Microbiological Hydroxylation. Part XIV.[1] Hydroxylation in the Terminal Rings of Dioxygenated 5α-Androstanes with the Fungi *Wojnowicia graminis* and *Ophiobolus herpotrichus*

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Dioxo-5α-androstanes having one keto-group in a terminal ring (at position 3 or 2, or at position 17) and the second at a middle ring position (7 or 11) are hydroxylated in the other terminal ring (at 17 or 16, or at 3 or 2) by the fungi *Wojnowicia graminis* and *Ophiobolus herpotrichus*. Efficient transformations include the 2α-hydroxylation of 5α-androstane-7,17-dione (with *W. graminis*), the 16-substitution of the 3,11-dione (with *O. herpotrichus*), and the 17β-hydroxylation of 3,7-dioxygenated substrates (with both fungi).

OUR previous work on steroid hydroxylation has been largely concerned with the effect of the substrate's functional groups on the hydroxylation patterns associated with the fungi *Aspergillus ochraceus* and *Calonectria decora*, and those of the *Rhizopus* species. While *A. ochraceus* has a marked propensity for attacking the middle steroidal rings (usually at the 11α-position) hydroxylation by the others can be directed to the middle or the terminal rings by appropriately placed substituents. In extending these studies we have examined three fungi which, from the scant literature, appeared to be promising for terminal ring hydroxylation. (Such processes might be useful with synthetic polycyclic substrates for introducing oxygen groups at positions corresponding to those of the normal steroid substituents.) Of the two micro-organisms whose behaviour is reported here, the first, *Wojnowicia graminis*, is known to hydroxylate pregnenolones at position 21 [2] and androst-4-ene-3,17-dione at the 6β-, 12α-, 14α-, 16α-, and 16β-positions;[3] the second, *Ophiobolus herpotrichus*, also hydroxylates pregnane derivatives at position 21 and has been used for this purpose in a synthesis of aldosterone,[4] but there is little information about its activity towards oxygenated androstanes. (The third fungus, *Daedalea rufescens* will be the subject of a later paper.)

A summary of the present results is shown in Table 1 (which contains some potentially useful hydroxylations) and Table 2 (which lists the unsatisfactory incubations). Table 3 lists the n.m.r. spectra of the steroids, substrates and products, involved here for which spectroscopic data have not appeared in the earlier publications: the arabic serial number sequence of steroids discussed earlier [5] is used in this Table which contains steroids nos. 701—731. New compounds were identified by a combination of spectrometric and chemical methods; for new compounds the n.m.r. signals appear in Table 3, and the other information required for their characterisation is given in Table 4. The microbiological and chemical operations of the present work are routine applications of techniques fully described in earlier parts. This being so, and with the new compounds adequately reported in the Tables, the whole of the Experimental section has been deposited as a Supplementary Publication (No. SUP 21134).†

The first group of substrates in Table 2 are androstanes with a single oxygen (usually a keto-) group at various nuclear positions. In all cases more than half the starting material was unchanged and the hydroxylations led to complex mixtures, most of which were not investigated. Even with androst-4-en-3-one in dimethyl sulphoxide, a system which generally shows high and specific reactivity,[5] only a modest amount of dihydroxylated product was obtained. *O. herpotrichus* was similarly unable to hydroxylate the dioxygenated substrates (the 3,17-compounds, lower part of Table 2) having both substituents in terminal rings. In contrast, these substrates were almost completely utilised by *W. graminis;* although small amounts of several products were isolated the poor total steroid recoveries suggest that many products are being formed. This activity of *W. graminis* is markedly reduced when

† For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1973, Index issue.

[1] Part XIII, M. J. Ashton, A. S. Bailey, and Sir Ewart R. H. Jones, *J.C.S. Perkin I*, 1974, 1665.

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

[3] H. L. Herzog, M. J. Gentles, A. Basch, W. Carcarelli, M. E. A. Zertz, and W. Charney, *J. Org. Chem.*, 1959, **24**, 691.

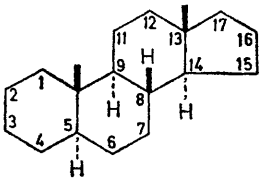
[4] C. Meystre, E. Vischer, and A. Wettstein, *Helv. Chim. Acta*, 1954, **37**, 1548.

[5] 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.

both oxo-groups are in central rings (as shown by the results with the 6,11- and 7,11-diketones).

The main result to emerge from Table 1 is that dioxo-5α-androstanes having one keto-group in a terminal ring (at position 3 or 2, or at position 17) and the second at a middle ring position (7 or 11) are hydroxylated in the other terminal ring (at 17 or 16, or at 3 or 2). With *W. graminis* and possibly also with *O. herpotrichus* replacement of the substrate's terminal keto-group by a hydroxy-group has only a minor effect on the hydroxylation process. Although there are significant differences between the micro-organisms' modes of action it seems that the results can be interpreted broadly along the lines suggested previously for the

Hydroxylations with *Wojnowicia graminis* and *Ophiobolus herpotrichus*



The substrates, all derivatives of 5α-androstane, are indicated by trivial names, *e.g.* 3α-OH–17-CO represents 3α-hydroxy-5α-androstan-17-one. In the ' products ' columns those oxygen functions introduced during the incubation are in bold type; a dash indicates that the substrate was not incubated with the micro-organism specified, and n.i. that no product was isolated (or that a small amount of a mixture of products was obtained). The substrates were introduced as solutions in ethanol (except for one case marked with an asterisk in Table 2), and incubated for the times (usually 3 or 4 days) specified in the Experimental section. The yields are calculated after making an allowance for recovered starting material.

TABLE 1

	Wojnowicia graminis						*Ophiobolus herpotrichus*				
Substrate	Substrate recovered (%)	Main monohydroxylation product(s)		Other products			Substrate recovered (%)	Main monohydroxylation product(s)		Other products	
2,7-$(CO)_2$	15	2β,	17β-$(OH)_2$ 19%	2β,	18-$(OH)_2$ 17β-OH	10% 9%					
3,7-$(CO)_2$	32	3β,	17β-$(OH)_2$ 41%				12	3β,17β-$(OH)_2$	62%		
		3α,	17β-$(OH)_2$ 27%								
3α-OH–7-CO	6		17β-OH 55%								
3β-OH–7-CO	4		17β-OH 63%								
					17-CO	5%					
				3-CO,	17β-OH	3%					
					7α,17β$(OH)_2$	2%					
3,11-$(CO)_2$	24		17β-OH 28%	3α,	17β-$(OH)_2$	8%	46	16-CO	38%	16β-OH	11%
		3β,	17β-$(OH)_2$ 13%	3β,	16β-$(OH)_2$	6%		3β-OH,16-CO	21%	17β-OH	11%
										3β,16β-$(OH)_2$	5%
										3β,17β-$(OH)_2$	5%
3β-OH–11-CO	48		16β-OH 48%								
			17β-OH 22%								
7,17-$(CO)_2$	13	2α-	OH 34%	3β-	OH	11%	38	3β,17β-$(OH)_2$	43%		
		2α,	17β-$(OH)_2$ 30%	3β,	17β-$(OH)_2$	5%		3β- OH	32%		
7-CO–17β-OH	14	2α-	OH 58%	2α, 7α,	$(OH)_2$	15%					
				3β-	(OH)	11%					
				3β, 7α-	$(OH)_2$	5%					
11,17-$(CO)_2$							63	3α- OH	45%		
								3α,17β-$(OH)_2$	44%		
11-CO–17β-OH	39	3β-	OH 35%	3α-	OH	11%	72	3α- OH	95%		

TABLE 2

	Wojnowicia graminis				*Ophiobolus herpotrichus*		
Substrate	Substrate recovered (%)	Main monohydroxylation product(s)	Other products		Substrate recovered (%)	Main monohydroxylation product(s)	
3-CO	72	9α, 17β-$(OH)_2$ 13%			85	n.i.	
3β-OH	22	9α, 17β-$(OH)_2$ 14%					
3-CO-$\Delta^{4,5}$	63	6β,11α- $(OH)_2$ 21%	4,5α-H_2	12%			
7-CO	64	n.i.			68	n.i.	
11-CO	58	n.i.			50	n.i.	
17-CO	74	n.i.			87	n.i.	
3,17-$(CO)_2$	7	1α, 17β-$(OH)_2$ 7%	1α,3α, 9α,17β-$(OH)_4$	1%	80	n.i.	
		1α,3β- $(OH)_2$ 6%	7α- OH	1%			
		3β, 9α-$(OH)_2$ 5%					
		9α-OH 5%					
6-11-$(CO)_2$	76	n.i.					
7,11-$(CO)_2$	65	n.i.					
3β-OH–17-CO	5	1α- OH 22%	9α- OH	7%	79	n.i.	
		14α-OH 13%	5α- OH	7%			
			1α, 7α- $(OH)_2$	2%			
3-CO–17β-OH	7	1α- OH 5%	1α,3β- $(OH)_2$	2%	81	n.i.	
			1α,3α, 9α- $(OH)_2$	2%			
			6β- OH	1%			

* As solution in dimethyl sulphoxide.

fungi *C. decora*[5] and *R. nigricans*.[6] A triangular arrangement on the enzyme surface of three sites is again assumed (see Scheme), but while the two terminal ones are thought to have both binding and hydroxylating capabilities the central site appears to be suitable for binding only. Attachment to the enzyme surface of the 3,11- and 11,17-dioxygenated substrates in the normal mode is followed by hydroxylation in the unsubstituted terminal ring; with 2,7- and 3,7-disubstituted androstanes similar processes involve binding in the reverse mode. The notion that the central site has only low hydroxylating activity is based on the results with the 3,17-dioxygenated substrates. Here binding is not followed by the specific hydroxylation at position 7 or 11 which would be expected from the presence of a dual-purpose central site. Little hydroxylation occurs with *O. herpotrichus*, but the more active micro-organism *W. graminis* gives a range of products presumably formed by reaction at a number of

TABLE 3

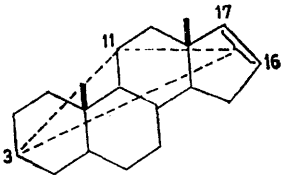
N.m.r. signals

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	
701	5α-Androstane-1,3,17-trione	19	8·73	8·74			
		18	9·10	9·10			
702	2α-Hydroxy-5α-androstane-7,17-dione	19	8·89	8·90	H-2	6·20	m (22)
		18	9·13	9·13			
703	7α-Hydroxy-5α-androstane-3,17-dione	19	8·97	8·96	H-7	6·00	m (7)
		18	9·11	9·09			
704	14-Hydroxy-5α-androstane-3,17-dione	19	8·95	8·96			
		18	8·99	8·98			
705	2,7-Dioxo-5α-androstan-17β-yl acetate	19	8·96	8·95	H-17	5·35	*4* (9, 8)
		18	9·22	9·21			
706	17β-Hydroxy-5α-androstane-3,7-dione	19	8·71	8·70	H-17	6·34	t (8)
		18	9·22	9·23			
707	17β-Hydroxy-5α-androstane-3,11-dione	19	8·77	8·76	H-17	6·14	t (8)
		18	9·28	9·27			
708	1α,3β-Dihydroxy-5α-androstan-17-one	19	9·18	9·16	H-1	6·17	m (4)
		18	9·15	9·13	H-3	5·95	m (20)
709	1α-Hydroxy-17-oxo-5α-androstan-3β-yl acetate	19	9·16	9·14	H-1	5·16	m (8)
		18	9·13	9·12	H-3	4·92	*7* (10, 10, 5, 5)
710	1α,3β-Diacetoxy-5α-androstan-17-one	19	9·08	9·09	H-1	5·05	t (3)
		18	9·15	9·14	H-3	5·05	*7* (10, 10, 5, 5)
711	1α,17β-Dihydroxy-5α-androstan-3-one	19	8·99	8·97	H-1	5·86	m (7)
		18	9·23	9·24	H-17	6·30	t (8)
712	1α,19β-Diacetoxy-5α-androstan-3-one	19	8·93	8·90	H-1	4·81	t (2)
		18	9·20	9·20	H-17	5·40	*4* (9, 8)
713	2α,17β-Dihydroxy-5α-androstan-7-one *	19	8·91	8·92	H-2	6·22	m (23)
		18	9·26	9·27	H-17	6·30	t (8)
714	2α,17β-Diacetoxy-5α-androstan-7-one	19	8·86	8·86	H-2	5·08	m (23)
		18	9·25	9·23	H-17	5·34	*4* (9, 8)
715	2β,17β-Dihydroxy-5α-androstan-7-one	19	8·68	8·68	H-2	5·80	m (8)
		18	9·26	9·27	H-17	6·32	t (8)
716	2β,17β-Diacetoxy-5α-androstan-7-one	19	8·78	8·78	H-2	4·88	m (8)
		18	9·20	9·22	H-17	5·38	*4* (9, 8)
717	2β,18-Diacetoxy-5α-androstan-7-one	19	8·78	8·77	H-2	4·85	m (9)
		18			H-18	5·86 6·30	d (10) d (10)
718	3β,5-Dihydroxy-5α-androstan-17-one	19	8·98	8·99	H-3	5·90	m (20)
		18	9·13	9·13			
719	3β,14-Dihydroxy-5α-androstan-17-one	19	9·16	9·17	H-3	6·40	m (20)
		18	9·01	9·01			
720	3β,17β-Diacetoxy-5α-androstan-7-one	19	8·89	8·87	H-3	5·35	m (25)
		18	9·22	9·20	H-17		
721	3β,17β-Dihydroxy-5α-androstan-11-one	19	8·97	8·97	H-3	6·45	m (20)
		18	9·31	9·30	H-17	6·36	t (8)
722	3β,17β-Diacetoxy-5α-androstan-11-one	19	8·95	8·94	H-3	5·20	m (20)
		18	9·26	9·25	H-17	5·32	*4* (9, 8)
723	6β,17β-Dihydroxy-5α-androstan-3-one	19	8·79	8·77	H-6	6·25	t (3)
		18	9·21	9·20	H-17	6·35	t (8)
724	9α,17β-Dihydroxy-5α-androstan-3-one	19	8·86	8·85	H-17	6·30	t (8)
		18	9·22	9·21			
725	9α-Hydroxy-3-oxo-5α-androstan-17β-yl acetate	19	8·86	8·84	H-17	5·30	*4* (9, 8)
		18	9·18	9·17			
726	1α,3β-7α-Triacetoxy-5α-androstan-17-one	19	9·08	9·07	H-1	5·02	m (10)
					H-3	5·03	m (25)
		18	9·16	9·15	H-7	4·97	m (3)
727	3β,17β-Diacetoxy-5α-androstan-1α-ol	19	9·17	9·16	H-1	6·17	m (8)
					H-3	4·90	*7* (10, 10, 5, 5)
		18	9·21	9·21	H-17	5·40	4 (9, 8)
728	5α-Androstane-3β,7α,17β-triol	19	9·18	9·19	H-3 H-7	6·28	m (25)
		18	9·26	9·26	H-17		
					H-3	5·30	m (20)
729	3β,7α,17β-Triacetoxy-5α-androstane	19	9·14	9·13	H-7	5·09	m (8)
		18	9·21	9·19	H-17	5·38	*4* (9, 8)
730	5α-Androstane-3β,9α-17β-triol	19	9·05	9·06	H-3	6·32	m (23)
		18	9·24	9·24	H-17		
					H-1	4·93	m (8)
731	1α,3α,17β-Triacetoxy-5α-androstan-9α-ol	19	9·00	9·00	H-3	5·10	m (8)
		18	9·18	9·19	H-17	5·38	*4* (9, 8)

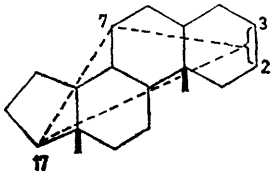
* Not fully characterised.

[a] Ref. 5.



Normal mode

Hydroxylation of 3,11-dioxygenated substrates at 17 or 16, and of 11,17-compounds at 3



Reverse mode

Hydroxylation of 2,7- and 3,7-dioxygenated substrates at 17, and of 7,17-compounds at 3 or 2

SCHEME Possible directing effects of substituents

TABLE 4

Characterisation of new compounds

Compound	M.p. (°C) *	$[\alpha]_D$ (*c*) †	Analytical figures (%)		
				C	H
2α-Hydroxy-5α-androstane-7,17-dione	201—203	−2° (0·8)	Found $C_{19}H_{28}O_3$ req.	75·0 75·0	9·1 9·3
7α-Hydroxy-5α-androstane-3,17-dione	158—160	+81 (0·7)	Found $C_{19}H_{28}O_3$ req.	75·2 75·0	9·3 9·3
9α-Hydroxy-5α-androstane-3,17-dione	212—214	+71 (0·1)	Found $C_{19}H_{28}O_3$ req.	74·8 75·0	9·2 9·3
2,7-Dioxo-5α-androstan-17β-yl acetate	180—181·5	−31 (0·6)	Found $C_{21}H_{30}O_4$ req.	72·5 72·8	8·7 8·7
2α,17β-Diacetoxy-5α-androstan-7-one	152—154	−83 (0·5)	Found $C_{23}H_{34}O_5$ req.	70·8 70·75	8·7 8·8
2β,17β-Dihydroxy-5α-androstan-7-one	198—200 ‡	−52 (0·5)	Found $C_{19}H_{30}O_3$ req.	74·55 74·5	10·0 9·9
2β,17β-Diacetoxy-5α-androstan-7-one	218—221	−50 (0·3)	Found $C_{23}H_{34}O_5$ req.	70·9 70·75	8·6 8·8
2β,18-Diacetoxy-5α-androstan-7-one	188—191	−36·5 (·04)	(Found M^+ ($C_{23}H_{34}O_5$ req. M	390·2409) 390·2406)	
3β,9α-Dihydroxy-5α-androstan-17-one	192—194	+73 (0·5)	Found $C_{19}H_{30}O_3$ req.	74·6 74·5	9·9 9·9
3β,14-Dihydroxy-5α-androstan-17-one	218—220	+69 (0·9)	Found $C_{19}H_{30}O_3$	74·5 74·5	9·65 9·9
9α-Hydroxy-3-oxo-5α-androstan-17β-yl acetate	201—204	+14 (0·3)	Found $C_{21}H_{34}O_4$ req.	72·2 72·4	9·1 9·25
1α,3β,7α-Triacetoxy-5α-androstan-17-one	214—215	−93 (0·2)	Found $C_{25}H_{36}O_7$ req.	66·9 66·9	7·8 8·1
3β,17β-Diacetoxy-5α-androstan-1α-ol	199—201	+9 (0·65)	Found $C_{23}H_{36}O_5$ req.	70·4 70·4	9·1 9·2
5α-Androstane-3β,7α,17β-triol	225—226 §	−9 ¶ (0·7)	Found $C_{19}H_{32}O_3$ req.	74·1 74·0	10·25 10·45
5α-Androstane-3β,9α,17β-triol	202—203 ‖	−5 (1·0)	Found $C_{19}H_{32}O_3$ req.	74·2 74·0	10·35 10·45
1α,3α,17β-Triacetoxy-5α-androstan-9α-ol	113—115	−35 (0·75)	Found $C_{25}H_{38}O$ req.	66·9 66·6	8·6 8·5

* From acetone–hexane unless otherwise specified. † Rotations determined with $CHCl_3$ as solvent unless otherwise indicated. ‡ From EtOAc. § From EtOH–H_2O. ¶ Rotation determined with EtOH as solvent. ‖ From MeOH.

[6] (*a*) 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. (*b*) V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. Pragnell, *ibid.*, p. 1500.

sites having comparable activities. This simple model does not, of course, account for more subtle features such as the contrast between the 3β-hydroxylation of 5α-androstane-7,17-dione and the 3α-substitution of the 11,17-dione by *O. herpotrichus*.

For synthetic purposes the most useful transformations are the introduction of a 2α-hydroxy-group † into 5α-androstane-7,17-dione (with *W. graminis*) and the 16-substitution of the 3,11-dione (with *O. herpotrichus*); both fungi effectively hydroxylate 3,7-dioxygenated androstanes at the 17β-position.

† Previously microbiological 2α-hydroxylation has been encountered only once, with *Norcadia italica*.[7]

[7] R. Modelli, *Ann. Chim.* (*Italy*), 1965, **55**, 310.

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