(b) **Cholesterol-24-C^{14}.**—Four separate experiments were performed. 1. A freshly prepared sample (m.p. 145–146°, s.a. 0.18 μc./mg. cholesterol) was analyzed by the above described procedure for cholesterol and 7-ketocholesterol. Within the limits of experimental error, the entire activity resided in the cholesterol fraction; a trace (<1%) was found in the 7-ketocholesterol fraction.

2. A portion of the same sample (m.p. 145–146°) was first dried in a high vacuum at 70° for 12 hours and then sealed in a vial and stored in the dark for 15 months. After this period of time, the m.p. had dropped to 129–130° and the infrared spectrum showed the broad band in the 2.8–3.1 μ region, characteristic of the decomposition products. The material was analyzed for cholesterol and 7-ketocholesterol. The cholesterol was found to possess 93% of the total activity and the 7-ketocholesterol 3%.

3. A portion of the same sample was kept for a month in a screw cap vial filled with air and then sealed into a vial under reduced pressure and stored for 14 months in the darkness. The aged material sintered 120° and melted at 129–130°. The infrared spectrum showed the broad band at 2.8–3.1 μ and a questionable indication of a carbonyl band. The sample was analyzed for cholesterol and 7-ketocholesterol and was found to have 87% of the total activity in the former and 7% of the total activity in the latter.

4. A portion of the sample was stored in a screw cap vial and the vial was opened at least once a week for 15 months and the sterol stirred with a glass rod. After this period, the m.p. was 126–127° and the infrared spectrum showed the broad band at 2.8–3.1 μ. The sample was analyzed for only three components and the results were: 83% cholesterol, 5% 7-ketocholesterol and 5% 7β-hydroxycholesterol.

(c) **Cholesterol-4-C^{14}.**—Two separate experiments were performed. 1. A sample of freshly prepared cholesterol (m.p. 146–147°, s.a. 3.83 μc./mg. cholesterol) was stored in a screw cap vial filled with air for a period of three months. After this period the m.p. was 129–130° and the infrared spectrum showed the broad band at 2.8–3.1 μ and a weak band at 6.0 μ. The sample was only analyzed for cholesterol and 7-ketocholesterol and these compounds possessed 92 and 7%, respectively, of the total activity.

2. A portion of the same sample was sublimed in a high vacuum and sealed directly in a vial without changing the pressure. The material was stored for 10 weeks in the darkness. At the end of this period, the material had a m.p. of 146–147° and within experimental error, all the activity was found to reside in the cholesterol when the sample was analyzed.

Experiments with Unlabeled Cholesterol. (a).—Purified cholesterol (m.p. 146–147°) was sealed in an evacuated vial and stored for one year in the darkness. At the end of this period the m.p. had not changed and the infrared spectrum was identical with that of the material at the start of the experiment.

(b).—Purified cholesterol (m.p. 146–147°) was stored for one year in a screw cap vial in the dark. At least once a week the vial was opened and the contents stirred with a glass rod. After one year, the cholesterol was still colorless but the crystals stuck together. After drying the sample, both the m.p. and the infrared spectrum were identical with that obtained at the start of the experiment.

(c).—Purified cholesterol (m.p. 146–147°) was stored for 18 months in a dark bottle equipped with a stirring device. The cholesterol was exposed to the atmosphere of the laboratory, only protected from the dust. The material was stirred intermittently. At the end of the experiment, the cholesterol was yellowish but the m.p., rotation and the infrared spectrum were identical with that obtained at the start of the experiment.

BERKELEY 4, CALIFORNIA

[CONTRIBUTION FROM THE DIVISION OF CANCER RESEARCH, DEPARTMENT OF SURGERY, UNIVERSITY OF ROCHESTER MEDICAL CENTER]

The Preparation of Δ^5-Androsten-17β-ol-3,7-dione 17-Acetate 3-Ethylene Ketal and Some of its Reactions

BY P. N. RAO[1] AND P. KURATH

RECEIVED APRIL 10, 1956

After the oxidation of Δ^5-androsten-17β-ol-3-one 17-acetate 3-ethylene ketal with *t*-butyl chromate, the starting material and Δ^5-androsten-17β-ol-3,7-dione 17-acetate 3-ethylene ketal were isolated from the reaction mixture. Several reactions carried out with the latter proved the assigned 7-ketone structure.

In view of a recent publication by Lenhard and Bernstein[2] describing a method for the preparation of 7-keto derivatives of ethylene ketals of Δ^4-3-ketosteroids, it seems desirable to report a different approach to the synthesis of such compounds.

After the oxidation of Δ^5-androsten-17β-ol-3-one 17-acetate 3-ethylene ketal (I)[3] with *t*-butyl chromate, the starting material I and an α,β-unsaturated ketone II with a high ultraviolet absorption maximum (log ε 4.1) at 241 mμ were isolated. The high absorption maximum of II indicated that the oxidation of I occurred on the allylic center of C-7 rather than of C-4[4] and the new product was, therefore, formulated as Δ^5-androsten-17β-ol-3,7-dione 17-acetate 3-ethylene ketal (II).

Following the alkaline hydrolysis of II, the Δ^{3,5}-androstadiene-3-(β-hydroxy)-ethoxy-17β-ol-7-one (III) was isolated. Treatment of II with methanolic sulfuric acid, followed by alkaline hydrolysis to ensure complete saponification of the 17-acetoxy group, yielded Δ^{3,5}-androstadiene-3-methoxy-17β-ol-7-one (IV) as the only crystalline product. This compound had the expected ultraviolet absorption maximum at 311 mμ, similar to the ultraviolet of III.[2,5]

These two hydrolysis experiments indicated that the formulation of II was correct. Oppenauer oxidation of the 17-hydroxy group in IV yielded the Δ^{3,5}-androstadiene-3-methoxy-7,17-dione (V). Following a similar oxidation of the Δ^5-androstene-3β,17β-diol-7-one (VI),[6] the Δ^{3,5}-androstadiene-3-ol-7,17-dione (VII) was obtained which, on treatment with methanolic sulfuric acid, yielded a compound identical with V. Inasmuch as V was

(1) Fulbright Fellow 1954–1955. Division of Organic Chemistry, National Chemical Laboratory, Poona, India.

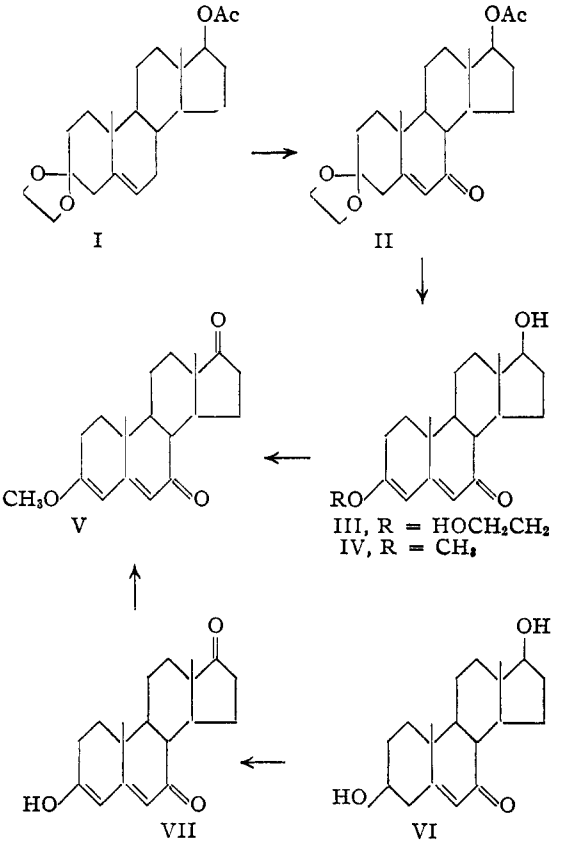
(2) R. H. Lenhard and S. Bernstein, THIS JOURNAL, **78**, 989 (1956).

(3) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952).

(4) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, p. 191; *cf.*, L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(5) (a) J. Barnett, B. E. Ryman and F. Smith, *J. Chem. Soc.*, 526 (1946); (b) C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *ibid.*, 2375 (1952); L. Dorfman, ref. 4.

(6) A. Butenandt, E. Hausmann and J. Paland, *Ber.*, **71**, 1316 (1938); K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).



obtained by two independent syntheses (I→II→IV →V←VII←VI), it is evident that upon oxidation of I the allylic center at C-7 was attacked.

Analogy to the corresponding Δ³,⁵-cholestadiene-3-methoxy-7-one[5] led to the formulation of III, IV and V as enol ethers on C-3. The high negative values found for the optical rotations[2,5b] of III, IV and V, and the similarity of their ultraviolet absorption spectra[2,5] justified their assigned structures.

Experimental[7,8]

Δ⁵-Androsten-17β-ol-3,7-dione 17-Acetate 3-Ethylene Ketal (II).—The solution of 3 g. of Δ⁵-androsten-17β-ol-3-one 17-acetate 3-ethylene ketal (I)[9] in 24 ml. of carbon tetrachloride was stirred and warmed to 80°. Then a mixture of 31 ml. of *t*-butyl chromate solution in carbon tetrachloride,[9] 3.6 ml. of acetic acid and 9.6 ml. of acetic anhydride was added dropwise over a period of 15 minutes to the warmed steroid solution. The reaction mixture was stirred and kept at 80° for 12 hr. The contents of the flask were cooled in an ice-bath and a solution of 7.2 g. of oxalic acid in 56 ml. of water was added dropwise with continued cooling and stirring. Later 3 g. of solid oxalic acid was added. The carbon tetrachloride layer was separated, washed with water, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. A residue of 3.13 g. of crude reaction product was obtained. This was further purified by chromatography on 80 g. of alumina (grade II/III). The early fractions with petroleum ether–benzene 1:1 yielded a total of 1044 mg. of a mixture of starting material and of the desired compound II as shown by further chromatographic purification of these products. From the later eluates of petroleum ether–benzene 1:1 and benzene, a total of 1190 mg. of Δ⁵-androsten-17β-ol-3,7-dione 17-acetate 3-ethylene ketal (II) was obtained. This, on crystallization from acetone, gave 1115 mg. of II, m.p. 255–258°, corresponding to a 36% yield. An analytical sample was obtained after three additional recrystallizations from acetone; m.p. 260–262°, $[\alpha]^{23}D$ −95° (*c* 1.25), λ_{max}^{EtOH} 241 mμ (log ε 4.1).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.24; H, 8.24.

Δ³,⁵-Androstadiene-3-(β-hydroxy)-ethoxy-17β-ol-7-one (III).—A solution of 405 mg. of II and 288 mg. of sodium hydroxide in 15 ml. of ethylene glycol was heated to 85° for 1 hr. in an atmosphere of nitrogen. Upon cooling, a saturated solution of sodium chloride was added, the mixture acidified with 2 *N* HCl and extracted with ether. The ether extract was washed with a saturated salt solution until neutral, dried, concentrated and evaporated to dryness. Following chromatographic purification of 396 mg. of the crude residue on 11 g. of alumina (grade II/III), 240 mg. of crude compound was isolated from the eluates of benzene–ether 1:1. After several recrystallizations from methanol–water, 63 mg. of III was isolated, m.p. 223–225°. An analytical sample, m.p. 224.5–226.5°, $[\alpha]^{28}D$ −372° (*c* 0.97), λ_{max}^{EtOH} 311 mμ (log ε 4.39), was obtained.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.64; H, 8.82.

Δ³,⁵-Androstadiene-3-methoxy-17β-ol-7-one (IV).—A solution of 1 g. of II in 112 ml. of methanol and 11.2 ml. of 8.5% (v./v.) sulfuric acid was refluxed for 1 hr. in an atmosphere of nitrogen. After working up as described for III, a residue of 880 mg. of crude compound was obtained. The latter was then refluxed in 33 ml. of 2% methanolic potassium hydroxide for 15 minutes. The mixture was worked up as before and 829 mg. of crude reaction product was isolated. Chromatographic purification on 25 g. of alumina (grade II/III) yielded 446 mg. of the compound IV in the eluates of benzene and benzene–ether 8:2. The compound was recrystallized from methanol–water to yield 279 mg. of crystals, m.p. 161–164°. Upon concentration of the mother liquors an additional 85 mg. of a less pure product was obtained, m.p. 159–162°. A portion of the first crop was recrystallized for analysis, m.p. 167–168°, $[\alpha]^{25}D$ −413° (*c* 1.28); λ_{max}^{EtOH} 311 mμ (log ε 4.35).

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.72; H, 8.96.

Δ³,⁵-Androstadiene-3-methoxy-7,17-dione (V).—The compound was prepared by the modified Oppenauer oxidation[10] of 192 mg. of IV (m.p. 164–166°) in 44 ml. of toluene and 3.5 ml. of cyclohexanone containing 0.32 g. of aluminum isopropoxide. After working up as previously described,[10] the reaction mixture yielded 200 mg. of a crude product. Chromatographic purification on 7 g. of alumina (grade II/III) yielded, in the eluates of petroleum ether–benzene 8:2, 1:1 and benzene, a total of 152 mg. of a crystalline product. After one crystallization from methanol–water, 124 mg. of V, m.p. 202–203°, was obtained. This was recrystallized twice from benzene–high boiling petroleum ether and again from aqueous methanol for analysis, m.p. 207–208°, $[\alpha]^{26}D$ −383° (*c* 1.00), λ_{max}^{EtOH} 311 mμ (log ε 4.35).

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.30; H, 8.31.

Δ³,⁵-Androstadien-3-ol-7,17-dione (VII) and Δ³,⁵-Androstadiene-3-methoxy-7,17-dione (V).—A solution of 400 mg. of Δ⁵-androstene-3β,17β-diol-7-one (VI)[6] in 64 ml. of toluene and 5 ml. of cyclohexanone was treated as previously described with 0.6 g. of aluminum isopropoxide.[10] Recrystallization of the product from ethyl acetate gave an analytical sample of Δ³,⁵-androstadien-3-ol-7,17-dione (VII), m.p. 225–227°, $[\alpha]^{26}D$ −9.6° (*c* 0.81), λ_{max}^{EtOH} 322 mμ (log ε 4.3), $\lambda_{max}^{0.1\%\ NaOEt}$ 392 mμ (log ε 4.7).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.75; H, 7.81.

Upon standing the compound was found to be unstable, changing in color from white to yellow to brown.

(7) Microanalyses by Mr. C. W. Beazley, Skokie, Illinois.

(8) The melting points were corrected. All optical rotations were measured in chloroform solution. The analytical samples were dried in high vacuum over phosphorus pentoxide at 80° for 10 days.

(9) This solution was prepared according to K. Heusler and A. Wettstein, ref. 6.

(10) S. Bernstein, M. Heller and S. M. Stolar, THIS JOURNAL, **76**, 5674 (1954); *cf.* H. H. Inhoffen, W. Logemann, W. Hohlweg and A. Serini, *Ber.*, **71**, 1024 (1938).

The oily mother liquors (192 mg.) of the above sample were dissolved in 20 ml. of methanol and 2 ml. of dilute sulfuric acid (8.5% v./v.) and refluxed under nitrogen for 1 hr. After working up as described before and purifying chromatographically, 82 mg. of $\Delta^{3,5}$-androstadiene-3-methoxy-7,17-dione (V) was obtained. This was recrystallized from benzene–high boiling petroleum ether and methanol–water for analysis, m.p. 207–208°, $[\alpha]^{24}D$ −379° (*c* 0.94), λ_{max}^{EtOH} 311 mμ (log ε 4.37).

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.55; H, 8.57.

Admixture of this compound with V, obtained from II, did not depress the melting point.

Acknowledgments.—This investigation was supported by the John J. Morton Cancer Fund. The authors are indebted to Dr. J. J. Morton and Dr. M. Radakovich for constant encouragement during the course of this investigation. We wish to thank Drs. M. Radakovich, W. H. Strain and F. Ganis for valuable discussions and help during the preparation of this manuscript. Our thanks go to Miss Margaret Capezzuto for valuable technical assistance during the experimental part of this work.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE ROLLIN H. STEVENS MEMORIAL LABORATORY OF THE DETROIT INSTITUTE OF CANCER RESEARCH]

The Partial Degradation and Reconstitution of the "A" Ring of Estradiol[1]

BY JOHN A. HARTMAN, ARTHUR J. TOMASEWSKI AND ANDRE S. DREIDING

RECEIVED JUNE 26, 1956

The ozonization of 19-nortestosterone-17-acetate produced the corresponding keto acid which was cyclized to the enol lactone. The latter was treated with methylmagnesium iodide and recyclized to 19-nortestosterone, purified as its 17-acetate. The aromatization into estradiol was accomplished with the use of N-bromosuccinimide, thereby providing a method for the preparation of the female sex hormone labeled at C_4.

At the present time, estrogen metabolism is not well defined. The studies using estrone-C_{16}^{14} have indicated that the "D" ring is partially destroyed. This is evidenced by the observation that of the total recovery of radiocarbon (45%), only *ca.* 4.5% was extractable with ether, the remainder being respiratory (2.4%) and water-soluble metabolites, the latter being presumably of low molecular weight.[2] Possible metabolites resulting from a complete saturation of the aromatic nucleus have been reported as being present in human non-pregnancy urine.[3] The desirability of an "A" ring labeled estrogen led to the investigation of various methods commonly used to aromatize the steroids containing the 1,4-dien-3-one structure in the "A" ring.[4] Since this approach seemed to be of little value, it was abandoned when 19-nortestosterone (IVa) became readily available by the Birch reduction of estradiol-3-ethers.[5] Methods for the incorporation of isotopic carbon into the "A" ring of the non-aromatic steroids have been well described[6,7] and it was assumed that IVa would behave in a similar manner since its stereochemistry at C_{10} is similar to that of testosterone.[5] With a new carbon at C_4, the objective could then be reached by aromatization of IV into estradiol (Ia).

The Wilds–Nelson procedure for the preparation of IVa[5] was modified slightly so that the steroid (Id) was added to the ether–ammonia–lithium mixture. Similar yields of the dihydro compound IId were obtained and an additional safety factor was introduced inasmuch as the most valuable component was added last.[8] The non-conjugated ketone IIIa and the conjugated ketone IVa were prepared and a number of derivatives of the latter examined. It has been noted that attempts to prepare the 17-propionate of IVa resulted in a mixture of mono and di(enol) acylation.[5] Direct acetylation of IVa gave a high yield of IVb; the latter was also obtained by partial saponification of the enol diacetate VIIIc. Ozonization of the 17-acetate IVb, 17-*p*-nitrobenzoate IVf and the 17-benzoate IVe gave the crystalline keto acids (Vb, f and e), respectively. Refluxing the keto acids with acetyl chloride gave the pseudo acid chlorides (VIb and e), only that of the 17-benzoate being a fairly stable solid. The infrared spectrum of the latter indicated a peak at 13.37μ which has been ascribed to structures of this type.[6] The enol lactone of the 17-benzoate VIIe was prepared by dehydrochlorination of VIe in refluxing collidine. One of the corresponding enol lactone-17-acetates VIIb was prepared by refluxing the keto acid Vb with acetic anhydride in the presence of anhydrous sodium acetate[9] to give a product which could be crystallized to a sharp melting (129–130°) solid exhibiting $[\alpha]^{25}D$ −37.9°.

Another isomer was obtained when prepared from Vb as described for testosterone.[6] This isomer was difficult to obtain in good yields and could

(1) This work was supported in part by a Research and Development contract between the Detroit Institute of Cancer Research and the United States Atomic Energy Commission. Additional support was provided by institutional grants from the American Cancer Society, Inc., the American Cancer Society, Southeastern Michigan Division and the Kresge Foundation. A generous supply of estradiol was furnished by the Schering Corporation, Bloomfield, N. J.

(2) R. D. H. Heard, *et al.*, "Recent Progress in Hormone Research," Vol. IX, Academic Press, New York, N. Y., 1954, p. 383.

(3) R. E. Marker, E. Rohrmann, E. L. Wittle and E. J. Lawson, THIS JOURNAL, **60**, 1901 (1938).

(4) A. S. Dreiding and A. Voltman, *ibid.*, **76**, 537 (1954); and previous papers referred to therein.

(5) A. L. Wilds and N. A. Nelson, *ibid.*, **75**, 5366 (1953). A superior method is described using estradiol-3-methyl ether in ether–liquid ammonia and lithium metal. The original method as described by A. J. Birch, *J. Chem. Soc.*, 2531 (1949), gives low yields.

(6) R. B. Turner, THIS JOURNAL, **72**, 579 (1950).

(7) (a) G. Fujimoto, *ibid.*, **73**, 1856 (1951); (b) R. D. H. Heard and P. Ziegler, *ibid.*, **73**, 4036 (1951).

(8) This modification was also used in the reduction (89% yield) of estrone-3-methyl ether-17-dioxalane.

(9) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952).