in vacuo and the residue, **2-ethylhexanol-1-C^{14}**, was directly acetylated with 6.0 g. (0.77 mole) of acetyl chloride and 30 ml. of pyridine by a procedure described previously.[19] The product, **2-ethylhexyl acetate-1-C^{14}**, was purified by distillation: b.p. 94.5° (21 mm.). The acetate was dissolved in about 4 ml. of 3-methylheptane and pyrolyzed at 550° as described previously,[11] yielding **3-methyl-C^{14}-heptenes**. Hydrogenation of the olefins at room temperature with 5% palladium-on-charcoal catalyst was done in a Parr hydrogenation apparatus. The 3-methyl-C^{14}-heptane weighed 6.8 g. and was over 99% pure by v.p.c. The over-all yield based on starting barium carbonate activity was 12%.

4-Methyl-C^{14}-heptane.—The reaction between *n*-propylmagnesium bromide (0.6 mole) and *n*-butyraldehyde (36 g., 0.65 mole) gave 48.4 g. (yield 83.5%) of **4-heptanol**, of over 99% purity, b.p. 72–74° (26 mm.). **4-Heptyl bromide** was made from 4-heptanol *via* the tosylate as described above for 3-heptyl bromide. The yield, starting with 29 g. (0.25 mole) of 4-heptanol, was 63.8% (28.5 g.), b.p. 64° (26 mm.). The procedure used to prepare the 4-methyl-C^{14}-heptane from 4-heptyl bromide starting with 10 mc. of barium carbonate-C^{14} was the same as described for the preparation of 3-methyl-C^{14}-heptane from 3-heptyl bromide. The hydrocarbon, which was prepared in an over-all yield of 41.6% based on the 10 mc. of barium carbonate used, was over 99% pure by v.p.c.

Catalyst.—The chromia–alumina catalyst was made according to the procedure described previously.[20] The alumina was precipitated from sodium aluminate and impregnated with chromic acid. The catalyst contained 13.8 wt. % of Cr_2O_3, its surface area was 89 $m.^2$/g., and the average pellet weight was 0.022 g.

Apparatus and Procedure.—The apparatus and procedure used were the same as those described previously.[9]

Separation of the Aromatic Hydrocarbons from the Dehydrogenation Products.—The separation of the aromatic hydrocarbons from the aromatization of 4-methyl-C^{14}-heptane was accomplished using an F & M Model 300 programmed-temperature gas chromatograph[21] with an 11 mm. × 2.5 m. preparative v.p.c. column filled with a 5% 7,8-benzoquinoline on 30–60-mesh Chromosorb. The column temperature was 75° with a helium flow of 100 cc./min. The sample recovery was the same as described previously.[7] The separation of the aromatics from 3-methyl-C^{14}-heptane was done using two separate preparative gas chromatography columns. First, a 7,8-benzoquinoline preparative column, as described above, was used to separate toluene and *o*-xylene in pure form. The ethylbenzene and *m*- and *p*-xylene, collected in one fraction, were separated using a Wilkens Autoprep gas chromatograph[22] with a $^3/_8$ in. × 20 ft. column with 5% SE-52 and 15% bentone clay on 60–80-mesh Chromosorb W. The column temperature was 80° and a helium flow of 40 cc./min. Sample injection sizes were 100 μl. The ethylbenzene was diluted with inactive ethylbenzene and the specific activity was determined. The loss in activity when the ethylbenzene is oxidized to benzoic acid corresponds to the activity on the β-carbon of the side chain.

Oxidation of the Aromatics.—After separation, the aromatics were diluted from 5 to 25 times with their corresponding inactive aromatic compounds and oxidized to their respective acids with hot alkaline potassium permanganate as described previously.[7]

Radiochemical Assay.—The radiochemical assay of the resulting aromatic acids and their decarboxylation products was the same as that reported previously[23] (Tables V and VI).

Acknowledgment.—The authors wish to acknowledge the help given by Mr. Miron Abramovici and his valuable assistance during part of the radiochemical assay work.

(20) H. Pines and C. T. Chen, *J. Am. Chem. Soc.*, **82**, 3562 (1960).

(21) F & M Scientific Corp., Avondale, Pa.

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Microbiological Hydroxylation of Saturated 17-Keto Steroids[1]

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Penicillium sp. ATCC 12,556 was previously shown to hydroxylate 3α-hydroxy-5β-androstan-17-one in the 7β position. Fermentation of 3α-hydroxy-5α-androstan-17-one with this same mold afforded the 12β-hydroxylated derivative as the principal product, whereas the 3β-OH,5α-H epimer was hydroxylated at 1α. 3β-Hydroxy-5β-androstan-17-one yielded two products, 3β,7β-dihydroxy-5β-androstan-17-one and 7β-hydroxy-5β-androstane-3,17-dione.

3α,7β-Dihydroxy-5β-androstan-17-one (7β-hydroxyetiocholanolone)[3] has recently been isolated from human urine and established as a metabolite of testosterone as well as of etiocholanolone.[4] The new metabolite was prepared by the microbiological hydroxylation of etiocholanolone with *Penicillium* sp. ATCC 12,556 and found to be identical with the urinary steroid. It was therefore of interest to study the site of hydroxylation by this organism of other saturated 17-keto steroid metabolites of testosterone, *i.e.*, androsterone, isoandrosterone, and 3β-hydroxy-5β-androstan-17-one.

Fermentation of the saturated steroids was carried out according to the procedure reported earlier.[4,5] It was expected that incubation of androsterone (I) with *Penicillium* sp. ATCC 12,556 would yield the 1α-hydroxylated derivative since Dodson and coworkers[5] have reported 1α-hydroxylation of 5α-androstane-3,17-dione and dehydroisoandrosterone with this organism. However, the principal product IIa isolated from the fermentation of androsterone did not appear to be the expected compound. Molecular rotation differences, determined for the dihydroxy ketone IIa (ΔM_D OH = −62) and its diacetate IIb (ΔM_D OAc = −107), were incompatible with 1α-hydroxyandrosterone although consistent with a 1β-hydroxy derivative.[6] Oxidation of IIa to a triketone III (Scheme I) gave a different product from that obtained by oxidation of 1α-hydroxy-5α-androstane-3,17-dione, a compound which had been prepared earlier from 5α-

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(2) On leave from Takeda Chemical Industries, Ltd., Osaka, Japan, 1964–1965.

(3) The following trivial names have been used: 7β-hydroxyetiocholanolone, 3α,7β-dihydroxy-5β-androstan-17-one; etiocholanolone, 3α-hydroxy-5β-androstan-17-one; testosterone, 17β-hydroxy-Δ^4-androsten-3-one; androsterone, 3α-hydroxy-5α-androstan-17-one; isoandrosterone, 3β-hydroxy-5α-androstan-17-one; dehydroisoandrosterone, 3β-hydroxy-Δ^5-androsten-17-one.

(4) D. K. Fukushima, *J. Biol. Chem.*, **239**, 1748 (1964).

(5) R. M. Dodson, A. H. Goldkamp, and R. D. Muir, *J. Am. Chem. Soc.*, **82**, 4026 (1960).

(6) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959.

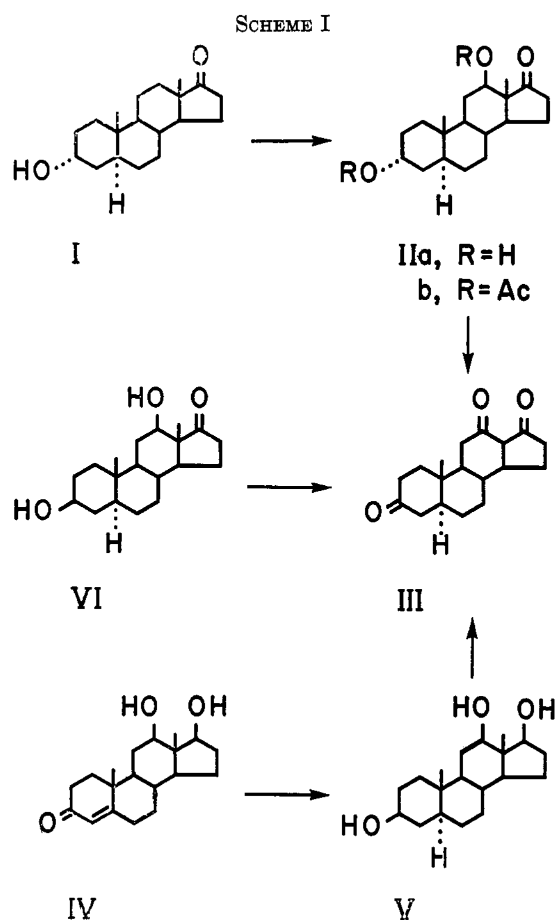
Table I

Molecular Rotatory Contributions of C-12 Substituents

Steroids	M_D[a]	ΔM_D (12-H → OR) R = H α	R = H β	R = Ac α	R = Ac β	Ref.
3α-Hydroxy-5α-androstan-17-one	+264					b
3α,12β-Dihydroxy-5α-androstan-17-one (IIa)	+202		−62			c
3β-Hydroxy-5α-androstan-17-one	+261					b
3β,12α-Dihydroxy-5α-androstan-17-one	+450	+189				7
3β,12β-Dihydroxy-5α-androstan-17-one	+193		−68			7, 10
3α-Acetoxy-5α-androstan-17-one	+302					b
3α,12β-Diacetoxy-5α-androstan-17-one (IIb)	+195				−107	c
3β-Acetoxy-5α-androstan-17-one	+219					b
3β-Acetoxy-12α-hydroxy-5α-androstan-17-one	+421	+202				10
3β,12α-Diacetoxy-5α-androstan-17-one	+534			+315		7, 10
3β,12β-Diacetoxy-5α-androstan-17-one	+101				−118	b
Fieser and Fieser		+93	+50	+280	+76	6

[a] Chloroform solution. [b] Determined in this laboratory. [c] Present investigation.

Scheme I



androstane-3,17-dione with *Penicillium* sp.[5] In fact, triketone III was not identical with any oxidation product of a hydroxylated androsterone predicted from molecular rotation differences.[6] However the melting point and specific rotation were similar to those of 5α-androstane-3,12,17-trione (III) prepared by Adams and coworkers from hecogenin.[7] The n.m.r. spectrum of the triketone afforded evidence that the new oxygen function was at C-12. The values of 0.35 and 0.08 p.p.m. for the shielding of the C-18 and C-19 methyl protons were in agreement with those of 0.34 and 0.10 p.p.m. for the C-12 carbonyl group.[8] The identity of the triketone obtained from the mold metabolite with 5α-androstane-3,12,17-trione (III) was established by partial synthesis of an authentic sample. 12β-Hydroxytestosterone,[9] 12β,17β-dihydroxy-Δ^4-androsten-3-one (IV) was reduced with lithium and ammonia to 5α-androstane-3β,12β,17β-triol (V) which was then oxidized to 5α-androstane-3,12,17-trione (III) with Jones reagent. 3β,12β-Dihydroxy-5α-androstan-17-one (VI) was recently prepared by Nambara.[10] Oxidation of this dihydroxy ketone also afforded triketone III. The newly introduced function is therefore at C-12 and the product of microbiological hydroxylation of androsterone is 3α,12-dihydroxy-5α-androstan-17-one.

Nuclear magnetic resonance (n.m.r.) spectrometry is a powerful tool, for the assignment of orientation of hydroxyl groups on the steroid nucleus. However, in the present instance an unambiguous assignment of the orientation of the C-12 hydroxyl group could not be made since the values reported[11−13] for the effect on the chemical shifts of the C-18 and C-19 methyl protons by the C-12 acetoxyl and hydroxyl groups were inconsistent. The orientation of the C-12 oxygen function in IIa and IIb was therefore assigned by molecular rotation difference (Table I). Comparison of molecular rotatory contributions of the C-12 hydroxyl (ΔM_D −62) and the acetoxyl (ΔM_D −107) groups was made with the values obtained from the C-12 substituted derivatives of the epimeric 3β-hydroxy-17-keto steroid.[14] The values are in good agreement with the 12β epimers and the metabolite is therefore 3α,12β-dihydroxy-5α-androstan-17-one (IIa).

Fermentation of the C-3 epimer of etiocholanolone (3β-hydroxy-5β-androstan-17-one, VII) with *Penicillium* sp. ATCC 12,556 afforded two main products. The less polar proved to be 7β-hydroxy-5β-androstane-3,17-dione (VIIIa), a compound previously prepared

(7) W. J. Adams, D. N. Kirk, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J. Chem. Soc.*, 2209, 2298 (1954); 871 (1955).

(8) A. J. Cohen and S. Rock, *Steroids*, **3**, 243 (1964).

(9) The authors are grateful to Dr. Stephen Kraychy, G. D. Searle and Co., Chicago, Ill., for a generous supply of this steroid.

(10) T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **12**, 1253 (1964).

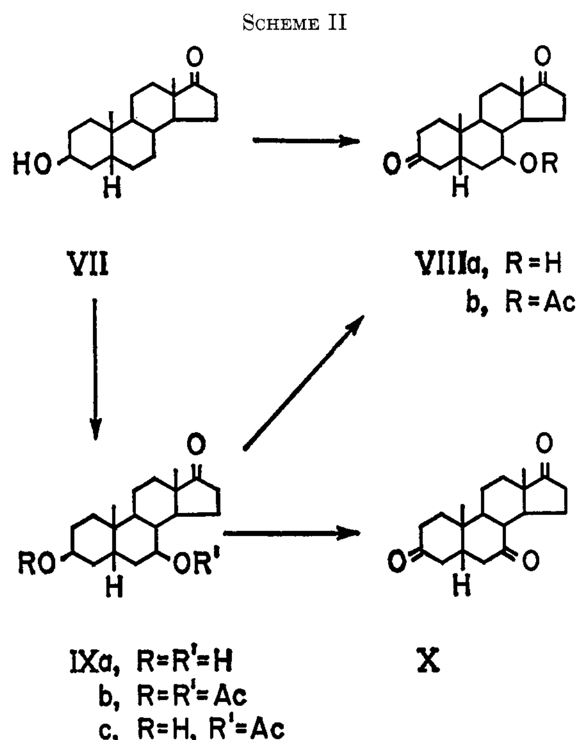
(11) R. F. Zurcher, *Helv. Chim. Acta*, **44**, 1380 (1961); **46**, 2054 (1963).

(12) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964.

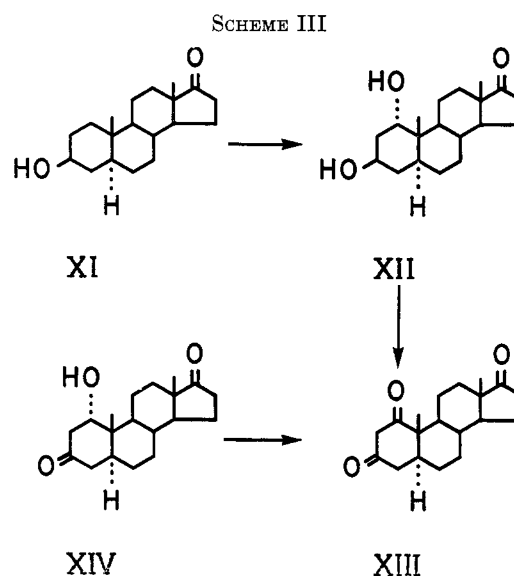
(13) L. L. Smith, *Steroids*, **4**, 395 (1964).

(14) Disagreement of the values obtained from the 17-keto steroids with those reported by Fieser and Fieser (Table I) must be due to the presence of the 17-keto group. The values reported by these investigators were derived principally from steroids with a side chain at C-17, *i.e.*, bile acids, sapogenins, and their C_2 degradation products. Derivatives of testosterone[15] and cardenolides [M. Schüpbach and Ch. Tamm, *Helv. Chim. Acta*, **47**, 2217, 2226 (1964)] afforded molecular rotatory contribution values in agreement with Fieser and Fieser.

SCHEME II



SCHEME III



chemically from 3α,7β-dihydroxy-5β-androstan-17-one.[4] The other product was 3β,7β-dihydroxy-5β-androstan-17-one (IXa), characterized by oxidation to 5β-androstane-3,7,17-trione (X) (Scheme II). Acetylation of the dihydroxy ketone IXa followed by partial saponification of the 3-acetoxyl group afforded IXc. Oxidation of this monohydroxy compound with Jones reagent yielded 7β-acetoxy-5β-androstane-3,17-dione (VIIIb). The latter was identical with the acetoxy diketone previously synthesized from 3α,7β-dihydroxy-5β-androstan-17-one and 7β-hydroxy-Δ^4-androstene-3,17-dione.[4] Oxidation of a hydroxyl group by *Penicillium* sp. ATCC 12,556 has been observed in only one other compound; Dodson and co-workers isolated 6β-hydroxy-Δ^4-pregnene-3,15,20-trione following incubation of progesterone.[15]

Since etiocholanolone and its 3β epimer were hydroxylated in the same position, it was interesting to determine whether the hydroxylation of the 3β epimer of androsterone would occur in the same site as the 3α derivative. This proved not to be the case; fermentation of 3β-hydroxy-5α-androstan-17-one (XI) afforded 1α,3β-dihydroxy-5α-androstan-17-one (XII) as the main product (Scheme III). The physical constants were identical with those of the minor product in the fermentation of 5α-androstane-3,17-dione.[5] Oxidation of the mold metabolite gave 5α-androstane-1,3,17-trione (XIII) which was also prepared from 1α-hydroxy-5α-androstane-3,17-dione (XIV), the major hydroxylation product of 5α-androstane-3,17-dione. The triketone had the expected absorption at 254 mμ characteristic of a β diketone. The crystalline triketone could not be characterized by melting point or infrared spectrum in potassium bromide dispersion which differed from sample to sample. The infrared spectrum of different samples were identical in carbon tetrachloride solution. These effects on the physical properties are the result of the presence of variable amounts of the enolic forms achieved in the crystallization process.

It has been shown in recent years that steroid sulfates can be transformed by mammalian tissues without rupture of the ester.[16] Fermentation of sodium etiocholanolone sulfate was therefore studied under conditions employed for the microbiological oxidations of the unesterified steroid; little or no hydroxylation was observed and the substrate was recovered essentially unchanged.

Since 5β-saturated-17-keto steroids were good substrates for introduction of a 7β-hydroxyl function, it seemed of value to ascertain whether the same reaction would take place with a typical bile acid. Ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanic acid, is rare in nature and its synthetic production from readily available cholic acid requires many steps. Lithocholic acid and the methyl ester were fermented with *Penicillium* sp. ATCC 12,556, but despite repeated attempts there was no evidence of any substantial hydroxylation of either substrate.

Experimental Section[17]

The fermentation procedure followed that previously reported.[4,5] A sterile mixture of 6 g. of glucose, 1.25 g. of cottonseed flour, 30 ml. of corn steep liquor, 10 g. of potassium dihydrogen phosphate, 0.6 g. of antifoam AF emulsion, and 8 l. of tap water inoculated with *Penicillium* sp. ATCC 12,556 was incubated at 25° with aeration and agitation for 24–40 hr. until good growth was established. A solution of 1 g. of steroid in 60 ml. of acetone was added and the fermentation was continued for an additional 24 hr. The mixture was extracted with methylene chloride and the organic layer was washed with water and dried. Evaporation of the solvent afforded the hydroxylated mixture.

A small portion of the extract was chromatographed on a thin layer of silica gel GF with ethyl acetate–cyclohexane (7:3) and stained with Zimmerman reagent. In all cases there was a number of products more polar than the starting material. However, there was usually only one product which was present

(15) R. C. Tweit, R. M. Dodson, and R. D. Muir, *J. Org. Chem.*, **27**, 3654 (1962).

(16) K. D. Roberts, L. Bandi, H. I. Calvin, W. D. Drucker, and S. Lieberman, *Biochemistry*, **3**, 1983 (1964).

(17) Melting points were taken on a micro hot stage and are corrected. Optical rotations were determined in chloroform unless otherwise stated. N.m.r. spectra were determined in deuteriochloroform on a Varian A-60 spectrometer using tetramethylsilane as internal reference. Infrared spectra were determined on a Beckman IR-9 spectrophotometer; br = broad, vs = very strong.

in the extract in large amount other than the 17-keto steroid incubated as judged by the size and intensity of the stained area. The mobility (R_f) of this compound was noted and the remainder of the extract was chromatographed on a column of silica gel for the isolation of this product.

Hydroxylation of Androsterone (I).—The extract from the fermentation of 1.0 g. of androsterone was chromatographed on 80 g. of silica gel. Elution with ethyl acetate–benzene (2:5) afforded fractions (400 mg.) containing the principal hydroxylation product (R_f 0.25) and small amounts of other material. Recrystallizations from ethyl acetate and acetone yielded 128 mg. of 3α,12β-dihydroxy-5α-androstan-17-one (IIa), m.p. 210–214°. The analytical sample had m.p. 213–214°; $[\alpha]^{25}D$ +65.6°, +74.1° (ethanol); ν_{max}^{KBr} 3520, 3422, 1720, 1407, 1095, 1057, 998, and 905 cm.$^{-1}$; n.m.r., 0.93 and 0.80 p.p.m. (18 and 19 CH_3).

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.72; H, 9.90. Found: C, 74.85; H, 9.72.

Acetylation with acetic anhydride and pyridine at room temperature afforded 3α,12β-diacetoxy-5α-androstan-17-one (IIb): m.p. 187–189°; $[\alpha]^{24}D$ +49.8°; ν_{max}^{KBr} 1748, 1738, 1724, 1410, 1245, 1161, and 1026 cm.$^{-1}$; n.m.r., 0.99 and 0.84 p.p.m. (18 and 19 CH_3); R_f 0.62 on a thin layer of silica gel GF with ethyl acetate–cyclohexane (7:3).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.71; H, 8.69.

5α-Androstane-3,12,17-trione (III).—A solution of 30 mg. of 3α,12β-dihydroxy-5α-androstan-17-one (IIa) in 10 ml. of acetone was oxidized with 0.1 ml. of Jones reagent under nitrogen atmosphere for 3 min. Recrystallization of the product from ethyl acetate yielded 20 mg. of 5α-androstane-3,12,17-trione (III): m.p. 178–181°; $[\alpha]^{24}D$ +231°; ν_{max}^{KBr} 1757, 1719, 1706, 1420, 1415, 1404, and 1016 cm.$^{-1}$; n.m.r., 1.18 and 1.11 p.p.m. (18 and 19 CH_3); R_f 0.39 on thin layer of silica gel GF with ethyl acetate. Adams and coworkers[7] have reported m.p. 179–181°, $[\alpha]^{19.5}D$ +227°, for III.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.57; H, 8.83.

To a solution of lithium in 10 ml. of liquid ammonia was added a solution of 50 mg. of **12β,17β-dihydroxy-Δ⁴-androsten-3-one** (IV)[9] in 2 ml. of tetrahydrofuran–ether (1:1). The reaction mixture was stirred for 30 min., 2 ml. of methanol was added, and the solution was stirred for an additional hour. Ammonia was removed and the residue extracted with ethyl acetate. The reduction product was recrystallized from acetone to yield 18 mg. of **5α-androstane-3β,12β,17β-triol** (V): m.p. 226–233°; R_f 0.31 on thin layer of silica gel GF with ethyl acetate; the infrared spectrum was consistent for the triol. Without further characterization, a solution of 10 mg. of 5α-androstane-3β,12β,17β-triol (V) in 2 ml. of acetone was oxidized with Jones reagent. The product was purified on a thin layer of silica gel GF with ethyl acetate to give 8 mg. of 5α-androstane-3,12,17-trione (III), m.p. 156–162°. Recrystallization from methylene chloride–cyclohexane afforded a product of m.p. 163–164°, 178–181°; the infrared spectrum was identical with that of the trione obtained above.

A solution of 3 mg. of **3β,12β-dihydroxy-5α-androstan-17-one** (VI)[18] in 0.5 ml. of acetone was oxidized with Jones reagent. The oxidation product was recrystallized from methylene chloride–cyclohexane to give 5α-androstane-3,12,17-trione (III), m.p. 165°; the infrared spectrum was identical with that of the trione obtained above.

Hydroxylation of 3β-Hydroxy-5β-androstan-17-one (VII).—The extract from the fermentation of 1.0 g. of 3β-hydroxy-5β-androstan-17-one was chromatographed on 700 g. of silica gel. Elution with ethyl acetate yielded 90 mg. of 7β-hydroxy-5β-androstane-3,17-dione (VIIIa), R_f 0.22. Recrystallization from ethyl acetate–cyclohexane afforded 56 mg. of hydroxy diketone VIIIa: m.p. 175–176°, $[\alpha]^{24}D$ +145°; lit.[4] m.p. 170–171°, $[\alpha]^{26}D$ +148°. The infrared spectrum in potassium bromide dispersion was identical with that of the authentic sample.

Further elution with ethyl acetate yielded fractions (98 mg.) containing 3β,7β-dihydroxy-5β-androstan-17-one (IXa), R_f 0.10. Rechromatography on silica gel and trituration with ethyl acetate yielded 44 mg. of amorphous 3β,7β-dihydroxy-5β-androstan-17-one: $[\alpha]^{25}D$ +97.8°; ν_{max}^{KBr} 3430 (br), 1737, 1406, 1037, 959, 788 (vs), and 768 (vs) cm.$^{-1}$.

A solution of 5 mg. of dihydroxy ketone (IXa) in 0.5 ml. of acetone was oxidized with Jones reagent. The product was recrystallized from acetone to give 3 mg. of 5β-androstane-3,7,17-trione (X), m.p. 221–222°, lit.[4] m.p. 223–223.5°. The infrared spectrum in potassium bromide dispersion and the mobility, R_f 0.42, on a thin layer of silica gel GF with ethyl acetate–cyclohexane (7:3) were identical with those of the authentic sample.

Acetylation of 5 mg. of dihydroxy ketone IXa with pyridine and acetic anhydride at room temperature overnight afforded 4.5 mg. of 3β,7β-diacetoxy-5β-androstan-17-one (IXb) which could not be crystallized, R_f 0.60 on silica gel GF with ethyl acetate–cyclohexane (7:3). The diacetate ketone IXb in 1.9 ml. of a solution containing 98 mg. of sodium hydroxide in 261 ml. of ethanol and 79 ml. of water was stored at room temperature for 50 hr. The mixture of the diacetate IXb and a monoacetate, R_f 0.25 on silica gel GF with ethyl acetate–cyclohexane (7:3), was separated by thin layer chromatography to give 1.5 mg. of 3β-hydroxy-7β-acetoxy-5β-androstan-17-one (IXc). Oxidation with Jones reagent afforded 7β-acetoxy-5β-androstane-3,17-dione (VIIIb); the infrared spectrum in potassium bromide dispersion was identical with that of an authentic sample.

Hydroxylation of Isoandrosterone (XI).—The extract from the fermentation of 1.0 g. of isoandrosterone was chromatographed on 700 g. of silica gel. Elution with ethyl acetate afforded 126 mg. of 1α,3β-dihydroxy-5α-androstan-17-one (XII). Recrystallization from methanol–ethyl acetate yielded diolone XII: m.p. 200–202°; $[\alpha]^{25}D$ +93.5°; ν_{max}^{KBr} 3500, 3420, 1730, 1407, and 1026 cm.$^{-1}$; R_f 0.16 on thin layer of silica gel GF with ethyl acetate–cyclohexane (7:3); Dodson and co-workers[5] reported m.p. 202–203.5°, $[\alpha]D$ +90°.

5α-Androstane-1,3,17-trione (XIII).—A solution of 10 mg. of 1α,3β-dihydroxy-5α-androstan-17-one (XII) in 1 ml. of acetone was oxidized with Jones reagent. The oxidation product was recrystallized from methylene chloride–cyclohexane to give 5α-androstane-1,3,17-trione (XIII): m.p. 157–159°, 199–200°; $\lambda_{max}^{ethanol}$ 254 mμ (ε 12,000); $\nu_{max}^{CS_2,CCl_4}$ 1744, 1710, indefinite absorption 1425–1407, 1042, and 786 cm.$^{-1}$; R_f 0.29 on a thin layer of silica gel GF with ethyl acetate–cyclohexane (7:3).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.33; H, 8.56.

A solution of 10 mg. of 1α-hydroxy-5α-androstane-3,17-dione (XIV) in 1 ml. of acetone was oxidized with Jones reagent. Recrystallization from methylene chloride–cyclohexane yielded the 1,3-diketone XIII, m.p. 160–180°, $\lambda_{max}^{ethanol}$ 254 mμ (ε 12,000). The infrared spectrum in carbon tetrachloride solution was identical with that of the trione prepared above.

Hydroxylation of Sodium Etiocholanolone Sulfate.—The fermentation was carried out on one-fourth of the amounts of the general description using 300 mg. of sodium etiocholanolone sulfate which was dissolved in 20 ml. of ethanol–water (1:1). After 40-hr. fermentation, the mixture was filtered through glasswool, the pH was adjusted to 9, and the mixture was extracted three times with 1 l. of 1-butanol. Examination of the extract by chromatography on a thin layer of silica gel GF with chloroform–methanol (1:1) saturated with water and staining with Zimmerman reagent showed that the etiocholanolone sulfate was recovered unchanged. No other Zimmerman-staining material was observed. Solvolysis of a portion of the extract with ethyl acetate saturated with 1 *N* sulfuric acid yielded only etiocholanolone as judged by thin layer chromatography.

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