sulfoxide layer to dryness (100°/1 mm.) followed by trituration of the residue with dry ether gave 0.62–0.70 g. of digitonin.

The same procedure applied to cholestanyl digitonide (1.035 g.) gave cholestanol (0.240 g., 97% recovery) melting at 140–142° (m.m.p., infrared spectrum).

Cleavage of Cholesteryl Tomatide.—Treatment of the cholesterol–tomatine complex (1.000 g.) by the procedure described above gave 0.253 g. cholesterol (93% recovery).

Determination of the Molecular Ratio in Cholesterol Holothuride.—Treatment of cholesteryl holothuride[3] (1.000 g.) with dimethyl sulfoxide gave cholesterol (0.389 g.). These data give a value of 2:1 for the molar ratio of cholesterol: holothurin[7] in the complex.

(7) The calculations have been based on a molecular weight of 1190 for holothurin. See ref. 3.

The Synthesis of 3,19-Dioxo-4-androsten-17β-ol[1]

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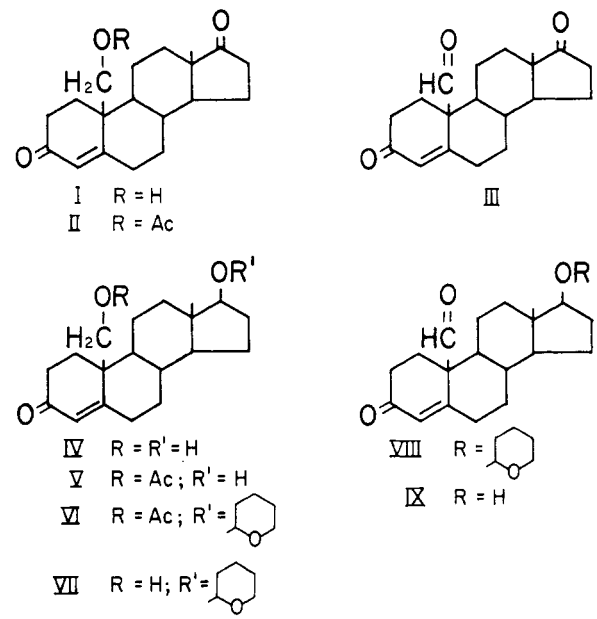
Earlier investigations have shown that incubation of C^{14}-labeled testosterone with normal human ovarian tissue converted it into C^{14}-labeled estradiol-17β.[2,3] In the biosynthetic transformation of androgens to estrogens the following sequence has been established: androgens → 19-hydroxy compounds → 19-aldo compounds → estrogens. However, when testosterone 4-C^{14} was incubated with certain human polycystic ovarian tissues, primarily 19 - hydroxy - 4 - androstene - 3,17 - dione (I), 19-hydroxytestosterone (IV), and 3,17,19-trioxo-4-androstene (III) were obtained and no estrogens were discernable.[3] Furthermore, radioactive metabolites isolated from these studies presented strong presumptive evidence for the presence of 3,19-dioxo-4-androsten-17β-ol (IX). A search of the literature revealed that this compound has not been synthesized and in view of its potential role in the steroid metabolism we wish to report its synthesis.

In the first instance we decided to study the reduction of 3,17,19-trioxo-4-androstene (III), with one equivalent of sodium borohydride under the conditions described by Norymberski[4] to achieve the specific reduction of the 17-keto group. In view



of the steric considerations and the tertiary nature of the C-19 aldehyde function, we anticipated that preferential reduction of the 17-ketone would occur without effecting the aldehyde group.

Oxidation of 19-hydroxy-4-androstene-3,17-dione (I) with 8 *N* chromic acid,[5] instead of chromium trioxide in acetic acid,[6] gave a much superior yield (90%) of 3,17,19-trioxo-4-androstene (III). Reduction of III with one equivalent of sodium borohydride in methanol,[4,7] contrary to our expectations (*supra*), gave a mixture of 19-hydroxytestosterone (IV) and 19-hydroxy-4-androstene-3,17-dione (I). Apparently, the primary attack of borohydride appears to be on the C-19 aldehyde function rather than on the C-17 ketone.

Another approach to the synthesis of IX was then investigated which resulted in its successful synthesis. 19 - Acetoxy - 4 - androstene-3,17 - dione (II),[8] was treated with one equivalent of sodium borohydride in methanol at 0° to give 17β-hydroxy-19-acetoxy-4-androsten-3-one (V, 19-acetoxytestosterone). The 17-hydroxyl function in V was then protected as the tetrahydropyranyl ether which is stable to alkali but can easily be hydrolyzed with acid. Compound V was stirred at room temperature with dihydropyran[9] in the presence of a cationic resin (Amberlite IR-120) to give 19-acetoxy-4-androsten - 3 - one - 17β - (2′ - tetrahydropyranyl) ether (VI). The 19-acetoxy group in VI was then

(1) This investigation was supported by a Grant A-3270 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

(2) B. Baggett, L. L. Engel, K. Savard, and R. I. Dorfman, *J. Biol. Chem.*, **221**, 931 (1956).

(3) L. R. Axelrod and J. W. Goldzieher, *Proc. Endocrinol. Soc.*, **43**, 24 (1961); *J. Clin. Endocrinol. and Metab.*, **22**, 431 (1962).

(4) J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).

(5) (a) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, **39** (1946); (b) A. Bowers, T. G. Halshall, E. R. H. Jones, and A. J. Lemin, *ibid.*, 2548 (1953); (c) C. Djerassi, R. R. Engel, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(6) H. Hagiwara, *Yakugaku Zasshi*, **80**, 965 (1960); *Chem. Abstr.*, **54**, 22724e (1960).

(7) M. Ehrenstein and K. Otto, *J. Org. Chem.*, **24**, 2006 (1959).

(8) A. S. Meyer, *Experientia*, **11**, 99 (1955).

(9) G. F. Woods and D. N. Kramer, *J. Am. Chem. Soc.*, **69**, 2246 (1947).

saponified with potassium carbonate to give 19-hydroxy-4-androsten-3-one-17β-(2′-tetrahydropyranyl) ether (VII). The 19-hydroxyl function in VII was oxidized with 8 *N* chromic acid reagent[5] to give 3,19-dioxo-4-androsten-17β-(2′-tetrahydropyranyl) ether (VIII). By refluxing VIII in ethanol containing a trace of hydrochloric acid the tetrahydropyranyl ether group was cleaved to give 3,19-dioxo-4-androsten-17β-ol (IX) in an over-all yield of 27%. Compound IX exists in two different polymorphic forms, one with a m.p. 124–126° (needles) and the other with a m.p. 167–168° (rectangular prisms). When the lower melting form was dissolved in acetone and seeded with a crystal of higher melting form, only the high melting form resulted.

Interestingly both 3,17,19-trioxo-4-androstene (III) and 3,19-dioxo-4-androsten-17β-ol (IX) exhibit a slight bathochromic shift in the ultraviolet absorption (246 mμ) when compared with the corresponding 19-hydroxy compounds (243 mμ). The 19-aldo compounds exhibited a weak but distinct band at 2725–2740 cm.$^{-1}$ in the infrared spectrum. This absorption was assigned to the presence of an aldehyde function in the steroid molecule.[10]

Experimental

Melting Points.—All melting points were determined on samples dried under high vacuum at 60° for 24 hr. and are uncorrected.

Analyses.—Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Absorption Spectra.—The ultraviolet absorption spectra were determined in methanol with a Cary recording spectrophotometer (Model 11MS). The infrared absorption spectra were determined in a potassium bromide disk on a Perkin–Elmer (Model 21) infrared spectrophotometer.

Optical Rotations.—All rotations were measured in chloroform solution at 25 ± 3° with a Zeiss–Winkel polarimeter.

Alumina.—Merck reagent grade aluminum oxide labeled as "Suitable for Chromatographic Adsorption" was treated with ethyl acetate and activated to give Brockmann Activity III.

Petroleum Ether.—Petroleum ether used was that of Mallinckrodt analytical reagent grade, b.p. 30–60°.

3,17,19-Trioxo-4-androstene (III).—To a cold solution (10–15°) of 19-hydroxy-4-androstene-3,17-dione (I, 504 mg.) in acetone (60 ml.; distilled over permanganate) in an atmosphere of nitrogen, 8 *N* chromium trioxide reagent was added dropwise until the reddish brown color persisted. After an additional 2 min. stirring, the mixture was diluted with water (500 ml.) and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, saturated sodium bicarbonate solution, and water until neutral and dried over anhydrous sodium sulfate. The solvent was removed and the residue was crystallized from acetone–petroleum ether to give 3,17,19-trioxo-4-androstene (III, 460 mg., 92%). The analytical sample was obtained after an additional crystallization from the same solvent mixture, m.p. 130–133°, $[\alpha]_D$ +313° (*c* 0.86), λ_{max} 246 mμ (ε 11,875), ν_{max} 2740, 1738, 1708, 1668, and 1614 cm.$^{-1}$; (lit.,[6] m.p. 129–133°).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.49; H, 8.25.

Reduction of 3,17,19-Trioxo-4-androstene with Sodium Borohydride.—A solution of 3,17,19-trioxo-4-androstene (III, 100 mg.) in methanol (20 ml.) was treated with sodium borohydride (14 mg., 1.1 equivalents) for 1 hr. at 0°. After decomposing the reaction mixture with a few drops of glacial acetic acid the solution was evaporated to dryness *in vacuo*. The residue was extracted with ethyl acetate, washed with water, sodium bicarbonate solution, and water until neutral, and dried over anhydrous sodium sulfate. The solvent was evaporated, the crystalline residue (100 mg.) was dissolved in chloroform (20 ml.) and was stirred with manganese dioxide[11] (500 mg.) for 4 hr. to oxidize any of the 3-hydroxy compounds possibly formed during borohydride reduction.[7] The chloroform solution was then filtered from the catalyst and evaporated to give a solid (100 mg.) which was chromatographed on alumina (4.5 g.). Elution with benzene–ether (4:1 and 1:1) gave 19-hydroxy-4-androstene-3,17-dione (I, 35 mg.) which crystallized from acetone–petroleum ether, m.p. 169–171°. Its identity was established by a mixture melting point determination and comparison of the infrared spectrum with that of an authentic sample.

Further elution of the column with ether gave 19-hydroxytestosterone (IV, 60 mg.) which crystallized from acetone–petroleum ether, m.p. 201–203°. There was no depression of melting point when mixed with an authentic sample, and the infrared spectra of both the samples were found to be identical.

17β-Hydroxy-19-acetoxy-4-androsten-3-one (V).—To a solution of 19-acetoxy-4-androstene-3,17-dione (II, 1.28 g.) in methanol (220 ml.) at 0°, sodium borohydride (0.155 g.) was added and stirred at the same temperature for 1 hr. After the addition of glacial acetic acid (1.5 ml.) the methanol solution was evaporated to dryness under vacuum and the residue was extracted with ethyl acetate. The ethyl acetate extract was washed with water, saturated sodium bicarbonate solution, and with water until neutral, and then dried over anhydrous sodium sulfate. The crystalline residue (1.25 g.) obtained after evaporating the solvent was treated with manganese dioxide[11] (6.5 g.) in chloroform (100 ml.) for 4 hr. to oxidize any 3-hydroxy compounds formed during the reduction. Evaporation of the chloroform solution after filtering from the manganese dioxide gave a residue (1.25 g.) which was chromatographed on alumina (38 g.). Elution of the column with benzene gave as a first fraction some unchanged 19-acetoxy-4-androstene-3,17-dione (422 mg.) whose identity was established by its infrared spectrum. Further elution of the column with benzene–ether (8:2 and 1:1) afforded 17β-hydroxy-19-acetoxy-4-androsten-3-one (V, 825 mg., 64%) which was crystallized from acetone–petroleum ether, m.p. 126–127°, $[\alpha]_D$ +175° (*c* 1.05), λ_{max} 240 mμ (ε 15,480); ν_{max} 3470, 1735, 1660, 1615, and 1225 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.99; H, 8.93.

19-Acetoxy-4-androsten-3-one-17β-(2′-tetrahydropyranyl Ether (VI).—To a solution of V (825 mg.) in dihydropyran (25 ml.), Amberlite IR-120 (H-form, dried at 70° for 24 hr., 2 g.) was added as the acid catalyst and the reaction mixture was stirred at room temperature (22°) for 20 hr. The resin was collected by filtration and washed with ether, the combined solvents were evaporated to dryness, and the residue (1.2 g.) was chromatographed on alumina (60 g.). Elution of the chromatographic column with benzene afforded 19-acetoxy-4-androsten-3-one-17β′-(2′-tetrahydro-

(10) A. L. Nussbaum, E. P. Yuan, C. H. Robinson, A. Mitchell, E. P. Oliveto, J. M. Beaton, and D. H. R. Barton, *J. Org. Chem.*, **27**, 20 (1962).

(11) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jensen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

pyranyl) ether (VI, 888 mg., 90%) as a viscous oil which resisted crystallization. The infrared spectrum showed no hydroxyl absorption, but exhibited absorption bands at 1735 cm.$^{-1}$ (acetate carbonyl), 1665 cm.$^{-1}$ (conjugated carbonyl), 1612 cm.$^{-1}$ (C=C stretching vibration) and 1225 cm.$^{-1}$ (C—O stretching vibration of the acetate group).

19-Hydroxy-4-androsten-3-one-17β-(2′-tetrahydropyranyl) Ether (VII).—To a solution of VI (875 mg.) in methanol (178 ml.), a solution of 75% methanolic 0.1 *N* potassium carbonate (670 ml.) was added and the contents were left under nitrogen for 20 hr. at room temperature. Most of the methanol was evaporated under reduced pressure at 40° and the contents were diluted with water (1500 ml.) and cooled in an ice box. The 19-hydroxy-4-androsten-3-one-17β-(2′-tetrahydropyranyl) ether (VII, 739 mg., 93%) which precipitated was filtered and crystallized from ethyl acetate to give the analytical product, m.p. 195–201°, $[\alpha]_D$ +140° (*c* 0.97), λ_{max} 243 mμ (ε 15,757); ν_{max} 3430, 1655, and 1610 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 74.19; H, 9.34. Found: C, 73.91; H, 9.45.

3,19-Dioxo-4-androstene-17β-(2′-tetrahydropyranyl) Ether (VIII).—To a cold solution (10–15°) of VII (693 mg.) in acetone (50 ml.) under nitrogen atmosphere, 8 *N* chromic acid was added dropwise until a reddish brown color persisted. After an additional 2 min. stirring, the mixture was diluted with water (500 ml.) and extracted with ethyl acetate. The extract was washed with brine, sodium bicarbonate solution, and with water until neutral, and dried over anhydrous sodium sulfate. The solvent was removed and the residue (608 mg.) was chromatographed on alumina (18 g.). Elution of the chromatographic column with benzene gave 3,19-dioxo-4-androstene-17β-(2′-tetrahydropyranyl) ether (VIII, 490 mg., 71%) which was crystallized from acetone–petroleum ether to give the analytical product, m.p. 114–118°, $[\alpha]_D$ +98° (*c* 0.57), λ_{max} 243 mμ (ε 13,426), ν_{max} 2725, 1710, 1673, and 1615 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.67; H, 9.14.

3,19-Dioxo-4-androsten-17β-ol (IX).—To a solution of VIII (439 mg.) in 90% ethanol (25 ml.), concentrated hydrochloric acid (0.4 ml.) was added and the contents were refluxed for 1 hr. in an atmosphere of nitrogen. The reaction mixture was cooled and neutralized with sodium bicarbonate solution. It was then diluted with water (500 ml.) and extracted with ethyl acetate. The ethyl acetate extract was washed with brine solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a resinous product (342 mg.) which was chromatographed on alumina (10 g.). After 3,19-dioxo-4-androsten-17β-ol (IX, 241 mg. 70%) was eluted with benzene–ether (8:2), it was crystallized (needles) from acetone–petroleum ether, m.p. 124–126°, $[\alpha]_D$ +186° (*c* 0.2), λ_{max} 246 mμ (ε 12,916), ν_{max} 3450, 2725, 1750, 1660, and 1610 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.11; H, 8.80.

The second polymorphic form was crystallized from acetone (rectangular prisms), m.p. 167–168°, $[\alpha]_D$ +195°; λ_{max} 246 mμ (ε 12,880), ν_{max} 3448, 2725, 1750, 1660, and 1610 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.51; H, 8.69.

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Reactions of Ortho Esters with Di-*t*-butyl Peroxide

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In reactions induced by dialkyl peroxides, compounds having ether functions undergo fragmentation by a free radical path yielding carbonyl-containing products. For example, acetals, the class of compounds that have received the most attention in such reactions,[1] yield esters as the major products in reactions induced with di-*t*-butyl peroxide. We have extended the investigation of the free radical reactions of compounds with ether functions to a study of the di-*t*-butyl peroxide induced reactions of trimethyl orthoformate, triethyl orthoformate, and triethyl orthoacetate. The orthoformates yield both carbonate and formate esters as reaction products whereas ethyl acetate was obtained from the orthoacetate.

Heating a mixture of an ortho ester and di-*t*-butyl peroxide at 135° results in the formation of the liquid and gaseous products in the quantities listed in Table I. Both the qualitative identifications and the quantitative determinations of these products were made by gas chromatographic analyses of the reaction mixtures and the gases evolved.

These products and their distribution suggest that the following sequence of free radical processes may be involved in these reactions.

$$((CH_3)_3CO)_2 \longrightarrow 2(CH_3)_3CO\cdot \quad (1)$$

$$(CH_3)_3CO\cdot \longrightarrow CH_3COCH_3 + CH_3\cdot \quad (2)$$

$$(CH_3)_3CO\cdot \text{ (or } CH_3\cdot) + \underset{\text{I}}{(RCH_2O)_3CH} \longrightarrow (CH_3)_3COH \text{ (or } CH_4) + \underset{\text{A}\cdot}{(RCH_2O)_3C\cdot} \quad (3)$$

$$(CH_3)_3CO\cdot \text{ (or } CH_3\cdot) + \text{I} \longrightarrow (CH_3)_3COH \text{ (or } CH_4) + \underset{\text{B}\cdot}{(RCH_2O)_2CHO\overset{\cdot}{C}HR} \quad (4)$$

$$\text{A}\cdot \longrightarrow (RCH_2O)_2C{=}O + \underset{\text{C}\cdot}{RCH_2\cdot} \quad (5)$$

$$\text{B}\cdot \longrightarrow RCHO + \underset{\text{D}\cdot}{(RCH_2O)_2\overset{\cdot}{C}H} \quad (6)$$

$$\text{D}\cdot \longrightarrow RCH_2OCHO + \text{C}\cdot \quad (7)$$

$$\text{D}\cdot + \text{I} \longrightarrow (RCH_2O)_2CH_2 + \text{A}\cdot \text{ or B}\cdot \quad (8)$$

$$\text{C}\cdot + \text{I} \longrightarrow RCH_3 + \text{A}\cdot \text{ or B}\cdot \quad (9)$$

R = H or CH_3

According to this proposed reaction scheme, certain quantitative relationships should exist

(1) L. P. Kuhn and C. Wellman, *J. Org. Chem.*, **22**, 774 (1956); E. S. Huyser, *ibid.*, **25**, 1820 (1960); C. L. Aldrich, J. B. Zachry, and E. A. Hunter, *ibid.*, **27**, 47 (1962); E. S. Huyser and Z. Garcia, *ibid.*, **27**, 2716 (1962).