Microbiological Hydroxylation of Steroids. Part IV.[1] The Pattern of Dihydroxylation of Mono-oxygenated 5α-Androstanes with Cultures of the Fungus *Calonectria decora*

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The work is concerned with the relation between the pattern of the dihydroxylation by *Calonectria decora* of mono-oxygenated 5α-androstane derivatives (mainly ketones), and the position of the oxygen function in the substrate. Terminal ring ketones (3, 4, 16, and 17) are converted, in useful yields, into one or two dihydroxy-ketones. (Ring B and C ketones are much less satisfactory as substrates.) The structures of most of the products followed from spectrometric investigations: this approach was supplemented by chemical correlations where necessary

The two hydroxy-groups are introduced on to carbon atoms separated by about 4 Å from one another. The distances of these centres from the carbonyl group are more variable, although with the 3-, 4-, 16-, and 17-ketones the correspondence is gratifyingly close and may have predictive value.

UPPERMOST among the objects of our microbiological hydroxylation studies was that of converting natural or synthetic materials into more useful products. In particular, hydroxylation by fungal cultures seemed promising for preparing relatively inaccessible steroids; some examples have already been described.[1,2] The introduction of one or more hydroxy-groups into synthetic materials could make polyfunctional compounds more readily available and we have achieved this in the hydrochrysene series.[3] A group at the Upjohn Company [4] has shown that *Sporotrichum sulfurescens* effectively monohydroxylates macrocyclic alcohols (*e.g.* cyclodecanol) and acyl derivatives of cyclic amines (cyclododecylamine) and azacycloalkanes (octamethyleneimine).

Nearly all the literature on the microbiological hydroxylation of steroids [5] refers to substrates having an oxygen atom at C-3. Further, most of the substrates studied contain the 3-oxo-Δ^4-system, since it confers useful physiological properties on steroids. These features, together with the equally ubiquitous presence of substituents, often complicated, at C-17 could well have a dominating influence on the position and extent of hydroxylation by micro-organisms. In order to ascertain whether there are more general patterns of hydroxylation it was essential to depart from this uniformity of substrate structure. The same idea had prompted the investigations of the Upjohn group,[6]

[1] Part III, 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.

[2] J. E. Bridgeman, P. C. Cherry, Sir Ewart R. H. Jones, and G. D. Meakins, *Chem. Comm.*, 1967, 482; A. S. Clegg, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *ibid.*, 1970, 1029.

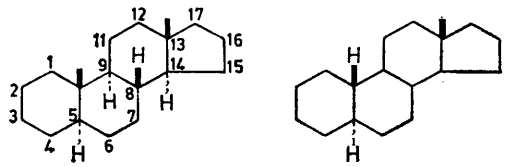
[3] M. J. Ashton, D.Phil. Thesis, Oxford, 1972.

[4] M. E. Herr, R. A. Johnson, W. C. Krueger, H. C. Murray, and L. M. Pschigoda, *J. Org. Chem.*, 1970, **35**, 3607, and references cited therein.

[5] *Inter alia*, W. Charney and H. L. Herzog, 'Microbial Transformations of Steroids,' Academic Press, New York, 1967, the most comprehensive of many reviews.

[6] G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Org. Chem.*, 1967, **32**, 672.

Hydroxylation of androstanes and estranes by *Calonectria decora*



5α-Androstane 5α-Estrane

In the ' Products ' column those oxygen functions introduced during the incubation are in bold type. 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).

TABLE 1

Substantial conversions into one or two products

Substrate	Conditions	Substrate recovered	Main product		Other products	
3-CO	E5	23%	**12β,15α**-$(OH)_2$	52%	3β,**12β,15α**-$(OH)_3$	13%
	D4	0	**6α,12β,15α**-$(OH)_3$	28		
3-CO-Δ^1	D6	14	**12β,15α**-$(OH)_2$	21	**6α,11α**- $(OH)_2$	2
3-CO-Δ^4	E2	31	**12β,15α**-$(OH)_2$	55		
3β-OH	E6	33	**12β,15α**-$(OH)_2$	18		
3β-OH-Δ^1	D6	23	**12β,15α**-$(OH)_2$—3-CO	23		
3β-OH-Δ^4	D6	0	**12β,15α**-$(OH)_2$—3-CO	38	3-CO	13
Estran-3-one	D6	18	**12β,15α**-$(OH)_2$	13	**11α,15α**-$(OH)_2$	3
Estr-4-en-3-one	D6	3	**12β,15α**-$(OH)_2$	35	**6β,11α**- $(OH)_2$	12
4-CO	D4	0	**11α,15α**-$(OH)_2$	36		
			12β,15α-$(OH)_2$	36		
2-CO	D4	12	**6α,12β**- $(OH)_2$	26	**6α,11α**- $(OH)_2$	11
15-CO	D6	4	14β-**6α,12β**- $(OH)_2$	21	**2α, 12β**- $(OH)_2$	8
14β-15-CO	D6	23	14β-**7β,12β**- $(OH)_2$	34	**7β,12β,14β**-$(OH)_3$	10
17-CO	E2	40	**1β,6α**- $(OH)_2$	47		
17β-OH	E6	41	**1β,6α**- $(OH)_2$	17	**6α,11α**- $(OH)_2$	7
3-CH_2—17β-OH	E2	54	**1β,6α**- $(OH)_2$	82		
16-CO	D4	31	**6α,11α**- $(OH)_2$	33	**1β,6α**- $(OH)_2$	9

TABLE 2

Modest or zero conversions

Substrate	Conditions	Substrate recovered	Products	
1-CO	D6	75%	none isolated (n.i.)	
1-CO—Δ^2	D6	27	6α-OH-**16**-CO	15%
			6α, 16β-$(OH)_2$	4
A-nor-2-CO	D4	40	**12β,15α**-$(OH)_2$	11
6-CO	D6	90	n.i.	
7-CO	D7	72	x,y-$(OH)_2$	14
			12β- OH	3
7-CO—Δ^5	E2	80	**3β, 12β**- $(OH)_2$	20
			4β, 12β- $(OH)_2$	10
			12β- OH	10
11-CO	E2	38	**1β,6α**- $(OH)_2$	11
			6α- OH	3
12-CO	D6	8	**6α, 15α**-$(OH)_2$	15
			1β,6α, 15α-$(OH)_3$	12
D-homo-17-CO	D4		**6α,11α**- $(OH)_2$	10
			7β,12β,15α-$(OH)_3$	11
			1β,7β, 15α-$(OH)_3$	6
5β-17-CO	E2	55	**12β,15α**-$(OH)_2$	2
Estran-17-one	D6	47	n.i.	
3-CH_2—17-CO	D2	14	**1β,6α**- $(OH)_2$	18
5α-Androstane	E2	45	n.i.	

TABLE 3

Hydroxylation of some 3-substituted 5α-androstanes

Substrate			Products	
3β-O·CH_2·CH=CH_2	D6	38%	**7β,12β,15α**-$(OH)_3$	16%
3β-O·CO·$[CH_2]_2$·CO_2Me	D4	41	**3β,12β,15α**-$(OH)_3$	2
3β-O·CH_2·CO_2Et	D4	49	**6α,12β,15α**-$(OH)_3$	8
3α-O·$[CH_2]_2$·OAc	D4	24	3α-O·$[CH_2]_2$·OH-**12β,15α**-$(OH)_2$	18
3β-O·$[CH_2]_2$·OAc	D4	33	3β-O·$[CH_2]_2$·OH-**12β,15α**-$(OH)_2$	37
3β-OMe	E2	84	n.i.	
3β-OMe	D4	81	n.i.	
3β-O·CH_2Ph	D4		n.i.	
3β-O·CO-α-furyl	D6	80	n.i.	
3β-O·CO_2Et	D6	64	n.i.	
3β-O·CO·O·CH_2·CCl_3	D6	40	n.i.	

who had been ' struck by the apparent lack of a rational explanation for the selection by a given micro-organism of the particular carbon atom to be oxygenated.'

The first stage of our studies was to screen a range of micro-organisms, known to hydroxylate steroids, with as substrates a series of mono- and dioxygenated 5α-androstanes in which the positions of the substituents around the steroid nucleus varied systematically. This paper describes the results obtained with several mono-substituted 5α-androstanes, and a few androstenes, and cultures of the fungus *Calonectria decora* (Wallr.), Sacc.[7]

Explanation of the form and order used in presenting the results. Our intention is to report most of the microbiological work, under the headings of the organisms used, in papers (such as the present one) of a standard form. The following paragraphs explain the form, and show how this paper links up with the earlier publications.

The basis is the assignment of (arabic) serial numbers to the steroids (about 600 so far, many of them new) which have been used as substrates or obtained as products. These enable the details of the particular

[7] Preliminary report, J. E. Bridgeman, J. W. Browne, P. C. Cherry, M. G. Combe, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, Y. Morisawa, and P. D. Woodgate, *Chem. Comm.*, 1969, 463.

compounds and the chemical transformations to be located. In each paper the steroids involved are arranged in the order described earlier.[8] So far the spectra (n.m.r. and, for the more important compounds, i.r.) of the following compounds have been, or are now, reported: nos. 1—344, ref. 8; 345—374, ref. 9; 375, ref. 1; 376—393, ref. 10; 394—411, ref. 11; 412—482, present paper. Apart from the first two papers, which are confined to spectrometric details, the serial numbers appear in the Experimental sections. The last four papers give complete descriptions (*i.e.*, spectra, preparations, and constants) of the compounds with the numbers shown: they also contain the preparations and constants of some of the new compounds with

FIGURE 1 Hydroxylation of:

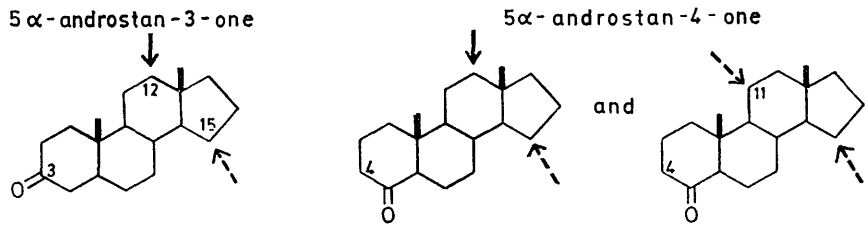
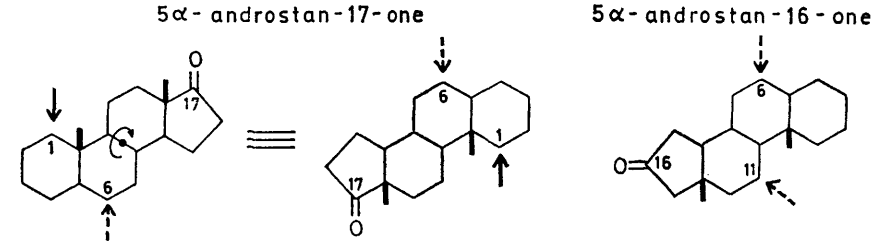
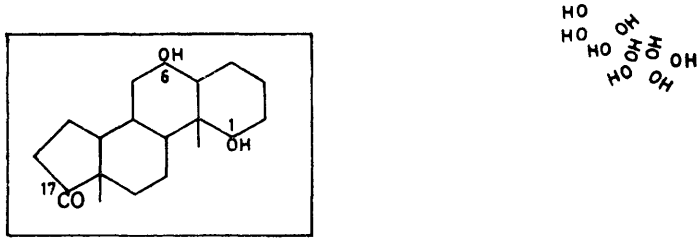


FIGURE 2 Hydroxylation of:





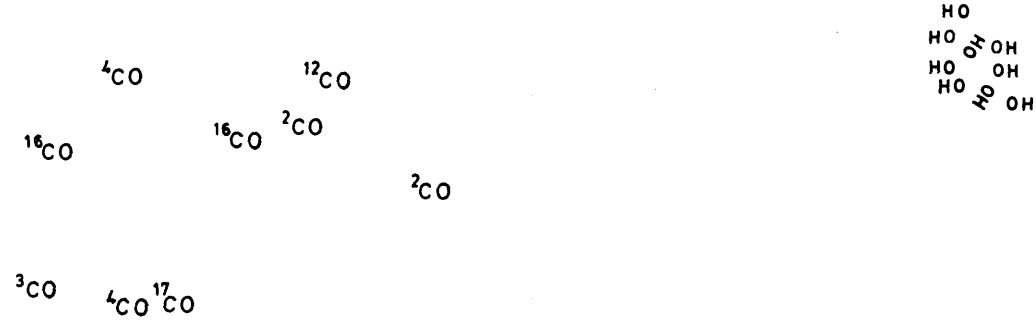


FIGURE 3 Distances between carbon atoms hydroxylated by *C. decora* and the directing carbonyl group

[8] 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.

[9] 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.

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

[11] I. M. Clark, A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and A. Pendlebury, *J.C.S. Perkin I*, 1972, 499.

numbers below 375. (The form appropriate for reporting a compound as new is used in the Experimental section giving the preparation of that compound even though its spectrometric characteristics may have appeared earlier.)

Since the main purpose of the work is to study microbiological transformations, the results of these are summarised in Tables 1—3, and discussed *before* the chemical background is considered. Most of the substrates are derivatives of 5α-androstane, and are indicated in the Tables of microbiological results by abbreviated names. Substrates derived from other parents are named fully. With the products only the groups which have been introduced (bold type) or modified are specified. The yields are calculated after making allowance for recovered starting material (*i.e.* they refer to the composition of the steroidal material obtained after incubation and removal of starting material, and are therefore the yields that would be obtained by recycling the substrate).

The structures of many of the products follow unequivocally from the combination of spectrometric and chemical methods as explained earlier.[8,9] With others, further operations were necessary to confirm the structural features. These generally involved the conversion of selected products into simpler, known steroids, and/or the establishment of chemical interrelations. Although detailed discussion is unwarranted it is necessary to show that the structural conclusions are soundly based. The salient features of the additional work are therefore presented briefly in the Scheme; points of interest which emerged during this work are also shown there. The serial numbers of the steroids are used in the Scheme and repeated in Table 4 (n.m.r. results, immediately before the Experimental section) in order to facilitate cross-reference.

RESULTS AND DISCUSSION

There is considerable variation in the behaviour of the substrates. Some are rapidly hydroxylated whereas others are largely unchanged after 6 days incubation; some give rise to complex mixtures, others give one or two products in reasonable yields. Table 1 shows the cases in which substantial conversions occur, and give mainly single products. When allowance is made for recovered substrates, the yields are seen to be in the 15—80% range; we have not tried to find optimal conditions and it is likely that appreciable improvements could be made. The introduction of *two* hydroxy-groups is the normal pattern, as observed by Schubert and Siebert with progesterone and 5α-pregnanolone,[12] and almost all these groups have the equatorial conformation. Monohydroxylated products cannot be obtained in reasonable amounts by using shorter incubation times. (It is notable that with dimethyl sulphoxide in the medium androstan-3-one gives a trihydroxy-ketone as major product; products of further hydroxylation would probably be formed from other substrates in Table 1 under appropriate conditions.

In the literature on steroid hydroxylation, fungi are generally classified according to which position in 3-oxo-Δ^4-steroids (*e.g.* progesterone) they attack most frequently; on this basis *Calonectria decora* is recorded as a 12β,15α-dihydroxylator. Our results confirm this for 3-oxygenated steroids but it is clear (from Tables 1 and 2) that the use of the conventional substrates has masked the versatility of this organism, and that by varying the location of a single oxygen group in the substrate, hydroxylation can be effected in other positions.

Schubert *et al.*[12] obtained 12β,15α-dihydroxylation exclusively with 3-oxygenated pregnane substrates. The 3-substituted androstane and estrane derivatives behave similarly, and the absence of a 17-substituent does not influence the result. With the 4-ketone the production of an equal amount of the 11α,15α-diol is a minor deviation from this pattern; the substitution at 11 is again equatorial and the distances between the centres involved (*cf.* Figure 1) are not very different.

The 16- and 17-oxygenated substrates (Table 1) are dihydroxylated (equatorially) in a manner akin to that of the 3- and 4-ketones, *i.e.* two equatorial hydroxy-groups are introduced at distances from one another, and from the oxygen substituent in the substrate, which are closely comparable. This is illustrated in Figure 2, and can be demonstrated by rotating models through 180° about an axis through positions 8 and 9. With the 16-ketone, and to a lesser extent with the 17β-alcohol, the 6α-hydroxylation is accompanied by substitution at the 11α-position in preference to 1β-attack (Table 1 and Figure 2). Although C-1 and C-11 are 2·9 Å apart, equatorial oxygen atoms attached to them are similarly situated with regard to the steroid molecule as a whole.

Most cases in Table 1 show a close correspondence in the distances between the carbon atoms attacked; these are 12,15-, 3·8 Å; 1,6- 3·9; 6,11-, 4·4; 11,15- 4·5. There is also similarity, though to a lesser degree, in the distances between the carbon atoms hydroxylated and the site of the original oxygen substituent. [The 15-ketone (14α and 14β) results cannot strictly be compared with the others since the major products have the more stable but less usual 14β-configuration. Nevertheless, diequatorial substitution on carbon atoms at about the usual distances from one another is again observed.] Figure 3 depicts the relative positions of the carbon atoms hydroxylated and the substrate carbonyl group. Nine formulae have been superimposed (one is indicated in the inset), matching up the substituted carbon atoms (represented by OH) and bringing the carbonyl groups as close together as possible. The coincidence between the hydroxylated sites is very close and, although the variation in the orientation of the carbonyl group is greater, there is a

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

strong suggestion of a rough geometrical relationship.* Studies with the A-nor- and D-homo-ketones were not extensive (Table 2) but the patterns of disubstitution appear to follow those of the 3- and 16-ketones, respectively.

Substantial conversion into a major product occurs only when the oxygen function of the substrate is in ring A or ring D. With the exception of 5α-androstan-12-one, no clear patterns emerge with the ring B and C ketones (Table 2), but the susceptible 1β, 6α-, 11α-, 12β-, and 15α-positions (Table 1) are frequently involved. (Attention has already been drawn [13] to the microbiological introduction of a 3-oxygen function.)

The polar group in the substrate may have several functions. Hydroxylation probably occurs within the cell and one of the limiting factors must be the ability of the substrate to penetrate the cell wall; thus, the solubilising effect of the polar group is likely to be an important feature. (This would explain the unreactivity of 5α-androstane and its 3β-methoxy-derivative.) The variation caused by changing the nature and the amount of the solvent used to introduce the steroid into the medium may arise from this effect.

The polar group also has a directing influence on the course of hydroxylation. It could be that the organism has a predilection for attacking certain positions in the steroid nucleus (*e.g.*, 1, 6, 12, and 15) and that the polar group acts in a negative way, directing the attack to more remote positions; hydroxylation might stop, or proceed less vigorously, when a sufficient number of hydroxy-groups (apparently two) has been introduced to give a product which is much more soluble in the medium. An alternative is that the polar group becomes associated with a hydrophilic region of the hydroxylating enzyme system and thereby determines the orientation in which the steroid is presented at complementary hydrophobic enzyme sites. Polar groups in rings A and D would then lead to specific arrangements of the substrate on the enzyme surface: involvement of the same sites (*e.g.* a triangular arrangement of one binding and two hydroxylating centres) could be the basis of the observed reversal in the directing effects of terminal ring carbonyl groups. Distinction between these alternatives cannot be made from results such as those reported here, and must await studies with isolated enzyme systems.

In practical terms ketones are better than alcohols as substrates, indicating that the carbonyl group is a more effective directing and/or binding site. The 1β,6α-dihydroxylation observed with 3-methylene-5α-androstan-17β-ol (Table 1) shows that the π-electrons have much less effect than the 17β-hydroxy-group. The failure of 5α-androstan-3α-ol, with an axial hydroxy-group, to hydroxylate suggests that the orientation of the carbon–oxygen bond is also an important factor. Steroids with oxygenated C-3 side-chains were made to study the effect of a polar group further from the steroid nucleus. Generally these were converted inefficiently (Table 3) but the results with the 3α- and 3β-(β-acetoxyethoxy)-substituents may be significant.

Hydroxylation of mono-substituted steroids with *Calonectria decora* is of limited preparative value. Although it leads to hydroxy-steroids of unusual types (*e.g.* 1β- and 15α-OH groups), the invariable dihydroxylation means that selective reactions are necessary to remove the second hydroxy-group. The scope for utilising this organism in synthetic work is greatly improved by using dioxygenated steroids as substrates, as will be described later.

EXPERIMENTAL

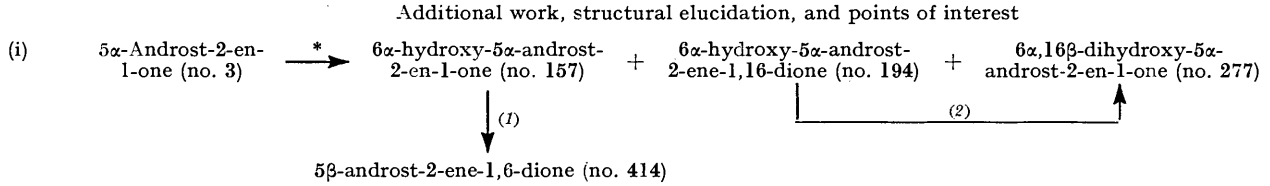
Unless otherwise stated, spectra were measured using a Perkin-Elmer R14 (100 MHz) spectrometer with $CDCl_3$ solutions (n.m.r.), a Perkin-Elmer 237 with CS_2 or CCl_4 (routine i.r.), and a Cary 14-M with EtOH (u.v.). An asterisk indicates that the n.m.r. signals, and possibly also the i.r. absorptions, have already been reported in the papers listed earlier. Optical rotations were determined on a Perkin-Elmer 141 polarimeter for $CHCl_3$ solutions at 20°C. Al_2O_3 refers to 'Camag' material, activity 1; deactivated Al_2O_3 was obtained by treatment with 5% of H_2O. Petrol refers to light petroleum, b.p. 60—80°. Details of the microbiological and preparative layer chromatography (p.l.c.) techniques, and an explanation of the abbreviations used in reporting the results, are

* Microbiological hydroxylation of a variety of macrocyclic alcohols and related compounds containing sulphur and nitrogen generally gives monohydroxylated products in which there is a roughly constant distance (5·5 Å) between the carbon atom substituted and the hetero-atom group.[6] Although there is some similarity between these results and ours, the dihydroxylation with *C. decora* and the lower conformational mobility of the steroids preclude precise comparison.

[13] P. C. Cherry, Sir Ewart R. H. Jones, and G. D. Meakins, *Chem. Comm.*, 1966, 587.

SCHEME

Additional work, structural elucidation, and points of interest



* Table 2. Reagents: (*1*), H_2CrO_4–Me_2CO; (*2*), $NaBH_4$.

No. 3, $J_{3,4\alpha} = J_{3,4\beta}$ (= 2 Hz); nos. 157, 194, and 277, $J_{3,4\alpha} \neq J_{3,4\beta}$: suggests that substituents are in ring B. No. 157, ΔM_D of OH = +35°: suggests 6α-OH (lit.,[a] +55°) rather than 7β-OH (lit.,[a] +110°). No. 414, Δ_1^3 +0·16 (19-H) and +0·20 (18-H): agrees with structure proposed (calc. +0·17 and +0·20 [b]) but not with that of corresponding 1,7-dione (calc. +0·44 and +0·11). No. 194, $\nu_{max.}$ 1740 cm^{-1} and large negative Cotton effect: suggests 16-oxo-group (lit.,[c] large positive effects of 15-and 17-oxo-groups).

SCHEME—*continued.*

(ii) Androst-5-en-7-one (no. 346) —*→ 12β-hydroxyandrost-5-en-7-one (no. 169) + 3β,12β-dihydroxyandrost-5-en-7-one (no. 257) + 4β,12β-dihydroxyandrost-5-en-7-one (no. 440)

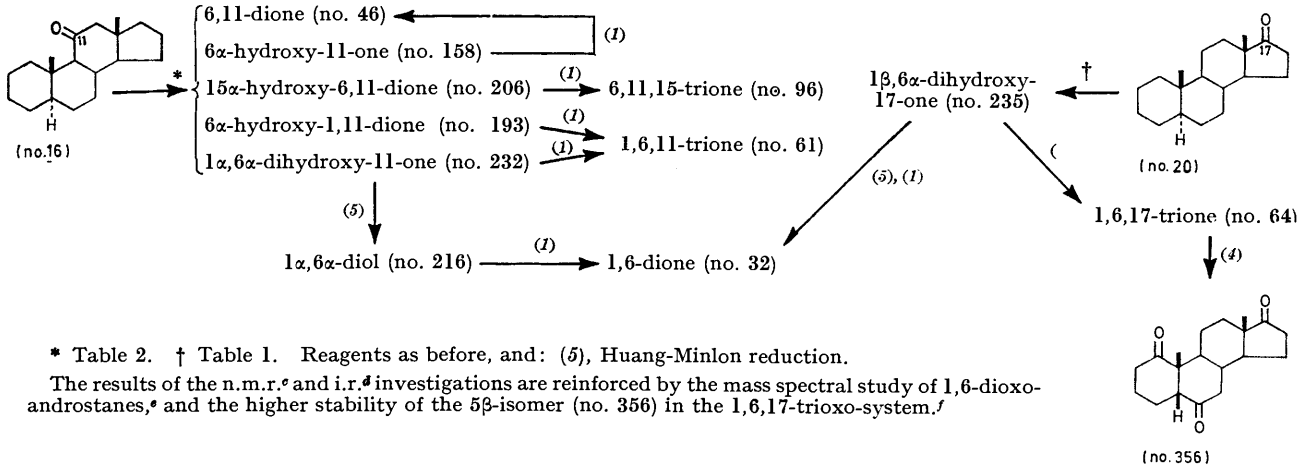
(3), (4) ↓

12β-hydroxyandrosta-3,5-dien-7-one (no. 170)

* Table 2. Reagents: (*3*), Ac_2O–C_5H_5N; (*4*), KOH–MeOH.

No. 257, $\lambda_{max.}$ (EtOH) 237 nm and (KOH–EtOH) 281 nm: suggestion of 3-OH confirmed by conversion into no. 170 (the β-configuration then follows from n.m.r.[b]). No. 440, H-4 and H-6 signals at τ 5·28 (t, *J* 2·8 Hz) and 4·23 (s, $W_{\frac{1}{2}}$ 1·5 Hz), respectively: suggests 4β-OH [the H-6 signal of androst-5-en-7-one (no. 346) has $W_{\frac{1}{2}}$ 3·0 OHz due to extra 4β,6-coupling].

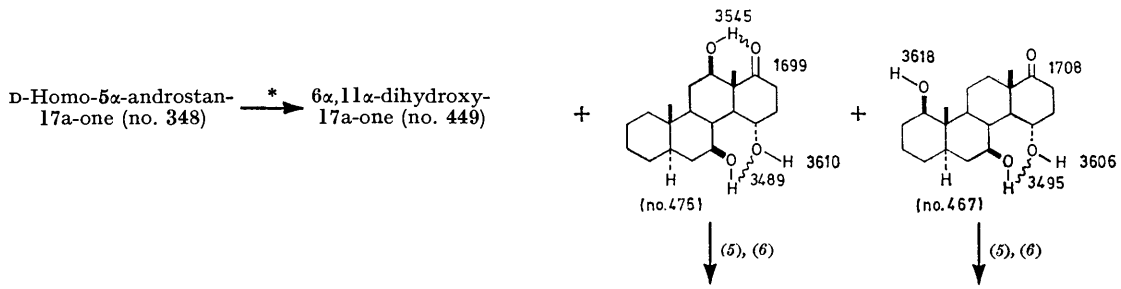
(iii) (The 5α-configuration is implied in the abbreviated names)



* Table 2. † Table 1. Reagents as before, and: (*5*), Huang-Minlon reduction.

The results of the n.m.r.[c] and i.r.[d] investigations are reinforced by the mass spectral study of 1,6-dioxoandrostanes,[e] and the higher stability of the 5β-isomer (no. 356) in the 1,6,17-trioxo-system.[f]

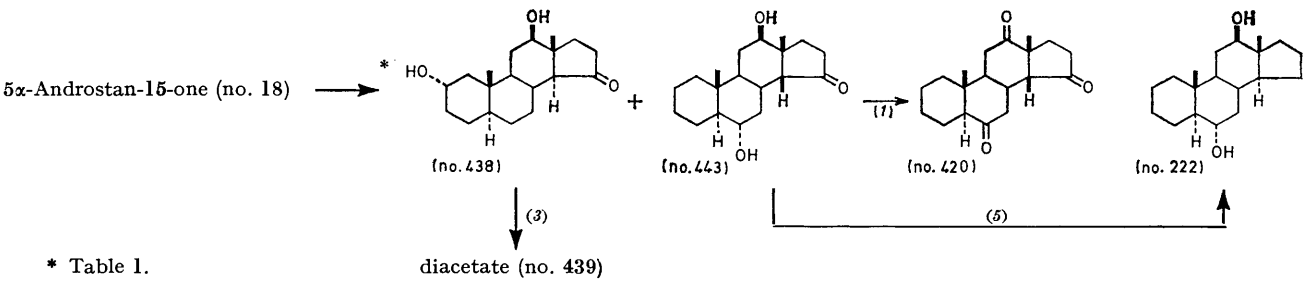
(iv) (The figures on the formulae are O—H and C=O frequencies, obtained under the conditions described earlier [d])



* Table 2. Reagents as before, and: (*6*), HCl–Me_2CO.

High resolution i.r. indicates a 12β-OH–17a-CO system in no. 476, and a 7β-15α-$(OH)_2$ system in nos. 476 and 467: chemical evidence supports this in that both compounds have a pair of hydroxy-groups sufficiently close for acetal formation. (The bonding in nos. 476 and 467 could be 15 ⟶ 7 rather than the 7 ⟶ 15 arrangement shown.)

(v)



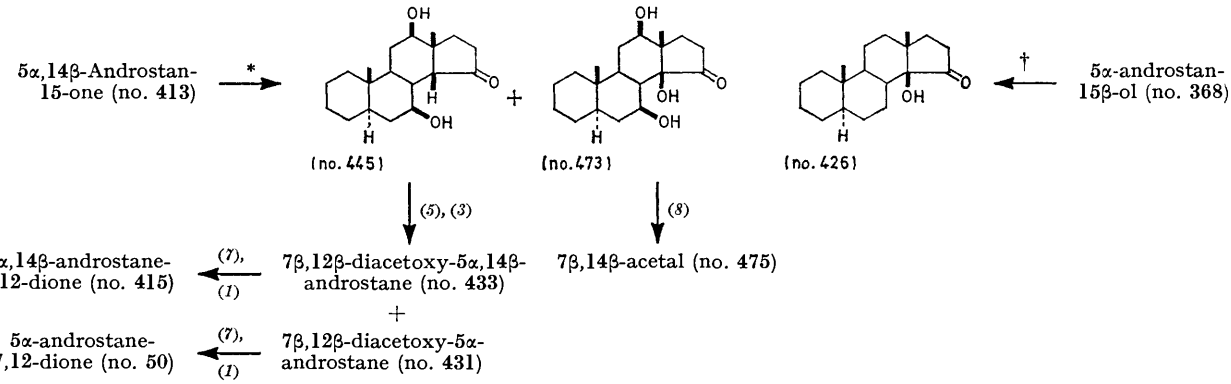
* Table 1.

Cotton effects, positive for no. 439 and negative for no. 443: suggest 14α- and 14β-configurations respectively.[g] Huang-Minlon reduction of no. 443 involves partial epimerisation at position 14 and gives, in low yield, the 6α,12β-diol (no. 222) previously encountered in work on the normal (14α) compounds.

SCHEME—*continued.*

(vi)

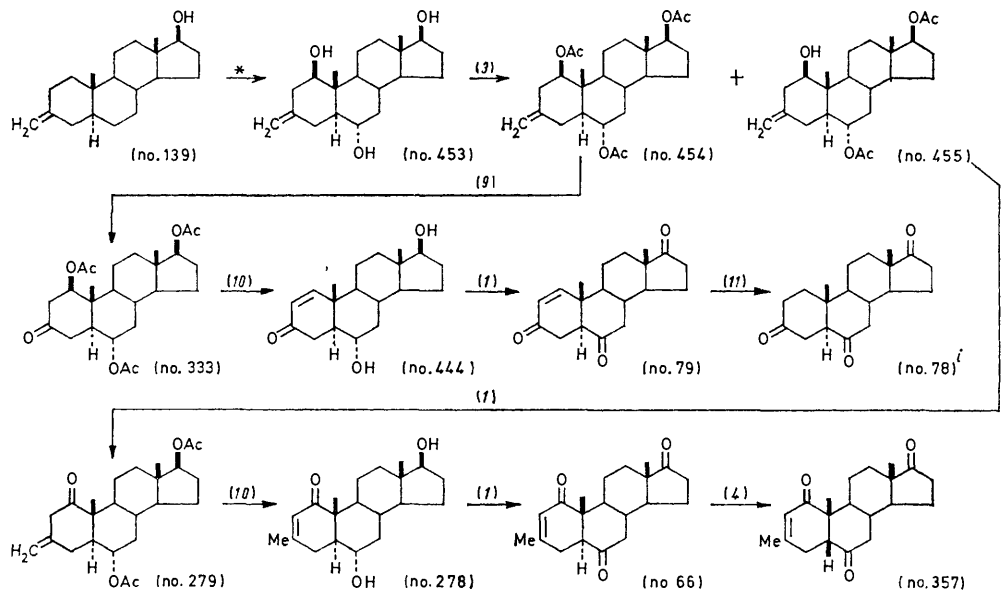
c.d.	301 nm($\Delta\varepsilon$ − 2·70)	327(−0·50)	319(−1·36)
$\nu_{max.}$	3626, 1733 cm^{-1}	3616, 3405, 1738	3601, 1741
m/e		266 (M^+ −56) (100%)	234 (M^+ − 56) (100%)



* Table 1. † Synthesis in Experimental section. Reagents as before, and: (7), $LiAlH_4$; (8), $TsOH–Me_2C(OMe)_2$.

Nos. 445 and 473, one >C*H*·OH signal at unusually low field: suggests 7α-H deshielded by 15-oxo-group in 14β-system. Similarity between no. 473 and authentic 14β-hydroxy-15-ketone (no. 426), especially in mass spectral base peaks arising from ready loss of ring D[h]: suggests presence of 14β-OH in no. 473. Strong OH · · · OH bonding in no. 473, and formation of an acetal: confirms 7β,14β-dihydroxy-system.

(vii)



* Table 1. Reagents as before, and: (9), O_3; (10), KOH–EtOH; (11), H_2–Pt.

Microbiological hydroxylation of no. 139 is efficient and clean; an appreciable quantity of the product (no. 453) is obtained readily. The sequences, one terminating in the known 3,6,17-triketone (no. 78),[i] confirm the positions of the hydroxy-groups in the product; they also provide a series of androstane derivatives which are useful as reference compounds, and as starting materials for further work.

[a] L. F. and M. Fieser, 'Steroids,' Reinhold, New York, 1959, p. 179. [b] Ref. 8. [c] P. Crabbé, 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry,' Holden-Day, San Francisco, 1965, p. 39. [d] Ref. 9. [e] R. T. Aplin and P. C. Cherry, *Chem. Comm.*, 1966, 628. [f] J. E. Bridgeman, P. C. Cherry, W. R. T. Cottrell, Sir Ewart R. H. Jones, P. W. LeQuesne, and G. D. Meakins, *Chem. Comm.*, 1966, 561. [g] C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Amer. Chem. Soc.*, 1965, **87**, 817. [h] R. Tschesche, H. G. Berscheid, H. Fehlhaber, and G. Snatzke, *Chem. Ber.*, 1967, **100**, 3289. [i] Ref. 15.

given in ref. 14. The abbreviation s.m. is used for starting material. Two forms are used in stating yields: the weight of a homogeneous chromatographic fraction is given immediately after the compound number, whereas the weight of crystallised material is given after the m.p. and the solvent used. References are not given to well known steroids which are readily located in Elsevier's 'Encyclopaedia of Organic Chemistry', vol. 14 and supplements.

5α-*Androst-2-en*-1-*one* (no. 3).* (a) *Incubation*: 2·08 g in

[14] 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.

[15] K. Tanabe, R. Takasaki, and R. Hayashi, *Chem. and Pharm. Bull.* (*Japan*), 1961, **9**, 7.

TABLE 4

N.m.r. signals

Solutions were examined at 100 MHz. Arabic numerals subscript to τ refer to the solvent [1, CCl_4; 2, $CDCl_3$; 3, C_6H_6]. $\Delta_1^3 = \tau(C_6H_6) - \tau(CCl_4)$. τ_2(calc.) values were obtained, where possible from refs. 8 and 9. Some signals are described as s (singlet) d (doublet), t (triplet), *etc.*, or m (unresolved multiplet): the letters d, t, *etc.* are followed, in parentheses, by the coupling constants (J in Hz); m is followed by the half-height width ($W_{\frac{1}{2}}$ in Hz). Where these terms are inappropriate the number of lines is indicated by an italicised number: this is followed, in parentheses, by a set of 'apparent J values'.[a]

No.	Compound		τ_1	τ_2	τ_2(calc.)	τ_3	Δ_1^3
(412)	5α,Androst-14-ene	19	8·99				
		18	9·19				
(413)	5α,14β-Androstan-15-one	19	9·14	9·26	9·26	9·17	+0·03
		18	8·64	8·84	8·83	8·93	+0·29
(414)	5β-Androst-2-ene-1,6-dione	19	8·98	8·93		9·14	+0·16
		18	9·26	9·26		9·46	+0·20
(415)	5α,14β-Androstane-7,12-dione	19	8·96	8·95		9·48	+0·53
		18	8·83	8·79		9·04	+0·21
(416)	Androst-5-ene-7,12-dione	19		8·72	8·71		
		18		8·94	8·94		
(417)	5α-Androst-1-ene-3,12,15-trione	19		8·88	8·88		
		18		8·85	8·81		
(418)	5α,14β-Androst-1-ene 3,12,15-trione	19	8·63	8·59		9·15	+0·52
		18	8·99	8·97		9·80	+0·81
(419)	5α-Androstane-6,12,15-trione	19		9·15	9·16		
		18		8·88	8·84		
(420)	5α,14β-Androstane-6,12,15-trione	19	9·26	9·22	9·24	9·59	+0·33
		18	8·63	8·60	8·61	9·23	+0·60
(421)	5α,14β-Androstane-7,12,15-trione	19		9·02			
		18		8·62			
(422)	5α-Estrane-3,11,15-trione	18	9·28	9·28		9·58	+0·30
(423)	A-Nor-5α-androstane-2,12,15-trione	19	9·06	9·06		9·66	+0·60
		18	8·90	8·86		9·40	+0·50

No.	Compound		τ_2	τ_2(calc.)	>CH-OR (in $CDCl_3$)		
(424)	Methyl 5α-androstan-3β-yl succinate	19	9·17		H-3	5·28	7(10,10,5,5)
		18	9·31				
(425)	Ethyl 5α-androstan-3β-yloxyacetate	19	9·19		H-3	6·68	7(11,11,5,5)
		18	9·31				
(426)	14-Hydroxy-5α,14β-androstan-15-one	19	9·25				
		18	8·94				
(427)	12β-Hydroxyandrost-4-ene-3,15-dione	19	8·79	8·78	H-12	6·34	4(11,4)
		18	9·13	9·10			
(428)	12β-Hydroxy-14β-androst-4-ene-3,15-dione	19	8·81		H-12	6·60	4(11,4)
		18	8·83				
(429)	5α-Androstane-6α,11α-diol	19	9·06	9·07	H-6	6·60	6(10,10,5)
		18	9·28	9·28	H-11	6·06	6(10,10,5)
(430)	5α-Androstane-7β,12β-diol	19	9·18	9·18	H-7	6·72	m(20)
		18	9·27	9·23	H-12	6·66	4(12,5)
(431)	7β,12β-Diacetoxy-5α-androstane	19	9·16	9·15	H-7	5·45	6(10,10,5)
		18	9·21	9·25	H-12	5·40	4(11,5)
(432)	5α,14β-Androstane-7β,12β-diol	19	9·19		H-7 } H-12 }	6·59	m(25)
		18	9·03				
(433)	7β,12β-Diacetoxy-5α,14β-androstane	19	9·17		H-7 } H-12 }	5·31	m(28)
		18	8·96				
(434)	5α,14β-Androstane-14,15α-diol	19	9·25		H-15	5·84	d(7·5)
		18	9·00				
(435)	5α,14β-Androstane-14,15β-diol	19	9·18		H-15	5·87	s
		18	8·78				
(436)	14α,15α-Epoxy 5α-androstane	19	9·19		H-15	6·60	s
		18	9·08				
(437)	14,15β-Epoxy-5α,14β-androstane	19	9·19		H-15	6·62	s
		18	8·96				
(438)	2α,12β-Dihydroxy-5α-androstan-15-one	19	9·18	9·18	H-2	6·26	m(22)
		18	9·18	9·18	H-12	6·39	4(12,4)
(439)	2α,12β-Diacetoxy-5α-androstan-15-one	19	9·14	9·11	H-2	5·05	m(23)
		18	9·14	9·13	H-12	5·14	4(12,4)
(440)	4β,12β-Dihydroxyandrost-5-en-7-one	19	8·57	8·56	H-4	5·64	t(2·8)
		18	9·25	9·27	H-12	6·57	4(12,4)
(441)	6α,15α-Dihydroxy-5α-androstan-12-one	19	9·11	9·08	H-6	6·49	m(27)
		18	8·95	8·90	H-15	5·72	6(9,9,5)
(442)	6α,15α-Diacetoxy-5α-androstan-12-one	19	9·06	9·11	H-6	5·24	6(10,10,5)
		18	8·92	8·86	H-15	4·87	6(10,10,5)
(443)	6α,12β-Dihydroxy-5α,14β-androstan-15-one	19	9·23		H-6 } H-12 }	6·70	m(20)
		18	8·87				
(444)	6α,17β-Dihydroxy-5α-androst-1-en-3-one	19	8·93	8·95	H-6	6·59	6(10,10,5)
		18	9·21	9·22	H-17	6·35	t(8)
(445)	7β,12β-Dihydroxy-5α,14β-androstan-15-one	19	9·22		H-7	5·38	6(10,10,5
		18	8·85		H-12	6·20	4(12,4)
(446)	12β,14-Dihydroxy-5α,14β-androstan-15-one	19	9·23	9·23	H-12	6·78	4(12,4)
		18	8·98	8·96			
(447)	12β,15α-Dihydroxy-5β-androstan-17-one	19	9·04		H-12	6·13	4(12,5)
		18	9·05		H-15	5·52	t(8)
(448)	11α,15α-Dihydroxy-5α-estran-3-one	18	9·19		H-11	6·24	m(25)
					H-15	5·96	6(8,8,3)
(449)	6α,11α-Dihydroxy-D-homo-5α-androstan-17a-one	19	9·05	9·08	H-6	6·65	6(10,10,4)
		18	8·90	8·89	H-11	6·16	7(10,10,5,5)
(450)	12β,15α-Dihydroxy-A-nor-5α-androstan-2-one	19	9·13	9·12	H-12	6·49	4(9,6)
		18	9·23	9·19	H-15	5·79	6(8,8,3)
(451)	7α,14-Dihydroxy-5α,14β-androstane-12,15-dione	19	9·13				
		18	8·71				
(452)	5α-Androstane-1β,6α,17β-triol (n.m.r. after acetylation)	19	9·03	9·03	H-1	5·17	4(10,5)
		18	9·22	9·22	H-6	5·40	m(27)
					H-17	5·44	t(7)
(453)	3-Methylene-5α-androstane-1β,6α,17β-triol	19	9·05	9·07			
		18	9·25	9·26			
(454)	1β,6α,17β-Triacetoxy-3-methylene-5α-androstane	19	8·91	8·94	H-1 } H-6 } H-17 }	5·30	m
		18	9·21	9·20			
(455)	6α,17β-Diacetoxy-3-methylene-5α-androstan-1β-ol	19	9·03	9·03	H-1	6·53	4(12,5)
		18	9·21	9·21	H-6 } H-17 }	5·32	m

TABLE 4 (*Continued*)

No.	Compound		τ_2	τ_2(calc.)	>CH-OR (in $CDCl_3$)		
(456)	7β,15α-Isopropylidene-dioxy-D-homo-5α-androstan-1β-ol	19	9·12		H-1	6·32	4(16,7)
		18	9·03		H-7	6·29	m(25)
					H-15	6·08	6(9,9,5)
(457)	3β-(2-Hydroxyethoxy)-5α-androstane-6α-15-diol	19	9·16	9·14	H-3	6·63	7(10,10,5,5)
		18	9·26	9·27	H-6	6·36	m(25)
					H-15	5·84	6(9,9,4)
(458)	6α,15α-Diacetoxy-3β-(2-acetoxyethoxy)-5α-androstane	19	9·17	9·17	H-3	6·75	7(10,10,5,5)
		18	9·21	9·24	H-6	5·37	6(10,10,5)
					H-15	4·99	6(7,7,3)
(459)	3α-(2-Hydroxyethoxy)-5α-androstane-12β,15α-diol	19	9·18	9·19	H-3	6·53	m(7)
		18	9·26	9·23	H-12	6·57	4(10,5)
					H-15	5·85	6(9,9,4)
(460)	12β,15α-Diacetoxy-3α-(2-acetoxyethoxy)-5α-androstane	19	9·17	9·16	H-3	6·65	m(7)
		18	9·20	9·20	H-12	5·29	4(10,5)
					H-15	4·98	6(8,8,4)
(461)	5α-Androstane-3β,12β,15α-triol	19	9·17	9·17			
		18	9·21	9·22			
(462)	5α-Androstane-6α,11α,17β-triol (n.m.r. after acetylation)	19	9·05	9·09	H-6	5·40	m(30)
		18	9·17	9·16	H-11	4·93	6(10,10,5)
					H-17	5·41	t(7)
(463)	5α-Androstane-6α,12β,15α-triol	19	9·17	9·17			
		18	9·23	9·23			
(464)	7β,15α-Isopropylidene-dioxy-D-homo-5α-androstan-12β-ol	19	9·19		H-7	6·40	m(25)
		18	9·16		H-12	6·74	4(11,5)
					H-15	6·21	6(10,10,5)
(465)	1β,6α,15α-Trihydroxy-5α-androstan-12-one	19	9·05	9·03	H-1 } H-6 }	6·55	m(30)
		18	8·93	8·90			
					H-15	5·75	6(9,9,5)
(466)	1β,6α,15α-Triacetoxy-5α-androstan-12-one	19	8·93	8·95	H-1 } H-6 }	5·40	m(30)
		18	8·90	8·86			
					H-15	4·84	6(9,9,5)
(467)	1β,7β,15α-Trihydroxy-D-homo-5α-androstan-17a-one	19	9·12	9·14	H-1	6·10	4(13,8)
		18	8·84	8·85	H-7 } H-15 }	6·10	m(25)
(468)	3β-Allyloxy-7β,15α-dihydroxy-5α-androstan-12-one	19	9·06	9·06	H-3 } H-7 }	5·08	m(45)
		18	8·93	8·86			
					H-15	5·60	6(8,8,3)
(469)	6α,12β-15α,Trihydroxy-5α-androstan-3-one (>CH-OH signals in C_5H_5N)	19	8·93	8·93	H-6	6·35	m(20)
		18	9·20	9·19	H-12	6·35	4(12,4)
					H-15	5·30	6(8,8,3)
(470)	6α,12β-Diacetoxy-15α-hydroxy-5α-androstan-3-one	19	8·93	8·92	H-6	5·75	m(20)
		18	9·14	9·16	H-12	5·37	4(12,5)
					H-15	5·43	6(8,8,3)
(471)	12β,15α-Diacetoxy-6α-hydroxy-5α-androstan-3-one	19	8·94	8·93	H-6	6·55	m(20)
		18	9·12	9·12	H-12	5·29	4(12,4)
					H-15	4·77	6(8,8,3)
(472)	6α,12β,15α-Triacetoxy-5α-androstan-3-one	19	8·92	8·93	H-6	5·38	6(10,10,5)
		18	9·09	9·12	H-12	5·29	4(10,4)
					H-15	4·92	6(8,8,2)
(473)	7β,12β,14-Trihydroxy-5α,14β-androstan-15-one	19	9·19		H-7	5·00	m(27)
		18	8·96		H-12	6·82	4(12,4)
(474)	7β,12β-Diacetoxy-14-hydroxy-5α,14β-androstan-15-one	19	9·16		H-7	4·12	m(20)
		18	8·84		H-12	5·49	4(12,4)
(475)	12β-Hydroxy-7β,14-isopropylidenedioxy-5α,14β-androstan-15-one	19	9·18		H-7	4·92	m(25)
		18	9·01		H-12	6·86	4(11,4)
(476)	7β,12β,15α-Trihydroxy-D-homo-5α-androstan-17a-one	19	9·18		H-7	6·29	6(10,10,5)
		18	8·81		H-12	6·17	4(11,5)
					H-15	5·75	6(8,8,5)
(477)	Ethyl 6α,12β,15α-trihydroxy-5α-androstan-3β-yloxyacetate	19	9·14	9·14	H-3 } H-6 } H-12 }	6·66	m(20)
		18	9·24	9·23			
					H-15	5·79	m(20)
(478)	Ethyl 6α,12β,15α-triacetoxy-5α-androstan-3β-yloxyacetate	19	9·13	9·14	H-3	6·69	m(23)
		18	9·13	9·16	H-6	5·37	6(11,11,5)
					H-12	5·32	4(12,5)
					H-15	4·97	6(8,8,5)
(479)	3β-Allyloxy-5α-androstane-7β,12β,15α-triol	19	9·14	9·15	H-3 } H-7 } H-12 }	6·60	m(35)
		18	9·23	9·19			
					H-15	5·65	6(8,8,3)
(480)	3β-Allyloxy-7β,12β,15α-triacetoxy-5α-androstane	19	9·13	9·14	H-3	6·73	m(24)
		18	9·13	9·17	H-7 } H-12 }	5·4	m(40)
					H-15	4·75	m(20)
(481)	3β-Propyloxy-5α-androstane-7β,12β,15α-triol	19	9·02		H-3	6·80	m(25)
		18	9·10		H-7 } H-12 }	5·25	m(35)
					H-15	4·80	6(8,8,3)
(482)	5α,14β-Androstane-7β,12β,14,15α-tetraol	19	9·16		H-7	6·27	t(6)
		18	9·01		H-12	6·69	4(11,4)
					H-15	5·74	m(22)

[a] M. G. Combe, W. A. Denny, G. D. Meakins, Y. Morisawa, and E. E. Richards, *J. Chem. Soc.* (*C*), 1971, 2300.

Me_2SO (312 ml), 52 flasks, A, 6 d, extraction I ⟶ 3·2 g combined extract. Chromat. SiO_2 (100 g). C_6H_6 gave s.m. (540 mg). C_6H_6–EtOAc (7 : 3) gave 6α-*hydroxy*-5α-*androst*-2-*en*-1-*one* (no. 157),* m.p. 194—195° (from Me_2CO) (23 mg), $[\alpha]_D$ +142° (*c* 0·4) (Found: C, 79·0; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%), $\nu_{max.}$ 3610 and 1680 cm^{-1}, $\lambda_{max.}$ 225 nm (ε 8650). C_6H_6–EtOAc (3 : 2) gave 6α-*hydroxy*-5α-*androst*-2-*ene*-1,16-*dione* (no. 194),* m.p. 222—225° (from Me_2CO–hexane) (254 mg),

$[\alpha]_D$ −19° (*c* 0·9) (Found: C, 75·3; H, 8·5. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·6%), $\nu_{max.}$ 3595, 1735, and 1670 cm^{-1}, $\lambda_{max.}$ 225 nm (ε 7000), c.d. 304 nm (Δε − 2·1). C_6H_6–EtOAc (3 : 7) gave 6α,16β-*dihydroxy*-5α-*androst*-2-*en*-1-*one* (no. 277),* m.p. 215—217° (from Me_2CO) (60 mg) (Found: C, 74·7; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9 3%), $\nu_{max.}$ ($CHCl_3$) 3626 and 1681 cm^{-1}.

(b) *Transformations:* Oxidation of 6α-hydroxy-5α-androst-2-en-1-one (no. 157) (55 mg) in Me_2CO with 8N-H_2CrO_4 gave 5β-*androst*-2-*ene*-1,6-*dione* (no. 414) (50 mg), m.p. 144—145° (from hexane), $[\alpha]_D$ +64° (*c* 0·6) (Found: C, 80·0; H, 9·2. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·15%), $\nu_{max.}$ 1715 and 1680 cm^{-1}. Oxidation of 6α-hydroxy-5α-androst-2-ene-1,16-dione (no. 194) (50 mg) gave 5β-*androst*-2-*ene*-1,6,16-*trione* (no. 62) * (40 mg), m.p. 237—242° (from Me_2CO–hexane), $[\alpha]_D$ −88° (*c* 0·6) (Found: C, 76·0; H, 8·2. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·0%), $\nu_{max.}$ 1740, 1712, and 1678 cm^{-1}. A solution of $NaBH_4$ (35 mg) and 6α-hydroxy-5α-androst-2-ene-1,16-dione (no. 194) (70 mg) in EtOH (5 ml)–H_2O (1 ml) was stirred for 1 h at 0°C. Addition of AcOH followed by extraction with $CHCl_3$ gave a solid (67 mg) which was purified by p.l.c. [2 small plates, 1 × Et_2O]. The first band (higher R_F) gave s.m. (12 mg); the second gave 6α,16β-dihydroxy-5α-androst-2-en-1-one (no. 277) (29 mg), m.p. and mixed m.p. 215—217°.

5α-*Androstan*-2-*one* (no. 4).* (a) *Incubation:* 1·2 g in Me_2SO(180 ml), 30 flasks, B, 4 d, extraction II ⟶ 1·81 g total extract. Chromat. Al_2O_3 (deactivated; 150 g). Petrol–Et_2O (9 : 1) gave s.m. (159 mg). Et_2O–MeOH (19 : 1) gave an oil (643 mg) which on p.l.c. [2 large plates, 6 × petrol–Me_2CO (7 : 3)] gave two bands. That of higher R_F afforded 6α,11α-*dihydroxy*-5α-*androstan*-2-*one* (no. 270) * (130 mg), m.p. 117—119° (from Me_2CO–hexane), $[\alpha]_D$ +20° (*c* 2·0) (Found: C, 74·1; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3600 and 1703 cm^{-1}. The second band gave 6α,12β-*dihydroxy*-5α-*androstan*-2-*one* (no. 273) * (270 mg), m.p. 208—210° (from Me_2CO–hexane), $[\alpha]_D$ +60° (*c* 0·9) (Found: C, 74·2; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3605 and 1703 cm^{-1}.

(b) *Transformations:* Huang-Minlon reduction of 6α,11α-dihydroxy-5α-androstan-2-one (no. 270) (87 mg) gave 5α-*androstane*-6α,11α-*diol* (no. 429) (70 mg), m.p. 159—160° (from Me_2CO–hexane), $[\alpha]_D$ −5° (*c* 0·9) (Found: C, 77·8; H, 10·8. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3605 cm^{-1}. Oxidation of the diol (no. 429) (50 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-6,11-*dione* (no. 46) * (40 mg), m.p. 173—174° (from hexane), $[\alpha]_D$ +52° (*c* 0·9) (Found: C, 78·9; H, 9·9. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%). Huang-Minlon reduction of 6α,12β-dihydroxy-5α-androstan-2-one (no. 273) (60 mg) gave 5α-*androstane*-6α,12β-*diol* (no. 222) * (40 mg), m.p. 197·5—198·5° (from Me_2CO–hexane), $[\alpha]_D$ +23° (*c* 1·0) (Found: C, 78·1; H, 11·0. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3609 cm^{-1}. Oxidation of the diol (no. 273) (30 mg) gave 5α-*androstane*-6,12-*dione* (no. 47) * (25 mg), m.p. 181—183° (from Me_2CO–hexane), $[\alpha]_D$ +41° (*c* 0·4) (Found: C, 78·8; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%).

A-*Nor*-5α-*androstan*-2-*one* (no. 345).* (a) *Incubation:* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction I ⟶ 1·4 g total extract. P.l.c. [3 large plates, 6 × petrol–Me_2CO (4 : 1)] gave 2 bands. Band 1 (higher R_F) afforded s.m. (400 mg). Band 2 gave 12β,15α-dihydroxy-A-nor-5α-androstan-2-one (no. 450) (80 mg) as an oil, $\nu_{max.}$ 3610, 3450, and 1739 cm^{-1}.

(b) *Transformations:* Oxidation of the diol (no. 450) gave A-*nor*-5α-*androstane*-2,12,15-*trione* (no. 423), m.p. 171—173° (from Me_2CO–hexane), $[\alpha]_D$ +207° (*c* 0·6) (Found: C, 74·9; H, 8·4. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%), $\nu_{max.}$ 1744 and 1716 cm^{-1}.

5α-*Androstan*-3-*one* (no. 5).* (a) *Incubation:* 3 g in EtOH (300 ml), 60 flasks, medium A, 5 d, extraction III ⟶ 3·0 g total extract. Chromat. Al_2O_3 (deactivated; 180 g). C_6H_6 gave s.m. (671 mg), m.p. and mixed m.p. 102—103°, Et_2O–MeOH (5 : 1) gave 12β,15α-*dihydroxy*-5α-*androstan*-3-*one* (no. 299),* m.p. 173—174° (from EtOAc) (1·35 g), $[\alpha]_D$ +61° (*c* 0·4) (Found: C, 74·7; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%). Further elution with Et_2O–MeOH (5 : 1) gave 5α-*androstane*-3β,12β,15α-*triol* (no. 461), m.p. 247—248° (from MeOH) (220 mg, 6·5%), $[\alpha]_D$ +46° (*c* 0·4) (Found: C, 73·9; H, 10·5. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ (Nujol) 3290 cm^{-1}.

(b) *Transformations:* Huang-Minlon reduction of 12β,15α-dihydroxy-5α-androstan-3-one (no. 299) (870 mg) gave 5α-*androstane*-12β,15α-*diol* (no. 229) * (820 mg), m.p. 139—140° and 170—171° (from Me_2CO–hexane), $[\alpha]_D$ +40° (*c* 0·9) (Found: C, 77·8; H, 10·9. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%). Oxidation of the diol (no. 229) (90 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-12,15-*dione* (no. 55) * (70 mg), m.p. 192—193° (from EtOH), $[\alpha]_D$ +113° (*c* 0·3) (Found: C, 79·0; H, 9·9. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%). The above dione (no. 55) (100 mg) was heated under reflux in 5% KOH–MeOH (20 ml) for 2 h to give 5α,14β-*androstane*-12,15-*dione* (no. 56),* m.p. 127—128° (from EtOH) (80 mg), $[\alpha]_D$ +12° (*c* 0·8) (Found: C, 79·4; H, 9·7. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%).

Oxidation of 12β,15α-dihydroxy-5α-androstan-3-one (no. 299) (20 mg) gave 5α-*androstane*-3,12,15-*trione* (no. 86) * (15 mg), m.p. 203—205° (from EtOH), $[\alpha]_D$ +118° (*c* 1·0) (Found: C, 75·4; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). The above trione (no. 86) (100 mg) was heated under reflux in 5% KOH–MeOH (20 ml) for 2 h to give 5α,14β-*androstane*-3,12,15-*trione* (no. 87),* m.p. 241—243° (from EtOH) (85 mg), $[\alpha]_D$ +30° (*c* 1·0) (Found: C, 75·3; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1748, 1726, and 1716 cm^{-1}.

Acetylation of 5α-androstane-3β,12β,15α-triol (no. 461) with Ac_2O–C_5H_5N (10 : 1) gave 3β,12β,15α-*triacetoxy*-5α-*androstane* (no. 328),* m.p. 141—142° (from EtOH), $[\alpha]_D$ +14° (*c* 0·8) (Found: C, 69·1; H, 8·5. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%), $\nu_{max.}$ 1745 cm^{-1}.

A solution of 12β,15α-dihydroxy-5α-androstan-3-one (no. 299) (100 mg) and $NaBH_4$ (80 mg) in EtOH (16 ml)–H_2O (4 ml) was stirred for 30 min at 20°C. After the addition of AcOH, the solvents were removed and the crude product was acetylated with Ac_2O–C_5H_5N (10 : 1) for 5 d at 20°C to give 3β,12β,15α-triacetoxy-5α-androstane (no. 328) (90 mg), m.p. (from EtOH) and mixed m.p. 141—142°.

5α-*Androstan*-3-*one* (no. 5). (a) *Incubation:* 1·0 g in Me_2SO (100 ml), 30 flasks, medium B, 4 d, extraction II ⟶ 600 mg mycelial extract and 500 mg broth extract. The mycelial extract contained no steroid and was discarded. Crystallisation of the broth extract from EtOAc and filtration of the residues through Al_2O_3 (10% deactivated; 10 g) in EtOAc gave 6α,12β,15α-*trihydroxy*-5α-*androstan*-3-*one* (no. 469) (390 mg), m.p. 231—233° (from EtOAc), $[\alpha]_D$ +60° (*c* 0·2) (Found: C, 69·55; H, 9·0. $C_{19}H_{30}O_4$,0·5EtOAc requires C, 69·2; H, 8·9%), $\nu_{max.}$ 3600 and 1715 cm^{-1}.

(b) *Transformations:* Huang-Minlon reduction of the

trihydroxy-ketone (no. 469) (200 mg) gave 5α-*androstane*-6α,12β,15α-*triol* (no. 463) (90 mg), m.p. 235—236° (from Me_2CO–hexane), $[\alpha]_D$ +67° (*c* 0·8) (Found: C, 74·0; H, 10·6. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%), $\nu_{max.}$ 3580 cm^{-1}.

Acetylation of the trihydroxy-ketone (no. 469) (200 mg) with Ac_2O–C_5H_5N for 3 h at 20°C gave a mixture of 3 compounds. P.l.c. [Me_2CO–hexane (1 : 4)] gave, in order of increasing polarity, 6α,12β,15α-*triacetoxy*-5α-*androstan*-3-*one* (no. 472) (50 mg), m.p. 182—184° (from Et_2O), $[\alpha]_D$ +97° (*c* 0·4) (Found: C, 66·9; H, 8·1. $C_{25}H_{36}O_7$ requires C, 66·9; H, 8·1%), $\nu_{max.}$ 1735 and 1720 cm^{-1}; 6α,12β-*diacetoxy*-15α-*hydroxy*-5α-*androstan*-3-*one* (no. 470) (30 mg), m.p. 206—210° (from Me_2CO–hexane), $[\alpha]_D$ +74° (*c* 0·6) (Found: C, 67·75; H, 8·3. $C_{23}H_{34}O_6$ requires C, 67·95; H, 8·4%), $\nu_{max.}$ 3600 and 1730 cm^{-1}; and 12β,15α-*diacetoxy*-6α-*hydroxy*-5α-*androstan*-3-*one* (no. 471) (100 mg), m.p. 220—225° (from Me_2CO–hexane), $[\alpha]_D$ +76° (*c* 0·7) (Found: C, 68·2; H, 8·35. $C_{23}H_{34}O_6$ requires C, 67·95; H, 8·4%), $\nu_{max.}$ 3600 and 1730 cm^{-1}.

5α-*Estran*-3-*one* (no. 26).* (a) *Incubation:* 1·6 g in Me_2SO (240 ml), 40 flasks, medium B, 6 d, extraction I ⟶ 2·5 g total extract. P.l.c. [5 large plates, 15 × petrol–Me_2CO (5 : 1)] gave 3 bands. Band 1 (highest R_F) gave s.m. (294 mg). Band 2 gave 11α,15α-*dihydroxy*-5α-*estran*-3-*one* (no. 448), m.p. 192—194° (from Me_2CO–hexane) (60 mg), $[\alpha]_D$ +36° (*c* 1·0) (Found: C, 74·0; H, 9·5. $C_{18}H_{28}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ 3590 and 1708 cm^{-1}. Band 3 (812 mg), after further p.l.c. [2 large plates, 20 × petrol–Me_2CO(4 : 1)], gave 12β,15α-*dihydroxy*-5α-*estran*-3-*one* (no. 312),* m.p. 185·5—187° (from Me_2CO–hexane) (191 mg), $[\alpha]_D$ +83° (*c* 1·0) (Found: C, 74·1; H, 9·5. $C_{18}H_{28}O_3$ requires C, 73·9; H, 9·7%), $\nu_{max.}$ 3590 and 1708 cm^{-1}.

(b) *Transformations:* Oxidation of 11α,15α-dihydroxy-5α-estran-3-one (no. 448) (25 mg) with 8N-H_2CrO_4 gave 5α-*estrane*-3,11,15-*trione* (no. 422) (21 mg), m.p. 192—194° (from MeOH), $[\alpha]_D$ +36° (*c* 0·4) (Found: C, 75·0; H, 8·5. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%), $\nu_{max.}$ 1746, 1725, and 1717 cm^{-1}.

5α-*Androst*-1-*en*-3-*one* (no. 6).* (a) *Incubation:* 3·0 g in Me_2SO (900 ml), 60 flasks, medium A, 6 d, extraction III ⟶ 5 g total extract. Chromat. Al_2O_3 (deactivated; 200 g). Petrol–C_6H_6 (2 : 3) gave s.m. (405 mg), m.p. and mixed m.p. 101—103°. C_6H_6–Et_2O (2 : 3) gave an oil (1·87 g) which was rechromatographed on SiO_2 (100 g). C_6H_6–EtOAc (2 : 3) gave 6α,11α-dihydroxy-5α-androst-1-en-3-one (no. 271) * (63 mg) as an oil, $\nu_{max.}$ 3600 and 1680 cm^{-1}. Further elution of the SiO_2 column with the same solvent mixture gave 12β,15α-*dihydroxy*-5α-*androst*-1-*en*-3-*one* (no. 300),* m.p. 193—196° (from Me_2CO–hexane) (608 mg), $[\alpha]_D$ +76° (*c* 0·8) (Found: C, 74·9; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ ($CHCl_3$) 3600 and 1673 cm^{-1}; $\lambda_{max.}$ 230 nm (ε 8900).

(b) *Transformations:* Hydrogenation of 12β,15α-dihydroxy-5α-androst-1-en-3-one (no. 300) (90 mg) in EtOH over 10% Pd–C (10 mg) gave 12β,15α-dihydroxy-5α-androstan-3-one (no. 299) (60 mg), m.p. and mixed m.p. 172—174°.

Oxidation of 12β,15α-dihydroxy-5α-androst-1-en-3-one (no. 300) (50 mg) gave 5α-androst-1-ene-3,12,15-trione (no. 417) (41 mg), m.p. 178—182° (Me_2CO–hexane); $\nu_{max.}$ ($CHCl_3$) 1740, 1710, and 1675 cm^{-1}. A solution of this trione (55 mg) in 5% KOH–EtOH was heated under reflux for 2 h to give, after p.l.c. (1 small plate, 1 × petrol–EtOAc (9 : 1)], 5α,14β-*androst*-1-*ene*-3,12,15-*trione* (no. 418) (42 mg), m.p. 244—246° (from Me_2CO) (Found: C, 75·8; H, 7·9. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·05%), $\nu_{max.}$ ($CHCl_3$) 1740, 1720, and 1680 cm^{-1}.

Oxidation of 6α,11α-dihydroxy-5α-androst-1-en-3-one (no. 271) (22 mg) gave 5α-*androst*-1-*ene*-3,6,11-*trione* (no. 73),* m.p. 172—175° (from $CHCl_3$–hexane) (10 mg), $[\alpha]_D$ +48° (*c* 0·9) (Found: C, 76·0; H, 8·4. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·05%), $\nu_{max.}$ ($CHCl_3$) 1725, 1715, and 1690 cm^{-1}, $\lambda_{max.}$ 220 nm (ε 7780).

Androst-4-*en*-3-*one* (no. 7).* (a) *Incubation:* 2·2 g in EtOH (220 ml), 44 flasks, medium A, 2 d, extraction III ⟶ 3·0 g total extract. Chromat. Al_2O_3 (deactivated; 180 g). Petrol–C_6H_6 (5 : 1) gave s.m. (680 mg), m.p. and mixed m.p. 105—107°. Et_2O–MeOH (10 : 1) gave 12β,15α-*dihydroxyandrost*-4-*en*-3-*one* (no. 302),* m.p. 204—205° (from Me_2CO) (950 mg), $[\alpha]_D$ +149° (*c* 0·9) (Found: C, 75·2; H, 9·4. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3624 and 1679 cm^{-1}, $\lambda_{max.}$ 241 nm (ε 15,500).

(b) *Transformations:* Oxidation of 12β,15α-dihydroxy-androst-4-en-3-one (no. 302) (200 mg) gave *androst*-4-*ene*-3,12,15-*trione* (no. 88),* m.p. 186—187° (from EtOH) (120 mg), $[\alpha]_D$ +167° (*c* 0·7) (Found: C, 75·9; H, 8·0. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·05%), $\nu_{max.}$ 1752, 1722, and 1682 cm^{-1}, $\lambda_{max.}$ 238 nm (ε 16,100). A solution of this trione in 5% KOH–EtOH was heated under reflux for 2 h to give 15β-androst-4-ene-3,12,15-trione (no. 89),* m.p. 242—244° (from EtOH), $[\alpha]_D$ +116° (*c* 1·0) (Found: C, 76·2; H, 8·3. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·05%), $\nu_{max.}$ 1747, 1716, and 1682 cm^{-1}, $\lambda_{max.}$ 238 nm (ε 16,300).

Estr-4-*en*-3-*one* (no. 27).* (a) *Incubation:* 3·0 g in Me_2SO (1110 ml), 75 flasks, medium A, 6 d, extraction I ⟶ 3 g total extract. P.l.c. [6 large plates, 24 × petrol–Me_2CO (5 : 1)] gave 3 bands. Band 1 (highest R_F) gave s.m. (80 mg). Band 2 gave 12β,15α-*dihydroxyestr*-4-*en*-3-*one* (no. 313),* m.p. 202—202·5° (from Me_2CO–hexane), (1·2 g), $[\alpha]_D$ +97° (*c* 1·0) (Found: C, 74·2; H, 9·1. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%), $\nu_{max.}$ 3600 and 1675 cm^{-1}, $\lambda_{max.}$ 240 nm (ε 17,600). Band 3 gave 6β,11α-*dihydroxyestr*-4-*en*-3-*one* (no. 311),* m.p. 161—162° (from Me_2CO–hexane) (400 mg), $[\alpha]_D$ −188° (*c* 1·0) (Found: C, 74·3; H, 8·9. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%), $\nu_{max.}$ 3592 and 1675 cm^{-1}, $\lambda_{max.}$ 236 nm (ε 13,300).

(b) *Transformations:* Oxidation of 12β,15α-dihydroxyestr-4-en-3-one (no. 313) (200 mg) gave *estr*-4-*ene*-3,12,15-*trione* (no. 100),* m.p. 153·5—154·5° (from Me_2CO–hexane) (124 mg), $[\alpha]_D$ +126° (*c* 0·8) (Found: C, 75·2; H, 7·8. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%), $\nu_{max.}$ 1742, 1712, and 1675 cm^{-1}, $\lambda_{max.}$ 238 nm (ε 7650).

5α-*Androstan*-3β-*ol* (no. 112).* *Incubation:* 200 mg in EtOH (10 ml), 5 flasks, medium B, 6 d, extraction III ⟶ 233 mg total extract. P.l.c. (1 large plate, 6 × Et_2O] gave two bands. Band 1 (higher R_F) afforded s.m. (65 mg). Band 2 gave 5α-androstane-3β,12β,15α-triol (no. 461) (28 mg), m.p. (from MeOH) and mixed m.p. 246—248°.

5α-*Androst*-1-*en*-3β-*ol* (no. 113).* *Incubation:* 800 mg in Me_2SO (120 ml), 20 flasks, medium B, 6 d, extraction III ⟶ 0·76 g total extract. Chromat. Al_2O_3 (100 g). Petrol–EtOAc (7 : 3) gave s.m. (183 mg). Further elution with the same solvent mixture gave 12β,15α-dihydroxy-5α-androst-1-en-3-one (no. 300), m.p. and mixed m.p. 192—195° (from Me_2CO–hexane) (100 mg).

Androst-4-*en*-3β-*ol* (no. 114).* (a) *Incubation:* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction I ⟶ 287 mg mycelial extract and 1·0 g broth extract. P.l.c. of the mycelial extract [1 large plate, 1 × Et_2O]

gave androst-4-en-3-one (no. 7) (125 mg), m.p. and mixed m.p. 102—105°. P.l.c. of the broth extract [2 large plates, 1 × EtOAc–Et_2O (9 : 1)] gave three bands. That of highest R_F yielded 12β-hydroxy-14β-androst-4-ene-3,15-dione (no. 428) as an oil (30 mg), $\nu_{max.}$ 3620, 1738, and 1675 cm^{-1}. The second band gave 12β-hydroxyandrost-4-ene-3,15-dione (no. 427) as an oil (35 mg), $\nu_{max.}$ 3620, 1738, and 1676 cm^{-1}. The third band gave 12β,15α-dihydroxyandrost-4-en-3-one (no. 302) (425 mg), m.p. and mixed m.p. 202—204°.

(b) *Transformations:* Oxidation of 12β-hydroxy-14β-androst-4-ene-3,15-dione (no. 428) and of its 14α-epimer (no. 427) gave 14β-androst-4-ene-3,12,15-trione (no. 87), m.p. (from Me_2CO) and mixed m.p. 242—244°.

3β-*Allyloxy-5α-androstane* (no. 408). (a) *Incubation*: 4 g in Me_2SO (1200 ml), 80 flasks, medium A, 6 d, extraction III ⟶ 4·6 g total extract. Chromat. SiO_2 (3% deactivated; 150 g). Petrol–EtOAc (19 : 1) gave s.m. (1·53 g). EtOAc gave an oil (1·2 g) which was rechromatographed on Al_2O_3 (deactivated; 120 g). Elution of the Al_2O_3 column with C_6H_6–EtOAc (2 : 3) gave 3β-*allyloxy-5α-androstane*-7β,12β,15α-*triol* (no. 479), m.p. 159—162° (from CH_2Cl_2–petrol) (0·48 g) (Found: C, 72·3; H, 9·7. $C_{22}H_{36}O_4$ requires C, 72·5; H, 9·9%), $\nu_{max.}$ ($CHCl_3$) 3585 and 3350 cm^{-1}.

(b) *Transformations:* Oxidation of the triol (no. 479) (50 mg) with 8N-H_2CrO_4 at 20 °C gave 3β-allyloxy-7β,15α-dihydroxy-5α-androstan-12-one (no. 468) (10 mg), m.p. 144—146° (from $CHCl_3$–petrol), *m/e* 362 (M^+), $\nu_{max.}$ ($CHCl_3$) 3580, 3350, and 1700 cm^{-1}. Acetylation of the triol (no. 479) gave 7β,12β,15α-triacetoxy-3β-allyloxy-5α-androstane (no. 480) as an oil, $\nu_{max.}$ (CS_2) 1735 cm^{-1}. The triol (no. 479) (100 mg) in EtOH (15 ml) was hydrogenated over 5% Pd–C (15 mg) for 4 h to give 3β-propyloxy-5α-androstane-7β,12β,15α-triol (no. 481) (100 mg), m.p. 174—176° (from $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3590 cm^{-1}.

Methyl 5α-Androstan-3β-yl Succinate (no. 424). *Incubation:* 1·3 g in Me_2SO (315 ml), 26 flasks, medium A, 4 d, extraction III ⟶ 1·4 g total extract. Chromat. SiO_2 (5% deactivated; 100 g). Petrol–Et_2O (1 : 1) gave s.m. (536 mg). Et_2O–MeOH (9 : 1 and 3 : 2) gave a gum (188 mg). P.l.c. of this [1 large plate, 3 × petrol–Me_2CO (3 : 2)] gave 5α-androstane-3β,12β,15α-triol (no. 461) (15 mg), m.p. 244—245° (from $CHCl_3$–petrol) and mixed m.p. 247—248°.

Ethyl 5α-Androstan-3β-yloxyacetate (no. 425). *Incubation:* 1·3 g in Me_2SO (390 ml), 26 flasks, medium A, 4 d, extraction III ⟶ 1·26 g total extract. Chromat. SiO_2 (5% deactivated; 100 g). Petrol–Et_2O (9 : 1) gave s.m. (634 mg). Et_2O–MeOH (9 : 1 and 3 : 2) gave a gum (222 mg). P.l.c. of this [1 large plate, 3 × petrol–Me_2CO (3 : 2)] gave ethyl 6α,12β,15α-trihydroxy-5α-androstan-3β-yloxyacetate (no. 477) (54 mg) as an oil, *m/e* 410 (M^+), $\nu_{max.}$ ($CHCl_3$) 3610, 3480, and 1749 cm^{-1}. Acetylation of the metabolite (no. 477) gave ethyl 6α,12β,15α-triacetoxy-5α-androstan-3β-yloxyacetate (no. 478) as an oil, *m/e* 536 (M^+), $\nu_{max.}$ (CS_2) 1755, 1740, 1728, and 1720 cm^{-1}.

3α-(2-*Acetoxyethoxy*)-5α-*androstane* (no. 404).* *Incubation:* 1·5 g in Me_2SO(450 ml), 30 flasks, medium A, 4 d, extraction II ⟶ 1·63 g combined extracts. Chromat. SiO_2 (5% deactivated; 100 g). Petrol–Et_2O (4 : 1) gave s.m. (360 mg). Et_2O–MeOH (3 : 1) gave a mixture which, after p.l.c. [2 large plates, 3 × petrol–Me_2CO (3 : 2)] gave 3α-(2-hydroxyethoxy)-5α-androstane-12β,15α-diol (no. 459) (210 mg), *m/e* 352 (M^+), $\nu_{max.}$ 3600 cm^{-1}. Acetylation of the metabolite (no. 459) gave 12β,15α-diacetoxy-3α-(2-acetoxyethoxy)-5α-androstane (no. 460) as an oil, $\nu_{max.}$ (CS_2) 1738, 1730, and 1233 cm^{-1}.

3β-(2-*Acetoxyethoxy*)-5α-*androstane* (no. 406).* *Incubation:* 1·05 g in Me_2SO (390 ml), 26 flasks, medium A, 4 d, extraction II ⟶ 1·6 g combined extracts. Chromat. SiO_2 (5% deactivated; 100 g). Petrol–Et_2O (2 : 1) gave s.m. (435 mg). Et_2O–MeOH (3 : 2) gave a mixture which, after p.l.c. [2 large plates, 3 × petrol–Me_2CO (3 : 2)] gave 3β-(2-hydroxyethoxy)-5α-androstane-6α,15α-diol (no. 457) (321 mg), *m/e* 352 (M^+), $\nu_{max.}$ ($CHCl_3$) 3610 and 3440 cm^{-1}. Acetylation of the diol (no. 457) gave 6α,15α-diacetoxy-3β-(2-acetoxyethoxy)-5α-androstane (no. 458), $\nu_{max.}$ (CS_2) 1738, 1732, and 1233 cm^{-1}.

5α-*Androstan-4-one* (no. 11).* (a) *Incubation:* 1·0 g in Me_2SO (375 ml), 25 flasks, medium A, 4 d, extraction III ⟶ 1·76 g total extract. P.l.c. [4 large plates, 8 × petrol–Me_2CO (4 : 1)] gave two bands. The band of higher R_F afforded 11α,15α-*dihydroxy-5α-androstan-4-one* (no. 291) * (404 mg), m.p. 203—205° (from MeOAc), $[\alpha]_D$ +13° (*c* 0·7) (Found: C, 74·2; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3618 and 1713 cm^{-1}. The second band gave 12β,15α-*dihydroxy-5α-androstan-4-one* (no. 305) * (407 mg), m.p. 206—209° (from MeOAc), $[\alpha]_D$ +45° (*c* 0·7) (Found: C, 74·6; H, 10·0. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3625 and 1718 cm^{-1}.

(b) *Transformations:* Huang-Minlon reduction of 11α,15α-dihydroxy-5α-androstan-4-one (no. 291) followed by oxidation with 8N-H_2CrO_4 gave 5α-*androstane*-11,15-*dione* (no. 52),* m.p. 155—155·5° (from EtOAc), $[\alpha]_D$ +80° (*c* 1·0) (Found: C, 79·0; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%), $\nu_{max.}$ 1751 and 1717 cm^{-1}. Huang-Minlon reduction of 12β,15α-dihydroxy-5α-androstan-4-one (no. 305) followed by oxidation gave 5α-androstane-12,15-dione (no. 55), m.p. (from EtOAc) and mixed m.p. 180—183°.

Oxidation of 11α,15α-dihydroxy-5α-androstan-4-one (no. 291) (100 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-4,11,15-*trione* (no. 91),* m.p. 191—193° (from EtOAc) (80 mg), $[\alpha]_D$ +105° (*c* 0·9) (Found: C, 75·7; H, 8·4. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Oxidation of 12β,15α-dihydroxy-5α-androstan-4-one (no. 305) (240 mg) gave 5α-*androstane*-4,12,15-*trione* (no. 93),* m.p. 182—184° (from MeOH) (200 mg), $[\alpha]_D$ +109° (*c* 0·9) (Found: C, 75·3; H, 8·9. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%).

5α-*Androstan-7-one* (no. 15).* (a) *Incubation:* 2·0 g in Me_2SO (300 ml), 50 flasks, medium B, 7 d, extraction I ⟶ 2 g mycelial extract + 1·4 g broth extract. Chromat. of mycelial extract on SiO_2 (50 g). C_6H_6 gave s.m. (1·44 g). P.l.c. of broth extract [3 large plates, 3 × C_6H_6 + 1 × EtOAc] gave 2 bands. The band of higher R_F gave 12β-*hydroxy-5α-androstan-7-one* (no. 168) * (22 mg) as a glass (Found: C, 78·3; H, 10·3. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ ($CHCl_3$) 3610 and 1710 cm^{-1}. The second band gave an unidentified dihydroxyketone (83 mg), m.p. 175—177° (from Et_2O), $[\alpha]_D$ −60° (*c* 0·2).

(b) *Transformation:* Oxidation of 12β-hydroxy-5α-androstan-7-one (no. 168) with 8N-H_2CrO_4 gave 5α-*androstane*-7,12-*dione* (no. 50),* m.p. 168—170° (from MeOH–H_2O), $[\alpha]_D$ −31° (*c* 0·5) (Found: C, 78·7; H, 9·5. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%).

Androst-5-en-7-one (no. 346).* (a) *Incubation:* 2 g in EtOH (160 ml), 80 flasks, medium A, 2 d, extraction I ⟶ 1·85 g mycelial extract + 1·04 g broth extract. Mycelial extract contained only s.m. (1·6 g). P.l.c. of the

broth extract [2 large plates, 3 × $CHCl_3$] gave 3 bands. The first band (highest R_F) gave 12β-*hydroxyandrost-5-en-7-one* (no. 169),* m.p. 166—168° (from hexane–C_6H_6) (35 mg), $[\alpha]_D$ −201° (*c* 0·3) (Found: C, 79·3; H, 9·9. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%), $\nu_{max.}$ 3610 and 1673 cm^{-1}. The second band gave 4β,12β-dihydroxyandrost-5-en-7-one (no. 440) (47 mg), m.p. 207—213° (from C_6H_6), $\lambda_{max.}$ 234 nm (unchanged on warming with base). The third band afforded 3β,12β-*dihydroxyandrost-5-en-7-one* (no. 257),* m.p. 208—209° (from EtOAc) (87 mg), $[\alpha]_D$ −150° (*c* 1·1) (Found: C, 74·5; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3605 and 1675 cm^{-1}, $\lambda_{max.}$ 237 nm (ε 13,500), $\lambda_{max.}$ (after warming in KOH–EtOH) 281 nm.

(b) *Transformations:* Oxidation of 12β-hydroxyandrost-5-en-7-one (no. 169) gave *androst-5-ene-7,12-dione* (no. 416), m.p. 163—165° (from MeOH), $[\alpha]_D$ −165° (*c* 1·0) (Found: C, 79·2; H, 9·6. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·2%), $\nu_{max.}$ 1715 and 1680 cm^{-1}.

Treatment of 3β,12β-dihydroxyandrost-5-en-7-one (no. 257) with Ac_2O–C_5H_5N for 12 h at 20 °C gave 3β,12β-*di*cetoxyandrost-5-*en*-7-*one* (no. 258),* m.p. 158—162° (from MeOH), $[\alpha]_D$ −136° (*c* 0·5) (Found: C, 70·8; H, 8·2. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%), $\nu_{max.}$ 1738, 1678, and 1240 cm^{-1}, $\lambda_{max.}$ 233 nm (ε 14,500). A solution of the diacetate (no. 258) (80 mg) in 5% KOH–MeOH (25 ml) was heated under reflux for 1·5 h to give 12β-hydroxyandrosta-3,5-dien-7-one (no. 170),* which, after sublimation *in vacuo*, had m.p. 150—152°, $\nu_{max.}$ 3620, 1667, and 1627 cm^{-1}, $\lambda_{max.}$ 277 nm (ε 21,500).

5α-*Androstan*-11-*one* (no. 16).* (a) *Incubation:* 10 g in EtOH (900 ml), 450 flasks, medium A, 2 d, extraction I ⟶ 10·09 g mycelial extract +8·5 g broth extract. Mycelial extract chromat. on Al_2O_3 (350 g). Petrol–C_6H_6 (4 : 1) gave s.m. (3·80 g), m.p. and mixed m.p. 47—50°. Broth extract chromat. Al_2O_3 (10% deactivated; 700 g). Petrol–C_6H_6 (1 : 1) gave 5α-androstane-6,11-dione (no. 46) (21 mg), m.p. and mixed m.p. 173—174°. C_6H_6 gave material (750 mg) which, after rechromatography on Al_2O_3 and elution with C_6H_6, afforded 6α-*hydroxy*-5α-*androstan*-11-*one* (no. 158),* m.p. 139—141° (C_6H_6) (173 mg), $[\alpha]_D$ +82° (*c* 1·1) (Found: C, 78·3; H, 10·1. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ 3620 and 1715 cm^{-1}. Further elution of the original column with C_6H_6 gave a mixture (1·18 g) (see later). C_6H_6–Et_2O (1 : 9) afforded 1α,6α-*dihydroxy*-5α-*androstan*-11-*one* (no. 232),* m.p. 207—209° (from $CHCl_3$–hexane) (705 mg), $[\alpha]_D$ +73° (*c* 0·4) (Found: C, 74·7; H, 9·75. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3610 and 1710 cm^{-1}. The mixture (1·18 g) obtained from the later C_6H_6 eluates was separated by p.l.c. [2 large plates, 1 × $CHCl_3$–Me_2CO (1 : 1)] into 2 bands. The band of higher R_F gave, after further p.l.c., 6α-*hydroxy*-5α-*androstane*-1,11-*dione* (no. 193),* m.p. 217—219° (from Et_2O) (24 mg), $[\alpha]_D$ +87° (*c* 0·3) (Found: C, 74·7; H, 9·2. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3605, 1727, and 1709 cm^{-1}. The second band gave, after further p.l.c., 15α-*hydroxy*-5α-*androstane*-6,11-*dione* (no. 206),* m.p. 173—175° (from Et_2O) (23 mg), $[\alpha]_D$ +87° (*c* 0·1) (Found: C, 74·8; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3610 and 1715 cm^{-1}.

(b) *Transformations:* Oxidation of 6α-hydroxy-5α-androstan-11-one (no. 158) with 8N-H_2CrO_4 gave 5α-androstane-6,11-dione (no. 46), m.p. and mixed m.p. 173—174°. Oxidation of 1α,6α-dihydroxy-5α-androstan-11-one (no. 232) (150 mg) gave 5α-*androstane*-1,6,11-*trione* (no. 61) * (140 mg), m.p. 198·5—200° (from MeOH), $[\alpha]_D$ +73° (*c* 0·7) (Found: C, 75·1; H, 9·05. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Oxidation of 6α-hydroxy-5α-androstane-1,11-dione (no. 193) also gave 5α-androstane-1,6,11-trione (no. 61), m.p. and mixed m.p. 198—200°. Oxidation of 15α-hydroxy-5α-androstane-6,11-dione (no. 206) (76 mg) with 8N-H_2CrO_4 gave 5α-*androstane*-6,11,15-*trione* (no. 94) * (36 mg), m.p. 219—223° (from EtOH), $[\alpha]_D$ +89° (*c* 0·6) (Found: C, 75·4; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1750 and 1720 cm^{-1}.

A solution of 1α,6α-dihydroxy-5α-androstan-11-one (no. 232) (88 mg) in Ac_2O (8 ml)–C_5H_5N (1 ml) was heated at 100°C for 4 h. The product was purified by p.l.c. [1 medium plate, 1 × C_6H_6–EtOAc (3 : 2)] to give 1α,6α-*diacetoxy*-5α-*androstan*-11-*one* (no. 233),* m.p. 157—160° (from MeOH–H_2O) (56 mg), $[\alpha]_D$ +89° (*c* 1·0) (Found: C, 70·9; H, 8·8. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1745, 1715, and 1240 cm^{-1}.

A solution of 1α,6α-dihydroxy-5α-androstan-11-one (no. 232) (300 mg), hydrazine hydrate (100%; 3 ml), and hydrazine dihydrochloride (830 mg) in diethylene glycol (25 ml) was heated at 130°C for 2·5 h. KOH (1·2 g) was added to the cooled mixture, which was then heated under N_2 at 210°C for 5 h. The material isolated with $CHCl_3$ was chromatographed on Al_2O_3 (15% deactivated; 50 g). C_6H_6 eluted 5α-*androstane*-1α,6α-*diol* (no. 216),* m.p. 245—246° (from MeOH) (69 mg), $[\alpha]_D$ +34° (*c* 1·1) (Found: C, 77·9; H, 10·8. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ (Nujol) 3420 cm^{-1}. Oxidation of the diol (no. 216) (120 mg) with 8N-H_2CrO_4, and purification of the product by p.l.c. [2 small plates, 1 × C_6H_6] gave 5α-androstane-1,6-dione (no. 32),* m.p. 178—180° (from MeOH) (32 mg), $[\alpha]_D$ +85° (*c* 0·5) (Found: C, 79·35; H, 10·0. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%), $\nu_{max.}$ 1725—1715br cm^{-1}.

5α-*Androstan*-12-*one* (no. 17).* (a) *Incubation:* 360 mg in Me_2SO (54 ml), 9 flasks, medium B, 6 d, extraction II ⟶ 100 mg mycelial extract and 345 mg broth extract. Chromat. mycelial extract on Al_2O_3 (5 g) gave s.m. (28 mg) in C_6H_6 eluates. The broth extract was acetylated with Ac_2O–C_5H_5N and separated into two components by p.l.c. [1 large plate, 4 × petrol–Et_2O (1 : 1)]. The band of higher R_F gave 6α,15α-*diacetoxy*-5α-*androstan*-12-*one* (no. 442) (57 mg), m.p. 222—225° (from Me_2CO), $[\alpha]_D$ +60° (*c* 0·4) (Found: C, 70·4; H, 8·6. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1737 and 1717 cm^{-1}. The second band gave 1β,6α,15α-triacetoxy-5α-androstan-12-one (no. 466) (51 mg), as an oil, $\nu_{max.}$ 1739 and 1714 cm^{-1}.

(b) *Transformations:* A solution of 6α,15α-diacetoxy-5α-androstan-12-one (no. 442) (44 mg) in 5% KOH–MeOH (5 ml) was kept at 20°C for 12 h. Isolation with Et_2O gave 6α,15α-*dihydroxy*-5α-*androstan*-12-*one* (no. 441) (40 mg), m.p. 187—190·5° (from MeOH–H_2O), $[\alpha]_D$ +43° (*c* 0·2) (Found: C, 74·2; H, 9·6. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3615 and 1712 cm^{-1}. Similar hydrolysis of the triacetoxy-ketone (no. 466) gave 1β,6α,15α-trihydroxy-5α-androstan-17-one (no. 465), as a gum, $\nu_{max.}$ 3630 and 1712 cm^{-1}.

Oxidation of 6α,15α-dihydroxy-5α-androstan-12-one (no. 441) (27 mg) with 8N-H_2CrO_4 gave, after separation by p.l.c. [1 small plate, 1 × Et_2O], the less polar 5α,14β-*androstane*-6,12,15-*trione* (no. 420) (16 mg), m.p. 205—206° (from hexane) (Found: C, 75·3; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1749 and 1712 cm^{-1}, and the more polar 5α-androstane-6,12,15-trione (no. 419) (4 mg) as an oil, $\nu_{max.}$ 1747 and 1713 cm^{-1}.

5α-*Androstan*-15-*one* (no. 18). (a) *Incubation:* 1·0 g in

Me_2SO (150 ml), 25 flasks, medium A, 6 d, extraction II ⟶ 2·76 g combined extracts. Chromat. on Al_2O_3 (deactivated; 60 g). $CHCl_3$ eluted successively 3 fractions, A (320 mg), B (610 mg), and C (350 mg), which were further purified by p.l.c. Fraction A [1 large plate, 2 × petrol–Et_2O (9 : 1)] gave s.m. (39 mg) and 5α,14β-androstan-15-one (no. 413) (13 mg). Fraction B [2 large plates, 3 × petrol–Me_2CO (4 : 1)] gave 6α,12β-*dihydroxy*-5α,14β-*androstan*-15-*one* (no. 443) (228 mg), m.p. 149—151° (from Me_2CO–hexane), $[\alpha]_D$ +1·0° (*c* 0·9) (Found: C, 74·5; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3620 and 1740 cm^{-1}, c.d. 303 nm (Δε — 2·07). Fraction C [1 large plate, 3 × petrol–Me_2CO(2 : 1)] gave 2α,12β-*dihydroxy*-5α-*androstan*-15-*one* (no. 438) (90 mg), m.p. 189—191° (from Me_2CO–hexane), $[\alpha]_D$ +43° (*c* 0·25) (Found: C, 74·2; H, 10·1. $C_{19}H_{20}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ 3620 and 1740 cm^{-1}.

(b) *Transformations:* Huang-Minlon reduction of 6α,12β-dihydroxy-5α,14β-androstan-15-one (no. 443) (60 mg) under the forcing conditions described previously,[14] and fractional crystallisation of the product from Me_2CO–hexane gave 5α-androstane-5α,12β-diol (no. 222) (5 mg), m.p. and mixed m.p. 195—198°. The material recovered from the mother liquors of these crystallisations was oxidised with 8N-H_2CrO_4 to give 5α-androstane-6,12-dione (no. 47) (4 mg), m.p. (from hexane) and mixed m.p. 180—183°. Oxidation of 6α,12β-dihydroxy-5α,14β-androstan-15-one (no. 443) (100 mg) with 8N-H_2CrO_4 gave 5α,14β-androstane-6,12,15-trione (no. 420) (96 mg), m.p. (from hexane) and mixed m.p. 205—206°.

Acetylation of 2α,12β-dihydroxy-5α-androstan-15-one (no. 438) (40 mg) gave 2α,12β-*diacetoxy*-5α-*androstan*-15-*one* (no. 439) (43 mg), m.p. 172—174° (from hexane), $[\alpha]_D$ −11° (*c* 1·0) (Found: C, 70·6; H, 8·9. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1740 cm^{-1}, c.d. 295 nm (Δε + 3·29).

5α,14β-*Androstan*-15-*one* (no. 413). (a) *Incubation:* 2·0 g in Me_2SO (300 ml), 50 flasks, medium B, 6 d, extraction II ⟶ 2·3 g broth extract and 850 mg mycelial extract. The mycelial extract was filtered through Al_2O_3 (deactivated; 20 g) in petrol–Et_2O (9 : 1), and further purified by p.l.c. [1 large plate, 3 × petrol–Et_2O (9 : 1)] to give s.m. (450 mg) and 5α-androstan-15-one (no. 18) (12 mg). The broth extract was filtered through Al_2O_3 (deactivated; 20 g) in $CHCl_3$ and then separated into 3 bands by p.l.c. [2 large plates, 3 × petrol–Me_2CO (4 : 1)]. The band of highest R_F gave 7β,12β-*dihydroxy*-5α,14β-*androstan*-15-*one* (no. 445) (585 mg), m.p. 169—170° (from Me_2CO–hexane), $[\alpha]_D$ −34° (*c* 0·9) (Found: C, 74·3; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%); i.r. and c.d. see Scheme. The second band gave 12β,14-dihydroxy-5α,14β-androstan-15-one (no. 446) (29 mg) as an oil, *m/e* 306 (M^+), $\nu_{max.}$ 3625, 3600, and 1744 cm^{-1}. The third band gave 7β,12β,14-trihydroxy-5α,14β-androstan-15-one (no. 473) (179 mg), m.p. 146—148° (from Me_2CO–hexane), $[\alpha]_D$ −16·5° (*c* 0·5) (Found: C, 70·9; H, 9·3. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%); i.r. and c.d. see Scheme.

(b) *Transformations:* Vigorous Huang-Minlon reduction[14] of 7β,12β-dihydroxy-5α,14β-androstan-15-one (no. 445) (250 mg), and acetylation of the product afforded material which was purified by p.l.c. [1 large plate, 4 × petrol–Et_2O (9 : 1)]. The first band (higher R_F) gave 7β,12β-*diacetoxy*-5α,14β-*androstane* (no. 433) (157 mg), m.p. 104—106° (from MeOH–H_2O), $[\alpha]_D$ +33° (*c* 1·0) (Found: C, 73·5; H, 9·5. $C_{23}H_{36}O_4$ requires C, 73·4; H, 9·6%), $\nu_{max.}$ 1735 cm^{-1}. The second band gave 7β,12β-*diacetoxy*-5α-*androstane* (no. 431) (73 mg) as an oil (Found: C, 73·3; H, 9·5%), $\nu_{max.}$ 1735 cm^{-1}. Treatment of the 14β-diacetate (no. 433) (140 mg) with $LiAlH_4$ (25 mg) in refluxing Et_2O gave 5α,14β-*androstane*-7β,12β-*diol* (no. 432) (116 mg), m.p. 169—170° (from hexane), $[\alpha]_D$ +48·5° (*c* 0·8) (Found: C, 78·0; H, 11·0. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3620 cm^{-1}. Similar treatment of the 14α-diacetate (no. 431) gave 5α-*androstane*-7β,12β-*diol* (no. 430), m.p. 144—145° (from hexane), $[\alpha]_D$ +36° (*c* 0·4) (Found: C, 77·9; H, 11·1. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3620 cm^{-1}.

Oxidation of 5α,14β-androstane-7β,12β-diol (no. 432) (90 mg) with 8N-H_2CrO_4 gave 5α,14β-*androstane*-7,12-*dione* (no. 415) (80 mg), m.p. 142—144° (from hexane), $[\alpha]_D$ +125° (*c* 0·6) (Found: C, 78·9; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·6%), $\nu_{max.}$ 1710 cm^{-1}. Similar oxidation of 5α-androstane-7β,12β-diol (no. 430) gave 5α-androstane-7,12-dione (no. 50),* m.p. (from hexane) and mixed m.p. 168—170°. Similar oxidation of 7β,12β-dihydroxy-5α,14β-androstan-15-one (no. 445) gave 5α,14β-*androstane*-7,12,15-*trione* (no. 421), m.p. 175—177° (from Me_2CO–hexane), $[\alpha]_D$ −28° (*c* 0·2) (Found: C, 75·6; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1750 and 1712 cm^{-1}.

Acetylation of 7β,12β,14-trihydroxy-5α,14β-androstan-15-one (no. 473) (50 mg) with Ac_2O–C_5H_5N at 20°C gave 7β,12β-*diacetoxy*-14-*hydroxy*-5α,14β-*androstan*-15-*one* (no. 474) (43 mg), m.p. 97—99° (from MeOH–H_2O), $[\alpha]_D$ +91° (*c* 1·0) (Found: C, 67·8; H, 8·5. $C_{23}H_{34}O_6$ requires C, 67·9; H, 8·4%), $\nu_{max.}$ 3709, 3500, 1757, 1744, and 1740 cm^{-1}.

Oxidation of the trihydroxy-ketone (no. 473) (20 mg) with 8N-H_2CrO_4 at 0°C gave 7β,14-*dihydroxy*-5α,14β-*androstane*-12,15-*dione* (no. 451) (16 mg), m.p. 194—196° (from Me_2CO–hexane) (Found: C, 71·0; H, 8·7. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 3620, 1744, and 1712 cm^{-1}. A solution of the trihydroxy-ketone (no. 473) (60 mg) and TsOH,H_2O (8 mg) in $Me_2C(OMe)_2$ (freshly distilled; 6 ml) was stirred at 20°C for 30 min. Work-up and p.l.c. [1 small plate, 2 × petrol–Et_2O (1 : 1)] gave 12β-*hydroxy*-7β,14-*isopropylidenedioxy*-5α,14β-*androstan*-15-*one* (no. 475) (62 mg) as a glass, $[\alpha]_D$ −55° (*c* 0·6) (Found: C, 73·2; H, 9·3. $C_{22}H_{34}O_4$ requires C, 72·9; H, 9·4%), $\nu_{max.}$ 3630 and 1742 cm^{-1}. A solution of the trihydroxy-ketone (no. 473) (60 mg) and $LiAlH_4$ (30 mg) in THF (20 ml) was stirred at 0°C for 30 min to give 5α,14β-*androstane*-7β,12β,14,15β-tetraol (no. 482) (54 mg), m.p. 269—272° (from Me_2CO–hexane), $[\alpha]_D$ +7·5° (*c* 0·2) (Found: C, 70·5; H, 9·9. $C_{19}H_{32}O_4$ requires C, 70·3; H, 9·9%), $\nu_{max.}$ (Nujol) 3450 cm^{-1}.

Syntheses of 14-*Hydroxy*-5α,14β-*androstan*-15-*one* (no. 426) via 5α-*Androst*-14-*ene* (no. 412).—A mixture of 5α-androstan-15β-ol (no. 368) * (1·2 g) and $MeSO_2Cl$ (12 ml) in C_5H_5N (12 ml)–Me_2CO (25 ml) was kept at 20 °C for 18 h. The mixture was acidified slowly with 2N-HCl at 0° C, and extracted with Et_2O. Chromatography on SiO_2 (40 g) gave 5α-*androst*-14-*ene* (no. 412) (960 mg; eluted with petrol), m.p. 38—39° (from MeOH–H_2O), $[\alpha]_D$ +32° (*c* 0·9) (Found: C, 88·5; H, 11·5. $C_{19}H_{30}$ requires C, 88·3; H, 11·7%), $\nu_{max.}$ 3060 and 1647 cm^{-1}.

Ice-cold solutions of monoperoxyphthalic acid (6·4 g) in Et_2O (80 ml) and 5α-androst-14-ene (760 mg) in Et_2O (25 ml) were mixed, and stirred at 0°C for 1 h. The solution was washed successively with 10% aq. solutions of KI, $Na_2S_2O_5$, and $NaHCO_3$, dried, and evaporated to give an oil (830 mg). P.l.c. [2 large plates, 3 × petrol–

Me_3CO (49 : 1)] gave 14α,15α-*epoxy*-5α-*androstane* (no. 436) (252 mg; higher R_F), m.p. 75—76° (from MeOH–H_2O), $[\alpha]_D$ +21·5° (*c* 1·0) (Found: C, 83·2; H, 10·9. $C_{19}H_{30}O$ requires C, 83·2; H, 11·0%), $\nu_{max.}$ 3022 cm^{-1}; and 14,15β-*epoxy*-5α,14β-*androstane* (no. 437) (187 mg; lower R_F), m.p. 57—59° (from MeOH–H_2O), $[\alpha]_D$ −4° (*c* 1·1) (Found: C, 83·2; H, 10·9. $C_{19}H_{30}O$ requires C, 83·2; H, 11·0%), $\nu_{max.}$ 3035 cm^{-1}. A mixture (*ca.* 1 : 1) of the epoxides (251 mg) was obtained from the intermediate region of the plate.

A solution of the 14α,15α-epoxide (no. 436) (100 mg) in THF (10 ml)–H_2O(10 ml)-2N-HCl(2 ml) was kept at 20°C for 1 h. Extraction with Et_2O and p.l.c. [1 small plate, 2 × petrol–Et_2O (1 : 1)] gave 5α,14β-androstan-15-one (no. 413) (36 mg) and 5α,14β-*androstane*-14,15α-*diol* (no. 434) (53 mg), m.p. 168—169° (from MeOH–H_2O), $[\alpha]_D$ +107° (*c* 0·5) (Found: C, 78·0; H, 10·9. $C_{19}H_{32}O_2$ requires C, 78·0; H, 10·9%), $\nu_{max.}$ 3630 cm^{-1}. Similar treatment of the 14β,15β-epoxide (no. 437) (80 mg) for 2 h gave s.m. (52 mg) and 5α,14β-*androstane*-14,15β-*diol* (no. 435) (24 mg), m.p. 119—122° (from MeOH–H_2O), $[\alpha]_D$ −15° (*c* 0·5) (Found: C, 77·7; H, 10·9. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), $\nu_{max.}$ 3634 and 3520 cm^{-1}.

Oxidation of 5α,14β-androstane-14,15α-diol (no. 434) (70 mg) with 8N-H_2CrO_4 at 0°C gave 14-*hydroxy*-5α,14β-*androstan*-15-*one* (no. 426) (60 mg), m.p. 128—130° (from MeOH–H_2O), $[\alpha]_D$ +50° (*c* 0·2) (Found: C, 78·7; H, 10·5. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·3%); see Scheme for spectral data.

5α-*Androstan*-16-*one* (no. 19).* (a) *Incubation:* 2·8 g in Me_2SO (910 ml), 56 flasks, medium A, 4 d, extraction III ⟶ 3·0 g total extract. Chromat. Al_2O_3 (deactivated; 150 g). Petrol–Et_2O (1 : 1) gave s.m. (875 mg), m.p. and mixed m.p. 106—107°. Et_2O–MeOH (20 : 1) gave a mixture (1·43 g) which was separated by p.l.c. [3 large plates, 7 × C_6H_6–EtOH (1 : 1)] into 2 bands. The band of higher R_F gave 6α,11α-*dihydroxy*-5α-*androstan*-16-*one* (no. 272),* m.p. 207—208° (from EtOAc) (740 mg), $[\alpha]_D$ −170° (*c* 1·1) (Found: C, 74·6; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%). The second band afforded 1β,6α-*dihydroxy*-5α-*androstan*-16-*one* (no. 234),* m.p. 235—237° (from EtOAc) (195 mg), $[\alpha]_D$ −165° (*c* 1·2) (Found: C, 75·4; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ ($CHCl_3$) 1748, 1713, and 1711 cm^{-1}.

(b) *Transformations:* Oxidation of 6α,11α-dihydroxy-5α-androstan-16-one (no. 272) with 8N-H_2CrO_4 gave 5α-*androstane*-6,11,16-*trione* (no. 95),* m.p. 235—237° (from EtOH), $[\alpha]_D$ −118° (*c* 0·8) (Found: C, 75·4; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Similar oxidation of 1β,6α-dihydroxy-5α-androstan-11-one (no. 234) gave 5α-*androstane*-1,6,16-*trione* (no. 355),* m.p. 224—226° (from EtOH), $[\alpha]_D$ −73° (*c* 0·7) (Found: C, 75·7; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%).

The mixture of hydroxylated metabolites (250 mg) from the incubation was reduced by the usual Huang-Minlon procedure, and the crude product was oxidised with 8N-H_2CrO_4. Separation by p.l.c. [1 large plate, 3 × petrol–EtOAc (10 : 1)] gave 5α-androstane-6,11-dione (no. 46) (125 mg), m.p. and mixed m.p. 173—174°, and 5α-androstane-1,6-dione (no. 32) (32 mg), m.p. and mixed m.p. 180°.

5α-*Androstan*-17-*one* (no. 20).* (a) *Incubation:* 2·2 g in EtOH (220 ml), 44 flasks, medium A, 2 d, extraction III ⟶ 2·4 g total extract. Chromat. Al_2O_3 (deactivated; 100 g) C_6H_6–Et_2O (4 : 1) gave s.m. (875 mg), m.p. and mixed m.p. 117—117·5°. Et_2O–MeOH (20 : 1) gave 1β,6α-*dihydroxy*-5α-*androstan*-17-*one* (no. 235),* m.p. 200—203° (from hexane–Me_2CO) (700 mg), $[\alpha]_D$ +89° (*c* 1·0) (Found: C, 74·3; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%).

(b) *Transformations:* Oxidation of 1β,6α-dihydroxy-5α-androstan-17-one (no. 235) gave 5α-androstane-1,6,17-trione (no. 64),* m.p. 202—203° (from EtOH), $[\alpha]_D$ +174° (*c* 0·7) (Found: C, 75·4; H, 8·8. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). A solution of the trione (no. 64) (125 mg) in 5% KOH–MeOH (25 ml) was heated under reflux for 2 h to give 5β-*androstane*-1,6,17-*trione* (no. 356),* m.p. 243—244° (from EtOH) (100 mg), $[\alpha]_D$ −40° (*c* 0·5) (Found: C, 75·4; H, 8·8. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%).

Huang-Minlon reduction of the dihydroxy-ketone (no. 235) (100 mg), and oxidation of the product with 8N-H_2CrO_4 gave 5α-androstane-1,6-dione (no. 32) (45 mg), m.p. and mixed m.p. 177—179°.

5β-*Androstan*-17-*one* (no. 21).* *Incubation:* 3·6 g in EtOH (360 ml), 72 flasks, medium A, 2 d, extraction III ⟶ 4·0 g total extract. Chromat. Al_2O_3 (deactivated; 160 g). Petrol–Et_2O (1 : 1) gave s.m. (2·0 g). Et_2O–MeOH (10 : 1) gave a mixture (900 mg), separation of which was attempted by p.l.c. [3 large plates, 12 × petrol–EtOAc (19 : 1)]. Only one product was obtained pure. This was 12β,15α-*dihydroxy*-5β-*androstan*-17-*one* (no. 447) (25 mg), m.p. 191—193° (from EtOAc), $[\alpha]_D$ +85° (*c* 0·2) (Found: C, 74·4; H, 9·8. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ (conditions of ref. 9) 3631, 3609, 3568, and 1735 cm^{-1}.

5α-*Androstan*-17β-*ol* (no. 138).* (a) *Incubation:* 2·0 g in EtOH (100 ml), 50 flasks, medium B, 6 d, extraction II ⟶ 2·0 g mycelial extract + 2·2 g broth extract. Chromat. mycelial extract on Al_2O_3 (10% deactivated; 25 g) gave s.m. (810 mg) from the petrol–Et_2O (10 : 1) eluates. Et_2O–MeOH (10 : 1) gave a mixture (226 mg). Chromat. broth extract on Al_2O_3 (10% deactivated; 25 g) gave a mixture (760 mg) eluted with Et_2O–MeOH (10 : 1). P.l.c. of the combined mixtures from both columns [3 large plates, 3 × Et_2O] gave two bands. The band of higher R_F afforded 5α-*androstane*-1β,6α,17β-*triol* (no. 452) (230 mg), m.p. 249—250° (from Me_2CO), $[\alpha]_D$ +36° (*c* 0·7) (Found: C, 73·6; H, 10·0. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%). The second band gave 5α-*androstane*-6α,11α,17β-*triol* (no. 462) (78 mg), m.p. 225—226° (from MeOH), $[\alpha]_D$ +20° (*c* 0·5) (Found: C, 74·1; H, 10·5. $C_{19}H_{32}O_3$ requires C, 74·0; H, 10·5%).

(b) *Transformations:* Oxidation of 5α-androstane-1β,6α,17β-triol gave 5α-androstane-1,6,17-trione (no. 64), m.p. (from Me_2CO) and mixed m.p. 202—203°. Oxidation of 5α-androstane-6α,11α,17β-triol (no. 462) gave 5α-*androstane*-6,11,17-*trione* (no. 96),* m.p. 212—216° (from Et_2O), $[\alpha]_D$ +131° (*c* 1·0) (Found: C, 75·1; H, 8·9. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%).

D-*Homo*-5α-*androstan*-17a-*one* (no. 348).* (a) *Incubation:* 1·0 g in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction III ⟶ 1·2 g total extract. P.l.c. [3 large plates, 5 × petrol–Me_2CO (4 : 1)] gave 3 main bands. The first band (highest R_F) afforded 1β,7β,15α-trihydroxy-D-homo-5α-androstan-17a-one (no. 467) (75 mg), m.p. 204—206° (from Me_2CO–hexane), $[\alpha]_D$ +38° (*c* 0·5), *m/e* 336 (M^+); $\nu_{max.}$ see Scheme. The second band gave 6α,11α-*dihydroxy*-D-*homo*-5α-*androstan*-17a-*one* (no. 449) (110 mg), m.p. 210—211·5° (from Me_2CO–hexane), $[\alpha]_D$ −43° (*c* 0·5) (Found: C, 75·2; H, 10·0. $C_{20}H_{32}O_3$ requires C, 75·0;

H, 10·1%), $\nu_{max.}$ ($CHCl_3$) 3602 and 1708 cm^{-1}. The third band gave 7β,12β,15α-*trihydroxy*-D-*homo*-5α-*androstan*-17a-*one* (no. 476) (132 mg), m.p. 213—215° (from Me_2CO–hexane), $[\alpha]_D$ +42° (*c* 0·45) (Found: C, 69·9; H, 9·35. $C_{20}H_{32}O_4$,Me_2CO requires C, 70·0; H, 9·7%); $\nu_{max.}$ (after repeatedly dissolving in CCl_4 and evaporating) see Scheme.

(b) *Transformations:* 1β,7β,15α-Trihydroxy-D-homo-5α-androstan-17a-one (no. 467) (100 mg) was reduced by the Huang-Minlon method. A solution of the product in Me_2CO (20 ml) containing 10N-HCl (0·4 ml) was heated under reflux for 30 min to give 7β,15α-*isopropylidenedioxy*-D-*homo*-5α-*androstan*-1β-*ol* (no. 456) as an oil, $[\alpha]_D$ −10° (*c* 0·55) (Found: C, 76·0; H, 10·6. $C_{23}H_{38}O_3$ requires C, 76·2; H, 10·6%), $\nu_{max.}$ 3620 cm^{-1}. Similar treatment of 7β,12β,15α-trihydroxy-D-homo-5α-androstan-17a-one (no. 476) afforded 7β,15α-*isopropylidenedioxy*-D-*homo*-5α-*androstan*-12β-*ol* (no. 464), m.p. 148—150° (from hexane), $[\alpha]_D$ +20° (*c* 0·25) (Found: C, 76·1; H, 10·45. $C_{23}H_{38}O_3$ requires C, 76·2; H, 10·6%), $\nu_{max.}$ 3620 cm^{-1}.

3-*Methylene*-5α-*androstan*-17β-*ol* (no. 139).* (a) *Incubation:* 3·7 g in EtOH (370 ml), 74 flasks, medium A, 2 d, extraction III ⟶ 4·0 g total extract. Chromat. Al_2O_3 (deactivated; (160 g). Petrol–Et_2O (1 : 1) gave s.m. (2·0 g). Et_2O–MeOH (20 : 1) gave 3-*methylene*-5α-*androstane*-1β,6α,17β-*triol* (no. 453), m.p. 252—253° (from MeOH) (1·6 g), $[\alpha]_D$ +43° (*c* 0·5) (Found: C, 74·7) H, 10·1. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%), $\nu_{max.}$ (Nujol) 3290 and 891 cm^{-1}.

(b) *Transformations:* Oxidation of 3-methylene-5α-androstane-1β,6α,17β-triol (no. 453) (100 mg) with 8N-H_2CrO_4 gave 3-*methylene*-5α-*androstane*-1,6,17-*trione* (no. 65) * (80 mg), m.p. 177—178° (from EtOH), $[\alpha]_D$ +129° (*c* 0·7) (Found: C, 76·5; H, 8·4. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3%), $\nu_{max.}$ 1745, 1719, and 891 cm^{-1}, $\lambda_{max.}$ 298 nm (ε 176).

Acetylation of the triol (no. 453) (1·87 g) with Ac_2O (50 ml)–C_5H_5N (5 ml) for 5 h at 20°C gave an oil (1·8 g). Chromat. Al_2O_3 (deactivated; 50 g) and elution with petrol–Et_2O (1 : 1) gave 1β,6α,17β-*triacetoxy*-3-*methylene*-5α-*androstane* (no. 454) (100 mg), m.p. 198—199° (from EtOH), $[\alpha]_D$ +21° (*c* 0·9) (Found: C, 70·1; H, 8·7. $C_{26}H_{38}O_6$ requires C, 69·9; H, 8·6%), $\nu_{max.}$ ($CHCl_3$) 1740 and 895 cm^{-1}. Further elution with the same solvent mixture gave 6α,17β-*diacetoxy*-3-*methylene*-5α-*androstan*-1β-*ol* (no. 455) (400 mg), m.p. 201—203° (from EtOH), $[\alpha]_D$ +33° (*c* 0·6) (Found: C, 71·3; H, 8·7. $C_{24}H_{36}O_5$ requires C, 71·3; H, 9·0%), $\nu_{max.}$ ($CHCl_3$) 3621, 1740, and 895 cm^{-1}. Et_2O eluted s.m. (790 mg), m.p. and mixed m.p. 251—253°.

Sequence Leading to 3-*Methyl*-5β-*androst*-2-*ene*-1,6,17-*trione* (no. 357).—Oxidation of 6α,17β-diacetoxy-3-methylene-5α-androstan-1β-ol (no. 455) (350 mg) with 8N-H_2CrO_4 gave 6α,17β-*diacetoxy*-3-*methylene*-5α-*androstan*-1-*one* (no. 279) * (310 mg), m.p. 172—173° (from EtOH), $[\alpha]_D$ +61° (*c* 0·9) (Found: C, 71·4; H, 8·7. $C_{24}H_{34}O_5$ requires C, 71·6; H, 8·5%), $\nu_{max.}$ ($CHCl_3$) 1742, 1720, and 899 cm^{-1}. A solution of this diacetoxy-ketone (270 mg) in 10% KOH–EtOH (50 ml) was kept at 20°C for 12 h. Work-up gave 6α,17β-*dihydroxy*-3-*methyl*-5α-*androst*-2-*en*-1-*one* (no. 278) * (210 mg), m.p. 232—233° (from EtOAc), $[\alpha]_D$ +167° (*c* 1·0) (Found: C, 75·5; H, 9·3. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%), $\nu_{max.}$ 3624 and 1675 cm^{-1}.

Oxidation of 6α,17β-dihydroxy-3-methyl-5α-androst-2-en-1-one (no. 278) (50 mg) afforded 3-*methyl*-5α-*androst*-2-*ene*-1,6,17-*trione* (no. 66) * (43 mg), m.p. 178—179° (from EtOH), $[\alpha]_D$ +102° (*c* 0·1) (Found: C, 76·4; H, 8·4. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3%), $\nu_{max.}$ 1744, 1718, and 1676 cm^{-1}, $\lambda_{max.}$ 236 nm (ε 8160). A solution of this trione (25 mg) in 5% KOH–MeOH (10 ml) was heated under reflux for 2 h to give 3-*methyl*-5β-*androst*-2-*ene*-1,6,17-*trione* (no. 357) * (22 mg), m.p. 235—237° (from Me_2CO–hexane), $[\alpha]_D$ −11° (*c* 0·3) (Found: C, 76·5; H, 8·4. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3%), $\nu_{max.}$ 1745, 1716. and 1672 cm^{-1}, $\lambda_{max.}$ 236 nm (ε 8720).

Sequence Leading to 5α-*Androst*-1-*ene*-3,6,17-*trione* (no. 79).—A solution of 1β,6α,17β-triacetoxy-3-methylene-5α-androstane (no. 454) (1·14 g) in MeOH (100 ml) was treated with O_3 at −20°C for 1 h. Glacial AcOH (57 ml) and then Zn dust (23 g) were added to the stirred solution, and the temperature of the mixture was allowed to rise to about 35°C. The mixture was filtered, and the filtrate was concentrated to *ca.* 70 ml at 50° and 2 cmHg. Dilution with H_2O and extraction with CH_2Cl_2 gave 1β,6α,17β-*triacetoxy*-5α-*androstan*-3-*one* (no. 333) * (1·03 g), m.p. 175—177° (from $CHCl_3$–Et_2O), $[\alpha]_D$ +34° (*c* 1·1) (Found: C, 66·7; H, 8·0. $C_{25}H_{36}O_7$ requires C, 66·9; H, 8·1%), $\nu_{max.}$ ($CHCl_3$) 1737 cm^{-1}. A solution of this triacetoxy-ketone (400 mg) in 1% KOH–EtOH (50 ml) was kept at 20°C for 12 h. Isolation with Et_2O gave 6α,17β-*dihydroxy*-5α-*androst*-1-*en*-3-*one* (no. 444) (230 mg), m.p. 277—279° (from EtOAc–EtOH), $[\alpha]_D$ +70° (*c* 0·7) (Found: C, 75·1; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ (Nujol) 3290 and 1680 cm^{-1}, $\lambda_{max.}$ 228 nm (ε 9670).

Oxidation of the dihydroxy-ketone (no. 444) (50 mg) gave 5α-*androst*-1-*ene*-3,6,17-*trione* (no. 79),* m.p. 223—225° (from EtOAc) (42 mg), $[\alpha]_D$ +2° (*c* 0·3) (Found: C, 76·1; H, 8·1. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·1%), $\nu_{max.}$ ($CHCl_3$) 1740, 1718, and 1687 cm^{-1}. Hydrogenation of this triketone (no. 79) (100 mg) in EtOAc–HOAc (10 : 1; 20 ml) over PtO_2 (10 mg) at 20°C, followed by oxidation of the product, gave 5α-androstane-3,6,17-trione (no. 78) * (80 mg), m.p. 194—195° (from Me_2CO-hexane), $[\alpha]_D$ +71° (*c* 0·6) (lit.,[15] m.p. 191—193°, $[\alpha]_D$ +67°).

A solution of 1β,6α,17β-triacetoxy-5α-androstan-3-one (no. 333) (750 mg) in Et_2O (75 ml) was added to a stirred suspension of $LiAlH_4$ (750 mg) in Et_2O (75 ml). The mixture was stirred for 2 h at 20°C, and worked up to give 5α-*androstane*-1β,3β,6α,17β-*tetraol* (no. 482) (410 mg), m.p. 335—338° (from MeOH) (Found: C, 70·4; H, 9·8. $C_{19}H_{32}O_4$ requires C, 70·3; H, 9·9%), $\nu_{max.}$ (Nujol) 3360—3280 cm^{-1}.

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