45a) of essentially single spot material, m.p. 206–215°. Recrystallization from acetone–ether afforded material, m.p. 215–225°, $[\alpha]^{chf}D$ +170°, λ_{max}^{MeOH} 238 mμ (16,800); λ_{max}^{Nujol} 2.76, 2.84, 5.74, 5.78, 6.0, 6.1 μ.

Anal. Calcd. for $C_{24}H_{33}O_6F$: C, 66.03; H, 7.61; F, 4.35. Found: C, 66.12; H, 7.77; F, 4.22.

16β-Methyl-9α-fluorohydrocortisone (46a).—To a stirred solution of 135 mg. of 16β-methyl-9α-fluorohydrocortisone acetate (46b) in 5 ml. of methanol, under nitrogen, was added 0.3 ml. of 1.0 *M* sodium methoxide. The reaction mixture was stirred at 24° for 10 minutes, then quenched by the addition of aqueous acetic acid (dropwise, until just acid) and blown down to one-half volume under nitrogen. The residue was diluted with 25 ml. of 75% saturated salt solution and extracted with ethyl acetate. The ethyl acetate extract was washed with saturated salt solution, dried over magnesium sulfate and concentrated, *in vacuo*, until crystallization occurred. After aging at 0°, the crystals were filtered, washed with ether and dried to yield 87 mg. (68%) of needles, m.p. 220–224°, $[\alpha]^{acetone}D$ +152°, λ_{max}^{MeOH} 239 mμ (16700); λ_{max}^{Nujol} 2.85–2.90, 5.83, 6.06, 6.15 μ.

Anal. Calcd. for $C_{22}H_{31}O_5F$: C, 66.98; H, 7.92; F, 4.81. Found: C, 67.12; H, 8.19; F, 4.76.

Additional good 46a was obtained on concentration of the mother liquors.

RAHWAY, N. J.

[CONTRIBUTION FROM THE BIOLOGICAL AND CHEMICAL RESEARCH DIVISIONS OF G. D. SEARLE & CO.]

Microbiological Transformations. V. 1α- and 2β-Hydroxylations of C_{19}-Steroids

BY R. M. DODSON, ARTHUR H. GOLDKAMP AND R. D. MUIR

RECEIVED FEBRUARY 8, 1960

Microbiological hydroxylations of steroids at positions 1α and 2β are recorded. Proofs for both the positions and configurations of the new hydroxyl groups are presented in detail. The 1α-hydroxylated products have been correlated through suitable chemical transformations with a 1β-hydroxysteroid obtained by degradation of the steroidal sapogenin, ruscogenin.

We have reported recently the microbiological hydroxylations of typical C_{19}-steroids at positions C-1 and C-2 by use of a species of *Penicillium*.[1] Of a number of steroids incubated with *Penicillium* sp. A.T.C.C. 12,556, only three—all C_{19}-steroids—were found to hydroxylate to a significant extent at position 1. These were 4-androstene-3,17-dione (I), androstane-3,17-dione (V) and dehydroisoandrosterone (VII). One of the substrates, 4-androstene-3,17-dione (I), also was found to hydroxylate at position 2. With other C_{19}- and C_{21}-steroids, hydroxylations at positions 2, 6, 7 and 15 also have been observed with this organism and will be reported at a later date.[2] In view of the novelty of 1- and 2-hydroxylations,[1,3] we wish to restrict ourselves here to recording in full our examinations of these reactions and of those chemical transformations which are significant in establishing and confirming our earlier structural assignments.[1]

Structures of the 1-Oxygenated Products. A. Positional Assignments.—Incubation of 4-androstene-3,17-dione (I) with *Penicillium* sp. A.T.C.C. 12,556 and subsequent chromatography gave rise to four new trioxygenated C_{19}- steroids (II, III, IVa, Chart I; and XII, Chart II). The position of the new hydroxyl group in II was based on the very facile elimination of acetic acid from its acetate to give 1,4-androstadiene-3,17-dione (IX), identified by comparison with authentic[4] material. Since both 2-acetoxyandrostenediones were known to us and were different from II acetate, and since these should not be converted readily to androstadienedione (IX), only C-1 remained for the position of the newly introduced hydroxyl group.

Initial assignment of position in the other two 1-oxygenated products III and IVa depended primarily on relating them through simple chemical transformations to the major product VIII obtained by incubation of dehydroisoandrosterone (VII) with this same organism. Oppenauer oxidation of VIII gave 1,4-androstadiene-3,17-dione. This result was best interpreted as a combination of the usual Oppenauer oxidation of a 3-hydroxy-Δ^5- to a 3-keto-Δ^4-system[5] and subsequent β-elimination of the second hydroxyl group. This placed the new hydroxyl function at position 1. Stepwise chemical reduction of VIII to IVa and VI, also obtained from I and III, respectively, served to correlate all of the 1-hydroxylated fermentation products with respect to positional assignment. Furthermore, the configurations of the new hydroxyl functions in III, IVa and VIII, though without stereochemical designation at this point, were thus shown to be identical. The remaining possible exception, though an unlikely one by analogy with the other fermentation products, was that of II.

Proof that II belonged to the same configurational series as the other 1-oxygenated products was obtained by correlation of II and VIII through a common transformation product. This is illustrated in Chart II, which shows the conversion of both II and VIII to 1,17β-diacetoxy-4-androsten-

(1) A preliminary communication of these results appeared in THIS JOURNAL, **79**, 3921 (1957). This organism has been designated as *Penicillium* sp., A.T.C.C. 12,556; G. D. Searle & Co. collection number M31-277.

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

(3) (a) H. L. Herzog, M. J. Gentles, E. B. Hershberg, F. Carvajal, D. Sutter, W. Charney and C. P. Schaffner, THIS JOURNAL, **79**, 3921 (1957); (b) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog and E. B. Hershberg, *ibid.*, **79**, 3922 (1957); (c) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt and J. M. Chemerda, *J. Org. Chem.*, **23**, 508 (1958); (d) M. Shirasaka, M. Tsuruta and M. Nakamura, *Bull. Agr. Chem. Soc. Japan*, **22**, 273 (1958); (e) M. Shirasaka, R. Takasaki, R. Hayashi and M. Tsuruta, *Bull. Agr. Chem. Soc. Japan*, **23**, 245 (1959).

(4) (a) H. H. Inhoffen, G. Zühlsdorff and Huang-Minlon, *Ber.*, **73B**, 451 (1940); (b) C. Djerassi and C. R. Scholz, *J. Org. Chem.*, **13**, 697 (1948).

(5) (a) R. V. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937); (b) C. Djerassi, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 207.

CHART I

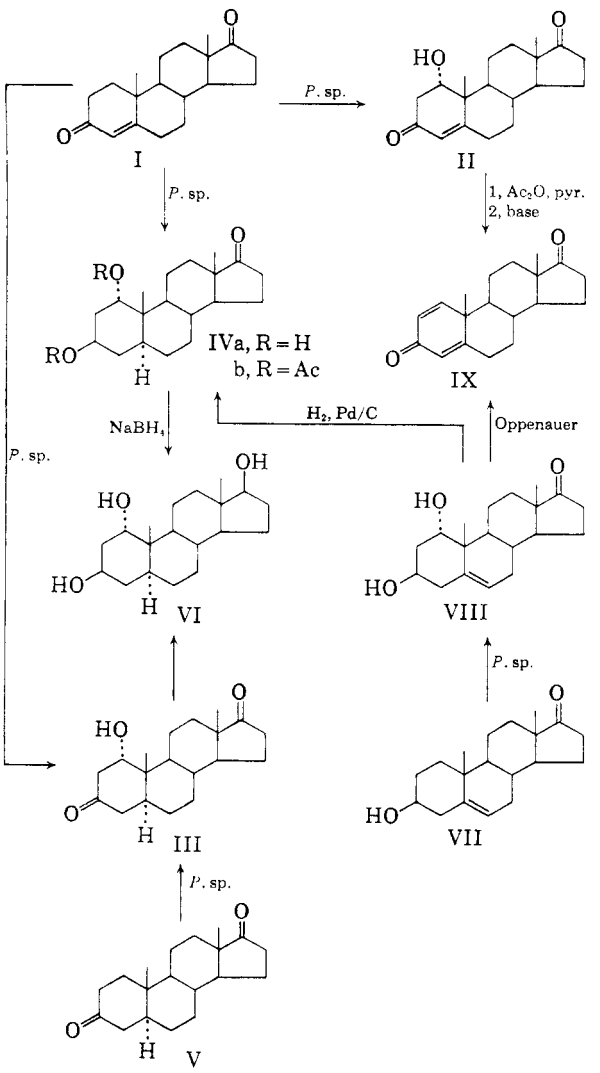
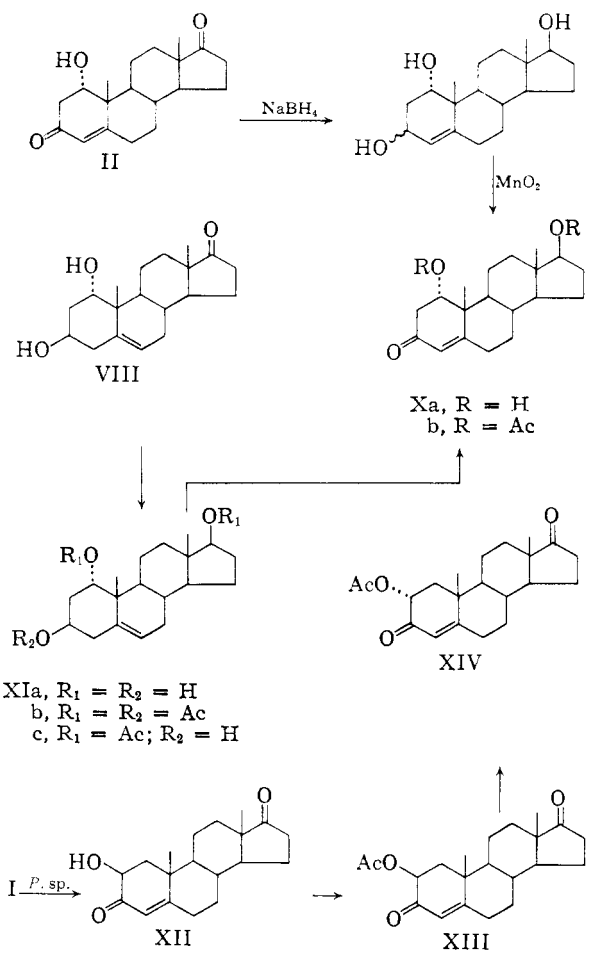


CHART II



3-one (Xb). Reduction of II with sodium borohydride gave a mixture of 4-androstene-1,3,17-triols epimeric at position 3, which in turn was oxidized, without prior separation, to 1α-hydroxytestosterone (Xa). Reduction of VIII, followed by triacetylation and then mild basic hydrolysis, gave XIc. Oxidation of the free hydroxyl group and isomerization of the double bond,[6] gave 1α-acetoxytestosterone acetate (Xb), which was identical with the product from direct acetylation of Xa obtained from II. This, incidentally, also confirmed our original interpretation of the Oppenauer oxidation of VIII (Chart I), which otherwise might be considered somewhat tenuous.

In view of the evidence (see below) for an α-assignment for the new 1-hydroxyl groups, it was interesting to note that the reduction of VIII to IVa (Chart I) was quite stereospecific and gave the A/B *trans* product in good yield. Thus, further reduction of IVa with sodium borohydride gave VI, which was correlated stereochemically at position 5 with III *via* microbiological hydroxylation of the known 5α-androstanedione (V). In view of the axial nature of the 1α-position and possible steric interference to reduction of a 5,6-double bond from the α-side, the observed stereospecificity in the reduction of VIII to IVa was somewhat unexpected.[7]

B. Configurational Assignment. Evidence from Molecular Rotatory Contributions.—In searching the literature, at the time this work was completed, for 1-hydroxylated-Δ^5- and 1-hydroxylated-Δ^4-systems, we found that there were no known reference compounds for comparison of optical rotations. On the other hand, in the saturated, A/B *trans* system, a number of reference compounds were available.[8] Comparison of molecular rotatory contributions in these compounds (A/B *trans* system) with those from our 1-oxygenated-Δ^5- and 3-keto-Δ^4-systems yielded ambiguous results, undoubtedly because of optical anomalies[9] resulting from the proximity of hydroxylated and unsaturated centers. For this reason, we turned our attention to our saturated 5α-androstanes for more reliable evidence of configuration.

(6) C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(7) The 1-keto function, on the other hand, exerted a profound effect on the reduction of a 5,6-double bond. In this case the major product had the A/B *cis* ring juncture; A. H. Goldkamp and R. M. Dodson, unpublished results.

(8) (a) P. Striebel and Ch. Tamm, *Helv. Chim. Acta*, **37**, 1094 (1954); (b) H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 3289 (1956); (c) *cf.* W. Schlegel and Ch. Tamm, *Helv. Chim. Acta*, **40**, 160 (1957), for a compilation of available data.

(9) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 783 (1948).

TABLE I

MOLECULAR ROTATORY CONTRIBUTIONS OF 1α-HYDROXY AND 1α-ACETOXY GROUPS

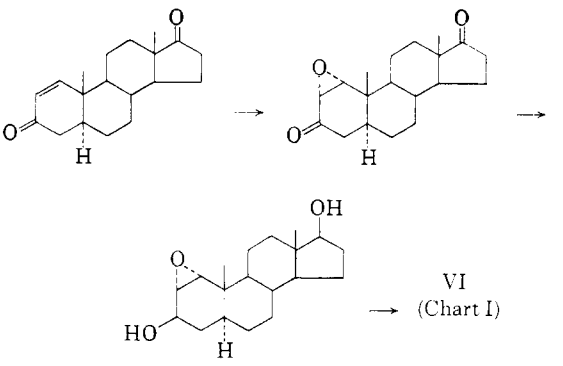
System	Parent compound[a]	ΔM_D[(1-OH) − (1-H)]	ΔM_D[(1-OAc) − (1-H)]
5α-Androstane	3β-Hydroxyandrostan-17-one	+18 (IVa)	
	3β-Acetoxyandrostan-17-one	+47[7]	+ 82 (IVb)
	Androstane-3β,17β-diol[b]	+44 (VI)	
5α-Androstan-3-one	Androstan-3-one	+ 6 (III)	
5-Androstene	3β-Hydroxy-5-androsten-17-one	+26 (VIII)	+123 (XVc)
	5-Androstene-3β,17β-diol[c]	+13 (XJa)	
	3β-Acetoxy-5-androsten-17-one	+ 2 (XVb)	+123 (XVa)
	3β,17β-Diacetoxy-5-androstene[c]		+108 (XIb)
	17β-Acetoxy-5-androsten-3β-ol		+123 (XIc)
4-Androsten-3-one	4-Androstene-3,17-dione	−14 (II)	+93
	17β-Hydroxy-4-androsten-3-one	−26 (Xa)	

[a] Values for parent compounds (unsubstituted at position 1) were taken from "Constants Selectionees Pouvoir Rotatoire Naturel. I. Steroids. Tables de Constantes et Donnees Numeriques Organisme Affilie de l'Union Internationale de Chimie Pure et Appliquee," Vol. 6, by J. P. Mathieu and A. Petit, unless otherwise noted. All rotation measurements were in $CHCl_3$. [b] A. L. Wilds and C. Djerassi, THIS JOURNAL, **68**, 2125 (1946). [c] Rotation of parent compound determined in these laboratories.

The values of ΔM_D for the 1-hydroxy-5α-androstanes (Table I) are in good agreement with the value of +35 ± 15 for 1α-hydroxycholestane.[8] This agreement can be reasonably contrasted with the value of −17 ± 15 for 1β-hydroxycholestane[8] and leads to an α-assignment in the present series. Confirmation of this assignment was obtained *via* 1α,3β-diacetoxy-5α-androstane-17-one, which gave a molecular rotatory contribution for the 1-acetoxy group of +82 (Table I). This compared favorably with 1α-acetoxycholestane [ΔM_D (1α-OAc) = +93 ± 16], but differed significantly from the 1β-epimer [ΔM_D(1β-OAc) = +27 ± 16].[8]

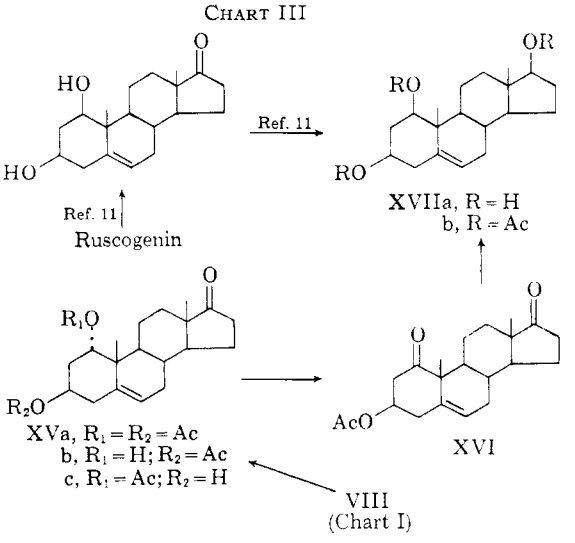
Evidence from Partial Synthesis.—In the course of our work, another group in these laboratories was investigating the structure of a new steroidal sapogenin, ruscogenin, which was first isolated by Lapin and Sannié, in 1955, and assigned the structure 19-hydroxydiosgenin.[10] Degradation of ruscogenin to an X-hydroxydehydroisoandrosterone and further conversion of this material to 1,4-androstadiene-3,17-dione[11a] by processes similar to ours (Chart I) showed that the X-hydroxyl group was, in fact, at position 1 rather than 19.[11] The hydroxydehydroisoandrosterone was reduced to an androstane-1,3β,17β-triol which was different from the androstane-1α,3β,17β-triol (VI), prepared by the route[11a,12] indicated in the adjoining chart. However, this partially synthetic androstane-1α,-3β,17β-triol (VI) was identical with the androstane-1,3β,17β-triol obtained in our work (Chart I). This firmly established the 1α-configuration of VI obtained *via* fermentation and, by the chemical interrelations discussed above (Charts I and II), of all the other 1-oxy compounds as well.

Since VI differed from the androstanetriol obtained by Benn, *et al.*, from ruscogenin, it was reasonably certain that their degradation product



was the 1β-epimer of VI. Direct confirmation that the two series of androstane derivatives differed only in configuration at position 1 is shown in Chart III. Monoacetylation of VIII, followed by chromium trioxide oxidation of the free hydroxyl group in XVb, gave 3β-acetoxy-5-androstene-1,17-dione (XVI). This, in turn, was reduced with either lithium aluminum hydride to give 5-androstene-1β,3β,17β-triol (XVIIa) or, alternatively, with sodium borohydride followed by saponification, to give both the 1β,3β,17β-triol XVIIa and its 1α-epimer XIa. The new triol XVIIa and its triacetate

CHART III



(10) (a) H. Lapin and Ch. Sannié, *Bull. soc. chim. France*, 1552 (1955); (b) Ch. Sannié and H. Lapin, *ibid.*, 1556 (1955).

(11) (a) W. R. Benn, F. Colton and R. Pappo, THIS JOURNAL, **79**, 3920 (1957). (b) After this work was completed, D. Burn, B. Ellis and V. Petrow [*Proc. Chem. Soc.*, 119 (1957)] also concluded that the hydroxyl group in ruscogenin was at C-1. However, they did not make a configurational assignment. See also Ch. Sannié and H. Lapin, *Bull. soc. chim. France*, **24**, 1237 (1957), and ref. 13.

(12) For proof of the stereochemical specificity for this sequence of reactions see ref. 8a and F. Sallmann and Ch. Tamm, *Helv. Chim. Acta*, **39**, 1340 (1956).

were identical, respectively, with the triol and triacetate obtained from ruscogenin[11] and differed conclusively from the 1α-epimers XIa and XIb.

Since the above comparisons definitively established the structure of ruscogenin as 1β-hydroxydiosgenin, it was of interest to compare the molecular rotatory contributions of the 1β-hydroxylated steroids derived from ruscogenin[13] with the molecular rotatory contributions of the 1α-hydroxylated steroids obtained here. For those cases for which the rotations in chloroform of the parent steroid were readily available,[14] the calculated rotatory contributions of the 1β-substituents were

Compound	ΔM_D[1β - OR) − (1 - H)][13,14]
1β,3β-Dihydroxy-5-pregnen-20-one	− 4.5
1β,3β-Dihydroxy-16α,17α-oxido-5-pregnen-20-one	− 36
1β-Hydroxy-4-pregnene-3,20-dione	−167
1β-Acetoxy-4-pregnene-3,20-dione	−371

In the first two of these, the values, when compared to the corresponding Δ^5-system of Table I, showed a somewhat lower contribution for 1β- than 1α-hydroxyl groups. The differences between the molecular rotatory contributions of the 1α- and 1β-hydroxyl and acetoxyl groups in the 3-keto-Δ^4-system are much more pronounced and should enable one to definitively assign configurations to 1-oxy-3-keto-Δ^4-steroids.

Structure of the 2-Oxygenated Product.—In addition to the 1-oxygenated products (II, III and IVa, Chart I) from the action of *Penicillium* sp. A.T.C.C. 12,556 on 4-androstene-3,17-dione, a fourth monohydroxylated product also was isolated. Its ultraviolet and infrared spectra confirmed the survival of the 3-keto-Δ^4-system and the 17-keto group, respectively. The presence of a hydroxyl function was also apparent from the infrared spectrum; its strongly negative molecular rotatory contribution [ΔM_D(OH) = −681] provided the first indication of its position. In general, contributions of hydroxyl groups, in the absence of unusual structural features, are of a low order of magnitude compared with those of more polarizable, unsaturated centers.[15] Thus, this high value suggested a vicinal type of interaction[9] between the new hydroxyl function and another functional group. This consideration would place the new hydroxyl group at positions 2, 4, 6 or 16.

The 6-position could be eliminated immediately, since both 6-hydroxy-4-androstene-3,17-diones are known[16] and differ markedly in properties from our material. Similarly, the properties of 16α-hydroxy-4-androstene-3,17-dione,[17] reported shortly after the isolation of our material, eliminated this substance from consideration. Two other positions, 2α and 4, show molecular rotatory increments of +25 and −30, respectively, for the hydroxyl groups; these were calculated from 2α[18]- and 4-hydroxytestosterone.[19] The 4-position may also be eliminated on the basis of the expected ultraviolet spectrum for this Δ^4-3,4-ketol, since it should absorb in the 280 mμ region of the ultraviolet[19,20] [the observed absorption was at 242 mμ (ε 14,200)]. The remaining 2β- and 16β-positions were both in accord with the observed positive "blue tetrazolium" test[21] characteristic of α-ketols.

Strong support for 2β-substitution was obtained by noting the characteristic change in ultraviolet spectrum with time in 0.1 *N* methanolic potassium hydroxide[22] (see Experimental). It remained, then, to establish the structure definitively by relating XII chemically to a compound of known structure. This was accomplished as shown at the bottom of Chart II. Conversion of XII to its acetate XIII gave material which had a molecular rotatory contribution for the 2β-acetoxy group of −556, in good agreement with that reported for 2β-acetoxytestosterone acetate (−581).[18] The acetate was then epimerized[23a] by heating with anhydrous potassium acetate in glacial acetic acid to give the known 2α-acetoxy-4-androstene-3,17-dione (XIV).[23b]

Experimental[24]

Fermentation of 4-Androstene-3,17-dione (I) with *Penicillium* sp. A.T.C.C. 12556.—A stainless steel fermentation tank of 40-l. capacity was charged with medium containing 1000 g. of dextrose, 200 g. of an enzymatic digest of whey protein, 90 ml. of corn steep liquor, 15 ml. of concentrated hydrochloric acid, 5.0 g. of silicone antifoam emulsion[25] and sufficient hot tap water to result in a final volume of approximately 30 l. after sterilization. The vessel and medium were sterilized by direct steam to a temperature of 120° and then were cooled to approximately 25° and maintained at that temperature during incubation.

The medium was inoculated with an aqueous suspension of spores of *Penicillium* sp. A.T.C.C. 12556. During an initial incubation period of 25.5 hours the culture was aerated at the rate of 35 l. of sterile air per minute through a perforated tube type of sparger located near the bottom of the fermentor. Continuous agitation of the culture was maintained by means of a vertically-mounted paddle type stirring device operated at 300 r.p.m.

4-Androstene-3,17-dione (I) (10.0 g.) dissolved in 750 ml. of ethanol was added to the culture and incubation was continued for 24 hours. During this second incubation period, aeration was reduced to 15 l. of sterile air per minute and agitation to 200 r.p.m. The steroids were recovered from the culture by extraction with an equal volume of methylene chloride in two portions.

(13) A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore and W. Charney, THIS JOURNAL, **79**, 4814 (1957); **81**, 5230 (1959).

(14) See footnote *a*, Table I.

(15) W. Klyne, in E. A. Braude and F. C. Nachod's "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, Chapter 3.

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

(17) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progr. Hormone Research*, XI, 149 (1955); *cf.* also J. Fried, D. Perlman, A. F. Langlykke and E. O. Titus, U. S. Patent 2,855,343, Oct. 7, 1958.

(18) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

(19) H. Levy and M. L. Mednick, U. S. Patent 2,762,818, Sept. 11, 1956.

(20) See L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(21) (a) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952); (b) A. S. Meyer and M. C. Lindberg, *ibid.*, **27**, 813 (1955).

(22) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(23) (a) R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, THIS JOURNAL, **77**, 661 (1955); (b) G. Rosenkranz, O. Mancera and F. Sondheimer, *ibid.*, **77**, 145 (1955).

(24) All melting points were taken on a Fisher–Johns melting point apparatus. 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.

(25) Antifoam AF Emulsion, Dow Chemical Corp., Midland, Mich.

The methylene chloride solution was evaporated to dryness. The residue was dissolved in 100 ml. of methanol, carbon-black was added, and the suspension was filtered to free the solution from insoluble silicone antifoaming agent. The methanol solution was evaporated to dryness, and the residue (10.54 g.) chromatographed on 1000 g. of silica gel. The column was washed with benzene, 5% ethyl acetate in benzene, 10% ethyl acetate in benzene, and 15% ethyl acetate in benzene. Elution of the column with 20% ethyl acetate in benzene gave 245 mg. of 4-androstene-3,17-dione (I), which after crystallization from aqueous acetone melted at 169–170° (no depression when mixed with an authentic sample).

Further elution of the column with 25 and 30% ethyl acetate in benzene yielded 387 mg. of 2β-hydroxy-4-androstene-3,17-dione (XII). By crystallization of this material from aqueous acetone and acetone–cyclohexane, there was obtained 144 mg. of pure 2β-hydroxy-4-androstene-3,17-dione, m.p. 143–145°, $[\alpha]_D$ −36.8°, λ_{max} 242 mμ, ε 14,200. The compound showed a positive "blue tetrazolium" test.[21] The ultraviolet spectrum of the compound in 0.1 *N* methanolic potassium hydroxide changed from λ_{max} 242 mμ (ε 14,200) to λ_{max} 230 mμ (ε 18,100); λ_{infl} 250 mμ (ε 7,120); λ_{max} about 360 mμ (ε 2,420) in 24 hours at room temperature.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.80; H, 8.71.

Further elution of the chromatographic column with 35% ethyl acetate in benzene yielded 227 mg. of material, which, after being crystallized from acetone–petroleum ether (b.p. 60–71°), aqueous methanol and acetone–cyclohexane, gave 85 mg. of 1α-hydroxyandrostane-3,17-dione (III), m.p. 204–206°, $[\alpha]_D$ +114°.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.95; H, 9.34.

Elution of the column with 40% ethyl acetate in benzene yielded 2.83 g. of crude 1α-hydroxy-4-androstene-3,17-dione. Crystallization of this material from methanol and acetone–cyclohexane gave 0.75 g. of pure 1α-hydroxy-4-androstene-3,17-dione (II), m.p. 215.5–218°, $[\alpha]_D$ +184°, λ_{max} 240 mμ (ε 15,000).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.32; H, 8.46.

Elution of the column with 100% ethyl acetate gave 415 mg. of crystalline material which, by crystallization from aqueous methanol and from acetone–cyclohexane, yielded 127 mg. of 1α,3β-dihydroxyandrostan-17-one (IVa), m.p. 200–201°, $[\alpha]_D$ +93°.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.52; H, 10.08.

Fermentation of Androstane-3,17-dione (V) with *Penicillium* sp. A.T.C.C. 12556.—A stainless steel fermenter of 40-l. capacity was charged with medium containing 25 g. of dextrose, 5.0 g. of cotton seed flour, 120 ml. of corn steep liquor, 40 g. of potassium dihydrogen phosphate, 2.5 g. of silicone antifoam emulsion[25] and sufficient hot tap water to result in a final volume of approximately 30 l. after sterilization. The vessel and medium were sterilized, cooled and inoculated as described for the fermentation of 4-androstene-3,17-dione.

Preliminary incubation was continued for a period of 24 hours with aeration at the rate of 5.0 l. of sterile air per minute and agitation at 200 r.p.m. Androstane-3,17-dione (V) (4.0 g.) dissolved in 250 ml. of acetone was added and incubation was continued for 16 hours. The steroids were recovered from the culture by extraction with an equal volume of methylene chloride in two portions.

The methylene chloride was removed and the residue chromatographed over silica gel (*ca.* 100:1 ratio) using benzene and benzene–ethyl acetate mixtures for elution. A crude mixture (872 mg.) eluted as a single peak with 35% ethyl acetate in benzene was crystallized from methylene chloride–ether to give 730 mg., m.p. 197–212°. Repeated recrystallization from this same solvent mixture ultimately afforded 290 mg. of 1α-hydroxyandrostane-3,17-dione (III), m.p. 211–213.5°, $[\alpha]_D$ +110°. The infrared spectra (chloroform) of this material and that isolated from the fermentation of 4-androstene-3,17-dione were identical.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.55; H, 9.37.

Ultraviolet spectra in 0.1 *N* methanolic potassium hydroxide taken at 0, 1, 4 and 24 hours after preparing the solution showed maximal absorption after 4 hours; λ_{max} 228 mμ, ε 8,760. Comparison with the spectrum of the authentic elimination product, 1-androstene-3,17-dione, indicated 82% elimination.[26]

Reduction of 30 mg. of 1α-hydroxyandrostane-3,17-dione in ethanol with sodium borohydride and isolation by dilution with water and extraction with chloroform gave a mixture of epimeric triols. Crystallization from ether–petroleum ether (b.p. 28–38°) gave 10 mg. of the crude 1α,3β,17β-triol (VI, see below), m.p. 225–237°. The infrared spectra (KBr pellet) of the crude and pure triols were identical.

Fermentation of Dehydroisoandrosterone (VII) with *Penicillium* sp. A.T.C.C. 12556.—A stainless steel fermentation tank having a capacity of 400 l. was charged with a medium containing 800 g. of sodium nitrate, 125 g. of magnesium sulfate heptahydrate, 0.65 g. of ferrous sulfate, 0.35 g. of manganous sulfate, 1,250 g. of sucrose, 30 ml. of 3% octadecanol in lard oil and 275 l. of hot tap water. The fermenter and medium were sterilized by heating with steam to 120° and then were cooled to approximately 25° and maintained at that temperature during incubation.

The medium was inoculated with an aqueous suspension of spores of *Penicillium* sp. A.T.C.C. 12556 and incubated for 55.5 hours during which time sterile air was passed through the growing culture by means of a perforated tube type of sparger at the rate of 30 l. of air per minute. The culture was mixed continuously by means of a vertically-mounted agitater operating at 165 r.p.m.

Dehydroisoandrosterone (VII, 100 g.) dissolved in 1200 ml. of acetone was added and incubation was continued for 12 hours. The steroids were recovered by extraction from the culture with 200 l. of methylene chloride in two portions.

The methylene chloride extract was concentrated to approximately 200 ml. with concurrent crystallization of the product. After further cooling in a Dry Ice–acetone-bath, the precipitate was separated by filtration and washed thoroughly with acetone, benzene and ether in that order to give 25.3 g. of 1α-hydroxydehydroisoandrosterone (VIII), m.p. 277–281°. Though material isolated in this manner was of sufficiently high purity for further synthetic transformations, it was found that recrystallization from either aqueous pyridine or tetrahydrofuran raised the melting point to 288–290°, $[\alpha]_D$ +10.6° ($CHCl_3$), $[\alpha]_D$ +31.5° (ethanol).

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.92; H, 9.21.

The mother liquors from the isolation of 1α-hydroxydehydroisoandrosterone were freed of solvent and the residue chromatographed over silica gel (*ca.* 100:1 ratio). Benzene and benzene–ethyl acetate mixtures containing progressively increasing proportions of ethyl acetate were used for elution. 1α-Hydroxy-4-androstene-3,17-dione (II) was obtained with 40% ethyl acetate and was recrystallized from methylene chloride–ether to give 2.83 g., m.p. 211–216°. By further recrystallization from methylene chloride–ether the melting point could be raised to 216–218°. This material had an infrared spectrum identical with that of material isolated from the fermentation of 4-androstene-3,17-dione (see above).

1α-Acetoxy-4-androstene-3,17-dione.—A solution of 51 mg. of 1α-hydroxy-4-androstene-3,17-dione in 1.00 ml. of pyridine and 1.00 ml. of acetic anhydride was allowed to stand at room temperature overnight. The solution was then diluted with petroleum ether (b.p. 60–71°), but since no precipitate separated, the resulting solution was evaporated to dryness under a jet of nitrogen. Crystallization of the residue from aqueous methanol, then aqueous acetone yielded 27 mg. of 1α-acetoxy-4-androstene-3,17-dione, m.p. 112.5–113.5°, $[\alpha]_D$ + 191.7°, λ_{max} 239.5 mμ (ε 16,500).

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.71; H, 8.07.

1,4-Androstadiene-3,17-dione (IX) from 1α-Acetoxy-4-androstene-3,17-dione.—A solution of 100 mg. of 1α-acetoxy-4-androstene-3,17-dione in 50 ml. of 4:6 water–methanol containing 100 mg. of sodium hyroxide was allowed

(26) The spectrum of authentic 1-androstene-3,17-dione was kindly supplied by Dr. Willard M. Hoehn of these laboratories.

to stand at room temperature for 4 hours. The solution was extracted with 100 ml. of ethyl acetate in three portions. The ethyl acetate solution was dried over sodium sulfate, then evaporated to dryness. Crystallization of the residue from acetone–cyclohexane, then from aqueous acetone yielded 25.3 mg. of 1,4-androstadiene-3,17-dione (IX), m.p. 139–140°, $[\alpha]D$ +118°, λ_{max} 242.5 mμ (ε 15,100). The identity of this material with an authentic sample of 1,4-androstadiene-3,17-dione[4] was established by comparison of infrared spectra and by the lack of depression in the melting point of a mixture of the two.

Anal. Calcd. for $C_{19}H_{24}O_2$: C, 80.23; H, 8.51. Found: C, 79.97; H, 8.59.

1,4-Androstadiene-3,17-dione (IX) from 1α-Hydroxydehydroisoandrosterone (VIII).—A solution of 1.00 g. of 1α-hydroxydehydroisoandrosterone in 40 ml. of toluene and 15 ml. of cyclohexanone was distilled at atmospheric pressure to ensure dryness of the solution (25 ml. of distillate was collected). Then 7.5 ml. of a toluene solution containing 1.9 g. of aluminum isopropoxide was added and the resulting solution was heated under reflux for 0.5 hour. The reaction mixture was poured into a saturated Rochelle salt solution and the toluene and cyclohexanone removed by steam distillation. The semi-solid product was separated by filtration and washed with water. The residue was chromatographed on 85 g. of silica gel and the chromatogram was developed with benzene containing increasing quantities of ethyl acetate. Elution of the column with 15% ethyl acetate in benzene yielded 458 mg. of crystalline 1,4-androstadiene-3,17-dione (IX), Crystallization of a center fraction (124 mg.) of this material from acetone–petroleum ether (b.p. 60–71°), then ether gave pure 1,4-androstadiene-3,17-dione, m.p. 140–141.5° (identity established by mixed m.p. and comparison of infrared spectra).

Further elution of the column with 25% ethyl acetate in benzene gave a small quantity of material, m.p. 219–221°, which, because of the quantity produced, was not further identified.

1α,3β-Dihydroxyandrostan-17-one (IVa).—1α-Hydroxydehydroisoandrosterone (5.0 g.) was suspended in 75 ml. of ethanol and reduced at atmospheric pressure in the presence of 5% palladium-on-carbon. The catalyst was separated by filtration and the ethanol removed to give 4.8 g. of residue melting at 195–199°. Crystallization of this material from methanol–benzene gave 3.5 g. of 1α,3β-dihydroxyandrostan-17-one, m.p. 202–203.5°, $[\alpha]D$ +90°. The infrared spectrum of this material was identical with that of material produced through the action of *Penicillium* sp. A.T.C.C. 12556 on androstene-3,17-dione (see above).

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.32; H, 9.74.

1α,3β-Diacetoxyandrostan-17-one (IVb).—1α,3β-Dihydroxyandrostan-17-one (500 mg.) was dissolved in 3 ml. of pyridine and 2 ml. of acetic anhydride. The solution was kept at room temperature for 24 hours. Attempted workup at this point indicated incomplete acetylation, m.p. of crude product (640 mg.) was 115–140° (see below). Retreatment of the material with 2 ml. of acetic anhydride in 3 ml. of pyridine at 100° for 2.5 hours and dilution with ice-water gave 580 mg. of crude product, m.p. 235–244°. Crystallization from methylene chloride–ether gave 390 mg., m.p. 244–246.5°, $[\alpha]D$ +77°.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.67; H, 8.71.

Androstane-1α,3β,17β-triol (VI).—Reduction of 500 mg. of 1α,3β-dihydroxyandrostane-17-one with sodium borohydride in aqueous ethanol gave 330 mg. of androstane-1α,3β,17β-triol, m.p. 238–239°, $[\alpha]D$ +20°. A mixture with a sample (m.p. 236–238°) prepared by stepwise reduction[11a] of 1α,2α-oxidoandrostane-3,17-dione[27] showed no depression in melting point. The infrared spectra (KBr pellet) of the two samples were identical.

Anal. Calcd. for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 73.99; H, 10.47.

5-Androstene-1α,3β,17β-triol (XIa).—1α-Hydroxydehydroisoandrosterone (6.25 g.) was dissolved in 400 ml. of tetrahydrofuran at reflux. A solution of 9.0 g. of sodium borohydride in 15 ml. of water and 100 ml. of ethanol was then added over approximately 10 minutes with stirring. After an additional 10 minutes, the reaction mixture was diluted with 300 ml. of water, acidified with dilute hydrochloric acid, and the tetrahydrofuran and ethanol were removed under reduced pressure. The product crystallized during this distillation. The mixture was further cooled, filtered, and the product thoroughly dried under reduced pressure to give 5.5 g. (88%) of 5-androstene-1α,3β,17β-triol. It exhibited a double melting point with partial melting at approximately 130° and a final melting point after resolidification at 211–217°. The melting point could be raised to 216–218.5° by recrystallization from acetone–benzene; $[\alpha]D$ −55° ($CHCl_3$), −29° (CH_3OH).

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.30; H, 10.17.

1α,3β,17β-Triacetoxy-5-androstene (XIb).—A solution of 4.0 g. of 5-androstene-1α,3β,17β-triol in 15 ml. of pyridine and 15 ml. of acetic anhydride was heated on the steam-bath for 3 hours. Dilution with ice-water gave 4.21 g. (74%) of the triacetate, m.p. 179–184°. Crystallization from ether–petroleum ether (b.p. 28–38°) gave material of m.p. 179–181°, $[\alpha]D$ −34°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.11; H, 8.24.

1α,17β-Diacetoxy-5-androsten-3β-ol (XIc).—A mixture of 1.50 g. of 1α,3β,17β-triacetoxy-5-androstene, 0.40 g. of potassium bicarbonate and 20 ml. of methanol was maintained at reflux for 20 minutes. Dilution with ice-water gave a crude product which, after thorough drying, was chromatographed over silica gel using benzene and benzene–ether mixtures for elution. Elution with 5–15% ether in benzene gave 350 mg. of starting material. A total of 660 mg. of desired product was obtained by elution with 25 and 35% ether in benzene. Crystallization from ether–petroleum ether (b.p. 28–38°) gave 550 mg. of pure 1α,17β-diacetoxy-5-androsten-3β-ol, m.p. 195–196.5°, $[\alpha]D$ −26°.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.64; H, 8.90.

1α,17β-Diacetoxy-4-androsten-3-one (Xb).—1α,17β-Diacetoxy-5-androsten-3β-ol (250 mg.) in 5 ml. of acetone was treated with 0.22 ml. of chromium trioxide reagent[6,28] (2.67 *M*) over a 1–2 minute period. Dilution with ice-water gave 250 mg. of material melting at 159–168°. Its infrared spectrum showed absence of any hydroxyl function and absorption at 5.82 μ due to the newly formed 3-carbonyl group. The ultraviolet spectrum indicated approximately 30% of the 3-keto-Δ^4 and/or the 3-keto-$\Delta^{1,4}$-system (λ_{max} 240 mμ [ε 4,000]).

An aqueous solution of oxalic acid (1.0 ml. of 1.5%) was added to a solution of 190 mg. of the above material in 5 ml. of methanol. The resulting solution was heated on a steam-plate for 45 minutes and then diluted with ice-water to give 175 mg. of material melting at 171–178° dec. Three recrystallizations from methylene chloride–petroleum ether (b.p. 28–38°) gave 90 mg. of 1α,17β-diacetoxy-4-androsten-3-one (Xb), m.p. 188–189°, with slight decomposition; λ_{max} 240 mμ (ε 12,700). The infrared spectrum had the expected absorption due to the conjugated keto function at 5.96 μ.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.60; H, 7.97.

Chromatography of 75 mg. of this material over silica gel, on elution with benzene and 5% ether–benzene followed by crystallization from ether–petroleum ether (b.p. 60–71°), gave 21.5 mg. of 1α,17β-diacetoxy-4-androsten-3-one, m.p. 191–194°, λ_{max} 241 (ε 13,900). The infrared spectrum (KBr pellet) was identical with that of the unchromatographed material.

1α,3β-Dihydroxy-4-androsten-3-one (1α-Hydroxytestosterone) (Xa).—A solution of 1.67 g. of 1α-hydroxyandrostenedione in 20 ml. of ethanol was added over 10 minutes to a solution of 2.80 g. of sodium borohydride in 5 ml. of water and 10 ml. of ethanol. After having stood another 5 minutes, the reaction mixture was diluted with water, neutralized with glacial acetic acid and extracted four times with chloroform. The combined chloroform extracts were dried and the organic solvents removed under reduced pressure.

(27) W. M. Hoehn, *J. Org. Chem.*, **23**, 929 (1958).

(28) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

The residue was taken up in 110 ml. of chloroform and treated with 10 g. of manganese dioxide at room temperature with stirring for 6.25 hours. The reaction mixture then was filtered through Super-cel and the chloroform evaporated under a jet of nitrogen.

The residue was crystallized from acetone to give 370 mg. of 1α-hydroxytestosterone, m.p. 234–252° dec. Though the infrared spectrum (KBr pellet) was identical with that of purer material (see below), paper chromatography indicated the presence of approximately 10% of a more polar component—probably unoxidized 1,3,17-triol. Recrystallization of 220 mg. of this crude product from acetone gave 170 mg. of pure 1α-hydroxytestosterone (Xa), m.p. 250–254° dec. on a block preheated to 240°, λ_{max} 242 mμ (ε 14,800), $[\alpha]_D$ +103°. Paper chromatography showed only one component.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.90; H, 9.34.

Acetylation of 1 mg. of this material in 2 drops of acetic anhydride and 3 drops of pyridine (24 hours at room temperature) gave, after isolation by addition of water and one crystallization from methanol–water, 0.7 mg. of the diacetate Xb, m.p. 190–203°. The infrared spectrum of this material (KBr pellet) was identical with that of material prepared from 1α,17β-diacetoxy-5-androsten-3β-ol (XIc) as described above.

1α,3β-Diacetoxy-5-androsten-17one (XVa) and 3β-Acetoxy-1α-hydroxy-5-androsten-17-one (XVb).—A mixture of 10 g. of 1α-hydroxydehydroisoandrosterone (VIII), 3.62 ml. (50% excess for monoacetylation) of acetic anhydride and 50 ml. of pyridine was stirred at room temperature for 3 days. The pyridine and residual acetic anhydride then were removed under reduced pressure using toluene for co-distillation of the last traces of these materials.

The residue was chromatographed over silica gel (*ca.* 100:1 ratio) using benzene and then benzene–ethyl acetate mixtures for elution. The diacetate, 1α,3β-diacetoxy-5-androsten-17-one (XVa, 1.27 g., 10%) was eluted with 10–12% ethyl acetate in benzene. Though sufficiently pure (infrared) for subsequent transformations, this material could be purified further by crystallization from methylene chloride–ether. In this manner, material of m.p. 230–231°, $[\alpha]_D$ +25°, was obtained.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.88; H, 8.33.

The monoacetate 3β-acetoxy-1α-hydroxy-5-androsten-17-one (XVb, 5.62 g., 50%) was also obtained in an essentially pure state (infrared) by elution with 15–25% ethyl acetate in benzene. Further purification could be effected by recrystallization from acetone–ether to give material of m.p. 243–244°, $[\alpha]_D$ −6.7°.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.58; H, 8.83.

Elution with 80% ethyl acetate gave 1.7 g. (17%) of starting material.

1α-Acetoxy-3β-hydroxy-5-androstene-17-one (XVc).—To a solution of 2.15 g. of 1α,3β-diacetoxy-5-androsten-17-one in 50 ml. of ethanol was added 2.2 ml. of 2.5 *N* aqueous sodium hydroxide. After heating for 50 minutes under reflux the reaction mixture was diluted with ice-water (*ca.* 12 volumes) and the crude product mixture collected. Attempted fractional crystallization from methanol–benzene gave, as the only pure material, 210 mg. of 1α-hydroxydehydroisoandrosterone, m.p. 279–284°, which was identified by its infrared spectrum.

The methanol–benzene mother liquors from the above operations were combined, solvent removed and the residue chromatographed over silica gel (*ca.* 100:1 ratio) using benzene and benzene–ethyl acetate mixtures for elution. Fractions of the desired product totaling 780 mg. (47%), with melting points in the range of 206–214°, were eluted with 20% ethyl acetate in benzene. Crystallization of this material from methylene chloride–ether gave 540 mg. of pure 1α-acetoxy-3β-hydroxy-5-androsten-17-one, m.p. 213–215°, $[\alpha]_D$ +37°.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.60; H, 8.74.

3β-Acetoxy-5-androstene-1,17-dione (XVI).—Oxidation of 1.0 g. of 3β-acetoxy-1α-hydroxy-5-androsten-17-one (XVb) in 20 ml. of acetone with 1.0 ml. of 2.67 *M* chromium trioxide reagent[28] and then crystallization from acetone–petroleum ether (b.p. 60–71°) gave 730 mg. of 3β-acetoxy-5-androstene-1,17-dione, m.p. 154–156°. The melting point was raised to 156.5–159° by further recrystallization from ether–petroleum ether (b.p. 60–71°), $[\alpha]_D$ +40.7°; λ_{max}^{KBr} 5.75, 5.83, and 8.0 μ. The ultraviolet spectrum showed no selective absorption.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.27; H, 8.27.

5-Androstene-1β,3β,17β-triol (XVIIa). A.—A solution of 410 mg. of 3β-acetoxy-5-androstene-1,17-dione in 80 ml. of anhydrous ether was added over 30 minutes to 700 mg. of lithium aluminum hydride in 150 ml. of anhydrous ether maintained at reflux. After a total of 2.5 hours, the reaction was quenched with acetone and water in that order. After washing with saturated ammonium chloride solution and then water, the ether solution was dried and the ether removed. Crystallization of the residue from methanol–benzene gave 200 mg. of 5-androstene-1β,3β,17β-triol (XVIIa), m.p. 256–274° dec.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.23; H, 9.77.

Its infrared spectrum (KBr pellet) was identical with that of a sample obtained by sodium borohydride reduction of XVI followed by hydrolysis (see below) and also with that of a sample prepared from ruscogenin[11a] by Benn, *et al.* There was no indication of the presence of any of its 1α-epimer (infrared).

Further recrystallization from methanol–benzene and finally from methanol–chloroform (in which the 1α-epimer is considerably more soluble) gave material melting at 259–274° dec. (block preheated to 245°) and 274–278° dec. (block preheated to 270°), $[\alpha]_D$ −40° (CH_3OH). Benn, *et al.*, report m.p. 275–280° dec.[11a]

B.—To a solution of 1.3 g. of 3β-acetoxy-5-androstene-1,17-dione in 20 ml. of methanol was added a solution of 500 mg. of sodium borohydride in 2 ml. of water and 10 ml. of methanol. After 2 hours, the reaction mixture was diluted with ice-water and the crude product collected. It was dissolved in 20 ml. of methanol and 2 ml. of 2.5 *N* aqueous sodium hydroxide and the solution heated under reflux for 30–45 minutes. The aqueous filtrate from isolation of the crude reduction product was treated similarly with 5 ml. of 2.5 *N* sodium hydroxide. The two hydrolysis mixtures then were combined and cooled to give 650 mg. (56%) of 5-androstene-1β,3β,17β-triol, m.p. 270–278° dec. Chromatography of this material over silica gel, on elution with 65% ethyl acetate in benzene, also gave material of m.p. 270–278° dec. This compound was identical (m.m.p. and infrared) with the triol (m.p. 275–280° dec.) obtained from ruscogenin by Benn, Colton and Pappo[11a]; its infrared spectrum (KBr pellet) was also identical with that of the triol prepared above by the lithium aluminum hydride reduction.

Extraction of the aqueous filtrate from isolation of the 1β,3β,17β-triol with chloroform gave, after drying and removal of the solvent, a residue of the crude 1α-epimer, m.p. 195–210°. Crystallization of this material from acetone–benzene gave 300 mg. (26%) of 5-androstene-1α,-3β,17β-triol (XIa), m.p. 214–217°. Its infrared spectrum (KBr pellet) was identical with that of the triol obtained by reduction of 1α-hydroxydehydroisoandrosterone; mixed m.p. 214–219°.

1β,3β,17β-Triacetoxy-5-androstene (XVIIb).—A solution of 25 mg. of 5-androstene-1β,3β,17β-triol, obtained by lithium aluminum hydride reduction of 3β-acetoxy-5-androstene-1,17-dione, in 1 ml. of pyridine and 0.5 ml. of acetic anhydride was heated on the steam-bath for 1 hour. Dilution with ice-water gave 1β,3β,17β-triacetoxy-5-androstene, m.p. 140–142°. Crystallization of this material from ether–petroleum ether (b.p. 28–38°) raised the m.p. to 147.5–148.5°, $[\alpha]_D$ −32.4°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.11; H, 8.54.

Acetylation of a sample of 5-androstene-1β,3β,17β-triol obtained by degradation[11a] of ruscogenin and crystallization of the product from ether–petroleum ether (b.p. 28–38°) gave the identical 1β,3β,17β-triacetoxy-5-androstene, m.p. 145–147°; mixed m.p. with the sample prepared above, 146–148°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.24; H, 8.57.

The infrared spectra (KBr pellet) of the two preparations were identical and had a band at 12.3 μ not present in the infrared spectrum of the 1α-epimer. The 1α-epimer, on the other hand, absorbed at 12.5 μ. These bands probably are the result of C–H out-of-plane deformation at position 6.

2β-Acetoxy-4-androstene-3,17-dione (XIII).—A solution of 51 mg. of 2β-hydroxy-4-androstene-3,17-dione in 1 ml. of pyridine and 1 ml. of acetic anhydride was allowed to stand at room temperature for 1.5 hours. It then was diluted with ice and water and the precipitate was separated by filtration. Crystallization of this material from aqueous acetone yielded 42.5 mg. of 2β-acetoxy-4-androstene-3,17-dione, m.p. 157–158°, $[\alpha]D$ −5.9°, λ_{max} 243 mμ (ϵ 15,300).

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.47; H, 8.04.

2α-Acetoxy-4-androstene-3,17-dione (XIV).—The 2β-acetoxy-4-androstene-3,17-dione, obtained from 48 mg. of 2β-hydroxy-4-androstene-3,17-dione by the procedure described above, was heated under reflux for 4 hours in a solution of 2.00 g. of anhydrous potassium acetate in 10 ml. of acetic acid. The excess acetic acid was removed under vacuum, the residue was diluted with 40 ml. of water, and the resulting suspension was extracted with 50 ml. of ether in three portions. The ether solution was washed with water, dilute aqueous sodium carbonate, then water. The ether solution then was dried over sodium sulfate and evaporated to dryness. Crystallization of the residue (34 mg.) from acetone–cyclohexane, then aqueous acetone gave 13.8 mg. of 2α-acetoxy-4-androstene-3,17-dione (XIV), m.p. 209–211°, $[\alpha]D$ +147°, λ_{max} 239.5 mμ (ϵ 15,500). The reported constants[23b] are m.p. 209–210°, $[\alpha]D$ +146° ($CHCl_3$), λ_{max} 241 mμ (log ϵ 4.21).

CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIF.]

The Introduction of Oxygen and Nitrogen into the B Ring of the Steroid Nucleus[1]

BY THOMAS L. JACOBS AND ROBERT B. BROWNFIELD

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The degradation of cholesterol to suitable intermediates open in the B ring was accomplished by the ozonization of 7-ketocholesteryl acetate to give either 5-keto-5,7-seco-6-nor-3-cholesten-7-oic acid or 3β-acetoxy-5-keto-5,7-seco-6-norcholestan-7-oic acid depending upon the method of isolation employed. Treatment of either of the δ-keto acids with ethanolic ammonia afforded 6-aza-2,4-cholestadien-7-one. The reaction of 5-keto-5,7-seco-6-norcholestan-7-oic acid with ethanolic ammonia under pressure or with benzylamine gave 6-aza-4-cholesten-7-one and the corresponding N-benzylated enamine lactam, respectively. Treatment of these enamine lactams with N-bromosuccinimide resulted in C_4-vinyl rather than C_3-allyl bromides. Lithium aluminum hydride reduction of 6-aza-4-cholesten-7-one and its hydrogenation product, 6-aza-cholestan-7-one, gave 6-aza-5-cholestene and 6-aza-cholestane, respectively. 6-Oxacholestan-7-one was obtained by sodium borohydride reduction of methyl 5-keto-5,7-seco-6-norcholestan-7-oate. 6-Oxacholestane was prepared by the cyclodehydration of 5,7-seco-6-norcholestane-5β,7-diol.

The synthetic modification of naturally occurring steroids during the past decade has resulted in the discovery of a number of potent, highly specific, commercially important therapeutic agents. Excepting for the 19-nor- and 18,19-bisnor-series, none of these has involved modification of the basic carbon skeleton of the steroid nucleus itself. Actual examples of the substitution of nitrogen or oxygen for carbon in the otherwise intact ring system are comparatively few. Although no attempt will be made here to name individually all of the oxa- and aza-steroids in the literature, it does seem appropriate to give a reasonably complete list of general types. These are: 2-aza-,[2] 3-aza-,[2,3] 3-aza-A-homo-,[3–5] 3,4-diaza-,[6] 3-oxa-A-homo-,[4,5,7] 3-oxa-A-nor-,[6,8] 4-aza-,[3,9–12a] 4-aza-A-homo-,[3] 4-oxa-,[5,9,13–17] 4-oxa-A-homo-,[4,5,18] 6-oxa-B-homo-,[19] 7-aza-B-homo-,[20] 7-oxa-B-homo-,[20,21] 7a-aza-B-homo-,[20,22] 7a-oxa-B-homo-,[20,23] 12a-aza-C-homo-,[24] 15-aza-D-homo-,[25] 16-aza-,[26] 16-aza-D-homo-,[25] 17-aza-D-homo-,[25,27,28] 17a-aza-D-homo-[27,29,30] and 17a-oxa-D-homo-.[16,31] Inspection of this list re-

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