12	**1**β,**6**α-$(OH)_2$ 41	**6**α,**11**α-$(OH)_2$ 7
				11α,**15**α-$(OH)_2$ 5
				1β,**15**α-$(OH)_2$ 4
				12β,**15**α-$(OH)_2$ 3
	Rn (6)	32	**3**-CO-**11**α- OH 12	**3**β,**7**β-$(OH)_2$ 9
			3β,**11**α-$(OH)_2$ 11	**3**-CO-**6**α- OH 5
	Ao (4)	18	**7**β,**11**α-$(OH)_2$ 41	**11**α-(OH) 18
17-CO-3β-F *	Rn (6)	28	**6**α,**11**α-$(OH)_2$ 24	**3**β,**7**β-$(OH)_2$ 8
			3-CO-**11**α- OH 12	**11**α- OH 5
				3β,**11**α-$(OH)_2$ 4
	Ao (4)	18	**7**β,**11**α-$(OH)_2$ 40	**11**α- OH 18
17-CO-3,3-F_2	Cd (4)	0	**6**α,**11**α-$(OH)_2$ 30	**1**β,**6**α-$(OH)_2$ 20
	Rn (4)	9	**6**α,**11**α-$(OH)_2$ 15	
	Ao (4)	28	**11**α- OH 45	
17-CO-6α-F	Ao (6)	67	**11**α- OH 54	
17-CO-6,6-F_2	Cd (4)	47	n.i.	
	Ao (6)	67	n.i.	
17-CO-7,7-F_2	Rn (4)	0	**3**α,**11**α-$(OH)_2$ 18	**4**α,**11**α-$(OH)_2$ 11
17-CO-12,12-F_2	Cd (4)	8	**1**β,**6**α-$(OH)_2$ 22	**7**β,**15**α,17β-$(OH)_3$ 6
			1β,**6**α,17β-$(OH)_3$ 20	
	Rn (4)	30	n.i.	
	Ao (6)	63	**7**β- OH 43	

* Results from ref. 2.

TABLE 2

N.m.r. results, presented in the usual form,[a] were obtained using solutions in $CDCl_3$. For ^1H signals (examined at 100 MHz) the τ_2(calc.) values are based on earlier work.[b] For ^{19}F signals [examined at 84.6 MHz and assigned, where possible, to equatorial (eq) and axial (ax) substituents] the shifts are p.p.m. from external trifluoroacetic acid. All but three of the products (nos. 1055, 1068, and 1069) are, or have been chemically related to, fully characterised compounds (Table 3)

		^1H Signals (J or $w_{\frac{1}{2}}$ /Hz)				^{19}F Signals (J or $w_{\frac{1}{2}}$/Hz)
No.	Compound		τ_2	τ_2(calc.)		
1028	16,16-Difluoro-5α-androstane-3,11-dione	19	8.77	8.75		−3.8 m(42)
		18	9.11	9.09		
1029	12,12-Difluoro-5α-androstane-7,17-dione	19	8.92	8.90		
		18	8.94	8.94		
1030	12,12-Difluoro-5α-androstane-1,6,17-trione	19	8.86	8.85		
		18	8.95	8.91		
1031	16,16-Difluoro-5α-androstane-3,7,12-trione	19	8.63	8.59		
		18	8.77	8.67		
1032	7,7-Difluoro-5α-androstane-3,11,17-trione	19	8.71	8.69		
		18	9.13	9.10		
1033	3α-Fluoro-5α-androstane-6,11,16-trione	19	8.99	9.00	H-3 {4.80 m(7) / 5.35 m(7)	
		18	9.13	9.15		
1034	3α-Fluoro-5α-androstane-7,11,16-trione	19	8.70	8.67	4.95 m(7) / 5.45 m(7)	
		18	9.18	9.16		
1035	12,12-Difluoro-5α-androstane-7,15,17-trione	19	8.83	8.88		
		18	8.83	8.86		
1036	16,16-Difluoro-11α-hydroxy-5α-androstan-3-one	19	8.85	8.86	H-11 6.00 sextet (10,10,5)	
		18	9.04	9.03		
1037	11α-Acetoxy-16,16-difluoro-5α-androstan-3-one	19	8.87	8.89	H-11 4.77 sextet (10,10,5)	
		18	9.00	9.00		
1038	3α-Fluoro-11α-hydroxy-5α-androstan-16-one	19	9.02	9.05	H-3 {4.95 m (7) / 5.45 m (7)	
		18	9.10	9.11	H-11 6.00 sextet (10,10,5)	
1039	12,12-Difluoro-7β-hydroxy-5α-androstan-17-one	19	9.16	9.14	H-7 6.51 m (20)	eq {−27.2 t(4) / −30.0 t(4)} ax {−31.2 q(30,14) / −34.0 q(30,14)}
		18	8.93	8.92		
1040	7β-Acetoxy-12,12-difluoro-5α-androstan-17-one	19	9.14	9.13	H-7 5.36 m (7)	
		18	8.91	8.92		
1041	3,3-Difluoro-11α-hydroxy-5α-androstan-17-one	19	9.01	9.04	H-11 6.03 sextet (10,10,5)	eq {−8.7 s / −11.6 s} ax {−20.5 m / −23.4 m}
		18	9.04	9.10		
1042	6α-Fluoro-11α-hydroxy-5α-androstan-17-one	19	9.02	9.06	H-6 {5.46 sextet (10,10.5) / 5.90 m (20)}	
		18	9.12	9.10	H-11 6.00 m (20)	
1043	17,17-Difluoro-16β-hydroxy-5α-androstane-3,11-dione	19	8.76	8.74	H-16 {5.67 t (7) / 5.86 t (7)}	
		18	9.13	8.88		
1044	6α-Hydroxy-5α-androstane-3,16-dione	19	8.92	8.91	H-6 6.60 sextet (10,10,5)	
		18	9.08	9.10		
1045	7α-Hydroxy-5α-androstane-3,16-dione	19	8.95	8.94	H-7 6.15 m (7)	
		18	9.09	9.09		
1046	3α-Fluoro-11α-hydroxy-5α-androstane-6,16-dione	19	9.09	9.10	H-3 {4.82 m (7) / 5.32 m (7)}	
		18	9.07	9.07	H-11 5.91 sextet (10,10,5)	
1047	3,3-Difluoro-1β,6α-hydroxy-5α-androstan-17-one [c]	19	8.86		H-1 6.07 q (8,5)	eq {−5.0 s / −7.7 s} ax {13.9 m / 16.6 m}
		18	9.15		H-6 6.17 sextet (11,11,5)	
1048	12,12-Difluoro-1β,6α-dihydroxy-5α-androstan-17-one [d]	19	9.08	9.10		
		18	8.95	8.95		
1049	1β,6α-Diacetoxy-12,12-difluoro-5α-androstan-17-one	19	8.95	8.99	H-1 5.42 q (10,5)	eq {−26.1 t (8) / −30.0 t (8)} ax {−31.1 t (19) / −34.0 t (19)}
		18	8.95	8.94	H-6 5.20 sextet (11,11,5)	
1050	12,12-Difluoro-1β,16β-dihydroxy-5α-androstan-3-one	19	8.95	8.88	H-1 6.46 q (10,5)	
		18	8.86	8.83	H-16 5.58 m (18)	
1051	7,7-Difluoro-3α,11α-dihydroxy-5α-androstan-17-one	19	9.02	9.04	H-3 / H-11 } 6.0 m (26)	
		18	9.11	9.08		
1052	3α,11α-Diacetoxy-7,7-difluoro-5α-androstan-17-one	19	9.06	9.06	H-3 4.9 m (23)	eq {−12.1 s / −15.0 s} ax {−33.5 m / −36.4 m}
		18	9.02	9.04	H-11 4.77 sextet (10,10,5)	
1053	7,7-Difluoro-4α,11α-dihydroxy-5α-androstan-17-one	19	9.01	9.02	H-4 6.58 m (19)	
		18	9.11	9.09	H-11 6.04 sextet (10,10,5)	
1054	4α,11α-Diacetoxy-7,7-difluoro-5α-androstan-17-one	19	8.97	9.02	H-4 5.32 sextet (11,11,5)	eq {−12.3 s / −15.1 s} ax {−33.7 m / −36.6 m}
		18	9.06	9.03	H-11 4.81 sextet (10,10,5)	
1055	4α,16β-Diacetoxy-12,12-difluoro-5α-androstan-3-one	19	8.89	8.88	H-4 4.09 d (10)	eq {−24.0 s / −26.8 s} ax {−34.9 q (32,16) / −38.7 q (32,16)}
		18	8.94	8.90	H-16 4.80 m (18)	
1056	16,16-Difluoro-6β,11α-dihydroxy-5α-androstan-3-one [c,d]	19	8.39			
		18	9.06			
1057	6β,11α-Diacetoxy-16,16-difluoro-5α-androstan-3-one	19	8.70	8.73	H-6 5.10 m (7)	
		18	8.96	8.93	H-11 4.77 sextet (10,10,5)	
1058	3α-Fluoro-6α,11α-dihydroxy-5α-androstan-16-one	19	9.01	9.02	H-3 {4.85 m (7) / 5.35 m (7)}	
		18	9.12	9.09	H-6 6.63 sextet (10,10,5)	
					H-11 6.01 sextet (10,10,5)	

TABLE 2 (*Continued*)

No.	Compound		^{1}H Signals (J or $w_{\frac{1}{2}}$/Hz) τ_2	τ_2(calc.)		^{19}F Signals (J or $w_{\frac{1}{2}}$/Hz)
1059	6α,11α-Diacetoxy-3α-fluoro-5α-androstan-16-one	19	8.98	9.06	H-3 {4.92 m (7) / 5.42 m (7)}	
		18	9.05	9.05	H-6 5.32 sextet (10,10,5)	
					H-11 4.81 sextet (10,10,5)	
1060	3,3-Difluoro-6α,11α-dihydroxy-5α-androstan-17-one	19	9.01	9.01	H-6 6.57 sextet (11,11,5)	
		18	9.12	9.11	H-11 6.05 sextet (10,10,5)	
1061	6α,11α-Diacetoxy-3,3-difluoro-5α-androstan-17-one	19	8.98	9.05	H-6 5.27 sextet (11,11,5)	
		18	9.06	9.07	H-11 4.78 sextet (10,10,5)	
1062	3α-Fluoro-6β,11α-dihydroxy-5α-androstan-16-one	19	8.81	8.82	H-3 {4.82 m (7) / 5.32 m (7)}	
		18	9.06	9.05	H-6 6.20 m (6)	
					H-11 5.90 sextet (10,10,5)	
1063	3α-Fluoro-7β,11α-dihydroxy-5α-androstan-17-one [d,e]	19	9.00			
		18	9.10			
1064	7β,11α-Diacetoxy-3α-fluoro-5α-androstan-16-one	19	9.01	9.04	H-3 {4.96 m (7) / 5.44 m (7)}	
		18	9.04	9.07	H-7 5.30 sextet (10,10,5)	
					H-11 4.75 sextet (10,10,5)	
1065	16,16-Difluoro-7β,12β-dihydroxy-5α-androstan-3-one	19	8.93	8.94	H-7, H-12 6.60 m (26)	
		18	9.04	8.98		
1066	7β,15α-Diacetoxy-12,12-difluoro-5α-androstan-3-one	19	8.91	8.92	H-7 5.38 m (19)	
		18	8.98	8.97	H-15 4.97 m (16)	
1067	17,17-Difluoro-11α,16β-dihydroxy-5α-androstan-3-one	19	8.85	8.84	H-11, H-16 6.00 m (26)	'ax' {−29.6 d (19) / −32.4 d (19)} 'eq' {−46.9 s / −49.7 s}
		18	8.99	8.82		
1068	7,7-Difluoro-12β,15α-dihydroxy-5α-androstan-3-one	19	8.91	8.92	H-12 6.50 q (10,5)	
		18	9.20	9.17	H-15 5.63 m (17)	
1069	12β,15α-Diacetoxy-7,7-difluoro-5α-androstan-3-one	19	8.92	8.92	H-12 5.30 q (10,5)	
		18	9.11	9.10	H-15 4.79 m (17)	
1070	17-Fluoro-12β,15α-dihydroxy-5α-androst-16-en-3-one	19	8.94	8.93	H-12 6.10 q (10,5)	
		18	8.92	8.92	H-15 5.42 t (8,8)	
					H-16 5.00 m (5)	
1071	1β,6α,17β-Triacetoxy-12,12-difluoro-5α-androstane	19	8.97	9.00	H-1 5.45 q (10,5)	eq {−24.7 s / −27.5 s} ax {−34.8 t (19) / −37.6 t (19)}
		18	9.00	9.03	H-6 5.25 sextet (11,11,5)	
					H-17 4.73 q (10.8)	
1072	16,16-Difluoro-5α-androstane-3β,7β,12β-triol [c,d]	19	8.92			
		18	9.10			
1073	3β,7β,12β-Triacetoxy-16,16-difluoro-5α-androstane	19	9.09	9.09	H-3, H-7, H-12 5.40 m (27)	
		18	9.00	9.02		
1074	3β,7β,15α-Triacetoxy-12,12-difluoro-5α-androstane	19	9.09	9.10	H-3, H-7 5.35 m (22)	eq {−28.2 s / −31.0 s} ax {−35.4 q (32,16) / −38.2 q (32,16)}
		18	9.01	8.99	H-15 4.96 m (17)	
1075	3β,11α,16β-Triacetoxy-17,17-difluoro-5α-androstane	19	9.05	9.07	H-3 5.33 m (25)	'ax' {−27.6 d (19) / −30.5 d (19)} 'eq' {−42.6 s / −45.5 s}
		18	9.02	8.89	H-11, H-16 4.85 m (26)	
1076	7,7-Difluoro-5α-androstane-3β,12β,15α-triol	19	9.11	9.13	H-3, H-12 6.5 m (25)	eq {−7.5 s / −10.2 s} ax {−29.3 m / −32.1 m}
		18	9.22	9.20	H-15 5.65 m (18)	
1077	3β,12β,15α-Triacetoxy-7,7-difluoro-5α-androstane	19	9.10	9.11	H-3, H-12 5.3 m (28)	eq {−11.3 s / −14.2 s} ax {−28.3 m / −31.2 m}
		18	9.12	9.12	H-15 4.82 m (17)	
1078	17-Fluoro-5α-androst-16-ene-3β,12β,15α-triol [c,d]	19	8.68			
		18	9.11			
1079	3β,12β,15α-Triacetoxy-17-fluoro-5α-androst-16-ene	19	9.12	9.12	H-3 5.35 m (23)	
		18	8.86	8.88	H-12 4.97 q (10,5)	
					H-15 4.55 t (6,6)	
					H-16 5.03 m (5)	
1080	7β,15α,17β-Triacetoxy-12,12-difluoro-5α-androstane	19	9.06	9.13	H-7 5.54 m (20)	
		18	8.96	8.94	H-15 5.13 m (18)	
					H-17 4.72 t (9)	

[a] Chemical shift and multiplicity; ref. 3*b*. [b] Ref. 6 (and earlier papers cited there). [c] Solution in C_5H_5N. [d] Dilute solution, only Me signals observed. [e] Solution in $(CD_3)_2CO$.

major product involves attack at C_x of a monofluoride (3α-fluoro-5α-androstan-17-one with *Rn*) and one similar case (the 3β-isomer with *Rn*) where a minor product is so formed. The corresponding *gem*-difluoride appears to retain its fluorine although the yield of the one product isolated was low. The sole example of C_y hydroxylation also occurs with *Rn* (in the incubation of the 17,17-difluoro-3-ketone). It may be noted that the three exceptional cases result in substitution at the two appropiate standard sites (*viz.*, positions 3 and 11 with 17-ketones, and positions 11 and 16 with 3-ketones) and involve substrates whose keto-groups would be expected to exert strong directing effects towards *Rn*.[4a] In the majority of cases, where neither C_x nor C_y is attacked, the

TABLE 3
Characterisation of new compounds

Compound	M.p. (°C) [a]	$[\alpha]_D$ (°) [b] (*c*)	Analytical figures (%)	C	H
16,16-Difluoro-5α-androstane-3,11-dione	148—149	+50 (0.1)	Found	69.8	7.95
			$C_{19}H_{26}F_2O_2$ req.	70.3	8.1
12,12-Difluoro-5α-androstane-7,17-dione	195—196	+9 (0.3)	Found	70.3	8.1
			$C_{19}H_{26}F_2O_2$ req.	70.3	8.1
12,12-Difluoro-5α-androstane-1,6,17-trione	245—247	+173 (0.4)	Found	67.6	7.0
			$C_{19}H_{24}F_2O_3$ req.	67.4	7.15
16,16-Difluoro-5α-androstane-3,7,12-trione	239—240	0 (0.1)	Found	67.6	7.2
			$C_{19}H_{24}F_2O_3$ req.	67.4	7.15
7,7-Difluoro-5α-androstane-3,11,17-trione	204—205	+126 (0.3)	Found	67.5	7.1
			$C_{19}H_{24}F_2O_3$ req.	67.4	7.15
3α-Fluoro-5α-androstane-7,11,16-trione	219—221	−178 (0.1)	Found	71.1	7.8
			$C_{19}H_{25}FO_3$ req.	71.2	7.9
11α-Acetoxy-16,16-difluoro-5α-androstan-3-one	190—192	−10 (0.4)	Found	68.5	8.3
			$C_{21}H_{30}F_2O_3$ req.	68.45	8.2
3α-Fluoro-11α-hydroxy-5α-androstan-16-one	211—212	−186 (0.4)	Found	73.8	9.5
			$C_{19}H_{29}FO_2$ req.	74.0	9.5
12,12-Difluoro-7β-hydroxy-5α-androstan-17-one	208—209	+146 (0.4)	Found	70.2	8.7
			$C_{19}H_{28}F_2O_2$ req.	69.9	8.65
3,3-Difluoro-11α-hydroxy-5α-androstan-17-one	185—186	+46 (0.4)	Found	70.05	8.5
			$C_{19}H_{28}F_2O_2$ req.	69.9	8.65
6α-Fluoro-11α-hydroxy-5α-androstan-17-one	193—194	+70 (0.6)	Found	74.1	9.45
			$C_{19}H_{29}FO_2$ req.	74.0	9.5
17,17-Difluoro-16β-hydroxy-5α-androstane-3,11-dione	202—204	+47 (0.3)	Found	67.0	7.7
			$C_{19}H_{26}F_2O_3$ req.	67.0	7.7
6α-Hydroxy-5α-androstane-3,16-dione	205—208	+119 (0.4)	Found	74.8	9.15
			$C_{19}H_{28}O_3$ req.	75.0	9.3
7α-Hydroxy-5α-androstane-3,16-dione	216—218	−161 (0.2)	Found	75.0	9.3
			$C_{19}H_{28}O_3$ req.	75.0	9.3
3α-Fluoro-11α-hydroxy-5α-androstane-6,16-dione	216—220	−96 (0.4)	Found	70.7	8.4
			$C_{19}H_{27}FO_3$ req.	70.8	8.4
3,3-Difluoro-1β,6α-dihydroxy-5α-androstan-17-one	268—269	+71 (0.5)	Found	66.6	8.2
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
12,12-Difluoro-1β,6α-dihydroxy-5α-androstan-17-one	259—262	+92 [c] (0.6)	Found	66.6	8.3
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
1β,6α-Diacetoxy-12,12-difluoro-5α-androstan-17-one	176—178	+106 (0.5)	Found	65.0	7.5
			$C_{23}H_{32}F_2O_5$ req.	64.8	7.6
12,12-Difluoro-1β,16β-dihydroxy-5α-androstan-3-one	249—251	+30 [c] (0.3)	Found	66.6	8.2
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
7,7-Difluoro-3α,11α-dihydroxy-5α-androstan-17-one	189—191	+56 [c] (0.2)	Found	66.7	8.3
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
4α,11α-Diacetoxy-7,7-difluoro-5α-androstan-17-one	193—195	0 (0.2)	Found	64.7	7.7
			$C_{23}H_{32}F_2O_5$ req.	64.8	7.6
16,16-Difluoro-6β,11α-dihydroxy-5α-androstan-3-one	228—233		Found	66.4	8.1
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
3α-Fluoro-6α,11α-dihydroxy-5α-androstan-16-one	213—215	−150 (0.5)	Found	70.4	8.9
			$C_{19}H_{29}FO_3$ req.	70.3	9.0
6α,11α-Diacetoxy-3α-fluoro-5α-androstan-16-one	163—164	−115 (0.5)	Found	67.9	8.1
			$C_{23}H_{33}FO_5$ req.	67.6	8.1
6α,11α-Diacetoxy-3,3-difluoro-5α-androstan-17-one	217—219	+50 (0.4)	Found	64.8	7.5
			$C_{23}H_{32}F_2O_5$ req.	64.8	7.6
3α-Fluoro-6β,11α-dihydroxy-5α-androstan-16-one	211—214	−172 (0.4)	Found	70.15	9.0
			$C_{19}H_{29}FO_3$ req.	70.3	9.0
3α-Fluoro-7β,11α-dihydroxy-5α-androstan-16-one	255—257	−118 (0.1)	Found	70.35	9.1
			$C_{19}H_{29}FO_3$ req.	70.3	9.0
7β,11α-Diacetoxy-3α-fluoro-5α-androstan-16-one	188—191	−95 (0.6)	Found	67.7	8.1
			$C_{23}H_{33}FO_5$ req.	67.6	8.1
16,16-Difluoro-7β,12β-dihydroxy-5α-androstan-3-one	247—249	+48 (0.05)	Found	66.8	8.1
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
7β,15α-Diacetoxy-12,12-difluoro-5α-androstan-3-one	165—167	+116 (0.2)	Found	64.9	7.6
			$C_{23}H_{32}F_2O_5$ req.	64.8	7.6
17,17-Difluoro-11α,16β-dihydroxy-5α-androstan-3-one	222—224	−7 (0.5)	Found	66.4	8.3
			$C_{19}H_{28}F_2O_3$ req.	66.6	8.2
17-Fluoro-12β,15α-dihydroxy-5α-androst-16-en-3-one	175—177	+110 (0.2)	Found	70.8	8.4
			$C_{19}H_{27}FO_3$ req.	70.8	8.4
1β,6α,17β-Triacetoxy-12,12-difluoro-5α-androstane	237—238	+61 (0.5)	Found	64.1	7.5
			$C_{25}H_{36}F_2O_6$ req.	63.8	7.7
16,16-Difluoro-5α-androstane-3β,7β,12β-triol	246—247		Found	66.2	8.8
			$C_{19}H_{30}F_2O_3$ req.	66.25	8.8
3β,11α,16β-Triacetoxy-17,17-difluoro-5α-androstane	127—128 [e]	−10 (0.4)	Found	63.9	7.6
			$C_{25}H_{36}F_2O_6$ req.	63.8	7.7
7,7-Difluoro-5α-androstane-3β,12β,15α-triol	223—225	+13 (0.1)	Found	66.0	8.8
			$C_{19}H_{30}F_2O_3$ req.	66.25	8.8
3β,12β,15α-Triacetoxy-7,7-difluoro-5α-androstane	218—220 [c]	+3 (0.5)	Found	64.1	7.8
			$C_{25}H_{36}F_2O_6$ req.	63.8	7.7
17-Fluoro-5α-androst-16-ene-3β,12β,15α-triol	273—275 [f]	+97 (0.5)	Found	70.4	8.9
			$C_{19}H_{29}FO_3$ req.	70.3	9.0
7β,15α,17β-Triacetoxy-12,12-difluoro-5α-androstane	156—157	+66 (0.3)	Found	64.05	7.7
			$C_{25}H_{36}F_2O_6$ req.	63.8	7.7

[a] From Me_2CO–light petroleum or Me_2CO–hexane unless otherwise indicated. [b] $CHCl_3$ as solvent unless otherwise indicated. [c] EtOH as solvent. [d] MeOH as solvent. [e] From MeOH–H_2O. [f] From EtOAc–MeOH.

fluorine may act merely as a blocking group and thereby prevent hydroxylation at a standard site. Such blocking is more common with *Ao* than with the other micro-organisms; its occurrence is illustrated by the contrast between the 7β,11α-dihydroxylation of the parent 17-ketone and the monohydroxylation of the 6α-fluoro-17-ketone at the 11α-position by *Ao*. However, the more general outcome is that hydroxylation still occurs, but at an alternative site. For example, with *Cd* the 12β,15α-dihydroxylation mediated by a 3-keto-group is supplanted by 7β-15α-dihydroxylation in the 12,12-difluoro-3-ketone and by 7β-12β-dihydroxylation in the 16,16-difluoro-3-ketone. A striking demonstration of this site shift is found with *Ao* which, although having a marked propensity for 11α-hydroxylation,[5] converts 12,12-difluoro-5α-androstan-17-one into the 7β-hydroxy-derivative. (This result is the more surprising in that with standard substrates 6β- and 7α-hydroxylation is known to require the presence of 11α-hydroxy-compounds for inducing formation of the appropriate enzyme systems [7] and the monohydroxylated products are invariably the 11α-hydroxy-compounds.[5])

(*ii*) The standard sites correspond to centres, depicted as C_z, more distant from the fluorine substituent(s) than C_x and C_y. In these cases the effects of the fluorine substituents should be small provided that hydroxylation of the parent ketones and the fluoro-ketones involves the same enzyme systems acting by broadly similar mechanisms. Examination of the results with all three micro-organisms shows that the introduction of remote fluorines leaves the basic hydroxylation patterns [3-5] unchanged. For example, the 7,7-difluoro- and the 17-fluoro-3-ketones undergo 12β,15α-dihydroxylation with *Cd*. The predilection of *Ao* for 11α-hydroxylation [5] is seen to apply also to the fluoro-ketones, and the extent of hydroxylation with these substrates illustrates a general tendency adumbrated in previous work,[2] *viz.*, that the presence of remote fluorine generally facilitates hydroxylation. Thus, while the parent ketones are largely unaffected by *Ao* under the conditions used here, several of the fluorinated substrates (*e.g.* 3,3-difluoro-5α-androstan-17-one) give the 11α-hydroxy-derivatives cleanly and in yields which are satisfactory for preparative work. These results, together with the convenient routes to a range of steroidal fluoro-ketones,[6] suggest that a sequence of chemical and microbiological stages may provide an efficient means of converting simple steroidal ketones into polyoxygenated fluoro-steroids.

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