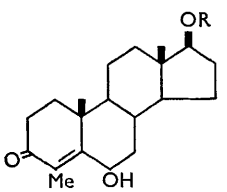
768. *Modified Steroid Hormones. Part XVIII.* The Microbiological Hydroxylation of 4-Methyltestosterone with* Rhizopus nigricans.

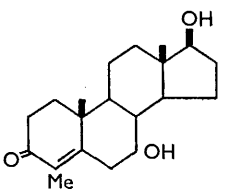
By D. N. Kirk, V. Petrow, and (Mrs.) M. H. Williamson.

Microbiological hydroxylation of 4-methyltestosterone with *Rhizopus nigricans* yields 7β-hydroxy- (IIb) as main product and 11α-hydroxy-4-methyltestosterone (III) as subsidiary product. Smaller quantities of the 7α- (IIa) and 6β-hydroxy-derivatives (Ib; R = H) are also formed.

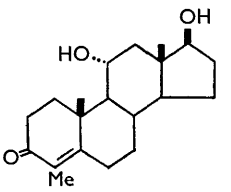
For studies on 4-methylated steroid hormones we required 11α-hydroxy-4-methyltestosterone (III). In seeking a route to this compound we examined, *inter alia*, the microbiological hydroxylation of 4-methyltestosterone with *Rhizopus nigricans*. The oxygenation of testosterone by *Rhizopus* species had previously been studied by Eppstein *et al.*[1] who had shown that 11α- and 6β-hydroxytestosterone respectively form the major and the the minor product of oxidation. In the case of 4-methyltestosterone, however, it was hoped



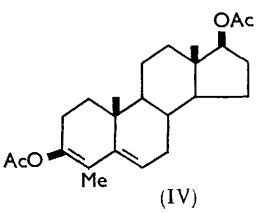
(Ia) 6α-OH
(Ib) 6β-OH



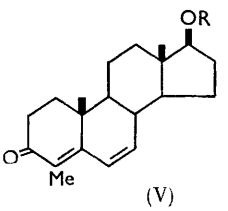
(IIa) 7α-OH
(IIb) 7β-OH



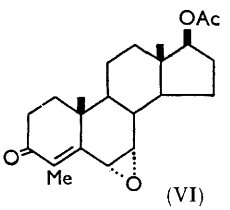
(III)



(IV)



(V)



(VI)

that the presence of the 4-methyl substituent might interfere with hydroxylation at the adjacent $C_{(6)}$. This expectation was fulfilled in that 6β-hydroxy-4-methyltestosterone

* Part XVII, *J.*, 1960, 2828.

[1] Eppstein, Meister, Leigh, Peterson, Murray, Reineke, and Weintraub, *J. Amer. Chem. Soc.*, 1954, **76**, 3174.

formed only a minute fraction of the fermentation products. These consisted, however, of 7β-hydroxy-4-methyltestosterone (IIb) and smaller quantities of the required 11α-hydroxy-derivative (III), together with subsidiary products as detailed below. 7β-Hydroxylation by *Rhizopus nigricans* has not been reported previously for the androgen series, but has been shown to occur with 3β-hydroxypregn-5-en-20-one.[2]

Chromatography on alumina separated the neutral hydroxylation products of 4-methyltestosterone into 6 fractions (see p. 3875). Fraction 1 consisted of a small quantity of starting material. Fractions 2—5 had mobilities on paper chromatograms indicating the presence of an additional hydroxy-group in the molecule (cf. Table). The small final fraction 6 behaved in a manner consistent with di- or poly-hydroxylated material and was not investigated further.

The presence of a 6-hydroxyl group in product A (see Table) was indicated by its ultraviolet absorption at a wavelength slightly below that for the parent compound.[1,3] Its constitution as 6β-hydroxy-4-methyltestosterone (Ib; R = H) was unequivocally established by its alternative preparation from 4-methyltestosterone. Enol acetylation of the last compound, followed by treatment of the product with monoperphthalic acid,[4] yielded, after chromatography, 17β-acetoxy-6β- (Ib; R = Ac) and 17β-acetoxy-6α-hydroxy-4-methylandrost-4-en-3-one (Ia; R = Ac), which were differentiated on the basis of their optical rotations [3] and ultraviolet absorption, including the *R*-band regions [3] (see p. 3875). Careful alkaline hydrolysis of the 6β-hydroxy-isomer furnished 6β-hydroxy-4-methyltestosterone (Ib; R = H), identical with product A.

Fraction no.	Product	4-Methyltestosterone derivative	M. p.[a]	$[\alpha]_D^{25}$	$\lambda_{max.}$ in EtOH (mμ)	R_F [b]	$\nu_{max.}$ (cm.$^{-1}$)	Found (%) [c] C	H	Yield (%)
2	A	6β-Hydroxy-	218—220°	+10° ($CHCl_3$)	247 (ε 11,655)	0·57	3250, 1643, 1600	75·4	9·2	0·2
3	B	7β-Hydroxy-	189—190·5	+106° ($CHCl_3$)	251·5 (ε 15,090)	0·48	3451, 1649, 1608	75·2	9·6 [d]	12·2
4	C	7α-Hydroxy-	190—192	+105° (dioxan)	252 (ε 14,520)	0·48	3370, 1658, 1604	75·8	9·7	2·0
5	D	11α-Hydroxy-	179·5—180·5	+72° ($CHCl_3$)	252 (ε 14,200)	0·40	3416, 1643, 1594	75·3	9·5 [d]	7·5

[a] After crystallisation from acetone–hexane. [b] Determined in the Bush B5 solvent system (Bush, *Biochem. J.*, 1952, **50**, 370). [c] $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%. [d] After drying at 115° *in vacuo*.

Methanolic hydrochloric acid converted 6β-hydroxy-4-methyltestosterone (Ib; R = H) into a mixture from which 17β-hydroxy-4-methylandrosta-4,6-dien-3-one (V; R = H) was isolated by chromatography. The identity of this was established by its alternative preparation from 4-methyltestosterone by dehydrogenation with chloranil.[5] The more polar fractions resisted purification. Their infrared spectra, however, showed the presence in them of a small proportion of saturated ketonic material ($\nu_{max.}$ *ca.* 1700 cm.$^{-1}$). The 4-methylated hydroxy-ketone (Ib; R = H) consequently differs from unsubstituted 6β-hydroxy-3-oxo-Δ^4-steroids, which are smoothly converted by acid into the corresponding 5α-3,6-diones.[1,6]

Oxidation of the dihydroxy-ketone (Ib; R = H) with chromic acid furnished 4-methylandrost-4-ene-3,6,17-trione. Its ultraviolet absorption spectrum (in ethanol) which was similar to that reported for 4-methylcholest-4-ene-3,6-dione [7] was unaffected by the

[2] Eppstein, Meister, Murray, and Peterson, "Vitamins and Hormones," Vol. XIV, Academic Press Inc., New York, 1956, p. 359; see also Dodson, Nicholson, and Muir, *J. Amer. Chem. Soc.*, 1959, **81**, 6295.
[3] Bush, *Biochem. J.*, 1952, **50**, 370.
[3] Bird, Cookson, and Dandegaonker, *J.*, 1956, 3675.
[4] Romo, Rosenkranz, Djerassi, and Sondheimer, *J. Org. Chem.*, 1954, **19**, 1509.
[5] Chas. Pfizer & Co. Inc., B.P. 794,392.
[6] Herzig and Ehrenstein, *J. Org. Chem.*, 1951, **16**, 1050.
[7] Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4386.

presence of potassium hydroxide or hydrogen chloride. As 4-unsubstituted 4-ene-3,6-diones pass readily into enolic structures,[8] the 4-methyl group must be regarded as inhibiting the enolisation of this particular system.

Products B and C (see Table) were converted into the same unsaturated trione on oxidation. This, coupled with their ready conversion into 17β-hydroxy-4-methylandrosta-4,6-dien-3-one (V; R = H) on treatment with acid or alkali, permits their formulation as epimeric 7-hydroxy-derivatives (II).

Their stereochemistry about $C_{(7)}$ was established by direct comparison with the authentic 7α-hydroxy-isomer (IIb), which was prepared by an alternative route from 17β-acetoxy-4-methylandrosta-4,6-dien-3-one (V; R = Ac). The last compound was treated with monoperphthalic acid to give the corresponding 6α,7α-epoxide (VI). Its reduction with lithium aluminium hydride gave the 3ξ,7α,17β-trihydroxy-derivative,[9] converted directly by 2,3-dichloro-5,6-dicyanobenzoquinone[10] into the 7α-hydroxy-derivative (IIa), which was identical with product C. Had the above 6,7-epoxide possessed the β-configuration, its reduction with lithium aluminium hydride would have furnished, by diaxial opening, the 6β-hydroxy- and not a 7-hydroxy-derivative. Product B is consequently regarded as the 7β-hydroxy-isomer (IIb).

The inhibiting effect of the 4-methyl substituent on the enolisation of neighbouring oxo-groups (see above) was again apparent in the behaviour of 4-methylandrost-4-ene-3,7,17-trione [obtained by oxidation of (II)]. This compound appeared to exist normally in the triketo-form, which passed into an enol only in the presence of alkali. The 4-demethyl analogues, in contrast, exist wholly in the enolic forms, probably as the 3-hydroxy-$\Delta^{3,5}$-7-ones.[11]

Product D (see Table) was identified as 11α-hydroxy-4-methyltestosterone (III) by comparison with authentic material which had become available through the development of a new 4-methylation technique and its application to 11α-hydroxytestosterone (forthcoming publication). Its oxidation gave 4-methyladrenosterone.

Experimental

Ultraviolet (in EtOH) and infrared spectra were kindly determined by Mr. M. T. Davies, B.Sc., and Miss D. F. Dobson, B.Sc. Optical rotations refer to $CHCl_3$ solutions in a 1 dm. tube unless otherwise stated.

Oxidation of 4-Methyltestosterone with Rhizopus nigricans (with Mrs. A. S. Carmichael, B.Sc.). —100 l. of a medium containing malt extract 5%, peptone 0·5%, and glucose 2% in tap-water were brought to pH 5·54 by the addition of 50% sodium hydroxide solution and sterilised at its b. p. at 15 lb. in.$^{-2}$ for 20 min., then cooled to 26° and inoculated with an 18 hr. vegetative mycelium of *Rhizopus nigricans* (B.D.H. culture No. 153) (0·6% v/v). The fermentation was carried out at 26° at a pressure of 10 lb. in.$^{-2}$ in a vessel equipped with a paddle and baffles, and air was introduced at 50 l./min., with stirring at 250 r.p.m. and addition of silicone " B " antifoam (Midland Silicones, Ltd.) (15% in water) as required. After 24 hours' growth 4-methyltestosterone (100 g.) in absolute alcohol (600 ml.) was added, and the fermentation was continued for a further 48 hr.

The solids were collected, washed with water (15 l.), and sucked dry. Extraction of the solids with boiling 4 : 1 chloroform–ethanol (4 × 20 l.) and evaporation gave a total of *ca.* 70 g. of material which was treated with boiling light petroleum (b. p. 60—80°) (300 ml.). The mixture was cooled and filtered and the filtrate rejected. The residual solids (23 g.) were purified from acetone–hexane (charcoal), to give 4-methyltestosterone (12 g.; m. p. 164—170°). The acetone–hexane filtrates were added to the extracts from the filtered fermentation liquors (see below).

The filtered fermentation liquors were extracted with ethyl acetate in a continuous liquid–liquid extractor, and afforded 67 g. of gum on evaporation of the solvent. This material,

[8] Windaus, *Ber.*, 1907, **40**, 257; Meyer, *J. Org. Chem.*, 1955, **20**, 1240.
[9] Cf. Nussbaum, Brabazon, Popper, and Oliveto, *J. Amer. Chem. Soc.*, 1958, **80**, 2722.
[10] Burn, Petrow, and Weston, *Tetrahedron Letters*, 1960, No. 9, 14.
[11] Barnett, Ryman, and Smith, *J.*, 1946, 526; Greenhalgh, Henbest, and Jones, *J.*, 1952, 2375.

combined with the residual material extracted from the mycelium, was dissolved in benzene (2 l.) and ether (1 l.), and acidic substances were removed by shaking with potassium hydroxide (20 g.) in water (1 l.). Evaporation of the benzene gave 49 g. of brown gum.

This gum (30 g.) in benzene (270 ml.) was chromatographed on alumina (840 g.; Grade III, Brockmann classification).

The eluted fractions were: (1) benzene–ether (1 : 1) and ether, 4·4 g. (recryst. 3·2 g.), 4-methyltestosterone; (2) ether, 0·8 g. (recryst. 0·12 g.) (Ib); (3) ether and ether–acetone (4 : 1), 10·7 g. (recryst. 7·5 g.), (IIb); (4) ether–acetone (1 : 2) and acetone, 3·8 g. (recryst. 1·2 g.), (IIa); (5) acetone–methanol (4 : 1), 7·5 g. (recryst. 4·6 g.), (III); (6) acetone–methanol (1 : 1), 1·0 g. The properties of the purified products are recorded in the Table on p. 3873.

Enol Acetylation of 4-*Methyltestosterone Acetate* (by Dr. B. Ellis).—4-Methyltestosterone acetate (10 g.) was suspended in acetic anhydride (50 ml.), and toluene-*p*-sulphonic acid (5 g.) was added. The mixture, on stirring (10 min.), became homogeneous and thereafter deposited crystals. After 16 hr. these were collected, washed with methanol, and purified from methanol, to give 3,17β-*diacetoxy*-4-*methylandrosta*-3,5-*diene* (IV), m. p. 174—175°, $[\alpha]_D^{24}$ −139° (*c* 0·99) $\lambda_{max.}$ 236 mμ (ε 19,930) in EtOH (Found: C, 75·0; H, 8·7. $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·9%).

6α- *and* 6β-*Hydroxy*-4-*methyltestosterone* 17-*Acetates* (Ia and b; R = Ac).—The foregoing enol acetate (8·46 g.) in chloroform (20 ml.) was treated with ethereal monoperphthalic acid (6·4 g. in 60 ml.) for 4 hr. at room temperature, then the solution was poured into excess of sodium hydrogen carbonate solution and the products were extracted with chloroform and chromatographed on alumina. Benzene eluates gave 6β-*hydroxy*-4-*methyltestosterone* 17-*acetate* which separated from aqueous methanol in needles, m. p. 207—209°, $[\alpha]_D^{23}$ +21° (*c* 0·31 in dioxan), $\lambda_{max.}$ 248·5 mμ (ε 13,555), $\nu_{max.}$ (in CCl_4) 3601, 1735, 1676, 1602, and (in CS_2) 1245 cm.$^{-1}$ (Found: C, 73·3; H, 8·7. $C_{22}H_{32}O_4$ requires C, 73·3; H, 8·95%). Further elution with benzene–ether (19 : 1 and 5 : 1) gave 6α-*hydroxy*-4-*methyltestosterone* 17-*acetate* which crystallised from aqueous methanol as needles, m. p. 182—185°, $[\alpha]_D^{22}$ +86° (*c* 0·27 in dioxan), $\lambda_{max.}$ 252·5 mμ (ε 13,770), $\nu_{max.}$ (in Nujol) 3463, 1740, 1651, 1595, and 1244 cm.$^{-1}$ (Found: C, 73·6; H, 8·7. $C_{22}H_{32}O_4$ requires C, 73·3; H, 8·95%).

6β-*Hydroxy*-4-*methyltestosterone* (Ib; R = H).—6β-Hydroxy-4-methyltestosterone 17-acetate (3·1 g.) in methanol (120 ml.) was treated with potassium carbonate (1·5 g.) in water (20 ml.) overnight at room temperature, then at 50° for 1 hr.; the product was precipitated with water. Purification from acetone gave 6β-hydroxy-4-methyltestosterone, m. p. 218—220°, not depressed in admixture with the material obtained from the fermentation. The identity of the samples was confirmed by comparison of their infrared spectra [$\nu_{max.}$ (in Nujol) 3250, 1643, and 1600 cm.$^{-1}$; identical " fingerprint "].

6α-*Hydroxy*-4-*methyltestosterone* (Ia; R = H).—Similar hydrolysis of the 17β-acetate (Ia; R = Ac) gave 6α-*hydroxy*-4-*methyltestosterone*, prisms (from acetone), m. p. 224—227°, $[\alpha]_D^{27}$ +98° (*c* 0·56), $\lambda_{max.}$ 252·5 mμ (ε 12,545), $\nu_{max.}$ (in Nujol) 3380, 3245, 1641, and 1595 cm.$^{-1}$ (Found: C, 75·9; H, 9·6. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%).

Treatment of 6β-*hydroxy*-4-*methyltestosterone with Methanol–Hydrochloric Acid.*—6β-Hydroxy-4-methyltestosterone (560 mg.) was heated under reflux in methanol (20 ml.) and concentrated hydrochloric acid (6 drops) for 3 hr. Evaporation of the solvents *in vacuo* left a gum which was chromatographed on alumina (17 g.; Brockmann Grade III). Elution with benzene gave 17β-*hydroxy*-4-*methylandrosta*-4,6-*dien*-3-*one*, which separated from aqueous methanol in solvated plates, m. p. 132—134° raised by drying at 100° for 24 hr. *in vacuo* to 154—155°, $[\alpha]_D^{24}$ +117° (*c* 0·64), $\lambda_{max.}$ 289·5 mμ (ε 29,440) (Found: C, 79·8; H, 9·4. $C_{20}H_{28}O_2$ requires C, 79·95; H, 9·4%).

4-*Methylandrost*-4-*ene*-3,6,17-*trione.*—6β-Hydroxy-4-methyltestosterone (250 mg.) in " AnalaR " acetone (20 ml.) was treated with the chromic acid–sulphuric acid reagent (1·3 ml. of a solution containing 240 g. of chromium trioxide and 230 ml. of concentrated sulphuric acid, diluted to 1 l.), and the mixture was poured into water (200 ml.). The acetone was removed under reduced pressure and the precipitated solids were collected and purified from acetone–hexane. 4-*Methylandrost*-4-*ene*-3,6,17-*trione* formed prisms, m. p. 171—173°, $[\alpha]_D^{24}$ +128° (*c* 0·45), $\lambda_{max.}$ 257 mμ (ε 10,970),[7] $\nu_{max.}$ (in CCl_4) 1746 and 1688 cm.$^{-1}$ (Found: C, 76·4; H, 8·1. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3%).

Dehydration of 7-*Hydroxy*-4-*methyltestosterones* (IIa and b).—The 7-hydroxy-compound (250 mg. of IIa or b) in methanol (10 ml.) containing concentrated hydrochloric acid (3 drops) or potassium hydroxide (50 mg.) was heated under reflux for 3 hr., and the solvent was removed

under reduced pressure. Purification from acetone–hexane gave 17β-hydroxy-4-methylandrosta-4,6-dien-3-one in each case.

Acetylation of 7β-*Hydroxy*-4-*methyltestosterone*.—Acetylation in acetic anhydride–pyridine (1 : 1) at 90° for 30 min. gave an amorphous product which was chromatographed on alumina (Brockmann Grade II). Elution with benzene and benzene–ether gave 17β-*acetoxy*-4-*methylandrosta*-4,6-*dien*-3-*one* which separated from aqueous methanol in prisms, m. p. 154—155°, $[\alpha]_D^{24}$ +87° (*c* 0·11), $\lambda_{max.}$ 289 mμ (ε 29,180) (Found: C, 76·8; H, 8·85. $C_{22}H_{30}O_3$ requires C, 77·15; H, 8·8%).

Preparation of 17β-*Acetoxy*-4-*methylandrosta*-4,6-*dien*-3-*one from* 4-*methyltestosterone Acetate*.—(a) *Dehydrogenation with chloranil*. 4-Methyltestosterone acetate (50 g.) and chloranil (40 g.) in isobutyl alcohol (500 ml.) were heated under reflux in nitrogen for 8 hr. The dark solution was poured into water (2 l.) containing potassium hydroxide (50 g.), and the product was extracted with ether–benzene (1 : 1) which was washed, dried (Na_2SO_4), and evaporated. The residual gum was treated with acetic anhydride (50 ml.) and pyridine (50 ml.) for ½ hr. at 90°, then the mixture was poured into water. The product was isolated with benzene, dissolved in hexane (charcoal), and after concentration of the solution obtained as pale yellow prisms, m. p. 146—150°. Recrystallisation from aqueous methanol gave pure 17β-acetoxy-4-methylandrosta-4,6-dien-3-one, identical with the sample described above.

(b) *Dehydrogenation by bromosuccinimide* (by Dr. B. Ellis). 4-Methyltestosterone acetate (5 g.) and *N*-bromosuccinimide (2·7 g.) in freshly distilled carbon tetrachloride (50 ml.) were heated under reflux for 40 min. The mixture was cooled and filtered, and the solvent removed *in vacuo*. Trituration of the residue with hexane gave the 6-bromo-compound [4·4 g.; m. p. 149—150° (decomp.)] which was treated directly with boiling collidine (10 ml.) for 5 min. The cooled mixture was poured into water and the product was isolated with ether and purified from aqueous ethanol.

(c) *Dehydrogenation by bromine*. 3,17β-Diacetoxy-4-methylandrosta-3,5-diene (IV) (20 g.) in acetic acid (150 ml.) containing anhydrous sodium acetate (5 g.) was treated dropwise with bromine in acetic acid (50 ml.; 1·09M-solution; 1·04 mol.). Decolorisation was almost instantaneous. The solution was diluted with water, to precipitate the 6-bromo-compound which was collected, washed, and dried at 30° *in vacuo*. Dehydrobromination of this material as in (b) above gave an identical product.

Oxidation of 7α *and* 7β-*Hydroxy*-4-*methyltestosterone* (IIa and b).— (*a*) Oxidation of either isomer with chromic acid–sulphuric acid in acetone according to the procedure used for 6β-hydroxy-4-methyltestosterone (above) gave a *product* which was purified from acetone and formed needles, m. p. 213—214°, $[\alpha]_D^{19}$ +12° (*c* 0·34) $\lambda_{max.}$ 244 mμ ($E_{1\,cm.}^{1\%}$ 292), $\lambda_{infl.}$ 269 mμ ($E_{1\,cm.}^{1\%}$ 94), $\nu_{max.}$ (in Nujol) 1746, 1695, 1669, and 1587 cm.$^{-1}$. A satisfactory analysis could not be obtained, but the physical data suggest that the product is 4-methylandrost-4-ene-3,7,17-trione.

(*b*) The 7-hydroxy-compound (either isomer) (1 part) in anhydrous pyridine (10 parts) was oxidised with chromium trioxide (1 part) in pyridine (10 parts) for 24 hr. at room temperature. The mixture was diluted with warm benzene and filtered, the filtrate was washed with dilute sulphuric acid and water and stirred with charcoal, and the solvent was evaporated. Purification from acetone gave a product identical with that obtained as under (*a*) (mixed m. p. and infrared spectra).

Preparation of 7α-*Hydroxy*-4-*methyltestosterone* (IIa).—17β-Acetoxy-4-methylandrosta-4,6-dien-3-one (5 g.) in chloroform (25 ml.) was left with monoperphthalic acid (8·5 g.) in ether (80 ml.) at 20—25° for 48 hr., then poured into dilute sodium hydrogen carbonate solution, the organic layer was washed until neutral and dried, and the solvents were evaporated. Purification of the residue from methanol gave 17β-*acetoxy*-6α,7α-*epoxymethylandrost*-4-*en*-3-*one*, needles, m. p. 182—84°, or plates, m. p. 152—154° $[\alpha]_D^{25}$ +85° (*c* 0·41), $\lambda_{max.}$ 248 mμ (ε 13,766), $\nu_{max.}$ (in CCl_4) 1742, 1678, 1617, and 1422 cm.$^{-1}$, (in CS_2) 1243, 898, and 818 cm.$^{-1}$ (Found: C, 73·6; H, 8·4. $C_{22}H_{30}O_4$ requires C, 73·7; H, 8·4%).

The 6α,7α-epoxide (2·46 g.) in tetrahydrofuran (50 ml.); (freshly distilled over sodium) was treated with lithium aluminium hydride (2 g.) under reflux with stirring for 4 hr. After decomposition of excess of reagent with ethyl acetate (10 ml.) in ether (100 ml.) the mixture was poured into a saturated solution of potassium sodium tartrate, and the product was extracted with chloroform. The crude 3,7α,17β-triol was stirred with 2,3-dichloro-5,6-dicyanobenzoquinone (2 g.) in anhydrous dioxan (25 ml.) for 1 hr., then left overnight. The resulting mixture

was poured into saturated sodium hydrogen carbonate solution, and the steroid extracted with methylene chloride and purified from acetone–hexane, to give 7α-hydroxy-4-methyltestosterone, identical with fermentation product C.

Oxidation of 11α-*Hydroxy*-4-*methyltestosterone* (III).—The oxidation was carried out with chromium trioxide–pyridine as described for the 7-hydroxy-compounds. The crude product (m. p. 146—180°) was shown by paper chromatography to be a mixture. Chromatography on alumina (Brockmann Grade III), and elution with benzene and benzene–ether (3 : 1) gave 4-*methylandrost*-4-*ene*-3,11,17-*trione* (4-*methyladrenosterone*), needles (from acetone–hexane), m. p. 166—168°, $[\alpha]_D^{24}$ +311° (*c* 0·66), $\lambda_{max.}$ 247 mμ (ε 13,645), $\nu_{max.}$ (in CCl_4) 1749, 1714, 1673, and 1610 cm.$^{-1}$ (Found: C, 76·0; H, 8·4. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·4%). Elution with benzene–ether (1 : 1) gave a small quantity of a compound believed to be 17β-*hydroxy*-4-*methylandrost*-4-*ene*-3,11-*dione*. It separated from acetone–hexane as prisms, m. p. 228—230°, $[\alpha]_D^{24}$ +241° (*c* 0·64), $\lambda_{max.}$ 248 mμ (ε 16,650), $\nu_{max.}$ (in Nujol) 3390, 1699, 1649, and 1608 cm.$^{-1}$ (determined on a Perkin–Elmer " Infracord ") (Found: C, 75·7; H, 8·9. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%).

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