and its rate of isomerization. The extent of this influence is under investigation.

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Microbiological Transformations. IX. The 1β-Hydroxylation of Androstenedione

R. M. DODSON,[1] STEPHEN KRAYCHY, R. T. NICHOLSON, AND SETH MIZUBA

Biological and Chemical Research Divisions of G. D. Searle and Co., Chicago 80, Illinois

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Incubation of 4-androstene-3,17-dione (I) with *Xylaria sp.* produced 1β-hydroxy-4-androstene-3,17-dione (II), 15β-hydroxy-4-androstene-3,17-dione (IV), 7β-hydroxy-4-androstene-3,17-dione (V), and 1β,6β-dihydroxy-4-androstene-3,17-dione (VI). Incubation of I with *Haplosporella sp.* also produced II, along with V and 6β-hydroxyandrostenedione. 1β-Hydroxytestosterone (XII) was prepared from 1β-acetoxyandrostenedione by reaction with lithium aluminum hydride and manganese dioxide. The structures of the 1β-hydroxy steroids (II, VI, and XII) were established by their conversion to the corresponding 1-dehydro steroids. 6β-Hydroxy-1,4-androstadiene-3,17-dione (XIV) was independently synthesized from 6β-acetoxy-4-androstene-3,17-dione (XVI) by dehydrogenation with dichlorodicyanobenzoquinone, then saponification.

The microbiological hydroxylation of steroids at C-1 is still a relatively rare phenomenon. 1α-Hydroxylation by the *Penicillium sp.*, A.T.C.C. 12,556, on which we initially reported,[2] was limited to 4-androstene-3,17-dione (I), androstane-3,17-dione, and dehydroisoandrosterone. With other C_{19} and C_{21} steroids hydroxylations at positions 2, 6, 7, and 15 have been observed with this organism,[3] but not hydroxylation at C-1. McAleer and co-workers[4] have reported the 1ξ-hydroxylation of 9α-fluorohydrocortisone with a *Streptomyces sp.*, but in this case 1-hydroxylation was limited to this one substrate. Hydrocortisone, cortisone, Reichstein's compound S (17α,21-dihydroxyprogesterone), and progesterone were not hydroxylated at C-1. Greenspan *et al.*[5] have reported the 1β-hydroxylation of Reichstein's compound S (17α,21-dihydroxyprogesterone) with *Rhizoctonia ferrugena* (CBS, Holland). In this paper, we wish to report the 1β-hydroxylation of 4-androstene-3,17-dione using a species of *Xylaria* (M40-6)[6] and a species of *Haplosporella* (M1086).[6] Again, the C-1 hydroxylation with *Xylaria* was substrate-specific. Progesterone on similar treatment gave no 1-hydroxy steroid.[7]

Incubation of androstenedione (I) with *Xylaria sp.* (M40-6) and subsequent chromatography of the isolated steroids gave amorphous 1β-hydroxy-4-androstene-3,17-dione (II) (25% yield) along with smaller quantities of 15β-hydroxy-4-androstene-3,17-dione[8] (IV), 7β-hydroxy-4-androstene-3,17-dione[9] (V), and 1β,6β-dihydroxy-4-androstene-3,17-dione (VI). The structure of the amorphous 1β-hydroxy-4-androstene-3,17-dione (II) was initially indicated by its conversion and by the conversion of its noncrystalline acetate (III) to 1,4-androstadiene-3,17-dione, by its analysis and infrared spectrum which closely corresponded to that expected for a monohydroxyandrostenedione, and by the molecular rotatory contribution of the newly introduced hydroxyl group (see Table I). These suppositions were placed upon a solid basis, by the isolation of crystalline 1β-hydroxy-4-androstene-3,17-dione (II), m.p. 155–156.5°, from the fermentation of androstenedione (I) with *Haplosporella sp.* (M1086). The crystalline 1β-hydroxy-4-androstene-3,17-dione (II) proved to be identical (infrared spectra, infrared spectra of the noncrystalline acetates, rotations) with the amorphous material previously isolated. It, too, was readily converted to 1,4-androstadiene-3,17-dione. Since this hydroxyandrostenedione differed from the

(1) Present address: University of Minnesota, Minneapolis 14, Minnesota.

(2) R. M. Dodson, A. H. Goldkamp, and R. D. Muir, *J. Am. Chem. Soc.*, **79**, 3921 (1957); **82**, 4026 (1960).

(3) R. M. Dodson, R. C. Tweit, and R. D. Muir, unpublished results.

(4) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt, and J. M. Chemerda, *J. Org. Chem.*, **23**, 508 (1958).

(5) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog, and E. B. Hershberg, *J. Am. Chem. Soc.*, **79**, 3922 (1957). A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore, and W. Charney, *ibid.*, **81**, 5230 (1959).

(6) Searle Isolation number.

(7) R. M. Dodson, R. T. Nicholson, and Seth Mizuba, unpublished results. Recently, Y. Nozaki and T. Okumura have reported the 1β-hydroxylation of digitoxigenin using *A. orchidis* [*Agr. Biol. Chem. (Tokyo)*, **25** (6), 515 (1961); *Index Chemicus*, 11,515].

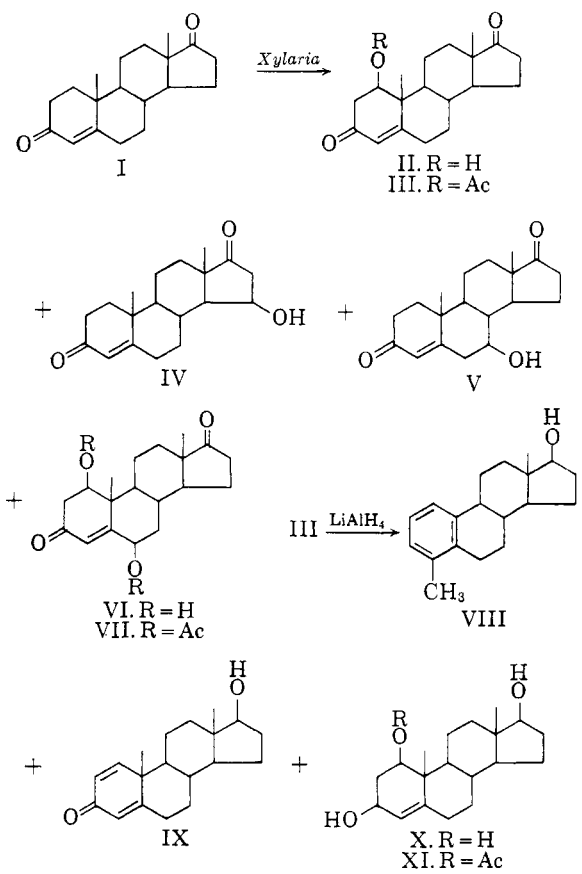
(8) S. Bernstein, L. I. Feldman, W. S. Allen, R. H. Blank, and C. E. Linden, *Chem. Ind.* (London), 111 (1956). H. L. Herzog, M. J. Gentles, W. Charney, D. Sutter, E. Townley, M. Yudis, P. Kabasakalian, and E. B. Hershberg, *J. Org. Chem.*, **24**, 691 (1959).

(9) R. C. Tweit, A. H. Goldkamp, and R. M. Dodson, *ibid.*, **26**, 2856 (1961). S. Bernstein, W. S. Allen, M. Heller, R. H. Lenhard, L. I. Feldman, and R. H. Blank, *ibid.*, **24**, 286 (1959).

previously described 1α,2α, or 2β-hydroxyandrostenediones,[2,10] it must be the 1β-isomer.

In addition to the 1β-hydroxy-4-androstene-3,17-dione (II), incubation of androstenedione (I) with *Haplosporella sp.* (M1086) also yielded 6β-hydroxy-4-androstene-3,17-dione[11] and 7β-hydroxy-4-androstene-3,17-dione (V)[9] (major product).

Further evidence for the position and configuration of the newly introduced hydroxyl group in the monohydroxyandrostenedione was obtained by the conversion of the noncrystalline 1β-acetoxy-4-androstene-3,17-dione (III) to 1β-hydroxytestosterone (XII) and 1β-acetoxytestosterone (XIII). Reduction of 1β-acetoxy-4-androstene-3,17-dione (III) with lithium aluminum hydride in ether,



followed by chromatography of the organic products on silica gel, produced 4-methyl-1,3,5(10)-estratrien-17β-ol (VIII),[12] testosterone,[13] 1-dehydrotestosterone (IX),[14] 1β-acetoxy-4-androstene-3β,17β-diol (XI), and 4-androstene-1β,3β,17β-triol (X). The 4-androstene-1β,3β,17β-triol (X) was oxidized with manganese dioxide[15] in isopropyl alcohol to 1β-hydroxytestosterone (XII). In a similar manner the 1β-acetoxy-4-androstene-3β,17β-diol (XI) was oxidized to 1β-acetoxytestosterone (XIII). The presence of a group at C-1 in both compounds (XII and XIII) was readily established by their conversion to 1-dehydrotestosterone (IX). The position of acetylation in XI and XIII was established by the conversion of XIII to 1-dehydrotestosterone (IX) by means of potassium acetate in acetic acid. The molecular rotatory contributions of the 1-hydroxy and 1-acetoxy groups in the substituted testosterones, XII and XIII, agreed well with those previously determined for groups with a β-configuration (see Table I). The β-configuration was assigned to the hydroxyl group at C-3 in both X and XI because of the expected predominance of the 3β-configuration in compounds obtained by the reduction of a Δ^4-3-keto system with lithium aluminum hydride[16] and because of the negative molecular rotatory difference, ΔM_D-[(3-OH)− (3 C=O)], obtained from X and XII (see Table I).[17]

The structure of the 1β,6β-dihydroxy-4-androstene-3,17-dione (VI) was first surmised from its analysis, infrared spectrum (λ_{max}^{KBr} 2.84–2.92, 5.74, 5.92, and 6.18 μ), ultraviolet spectrum (λ_{max} 233 mμ, ϵ 13,530), and the immediate change of its ultraviolet spectrum in 1 *N* methanolic potassium hydroxide (λ_{max} 246 mμ, ϵ 16,550). A maximum at *ca.* 235 mμ is often indicative of a 6β-hydroxyl group in Δ^4-3-keto steroids.[11] The lack of effect of the 6β-hydroxyl group on the ultraviolet spectrum of the $\Delta^{1,4}$-3-keto steroid, XIV, however, was a surprise to us.

Further evidence for the structure of VI was obtained from its n.m.r. spectrum[18] and the n.m.r. spectrum of the 6β-hydroxy-1,4-androstadiene-3,17-dione (XIV), prepared from VI by treatment with base. The n.m.r. spectrum of VI showed the following resonance bands: 57 c.p.s. (18–CH_3), 85 c.p.s. (19–CH_3), 242.5 c.p.s. (axial-H on carbon atom holding hydroxyl group; peak width at half-height, W_H 23.5 c.p.s.), 268 c.p.s. (equatorial-H

(10) The infrared spectra of the 2α-acetoxy-4-androstene-3,17-dione and the new acetoxyandrostenedione were compared, rather than the hydroxy derivatives. G. Rosenkranz, O. Mancera, and F. Sondheimer, *J. Am. Chem. Soc.*, **77**, 145 (1955).

(11) C. P. Balant and M. Ehrenstein, *J. Org. Chem.*, **17**, 1587 (1952).

(12) H. Dannenberg and C. H. Doering, *Z. physiol. Chem.*, **311**, 84 (1958). M. J. Gentles, J. B. Moss, H. L. Herzog, and E. B. Hershberg, *J. Am. Chem. Soc.*, **80**, 3702 (1958).

(13) The small quantity of testosterone obtained was probably formed by the reduction of the Δ^1-bond (with the lithium aluminum hydride) of the 1-dehydrotestosterone (formed in the reaction) or of some 1,4-androstadiene-3,17-dione (formed during the acetylation).

(14) (a) J. Fried, R. W. Thoma, and A. Klingsberg, *J. Am. Chem. Soc.*, **75**, 5764 (1953). (b) H. H. Inhoffen, G. Zühlsdorff, and Huang-Minlon, *Ber.*, **73**, 451 (1940).

(15) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953). F. Sondheimer, C. Amendolla, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 5930 (1953). R. M. Evans, *Quart. Rev.*, **13**, 61 (1959).

(16) W. G. Dauben, R. A. Micheli, and J. F. Eastham, *J. Am. Chem. Soc.*, **74**, 3852 (1952). Pl. A. Plattner, H. Heusser, and A. B. Kulkarni, *Helv. Chem. Acta*, **32**, 265 (1949).

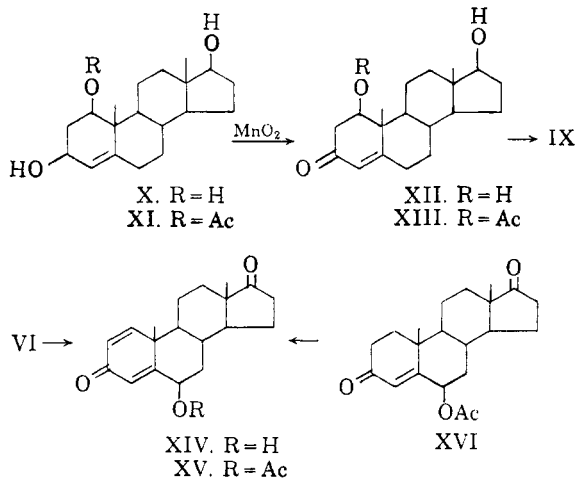
(17) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4631 (1961).

(18) The n.m.r. spectrum of 1β,6β-dihydroxy-4-androstene-3,17, dione (VI) was run in tetradeuteroacetic acid by Dr. Neal McNiven, Worcester Foundation for Experimental Biology. The n.m.r. spectra of the other compounds were run in deuterochloroform by Dr. Roy H. Bible and Mr. A. J. Damascus, G. D. Searle and Co. All spectra were run at 60 Mc./sec. using tetramethylsilane as an internal standard.

TABLE I[a]

	MD	ΔMD[(1β-OR) − (1-H)] 1β-OH	1β-OAc	ΔMD[(3β-OH) − (3C=O)]
1β-Hydroxy-4-androstene-3,17-dione	+386	−182		
1β-Acetoxy-4-androstene-3,17-dione (noncrystalline)	+210		−358	
1β-Hydroxytestosterone	+122	−218		
1β-Acetoxytestosterone	− 50		−390	
4-Androstene-1β,3β,17β-triol	− 9	−150[b]		−131
1β-Acetoxy-4-androstene-3β,17β-diol	− 14		−155[b]	− 64
1β,6β-Dihydroxy-4-androstene-3,17-dione	+214	−116		
1β-Hydroxyprogesterone[c]		−167		
1β-Acetoxyprogesterone[c]			−371	

[a] Rotations of the parent compounds taken from J. P. Mathieu and A. Petit, "Constants Selectionees Pouvoir Rotatoire Natural, I. Steroids," Masson et Cie., Editeurs, Paris, 1956. [b] Reference compound in ethanol. [c] A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore, and W. Charney, *J. Am. Chem. Soc.*, **79**, 4814 (1957). Also see ref. 5.



on carbon atom holding hydroxyl group, W_H 11.7 c.p.s.),[19] 359 c.p.s. (C_4–H). 6β-Hydroxy-1,4-androstadiene-3,17-dione (XIV) showed the expected resonance band for the C-18 methyl group (59 c.p.s.). The band for the C-19 methyl group was shifted to 88 c.p.s. The resonance band for the axial proton attached to carbon holding the hydroxyl group had disappeared, and the band for the equatorial proton attached to carbon holding the hydroxyl group had shifted down field to 275 c.p.s. This band, after exchange of the hydroxyl hydrogen for deuterium, was a symmetrical triplet, J = 2.75 c.p.s. This evidence confirmed the β-configuration assigned above to the hydroxyl group at C_6 in XIV. In addition, resonance bands at 368 and 376 c.p.s. (C_2—H, "doublet"; C_4—H, "singlet")[19a] and at 420 and 430 c.p.s. (C_1—H) confirmed the structural assignment of XIV. To substantiate further the configuration assigned to the C-6 hydroxyl in XIV, the n.m.r. spectrum of 6α-hydroxy-4-androstene-3,17-dione was determined. The resonance band of the C_6—H (axial H) was a wide multiplet centered at 265 c.p.s. (W_H *ca.* 20 c.p.s.). This band, after exchange of the hydroxyl hydrogen for deuterium, became a quadruplet, J_{ae} = 5 c.p.s., J_{aa} = 12 c.p.s.

The structure of VI was proved by the independent synthesis of 6β-hydroxy-1,4-androstadiene-3,17-dione (XIV), obtained from VI on treatment with base, from 6β-acetoxy-4-androstene-3,17-dione (XVI). Reaction of XVI with 2,3-dichloro-5,6-dicyanobenzoquinone[20] gave 6β-acetoxy-1,4-androstadiene-3,17-dione (XV), identical with the material obtained from the acetylation of XIV from VI. This same 6β-acetoxy-1,4-androstadiene-3,17-dione (XV) could be obtained by the oxidation of 6β-acetoxy-4-androstene-3,17-dione (XVI) with selenous acid[21] in *t*-butyl alcohol containing a small amount of pyridine. However, the yield was very much lower than that obtained by using the dichlorodicyanoquinone. It is interesting to note that 6β-hydroxy-1,4-androstadiene-3,17-dione (XIV) was not converted to the 6α-isomer with alkali at room temperature.

Experimental[22]

Fermentation of 4-Androstene-3,17-dione (I) with *Xylaria sp.* (M4O-6).[6]—A stainless steel fermentation tank of 40-l. capacity was charged with medium containing 1000 g. of commercial dextrose, 150 g. of cotton seed flour, 90 ml. of corn steep liquor, 5 g. of silicone antifoam emulsion,[23] 20 g. of yeast extract, and sufficient hot tap water to result in a final volume of approximately 35 l. after sterilization. The vessel and medium were sterilized by heating to 120°

(19) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958). For an analysis of the relationship between the conformation of a proton and its coupling constants (J or W_H, where coupling constants cannot be directly determined) with adjacent protons see K. L. Williamson and W. S. Johnson, *ibid.*, **83**, 4623 (1961), and the many references contained therein.

(19a) NOTE ADDED IN PROOF. There is very weak coupling between the C-2 and C-4 protons J < 2 c.p.s. The C_4—H absorbs at 368

(20) D. Burn, D. N. Kirk, and V. Petrow, *Proc. Chem. Soc.*, 14 (1960).

(21) Ch. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chem. Acta*, **39**, 734 (1956). S. A. Szpilfogel, T. A. P. Posthumus, M. S. de Winter, and D. A. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956).

(22) Melting points were taken on a Fisher-Johns melting point apparatus except as otherwise noted. Unless stated differently, the rotations were taken in chloroform at 24 ± 2° and the ultraviolet spectra in methanol. We are indebted to Drs. R. T. Dillon and H. W. Sause of the Analytical Division of G. D. Searle and Co. for the analytical and optical data reported, and to Mr. Ernest Kopka for technical assistance.

(23) Antifoam AF Emulsion, Dow Chemical Corp., Midland,

with direct steam, cooled to 25°, and maintained at this temperature during incubation.

The medium was inoculated with an aqueous suspension of spores and mycelium of *Xylaria sp.* (M40-6) which had been mixed in a Waring Blendor for 1 min. Throughout the incubation period, 10 l. of sterile air per minute was introduced into the tank through a perforated tube-type sparger located near the bottom of the tank. Continuous agitation of the culture was maintained by means of a vertically mounted paddle-type stirring device operated at 200 r.p.m.

After an initial incubation period of 51 hr., 4-androstene-3,17-dione (10 g.) dissolved in 250 ml. of acetone was added to the culture and incubation was continued for another 23 hr. The steroids were recovered from the medium and culture by using 15 l. of methylene chloride in each of two extractions.

The methylene chloride solution was evaporated to dryness and the residue (23.8 g.) was chromatographed on 1700 g. of silica gel. 4-Androstene-3,17-dione (618 mg.) was eluted with 25% ethyl acetate in benzene. The column was washed further with 30%, 35%, and 40% ethyl acetate in benzene. Elution with 45% ethyl acetate in benzene produced, as an amorphous solid, 2.66 g. of 1β-hydroxy-4-androstene-3,17-dione (II), $[\alpha]_D$ +126°; λ_{max} 240 mμ (ε 14,200); $\lambda_{max}^{CHCl_3}$ 2.76, 2.90, 5.74, 5.98, and 6.18 μ. Because of our inability to crystallize this material at the time of isolation, analyses on the amorphous product, while close to the theoretically calculated values, were not within the usually accepted limits. The infrared spectrum and rotation of this material were identical with that of the crystalline 1β-hydroxy-4-androstene-3,17-dione (II) described below.

The column was washed next with 50% ethyl acetate in benzene. Further elution of the silica gel column with 75% ethyl acetate in benzene gave 431 mg. of 15β-hydroxy-4-androstene-3,17-dione (IV), which, after crystallization from acetone–hexane melted at 192–197°; $[\alpha]_D$ + 136°; λ_{max} 239 mμ (ε 17,500); $\lambda_{max}^{CHCl_3}$ 2.92, 5.72, 6.02–6.06, and 6.18 μ. The infrared spectrum of this material was identical with that of an authentic sample,[24] m.p. 201–203°.

Further elution of the column with 75% ethyl acetate in benzene produced 1.12 g. of 7β-hydroxy-4-androstene-3,17-dione, m.p. 218–223°. This material was identical in all respects with the authentic sample of 7β-hydroxy-4-androstene-3,17-dione[9] previously described.

Final elution of the column with ethyl acetate produced 630 mg. of material which, after four crystallizations from acetone–hexane, yielded 155 mg. of pure 1β,6β-dihydroxy-4-androstene-3,17-dione (VI), m.p. 237–239°; $[\alpha]_D$ + 68°, +66°; λ_{max} 233 mμ (ε 13,530); λ_{max}^{KBr} 2.84–2.92, 5.74, 5.92, and 6.18 μ. A solution of this compound in 1 *N* methanolic potassium hydroxide showed an immediate maximum at 246 mμ in the ultraviolet. The position of this maximum remained unchanged over a 24-hr. period.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.66; H, 8.23. Found: C, 71.38; H, 8.18.

Acetylation of 1β,6β-dihydroxy-4-androstene-3,17-dione with acetic anhydride and pyridine at room temperature produced 1β,6β-diacetoxy-4-androstene-3,17-dione, a compound that, in our hands, resisted crystallization: λ_{max} 235 mμ (ε 10,790); $\lambda_{max}^{CHCl_3}$ 5.73, 5.92, 5.98 (w), 6.25, and 8.02 (s) μ.

1,4-Androstadiene-3,17-dione from 1β-Hydroxy-4-androstene-3,17-dione.—Acetylation of 2.66 g. of amorphous 1β-hydroxy-4-androstene-3,17-dione with 26 ml. of acetic anhydride in 26 ml. of pyridine at room temperature, overnight, yielded 2.85 g. of 1β-acetoxy-4-androstene-3,17-dione, $[\alpha]_D$ + 61°; λ_{max} 240 mμ (ε 14,530); $\lambda_{max}^{CHCl_3}$ 5.72, 5.98, 6.14, 6.22, and 8.02 μ. Attempts to crystallize this material were unsuccessful.[25]

A solution of 54 mg. of 1β-acetoxy-4-androstene-3,17-dione and *ca.* 50 mg. of potassium hydroxide in 5 ml. of 80% methanol was allowed to stand at room temperature for 4 hr. The reaction mixture was diluted with water and extracted with methylene chloride. The methylene chloride solution was washed with water and then dried over sodium sulfate. The solvent was removed under reduced pressure. Crystallization of the residue (50 mg.) from hexane yielded 1,4-androstadiene-3,17-dione, m.p. 138–143°, identical in all respects (m.m.p. and infrared spectra) with an authentic sample.

Fermentation of 4-Androstene-3,17-dione (I) with *Haplosporella sp.* (M1086).[6]—A stainless steel fermentation tank of 40-l. capacity was charged with medium containing 1000 g. of commercial grade dextrose, 150 g. of cotton seed flour, 90 ml. of corn steep liquor, 5 g. of silicone antifoam emulsion, and sufficient hot tap water to result in a final volume of approximately 35 l. after sterilization. The vessel and medium were sterilized, cooled, and inoculated in a manner similar to that previously described for the fermentation of 4-androstene-3,17-dione with *Xylaria sp.* (M40-6).

Preliminary incubation was continued for 43.5 hr. with aeration at the rate of 10 l. of sterile air per minute and agitation at 200 r.p.m. 4-Androstene-3,17-dione (10 g.) dissolved in 250 ml. of acetone was added and incubation was continued for 6.75 hr. The steroids were recovered from the medium and culture using 15 l. of methylene chloride in each of two extractions.

The methylene chloride solution was evaporated to dryness and the residue (14 g.) was washed twice by shaking with 100-ml. portions of pentane and decanting the solution. The steroid residue thus obtained was dried (10.4 g.), boiled for several minutes with a mixture of 60 ml. of ethyl acetate and 240 ml. of benzene, and separated from the insoluble portion by filtration. This latter was recrystallized from methanol giving 0.47 g. of solid, m.p. 222–228°, λ_{max} 241 mμ (ε 15,600), identified as 7β-hydroxy-4-androstene-3,17-dione by comparison of its infrared spectrum (KBr) with that of an authentic sample.

The mother liquors were chromatographed on 800 g. of silica gel. Elution with 20% ethyl acetate in benzene afforded 2.48 g. (after recrystallization) of 4-androstene-3,17-dione, m.p. 174.5–177.5° (Hershberg m.p. apparatus, corrected for stem exposure). The column was washed further with 25% ethyl acetate in benzene (6 l.). Elution with 35% ethyl acetate in benzene afforded 1.02 g. of amorphous material. Crystallization of 0.73 g. of this product from aqueous methanol, after numerous attempts from a variety of solvents and mixtures of solvents, produced a gel-like solid, m.p. 138–153° (Hershberg, corrected). This was recrystallized from isopropyl ether containing a small amount of 95% ethanol, and yielded, in two crops, a small amount (18.5 mg.) of pure 1β-hydroxy-4-androstene-3,17-dione (II), m.p. 155–156.5° (Hershberg, corrected); $[\alpha]^{27}_D$ +127.5°; λ_{max} 239 mμ (ε 14,140); $\lambda_{max}^{CHCl_3}$ 2.75, 2.89 (OH), 5.74 (17–CO), 5.98 (3-CO) and 6.19 μ (Δ^4).

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.78; H, 8.59.

Acetylation of 0.40 g. of amorphous material obtained from the mother liquors with 2 ml. of pyridine and 1 ml. of acetic anhydride at room temperature overnight (worked up by evaporation of the volatile components at room temperature *in vacuo*) afforded noncrystalline 1β-acetoxy-4-androstene-3,17-dione (III). The infrared spectrum of this product, in chloroform, was identical with that obtained above.

(24) We are indebted to Dr. C. E. Holmlund of the Lederle Laboratories for the authentic sample of 15β-hydroxy-4-androstene-3,17-dione.

(25) Very recently, Dr. Robert C. Tweit of these laboratories succeeded in crystallizing 1β-acetoxy-4-androstene-3,17-dione, m.p. 121.5–122°, using ether–hexane. The infrared spectrum of this material in chloroform was identical with that of the noncrystalline acetate described above.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.16; H, 8.07.

Further elution of the column with 35% ethyl acetate in benzene (1 l.) and with 50% ethyl acetate in benzene (3 l.) afforded 1.11 g. of crude 6β-hydroxy-4-androstene-3,17-dione. This, upon recrystallization from acetone–hexane gave 0.37 g. of 6β-hydroxyandrostenedione, m.p. 183–187° [infrared spectrum (KBr) identical with that of an authentic sample] and 0.25 g. of lower melting (168.5–177°) material.

Finally, elution with 13 additional liters of 50% ethyl acetate–benzene mixture produced 1.78 g. of crude 7β-hydroxy-4-androstene-3,17-dione (V). Recrystallization of this from acetone (*ca.* 10% was lost during work-up) gave 0.83 g. of 7β-hydroxyandrostenedione, m.p. 225.5–228.5°, and 0.26 g. of product, m.p. 223.5–225.5°. The infrared spectrum (KBr) was identical with that of an authentic sample.

Reduction of 1β-Acetoxy-4-androstene-3,17-dione (III) with Lithium Aluminum Hydride.—To a solution of 2.435 g. (7.1 mmoles) of amorphous 1β-acetoxy-4-androstene-3,17-dione (III), prepared from amorphous 1β-hydroxy-4-androstene-3,17-dione, in 50 ml. of ether was slowly added a solution of 296 mg. (7.8 mmoles) of lithium aluminum hydride in 20 ml. of ether. The evolution of hydrogen and the precipitation of a white solid resulted. The reaction mixture was allowed to stand at room temperature overnight; then 150 ml. of ethyl acetate was cautiously added, and the mixture was poured slowly into 100 ml. of a saturated aqueous Rochelle salt solution. The two phase system was filtered in order to break up a small amount of emulsion. The layers were separated, and the organic phase was washed with 50 ml. of a saturated aqueous Rochelle salt solution, then with two 50-ml. portions of water. The solution was dried over sodium sulfate and then evaporated to dryness *in vacuo* to yield 2.049 g. of a residual oil. This material in benzene was chromatographed on 200 g. of silica gel. Elution of the column with 5% ethyl acetate in benzene produced 264 mg. of material which, after crystallization from hexane, yielded 124 mg. of 4-methyl-1,3,5(10)-estratrien-17β-ol (VIII), m.p. 114–115°; λ_{max} 263 mμ (ε 265); $[\alpha]_D$ +62°; λ_{max}^{KBr} 2.96, 6.30 (w), 8.25, 12.84, and 13.53 μ; (reported m.p. 114–116°; λ_{max} 262.5 mμ (ε 309); $[\alpha]_D$ +66°; λ_{max}^{KBr} 12.84 and 13.53 μ.).[12] 17β-Acetate, m.p. 175–182°. (reported 174–176°).[12]

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.42; H, 9.65.

The column was next washed with 10% ethyl acetate in benzene. After the elution of 51 mg. of an unidentified crystalline material, m.p. 196–200°, with 15% ethyl acetate in benzene, 105 mg. of crude testosterone, m.p. 133–138° after crystallization from acetone–hexane, was eluted with 20% ethyl acetate in benzene. The testosterone was identified by comparison of its infrared spectrum in chloroform with that of an authentic sample.

The column was washed with 25% ethyl acetate in benzene. Elution with 30% ethyl acetate in benzene yielded 133 mg. of crystalline material, which, after two crystallizations from acetone–hexane, gave pure 1-dehydrotestosterone (IX), m.p. 171.5–172.5°, $[\alpha]_D$ +21°; λ_{max} 244 mμ (ε 16,300); (reported m.p. 168–169°, $[\alpha]_D$ +22.5°).[14b] This material proved to be identical with an authentic sample (m.m.p. and infrared spectra).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.67; H, 9.15. Found: C, 79.63; H, 9.35.

Immediately following the elution of the 1-dehydrotestosterone, there was obtained by further elution with 30% ethyl acetate in benzene 374 mg. of crystalline material. Four crystallizations from acetone–hexane yielded 120 mg. of 1β-acetoxy-4-androstene-3β,17β-diol, m.p. 183–185°; $[\alpha]_D$ −4°; λ_{max}^{KBr} 2.78 (w), 3.00 (s), 5.76, 5.80, 6.00 (w), and 7.98 (s) μ.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.21; H, 9.41.

After washing the column further with 50% ethyl acetate in benzene, the final product (512 mg.) was eluted from the column with 70% ethyl acetate in benzene. Crystallization of this material from dilute methanol yielded 191 mg. of 4-androstene-1β,3β,17β-triol (X), m.p. 121–123°, resolidified, then m.p. 170–188°; $[\alpha]_D$ −3°; λ_{max}^{Kbr} 2.95 (s), 6.02 (w), and 6.90 μ. After being dried under vacuum at 139° for 2 hr., the compound melted at 177–180°.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.51; H, 9.85.

1β-Hydroxytestosterone (XII).—To a solution of 131 mg. of 4-androstene-1β,3β,17β-triol in 6.5 ml. of isopropyl alcohol was added 1.31 g. of manganese dioxide. The reaction mixture was stirred at room temperature for 3 hr. The suspension was filtered through a bed of Supercel; the residue was washed thoroughly with isopropyl alcohol; then the filtrate was evaporated to dryness. The residue (115 mg.) was crystallized twice from acetone–hexane to yield 97 mg. of 1β-hydroxytestosterone, m.p. 212–213.5°; $[\alpha]_D$ +40.5°; λ_{max} 241 mμ (ε 14,300); λ_{max}^{KBr} 2.88–2.90, 5.98, and 6.18 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.12; H, 9.26.

1-Dehydrotestosterone (IX) from 1β-Hydroxytestosterone (XII).—A solution of 1β-hydroxytestosterone (15 mg.) in 0.8 ml. of methanol was treated with 15 mg. of potassium hydroxide in 0.2 ml. of water. After having stood at room temperature for 4 hr., the reaction mixture was diluted with water and the resulting crystals were collected by filtration. The 1-dehydrotestosterone so obtained, m.p. 169–171°, proved to be identical with that prepared above (infrared spectra).

1β-Acetoxytestosterone (XIII).—A solution of 80 mg. of 1β-acetoxy-4-androstene-3β,17β-diol (XI) in 4.0 ml. of isopropyl alcohol was treated with 800 mg. of manganese dioxide and then stirred at room temperature for 2 hr. The reaction mixture was filtered through a bed of Supercel; the residue was washed with isopropyl alcohol and the filtrate was evaporated to dryness *in vacuo*. The residual oil (74 mg.) would not crystallize, $[\alpha]_D$ −14.5°; λ_{max} 241.5 mμ ε 13,850); $\lambda_{max}^{CHCl_3}$ 2.76, 5.76, 5.95, 6.18, and 8.00 (s) μ.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 73.12; H, 8.75.

1-Dehydrotestosterone (IX) from 1β-Acetoxytestosterone (XIII).—A solution of 14 mg. of 1β-acetoxytestosterone and 50 mg. of potassium acetate in 1.00 ml. of glacial acetic acid was heated on the steam bath for 1 hr. The clear solution was blown to dryness with a stream of nitrogen, and water was added to the residue to dissolve the potassium acetate. The remaining precipitate was removed by filtration and washed with water. After being crystallized from acetone–hexane, the 1-dehydrotestosterone so obtained, m.p. 169–171°, proved to be identical with that previously prepared (infrared spectrum).

6β-Hydroxy-1,4-androstadiene-3,17-dione (XIV).—A solution of 111 mg. of 1β,6β-dihydroxy-4-androstene-3,17-dione (VI) in 8 ml. of methanol was treated with 100 mg. of potassium hydroxide in 2 ml. of water. After having stood at room temperature for 4 hr., the reaction mixture was diluted with water, but no product separated. The clear, aqueous solution was extracted with methylene chloride; the methylene chloride solution was washed with water and then dried over sodium sulfate. Evaporation of the methylene chloride left 111 mg. of a glassy residue. Two crystallizations of this material from acetone–hexane yielded 46 mg. of 6β-hydroxy-1,4-androstadiene-3,17-dione (XIV) as silvery plates, m.p. 211–212°. A sample of XIV previously prepared from the diacetate VII by a similar procedure crystallized as needles, m.p. 203.5–204.5°; $[\alpha]_D$ +68°; λ_{max} 244 mμ (ε 15,600); $\lambda_{max}^{CHCl_3}$ 2.75, 2.90, 5.73, 6.00, 6.18, and a shoulder at 6.22 μ. The infrared spectra of these two materials in chloroform were identical.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.60; H, 7.97.

6β-Acetoxy-1,4-androstadiene-3,17-dione (XV).—A solution of 60 mg. of 6β-hydroxy-1,4-androstadiene-3,17-dione

in 1.0 ml. of pyridine and 1.0 ml. of acetic anhydride was allowed to stand overnight at room temperature. The excess acetic anhydride was decomposed by the addition of ice and water, and the crystalline product (47 mg., m.p. 181–182.5°) was isolated by filtration and then washed with water. One crystallization of this material from acetone–hexane gave the desired 6β-acetoxy-1,4-androstadiene-3,17-dione, m.p. 184–185.5°; $[\alpha]_D$ +59°; λ_{max} 244 mμ (ε 15,300); $\lambda_{max}^{CHCl_3}$ 5.74, 5.98, 6.15, 6.22 (shoulder), and 8.02 μ.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.65; H, 7.65. Found: C, 73.48; H, 7.48.

6β-Acetoxy-1,4-androstadiene-3,17-dione (XV) from 6β-Acetoxy-4-androstene-3,17-dione (XVI).—A solution of 0.500 g. (1.45 mmoles) of 6β-acetoxy-4-androstene-3,17-dione in 25 ml. of benzene was heated to boiling to free it from traces of water. To the dry solution was added 0.395 g. (1.74 mmoles, 1.20 equiv.) of 2,3-dichloro-5,6-dicyanobenzoquinone. The resulting solution was heated under reflux for 48 hr. It was then cooled, and the solution decanted from the precipitated hydroquinone. The hydroquinone was washed with benzene and then with ether. The combined organic solution was washed with saturated aqueous sodium sulfite, water, saturated aqueous sodium sulfite, then finally water in that order, dried over sodium sulfate, and then evaporated to dryness. The residue (388 mg.), after crystallization from dilute acetone then acetone–cyclohexane, yielded 308 mg. of 6β-acetoxy-1,4-androstadiene-3,17-dione (XV), m.p. 179.5–181°; $[\alpha]_D$ +58°, +60°; λ_{max} 243.4 mμ (ε 16,500). This material proved to be identical (m.m.p. and infrared spectrum) with that prepared from the fermentation product.

Hydrolysis of this 6β-acetoxy-1,4-androstadiene-3,17-dione (XV) with sodium hydroxide in aqueous methanol produced 6β-hydroxy-1,4-androstadiene-3,17-dione, m.p. 202–205°, identical (infrared spectrum) with that produced from the fermentation product.

The Reaction of Sulfur Tetrafluoride with Steroids[1]

DAVID G. MARTIN AND FRED KAGAN

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

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Sulfur tetrafluoride containing 20% hydrogen fluoride effected room temperature transformations of a variety of carbonyl-containing steroids into fluorinated derivatives in moderate to high yield. Included were the conversions of a carboxylic acid into its trifluoromethyl derivative and of a formyl group into a difluoromethyl group as well as of ketones at C-3, C-17, and C-20 into *gem*-difluoro groupings. This relatively mild procedure permitted the selective fluorination of the carbonyl groups mentioned above in steroids also containing acetate and α,β-unsaturated ketone functions.

The profound effects on the biological properties of certain steroids elicited by the introduction of fluorine[2] combined with the introduction of sulfur tetrafluoride as a reagent for the replacement of carbonyl oxygen with fluorine[3] prompted our investigations on steroid fluorination with this new reagent. Since the completion of our work, a report has appeared[4] on the conversion of cholestanone and several steroid diketones into *gem*-difluoro steroids with this reagent; however, low yields and restriction to comparatively simple steroids containing only ketone functions limit the utility of the reported procedure. The investigations reported herein led to procedures compatible with the preparation of relatively complex fluorinated steroids in moderate to good yields. An example of the degree of complexity feasible was the fluorination of a cortical steroid intermediate containing a dihydroxy acetone side chain protected as its bismethylenedioxy derivative.[5] A modified procedure was vigorous enough to convert lithocholic acid 3-acetate into its trifluoromethyl derivative Ib in moderate yield. To our knowledge this is the first report of the conversion of a steroid carboxylic acid into its trifluoromethyl derivative.

Although catalytic amounts of hydrogen fluoride have been found by other investigators[3,4] to favor the reaction of sulfur tetrafluoride with certain carbonyl groups, we found that the presence of *substantial amounts of hydrogen fluoride* was essential for the successful fluorination of a variety of steroids. Considerations of expediency and convenience in the exploration of steroid fluorination prompted our attempts at adapting the conditions of Hasek, Smith, and Engelhardt[3] to the fluorination of comparatively small samples. Investigations on the fluorination of relatively small amounts of stearic acid dramatically indicated that more than catalytic amounts of hydrogen fluoride were required for the successful conversion of a carboxyl group into its trifluoromethyl derivative. This transformation has been reported to proceed stepwise *via* an initial facile conversion into an acyl fluoride[3] with the liberation of an equivalent of hydrogen fluoride, *e.g.*,

$$-CO_2H + SF_4 \longrightarrow -COF + HF + SOF_2 \quad (1)$$

$$-COF + SF_4 \longrightarrow -CF_3 + SOF_2 \quad (2)$$

Significant results of three pertinent experiments on the fluorination of stearic acid are tabulated in Table I. These data made it readily apparent that a critical concentration of hydrogen fluoride was

(1) Presented at the Symposium on Fluorine Containing Compounds of Biological Interest, at the 140th Meeting of the American Chemical Society, September 6, 1961, Chicago, Illinois.

(2) *Cf.* L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, 1959, pp. 593, 682–686.

(3) W. R. Hasek, W. C. Smith, and V. A. Engelhardt, *J. Am. Chem. Soc.*, **82**, 543 (1960).

(4) J. Tadanier and W. Cole, *J. Org. Chem.*, **26**, 2436 (1961).

(5) D. G. Martin and J. E. Pike, to be published.