Microbiological Hydroxylation of Steroids. Part X.[1] 1β,11α-Dihydroxylation of 3β-Hydroxy-5α-pregnan-20-one and the Hydroxylation of Other 20-Oxo-5α-pregnanes with the Fungus *Aspergillus ochraceus* [2]

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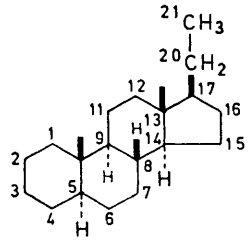
Hydroxylation of 3β-hydroxy-5α-pregnan-20-one with *Aspergillus ochraceus*, in shake-flasks or using 10 g batch fermentation, gives 1β,3β,11α-trihydroxy-5α-pregnan-20-one as the main product (*ca.* 50% yield); this trihydroxy-ketone is readily converted into 5α-pregn-1-ene-3,11,20-trione. With some other 20-oxo-5α-pregnanes containing a second oxygen group, the initial 11α-hydroxylation is followed by a variety of processes which lead to mixtures of products.

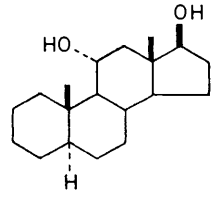
THE fungus *Aspergillus ochraceus* is known to introduce an 11α-hydroxy-group into many steroids;[3] the 6β-position is an additional, or occasionally an alternative, site for hydroxylation.[4] These processes are caused by different enzyme systems, which can be induced independently by many of the usual substrates.[5] Previous work in this series[6] showed that while most 5α-androstane monoketones are not hydroxylated, 5α-androstan-3-one and 5α-estran-3-one (and the corresponding Δ^4-ketones) give 6β,11α-dihydroxy-products; incubation of a variety of dioxygenated 5α-androstanes with *A. ochraceus* resulted in 11α-hydroxylation, generally in high yield.

TABLE 1

Hydroxylation of 5α- and Δ^5-20-oxopregnanes by *Aspergillus ochraeus*



5α-Pregnane



5α-Androstane-11α,17β-diol

Substrates are indicated by abbreviated names, *e.g.* 3β-OH–20-CO represents 3β-hydroxy-5α-pregnan-20-one; with one exception (the 11α,17β-diol shown) all the steroids are deviatives of pregnane. In the Products columns those oxygen functions introduced during the incubation are indicated by use of bold type. Incubations (in shake-flasks, apart from the one case specified) were carried out for 6 days, dimethyl sulphoxide being used to introduce the substrates. The nutrient medium was more concentrated than usual in the second incubation of the 3,20-diketone. The yields are calculated after making allowance for recovered starting material, *i.e.* they refer to the composition of the steroidal material after incubation and removal of the substrate.

Substrate	Substrate recovered	Main products		Other products	
20-CO	91%				
3β-OH–20-CO	3	**1β**, **11α**-$(OH)_2$	57%	3-CO-**1β**, **11α**- $(OH)_2$	8%
(in fermentor)	36	**1β**, **11α**-$(OH)_2$	60	**11α**- OH	10
3,20-$(CO)_2$	28	**11α**- OH	38	**11α,17β**-$(OH)_2$ *	9
		4-oxa-A-homo-**11α**- OH	30	Δ^1- **11α**- OH †	8
				1β,3β, **11α**- $(OH)_3$ ‡	8
				6β,11α- $(OH)_2$ *	4
				3β,11α- $(OH)_2$ *	1
3,20-$(CO)_2$	73	**11α**- OH	68	Δ^1- **11α**- OH †	10
				11α,17β-$(OH)_2$ *	2
3β-OH–Δ^5–20-CO	2	3-CO-Δ^4-**6β**, **11α**-$(OH)_2$	22	**5β,6β**-epoxy-**11α**- OH	11
		7β,11α-$(OH)_2$	22	**1β**, **11α**- $(OH)_2$	8
				7α,11α- $(OH)_2$	6
2,20-$(CO)_2$	0	**11α**- OH	50		

* Isolated as corresponding diacetate. † Probably formed, during the acetylation stage of the separation, from the 1β,11α-$(OH)_2$-3,20-$(CO)_2$, which may then be regarded as the product of the hydroxylation. ‡ Obtained, after acetylation, as a mixture of monohydroxy-diacetoxy-20-ketones whose identification is based on n.m.r. evidence only (see Experimental section).

[1] Part IX, A. M. Bell, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, W. E. Müller, and E. E. Richards, preceding paper.

[2] (*a*) Preliminary account, A. S. Clegg, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *Chem. Comm.*, 1970, 1029; (*b*) routine technical operations not fully described in the Experimental section are recorded by A. S. Clegg (D.Phil. Thesis, Oxford, 1970).

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

[4] (*a*) T. Okumura, Y. Nozaki, and D. Satoh, *Chem. and Pharm. Bull.* (*Japan*), 1962, **12**, 1143; (*b*) L. L. Smith, G. Greenspan, R. Rees, and T. Foell, *J. Amer. Chem. Soc.*, 1966, **88**, 3120.

[5] M. Shibahara, J. A. Moody, and L. L. Smith, *Biochim. Biophys. Acta*, 1970, **202**, 172; L. Tan and P. Falardeau, *J. Steroid Biochem.*, 1970, **1**, 221.

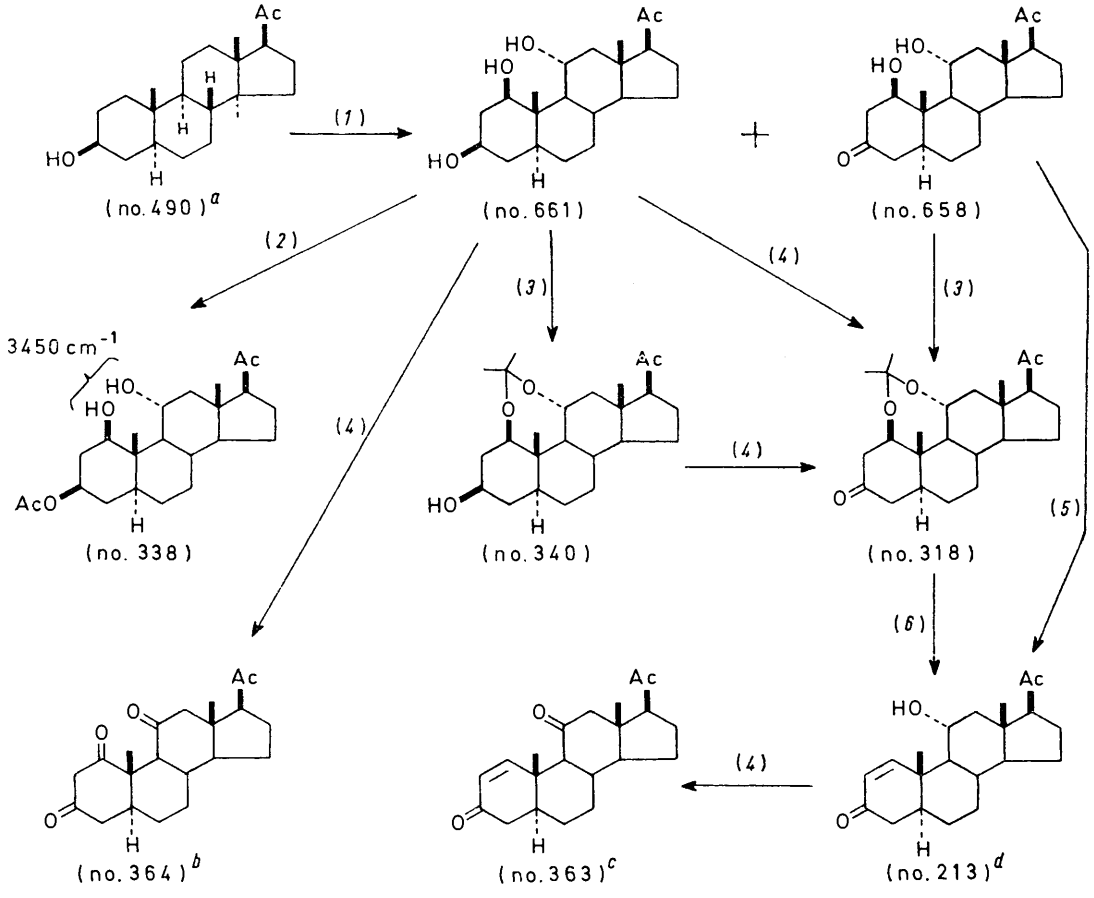
[6] A. M. Bell, J. W. Browne, W. A. Denny, Sir Ewart R. H. Jones, A. Kasal, and G. D. Meakins, *J.C.S. Perkin I*, 1972, 2930.

From these studies it was concluded that *A. ochraceus* has a predilection for attacking the 11α- and, less readily, the 6β-positions, irrespective of the substrate's structure. Thus, site specificity appeared to dominate the hydroxylation processes, and to supervene over the directing effect of the substrates' oxygenated groups which had been found to operate with other fungi (*Calonectria decora* [7] and *Rhizopus nigricans* [8]). In the present work some 20-oxopregnanes have been examined: it was thought that this more marked variation in substrate

651—665 (listed in Table 2) and some of the new steroids with numbers below 375 are described here.]

As expected from studies of other mono-ketones,[6] 5α-pregnan-20-one was not attacked by *A. ochraceus*. However, 3β-hydroxy-5α-pregnan-20-one was hydroxylated remarkably cleanly when either the usual shake-flask method [9] or the 10 g batch fermentation technique [10] was used. Both procedures gave the 1β,3β,11α-trihydroxy-20-ketone (no. 661; see Scheme) as the main product. There appears to be no precedent for this

SCHEME



Reagents: (1), *A. ochraceus*; (2), Ac_2O–*s*-collidine; (3), Me_2CO–HCl; (4) H_2CrO_4–Me_2CO; (5), Ac_2O–C_5H_5N; (6), HCl–H_2O–dioxan, reflux.

[a] Ref. 16. [b] Ref. 19. [c] Ref. 20. [d] Ref. 17.

structure would test the notion that hydroxylations with *A. ochraceus* all follow the same pattern.

Table 1 and the Scheme summarise the results. [The use of the (arabic) serial number sequence of steroids throughout this work, and considerations about the structural elucidation and the reporting of new compounds have been explained earlier.[7] Compounds nos.

1β,11α-dihydroxylation. Although the 1β- and 11α-positions are considered to be equivalent in microbiological work [4a] and the extent to which one or the other is attacked can be influenced by the geometry of the substrate,[4b] they have been envisaged as alternative sites for hydroxylation. For example, the 11α- or 1β-hydroxylations of steroids by *Absidia orchidis* have been regarded as mutually exclusive.[11]

[7] 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.

[8] 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.

[9] J. W. Blunt, I. M. Clark, J. M. Evans, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *J. Chem. Soc.* (*C*), 1971, 1136.

[10] Details of the technique will be described later.

[11] V. Schwarz, M. Ulrich, and K. Syhora, *Steroids*, 1964, **4**, 645.

Under conditions of complete utilisation of 3β-hydroxy-5α-pregnan-20-one (shake-flasks) a small amount of the 1β,11α-dihydroxy-3,20-diketone (no. 658) is formed by oxidation of the main product; when hydroxylation is incomplete (fermentor) the 3β,11α-dihydroxy-20-ketone is the minor product. These results and those obtained using shorter incubation times [2b] suggest that the dihydroxylation is a sequential process, the initial 11α-attack being followed rapidly by 1β-substitution, and that the same enzyme site is involved in both hydroxylations. The alternative sequence, induction by the 11α-monohydroxy-product of a new enzyme system responsible for further substitution, would have been expected to lead to 6β,11α-dihydroxylation, and there would have been a longer interval between the completion of the first stage and the formation of an appreciable amount of the final product.[5,6]

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. For compounds of low solubility references to the spectra of soluble derivatives are given.

No.	Compound		τ_2	τ_3(calc).	>C*H*-OR and other characteristic signals		
651	3β-Hydroxypregn-5-en-20-one	19	8·98	8·97	H-3	6·49	*m*(25)
		18	9·36	9·36			
652	11α-Hydroxy-5α-pregnane-2,20-dione	19	9·13	9·13	H-11	6·06	*6*(10,10,5)
		18	9·38	9·38			
653	11α-Hydroxy-4-oxa-A-homo-5α-pregnane-3,20-dione	19	8·95		H-11	6·09	*6*(10,10,5)
		18	9·37		H-4aα	6·36	d(14)
					H-4aβ	5·68	*4*(14,8)
654	3,20-Dioxo-5α-pregn-1-en-11α-yl acetate	19	8·93	8·92	H-11	4·71	*6*(10,10,5)
		18	9·25	9·29	H-1	1·64	d(11)
					H-2	4·18	d(11)
655	3β,11α-Dihydroxy-5α-pregnan-20-one	19	9·09	9·08	H-11	6·01	*6*(10,10,5)
		18	9·25	9·26			
656	3β,11α-Diacetoxy-5α-pregnan-20-one *	19	9·07	9·10	H-11	4·85	*6*(10,10,5)
		18	9·34	9·31			
657	5,6β-Epoxy-3β,11α-dihydroxy-5β-pregnan-20-one	[see the 3β,11α-diacetate (no. 342) [a]]					
658	1β,11α-Dihydroxy-5α-pregnane-3,20-dione	[see the 1β,11α-acetonide (no. 318) [a]]					
659	6β,11α-Diacetoxy-5α-pregnane-3,20-dione *	19	8·71	8·75	H-6	5·01	m(7)
		18	9·23	9·23	H-11	4·70	*6*(10,10,5)
660	6β,11α-Dihydroxypregn-4-ene-3,20-dione	[see the 6β,11α-diacetate (no. 319) [a]]					
661	1β,3β,11α-Trihydroxy-5α-pregnan-20-one	[see the 1β,11α-acetonide (no. 340) [a]]					
662	1β 3β,11α-Trihydroxypregn-5-en-20-one	[see the 1β,3β,11α-triacetate (no. 339) [a]]					
663	3β-Hydroxy-1β,11α-isopropylidenedioxy pregn-5-en-20-one	19	8·89	8·91	H-1	6·38	*4*(12,5)
		18	9·37	9·37	H-3	6·43	m(25)
					H-11	6·00	*6*(11,9,6)
664	3β,7α,11α-Trihydroxy pregn-5-en-20-one	[see the 3β,7α,11α-triacetate (no. 343) [a]]					
665	3β,7β,11α-Trihydroxy pregn-5-en-20-one	[see the 3β,7β,11α-tracietate (no. 344) [a]]					

* Not fully characterised.

[a] N.m.r. signals given in *J. Chem. Soc.* (*C*), 1970, 250.

The behaviour of 5α-pregnane-3,20-dione contrasts sharply with that of the 3β-hydroxy-20-ketone already discussed, and with that of the Δ^4-3,20-diketone (progesterone), which is converted quantitatively into 6β,11α-dihydroxyprogesterone.[12] Depending on the conditions used the 3,20-diketone is attacked either slowly, the 11α-hydroxy-derivative then being the main product isolated, or more rapidly to give a complex mixture resulting from different processes succeeding the initial 11α-hydroxylation. Formation of 11α,17β-dihydroxy-5α-androstan-3-one involves the microbiological equivalent of a Baeyer–Villiger oxidation of the 17β-acetyl side-chain, a conversion well documented in the case of *A. ochraceus*.[3] Similar microbiological oxidation of the 3-oxo-group, to give the 11α-hydroxy-A-homo-lactone, has been observed previously with triterpenoid 3-ketones [13] but not with steroids. [Formulation of the lactone (no. 653; see Table 2) as the 4-oxa- rather than the 3-oxa-isomer is based on (i) the similarity between its 11β-H and 18-H n.m.r. signals and those of 11α-hydroxy-5α-pregnane-3,20-dione [9] (no. 212), and (ii) its possession of an $-O{\cdot}CH_2{\cdot}CH<$ rather than an $-O{\cdot}CH_2{\cdot}CH_2-$ unit.]

In the earlier hydroxylations [14] of 3β-hydroxypregn-5-en-20-one only progesterone derivatives were isolated (*i.e.* those in which substitution at the 11α- and 6β-positions had been accompanied by 3β-OH–Δ^5 ⟶ 3-oxo-Δ^4 transformation). 6β,11α-Dihydroxylation with isomerisation, and 7β,11α-dihydroxylation without isomerisation predominated in the present incubation/minor products included the trihydroxy-ketone arising from 1β,11α-dihydroxylation and an 11α-hydroxy-5β,6β-epoxide. Formation of the last compound supports the proposal [15] that micro-organisms capable of axial hydroxylation (here 6β) may also cause stereochemically equivalent epoxidation (here 5β,6β). With 5α-pregnane-2,20-dione,[16] which was not investigated in detail, only the 11α-hydroxy-derivative was isolated.

This work shows that the initial 11α-hydroxylation of steroids by *A. ochraceus* may be followed by a variety of processes whose nature is determined by the structure of the substrate. Of the present results the most interesting is the clean 1β,11α-dihydroxylation of 3β-hydroxy-5α-pregnan-20-one. Chemical transformations of the main product (no. 661) and of the corresponding 3-ketone (no. 658) are shown in the Scheme. The remarkable stability of the 1,11-acetonides is paralleled by the strong intramolecular hydrogen bonding of the parent 1,11-diol system. These features are exemplified by the recovery of the 3,20-dioxo-1,11-acetonide (no. 318) after being boiled with 2N-hydrochloric acid in dioxan, and by the i.r. absorption at 3450 cm^{-1} of a dilute solution of the 3-acetoxy-1,11-dihydroxy-20-ketone (no. 338). With stronger acid the acetonide gave the known 11α-hydroxy-diketone [17] (no. 213) by the expected β-elimination of the 1-alkoxy-group. Attempted acetylation of the dihydroxy-diketone (no. 658) caused dehydration; in the product (no. 213), proximity of the 11α-OH and the C(1)–H is suggested by the small extent to which the hydroxy-group is acetylated under standard conditions and by the resonance of the olefinic proton at unusually low field.

[12] E. L. Dulaney, W. J. McAleer, M. Koslowski, E. O. Stapley, and J. Jaglom, *Appl. Microbiol.*, 1955, **3**, 336.

[13] A. I. Laskin, P. Grabowich, C. de L. Meyers, and J. Fried, *J. Medicin. Chem.*, 1964, **7**, 406.

[14] (*a*) A. Capek, O. Hans, and H. Paula, *Cesk. Microbiol.*, 1957, **2**, 168; (*b*) L. L. Smith and L. Tan, *Biochim. Biophys. Acta*, 1968, **164**, 389.

[15] B. M. Bloom and G. M. Shull, *J. Amer. Chem. Soc.*, 1955, **77**, 5767.

[16] A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, V. Kumar, G. D. Meakins, and V. E. M. Thomas, *J.C.S. Perkin I*, 1972, 492.

[17] C. Meystre, J. Kalvoda, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, 1963, **46**, 2844.

EXPERIMENTAL

For general directions and use of an asterisk to indicate that the n.m.r. signals, and possibly also the i.r. absorptions, of a compound have already been reported, see ref. 7. Where compounds with serial numbers below 651 are stated to have been identified by mixed m.p., the original preparations are contained in, or can be found from, the papers cited. The microbiological procedures and the abbreviations used in reporting the results are given fully in ref. 9. I.r. spectra indicated by $\nu_{max.}$ (high resolution) refer to dilute solutions in CCl_4 examined at a spectral slit-width of 1·5—2 cm^{-1}. Petrol refers to light petroleum, b.p. 60—80°. The abbreviation s.m. indicates starting material.

3β-*Hydroxy*-5α-*pregnan*-20-*one* [16] (no. 490).—(*a*) Incubation in shake-flasks: 3 g in Me_2SO (1025 ml), 75 flasks, medium A, 6 d, extraction II ⟶ 3·8 g combined extracts. Chromat. Al_2O_3 (10% deactivated; 250 g). Petrol–$CHCl_3$ (2 : 1) eluted s.m. (100 mg). $CHCl_3$ eluted material which was purified by p.l.c. [1 large plate, 6 × petrol–Me_2CO (4 : 1)] to give 1β,11α-*dihydroxy*-5α-*pregnane*-3,20-*dione* (no. 658) (256 mg), m.p. 190—196° (from Me_2CO–hexane), $[\alpha]_D$ (MeOH) +75° (*c* 0·3) (Found: C, 72·3; H, 9·1. $C_{21}H_{32}O_4$ requires C, 72·4; H, 9·3%), $\nu_{max.}$ 3603, 3435, 1717, and 1704 cm^{-1}. $CHCl_3$–MeOH (20 : 1) eluted material which was purified by p.l.c. [5 large plates, 6 × petrol–Me_2CO (3 : 1)] to give 1β,3β,11α-*trihydroxy*-5α-*pregnan*-20-*one* (no. 661) (1·7 g), m.p. 212—215° (from Me_2CO–hexane), $[\alpha]_D$ (MeOH) +20° (*c* 0·5) (Found: C, 71·7; H, 9·7. $C_{21}H_{34}O_4$ requires C, 72·0; H, 9·8%), $\nu_{max.}$ (Nujol) 3550, 3320, and 1698 cm^{-1}.

Incubation using a Biotech fermentor: [10] 10 g in EtOH (100 ml)–Me_2SO (100 ml) fermented for 6 d, extraction II ⟶ 14 g combined extracts. Chromat. Al_2O_3 (5% deactivated; 200 g). Petrol–EtOAc (4 : 1) eluted s.m. (3·64 g). Petrol–EtOAc (1 : 1) eluted 3β,11α-dihydroxy-5α-pregnan-20-one (no. 655) (650 mg), m.p. 176—178° (from Me_2CO–hexane), $[\alpha]_D$ +62° (*c* 0·9) (lit.,[18] m.p. 177—179°, $[\alpha]_D$ +60°). EtOAc–MeOH (9 : 1) eluted 1β,3β,11α-trihydroxy-5α-pregnan-20-one (4·25 g).

(*b*) *Transformations*: Oxidation of 1β,3β,11α-trihydroxy-5α-pregnan-20-one (no. 661) (400 mg) with 8N-H_2CrO_4 gave 5α-pregnane-1,3,11,20-tetraone (no. 364) * (292 mg), m.p. (from Me_2CO–hexane) and mixed [19] m.p. 206—212°. A solution of the trihydroxy-ketone (no. 661) (160 mg) in Me_2CO (15 ml)–2N-HCl (0·3 ml) was kept at 20 °C for 2 h. Work-up and filtration of the product, in Et_2O–petrol (1 : 1), through Al_2O_3 (10% deactivated; 10 g) gave 3β-*hydroxy*-1β,11α-*isopropylidenedioxy*-5α-*pregnan*-20-*one* (no. 340) * (151 mg), m.p. 166—168° (from hexane), $[\alpha]_D$ +77° (*c* 0·4) (Found: C, 73·8; H, 9·7. $C_{24}H_{38}O_4$ requires C, 73·8; H, 9·8%), $\nu_{max.}$ 3615 and 1708 cm^{-1}. Similarly, 1β,11α-dihydroxy-5α-pregnane-3,20-dione (no. 658) (200 mg) gave 1β,11α-*isopropylidenedioxy*-5α-*pregnane*-3,20-*dione* (no. 318) * (189 mg), m.p. 182—183° (from Me_2CO–hexane), $[\alpha]_D$ +106° (*c* 0·7) (Found: C, 74·0; H, 9·2. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·3%), $\nu_{max.}$ 1721 and 1708 cm^{-1}. A solution of the trihydroxy-ketone (no. 661) (450 mg) in *s*-collidine (24 ml) was treated with Ac_2O (8 ml) and kept at 20 °C for 3·5 h. Work-up and p.l.c. [1 large plate, 6 × petrol–Me_2CO (6 : 1)] gave 1β,11α-*dihydroxy*-20-*oxo*-5α-*pregnan*-3β-*yl acetate* (no. 338) * (180 mg), m.p. 181·5—183°, $[\alpha]_D$ +40° (*c* 0·6) (Found: C, 70·2; H, 9·3. $C_{23}H_{36}O_5$ requires C, 70·4; H, 9·2%), $\nu_{max.}$ (high resolution) 3602, 3450, 1737, and 1710 cm^{-1}.

Oxidation of the 3β-hydroxy-acetonide (no. 340) (200 mg) with 8N-H_2CrO_4 gave the 3-keto-acetonide (no. 318) (182 mg). Similar oxidation of the trihydroxy-ketone (no. 661) (400 mg) and chromatography of the products on Al_2O_3 (10% deactivated; 50 g) gave the 3-keto-acetonide (no. 318) [eluted with petrol–Et_2O (3 : 1); 160 mg]. A solution of the 3-keto-acetonide (200 mg) in dioxan (20 ml)–2N-HCl (0·6 ml) was boiled under reflux for 1 h. T.l.c. showed that the s.m. was unchanged. More 2N-HCl (4 ml) was added and the solution was refluxed gently under an air condenser for 3 h, during which time the volume of the solution decreased to *ca.* 18 ml. Work-up and p.l.c. [2 small plates, 6 × petrol–Me_2CO (6 : 1)] gave 11α-hydroxy-5α-pregn-1-ene-3,20-dione (no. 213) * (121 mg), m.p. 202—203° (from Me_2CO–hexane), $[\alpha]_D$ +98° (*c* 0·2) (Found: C, 76·1; H, 9·3. Calc. for $C_{21}H_{30}O_3$: C, 76·3; H, 9·15%), $\lambda_{max.}$ 231 nm (ε 10,800), $\nu_{max.}$ 3605, 1707, and 1689 cm^{-1} (lit.,[17] m.p. 193—195°, $[\alpha]_D$ +75°). Treatment of the dihydroxy-diketone (no. 658) (80 mg) with Ac_2O (2 ml)–C_5H_5N (6 ml) at 20 °C for 24 h gave the 11-hydroxy-diketone (no. 213) (51 mg). Oxidation of the 11-hydroxy-diketone (no. 213) (100 mg) with 8N-H_2CrO_4 gave 5α-pregn-1-ene-3,11,20-trione (no. 363) * (90 mg), m.p. 219—222° (from Me_2CO–hexane), $[\alpha]_D$ +149° (*c* 1·0), $\lambda_{max.}$ 227 nm (ε 10,500) (lit.,[20] m.p. 208—213°, $[\alpha]_D$ +150°).

5α-*Pregnane*-3,20-*dione* (no. 59).—(*a*) First incubation: 3·92 g in Me_2SO (1470 ml), 98 flasks, 6 d, medium A, extraction II ⟶ combined extracts. Chromat. Al_2O_3 (10% deactivated; 300 g). Petrol–$CHCl_3$ (5 : 1) eluted s.m. (1·1 g). Petrol–$CHCl_3$ (2 : 1) eluted 11α-hydroxy-5α-pregnane-3,20-dione (1·1 g) [no. 212; * see ref. 7 for main n.m.r. signals, τ 1·63 (d, *J* 10 Hz, H-1), and 4·17 (d, *J* 10 Hz, H-2)], m.p. 201—202° (from Me_2CO–hexane), $[\alpha]_D$ +86° (*c* 0·9) (lit.,[21] m.p. 197—200°, $[\alpha]_D$ +83°). Petrol–$CHCl_3$ (1 : 1) eluted 11α-*hydroxy*-4-*oxa*-A-*homo*-5α-*pregnane*-3,20-*dione* (no. 653) (900 mg), m.p. 199—200° (from Me_2CO–hexane), $[\alpha]_D$ +45° (*c* 1·0) (Found: C, 72·4; H, 9·4. $C_{21}H_{32}O_4$ requires C, 72·4; H, 9·3%), $\nu_{max.}$ 3615, 1742, and 1708 cm^{-1}. $CHCl_3$ eluted material which was acetylated [Ac_2O–C_5H_5N (1 : 1) for 2 d at 20 °C] and separated by p.l.c. [2 large plates, 3 × petrol–Me_2CO (6 : 1)] to give, in order of decreasing R_F, 3β,11α-diacetoxy-5α-pregnan-20-one (no. 655) (35 mg), as a gum, *m/e* 418 (M^+), $\nu_{max.}$ 1734 and 1709 cm^{-1}; 3,20-*dioxo*-5α-*pregn*-1-*en*-11α-*yl acetate* (no. 654) (100 mg), m.p. 148—151° (from Me_2CO–hexane), $[\alpha]_D$ +85° (*c* 0·3) (Found: C, 74·1; H, 8·8. $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·7%), $\nu_{max.}$ 1738, 1709, and 1680 cm^{-1}; and a mixture which was separated by p.l.c. [one large plate, 15 × petrol–Me_2CO (8 : 1)] into 6β,11α-diacetoxy-5α-pregnane-3,20-dione (no. 659) (higher R_F) (150 mg), a glass, $[\alpha]_D$ +17° (*c* 0·8), *m/e* 432 (M^+), $\nu_{max.}$ 1740, 1722, and 1710 cm^{-1}, and 11α-hydroxy-5α-pregn-1-ene-3,20-dione (no. 213) (lower R_F) (250 mg). $CHCl_3$–MeOH (20 : 1) eluted material which was similarly acetylated and separated [1 large plate, 4 × $CHCl_3$] to give 11α,17β-*diacetoxy*-5α-*androstan*-3-*one* (no. 297) * (higher R_F) (304 mg), m.p. 193—195° (from

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Me_2CO–hexane), $[\alpha]_D$ −27° (*c* 1·1) (Found: C, 70·5; H, 8·7. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1735 and 1718 cm^{-1}, and a mixture (lower R_F) (310 mg) thought to contain 3β,11α-diacetoxy-1β-hydroxy- and 1β,3β-diacetoxy-11α-hydroxy-5α-pregnan-20-one in a ratio of 2 : 1 [n.m.r. signals at τ 8·0 (OAc, both components), at τ 4·92 (*J* 10, 10, and 6 Hz, 11β-H), 5·31 (*J* 10, 10, 5, and 5 Hz, 3α-H), 6·38 (*J* 12 and 5 Hz, 1α-H), 9·09 (19-H), and 9·38 (18-H) (major component), and at τ 5·19 (*J* 12 and 5 Hz, 1α-H), 5·31 (*J* 10, 10, 5, and 5 Hz, 3α-H), 6·15 (*J* 10, 10, and 6 Hz, 11β-H), 8·86 (19-H), and 9·41 (18-H) (minor component).

Second incubation: 2·8 g in Me_2SO (420 ml), 70 flasks, medium B, 6 d, extraction II ⟶ combined extracts. Chromat. Al_2O_3 (10% deactivated; 300 g). Petrol–$CHCl_3$ (5 : 1) eluted s.m. (2·05 g). Petrol–$CHCl_3$ (2 : 1) eluted 11α-hydroxy-5α-pregnane-3,20-dione (no. 212) (530 mg). $CHCl_3$–MeOH (19 : 1) eluted material which, after treatment with Ac_2O–C_5H_5N and p.l.c., afforded 11α,17β-diacetoxy-5α-androstan-3-one (no. 297) (20 mg). Similar treatment of the material eluted with $CHCl_3$–MeOH (9 : 1) gave 11α-hydroxy-5α-pregn-1-ene-3,20-dione (no. 213) (80 mg).

3β-*Hydroxypregn-5-en*-20-*one* (no. 651).—(*a*) Incubation: 3 g in Me_2SO (1110 ml), 75 flasks, medium A, 6 d, extraction II ⟶ combined extracts. Chromat. Al_2O_3 (10% deactivated; 500 g). Petrol–$CHCl_3$ (1 : 1) eluted s.m. (60 mg). $CHCl_3$ eluted a mixture which was separated by p.l.c. [2 large plates, 2 × Et_2O–MeOH (49 : 1)] into 6β,11α-dihydroxypregn-4-ene-3,20-dione (no. 660) (higher R_F) (721 mg), m.p. 244—247° (from Me_2CO–hexane), $[\alpha]_D$ (MeOH) +96° (*c* 0·5) {lit.,[12,22] m.p. 245—248°, $[\alpha]_D$ (MeOH) [12] +100°}, $\lambda_{max.}$ 238 nm (ε 13,800), and 5,6β-*epoxy*-3β,11α-*dihydroxy*-5β-*pregnan*-20-*one* (no. 657) (lower R_F) (360 mg), m.p. 244—246° (from EtOH), $[\alpha]_D$ (MeOH) +67° (*c* 0·5) (Found: C, 72·7; H, 9·1. $C_{21}H_{32}O_4$ requires C, 72·8; H, 9·3%), $\nu_{max.}$ (Nujol) 1703 cm^{-1}. $CHCl_3$–MeOH (49 : 1) eluted 3β,7β,11α-*trihydroxypregn*-5-*en*-20-*one* ‡ (no. 665) (710 mg), m.p. 265—267° (from EtOAc–MeOH), $[\alpha]_D$ (EtOH) +68° (*c* 0·5) (Found: C, 72·5; H, 9·0. $C_{21}H_{32}O_4$ requires C, 72·8; H, 9·3%), $\nu_{max.}$ 1705 cm^{-1}. $CHCl_3$–MeOH (19 : 1) eluted material which was purified by p.l.c. to give 1β,3β,11α-*trihydroxypregn*-5-*en*-20-*one* (no. 662) (250 mg), m.p. 224—226° (from Me_2CO–hexane), $[\alpha]_D$ (EtOH) +8° (*c* 0·9) (Found: C, 73·0; H, 9·4. $C_{21}H_{32}O_4$ requires C, 72·8; H, 9·3%), $\nu_{max.}$ 1709 cm^{-1}, and then 3β,7α,11α-*trihydroxypregn*-5-*en*-20-*one* * (no. 664) (200 mg), m.p. 249—253° (from Me_2CO–hexane), $[\alpha]_D$ (EtOH) −45° (*c* 0·5) (Found: C, 72·8; H, 9·2. $C_{21}H_{32}O_4$ requires C, 72·8; H, 9·3%), $\nu_{max.}$ 1709 cm^{-1}.

(*b*) Transformations: Acetylation of the 6β,11α-dihydroxy-compound (no. 660) for 4 d at 20 °C gave 6β,11α-diacetoxypregn-4-ene-3,20-dione (no. 319),* m.p. 156—160° (from Me_2CO–hexane), $[\alpha]_D$ +84° (*c* 0·9) (lit.,[21] m.p. 153—154°, $[\alpha]_D$ +71°). Similarly the trihydroxy-compounds (nos. 662, 665, and 664) and the hydroxy-epoxide (no. 657) gave, respectively, 1β,3β,11α-*triacetoxypregn*-5-*en*-20-*one* (no. 339),* m.p. 158—159·5° (from Me_2CO–hexane), $[\alpha]_D$ −25° (*c* 0·9) (Found: C, 68·5; H, 8·0. $C_{27}H_{38}O_7$ requires C, 68·4; H, 8·1%), $\nu_{max.}$ 1740 and 1710 cm^{-1}; 3β,7β,11α-*triacetoxypregn*-5-*en*-20-*one* (no. 344),* m.p. 175—177° (from Me_2CO–hexane), $[\alpha]_D$ +69° (*c* 1·0) (Found: C, 68·1; H, 8·0%), $\nu_{max.}$ 1737 and 1707 cm^{-1}; 3β,7α,11α-triacetoxypregn-5-en-20-one (no. 343) * as an oil, *m/e* 474 (M^+), $\nu_{max.}$ 1735 and 1708 cm^{-1}; and 3β,11α-*diacetoxy*-5,6β-*epoxy*-5β-*pregnan*-20-*one* (no. 342),* m.p. 214—215° (from Me_2CO–hexane), $[\alpha]_D$ +17° (*c* 1·0) (Found: C, 69·5; H, 8·4. $C_{25}H_{36}O_6$ requires C, 69·4; H, 8·4%), $\nu_{max.}$ 1738 and 1708 cm^{-1}.

A solution of the 1β,3β,11α-trihydroxy-compound (no. 662) (65 mg) and TsOH (2 mg) in Me_2CO (20 ml) was boiled under reflux for 3 h. Work-up and p.l.c. [1 small plate, 12 × petrol–Me_2CO (12 : 1)] gave 3β-*hydroxy*-1β,11α-*isopropylidenedioxypregn*-5-*en*-20-*one* (no. 663) (39 mg), m.p. 246—248° (from Me_2CO–hexane), $[\alpha]_D$ −10° (*c* 0·8) (Found: C, 74·3; H, 9·3. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·3%), $\nu_{max.}$ 3610 and 1707 cm^{-1}.

5α-*Pregnane*-2,20-*dione* (no. 353).—Incubation: 320 mg in Me_2SO (120 ml), 8 flasks, medium A, 6 d, extraction II ⟶ combined extracts. P.l.c. [1 large plate, 4 × $CHCl_3$–MeOH (49 : 1)] gave, as the main product, 11α-*hydroxy*-5α-*pregnane*-2,20-*dione* (no. 652) (164 mg), m.p. 188—190° (from Me_2CO–hexane), $[\alpha]_D$ +93° (*c* 0·8) (Found: C, 75·6; H, 9·5. $C_{21}H_{32}O_5$ requires C, 75·9; H, 9·7%), $\nu_{max.}$ 3605, 1717sh, and 1711 cm^{-1}.

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* The compound [23] {m.p. 247—248°; $[\alpha]_D$ (MeOH) −41°; analytical figures not given} previously formulated as 3β,7β,11α-trihydroxypregn-5-en-20-one is probably the 7α-isomer (no. 664); the constants {m.p. 216—218°; $[\alpha]_D$ ($CHCl_3$–EtOH) −19°} of a compound [24] thought to be 3β,7α,11α-trihydroxypregn-5-en-20-one differ markedly from those of the 3β,7α,11α-triol reported here.

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