

## Microbiological Hydroxylation. Part XVI.<sup>1</sup> Incubation of Derivatives (mainly Acetals) of 5 $\alpha$ -Androstane Ketones with the Fungi *Calonectria decora*, *Aspergillus ochraceus*, and *Rhizopus nigricans*

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Acetals and enol ethers derived from oxoandrostanones are less reactive than the parent ketones towards the title fungi. None of the derivatives is hydroxylated by *Rhizopus nigricans*, and only one by *Aspergillus ochraceus*. With *Calonectria decora* the acetals generally give patterns of hydroxylation similar to, but less specific than, those of the corresponding ketones. 16,16'-Ethylenedioxy-5 $\alpha$ -androstane is exceptional in that its hydroxylation with *Calonectria decora* to a 6 $\alpha$ ,12 $\beta$ -dihydroxy-product is more efficient than the 6 $\alpha$ ,11 $\alpha$ -dihydroxylation of 5 $\alpha$ -androstane-16-one.

EARLIER papers in this series describe the hydroxylation of a range of mono- and di-oxygenated 5 $\alpha$ -androstanones with *Calonectria decora*<sup>2,3</sup> (*Cd*), *Aspergillus ochraceus*<sup>4</sup> (*Ao*), and *Rhizopus nigricans*<sup>5,6</sup> (*Rn*). Most of the androstane derivatives were ketones, alcohols, and hydroxy-ketones, and our main object was to examine the variations in the patterns of hydroxylation associated with changes in the positions of the substrates' oxygenated groups. The present work is concerned with the influence on hydroxylation of using simple derivatives (mainly acetals) of the ketones, *i.e.* the effect of modifying the ketone group chemically rather than changing its position in the steroid nucleus.

Table I summarises the microbiological results obtained

<sup>1</sup> Part XV, A. M. Bell, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. Pendlebury, *J.C.S. Perkin I*, 1975, 357.

<sup>2</sup> 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.

<sup>3</sup> 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.

by incubating ketone derivatives with the three fungi. The substrates contain an acetal group, either alone or in conjunction with a second (alcoholic or ketonic) oxygenated group. A few enol ethers and one thioacetal, and 11 $\alpha$ -hydroxy-5 $\alpha$ -androstane-3-one (which was not studied in the earlier work) are also included. Table 2 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<sup>2</sup> is used in this Table which contains steroids nos. 754—789. The structures of new compounds follow, as usual, from a combination of spectrometric and chemical methods.

<sup>4</sup> 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.

<sup>5</sup> 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.

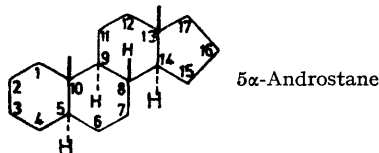
<sup>6</sup> V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. Pragnell, *J.C.S. Perkin I*, 1973, 1500.

For new compounds the n.m.r. signals appear in Table 2, and the other information required for their characterisation is given in Table 3. The microbiological and chemical operations of the present work<sup>7</sup> 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 Experimental section

of an acetal by *Ao*, the 7 $\beta$ ,11 $\alpha$ -dihydroxylation of 17,17-ethylenedioxy-5 $\alpha$ -androstan-3 $\beta$ -ol, may be due to the ability of this organism to metabolise steroids containing bulky C-17 substituents.<sup>8</sup>

The hydroxylations of the acetals by *Cd* are usually less specific than those of the parent ketones, but the patterns of substitution are generally similar. Thus,

TABLE 1  
Hydroxylation of 5 $\alpha$ -androstan derivatives with *Calonectria decora* (*Cd*), *Aspergillus ochraceus* (*Ao*), and *Rhizopus nigricans* (*Rn*)



Substrates are indicated by abbreviated names or symbols, e.g. 3-CO-17,17- $\text{O}$ ] represents 17,17-ethylenedioxy-5 $\alpha$ -androstan-3-one. In the 'products' column those oxygen functions introduced during the incubations are in bold type, and n.i. indicates no products isolated. The entries under conditions refer to the use of ethanol (E) and dimethyl sulphoxide (D) as solvents 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)
3,3- $\text{O}$ ]	<i>Cd</i>	D4	37%	<b>6<math>\alpha</math>,12<math>\beta</math>-</b> (OH) <sub>2</sub>	27% <b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> 14%
	<i>Ao, Rn</i>	D4	>90	n.i.	
3,3- $\text{S}$ ]	<i>Cd, Ao, Rn</i>	D4	>90	n.i.	
16,16- $\text{O}$ ]	<i>Cd</i>	D4	29	<b>6<math>\alpha</math>,12<math>\beta</math>-</b> (OH) <sub>2</sub>	71 <b>6-CO-12<math>\beta</math>-</b> OH 5
	<i>Ao, Rn</i>	D4	>90	n.i.	
17,17- $\text{O}$ ]	<i>Cd</i>	D4	53	<b>7<math>\beta</math>,12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>3</sub>	47 <b>7<math>\beta</math>,12<math>\beta</math>-</b> (OH) <sub>2</sub> 6
	<i>Rn</i>	D4	>90	<b>1<math>\beta</math>,7<math>\beta</math>,12<math>\beta</math>-</b> (OH) <sub>3</sub>	21
3-CO-17,17- $\text{O}$ ]	<i>Cd</i>	D4	12	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	25
17-CO-3,3- $\text{O}$ ]	<i>Cd</i>	D4	2	<b>1<math>\beta</math>,6<math>\alpha</math>-</b> (OH) <sub>2</sub>	22 <b>7<math>\beta</math>,12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>3</sub> 6
				<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	11 <b>3-CO-12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> 5
					<b>6<math>\alpha</math>,11<math>\alpha</math>-</b> (OH) <sub>2</sub> 5
					<b>1<math>\beta</math>, 15<math>\alpha</math>-</b> (OH) <sub>2</sub> 2
	<i>Ao, Rn</i>	D4	>90	n.i.	
3,3:17,17-( $\text{O}$ )] <sub>2</sub>	<i>Cd</i>	D4	10	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	25 <b>7<math>\beta</math>,12<math>\beta</math>-</b> (OH) <sub>2</sub> 13
	<i>Ao, Rn</i>	D4	>90	n.i.	
3 $\alpha$ ,OH-17,17- $\text{O}$ ]	<i>Cd</i>	D4	8	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	37 <b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> -17-CO 7
				<b>1<math>\beta</math>, 15<math>\alpha</math>-</b> (OH) <sub>2</sub> -17-CO 16	<b>1<math>\beta</math>,6<math>\alpha</math>-</b> (OH) <sub>2</sub> -17-CO 4
3 $\beta$ ,OH-17,17- $\text{O}$ ]	<i>Cd</i>	D4	0	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	29 <b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> -17-CO 6
	<i>Ao</i>	D4	0	<b>3-CO-12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	10 <b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> -3,17-(CO) <sub>2</sub> 3
				<b>7<math>\beta</math>,11<math>\alpha</math>-</b> (OH) <sub>2</sub>	34 <b>11<math>\alpha</math>-</b> OH 6
				<b>1<math>\beta</math>, 11<math>\alpha</math>-</b> (OH) <sub>2</sub>	16
11 $\alpha$ ,OH-3-CO	<i>Cd</i>	D4	4	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	24
11 $\alpha$ ,OH-3,3- $\text{O}$ ]	<i>Cd</i>	D4	20	<b>12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub>	28 <b>6<math>\alpha</math>-</b> OH 11
				<b>6<math>\alpha</math>,12<math>\beta</math>-</b> (OH) <sub>2</sub>	18
3-OMe- $\Delta^2$	<i>Ao, Rn</i>	D4	>90	n.i.	
	<i>Cd</i>	E4	0	3-CO	35 <b>3-CO-12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>2</sub> 6
					<b>3<math>\beta</math>, 12<math>\beta</math>,15<math>\alpha</math>-</b> (OH) <sub>3</sub> 5
3-OMe- $\Delta^{3,5}$	<i>Cd, Ao, Rn</i>	D4	>90	n.i.	
3-OMe-17-CO- $\Delta^{3,5}$	<i>Cd</i>	D4	0	3-CO- <b>6<math>\beta</math>-</b>	<b>15<math>\alpha</math>-</b> (OH) <sub>2</sub> - $\Delta^4$ 29 <b>3-CO-<math>\Delta^4</math></b> 5
				3-CO- <b>6<math>\beta</math>-</b>	<b>15<math>\alpha</math>-</b> (OH)- $\Delta^4$ 9 <b>3,6-(CO)<sub>2</sub>-15<math>\alpha</math>-OH</b> 7
				3-CO-	<b>15<math>\alpha</math>-</b> OH- $\Delta^4$ 9
	<i>Rn</i>	D4	>90	n.i.	

has been deposited as Supplementary Publication No. SUP 21363 (12 pp., 1 microfiche).\*

Acetals are generally less reactive than the corresponding ketones towards *Ao* and *Rn*. For example, the 3,17-diketone is hydroxylated at position 11 by *Ao*<sup>4</sup> and at positions 7 and 11 by *Rn*,<sup>3</sup> but neither fungus attacks the corresponding diacetal. The only appreciable conversion

while the 3-ketone gives clean 12 $\beta$ ,15 $\alpha$ -dihydroxylation,<sup>2</sup> the acetal forms both 12 $\beta$ ,15 $\alpha$ - and 6 $\alpha$ ,12 $\beta$ -dihydroxy-derivatives, the latter being the major product. A notable feature of the incubation of 3,3-ethylenedioxy-5 $\alpha$ -androstan-11 $\alpha$ -ol and 11 $\alpha$ -hydroxy-5 $\alpha$ -androstan-3-one is the formation of 12 $\beta$ -hydroxylated products; such

<sup>7</sup> A. detailed account is given by A. Pendlebury, D.Phil. Thesis, Oxford, 1972.

<sup>8</sup> 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.

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

TABLE 2  
N.m.r. signals

The results, presented in the form used earlier,<sup>a</sup> were obtained by examining solutions in CDCl<sub>3</sub> at 100 MHz

No.	Compound	$\tau_2$	$\tau_3$ (calc)	>CH-OR etc.		
754	3,3-Ethylenedithio-5 $\alpha$ -androstane	19	9.20	9.20		
		18	9.32	9.31		
755	16,16-Ethylenedioxy-5 $\alpha$ -androstane	19	9.22	9.22		
		18	9.10	9.10		
756	17,17-Ethylenedioxy-5 $\alpha$ -androstane-3-one	19	8.98	8.98		
		18	9.14	9.14		
757	3,3-Ethylenedioxy-5 $\alpha$ -androstane-17-one	19	9.17	9.17		
		18	9.15	9.15		
758	5 $\alpha$ -Androstane-1,7,12-trione	19	8.49	8.47		
		18	8.99	8.96		
759	5 $\alpha$ -Androstane-6,12,16-trione	19	9.12	9.14		
		18	8.78	8.80		
760	3-Methoxy-5 $\alpha$ -androst-2-ene	19	9.24	9.26	H-2	5.50
		18	9.30	9.30	OMe	6.50
761	3-Methoxyandrostane-3,5-diene	19	9.05	9.06	H-4	4.98
		18	9.25	9.27	H-6	4.89
762	3-Methoxyandrostane-3,5-dien-17-one	19	9.03	9.04	OMe	6.46
		18	9.13	9.11	H-4	4.97
763	17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\alpha$ -ol	19	9.22	9.22	H-3	6.00
		18	9.18	9.16	Acetal	6.16
764	17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\beta$ -ol	19	9.18	9.19	H-3	7 (10, 10, 5, 5)
		18	9.16	9.16	Acetal	6.10
765	3,3-Ethylenedioxy-5 $\alpha$ -androstane-11 $\alpha$ -ol	19	9.15	9.17	H-11	6.02
		18	9.28	9.29	Acetal	6.10
766	12 $\beta$ -Hydroxy-5 $\alpha$ -androstane-6,16-dione	19	9.22	9.21	H-12	6.26
		18	9.07	9.10		
767	15 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-3,17-dione	19	8.94	8.95	H-15	5.58
		18	9.07	9.07		
768	15 $\alpha$ -Hydroxy-5 $\alpha$ -androst-1-ene-3,17-dione	19	8.95	8.96	H-1	4.14
		18	9.06	9.07	H-2	2.89
769	16 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-3,6,17-trione	19	9.00	9.00	H-15	5.58
		18	9.06	9.05		
770	3,3-Dimethoxy-5 $\alpha$ -androstane	19	9.21	9.22	OMe	6.80
		18	9.32	9.30	OMe	6.86
771	6 $\alpha$ ,11 $\alpha$ -Diacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	19	9.00	9.04	H-6	5.34
		18	9.24	9.25	Acetal	6.05
772	6 $\alpha$ ,12 $\beta$ -Dihydroxy-5 $\alpha$ -androstane-16-one	19	9.15	9.14	H-6	6.55
		18	9.11	9.09	H-12	6.35
773	3,3-Ethylenedioxy-1 $\beta$ ,6 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	19	9.13	9.09	H-1	6.40
		18	9.14	9.15	Acetal	6.06
774	3,3-Ethylenedioxy-1 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	19	9.13	9.11	H-1	6.42
		18	9.13	9.12	H-15	5.56
775	3,3-Ethylenedioxy-6 $\alpha$ ,11 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	19	9.01	9.02	H-6	6.58
		18	9.12	9.12	Acetal	6.05
776	6 $\alpha$ ,11 $\alpha$ -Diacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane-17-one	19	8.99	9.01	H-6	5.31
		18	9.08	9.08	H-11	4.83
777	6 $\beta$ ,15 $\alpha$ -Dihydroxyandrost-4-ene-3,17-dione	19	8.58	8.55	Acetal	6.04
		18	9.03	9.02	H-4	4.20
778	7 $\beta$ ,12 $\beta$ -Dihydroxy-5 $\alpha$ -androstane-3,17-dione	19	8.92	8.92	H-7	6.53
		18	9.02	8.99	H-12	6.23
779	17,17-Ethylenedioxy-12 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-3-one	19	8.96	8.96	H-12	6.23
		18	9.04	9.02	H-15	5.95
780	3,3-Ethylenedioxy-12 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	19	9.14	9.14	H-12	6.17
		18	9.04	9.04	H-15	5.55
781	1 $\beta$ ,7 $\beta$ ,12 $\beta$ -Trihydroxy-5 $\alpha$ -androstane-17-one	19	9.04	9.06	Acetal	6.06
		18	9.11	9.08	H-1	6.42
782	3 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-17-one	19	9.18	9.18	H-7	6.42
		18	9.04	9.02	H-3	5.94
783	17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -triol	19	9.20	9.19	H-12	6.15
		18	9.08	9.11	H-3	5.52
784	17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\beta$ ,12 $\beta$ ,15 $\alpha$ -triol	19	9.17	9.16	H-12	6.15
		18	9.08	9.11	H-15	5.98
785	6 $\alpha$ ,11 $\alpha$ ,12 $\beta$ -Triacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	19	9.06	9.04	Acetal	6.00
		18	9.08	9.11	H-6	5.19
786	11 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-3-one	19	9.17	9.18	H-11	5.00
		18	8.96	8.96	H-12	5.31
787	11 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Triacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	19	9.04	9.07	H-11	5.00
		18	9.20	9.21	H-12	5.82
788	7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-3,17-dione	19	9.12	9.11	H-7	6 (9, 9, 3)
		18	8.90	8.91	H-11	4.73
789	3,3-Ethylenedioxy-7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -trihydroxy-5 $\alpha$ -androstane-17-one	19	9.13	9.12	H-12	6.22
		18	9.03	9.01	H-15	5.22

<sup>a</sup> Ref. 2.

hydroxylation, at a position adjacent to a hydroxy-function in the substrate, is an uncommon microbiological process. Dihydroxylations of the 16-oxygenated substrates (6 $\alpha$ ,11 $\alpha$ - with the ketone and 6 $\alpha$ ,12 $\beta$ - with the acetal) provide an interesting variation in having one

TABLE 3

Characterisation of new compounds

Compound	M.p. (°C) (cryst. solvent)	[ $\alpha$ ] <sub>D</sub> (°) (c)	Analyses (%)	C	H
5 $\alpha$ -Androstane-1,7,12-trione	250-251 (MeOH)	+62 Found (0.2)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	75.5	8.6
5 $\alpha$ -Androstane-6,12,16-trione	242-244 (MeOH)	-51 Found (0.7)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	75.3	8.6
3-Methoxy-5 $\alpha$ -androst-2-ene	80-81 (MeOH-C <sub>4</sub> H <sub>9</sub> N)	+57 Found (1.0)	C <sub>22</sub> H <sub>38</sub> O req.	83.3	11.0
3,3-Ethylenedioxy-5 $\alpha$ -androstane-11 $\alpha$ -ol	156-157.5 (Me <sub>2</sub> CO-hexane)	-23 Found (0.3)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	75.3	10.3
6 $\alpha$ -Hydroxy-5 $\alpha$ -androst-1-ene-3,17-dione	203-205 (Me <sub>2</sub> CO-hexane)	+143 Found (1.0)	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub> req.	75.5	8.8
12 $\beta$ -Hydroxy-5 $\alpha$ -androstane-6,16-dione	187-189 (Me <sub>2</sub> CO-hexane)	-174 Found (0.2)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	75.0	9.4
15 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-3,17-dione	171-172 (EtOAc-hexane)	+115 Found (0.9)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	74.9	9.3
15 $\alpha$ -Hydroxy-5 $\alpha$ -androst-1-ene-3,17-dione	213-215 (Me <sub>2</sub> CO-hexane)	+159 Found (0.9)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	75.4	8.5
15 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-3,6,17-trione	224-226 (EtOAc-hexane)	+103 Found (1.0)	C <sub>19</sub> H <sub>26</sub> O <sub>4</sub> req.	71.8	8.35
3,3-Ethylenedioxy-5 $\alpha$ -androstane-6 $\alpha$ ,12 $\beta$ -diol	185-188 (Me <sub>2</sub> CO-hexane)	+14 Found (0.9)	C <sub>21</sub> H <sub>34</sub> O <sub>4</sub> req.	72.1	9.6
6 $\alpha$ ,12 $\beta$ -Dihydroxy-5 $\alpha$ -androstane-17-one	214-216 (MeOH)	-136 Found (0.9)	C <sub>19</sub> H <sub>28</sub> O <sub>3</sub> req.	74.4	10.0
6 $\alpha$ ,11 $\alpha$ -Diacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	173-175 (Me <sub>2</sub> CO-hexane)	-22 Found (0.15)	C <sub>25</sub> H <sub>38</sub> O <sub>6</sub> req.	69.3	9.9
7 $\beta$ ,12 $\beta$ -Dihydroxy-5 $\alpha$ -androstane-17-one	178-179 (Me <sub>2</sub> CO-hexane)	+100 Found (1.0)	C <sub>19</sub> H <sub>28</sub> O <sub>3</sub> req.	74.4	9.8
3,3-Ethylenedioxy-5 $\alpha$ -androstane-12 $\beta$ ,15 $\alpha$ -diol	193-195 (Me <sub>2</sub> CO-hexane)	+32 Found (1.0)	C <sub>21</sub> H <sub>34</sub> O <sub>4</sub> req.	72.3	9.7
3,3-Ethylenedioxy-1 $\beta$ ,6 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	235-237 (Me <sub>2</sub> CO-hexane)	+91 † Found (0.8)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.3	8.6
3,3-Ethylenedioxy-1 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	219-221 (Me <sub>2</sub> CO-hexane)	+78 † Found (0.15)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.4	8.7
3,3-Ethylenedioxy-6 $\alpha$ ,11 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	260-262 (Me <sub>2</sub> CO-hexane)	+63 † Found (0.4)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.2	8.85
6 $\alpha$ ,11 $\alpha$ -Diacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane-17-one	232-234 (Me <sub>2</sub> CO-hexane)	+51 Found (0.2)	C <sub>25</sub> H <sub>38</sub> O <sub>7</sub> req.	66.8	8.0
6 $\beta$ ,15 $\alpha$ -Dihydroxyandrost-4-ene-3,17-dione	203-205 (Me <sub>2</sub> CO-hexane)	+30 Found (0.8)	C <sub>19</sub> H <sub>26</sub> O <sub>4</sub> req.	71.6	8.4
7 $\beta$ ,12 $\beta$ -Dihydroxy-5 $\alpha$ -androstane-3,17-dione	233-235 (Me <sub>2</sub> CO-hexane)	+109 Found (1.05)	C <sub>19</sub> H <sub>26</sub> O <sub>4</sub> req.	71.5	8.8
17,17-Ethylenedioxy-12 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-3-one	203-205 (Me <sub>2</sub> CO-hexane)	+30 Found (0.8)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.4	8.9
3,3-Ethylenedioxy-12 $\beta$ ,15 $\alpha$ -dihydroxy-5 $\alpha$ -androstane-17-one	218-220 (Me <sub>2</sub> CO-hexane)	+83 † Found (1.0)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.2	8.73
5 $\alpha$ -Androstane-7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -triol	229-230 (Me <sub>2</sub> CO)	+66 † Found (0.15)	C <sub>19</sub> H <sub>28</sub> O <sub>3</sub> req.	74.0	10.55
7 $\beta$ ,15 $\alpha$ -Isopropylidenedioxy-5 $\alpha$ -androstane-12 $\beta$ -ol	157-158 (hexane)	+14 Found (0.3)	C <sub>22</sub> H <sub>34</sub> O <sub>3</sub> req.	76.1	10.5
1 $\beta$ ,7 $\beta$ ,12 $\beta$ -Trihydroxy-5 $\alpha$ -androstane-17-one	235-238 (Me <sub>2</sub> CO-hexane)	+99 † Found (0.1)	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub> req.	70.7	9.15
3 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-17-one	223-225 (Me <sub>2</sub> CO-hexane)	+89 † Found (0.6)	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub> req.	70.8	9.4
17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -triol	184-186 (Me <sub>2</sub> CO-hexane)	+25 † Found (1.0)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	68.5	9.25
17,17-Ethylenedioxy-5 $\alpha$ -androstane-3 $\beta$ ,12 $\beta$ ,15 $\alpha$ -triol	232-234 (Me <sub>2</sub> CO-hexane)	+73 † Found (0.7)	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub> req.	69.1	9.5
6 $\alpha$ ,11 $\alpha$ ,12 $\beta$ -Triacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	168-170 (Me <sub>2</sub> CO-hexane)	+17 Found (0.1)	C <sub>27</sub> H <sub>40</sub> O <sub>8</sub> req.	65.8	8.2
7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-17-one	175-176 (Me <sub>2</sub> CO-hexane)	+167 Found (0.7)	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub> req.	70.8	9.7
11 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-3-one	110-112 (Me <sub>2</sub> CO-hexane)	+38 † Found (0.5)	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub> req.	71.0	9.5
11 $\alpha$ ,12 $\beta$ ,15 $\alpha$ -Triacetoxy-3,3-ethylenedioxy-5 $\alpha$ -androstane	203-204 (Me <sub>2</sub> CO-hexane)	+30 Found (0.4)	C <sub>27</sub> H <sub>40</sub> O <sub>8</sub> req.	65.9	8.1
7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -Trihydroxy-5 $\alpha$ -androstane-3,17-dione	246-248 (Me <sub>2</sub> CO-hexane)	+124 † Found (0.1)	C <sub>19</sub> H <sub>28</sub> O <sub>5</sub> req.	67.7	8.4
3,3-Ethylenedioxy-7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -trihydroxy-5 $\alpha$ -androstane-17-one	194-196 (Me <sub>2</sub> CO)	+98 † Found (0.4)	C <sub>21</sub> H <sub>34</sub> O <sub>6</sub> req.	66.2	8.4

\* In CHCl<sub>3</sub> unless otherwise indicated. † In EtOH. ‡ In dioxan.

common and one different point of attack. With the 17-oxygenated androstanes however the parallel between acetal and ketone disappears: the acetal is converted into the 7 $\beta$ ,12 $\beta$ -disubstituted derivative\* which is then further hydroxylated at the 1 $\beta$ - or 15 $\alpha$ -position, whereas the ketone undergoes 1 $\beta$ ,6 $\alpha$ -dihydroxylation.<sup>2</sup> (The behaviour of the 17-acetal resembles that of the 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one which also gives a 7 $\beta$ ,12 $\beta$ ,-15 $\alpha$ -trihydroxy-product, albeit in low yield.<sup>10</sup>)

The thioacetal was not utilised by any of the fungi, and the enol ethers were recovered unchanged from incubations with *Ao* and *Rn*. Two cases (Table I) in which enol ethers were attacked by *Cd* were investigated by varying the incubation times and the composition of the medium,<sup>2</sup> and by carrying out blank experiments with the substrates and media alone. It transpired that the

\* The importance of i.r. studies in establishing the structures of 7 $\beta$ ,12 $\beta$ -dihydroxy-5 $\alpha$ -androstan-17-one (no. 288) and 7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -trihydroxy-5 $\alpha$ -androstan-17-one (no. 337) was discussed earlier;<sup>9</sup> the presence of a hydrogen bonded 7 $\beta$ ,15 $\alpha$ -dihydroxy-system in the latter was confirmed by preparing the 7,15-acetonide from the derived 7 $\beta$ ,12 $\beta$ ,15 $\alpha$ -triol (no. 330).

microbial hydrolysis of the enol ethers precedes hydroxylation of the ketones so formed.

This investigation suggests that chemical modification of a steroidal substrate's ketone group is generally unprofitable in microbiological work. However, a notable exception is provided by the 16-oxygenated substrates, since the ketone and the acetal are both efficiently hydroxylated by *Cd*, but give dihydroxylated products of different types: conversion of the ketone into the acetal before hydroxylation thus leads to a convenient preparation of the uncommon 6,12,16-trioxygenated substrates.

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<sup>9</sup> 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.

<sup>10</sup> A. S. Clegg, D.Phil. Thesis, Oxford, 1970.