Reaction of I with methyl ethanethiolsulfonate (VII). To 0.2 mol. of I, 0.2 mol. of VII was added, and the mixture was allowed to stand at room temperature for two weeks. There was no change in color. The mixture was then distilled yielding 5.27 g. of methyl disulfide (58% recovery) and 1.39 g. of a fraction boiling 57–63° (12 mm.). The latter was diluted with ether and caused to react with *p*-toluidine and yielded 15.7 g. of ethanesulfon-*p*-toluidide, corresponding to a 40% yield of ethanesulfonyl chloride.

Reaction of I with ethyl ethanesulfinate (V). Ethyl ethanesulfinate, $C_2H_5S(=O)OC_2H_5$ (V) (0.2 mol.), was added dropwise to 0.2 mol. of I at −20°. The mixture gradually faded in color and was nearly colorless by the time it had warmed to room temperature. It was heated to 90° to drive off volatile matter and was then cooled to await distillation.

The volatile portion after purification consisted of ethyl chloride (3.0 g., 23%). Molecular wt.: Calcd., 64.52; found, 66. Boiling pt.: Reported, 12.3°; found, 12–13°.

Distillation of the residual reaction mixture yielded 8.5 g. of methyl disulfide, identified by boiling point and refractive index and 12.4 g. of ethanesulfonyl chloride (VI). The latter was identified by conversion to the *p*-toluidide which melted at 81° and unchanged when mixed with an authentic sample.

More than 20% of the original V was recovered unchanged.

Reaction of I with methanol. In an attempt to prepare methyl methanesulfenate, $CH_3S—O—CH_3$ (IX), 0.6 mol. of I was added slowly to 1.2 mol. of well stirred methanol at −20°. The resulting colorless reaction mixture was then distilled but no product with the properties expected of IX was found. There was obtained, however, 12.5 g. of III or 66% yield on the basis of the postulated reactions described above.

The reaction was repeated using 1.6 mol. I and 0.8 mol. of methanol under conditions which would insure the recovery of any methyl chloride and methyl disulfide formed. A 55% yield of methyl chloride, identified by boiling point and molecular weight, was obtained. Methyl disulfide, having properties identical to an authentic sample, was recovered in 70% yield.

Reaction of I with water. Water (0.25 mol.) was added dropwise to well stirred I (0.25 mol.) at −20°. At first there was little evidence of reaction but gradually the evolution of hydrogen chloride became apparent and when the last of the water had been added the mixture was only faintly yellow. After warming slowly to room temperature, the reaction mixture was distilled and yielded 8.0 g. of methyl disulfide and 12.0 g. of III. The yields were 64% and 72%, respectively, based on the reactions outlined above. Both products proved identical to authentic samples.

ORONO, ME.

[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE UNIVERSITY OF PENNSYLVANIA]

Investigations on Steroids. XXX. New Transformation Products of Strophanthidin: 19-Hydroxytestosterone, 19-Hydroxy-1-dehydrotestosterone Diacetate and Estradiol-17β[1-3]

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Improvements in the synthesis from strophanthidin of 19-hydroxy-Δ⁴-androstene-3,17-dione (II) are presented. The role of II as a possible key intermediate in the metabolic transformation of androgens into estrogens is pointed out. Under specific conditions, reduction of II with sodium borohydride gave mainly 19-hydroxytestosterone (IV) and, as a by-product, Δ⁴-androstene-3β,17β,19-triol (VI). By treatment with selenium dioxide, 19-hydroxytestosterone diacetate (V) was converted into 19-hydroxy-1-dehydrotestosterone diacetate (IX). Even under mild alkaline conditions, it was not possible to saponify IX to the free 19-hydroxy-1-dehydrotestosterone (VIII). By the action of mild alkali, IX is rapidly transformed into the 17-monoacetate of estradiol-17β (XI), whereas with stronger alkali, free estradiol-17β (X) is obtained. The physiological activities of IV and IX are discussed.

The synthesis from strophanthidin of analogs of steroid hormones oxygenated in the 19- position was reported from this laboratory some time ago.[6–10] A number of such products were subsequently isolated from various biological systems. Thus, several 19-hydroxy steroids have been isolated from adrenocortical extracts.[11] Hydroxyl groups have been introduced into the 19- position either by incubation with beef adrenal homogenates

(1) This paper is dedicated to the memory of Lyndon F. Small, former editor of this journal.

(2) This investigation was supported by research grants (CY757-C5 and CY757-C6) from the National Cancer Institute of the National Institutes of Health, Public Health Service. A part of the K-Strophanthin used in this investigation was kindly donated by S. B. Penick & Co., New York, N. Y.

(3) The essential findings of this paper were presented on September 5, 1958, at the 4th International Congress of Biochemistry in Vienna (*cf.* Maximilian Ehrenstein: Biochemistry of the Corticoids, Proceedings of the Fourth International Congress of Biochemistry, Vol. 4 (Symposium: Biochemistry of Steroids), Pergamon Press, p. 259 (1959).

(4) Dr. Klaus Otto was the recipient of a Fulbright Travel Grant and was on leave of absence from the Physiologisch-chemisches Institut der Universität Bonn, West Germany.

(5) In collaboration with G. Winston Barber.

(6) G. W. Barber and M. Ehrenstein, *J. Am. Chem. Soc.*, **76**, 2026 (1954).

(7) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 19, 1758 (1954).

(8) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 20, 1253 (1955).

(9) M. Ehrenstein and M. Dünnenberger, *J. Org. Chem.*, 21, 774 (1956).

(10) M. Ehrenstein and M. Dünnenberger, *J. Org. Chem.*, 21, 783 (1956).

(11) *Cf. e.g.*, Albert Wettstein: Biochemie der Corticoide, Proceedings of the Fourth International Congress of Biochemistry, Vol. 4 (Symposium: Biochemistry of Steroids), Pergamon Press, p. 233 (1959).

or by perfusion through bovine adrenal glands.[12] Recently a microorganism was described which is capable of producing the same effect.[13]

Of particular interest is 19-hydroxy-Δ^4-androstene-3,17-dione (II).[9,14] It is considered to be a metabolic intermediate in the transformation of Δ^4-androstene-3,17-dione into estrone.[15–18] In this connection it appears interesting that Δ^4-androstene-3,17-dione and its metabolic precursor 17α-hydroxyprogesterone have been identified in human follicles and corpora lutea.[19]

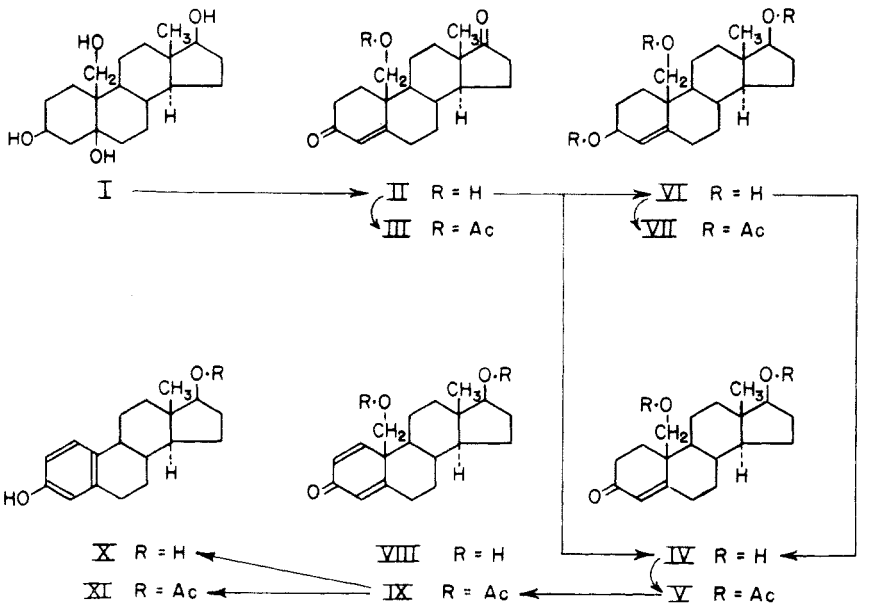
In view of the role of 19-hydroxy-Δ^4-androstene-3,17-dione (II) as a potential key intermediate in steroid metabolism, it was indicated to extend the chemical investigations on this compound. As was reported earlier,[9] II was prepared from 5β-androstane-3β,5,17β,19-tetrol (I) by oxidizing with 2.4 equivalents of *N*-bromoacetamide and subjecting the reaction product to dehydration with Girard's reagent T. On repeating this work, it was found that reproducible and satisfactory yields of II are obtained by increasing the amount of *N*-bromoacetamide to 3 equivalents. The rotatory dispersion curve of II[20] is in good agreement with that of a standard Δ^4-3-ketone.

In incubation experiments with human ovarian slices, testosterone-3-C^{14} has been converted into C^{14}-labeled estradiol-17β.[16] The question arises whether 19-hydroxytestosterone (IV) is an intermediate in such a conversion. With the intention of preparing IV, we had previously[9] subjected I to selective oxidation with 1.2 equivalents of *N*-bromoacetamide. However, the resulting product was not the desired 3-keto compound but rather 3β,5,19-trihydroxy-5β-androstan-17-one. Although the selective catalytic dehydrogenation of 3-hydroxyl groups with platinum has been achieved in several instances,[21–23] in the case of strophanthidol[23] rather poor yields are reported, because to some extent also the primary alcohol group was attacked.[23a] Therefore, it was decided not to apply this procedure to the rather scarce tetrol I.

We were able to prepare 19-hydroxytestosterone (IV) by a different approach. Norymberski found[24] that, under specific conditions, the reduction of Δ^4-androstene-3,17-dione with sodium borohydride gives testosterone in 60–70% yield. This method of selective reduction was applied to 19-hydroxy-Δ^4-androstene-3,17-dione (II). With a 1:1 molecular ratio of the steroid and the reducing agent a satisfactory yield (71.9%; 84.5%) of 19-hydroxytestosterone (IV) resulted. Reasoning by analogy, the hydroxyl group at carbon atom 17 was considered to possess the β-configuration. The correctness of this assumption was proved by the ultimate transformation of IV into estradiol-17β (*v. infra*). To a second reduction product was assigned the structure of Δ^4-androstene-3β,17β,19-triol (VI). Although the formation of a 3β-hydroxyl group (equatorial) appears favored on the basis of conformational considerations, mixtures of epimers, with the β-form prevailing, have been obtained in analogous instances.[22,25] Consequently, the configuration of the 3-hydroxyl group of VI is not claimed to be proved. By treatment with manganese dioxide,[26] VI could be converted into 19-hydroxytestosterone (IV). IV was characterized by the crystalline diacetate (V). The acetylation product of VI, probably representing the triacetate (VII), resisted all attempts at crystallization, even after chromatography.

Recent investigations performed with human placental tissue[18] indicate that 19-nortestosterone and $\Delta^{1,4}$-androstadiene-3,17-dione are not likely intermediates in the biosynthesis of estrogens. Since the role of 19-hydroxy-Δ^4-androstene-3,17-dione (II) as a possible intermediate has been demonstrated, one may assume[17] that the hydroxylation of the C-19 angular methyl group is probably the primary reaction in the conversion of 19-carbon steroids to estrogens. Subsequently a 1-2 dehydrogenation may occur leading to another intermediate, *viz.*, a 19-hydroxy-$\Delta^{1,4}$-dien-3-one. Such a compound should be very easily convertible into a compound of estrogen type even by ordinary organic chemical means.[17] In order to make compounds of this type available for the study of intermediary metabolism, it was decided to convert the diacetate of 19-hydroxytestosterone (V) into the corresponding 1-dehydro compound (IX) with the aim of hydrolyzing the latter to 19-hydroxy-1-dehydrotestosterone (VIII).

(12) Lit. *cf.* ref. 9.
(13) M. Nishikawa and H. Hagiwara, *Chemical and Pharmaceutical Bulletin*, **6**, 226 (1958).
(14) A. S. Meyer, *Experientia*, **11**, 99 (1955).
(15) A. S. Meyer, *Biochim. et Bioph. Acta*, **17**, 441 (1955).
(16) B. Baggett, L. L. Engel, K. Savard, and R. I. Dorfman, *J. Biol. Chem.*, **221**, 931 (1956).
(17) P. Talalay, *Physiol. Reviews*, **37**, 362, *v.p.* 374 (1957).
(18) K. J. Ryan, *J. Biol. Chem.*, **234**, 268 (1959).
(19) J. Zander, *J. Biol. Chem.*, **232**, 117 (1958).
(20) Determined through the courtesy of Professor Carl Djerassi at Wayne State University, Detroit 2, Mich. *Cf.* the chapter on Rotatory Dispersion, pp. 180–185 in "Steroids" by Louis F. Fieser and Mary Fieser, Reinhold Publishing Corp., New York, 1959.
(21) R. P. A. Sneeden and R. B. Turner, *J. Am. Chem. Soc.*, **77**, 130 (1955).
(22) A. Katz, *Helv. Chim. Acta*, **40**, 831 (1957).
(23) Ch. Tamm and A. Gubler, *Helv. Chim. Acta*, **42**, 239 (1959).
(23a) Addition (September 15, 1959): In contrast, treatment of methyl 3β,5,12β(?),14,19-pentahydroxy-5β,14β-etianate with platinum has been reported to give a good yield of 3-oxo-5,12β(?),14,19-tetrahydroxy-5β,14β-etianate. *Cf.* R. P. Martin and Ch. Tamm, *Helv. Chim. Acta*, **42**, 696 (1959).
(24) J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).
(25) P. Th. Herzig and M. Ehrenstein, *J. Org. Chem.*, **17**, 713 (1952).
(26) F. Sondheimer, C.Amendolla, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 5930 (1953).



Treatment of V with selenium dioxide[27] provided 19-hydroxy-1-dehydrotestosterone diacetate [17β,-19-diacetoxy-$\Delta^{1,4}$-androstadien-3-one] (IX) in good yield. As has been demonstrated in the case of 19,21-diacetoxy-17-hydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione,[13] compounds of this type aromatize easily when treated with ethanolic potassium hydroxide or with ethanolic hydrochloric acid. Therefore, in attempting the conversion of IX into the free 19-hydroxy-1-dehydrotestosterone (VIII), it was essential to select the mildest possible conditions.

In previous work from this laboratory, the saponification of 19-acetoxy groups has been achieved in some instances with potassium bicarbonate,[7] but more generally with potassium carbonate.[7,9,10] Therefore, in an orienting experiment, IX was subjected to treatment with potassium carbonate at room temperature.[28] As indicated by the ultraviolet absorption curve, extensive aromatization took place within a period of 1 hr. On the basis of this observation, the behavior of IX in various concentrations of alkali was studied on a microscale, using as criteria paper chromatographic and ultraviolet absorption data.[5] In these studies under mild conditions the formation of only the 17-monoacetate of estradiol-17β (XI)[29,30] was demonstrated, whereas under slightly more vigorous conditions, estradiol-17β (X) itself was obtained.

In 0.01*N* sodium carbonate solution containing 10% ethanol, conversion of IX to XI was essentially complete in 30 min., and in 0.1*N* sodium hydroxide containing 10% ethanol, conversion of IX to X was nearly instantaneous. Even in 0.01*N* sodium bicarbonate containing 10% ethanol, on standing overnight, partial conversion of IX to XI was indicated by paper chromatography, although here the ultraviolet studies were inconclusive. The possibility of confusing XI with a compound such as the 17-monoacetate of VIII having the same mobility in the paper chromatographic system is not very likely (*cf.* Experimental). It follows, therefore, that the preparation of VIII from the diacetate IX is probably not possible.[30a]

After these orienting studies, IX was converted on a preparative scale with aqueous methanolic potassium carbonate into XI, and with ethanolic sodium hydroxide into X.

Physiological activity. According to preliminary bioassays, conducted by Dr. Ralph I. Dorfman at the Worcester Foundation for Experimental Biology, 19-hydroxytestosterone (IV) possesses little, if any, androgenic activity (chick inunction test). Similar findings had been obtained with 19-hydroxy-Δ^4-androstene-3,17-dione (II).[14] The estrogenic activity (20-day-old female mice; uterine weight) of 19-hydroxy-1-dehydrotestosterone diacetate (IX) is about 1% that of estradiol-17β (X) and less than 1% that of the 17-monoacetate of estradiol-17β (XI). The details of the bioassay and a series of biochemical studies on compound IX will be published later. Furthermore, in particular, compounds IV and IX will be tested for androgen and estrogen inhibitory activity.

(27) For method, *cf.* Ch. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

(28) For method, *cf.* J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, **40**, 2291, *v.p.* 2319 (1957).

(29) K. Miescher and C. Scholz, *Helv. Chim. Acta*, **20**, 263, *v.p.* 270 (1937).

(30) C. Djerassi, G. Rosenkranz, J. Romo, St. Kaufmann, and J. Pataki, *J. Am. Chem. Soc.*, **72**, 4534, *v.p.* 4539 (1950).

(30a) Addition (September 15, 1959): A 19-hydroxy-$\Delta^{1,4}$-dien-3-one (methyl 3,11-dioxo-14,19-dihydroxy-$\Delta^{1,4}$,14β-etiadienate) has recently been obtained in very small amounts as a by-product of a chemical reaction. It obviously undergoes facile aromatization. *Cf.* G. Volpp and Ch. Tamm, *Helv. Chim. Acta*, **42**, 1408 (1959).

EXPERIMENTAL

Melting points. The m.p.'s were determined with the Fisher-Johns melting point apparatus and are uncorrected.

Absorption spectra. Ultraviolet spectra were determined in 95% ethanol with a Beckmann Model DU spectrophotometer. The infrared studies pertaining to this paper were carried out on a Perkin-Elmer Model 21 double beam spectrometer in the Division of Steroid Metabolism of the Sloan-Kettering Institute for Cancer Research through the courtesy of Dr. Thomas F. Gallagher. The interpretation was done by Dr. David Fleischer and Mrs. Beatrice S. Gallagher. The correlations are based upon those summarized in the publication of Jones and Herling.[31] Only those bands are mentioned which appear to have a direct bearing upon the structure of the particular compound. Details of other correlations between spectrum and structure will be summarized at a later time by the group at the Sloan-Kettering Institute.

Analyses. Unless stated otherwise, the microanalyses were performed by Dr. E. W. D. Huffman, Wheatridge, Colo., on samples which were dried to constant weight in vacuo (P_2O_5; 80°) according to Milner and Sherman.[32] The percentage loss of weight on drying is recorded; there was in no instance a gain of weight on exposure of the sample to the atmosphere.

Optical rotations. No corrections for crystal solvent have been made. Unless stated otherwise, the sample was dissolved in chloroform to make 2 cc. of solution and the rotation was determined in a 2-dm. semimicro tube.

Chromatography. The alumina (activity II) used as adsorbent for chromatography has been described.[7]

Remarks concerning the preparation of the starting material: Δ^4-androstene-3,17-dione (II). Generally the procedures used and the yields obtained in the preparation of the various intermediates were in agreement with the data published earlier.[9] This applies in particular to the conversion of strophanthidin by way of strophanthidol into ethyl 3β,5,19-trihydroxy-Δ^{14}-etienate. Pertinent observations concerning some of the subsequent steps are recorded as follows:

Hydrogenation of ethyl 3β,5,19-trihydroxy-Δ^{14}-etienate. Average yield (15 expts.) of pure ethyl 3β,5,19-trihydroxyetianate, 88%. Recryst. from ethanol water; double m.p. 186° and 195–196°.

Saponification of ethyl 3β,5,19-trihydroxyetianate. Average yield (7 expts.) of pure 3β,5,19-trihydroxyetianic acid, 94%.

3β,19-Diacetoxy-5-hydroxyetianic acid. Recryst. from acetone-hexane; double m.p. 167–170° and 182–184°.

3β,19-Diacetoxy-5-hydroxy-21-diazo-5β-pregnan-20-one from 3β,19-diacetoxy-5-hydroxyetianic acid. Average yield (10 expts.) of crude crystalline diazoketone, as obtained after chromatography, 73%. Occasionally, without discernible reason, the reaction did not proceed according to schedule.

3β,19-Diacetoxy-5-hydroxy-5β-pregnan-20-one from 3β,19-diacetoxy-5-hydroxy-21-diazo-5β-pregnan-20-one. Relative to the previously reported data, the volume of the 48% hydriodic acid used in this reaction was reduced to two fifths and the shaking with this reagent to 45 sec. Average yield (8 expts.) of crude crystalline methyl ketone, as obtained after chromatography, 85%.

3β,5,19-Triacetoxy-5β-pregnan-20-one from 3β,19-diacetoxy-5-hydroxy-5β-pregnan-20-one. One part (g.) of the diacetate was refluxed with 200 parts (cc.) of acetic anhydride for 15 hr. Uniform, satisfactory yields of the pure triacetate resulted, average 78% (7 expts., range 0.100–1.0 g. of diacetate).

3β,5,17β,19-Tetraacetoxy-5β-androstane from 3β,5,19-triacetoxy-5β-pregnan-20-one. Average yield (4 expts.) of the pure crystalline product, 89%.

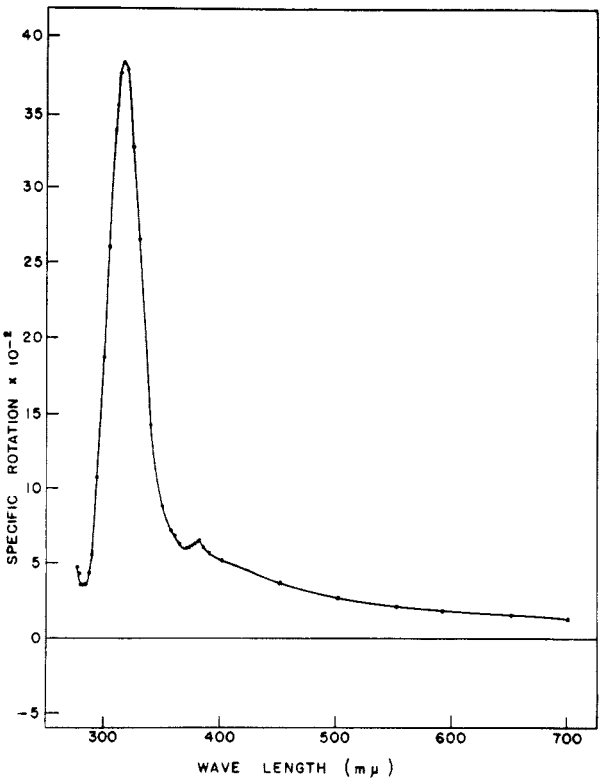


Fig. 1. Rotatory dispersion curve of 19-hydroxy-Δ^4-androstene-3,17-dione (II) (double m.p. 169–172° and 180–182°) in dioxane (c = 0.078, 700 ~ 305 mμ; c = 0.015, 300 ~ 277.5 mμ).

Saponification of 3β,5,17β,19-tetraacetoxy-5β-androstane. Average yield (4 expts.) of pure 5β-androstane-3β,5,17β,19-tetrol (I), 88%.

Conversion of 5β-androstane-3β,5,17β,19-tetrol (I) *into 19-hydroxy-Δ^4-androstene-3,17-dione* (II). Reproducible, satisfactory yields were obtained by using for the oxidation 3 rather than 2.4 equivalents of *N*-bromoacetamide. A revised and simplified procedure is given as follows: To 263.1 mg. of I, m.p. 205–207°, in 7 cc. of redistilled *tert*-butanol were added 336 mg. of *N*-bromoacetamide and 1.2 cc. of water. After keeping the solution at room temperature for 16 hr., 80 cc. of water was added and a sufficient amount of *N* sodium thiosulfate to destroy the bromine. Following extraction with ethyl acetate, drying, and evaporating the solvent, a partly crystalline reaction product resulted. This was dissolved in 12 cc. of ethanol and, after the addition of 800 mg. of Girard's reagent T and 0.27 cc. of glacial acetic acid, the solution was refluxed for 1 hr. and was subsequently concentrated *in vacuo* at room temperature to a volume of approximately 6 cc. Following the addition of 210 mg. of sodium carbonate, ice, and some water, the mixture was extracted with ethyl acetate, yielding only 2.4 mg. of nonketonic material. The aqueous phase was then acidified to Congo Red with 50% sulfuric acid and, after standing overnight, the ketonic material was isolated by extracting with ethyl acetate; yield, 181 mg. of yellowish, mostly crystalline material. Chromatography over 8 g. of alumina (diameter of column, 1.3 cm.) and elution with benzene ether gave 147.8 mg. (yield, 60%) of crude crystalline II. Recrystallization from acetone hexane furnished 135.9 mg. (yield, 55.5%) of pure II; double m.p. 169–172° and 180–182°. Elution with methanol gave 9 mg. of crystalline material, probably representing 3β,5,19-trihydroxy-5β-androstan-17-one.

19-Hydroxytestosterone [*17β,19-dihydroxy-Δ^4-androsten-3-one*] (IV) *and Δ^4-androstene-3β,17β,19-triol* (VI) *from 19*

(31) R. N. Jones and F. Herling, *J. Org. Chem.*, 19, 1252 (1954).

(32) R. T. Milner and M. S. Sherman, *Ind. Eng. Chem., Anal. Ed.*, 8, 427 (1936).

hydroxy-Δ⁴-androstene-3,17-dione (II). A solution of 30.2 mg. (0.1 mmol.) of II, m.p. 169–171°, in 6 cc. of redistilled methanol was treated with 5.7 mg. (0.15 mmol.) of sodium borohydride for 1 hr. at 0°. After the addition of 5 drops of glacial acetic acid, the solution was brought to dryness *in vacuo*. The crystalline residue was extracted with two 10-cc. portions of hot benzene and of ethyl acetate. After evaporation of the solvents, the resulting material (43.8 mg.) was taken up in 50 cc. of benzene (8.0 mg. remained undissolved) and chromatographed over 3 g. of alumina (diam. of column, 0.8 cm.). The following eluates were collected: (a) benzene (50 cc.), 0.2 mg. of resin; (b) benzene ether, range 9:1 to 3:2 (total 120 cc.), 0.5 mg. of resin; (c) benzene ether, range 3:7 to 1:9 (total 60 cc.), ether (20 cc.) and ether methanol, 99:1 (20 cc.), 14.3 mg. of crystalline material; (d) ether methanol, 39:1 (20 cc.), 1.3 mg. of resin; (e) ether methanol, 19:1 and 17:3 (20 cc. each), 12.1 mg. of crystalline product; (f) ether methanol, 3:1 and 1:1 (20 cc. each); 0.6 cc. of resin.

The fractions (c) (14.3 mg., m.p. range 200–204°, yield 47%) consisted essentially of 19-hydroxytestosterone (IV). Repeated recrystallization from acetone hexane gave pure IV, m.p. 201–203°, $[\alpha]_D^{20}$ +109.7° (23.41 mg. in 2 cc. of chloroform containing 5 drops of ethanol, α + 2.57°),[33] λ_{max}^{alc} 243 mμ, ε 14900.[33]

Infrared spectrum of IV ($CHCl_3$ suspension): 3613 cm.$^{-1}$, hydroxyl absorption (Note: comparing IV and II which have the same amount of CH_2 and CH_3 absorption, the ratio of the O—H stretching absorption to this C—H stretching absorption is approximately twice as large in IV. This is in agreement with the presence of two hydroxyl groups); 1710 cm.$^{-1}$, small unexplained absorption; 1665 cm.$^{-1}$, C=O absorption of the Δ⁴-3-ketone; 1617 cm.$^{-1}$, C=C absorption of the Δ⁴-3-ketone.

Anal. Calcd. for $C_{19}H_{28}O_3$(304.41): C, 74.96; H, 9.27. Found: C, 74.82; H, 9.26. Weight loss, 0.29.

The fractions (e) (12.1 mg., m.p. range 180–187°, yield 39.5%) represented crude Δ⁴-androstene-3β,17β,19-triol (VI) which is markedly less soluble in acetone than IV. Repeated recrystallization from acetone hexane gave 6.3 mg. of VI, m.p. 202–205°. The product gave no color with tetranitromethane. No ultraviolet absorption in the range 220–300 mμ. $[\alpha]_D^{21}$ +44.5° (11.25 mg. in 2 cc. of ethanol, α + 0.50°).[34]

Infrared spectrum of VI (KBr pellet): hydroxyl absorption present (very broad band); 1656 cm.$^{-1}$, C=C absorption of the Δ⁴-group. (Note: No conclusions can be drawn from the infrared spectrum regarding the configuration of the OH-- group at C-3.)

Anal. Calcd. for $C_{19}H_{30}O_3$(306.43): C, 74.47; H, 9.87. Found: C, 74.30; H, 9.88. Weight loss, 0.1.

Orienting experiments were carried out to determine to what extent the yields of IV and VI could be influenced by varying the molecular ratio of II and sodium borohydride. Summary of experiments; the yields apply to the pertinent chromatographic fractions, as weighed before recrystallization. (A) Ratio 1:1; 2 experiments: (a) 54.6 mg. of II in 10 cc. of methanol, 7 mg. of $NaBH_4$, yield of IV 39.4 mg. = 71.9% (eluted with ether methanol); (b) 60.0 mg. of II in 12 cc. of methanol, 8 mg. of $NaBH_4$, yield of IV 51 mg. = 84.5% (eluted with ether methanol). In both experiments the estimated yield of VI (eluted with ether methanol) was less than 5%. (B) Ratio 2:3, 1 experiment, in addition to the example described in detail above: 55.1 mg. of II in 10 cc. of methanol, 10.3 mg. of $NaBH_4$; yield of IV, 31.2 mg. = 56.3% (eluted with benzene ether); yield of VI, 9.4 mg. = 16.9% (eluted with ether methanol). (C) Ratio 1:2; 1 experiment: 45.4 mg. of II in 9 cc. of methanol, 11.5 mg. of $NaBH_4$; yield of IV, 17.3 mg. = 37.9% (eluted with benzene ether); yield of VI, 20.2 mg. = 43.9% (eluted with ether methanol). (D) Ratio 1:3; 1 experiment: 30.2 mg. of II in 6 cc. of methanol, 11.5 mg. of $NaBH_4$; yield of IV, 7.0 mg. = 23.0% (eluted with benzene ether); yield of VI, 12.1 mg. = 39.5% (eluted with ether methanol).

19-Hydroxytestosterone [*17β,19-dihydroxy-Δ⁴-androsten-3-one*] (IV) *from* Δ⁴-*androstene-3β,17β,19-triol* (VI). A solution of 4.7 mg. of VI, m.p. 199–201°, in 5 cc. of chloroform, containing 50 mg. of freshly prepared manganese dioxide,[35] was allowed to stand at room temperature for 3 days with occasional shaking. The oxide was then removed by filtration and washed with chloroform. Evaporation of the solvent gave 4.2 mg. of crude material which was chromatographed over 1 g. of alumina. The eluates obtained with ether yielded a crystalline product which was recrystallized from acetone-hexane; yield 2.1 mg., m.p. 201–205°. There was no depression of the m.p. when mixed with the analytical sample of IV. The determination of the ultraviolet absorption spectrum (λ_{max}^{alc} 244 mμ; ε 12,750) indicated that the reaction product was approximately 85% pure.

19-Hydroxytestosterone diacetate [*17β,19-diacetoxy-Δ⁴-androsten-3-one*] (V). To 30.4 mg. of 19-hydroxytestosterone (IV) in 1 cc. of pyridine was added 1 cc. of acetic anhydride. The solution was kept at room temperature for 16 hr. and was then evaporated to dryness *in vacuo* (30°). After the addition and evaporation of absolute ethanol and then of benzene, 36.7 mg. of a colorless resinous product resulted which did not crystallize. Chromatography over 4 g. of alumina yielded, by elution with petroleum ether benzene, a total of 32.1 mg. of crystalline residues. By treating this material with acetone hexane and seeding, crystals separated; 2 crops: 25.2 mg. (65%), m.p. 126–128°, 4.2 mg. (10.8%), m.p. 124–126°. By repeated crystallization from methylene chloride-hexane the m.p. was raised to 128.5–130° (analytical sample). In a repeat experiment, the crude acetylation product crystallized directly from acetone water after seeding; very minute colorless rods; m.p. 128.5° (sharp); $[\alpha]_D^{21}$ +133.6° (10.30 mg., α + 1.38°); λ_{max}^{alc} 239 mμ, ε 18,800.

Infrared spectrum of V (CS_2 and CCl_4 solutions): Hydroxyl absorption absent; 1745 cm.$^{-1}$, possibly C=O absorption of the 19-acetate; 1735 cm.$^{-1}$ (shoulder), C=O absorption of the 17-acetate; 1678 cm.$^{-1}$, C=O absorption of the Δ⁴-3-ketone; 1623 cm.$^{-1}$, C=C stretching vibrations of the Δ⁴-3-ketone; 1419 cm.$^{-1}$, due to CH_2 adjacent to the Δ⁴-3-ketone; 1246 cm.$^{-1}$ (shoulder), 1236 cm.$^{-1}$, and 1226 cm.$^{-1}$ (shoulder), combined absorption of the C—O stretching vibrations of the acetate groups.

Anal. Calcd. for $C_{23}H_{32}O_5$ (388.49): C, 71.11; H, 8.30. Found: C, 70.83; H, 8.43.

19-Hydroxy-1-dehydrotestosterone diacetate [*17β,19-diacetoxy-Δ¹,⁴-androstadien-3-one*] (IX) *from 19-hydroxytestosterone diacetate* (V).[27] A mixture consisting of 38.5 mg. of V, 4 cc. of *tert*-butanol, 0.04 cc. of glacial acetic acid and 12 mg. of freshly sublimed selenium dioxide was refluxed under nitrogen for 20 hr. Another 12 mg. of selenium dioxide was then added and the refluxing continued for 9 hr. After subsequent standing at room temperature for 15 hr. and the addition of ethyl acetate, the solution was filtered and evaporated to dryness *in vacuo*. The crystalline residue was taken up in 20 cc. of ethyl acetate and the solution was washed successively with the following: 3 cc. of a solution of potassium bicarbonate (5%), 3 cc. of water, two 3-cc. portions of a freshly prepared solution of ammonium sulfide, 3 cc. of ammonia (1%), 3 cc. of water, 3 cc. of *N* hydrochloric acid, three 3-cc. portions of water. After drying over sodium sulfate and evaporating the solvent, 37 mg. (theoretical yield: 38.3 mg.) of an almost colorless crystalline product resulted which was chromatographed over 5 g. of alumina.

(33) Derived from a sample of another experiment, m.p. 204.5–206.5°.

(34) Derived from a sample of another experiment, m.p. 206–209°.

(35) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

Elution with benzene ether gave a total of 33.8 mg. of uniform crystalline material which, after two recrystallizations from acetone-hexane gave 23.9 mg. (yield 77.5%) of IX with constant m.p. 169–171°. In a repeat experiment,[16] IX was obtained, without chromatography, by direct recrystallization of the crude reaction product; colorless needles (from acetone water), m.p. 170–171°; $[\alpha]_D^{20}$ +59.2° (15.39 mg., α + 0.91°); λ_{max}^{alc} 243 mμ; ε 13,700.

Infrared spectrum of IX (CS_2 and CCl_4 solutions): hydroxyl absorption absent; 1746 cm.$^{-1}$, possibly C=O absorption of the 19-acetate; 1735 cm.$^{-1}$ (shoulder), C=O absorption of the 17-acetate; 1671 cm.$^{-1}$, C=O absorption of the $\Delta^{1,4}$-3-ketone; 1650 cm.$^{-1}$, unexplained band; 1635 cm.$^{-1}$ and 1608 cm.$^{-1}$, C=C stretching vibrations of the $\Delta^{1,4}$-3-ketone; 1404 cm.$^{-1}$, usually found with a $\Delta^{1,4}$-3-ketone; 1245 cm.$^{-1}$ (shoulder), 1236 cm.$^{-1}$, and 1220 cm.$^{-1}$ (shoulder), combined absorption of the C—O stretching vibrations of the acetate groups. (Note: While all the necessary absorptions are present, the 1650 cm.$^{-1}$ band indicates the presence of some impurity.)

Anal.[36] Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82. Found: C, 71.03; H, 7.54. Weight loss, 0.83.

Treatment of 19-hydroxy-1-dehydrotestosterone diacetate (IX) *with alkaline reagents.*[5] *A. Treatment with 0.1N sodium hydroxide.* A solution of 0.15 mg. of IX in 1 cc. of 95% ethanol was diluted to 10 cc. with 0.1*N* aqueous sodium hydroxide. Measurement of the development of ultraviolet absorption at 298 mμ was attempted, but the value E = 0.103, obtained 2 min. after mixing, did not change during the following 24 hr., and the absorption curve was essentially the same as that determined for estradiol-17β (X)[37] in the same solvent medium[38]: λ_{min} 226 mμ, ε 8300; λ_{max} 238 mμ, ε 10,300; λ_{min} 270 mμ, ε 1200; λ_{max} 298 mμ, ε 3200.

One fourth of the above reaction mixture was made acid to litmus and extracted with ether, and the material so obtained was compared with authentic samples of estradiol-17β (X)[37] and estradiol-17α[37] by paper chromatography in the system, toluene–propylene glycol. After development for 48 hr., the air-dried chromatogram was sprayed with a mixture of equal parts of 1% solutions of ferric chloride and potassium ferricyanide.[39] Under these conditions, 25γ of estradiol-17β[37] moved 13.8 cm., 25γ of estradiol-17α[37] moved 18.2 cm., and the product of treatment of IX with 0.1*N* sodium hydroxide gave a single spot at 13.3 cm.

B. *Treatment with 0.01N sodium carbonate.* A solution of 0.10 mg. of IX in 1 cc. of 95% ethanol was diluted to 10 cc. with 0.01*N* aqueous sodium carbonate and the development of absorption at 298 mμ was followed. The value, E = 0.023, obtained 2 min. after mixing, increased to E = 0.064 after 60 min., and increased only to E = 0.065 after 3 hr. After 24 hr., the absorption curve was measured and was nearly the same as that obtained with authentic 17-monoacetate of estradiol-17β (XI)[40] in the same solvent medium: λ_{min} 230 mμ, ε 7000; λ_{max} 238 mμ, ε 7400; λ_{min} 265 mμ, ε 1200; λ_{max} 298 mμ, ε 2200.

One third of the reaction mixture was now extracted with ether and the product was compared with authentic samples of IX, XI,[40] and X[37] by paper chromatography in the system, methyl cyclohexane–propylene glycol. After development for 18 hr., the air-dried chromatogram was sprayed with a mixture of equal parts of 1% ferric chloride, 1% potassium ferricyanide, and 6*N* *hydrochloric acid.*[41] Under these conditions, the reaction product gave two spots, at 0 cm. (small), and 7.8 cm. (large), and 25γ samples of IX, XI, and X gave spots at 16.5 cm., 7.5 cm., and 0 cm., respectively.

C. *Treatment with 0.01N sodium bicarbonate.* A solution of 0.10 mg. of IX in 1 cc. of 95% ethanol was diluted to 10 cc. with 0.01*N* aqueous sodium bicarbonate. The ultraviolet absorption at 298 mμ 2 min. after mixing was E = 0.014, and did not change during 24 hr. The absorption curve then determined (λ_{max} 246 mμ, ε 13,000) did not differ greatly from that of IX in alcohol solution (λ_{max} 244 mμ, ε 14,000).

After 24 hr., one third of the reaction mixture was extracted with ether, and the product was compared with authentic samples of IX, XI,[40] and X[37] by paper chromatography in the system, methyl cyclohexane–propylene glycol. After development for 18 hr., the air-dried chromatogram was sprayed with the *acidified*[41] ferric chloride–ferricyanide reagent. The reaction product gave three spots, at 0 cm. (minute), 7.7 cm. (large), and 16.3 cm. (large) from the starting line, and 25γ samples of IX, XI, and X gave spots at 16.5 cm., 7.5 cm., and 0 cm. The latter experiment was repeated in identical fashion, except that the air-dried chromatogram, obtained from approximately 50γ of reaction product, was sprayed with *neutral* ferric chloride–potassium ferricyanide reagent. The reaction product gave two spots at 0 cm. (minute) and 12.1 cm. (large) from the starting line. A 12γ sample of XI gave a single spot at 12.4 cm.

Conversion of 19-hydroxy-1-dehydrotestosterone diacetate (IX) *into the 17-monoacetate of estradiol-17β* (XI).[5] To 3.4 mg. of IX, m.p. 172–174°, in 1 cc. of methylene chloride was added 2.5 cc. of 75% methanolic 0.1*N* potassium carbonate.[28] After standing at room temperature for 1 hr., the slightly yellow solution was evaporated to dryness *in vacuo*, and the residue was then taken up in chloroform and water. Drying of the organic layer over sodium sulfate and subsequent evaporation to dryness *in vacuo* gave 3.0 mg. of material which was chromatographed over a small column of alumina (diam., 0.5 cm.; height, 4.0 cm.). Elution with benzene ether gave approx. 3 mg. of crystalline product which on recrystallization from acetone hexane gave 1.8 mg. of XI, m.p. 222–223.5°.

The substance was compared by paper chromatography in the system methyl cyclohexane–propylene glycol with two authentic samples of the 17-monoacetate of estradiol-17β: Sample A,[30] m.p. 225–226°, obtained from Dr. Carl Djerassi (Syntex, Mexico City) and sample B,[29] m.p. 221–222°, supplied by Dr. Emil Schlittler (Ciba Laboratories, Summit, N. J.). After development for 19 hr., the air-dried chromatogram was sprayed with the ferric chloride–potassium ferricyanide reagent. The above reaction product (25γ) gave a single spot at 13.2 cm. from the starting line, and sample A gave a single spot at 13.2 cm. Sample B gave two spots, at 13.1 cm. (large) and 5.1 cm. (small).

Comparison of infrared spectra (CS_2 and CCl_4 solutions): In the same solvent, the spectrum of our compound XI is identical with that of an authentic sample of the 17-monoacetate of estradiol-17β.[30] (Note: Although the material is not too soluble and the spectra were weak, there were more bands to compare than by using a more concentrated $CHCl_3$ solution which limits the regions of absorption.)

Conversion of 19-hydroxy-1-dehydrotestosterone diacetate (IX) *into estradiol-17β* (X).[5] To 5.0 mg. of IX, m.p. 170–171°, in 2 cc. of 95% ethanol was added 8 cc. of 0.1*N* sodium hydroxide. The solution was kept at room temperature for 18 hr. and was then barely acidified by the addition of hydrochloric acid. After extracting with three 20-cc. portions of ether, washing of the solvent with a saturated solution of sodium chloride, drying over sodium sulfate, and evaporating, 5.1 mg. of a colorless resin was obtained. From acetone–petroleum ether microcrystalline material separated which, on recrystallization from acetone water yielded

(36) Dried at room temperature. Drying at 80° *in vacuo* is apparently connected with partial volatilization.

(37) Reference sample kindly supplied by Dr. Albert Wettstein, CIBA-A.G.-Laboratories, Basel, Switzerland.

(38) For comparison, *v.e.g.* the curves recorded by R. P. A. Sneeden R. B. and Turner, *J. Am. Chem. Soc.*, **77**, 130 (1955).

(39) *Cf.* L. R. Axelrod, *Recent Progress in Hormone Research*, 9, Academic Press Inc., New York, 1954, p. 69.

(40) Reference sample kindly supplied by Dr. Carl Djerassi, Syntex S.A., Mexico City. *Cf.* ref. 30.

(41) IX does not react with the neutral reagent.

2.2 mg. of colorless needles, m.p. 177–180°. Mixture m.p. with an authentic sample of estradiol-17β (m.p. 184–187°),[37] 184–188°.

Infrared spectrum of X (KBr pellet): hydroxyl absorption present (very broad band); 1733 cm.$^{-1}$ (relatively small) and 1638 cm.$^{-1}$, both unexplained absorptions, indicating the presence of some impurity. Frequency-wise the region from 1400–650 cm.$^{-1}$ agrees in general with a reference standard of estradiol-17β with two exceptions, *i.e.*, a small missing band at about 1300 cm.$^{-1}$ and a small added band at about 885 cm.$^{-1}$ Intensity-wise differences exist which are possibly due to the variability of the KBr technique.

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