acid (XIV). The analytical sample was purified by four recrystallizations from acetone: m.p. 192–194° [14]; t.l.c., ethyl acetate–acetone–methanol (3:3:1). Mixture melting point and infrared (KBr) were coincident with an authentic specimen.

Anal. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 73.67; H, 9.98.

Soluble Sodium Salt.—The alkaline aqueous phase containing the soluble sodium salts was not studied.

B. Neutral Part.—The ether layer, after removal of the acidic material and the insoluble sodium salt, was washed with water, dried, and evaporated. The residue was checked by t.l.c. (chloroform), after spraying with a 2% solution of cerium sulfate in 2 *N* sulfuric acid, and showed an intensely blue colored spot. All of the neutral crude material (*ca.* 50 mg.) was subjected to preparative thin layer chromatography (chloroform) and the material corresponding to the blue spot was eluted with acetone. It afforded 6 mg. of 3β-acetoxyandrostan-17-one (XIII), m.p. 89–92°,[15] melting point and infrared spectrum coincident with an authentic specimen.

Methyl 3β-Acetoxybisnorallocholanate (XIVa).—An ether solution of diazomethane was added to an ice-cold suspension of 3β-acetoxybisnorallocholanic acid (XIV, 21 mg.) and allowed to stand at room temperature for 45 min. After removal of the solvent at reduced pressure, the residue was dissolved in methanol, shaken with Norit, and filtered. After partial evaporation of the solvent, 100 mg. of crystalline (plates) methyl 3β-acetoxybisnorallocholanate was obtained, m.p. 128–130°[14]; the t.l.c. (benzene–ethyl acetate, 9:1), melting point, and infrared spectrum were coincident with an authentic specimen.[14]

Acknowledgment.—We are indebted to Mr. Fernando Diaz, Miss Liliana Vaccaro, and Miss Sonia Martinez of the Catholic University of Santiago, Chile, for providing us with the crude tomatillidine.

Transformation of Progesterone and Related Steroids by *Aspergillus tamarii*

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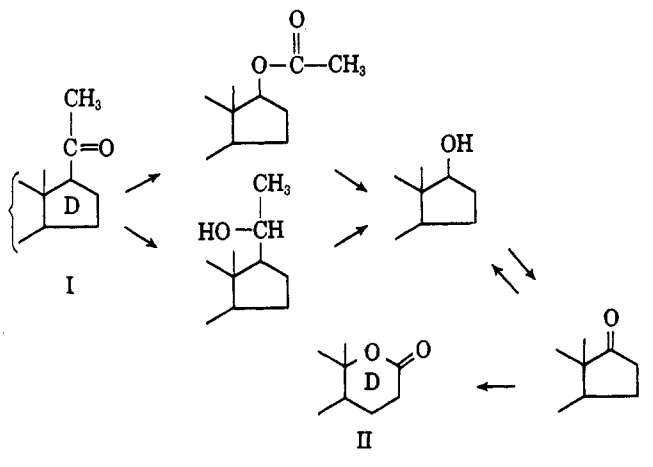
Progesterone is readily converted into testololactone and 11β-hydroxytestosterone by the mold *Aspergillus tamarii*. 11β-Hydroxytestosterone is an end product and is not converted into testololactone. 11β- and 11α-hydroxyprogesterone are transformed into 11β- and 11α-hydroxytestosterone, respectively, but not into the corresponding testololactone. Δ⁴-Androsten-11β-ol-3,17-dione was unaffected by *A. tamarii*, but Δ⁴-androstene-3,17-dione and testosterone were transformed into testololactone. Unlike related microbiological transformations, hydroxylation at C-11 inhibits oxidative cleavage of ring D by *A. tamarii*. Furthermore, this is the first report that an *Aspergillus* sp. of any kind produces 11β-hydroxylation of steroids.

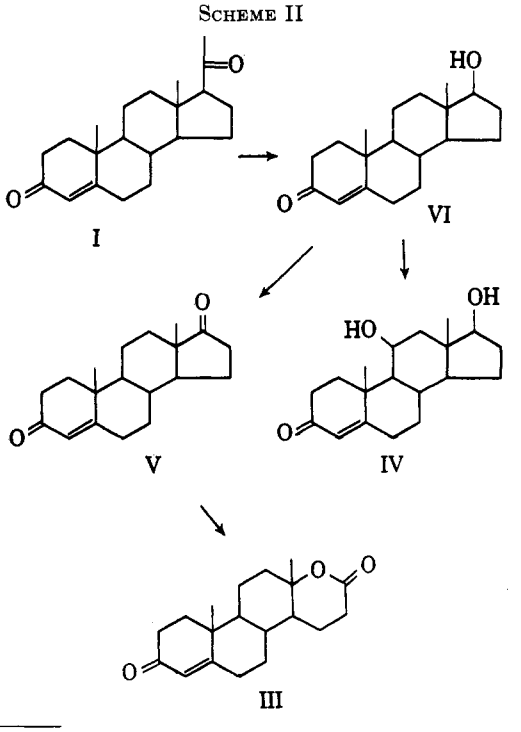
A number of microorganisms are known[1–4] to stereospecifically degrade the C-17 β-acetyl side chain of progesterone (I) and related compounds to give D-ring lactones (II), and the present evidence supports the pathway[4–7] outlined in Scheme I. Another well-known microbiological transformation is C-11 hydroxylation, undoubtedly the most important of the chemical changes carried out by microorganisms.[8]

SCHEME I



We have found that *Aspergillus tamarii* transforms progesterone by two independent paths. The major pathway leads to the end product testololactone (III), which is produced at an early stage *via* the intermediates testosterone (VI) and Δ⁴-androstene-3,17-dione (V) as previously postulated (see Scheme II). The second path leads to the end product 11β-hydroxytestosterone (IV). These findings differ from a previous report[9]

SCHEME II



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of steroidal transformations by *A. tamarii* which noted the 11α-hydroxylation of progesterone. However, the *A. tamarii*[10] used in this study was recently isolated from the soil which might account for the different fermentation paths.

Thin layer chromatography of the crude product obtained after incubation of progesterone with *A. tamarii* for 48 hr. showed the presence of three compounds (R_f 0.72, 0.25, and 0.09). Preparative chromatography on alumina provided the three compounds in pure form and showed that the least polar substance (R_f 0.72) was unchanged progesterone (13%), whereas the product with R_f 0.25 was testololactone (70%) and the most polar product (R_f 0.09) was 11β-hydroxytestosterone (14%). When the incubation was interrupted after 12 hr., the thin layer chromatogram of the crude product showed four compounds (R_f 0.95, 0.72, 0.42, and 0.25) identified as Δ⁴-androstene-3,17-dione (V), progesterone (I), testosterone (VI), and testololactone (III), respectively. After 24 hr. of incubation, the same four compounds were present but the relative amount of progesterone had decreased, whereas the amount of testololactone had greatly increased and Δ⁴-androstene-3,17-dione and testosterone were present in small amounts. When Δ⁴-androstene-3,17-dione (V) was incubated with *A. tamarii* for 48 hr., testololactone was the only product evident at this time. Incubation of testosterone (VI) under similar conditions gave 11β-hydroxytestosterone (IV) in 25% yield and testololactone (III) in 41% yield and unchanged testosterone in 31% yield. Testosterone is apparently an intermediate in the formation of both III and IV. When 11β- and 11α-hydroxyprogesterone were incubated with *A. tamarii*, the corresponding 11-hydroxytestosterones were produced but none of the corresponding testololactones were formed.

Thus it appears that *A. tamarii* is not capable of oxidatively cleaving the D-ring of 11-hydroxylated progesterones and testosterones. Δ⁴-Androsten-11β-ol-3,17-dione was unchanged by *A. tamarii* thus indicating that the oxidative cleavage of the D-ring of the 11-hydroxylated steroids is not inhibited solely by the lack of enzymes capable of oxidizing the 17-hydroxyl group to a keto group. The increased relative yield of 11β-hydroxytestosterone compared with testololactone, when testosterone was incubated with *A. tamarii* as compared with incubation of progesterone, suggests that in the latter case the intermediate testosterone is not completely free and the "bound" testosterone is preferentially transformed into testololactone. When testosterone itself is incubated with *A. tamarii*, the enzyme system which converts it to 11β-hydroxytestosterone successfully competes for it with the enzyme system which converts it to testololactone.

Sebek, *et al.*,[7] found that 11α-hydroxyprogesterone was converted into 11α-hydroxytestololactone by *Penicillium lilacinum;* Fried and Thoma[5] found that *Fusarium javanicum* transformed progesterone into 11α-hydroxytestololactone and a number of other products. Thus, *A. tamarii* appears unusual in its inability to oxidatively cleave the D-ring of 11-hydroxylated steroids.

(10) Kindly identified by Dr. George Meyer, Department of Microbiology, University of Texas, Austin, Texas.

Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Infrared spectra were recorded with a Beckman IR-5 spectrometer and n.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, using tetramethylsilane as an internal standard (δ = 0). Thin layer chromatograms (t.l.c.) were run on a 35-μ thick silica gel G coated glass plates using ethyl acetate as the mobile phase; iodine vapors were used for detection and R_f-values were reproducible within ±0.03.

General Methods. Fermentation.—*A. tamarii*[10] spores (maintained on Sabouraud agar slants) were inoculated aseptically into 250-ml. flasks containing 100 ml. of 3% malt extract medium (Difco). After a 48-hr. incubation period at 25° on a rotary shaker, 50 mg. of the steroid, dissolved in 0.4 ml. of dimethylformamide, was added to each flask and the incubation was continued for 48 hr. at 25° with shaking.

Extraction.—The flask contents were combined, the mycelium was filtered and washed with chloroform, and the washings were added to the filtrate that was extracted by stirring with a volume of chloroform equal to one-half its volume for 24 hr. The chloroform layer was removed and the extraction process was repeated. The combined chloroform extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator and finally under vacuum. The solvent-free extracts were often crystalline.

Progesterone Transformation by *A. tamarii*.—Progesterone[11] (1.2 g.) was added to 2400 ml. of a 48-hr. growth of *A. tarmarii* as described above. After 48 hr. of incubation, chloroform extraction gave 1.03 g. of crude crystalline transformation product. T.l.c. showed three spots, R_f 0.72, 0.25, and 0.09. The crude extract was chromatographed on 70 g. of Merck acid-washed alumina (activity III). The ether–benzene (1:9) eluate gave 128 mg. of amorphous solid which showed only one spot in t.l.c.; melting point (130–131° alone and on admixture) and infrared and n.m.r. spectra were identical with those of authentic progesterone.

Further ether–benzene (4:6) eluate gave 722 mg. of testololactone, m.p. 207–209° (lit.[1] m.p. 205.5–207°). T.l.c. of the eluate showed one spot, R_f 0.25; ν^{KBr} 1720, 1670, and 1620 cm.$^{-1}$; n.m.r. ($CDCl_3$) δ 1.17 (3H), 1.35 (3H), and 5.75 (1H). The infrared and n.m.r. spectra were superimposable on those of an authentic sample.[11]

The methanol–ether (1:99) eluate gave 139 mg. of 11β-hydroxytestosterone. T.l.c. of the eluate showed only one spot, R_f 0.09. Recrystallization from 1:1 methanol–ether gave, after drying at 0.1 mm., m.p. 241–243° (lit.[12] m.p. 241°); $[\alpha]^{23}D$ 142° (*c* 0.0164, $CHCl_3$), lit.[12] $[\alpha]^{24}D$ 159°; ν^{KBr} 3500, 1670, and 1620 cm.$^{-1}$; n.m.r. ($CDCl_3$) δ 1.03 (3H), 1.45 (3H), 2.10 (1H), 2.34 (1H), 3.56 (1H), 4.39 (1H), and 5.67 (1H).

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.97; H, 9.27. Found: C, 74.88; H, 9.40.

Oxidation of 11β-Hydroxytestosterone to Δ⁴-Androstene-3,11,-17-trione.—To 20 mg. of 11β-hydroxytestosterone in 3 ml. of dry pyridine was added dropwise a solution of 100 mg. of CrO_3 in 2 ml. of pyridine. After stirring at room temperature for 10 min. the pyridine was removed under vacuum and the dark-colored residue was poured into 20 ml. of water. The resulting solution was extracted with three 20-ml. portions of ether. The combined ether extracts were dried over magnesium sulfate and removal of the solvent gave 17 mg. of Δ⁴-androstene-3,11,17-trione: m.p. 219–220°, after recrystallization from methanol (lit.[13] m.p. 220–223°); ν^{KBr} 1740, 1710, 1670, and 1620 cm.$^{-1}$. This compound was identical in all respects with an authentic sample.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.96; H, 8.05. Found: C, 76.01; H, 8.32.

Progesterone Conversion after 12, 24, and 48 Hr. of Incubation.—To each of 24 250-ml. flasks containing 100 ml. of a 48-hr. growth of *A. tamarii* was added 50 mg. of progesterone. Eight of the flasks were incubated for 12 hr., eight for 24 hr., and eight for 48 hr. At each time interval the contents of the eight flasks

(11) Generously supplied by Dr. Durey H. Peterson, The Upjohn Co., Kalamazoo, Mich.

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were pooled and chloroform extracted, and the crude steroidal metabolite was isolated as previously described. The extracts from the 12-, 24-, and 48-hr. incubations were analyzed by t.l.c. T.l.c. of the 12-hr. incubation product showed four compounds identified as progesterone (R_f 0.72), androstenedione (R_f 0.95), testosterone (R_f 0.42), and testololactone (R_f 0.24) by comparison with authentic samples. The relative intensity of the iodine-detected spots showed progesterone to be present in large amount. T.l.c. of the 24-hr. incubation product showed the same four spots but the relative amount of progesterone had decreased, androstenedione and testosterone appeared only in small amounts, and testololactone had increased significantly. T.l.c. of the 48-hr. incubation product showed the presence only of progesterone, testololactone, and 11β-hydroxytestosterone (R_f 0.09). Androstenedione and testosterone had completely disappeared.

Conversion of 11α-Hydroxyprogesterone[11] by *A. tamarii*.—11α-Hydroxyprogesterone (1.20 g.) was incubated with *A. tamarii* for 48 hr. as previously described and 1.08 g. of crude amorphous product was obtained. T.l.c. showed two spots with R_f 0.37 and 0.20. The crude chloroform extract was chromatographed on 70 g. of Merck acid-washed alumina (activity III). The ether–benzene (1:9) eluate gave 157 mg. of 11α-hydroxyprogesterone (R_f 0.37): m.p. 169–172° (alone and on admixture with an authentic sample); ν^{KBr} 3450, 1710, 1670, 1620, and 870 cm.$^{-1}$. The ether eluate gave 655 mg. of 11α-hydroxytestosterone: m.p. 181–181.5° after recrystallization from ether (lit.[14] m.p. 181.5°); R_f 0.20; ν^{KBr} 3450, 3320, 1660, 1620, and 865 cm.$^{-1}$; $[\alpha]^{26}D$ +94° (*c* 0.0284, $CHCl_3$), reported[14] [α] +93°; n.m.r. ($CDCl_3$) δ 1.83 (3H), 1.32 (3H), 2.20 (2H, which disappeared upon addition of D_2O to the sample), 2.29 (1H), 2.35 (1H), and 5.74 (1H). None of the chromatography fractions showed six-membered lactone absorption, indicative of 11α-hydroxytestololactone, in the infrared.

Conversion of 11β-Hydroxyprogesterone by *A. tamarii*.—11β-Hydroxyprogesterone (167 mg.) was incubated with *A. tamarii* for 48 hr. as previously described. The usual work-up gave 145 mg. of crude product, whose t.l.c. showed only two spots, R_f 0.35 and 0.09. The crude extract was chromatographed on 35 g. of Merck acid-washed alumina (activity III) and the ether–benzene (3:7) eluate gave 87 mg. of unchanged 11β-hydroxyprogesterone, m.p. 185–187°, R_f 0.35, identical in all respects with authentic material. The ether–benzene (8:2) eluate afforded 46 mg. of 11β-hydroxytestosterone: R_f 0.09; m.p. 237–240; ν^{KBr} 3500, 1670, and 1620 cm.$^{-1}$. This material was identical in all respects with 11β-hydroxytestosterone obtained as described above. None of the chromatography fractions showed the presence of six-membered lactones in their infrared spectra.

Transformation of Δ⁴-Androstene-3,17-dione by *A. tamarii*.—Δ⁴-Androstene-3,17-dione (332 mg.) was incubated with *A. tamarii* for 48 hr. as previously described and chloroform extraction gave 235 mg. of crude product. T.l.c. showed only two spots (R_f 0.96 and 0.25). The crude extract was chromatographed on 65 g. of Merck acid-washed alumina (activity III). The ether–benzene (1:9) eluate gave 167 mg. of unchanged Δ⁴-androstene-3,17-dione: m.p. 169–172; R_f 0.96; ν^{KBr} 1735, 1670, and 1620 cm.$^{-1}$. The ether–benzene (6:4) eluate gave 66 mg. of testololactone (R_f 0.25).

Conversion of Testosterone by *A. tamarii*.—Testosterone (1.00 g.) was incubated with *A. tamarii* for 48 hr. as previously described and the usual work-up gave 877 mg. of crude product; the t.l.c. showed three spots (R_f 0.43, 0.25, and 0.09). The crude extract was chromatographed on 20 g. of Merck acid-washed alumina (activity IV); the benzene eluate gave 624 mg. of noncrystalline material, the t.l.c. of which showed two spots of R_f 0.43 and 0.25, corresponding, respectively, to testosterone and testololactone. Recrystallization of the combined chromatography fractions from benzene gave, after drying at 0.1 mm., 210 mg. of pure testosterone, m.p. 149–151°. Rechromatography of the residue from the recrystallization on 35 g. of Merck acid-washed alumina (activity IV) gave 363 mg. of pure testololactone, m.p. 205–207°, R_f 0.25.

The ether–benzene (8:2) eluate gave 217 mg. of 11β-hydroxytestosterone, m.p. 238–240°, R_f 0.09, identical in all respects with that obtained as previously described.

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Synthesis of 4-Demethyltetrahydroalantolactone

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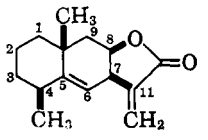
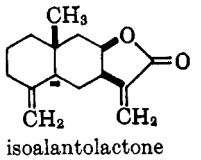
A synthesis of 4-demethyltetrahydroalantolactone (**17**) from 10-methyl-1(9)octal-2-one (**1**) is described. The most efficient route was found to be **1** → **2** → **3** → **4** → **10** → **12** → **13** → **15** → **16** → **17**. Isoalantolactone was degraded, *via* the ketone **18** and the thioketal **19**, to the natural isomer **17a** which was identical with the racemic lactone **17**.

We recently reported a new method for the synthesis of the α-methylene-γ-butyrolactone moiety found in numerous sesquiterpenes.[3] We now describe an application of this method to the synthesis of 4-demethyl-5,6-dihydroalantolactone (**16**) and 4-demethyltetrahydroalantolactone (**17**) a degradation product of isoalantolactone.



alantolactone



isoalantolactone

The required starting material, *trans*-10-methyl-2-decalone (**2**), was prepared from 2-methylcyclohexanone and methyl vinyl ketone, *via* octalone (**1**), in 35–40% over-all yield by an improved procedure.[4] Bromination of decalone **2** afforded the crystalline bromodecalone **3**[5] which was reduced using a slight excess of lithium aluminum hydride in ether. The resulting bromohydrin was treated with potassium hydroxide in refluxing isopropyl alcohol, according to steroid analogy,[6] giving a 9:1 mixture of β-oxide **4** and decalone **2** which was separated by fractional distillation. Reduction of bromo ketone **3** using a two- or threefold excess of lithium aluminum hydride for extended times (over-

(1) Fellow of the National Institute of General Medical Sciences, Public Health Service, 1963–1964.

(2) Participant in the National Science Foundation Summer Undergraduate Research Program, 1964.

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