Microbiological Hydroxylation. Part XXII.[1] Hydroxylation of 3,20-, 7,20-, and 11,20-Dioxygenated 5α-Pregnanes

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Eight 3,20-, 7,20-, and 11,20-dioxygenated 5α-pregnanes have been incubated with the fungi *Calonectria decora* and *Daedalea rufescens*, and with three *Rhizopus* species. In most cases complex mixtures are formed, and the hydroxylations are less satisfactory than those of dioxygenated androstane analogues. Although *Rhizopus nigricans* leads mainly to the 11α-hydroxylation of 5α-pregnane-3,20-dione, hydroxylation of 3β-hydroxy-5α-pregnan-20-one occurs predominantly at the 7β-position (not at the 11α-position as reported in the literature). The cleanest incubation studied here is that of 5α-pregnane-7,20-dione with *Calonectria decora*, which gives the 1α,12β-dihydroxy-7,20-dione (30%) and the 12β-hydroxy-1,7,20-trione (19%).

Convenient preparations of the 7,20- and 11,20-dioxygenated substrates have been developed.

THE important physiological activity of many pregnane derivatives has prompted numerous investigations into the preparation of these compounds by microbiological methods.[2] However, almost all the substrates used contain a 3-oxo-group, and apart from work with the fungus *Aspergillus ochraceus* [3] little is known about the relation-

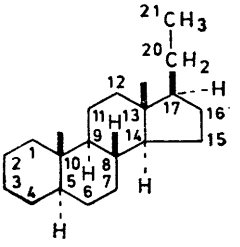
[1] Part XXI, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 2308.

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

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

TABLE 1

Hydroxylation of 3,20-, 7,20-, and 11,20-dioxygenated 5α-pregnanes by *Calonectria decora* (*Cd*), *Daedalea rufescens* (*Dr*), *Rhizopus arrhizus* (*Ra*), *Rhizopus circinans* (*Rc*), and *Rhizopus nigricans* (*Rn*)



5α-Pregnane

The substrates, all derivatives of 5α-pregnane, are indicated by abbreviated names, *e.g.* 3β-OH–20-CO represents 3β-hydroxy-5α-pregnan-20-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 complex mixture was obtained). The entries under conditions refer to the use of ethanol (E) or acetone (A) as solvent for the substrate and to the time of the incubation (in days). The yields are calculated after making allowance for recovered starting material.

Substrate	Fungus	Conditions	Recovered substrate	Main hydroxylation product(s)		Other products	
3,20-$(CO)_2$	*Cd* *	A2		**12β,15α**-$(OH)_2$			
	Dr	E4	0%	3β,**7α**-$(OH)_2$	4%	3β-OH	5%
				3β,**7β**-$(OH)_2$	5		
	Rn †	E4	25	**11α**-OH	22	3β,**7β,11α**-$(OH)_3$	7
						3β, **11α**-$(OH)_2$	5
						3β-OH	3
	Ra †	E6	14	**11α**-OH	12	3β,**7β,11α**-$(OH)_3$	5
	Rc	E5	7	**11α**-OH	9	**11α**-OH –**17**-CO	7
						3β, **11α**-$(OH)_2$–**17**-CO	4
						3-CO–**11α,17β**-$(OH)_2$	4
						3β, **11α**-$(OH)_2$	3
3β-OH–20-CO	*Cd* ‡	E6	13	**12β,15α**-$(OH)_2$	24	3-CO–**7β,12β,15α**-$(OH)_3$	8
	Dr	E5	17	**7α**-OH	9		
				7β-OH	9		
	Rn †	E4	10	**7β,12β**-$(OH)_2$	22	**7β,11α**-$(OH)_2$	10
						7β-OH	8
						11α-OH	5
		E2	12	**7β**-OH	15	**7β,12β**-$(OH)_2$	12
						11α-OH	7
						7β,11α-$(OH)_2$	7
	Ra †	E5	14	**7**-CO–**11α**-OH	16	**7β**-OH	11
						11α-OH	4
	Rc	E5	5	**7β**-OH	11	**11α**-OH	4
3β-OH–Δ^{16}–20-CO	*Cd*	E4	21	n.i.			
	Dr	E4	8	**14α,15β**-$(OH)_2$	21		
	Rn	E4	16	**7α**-OH	13		
				7α, 15α-$(OH)_2$	12		
				7-CO–**15β**-OH	12		
7,20-$(CO)_2$	*Cd*	E1	45	**12β**-OH	18		
		E6	10	**1α, 12β**-$(OH)_2$	33		
				1-CO–**12β**-OH	21		
	Dr	E4	30	**3β,7α**-$(OH)_2$	13	3β,**7β**-$(OH)_2$	3
	Rn	E4	21	**3α, 11α**-$(OH)_2$	10	**4α, 11α**-$(OH)_2$	12
						3β, 11α-$(OH)_2$	14
7α-OH–20-CO	*Cd*	E5	26	**12β**-OH	17		
	Dr	E5	9	**3β**-OH	27		
	Rn	E4	53	**3β**-OH	24		
11,20-$(CO)_2$	*Cd*	E5	58	n.i.			
	Dr	E5	10	**3β**-OH	35		
	Rn	E4	57	n.i.			
11α-OH–20-CO	*Cd*	E5	43	n.i.			
	Dr	E5	23	**3β**-OH	13		
	Rn	E5	56	n.i.			
11β-OH–20-CO	*Cd*	E5	64	n.i.			
	Dr	E5	62	n.i.			
	Rn	E5	71	n.i.			

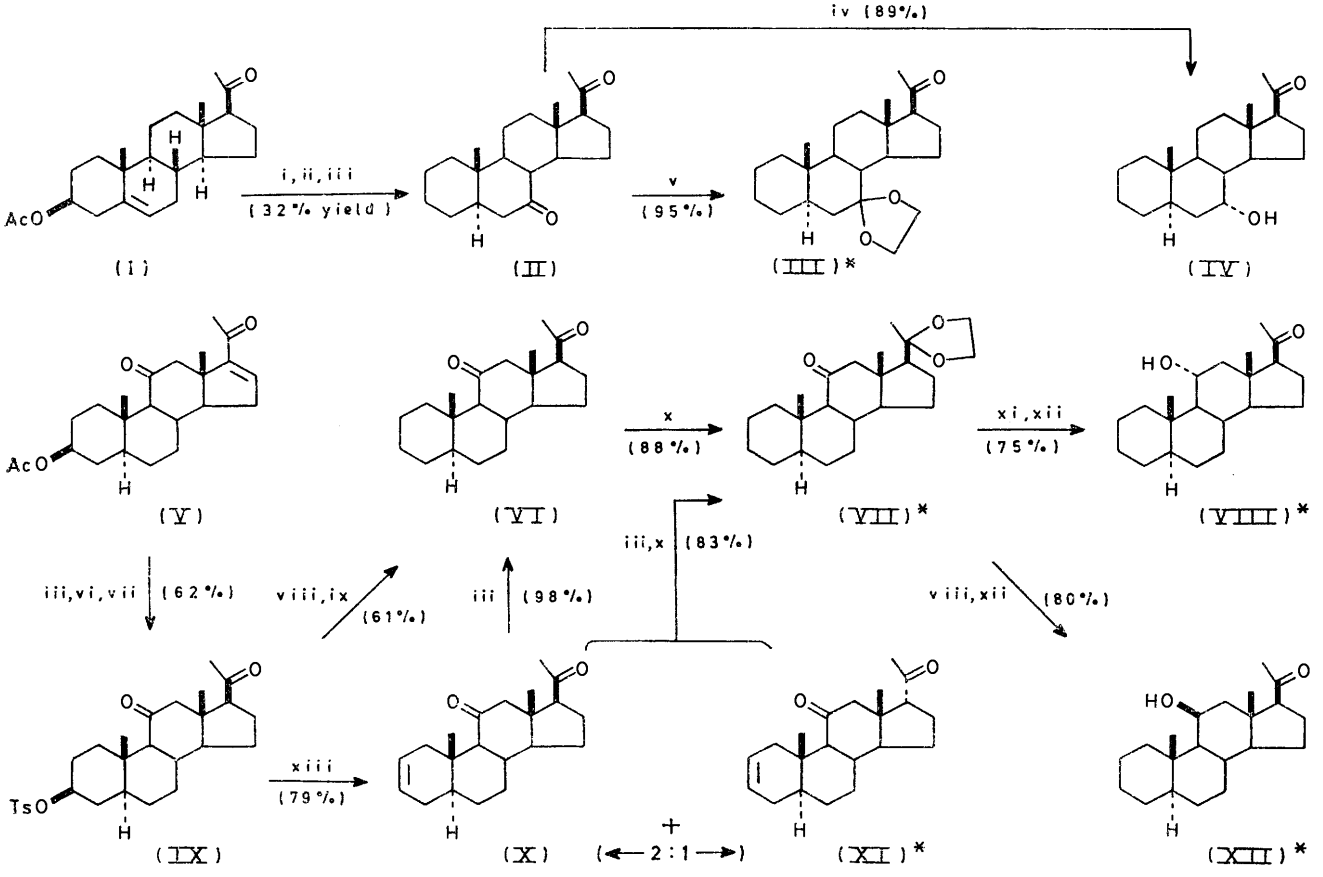
* Ref. 7. † *Cf.* Ref. 8. ‡ Ref. 4.

ship between substrate structure and the outcome of the microbiological process. Earlier parts of this series have been concerned mainly with the hydroxylation of mono- and di-oxygenated androstanes; with a number of fungi (notably *Calonectria decora* [4] and *Rhizopus nigricans* [5]) the hydroxylation patterns found with androstane derivatives are intimately connected with the positions and nature of the substrates' functional groups. The main object of the present work was to investigate the possibility that similar directing effects might also operate with *x*,20-dioxo- or *x*-hydroxy-20-oxo-5α-pregnanes which, unlike the androstanes, have a degree of conformational mobility arising from rotation of the 17-acetyl side-chain. In order to satisfy the geometric requirements of a particular enzyme system the side-chain would be free to adopt the orientation most favourable for the binding and hydroxylation processes. At first sight this feature appears advantageous in that it should enhance the directing influence of the substrate's functional groups; several enzyme systems are available in each of the commonly used fungi, however, and plurality of favourable paths might well lead to complex mixtures of hydroxylated products. The fungi used for this study, *Calonectria decora* [4] (*Cd*), *Daedalea rufescens* [6] (*Dr*), and *Rhizopus nigricans* [5] (*Rn*), were selected on the basis of the directing effects observed with androstane derivatives and their ability to metabolise many dioxygenated pregnanes.

SCHEME

Preparation of 7,20- and 11,20-dioxygenated 5α-pregnanes

References to known compounds are given in the Experimental section; new compounds are marked with an asterisk. Ts = *p*-$MeC_6H_4\cdot SO_2$



Reagents: i, CrO_3–Bu^tOH–Ac_2O–CCl_4; ii, HCl–EtOH; iii, H_2–Pd; iv, $NaBH_4$–EtOH; v, HO·$[CH_2]_2$·OH–C_6H_6–Amberlite resin; vi, KOH–EtOH; vii, TsCl–C_5H_5N; viii, $LiAlH_4$–tetrahydrofuran; ix, H_2CrO_4–Me_2CO; x, HO·$[CH_2]_2$·OH–C_6H_6–TsOH; xi, Na–Pr^iOH; xii, TsOH–Me_2CO; xiii, Me_2SO, heat.

A summary of the present results is shown in Table 1. (Earlier work on *Cd* with 3β-hydroxy-5α-pregnan-20-one [4a] and the 3,20-dione [7] is included although the yield of the 12β,15α-dihydroxy-derivative from the latter substrate is not reported.) The hydroxylations of the 3,20-

[4] (*a*) 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; (*b*) A. M. Bell, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and W. E. Müller, *ibid.*, p. 2759.

[5] V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. H. Pragnell, *J.C.S. Perkin I*, 1973, 1500.

[6] A. M. Bell, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. Pendlebury, *J.C.S. Perkin I*, 1975, 357.

[7] A. Schubert and R. Siebert, *Chem. Ber.*, 1958, **91**, 1856.

dioxygenated pregnanes with *Rn* were studied in the pioneering microbiological work of Murray and Peterson;[8] use of the more powerful methods for product separation and identification now available reveals that the earlier conclusions must be modified in one important respect (see later). Table 2 lists the n.m.r. spectra of the steroids, substrates, and products, involved here for which spectrometric data have not appeared in previous publications: the arabic serial number sequence discussed earlier[4] is used in this Table which contains steroids nos. **977—1016**. The structures of new compounds follow, as usual,[4] from a combination of spectrometric and chemical methods. [3β,14α,15β-Trihydroxy-5α-pregn-16-en-20-one, the product from 3β-hydroxy-Δ^{16}-20-oxopregnane and *Dr*, was acetylated to give a derivative which was shown to be a conjugated ketone

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]

No.	Compound		τ_2	τ_2(calc.) *	>C*H*-OR and other signals †
977	5α-Pregnane-7,20-dione	19	8.96	8.95	
		18	9.38	9.39	
978	Pregna-3,5-diene-7,20-dione	19	8.87	8.86	H-3, H-4 } 3.89m (5)
		18	9.36	9.36	H-6 4.41s
979	7,7-Ethylenedioxy-5α-pregnan-20-one	19	9.20	9.20	
		18	9.38	9.39	
980	5α-Pregnane-11,20-dione	19	8.99	9.01	
		18	9.43	9.43	
981	5α-Pregn-2-ene-11,20-dione	19	9.03	9.02	H-2, H-3 } 4.42m (5)
		18	9.41	9.41	
982	5α,17β-Pregn-2-ene-11,20-dione	19	9.04	9.02	H-2, H-3 } 4.45m (5)
		18	9.15	9.15	
983	20,20-Ethylenedioxy-5α-pregnan-11-one	19	9.00	9.00	H-21 8.76s
		18	9.30	9.30	
984	5α-Pregnane-3,7,20-trione	19	8.71	8.71	
		18	9.34	9.35	
985	5α-Pregnane-3,11,20-trione	19	8.80	8.77	
		18	9.40	9.39	
986	5α-Pregnane-1,7,12,20-tetraone	19	8.50	8.48	H-21 7.72s
		18	9.04	9.05	
987	5α-Pregnane-3,7,11,20-tetraone	19	8.53	8.49	
		18	9.36	9.38	
988	3β-Acetoxypregn-5-en-20-one	19	8.97	8.95	H-3 5.39m (23)
		18	9.36	9.35	H-6 4.61m (10)
989	7α-Hydroxy-5α-pregnan-20-one	19	9.22	9.23	H-7 6.18m (8)
		18	9.39	9.39	
990	11α-Hydroxy-5α-pregnan-20-one	19	9.08	9.11	H-11 6.10m (22)
		18	9.39	9.37	
991	20,20-Ethylenedioxy-5α-pregnan-11α-ol	19	9.08	9.10	H-11 Acetal } 6.14m (11)
		18	9.25	9.25	H-21 8.72s
992	11β-Hydroxy-5α-pregnan-20-one	19	8.98	9.00	H-11 5.68m (11)
		18	9.17	9.15	
993	20,20-Ethylenedioxy-5α-pregnan-11β-ol	19	8.99	8.99	H-11 5.76m (12)
		18	9.03	9.03	H-21 8.73s
994	3β-Acetoxypregn-5-ene-7,20-dione	19	8.78	8.77	H-3 5.25m (23)
		18	9.35	9.38	H-6 4.26s
995	3β-Hydroxy-5α-pregnane-11,20-dione	19	8.98	8.98	H-3 6.40m (22)
		18	9.42	9.42	
996	11,20-Dioxo-5α-pregnan-3β-yl toluene-*p*-sulphonate	19	9.02	9.00	H-3 5.54m (22)
		18	9.43	9.43	
997	3β-Hydroxy-5α-pregn-16-ene-11,20-dione	19	8.97	8.97	H-3 6.42m (23)
		18	9.17	9.15	H-16 3.24m (5)
					H-21 7.75s
998	3β-Acetoxy-5α-pregn-16-ene-11,20-dione	19	8.95	8.93	H-3 5.46m (23)
		18	9.14	9.14	H-16 3.26m (5)
					H-21 7.76s
999	12β-Hydroxy-5α-pregnane-7,20-dione	19	8.96	8.96	H-12 6.62m (16)
		18	9.29	9.29	H-21 7.78s
1 000	12β-Hydroxy-5α-pregnane-1,7,20-trione	19	8.58	8.56	H-12 6.48m (15)
		18	9.28	9.27	H-21 7.79s
1 001	3β,7α-Dihydroxy-5α-pregnan-20-one	19	9.19	9.20	H-3 6.38m (22)
		18	9.38	9.38	H-7 6.16m (7)
					H-3 6.36m (22)
1 002	3β,7α-Dihydroxy-5α-pregn-16-en-20-one	19	9.16	9.16	H-7 6.11m (7)
		18	9.11	9.10	H-16 3.31m (6)
					H-21 7.76s
1 003	3β,7β-Dihydroxy-5α-pregnan-20-one ‡	19	9.16	9.17	H-3, H-7 } 6.45m (24)
		18	9.36	9.36	
1 004	7α,12β-Dihydroxy-5α-pregnan-20-one	19	9.22	9.23	H-7 5.98m (8)
		18	9.29	9.29	H-12 6.52m (16)
					H-21 7.79s
1 005	1α,12β-Dihydroxy-5α-pregnane-7,20-dione	19	8.96	8.93	H-1 6.24m (6)
		18	9.29	9.29	H-12 6.57m (18)
					H-21 7.80s
1 006	3α,11α-Dihydroxy-5α-pregnane-7,20-dione	19	8.83	8.83	H-3, H-11 } 5.96m (9)
		18	9.37	9.35	
1 007	3β,11α-Dihydroxy-5α-pregnane-1,7,20-dione	19	8.80	8.80	H-3 6.45 *7* (10, 10, 5, 5)
		18	9.36	9.35	H-11 5.98 *6* (10, 10, 5)
1 008	3β,11α-Diacetoxy-5α-pregnane-7,20-dione	19	8.79	8.78	H-3 5.42 *7* (10, 10, 5, 5)
		18	9.34	9.34	H-11 4.78 *6* (10, 10, 5)
1 009	3β,15β-Dihydroxy-5α-pregn-16-ene-7,20-dione	19	8.89	8.91	H-3 6.38m (23)
		18	8.80	8.82	H-15 4.72 m(11)
					H-16 3.32d (3.5)
					H-21 7.69s
1 010	4α,11α-Dihydroxy-5α-pregnane-7,20-dione	19	8.81	8.81	H-4 6.50m (20)
		18	9.36	9.36	H-11 5.92m (22)
1 011	3β,7α,15α-Trihydroxy-5α-pregn-16-e -20-one	19	9.16	9.16	H-3 6.38m (22)
		18	9.04	9.04	H-7 5.90m (8)
					H-15 5.30m (12)
					H-16 3.48s
					H-21 7.73s
1 012	3β,7β,11α-Trihydroxy-5α-pregnan-20-one §	19	9.05	9.05	
		18	9.37	9.33	
1 013	3β,7β,11α-Triacetoxy-5α-pregnan-20-one	19	9. 2	9.05	H-3, H-7 } 5.40m (23)
		18	9.31	9.28	H-11 4.95 *6* (10, 10, 5)
1 014	3β,7β,12β-Trihydroxy-5α-pregnan-20-one §	19	9.16	9.16	H-21 7.79s
		18	9.27	9.26	
1 015	3β,7β,12β-Triacetoxy-5α-pregnan-20-one	19	9.12	9.12	H-3, H-7, H-12 } 5.42m (30)
		18	9.12	9.12	
1 016	3β,15β-Diacetoxy-14α-hydroxy-5α-pregn-16-en-20-one	19	9.08	9.08	H-3 5.36 *7* (10, 10, 5, 5)
		18	8.74	8.74	H-15 4.73d (3.5)
					H-16 3.28d (3.5)
					H-21 7.69s

[a] Ref. 4. [b] Ref. 12; 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.

* Calculated increments (H-19, H-18); 20,20-(O·[CH_2]$_2$·O) (0.00, −0.03), 12β-OH–17β-Ac (0.00, −0.01), 12β-OAc-17β-Ac (0.00, −0.14), 15α-OH-Δ^{16}-20-CO (−0.03, −0.25), 15β-OH–Δ^{16}-20-CO (−0.02, −0.47), 15β-OAc-Δ^{16}-20-CO (−0.09, −0.43). † H-21 resonance in region τ 7.87—7.93 unless otherwise indicated. ‡ Not isolated. § Dilute solution, only Me signals recorded.

Table 3

Characterisation of new compounds

Compound	M.p. (°C) *	[α]$_D$ (°) † (*c*)	Analytical figures (%)	C	H
7,7-Ethylenedioxy-5α-pregnan-20-one	146—149°	+49 (0.3)	Found	76.85	10.25
			$C_{23}H_{38}O_3$ req.	76.6	10.1
5α,17β-Pregn-2-ene-11,20-dione	137.5—139	+30 (0.75)	Found	80.0	9.7
			$C_{21}H_{32}O_3$ req.	80.2	9.6
20,20-Ethylenedioxy-5α-pregnan-11-one	117—119	+56 (0.5)	Found	76.5	10.2
			$C_{23}H_{38}O_4$ req.	76.6	10.1
5α-Pregnane-1,7,12,20-tetraone	297—298 ‡	+183 (0.55)	Found	73.3	8.05
			$C_{21}H_{28}O_4$ req.	73.2	8.2
5α-Pregnane-3,7,11,20-tetraone	255—257‡	+49 (0.65)	Found	73.0	8.35
			$C_{21}H_{28}O_4$ req.	73.2	8.2
11α-Hydroxy-5α-pregnan-20-one	165—168	+59 (0.35)	Found	78.95	10.75
			$C_{21}H_{34}O_2$ req.	79.2	10.75
20,20-Ethylenedioxy-5α-pregnan-11α-ol	121—123	+5 (1.1)	Found	76.1	10.4
			$C_{23}H_{38}O_3$ req.	76.2	10.5
11β-Hydroxy-5α-pregnan-20-one	181—183	+81.5 (1.3)	Found	79.05	10.75
			$C_{21}H_{34}O_2$ req.	79.2	10.75
20,20-Ethylenedioxy-5α-pregnan-11β-ol	153—155	+24.5 (0.35)	Found	76.4	10.35
			$C_{23}H_{38}O_3$ req.	76.2	10.5
12β-Hydroxy-5α-pregnane-7,20-dione	179—180	−82 (0.7)	Found	75.85	9.75
			$C_{21}H_{32}O_3$ req.	75.85	9.7
12β-Hydroxy-5α-pregnane-1,7,20-trione	181—182	+34 (0.75)	Found	72.8	8.85
			$C_{21}H_{30}O_4$ req.	72.8	8.75
3β,7α-Dihydroxy-5α-pregn-16-en-20-one	110—112	+14 (0.7)	Found	75.75	9.7
			$C_{21}H_{32}O_3$ req.	75.85	9.7
7α,12β-Dihydroxy-5α-pregnan-20-one	199—201	+1 (0.45)	Found	75.7	10.2
			$C_{21}H_{34}O_3$ req.	75.4	10.25
1α,12β-Dihydroxy-5α-pregnane-7,20-dione	170—171	−72 (0.9)	Found	72.45	9.35
			$C_{21}H_{32}O_4$ req.	72.4	9.25
3α,11α-Dihydroxy-5α-pregnane-7,20-dione	196—198	−7 (1.1)	Found	72.6	9.2
			$C_{21}H_{32}O_4$ req.	72.4	9.25
3β,11α-Dihydroxy-5α-pregnane-7,20-dione	245—247	−8 (0.3)	Found	72.35	9.3
			$C_{21}H_{32}O_4$ req.	72.4	9.25
3β,11α-Diacetoxy-5α-pregnane-7,20-dione	160—162	−3 (0.9)	Found	69.6	8.35
			$C_{25}H_{36}O_6$ req.	69.4	8.4
3β,15β-Dihydroxy-5α-pregn-16-ene-7,20-dione	261—263	−72 (0.5)	Found	72.6	8.9
			$C_{21}H_{30}O_4$ req.	72.8	8.75
4α,11α-Dihydroxy-5α-pregnane-7,20-dione	243—245	−43 (0.2)	Found	72.2	9.5
			$C_{21}H_{32}O_4$ req.	72.4	9.25
3β,7α,15α-Trihydroxy-5α-pregn-16-en-20-one	212—213	+71 (0.3)	Found	72.6	9.4
			$C_{21}H_{32}O_4$ req.	72.4	9.25
3β,7β,11α-Trihydroxy-5α-pregnan-20-one	211—213	+62 § (0.15)	Found	72.1	9.8
			$C_{21}H_{34}O_4$	71.95	9.8
3β,7β,11α-Triacetoxy-5α-pregnan-20-one	181—183	+40.5 (1.4)	Found	68.3	8.35
			$C_{27}H_{40}O_7$ req.	68.05	8.45
3β,7β,12β-Trihydroxy-5α-pregnan-20-one	250—253	+63 § (0.5)	Found	72.2	9.7
			$C_{21}H_{34}O_4$ req.	71.95	9.8
3β,7β,12β-Triacetoxy-5α-pregnan-20-one	182—185	+51 (0.25)	Found	67.8	8.25
			$C_{27}H_{40}O_7$ req.	68.05	8.45
3β,15β-Diacetoxy-14α-hydroxy-5α-pregn-16-en-20-one	188—191	−135 (1.3)	Found	69.7	8.3
			$C_{25}H_{34}O_6$ req.	69.4	8.4

* From Me_2CO-petrol or Et_2O-petrol unless otherwise indicated. † $CHCl_3$ as solvent unless otherwise indicated. ‡ From EtOH. § EtOH as solvent.

[8] H. C. Murray and D. H. Peterson, U.S.P. 2,602,769 (*Chem. Abs.*, 1952, **46**, 8331f).

containing two acetoxy-groups and a tertiary hydroxy-group. The n.m.r. features of this derivative, considerable deshielding of only one angular methyl group (that at C-13) and doublets (J 3.5 Hz) at τ 3.28 and 4.73, provide evidence for the 14α-hydroxy-15β-acetoxy-Δ^{16} system.] 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 most of the chemical operations of the present work are routine applications of techniques fully described in earlier parts.* However, the preparations of some of the 7,20- and 11,20-dioxygenated pregnanes (Scheme) have not been recorded previously, and these are described in the Experimental section.

A few points in the Scheme require brief comment. Our original intention was to prepare 7α-hydroxy-5α-pregnan-20-one (IV) by hydride reduction of a 7-oxo-20-acetal and subsequent hydrolysis. However, acetalisation of the 7,20-dione (II) using an ion-exchange resin (Amberlite), conditions employed effectively for selective reactions of several diketones,[9] gave the (*sic*) 20-oxo-7-acetal (III) in almost quantitative yield. The difference in steric hindrance suggested by this result was exploited in a selective reduction of the 7,20-dione (II) to the required 7α-hydroxy-20-ketone (IV). During preparation of 5α-pregnane-11,20-dione (VI) epimerisation[10] of the 17β-acetyl group was found to occur with surprising ease. For example, boiling the tosylate (IX) in dimethyl sulphoxide for 2 h gave a 2 : 1 mixture of 5α-pregn-2-ene-11,20-dione (X) and the 17-epimer (XI). [This difficulty is avoided in the alternative route from the tosylate (IX) to the diketone (VI).] Fortunately, acetalisation of a 20-oxo-17β-pregnane (*i.e.* a 17α-acetyl compound) is accompanied by inversion,[11] and the mixture of 17-epimers (X) and (XI) was converted efficiently into the 11-oxo-20-acetal (VII) with the natural 17-configuration.

Comparison of the results in Table 1 with those of similar incubations[4b,5,6] of the androstane analogues shows that there are broad similarities between the two series, *i.e.* hydroxylations of an *x*-oxygenated 20-oxopregnane and an *x*-oxygenated 17-oxoandrostane by a particular fungus usually occur at the same steroid position(s). However, the dioxygenated pregnanes are metabolised more slowly, generally give complex mixtures, and show less satisfactory steroid balances. Thus if a particular oxygenated pregnane is to be prepared by a sequence involving microbiological steps it may well be advisable to hydroxylate a 17-oxoandrostane and to introduce the side-chain by standard chemical methods at a later stage. Although the results in Table 1 are considered to be too complicated for detailed analysis, the following points emerge. (i) *Rn* leads mainly to the 11α-hydroxylation[8] of 5α-pregnane-3,20-dione, but hydroxylation of 3β-hydroxy-5α-pregnan-20-one occurs predominantly at the 7β-position (not at the 11α-position as reported earlier[8]). (ii) Comparison of the hydroxylations of 3β-hydroxy-5α-pregnan-20-one with those of 3β-hydroxy-5α-pregn-16-en-20-one shows that the presence of the 16,17-double bond has a dramatic effect; the differences probably stem more from the allylic nature of the 15-position in the unsaturated ketone than from restricted rotation of its 17-acetyl group. (iii) The differences between the three *Rhizopus* species in their hydroxylations of the present substrates are similar to, but less clear than those observed previously[12] with androstanes.

The 1α,12β- and 12β,15α-dihydroxylations (of the 7,20-diketone and the 3-hydroxy-20-ketone respectively) by *Cd* may be useful in connection with the synthesis of steroids not readily available by chemical methods.

EXPERIMENTAL

For general directions and details of preparative layer chromatography (p.l.c.) see ref. 4. For the n.m.r. signals and other constants of new compounds see Tables 2 and 3. The constants (m.p., $[\alpha]_D$) of known compounds are not given if the values found here correspond closely with those in the literature reference cited. Petrol refers to light petroleum, b.p. 60—80°.

7α-Hydroxy-5α-pregnan-20-one (IV) (no. 989).—$NaBH_4$ (240 mg) was added in 10 portions over 30 min to a stirred solution of 5α-pregnane-7,20-dione[13] (II) (no. 977) (5.6 g) [prepared[13,14] *via* the intermediates 3β-acetoxypregn-5-en-20-one (no. 988) ⟶ 3β-acetoxypregn-5-ene-7,20-dione (no. 994) ⟶ pregna-3,5-diene-7,20-dione (no. 978)] in MeOH–CH_2Cl_2 (1 : 1; 300 ml) at 0 °C. The solution was warmed to 20 °C and stirred for a further 45 min. (T.l.c. indicated that little starting material or diol was present.) Work-up gave material (5.5 g) which was chromatographed on neutral Al_2O_3 (200 g). Elution with Et_2O–petrol (2 : 1) gave starting material (0.97 g). Et_2O eluted 7α-hydroxy-5α-pregnan-20-one[15] (IV) (4.1 g), $[\alpha]_D$ +51.5 (*c* 1.0), $\nu_{max.}$ 3 620 and 1 715 cm^{-1}.

7,7-Ethylenedioxy-5α-pregnan-20-one (III) (no. 979).—A stirred solution of 5α-pregnane-7,20-dione (II) (1.89 g) and HO·$[CH_2]_2$·OH (4 ml) in dry C_6H_6 (150 ml) was boiled under reflux with Amberlite resin (IR120-H) (19 g) under a Dean–Stark separator for 3 h. The mixture was filtered, and the filtrate was washed with water, dried, and evaporated to give *7,7-ethylenedioxy-5α-pregnan-20-one* (2.04 g), $\nu_{max.}$ 1 705 cm^{-1}.

11,20-Dioxo-5α-pregnan-3β-yl Toluene-p-sulphonate (IX) (no. 996).—KOH (16 g) in water (50 ml) was added to a solution of 3β-acetoxy-5α-pregn-16-ene-11,20-dione † (V) (no. 998) (50 g) in warm EtOH (500 ml). The mixture was heated at 100 °C for 30 min and then kept at 20 °C for 12 h.

* An account of these operations is given in Supplementary Publication No. SUP 21771 (10 pp., 1 microfiche). For details of Supplementary Publications see Notice to Authors, No. 7 in *J.C.S. Perkin I*, 1975, Index issue.

† Supplied by Glaxo Research Ltd.

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Work-up gave 3β-hydroxy-5α-pregn-16-ene-11,20-dione [16] (no. 997) (30.1 g). Hydrogenation of this diketone (30.0 g) in EtOH–EtOAc (1 : 1, 450 ml) over 5% Pd–C (4 g) gave 3β-hydroxy-5α-pregnane-11,20-dione [17] (no. 995) (29.8 g). Treatment of this compound (29.7 g) with toluene-*p*-sulphonyl chloride (50 g) in pyridine (300 ml) for 48 h at 20 °C gave 11,20-dioxo-5α-pregnan-3β-yl toluene-*p*-sulphonate [18] (39.1 g).

5α-Pregn-2-ene-11,20-dione (X) (no. 981).—A solution of the foregoing tosylate (IX) (10.0 g) in Me_2SO (150 ml) was boiled under reflux for 2 h, cooled, diluted with water (500 ml), and extracted with $CHCl_3$ (2 × 250 ml). The brown oil (6.5 g) so obtained was chromatographed on neutral Al_2O_3 (250 g). Elution with Et_2O–petrol (1 : 1) gave material (5.2 g) shown by t.l.c. to be a mixture of two compounds. Separation of a sample (600 mg) by p.l.c. [2 large plates; petrol–Et_2O (65 : 35)] afforded *5α,17β-pregn-2-ene-11,20-dione* (XI) (no. 982) (180 mg), R_F 0.5, $\nu_{max.}$ 1 710 cm^{-1}, and 5α-pregn-2-ene-11,20-dione [18] (X) (366 mg), R_F 0.45.

5α-Pregnane-11,20-dione (VI) (no. 980).—(*a*) The unsaturated dione (X) (300 mg) in EtOH–EtOAc (1 : 1; 10 ml) was hydrogenated over Pd–C (40 mg) to give 5α-pregnane-11,20-dione [19] (296 mg), $\nu_{max.}$ 1 710 cm^{-1}.

(*b*) A solution of the tosylate (IX) (21.6 g) in dry tetrahydrofuran (250 ml) was added during 1 h to a suspension of $LiAlH_4$ (6 g) in tetrahydrofuran (250 ml) which was boiling under reflux, and the mixture was boiled for a further 36 h. Work-up gave material (19.2 g) which was chromatographed on Al_2O_3 (10% deactivated; 750 g). The diol fraction (9.8 g) eluted with Et_2O was dissolved in Me_2CO and oxidised with 8N-H_2CrO_4 to give the 11,20-dione (VI) (8.85 g after crystallisation from EtOH).

20,20-Ethylenedioxy-5α-pregnan-11-one (VII) (no. 983). (*a*) A solution of the dione (VI) (4.0 g) in dry C_6H_6 (250 ml) was boiled under reflux with $HO{\cdot}[CH_2]_2{\cdot}OH$ (2 ml) and toluene-*p*-sulphonic acid (0.25 g) under a Dean–Stark separator for 3 h. Work-up gave *20,20-ethylenedioxy-5α-pregnan-11-one* (4.14 g), $\nu_{max.}$ 1 710 cm^{-1}.

(*b*) A mixture (4.5 g) of the unsaturated diketones (X) and (XI) (see before) was hydrogenated over Pd–C (0.5 g). Acetalisation of the product as described in the preceding preparation gave the oxo-acetal (VII) (4.33 g).

11β-Hydroxy-5α-pregnan-20-one (XII) (no. 992).—The foregoing oxo-acetal (VII) (4.2 g) in dry Et_2O (100 ml) was boiled under reflux with $LiAlH_4$ (0.6 g) for 24 h. The excess of reagent was decomposed with wet Et_2O and the precipitated material was removed by filtration through Celite. Evaporation of the filtrate afforded *20,20-ethylenedioxy-5α-pregnan-11β-ol* (no. 993) (3.55 g), $\nu_{max.}$ 3 620 cm^{-1}. A solution of this alcohol (3.5 g) in Me_2CO–H_2O (50 : 1; 51 ml) was stirred with toluene-*p*-sulphonic acid (0.4 g) at 20 °C for 24 h. Work-up gave *11β-hydroxy-5α-pregnan-20-one* (3.05 g), $\nu_{max.}$ 3 620 and 1 710 cm^{-1}.

11α-Hydroxy-5α-pregnan-20-one (VIII) (no. 990).—Na (50 g) was added during 2 h to a solution, boiling under reflux, of the oxo-acetal (VII) (4.1 g) in Pr^iOH (350 ml). EtOH was added and the mixture was diluted carefully with H_2O (800 ml). Extraction with EtOAc (3 × 400 ml) gave *20,20-ethylenedioxy-5α-pregnan-11α-ol* (no. 991) (3.2 g), $\nu_{max.}$ 3 620 cm^{-1}. Hydrolysis of this hydroxy-acetal as described in the preceding experiment gave *11α-hydroxy-5α-pregnan-20-one* (2.8 g), $\nu_{max.}$ 3 620 and 1 710 cm^{-1}.

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