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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

## The Synthesis of Estradiol and 1-Methylestradiol from Cholesterol<sup>1</sup>

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Inhoffen and his co-workers have carried out a series of investigations with the aim of developing methods for the partial aromatization of ring A in various sterol derivatives, as a route to the synthesis of female sex hormones of the estrogenic type.<sup>4</sup> This research reached a climax in 1941, when Inhoffen and Zühlsdorff<sup>5</sup> reported the synthesis of the sex hormone  $\alpha$ -estradiol (VII) from 1,4-androstadien-17-ol-3-one (Vb), which in turn had been prepared from cholesterol. The over-all yield of the hormone was quite low; indeed, the last step, which consisted in heating the dienone Vb at 325° to effect elimination of the angular methyl group, resulted in less than a 5% yield of  $\alpha$ -estradiol. Nevertheless, this material was reported to be obtained in pure form and to be identical with the natural product in the melting points of the diol, 3-monobenzoate and diacetate, in absorption spectrum and in estrogenic activity.<sup>5</sup>

This synthesis of estradiol is of considerable interest for itself, and because it provided an interconversion of the two large classes of natural steroids—the non-aromatic, of which cholesterol is a member, and the partially aromatic, exemplified by estradiol. Due to the ready availability of cholesterol, the synthesis, if modified and improved in yields, might offer a practical method of preparing the natural estrogenic hormone, for which no synthesis has been available previously.<sup>6</sup>

(1) From part of the Ph. D. thesis of Carl Djerassi.

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(4) See Inhoffen, *Angew. Chem.*, **53**, 471 (1940), for a review of this work.

(5) Inhoffen and Zühlsdorff, *Ber.*, **74**, 1911 (1941).

(6) In a communication in 1936 Marker, Kamm, Oakwood and Laucius [*THIS JOURNAL*, **58**, 1503 (1936)] reported the synthesis of estrone from tetrahydrocholesterol, but without details. This synthesis was questioned by Windaus and Deppe [*Ber.*, **70**, 76 (1937)] on the grounds that reduction of the naphthol ring gave primarily non-phenolic material instead of the phenolic reduction

product necessary in the above synthesis. In a subsequent publication on the reduction of equilenin by Marker [*THIS JOURNAL*, **60**, 1897 (1938)] this discrepancy apparently was clarified, when it was stated that the minor reduction product (phenolic), rather than the major product (non-phenolic) was employed in the synthesis. However, until the experimental details appear, this work cannot be properly evaluated [*cf.*, Remesov, *Rec. trav. chim.*, **56**, 1093 (1937) and Ruzicka, Müller and Mörgeli, *Helv. Chim. Acta*, **21**, 1394 (1938)].

For preparing 1,4-androstadien-17-ol-3-one (Vb), the key intermediate in the synthesis of estradiol, we started with androstene-3,17-diol 3-acetate 17-benzoate (I) rather than the diacetate originally employed by Inhoffen.<sup>7</sup> The unsymmetrical ester is an important intermediate in the commercial preparation of testosterone from cholesterol. Hydrogenation of the nuclear double bond as well as the benzoate group to 3,17-androstenediol 3-acetate 17-hexahydrobenzoate, partial saponification to remove the 3-acetate group<sup>8</sup> and oxidation, were carried out by modifications of the procedures of Ruzicka and Kägi,<sup>9</sup> giving dihydrotestosterone hexahydrobenzoate (II), in 72–75% over-all yields from I.

Inhoffen and Zühlsdorff,<sup>10</sup> investigating the introduction of two bromine atoms into the ketone II, reported that the product formed initially was the 2,2-dibromo derivative III (m. p. 168–170°), which underwent rearrangement to the 2,4-dibromo isomer IV (m. p. 178–180°) upon standing in hydrogen bromide-acetic acid solution. No

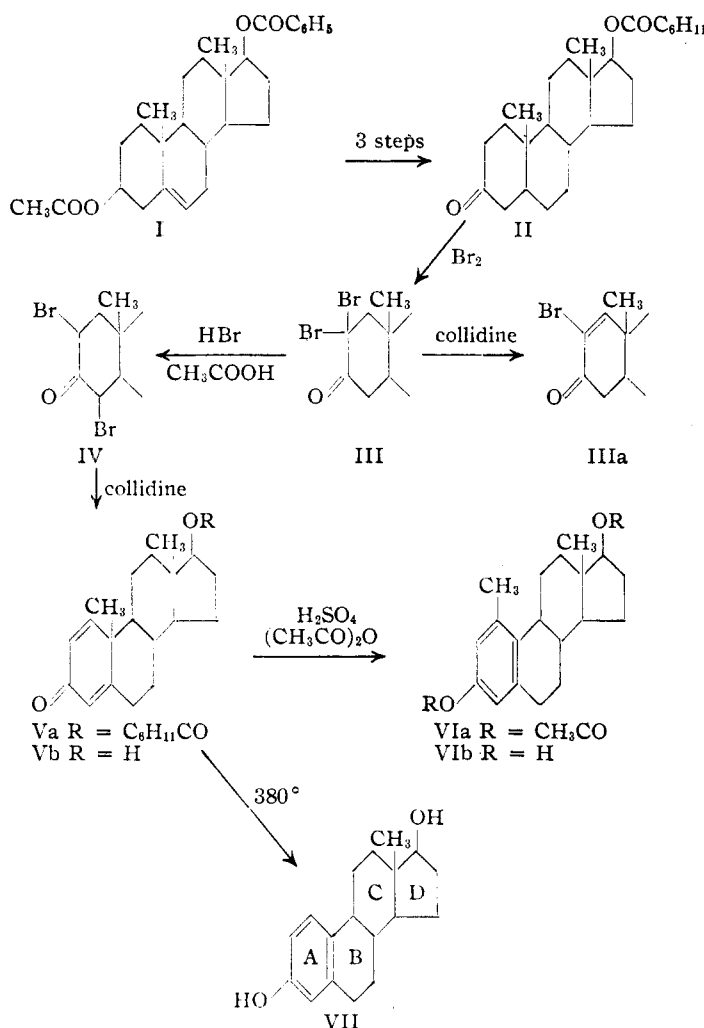
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(7) Inhoffen, Zühlsdorff and Huang-Minlon, *Ber.*, **73**, 451 (1940).

(8) The partial saponification is more selective with this unsymmetrical ester than with the diacetate; *cf.* Butenandt, Tscherning and Hanisch, *Ber.*, **68**, 2097 (1935), and Butenandt and Dannenberg, *ibid.*, **69**, 1161 (1936).

(9) Ruzicka and Kägi, *Helv. Chim. Acta*, **20**, 1557 (1937).

(10) Inhoffen and Zühlsdorff, *Ber.*, **76**, 233 (1943).



yields were reported. Repetition of these reactions gave products having melting points at variance with those reported by Inhoffen; the dibromo derivative obtained initially melted at  $185\text{--}186^\circ$ , and that obtained after rearrangement melted at  $175\text{--}176^\circ$ . Further investigation indicated the original product to be the 2,2-isomer and the rearrangement product the 2,4-isomer, thus confirming the course of the reaction reported by the German workers.<sup>10</sup> However, the material which they describe as the pure 2,2-isomer probably was instead the 2,4-isomer, resulting from rearrangement in the solvent (acetic acid) used for the recrystallization. The matter was complicated by the existence of three polymorphic forms for the 2,2-isomer, which, however, could readily be distinguished from the 2,4-isomer by their optical rotations. The procedures for preparing the dibromo ketones were improved considerably, so that the 2,2-isomer could be obtained in 71–74% yield and through it the 2,4-isomer in the same over-all yield from the dihydrotestosterone ester II.

Dehydrobromination of the 2,2-dibromo isomer

III by heating with  $\gamma$ -collidine gave the unsaturated bromo ketone (IIIa) reported by Inhoffen, thus confirming a crucial point in establishing the structure of the dibromo compound.<sup>10</sup>

A similar dehydrobromination of the 2,4-isomer IV gave the bromine-free 1,4-androstadien-17-ol-3-one ester Va.<sup>10</sup> Under the best conditions found, a 52% yield of satisfactory product was isolated after chromatographic separation on alumina. The remaining mixture, which could be partially separated by rechromatographing, was examined for light absorption in the ultraviolet, with the results shown in Fig. 1. The curve for Fraction A corresponded approximately to that for the unsaturated bromo ketone IIIa, and a small amount of the crystalline material could be isolated. The curve for fraction B, with a maximum at  $282.5\text{ m}\mu$ , indicated the presence of material with a longer conjugated system than the 1,4-androstadienolone ester Va (maximum at about  $244\text{ m}\mu$ ). This is very probably the 4,6-androstadienolone ester resulting from rearrangement. The corresponding 4,6-isomer in the acetate series has been isolated by Inhoffen,<sup>10</sup> and recently it was shown that a similar compound is formed as a by-product in the cholesterol series.<sup>11</sup>

Hydrolysis of the hexahydrobenzoate ester Va gave 1,4-androstadienolone (Vb) in good yield. The compound agreed in its properties with those reported by Inhoffen.<sup>7</sup> The over-all yield of this compound in the seven-step synthesis from I was about 25%.

Inhoffen and Zühlsdorff<sup>12</sup> reported the rearrangement of the dienone Vb to 1-methylestradiol (as the diacetate VIa) in 37% yield, by treatment with acetic anhydride and sulfuric acid at room temperature. We have confirmed this rearrangement and by a slight modification in procedure have increased the yield to 71%. Hydrolysis of the diacetate gave 1-methylestradiol (VIb) in 87% yield. This compound is reported to be inactive as an estrogen.<sup>12</sup>

The method reported by Inhoffen and Zühlsdorff<sup>8</sup> for effecting aromatization of ring A of 1,4-androstadienolone (Vb) with *elimination* of the angular methyl group involved heating the compound in a sealed tube for twelve minutes at  $325^\circ$ . By a laborious purification procedure they reported they were able to isolate pure  $\alpha$ -estradiol; the exact yield was not reported but it would appear to be somewhat less than 5%. They mentioned that the use of a solvent such as tetralin

(11) Wilds and Djerassi, *This Journal*, **68**, 1712 (1946).

(12) Inhoffen and Zühlsdorff, *Ber.*, **74**, 804 (1941).

markedly increased the yield of estradiol but reported no details at that time. In a recent patent Inhoffen<sup>13</sup> has described the use of several solvents and claimed to obtain a yield of 14.5% of estradiol with tetralin, and 20% with dihydronaphthalene. In these experiments the materials were heated in a sealed tube at 370–380° for thirty minutes. The isolation of  $\alpha$ -estradiol from the phenolic fraction was very greatly simplified by utilizing the observation of Priewe,<sup>14</sup> that in methanol solution the hormone forms an insoluble addition product with urea, which is readily cleaved to estradiol by warming with water.

In the present work the first attempts to duplicate the results of Inhoffen were made with dihydronaphthalene. Since the isomer was not specified in the patent, runs were made both with 1,4- and 1,2-dihydronaphthalene. In each case about 20% of crude alkali-soluble material was formed, from which 3 to 4% of estradiol could be isolated by means of the urea complex or as the dibenzoate. These runs were encouraging in that some estradiol was obtained, but the yields were considerably below the 20% claimed by Inhoffen.<sup>13,15</sup> A run was then made using tetralin, which Inhoffen reported to result in a 14% yield of crystalline estradiol. In our case the weight of material in the alkali-soluble fraction corresponded to about 20%, but no estradiol could be isolated. From the neutral fraction 57% of the crystalline starting material could be recovered unchanged.

Since these results were inferior to those reported, 9,10-dihydrophenanthrene was next considered as a solvent. Our best results have been obtained with this material, which does not appear to have been used by Inhoffen. The alkali-soluble fraction amounted to about 35%, from which approximately 10% of the crystalline estradiol could be isolated. At this point, it became apparent that different samples of the solvent gave differing yields of estradiol, although check runs on a given sample agreed satisfactorily. The best yields indicated above (10%) were obtained with an old but crystalline sample of dihydrophenanthrene. With a freshly distilled liquid sample the yield of crude phenolic material was smaller and that of estradiol was lowered to about one-half (3–4%). A third sample obtained by partial crystallization of the second sample of dihydrophenanthrene gave results (7% yield of estradiol) that were nearly equivalent to the best sample. These variations with dihydrophenanthrene indicate that small amounts of impurities in the solvent, possibly peroxides, may have a significant effect on the yield of estradiol formed by heating 1,4-androstadien-17-

ol-3-one. This also may be the explanation for the discrepancies between our results with tetralin and dihydronaphthalene and those of Inhoffen.<sup>13</sup> Unfortunately lack of material prevented our investigating this aspect of the reaction further at the present time.

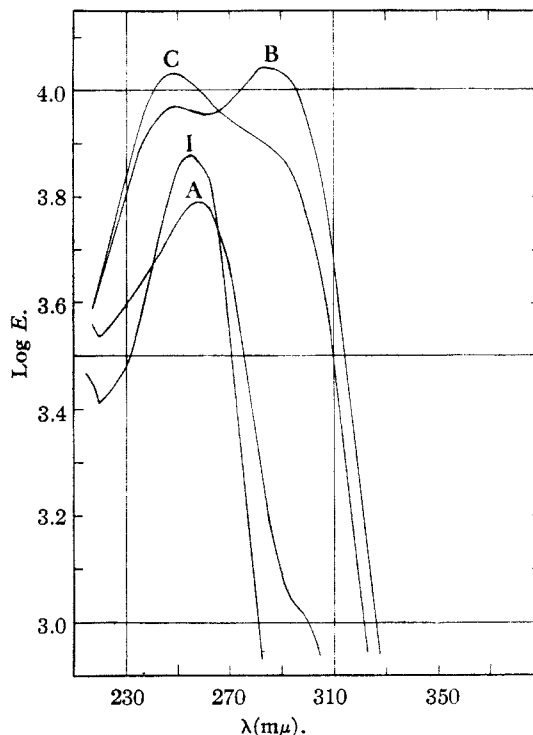


Fig. 1.—Ultraviolet absorption spectra (in absolute alcohol): curve I,  $\Delta^1$ -2-bromoandrost-17 $\alpha$ -ol-3-one 17-hexahydrobenzoate (IIIa); curve A, fraction A of chromatogram of the by-product from the preparation of Va; curve B, fraction B of by-product; curve C, fraction C of by-product.

The estradiol obtained from androstadienolone agreed satisfactorily in melting point, specific rotation and in the double melting point of the dibenzoate with the values for authentic samples of the natural hormone and its derivative, and showed no melting point depression upon admixture with these. This evidence, coupled with the comparisons in physical properties and estrogenic activity reported by Inhoffen and Zühlendorf,<sup>5</sup> establishes the identity of the synthetic material with natural estradiol. Thus, the reported synthesis of  $\alpha$ -estradiol from cholesterol<sup>16</sup> has been confirmed in the main. The failure to duplicate the yields claimed in the patent of Inhoffen for the last step can perhaps be attributed to relatively minor and pos-

(13) Inhoffen, U. S. Patent 2,361,847 (Oct. 31, 1944).

(14) Priewe, U. S. Patent 2,300,134 (Oct. 27, 1942).

(15) In this and some of the other runs described in the patent the yield appears to have been estimated from the weight of urea complex of estradiol obtained rather than from estradiol actually isolated. In our experiments the weight of complex has not been a reliable indication of yield due to contamination with excess urea.

(16) The synthesis of estradiol from cholesterol would indicate that the estrogenic hormones have the same steric configurations at the B:C and C:D ring junctures as the sterols, etc., provided no inversion occurred. However, this possibility can by no means be overlooked in the present synthesis, in view of the drastic conditions during the aromatization step (370–390°) and the lack of information about the nature of this reaction.

sibly unknown, but very significant differences in the solvents used for this reaction.<sup>16a</sup>

The neutral fractions from the aromatization runs which constituted the major product of this reaction, were investigated further in an effort to identify the constituents. A second heating with dihydrophenanthrene did not yield additional estradiol from those runs in which the higher yields (10%) had been obtained. Using a chromatographic separation it was possible to isolate some of the alkali-insoluble 1-methylestradiol (VIb) indicating that the elimination of the methyl group was accompanied by some migration. The amount of this material corresponded to a yield of 7 to 14%. The nature of the major portion of the neutral fractions is still unknown.

An aromatization run also was carried out with 1,4-cholestadienone,<sup>11</sup> using dihydrophenanthrene and a temperature of about 380–390°. The only crystalline material which could be isolated, in addition to 11% of the starting material, was 10% of the methyl migration product corresponding to VIb, 3-hydroxy-1-methyl-10-norcholestatriene-1,3,5. Inhoffen<sup>12</sup> has reported isolating a small amount of this product when the dienone was heated at 300° in a carbon dioxide atmosphere.

We are indebted to Dr. C. R. Scholz of Ciba Pharmaceutical Products, Inc., Summit, N. J., for the generous gift of the androstane derivatives used as the starting point in this work, and of a sample of natural estradiol for comparison purposes.

### Experimental<sup>17</sup>

**3 $\beta$ ,17 $\alpha$ -Androstenediol 3-Acetate 17-Hexahydrobenzoate.**—The use of acetic acid as the solvent, instead of a mixture of alcohol and acetic acid as described by Ruzicka and Kägi,<sup>9</sup> gave a smoother and more rapid reduction of the androstenediol diester. A solution of 2 g. of  $\Delta^5$ -androstene-3 $\beta$ ,17 $\alpha$ -diol 3-acetate 17-benzoate (I)<sup>18</sup> in 80 cc. of pure<sup>19a</sup> acetic acid was shaken with 200 mg. of Adams platinum oxide catalyst until the hydrogen uptake slowed down (thirty to forty minutes), at which point an additional 200 mg. of the catalyst was added and the solution shaken until the theoretical amount of hydrogen corresponding to four moles had been absorbed (two to three hours). The product, obtained after filtration of the catalyst and dilution of the filtrate, was dried at 80° and 0.2 mm. for sixteen hours; yield 1.96–2.00 g. (96 to 98%), m. p. 120–127° with some previous softening. As the material gave only a very faint Liebermann-Burchard test, it was used without purification in the next step of the synthesis. Recrystallization of a sample from ethanol gave rosetts of colorless needles in 70% recovery, m. p. 130–132° (Ruzicka and Kägi,<sup>9</sup> m. p. 134–135°).

**3 $\beta$ ,17 $\alpha$ -Androstenediol 17-Hexahydrobenzoate.**—Following the procedure of Ruzicka and Kägi<sup>9</sup> for partial

saponification, a suspension of 5.08 g. of the crude diester (m. p. 120–127°) in 114 cc. of 0.1 *N* methanolic sodium hydroxide was shaken mechanically for four and one-half hours, cooled and the crystalline solid filtered, washed with cold alcohol and dried. This product, 3.35 g. (73% yield) represented almost pure material, m. p. 165–166°. From the filtrate was isolated an additional 0.32 to 0.37 g. (m. p. 161–164°) bringing the total yield to 80–81%. Recrystallization of a sample from alcohol gave colorless, glistening leaves, m. p. 165–166°,  $[\alpha]_D^{20} + 4.5 \pm 0.5^\circ$  (11.3 mg. in 2 cc. of chloroform); reported<sup>9</sup> m. p. 167.5–168°.

No additional mono ester could be isolated from the mother liquors after repetition of the partial saponification treatment. However, complete saponification by refluxing with 50 cc. of 0.2 *N* methanolic potassium hydroxide followed by concentration, dilution with water and recrystallization of the solid from ethyl acetate gave 0.48 g. (14%) of 3 $\beta$ ,17 $\alpha$ -androstenediol, m. p. 160–162°,  $[\alpha]_D^{20} + 6 \pm 0.2^\circ$  (11.6 mg. in 2 cc. of chloroform), indicating that the original hydrogenation had gone substantially entirely to the product with the desired *allo* configuration. The pure diol is reported by Ruzicka, *et al.*,<sup>19</sup> to melt at 168° and by Butenandt, *et al.*,<sup>20</sup> at 164°,  $[\alpha]_D^{20} + 4.2^\circ$ .

**Androstan-17 $\alpha$ -ol-3-one Hexahydrobenzoate (Dihydrotestosterone Hexahydrobenzoate II).**—A solution of 3.5 g. of the monohexahydrobenzoate in 53 cc. of pure acetic acid was treated with 0.875 g. of chromic anhydride in 13 cc. of 90% acetic acid. After twenty-four hours at room temperature, the mixture was poured into saturated salt solution, extracted with ether and the latter washed with sodium carbonate (which removed a small amount of gelatinous acidic material), then with water, dried and evaporated. The colorless, crystalline residue, after drying at 90° and 0.2 mm. for eighteen hours, weighed 3.28 g. (94%), m. p. 161–163°, and was satisfactory for the next step.

Two recrystallizations from methanol gave the analytical sample as a colorless microcrystalline powder, m. p. 165–167°,  $[\alpha]_D^{20} + 24 \pm 0.5^\circ$  (8.4 mg. in 2 cc. of chloroform). The absorption spectrum<sup>21</sup> in absolute alcohol resembled that of cholestanone,<sup>21a</sup> maximum 280.5  $m\mu$  ( $\log E = 1.49$ ) and minimum 249  $m\mu$  ( $\log E = 1.33$ ).

*Anal.* Calcd. for  $C_{26}H_{40}O_3$ : C, 78.0; H, 10.1. Found: C, 78.3; H, 9.9.

Ruzicka and Kägi<sup>9</sup> reported the melting point 164–165°, but did not obtain an analytically pure sample.

**2,2-Dibromoandrostan-17 $\alpha$ -ol-3-one 17-Hexahydrobenzoate (III).**<sup>22, 22a</sup>—A solution of 2 g. of dihydrotestosterone hexahydrobenzoate in 40 cc. of pure<sup>19a</sup> acetic acid was treated with four drops of 4 *N* hydrogen bromide in acetic acid, and then dropwise with 25 cc. of acetic acid containing 1.6 g. of bromine, allowing each drop to decolorize before adding more. Colorless needles separated at the end of the addition; after standing for one hour at room temperature and one and one-quarter hours in cold water (*ca.* 10°), these were filtered, washed with water and dried at 45° and 0.2 mm. The yield was 1.99–2.07 g. (71–74%) of material melting at 185–186° (dec.). The rotations of two samples

(19) Ruzicka, Goldberg and Rosenberg, *Helv. Chim. Acta*, **18**, 1497 (1935).

(20) Butenandt, Tscherning and Hanisch, *Ber.*, **68**, 2101 (1935).

(21) The absorption spectra measurements were made using a Beckman Quartz Photoelectric Spectrophotometer;  $E = 1/c \log I_0/I$  for 1 cm. cell, where  $c$  is the concentration in moles per liter.

(21a) Klotz, *This Journal*, **66**, 90 (1944).

(22) The conditions of concentration and time of standing reported here represent the optimum for the isolation of the 2,2-dibromo derivative. The importance of time is illustrated by the following results: after 35 minutes, 47% yield; two and one-quarter hours, 71–74% yield; four hours, 65% yield; twenty-five hours, 19% yield.

(22a) Added in proof.—Recently one of us (C. D.) has found that the rate of bromination and especially of rearrangement is markedly dependent upon whether or not the acetic acid is purified. Using acetic acid which had not been distilled from permanganate, no 2,2-isomer separated and the 2,4-isomer was obtained directly after diluting and cooling the reaction mixture.

(16a) In an intelligence report on activities in Germany during the war (Report No. 245, Office of the Publication Board, U. S. Dept. of Commerce), which recently became available to us, it is stated that 15 kg. of synthetic estrone was prepared from cholesterol by Schering A. G. in 1944. While the exact synthesis used to prepare the intermediates is not clear from the report, it stated that the aromatization step consisted in passing 1,4-androstadien-3-ol-17-one with cyclohexane vapor over a nickel catalyst at 500–600° with the formation of estrone by elimination of methane.

(17) All melting points are corrected.

(18) Kindly furnished to us by Dr. C. R. Scholz.

(18a) Acetic acid distilled from potassium permanganate.

with the same melting point but from different runs were (a)  $[\alpha]^{25}_D + 101 \pm 0.7^\circ$  (9.7 mg. in 2 cc. of chloroform) and (b)  $[\alpha]^{25}_D + 95 \pm 0.6^\circ$  (6.2 mg. in 2 cc. of chloroform). Analysis indicated that neither sample was quite pure, but upon recrystallization they were converted into a different polymorphic form or into the 2,4-isomer (see below).

*Anal.* Calcd. for  $C_{26}H_{38}O_3Br_2$ : C, 55.9; H, 6.9. Found: (a) C, 57.0; H, 7.1; (b) C, 56.8; H, 6.7.

Dilution of the original mother liquor gave 0.60–0.72 g. (22–26%) of crude material, m. p. 90–130°, which, however, could be transformed into the 2,4-isomer.

The behavior of the 2,2-dibromo compound (m. p. 185–186°) upon recrystallization was rather complicated. Recrystallization from *alcohol-chloroform* gave needles (80% recovery), melting at 169.5–171°. After another recrystallization of the solid the melting point was 172.5–174° (dec.),  $[\alpha]^{25}_D + 110 \pm 0.6^\circ$  (7.9 mg. in 2 cc. of chloroform); a mixture with the crude 2,2-isomer (m. p. 185–186°) was raised in melting point to 180.5–181.5° dec., but the melting point of a mixture with the 2,4-isomer (m. p. 175–176° dec.) was depressed to 157–161°. A third recrystallization from the same solvent mixture gave needles, m. p. 164–165° (dec.),  $[\alpha]^{25}_D + 110^\circ$  (8.5 mg. in 2 cc. of chloroform); mixed m. p. with the 2,2-isomer (m. p. 185–186°) 165.5–166.5° (dec.), mixed m. p. with the 2,4-isomer, 153.5–156° dec. In another case this lowest melting form was obtained directly from the 185° material.

*Anal.* Calcd. for  $C_{26}H_{38}O_3Br_2$ : C, 55.9; H, 6.9. Found: C, 55.7; H, 6.9.

In view of their similar optical rotations, the three samples, m. p. 185–186°, 172.5–174° and 164–165°, are believed to be polymorphic forms of the 2,2-dibromo compound.

Recrystallization from *acetic acid* gave crystals (32% recovery) which melted at 168.5–172° (dec.). However, these had the rotation  $[\alpha]^{25}_D - 2.3 \pm 0.7^\circ$  (4.3 mg. in 2 cc. of chloroform) and depressed the melting point of the 2,2-isomer (m. p. 185–186°) to 146–151° (dec.), but gave no depression (m. p. 171.5–173°) with the 2,4-isomer described below (m. p. 175–176°,  $[\alpha]^{25}_D - 6.3^\circ$ ). Thus it is apparent that recrystallization from this solvent resulted in rearrangement.

Inhoffen and Zühlendorf<sup>10</sup> reported obtaining the 2,2-dibromo compound as the initial bromination product (in unspecified yield), but reported the m. p. 168–170° (dec.) for the pure compound (the m. p. of the unrecrystallized material was not stated). Since they obtained this material by recrystallization from acetic acid, it seems probable that they were actually describing the 2,4-isomer although their unrecrystallized material was undoubtedly the 2,2-isomer. Unfortunately they did not determine the rotations.

**$\Delta^1$ -2-Bromoandrosten-17 $\alpha$ -ol-3-one 17-Hexahydrobenzoate (IIIa).**—Inhoffen and Zühlendorf<sup>10</sup> reported obtaining this material from the 2,2-isomer upon treatment with collidine for fifteen minutes. In view of the discrepancies in melting point noted above, this reaction, critical for the structure of the isomers, was repeated by heating 300 mg. of the 2,2-isomer (m. p. 185–186°) in 3 cc. of  $\gamma$ -collidine at reflux under nitrogen for seventy minutes. The longer time was considered to be sufficient to allow the dehydrobromination to proceed as far as possible. The amount of collidine hydrobromide, isolated by filtering and washing with ether, corresponded to elimination of 1.28 moles of hydrogen bromide. From the filtrate, after extraction, washing with acid and alkali, was obtained a brown oil which was digested with methanol, the latter decanted, concentrated and allowed to cool slowly; 50 mg. (20%) of material, m. p. 137–145°, was obtained. After two recrystallizations from methanol, the melting point of the material was 155–156°;  $[\alpha]^{25}_D + 37.9^\circ$  (12.2 mg. in 2 cc. of chloroform). The absorption spectrum<sup>21</sup> was taken in absolute alcohol solution and is shown in Fig. 1; maximum 255  $m\mu$  (log  $E = 3.88$ ); minimum 220  $m\mu$  (log  $E = 3.41$ ). Inhoffen and Zühlendorf<sup>10</sup> reported the m. p. 154–155° and an absorption maximum in ether at 254  $m\mu$  (log  $E = 3.89$ ).

**2,4-Dibromoandrostan-17 $\alpha$ -ol-3-one 17-Hexahydrobenzoate (IV).**—Inhoffen and Zühlendorf<sup>10</sup> reported the preparation of the 2,4-isomer (A) by isolation and rearrangement of the 2,2-isomer and (B) directly from the ketone by bromination and allowing to stand overnight, each in unspecified yield. In the present work, in spite of attempts to modify procedure B, the best over-all yields were obtained by isolating the intermediate 2,2-isomer (procedure A).

**Procedure A. Rearrangement of the 2,2-Isomer.**—A solution of 2 g. of the 2,2-isomer III (m. p. 185–186°) in 200 cc. of acetic acid was treated with 9 cc. of a 4 N solution of hydrogen bromide in acetic acid and allowed to stand at room temperature (ca. 25°) for twenty-four hours. After diluting, filtering and drying at 45° (0.2 mm.), 1.9 g. (95% yield) of the crude 2,4-isomer was obtained, m. p. 160–167° (dec.). Recrystallization from alcohol-chloroform gave long colorless needles (1.51 g., 76% yield) with the melting point 175–176° (dec.) which was unchanged by further recrystallization;  $[\alpha]^{25}_D - 6.5 \pm 0.8^\circ$  (7.6 mg. in 2 cc. of chloroform).

A similar rearrangement of the impure second crop (0.60–0.72 g.) from the original bromination gave 0.46–0.54 g. of needles of the 2,4-dibromo compound of satisfactory purity, bringing the total over-all yield of the 2,4-isomer from II to 71–74%. Further rearrangement of material in the mother liquor from the pure 2,4-isomer did not yield additional pure material.

**Procedure B. Without Isolation of the 2,2-Isomer.**<sup>22a</sup>—A solution of 1 g. of the dihydrotestosterone ester II in 25 cc. of pure<sup>18a</sup> acetic acid was treated dropwise with 25 cc. of an acetic acid solution containing 0.8 g. of bromine and allowed to stand at room temperature (ca. 25°). The voluminous precipitate, which formed immediately, slowly redissolved in twenty-eight to thirty-five hours. After forty-eight hours, the clear solution was diluted with 10 cc. of water, chilled, filtered and the solid dried *in vacuo*; yield 1.0 g. (72%) of colorless material melting at 152–166° (dec.). An additional 0.36 g. (26%), m. p. 99–145° (dec.), obtained from the mother liquor by precipitation, was shown by analysis also to correspond to a dibromo derivative.

*Anal.* Calcd. for  $C_{26}H_{38}O_3Br_2$ : C, 55.9; H, 6.9. Found: C, 55.7; H, 6.8.

Recrystallization of the material from ethanol-chloroform gave 0.63 g. (45%) of needles, m. p. 172–174° (dec.);  $[\alpha]^{25}_D - 4.2 \pm 0.4^\circ$  (9.0 mg. in 2 cc. of chloroform). The melting point was not depressed by the sample (m. p. 175–176°) obtained in Procedure A. Inhoffen and Zühlendorf<sup>10</sup> reported the m. p. 178–180° (dec.) for the 2,4-dibromo compound but gave no rotation.

*Anal.* Calcd. for  $C_{26}H_{38}O_3Br_2$ : C, 55.9; H, 6.9. Found: C, 55.6; H, 6.7.

When the time of standing in Procedure B was reduced to twenty-four hours, 19% of the 2,2-isomer (m. p. 181–182°) was isolated and only 30% of the 2,4-isomer (m. p. 170–172°). Attempts were made to duplicate in Procedure B the conditions of concentration and amount of hydrogen bromide in the stages of bromination and rearrangement by Procedure A, but no increase in yield resulted.

**1,4-Androstadien-17 $\alpha$ -ol-3-one 17-Hexahydrobenzoate (Va).**—A solution of 2.1 g. of the 2,4-dibromo compound IV (m. p. 175–176° dec.) in 6.3 cc. of  $\gamma$ -collidine was refluxed for seventy minutes. The amount of collidine hydrobromide (1.38 g.) obtained by filtering the cooled mixture and washing with ether corresponded to the elimination of 1.81 moles of hydrogen bromide. The ether filtrate was washed thoroughly with 5% hydrochloric acid, 5% potassium hydroxide, water and dried over sodium sulfate. The brown oil (1.55 g.) obtained after evaporation was digested with boiling methanol and the latter decanted from a small amount (0.02 g.) of black oil. The methanol was evaporated and a solution of the product in petroleum ether (b. p. 40–60°) was adsorbed on 47 g. of alumina<sup>23</sup> in a 2 × 22 cm. column and fractionally eluted. The results in Table I were typical.

(23) Aluminum Co. of America, grade F-20, minus 80 mesh.

TABLE I

Solvent <sup>a</sup>	Fraction, wt. <sup>b</sup>	M. p., °C.	Remarks
Pet. ether-benzene (60/40)	1		
Pet. ether-benzene (60/40)	2		Very small amounts of oils
Pet. ether-benzene (50/50)	3		
Pet. ether-benzene (50/50)	4		
Pet. ether-benzene (25/75)	5 27 mg. (total)		
Pet. ether-benzene (25/75)	6		
Pet. ether-benzene (25/75)	7 68 mg.	(118) 124-133	Contained Br
Benzene	8	(98) 104-111	All fractions crystallized upon scratching
Benzene	9	(112) 114-119	
Benzene	10	(112) 114-119	
Benzene-ether (80/20)	11 507 mg.	(116) 118-123	
Benzene-ether (80/20)	12	(117) 119-123.5	All fractions crystallized upon scratching
Benzene-ether (80/20)	13		
Benzene-ether (60/40)	14		
Benzene-ether (60/40)	15		
Benzene-ether (60/40)	16		
Benzene-ether (50/50)	17 590 mg.	(121) 122-125	
Benzene-ether (50/50)	18 <sup>d</sup>		Tan colored solid
Acetone	19		
Acetone	20 165 mg.		Dark brown oil

<sup>a</sup> The amount of solvent used for each fraction was 100 cc. <sup>b</sup> This column gives the combined weight of the fractions in each group. <sup>c</sup> The temperature in parentheses denotes sintering. <sup>d</sup> The weight of this fraction is included in that for fractions 8-11.

A combination of the acetone eluates from several runs did not yield any more crystalline material upon rechromatographing.

Fractions 8 to 11 and fraction 18 were combined and recrystallized from petroleum ether (b. p. 60-68°) giving 0.34 g. of needles melting at 119-121.5°. This was combined with fractions 12 to 17 and recrystallized from petroleum ether to give 0.78 g. (52%) of the 1,4-androstadienolone ester as colorless, broad needles, m. p. 125-126.5°. This product was satisfactory for the saponification. The nature of the remaining material is considered below. The yields were somewhat lower for smaller runs than that described above (e. g. 42% yield, using 0.6 g. of dibromo derivative).

Further recrystallization of the 1,4-isomer raised the melting point to 127.5-128°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 45 ± 0.3° (8.8 mg. in 2 cc. of chloroform). Inhoffen and Zühlendorf<sup>10</sup> reported the melting point 126-127° for material prepared (in unspecified yield) by carrying out the dehydrobromination in two stages.

The time of reflux and the amount of collidine were found to be important factors in the above procedure. Forty-five minutes of refluxing (as used in the acetate series<sup>7</sup>) was insufficient, resulting in the elimination of only 1.55 moles of hydrogen bromide; heating for ninety minutes resulted in the same amount of reactions as for seventy minutes. The optimum amount of collidine was found to be 0.3 cc. per 100 mg. as described above. This is in agreement with the results found in the cholesterol series.<sup>11</sup> With 1 cc. of collidine per 100 mg. (the ratio used by Inhoffen in the acetate series<sup>7</sup>) the yield dropped to 13-23% of satisfactory material with an additional 10-20% of low-melting product.

**Characterization of the By-products from the Dehydrobromination.**—Since no additional pure material could be obtained by direct crystallization of the mother liquors, these were combined and the material (0.6 g. from two identical runs) rechromatographed as before using 20 g. of alumina. *Fraction A*, eluted by petroleum ether-benzene (25/75) gave a positive Beilstein test for halogen. The absorption spectrum<sup>21</sup> of this fraction in absolute alcohol, shown in Fig. 1, corresponded to that of  $\Delta^1$ -2-bromoandrost-17 $\alpha$ -ol-3-one hexahydrobenzoate (IIIa), except that the extinction coefficient was lower for the maximum at

257.5 m $\mu$  (log  $E$  = 3.79) and higher for the minimum at 220 m $\mu$  (log  $E$  = 3.54). Combination of this material with that in fractions 6 and 7 of the first chromatogram, and recrystallization three times from methanol gave colorless material melting at 151-154° and giving no depression in melting point with the  $\Delta^1$ -2-bromo derivative obtained above by dehydrobromination of the 2,2-isomer. Apparently it arose from a small amount of the latter compound present as an impurity in the 2,4-isomer. The amount of the  $\Delta^1$ -2-bromo derivative formed is estimated to be about 5%.

All the other fractions from the second chromatogram showed a wide melting point range (106-115°) which was not narrowed by recrystallization. The absorption spectra of two such fractions, *Fraction B* (a middle fraction eluted by benzene) and *Fraction C* (one of the last crystalline fractions) are shown in Fig. 1. The curves indicated that a partial separation had been effected; the second maximum at 282.5 m $\mu$  (log  $E$  = 4.05) shown by *Fraction B* clearly indicates the presence of 4,6-androstadien-17 $\alpha$ -ol-3-one hexahydrobenzoate (reported<sup>24</sup> for the free 17-hydroxy compound, maximum at 286 m $\mu$  in alcohol solution, log  $E$  = 4.47). *Fraction C* contained a larger amount of the 1,4-isomer and showed a single maximum at 249 m $\mu$ , corresponding to this isomer.

**1,4-Androstadien-17 $\alpha$ -ol-3-one (Vb).**—A solution of 610 mg. of the hexahydrobenzoate Va (m. p. 125-126.5°) was refluxed with 15 cc. of 5% methanolic potassium hydroxide for one and one-half hours. The solution was cooled, diluted with water, extracted with ether and the extract washed with dilute alkali and water, dried and evaporated. Trituration of the residue with petroleum ether yielded 387-403 mg. (88-92%) of the dienolone Vb, m. p. 162-167°, which was used directly for the subsequent aromatization experiments. Two recrystallizations of a sample from petroleum ether-acetone gave colorless needles (62% recovery) melting at 168.5-170°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 20 ± 0.4° (8.4 mg. in 2 cc. of chloroform). The absorption spectrum taken in absolute alcohol showed a maximum at 244 m $\mu$  (log  $E$  = 4.19). Inhoffen, *et al.*,<sup>7</sup> prepared the compound from the acetate and reported the melting point 168-169°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 22.5°, and a maximum in ether at about 236 m $\mu$  (log  $E$  = about 4.21); using the

(24) Wettstein, *Helv. Chim. Acta*, **23**, 390 (1940).

average shift<sup>25</sup> observed for this solvent the maximum in ether would correspond to approximately 242  $\mu$  in alcohol.

**1-Methyl- $\alpha$ -estradiol Diacetate (VIa).**—To a solution of 60 mg. of 1,4-androstadienolone in 1.5 cc. of acetic anhydride was added 30 mg. of concentrated sulfuric acid and the mixture was allowed to stand for four and one-half hours at about 25°. The solution was poured into 35 cc. of cold water and swirled until the acetic anhydride had hydrolyzed and most of the product had crystallized. After cooling in ice, the solid was filtered and dried at 45° under reduced pressure; yield, 55 mg. (71%), m. p. 133–136.5°. Recrystallization of a sample from methanol gave (73% recovery) rosetts of colorless needles melting at 138.5–139°. Inhoffen and Zühlendorf<sup>12</sup> obtained the diacetate in 37% yield, m. p. 135–136° (uncor.).

**1-Methyl- $\alpha$ -estradiol (VIb).**—Hydrolysis of 55 mg. of the diacetate (m. p. 133–136.5°) by heating for seventy-five minutes with 7 cc. of 5% methanolic potassium hydroxide gave, after dilution with 5% hydrochloric acid and filtering, 37 mg. (87%) of colorless crystals, m. p. 234–235.5°. Recrystallization from petroleum ether–acetone raised the melting point of the colorless needles to 235.5–236.5°,  $[\alpha]_D^{25} + 185 = 1^\circ$  (5.6 mg. in 2 cc. of dioxane); reported<sup>12</sup> m. p. 231–232° (uncor.),  $[\alpha]_D^{25} + 179.5^\circ$  (dioxane).

#### Aromatization of 1,4-Androstadien-17 $\alpha$ -ol-3-one to $\alpha$ -Estradiol

**Solvents.**—1,4-Dihydronaphthalene was prepared by reduction of naphthalene with sodium and alcohol as described by Cook and Hill.<sup>26</sup> The crude product was fractionated through a 22-cm. Vigreux column. The fraction b. p. 96.5° at 19 mm.,  $n_D^{20}$  1.5570 (49% yield) was used in the aromatization experiments (reported, b. p. 96° at 18 mm.;<sup>26</sup>  $n_D^{20}$  1.5549<sup>27</sup>). After standing for one and one-half months, the liquid had turned yellowish-green and had the  $n_D^{20}$  1.5650.

**1,2-Dihydronaphthalene** was prepared by isomerizing the 1,4-isomer with 10% sodium ethoxide solution, as described by Straus and Lemmel.<sup>28</sup> The fraction of b. p. 98° at 22 mm. (83% yield)  $n_D^{20}$  1.5800,<sup>27</sup> was used; reported b. p. 86° at 13 mm.,<sup>28</sup>  $n_D^{18}$  1.5833.

**9,10-Dihydrophenanthrene.**—Sample 1 had been prepared by the method of Fieser and Johnson,<sup>29</sup> and was approximately six years old. The material was a light yellow solid (which originally had the m. p. 32.2–33.0°, b. p. 154° at 7.5 mm.,  $n_D^{20}$  1.6430) with the  $n_D^{20}$  1.6425 (on the supercooled melt) and was used without purification.

**Sample 2**, prepared according to Durland and Adkins,<sup>30</sup> was a colorless liquid, b. p. 179° at 22 mm.,  $n_D^{20}$  1.6321.

**Sample 3** was prepared by freezing sample 2, washing several times with ice-cold methanol by decantation and filtering. The colorless solid had the m. p. 33–34°,  $n_D^{30}$  1.6380. For their purest sample Durland and Adkins<sup>30</sup> reported the m. p. 33.8–34.4°,  $n_D^{20}$  1.6406.

**Aromatization Experiments and the Isolation of Estradiol (VII).**—The following procedure was typical of all runs, except for variations in time, temperature and solvent as indicated in Table II.

A solution of 100 mg. of 1,4-androstadien-17 $\alpha$ -ol-3-one (m. p. 162–167°) in 1.3 cc. of 9,10-dihydrophenanthrene (Sample 1) was placed in a heavy-walled Pyrex tube (1.2  $\times$  16 cm.), evacuated to 25 mm. and sealed. The tube was heated in a cylindrical steel block (7.5  $\times$  17.5 cm.), equipped with a hole for the tube and a thermometer well of the same depth and heated with a burner. The time required to raise the temperature from 200 to 370° was about twenty minutes and it required approximately fifteen

minutes to cool the block from 390 to 240°. In the present run the material was heated at 380–390° for thirty minutes (not including the time required for heating and cooling). At the end of the run the tube was cooled in ice and opened. The contents were washed out with ether and the latter extracted with ten 25-cc. portions of 10% potassium hydroxide. The neutral fraction is considered in a later section.

The alkali-soluble material, obtained by acidification of the alkaline extracts with hydrochloric acid, was extracted thoroughly with ether, washed with sodium bicarbonate, water and dried. After evaporation of the ether and drying the resulting yellow-brown oil at 45° and 0.2 mm., the crude phenolic fraction weighed 34 mg.

The estradiol was isolated by Priewe's method.<sup>14</sup> The oil was treated with just enough methanolic urea solution (1–2 cc., saturated at room temperature) so that on warming, most of the material either dissolved or became crystalline. After standing for twenty-four to thirty-six hours at room temperature, the crystals of the molecular complex of urea and estradiol were filtered and washed with a small amount of the methanolic urea solution. The weight and melting point of the complex were not very significant, since it was contaminated with varying amounts of urea. In the present case 30–42 mg. of crystals was obtained, softening at 125–135° and melting at 198–213° (dec.); the pure complex is reported to melt at 221° (dec.).<sup>14</sup> The complex was cleaved by warming with 10 cc. of water, cooled and filtered to give (after drying at 45° and 0.2 mm.) 7–10 mg. (7.4–10.5% yield) of nearly colorless crystals of  $\alpha$ -estradiol, m. p. 170.5–173°.

The mother liquor from the urea complex also was treated with water and extracted with ether. The residue after evaporation was benzoyleated in 2 cc. of pyridine with 4 drops of benzoyl chloride, allowing to stand for twenty-four hours at room temperature under nitrogen. The mixture was poured into sodium bicarbonate solution, extracted with ether and washed well with bicarbonate solution, dilute acid and water. Crystallization of the oil from alcohol yielded 3–7.5 mg. (1.8–4.5%) of crude  $\alpha$ -estradiol dibenzoate, m. p. 125–136°. Recrystallization of 7.5 mg. from alcohol gave 2 mg., m. p. 138–142°.

Recrystallization of 25 mg. of synthetic estradiol (obtained from a number of runs through the urea complex) from dilute alcohol gave 7 mg. of pure  $\alpha$ -estradiol as needles, m. p. 175–176°,  $[\alpha]_D^{25} + 78 = 0.6^\circ$  (5.1 mg. in 1.8 cc. of dioxane). A mixture with an authentic sample of natural estradiol<sup>18</sup> (m. p. 176–177°) melted at 175–177°. Inhoffen and Zühlendorf<sup>18</sup> reported the m. p. 173–174° (uncor.),  $[\alpha]_D^{25} + 73.3^\circ$ , 71.7° (dioxane) for their synthetic sample. The values reported for the melting point of natural estradiol vary from 173° (uncor.) to 176–178° (cor.)<sup>31</sup> and for the specific rotation in dioxane solution from  $[\alpha]_D^{18} + 74^{31a}$  to  $[\alpha]_D^{25} + 82^{31d}$ .

The material in the filtrate from recrystallization of the estradiol was benzoyleated in pyridine as described above and crystallized from alcohol, yielding 17 mg. of  $\alpha$ -estradiol dibenzoate, m. p. 141.5–145°; the melt, after cooling, solidified and remelted at 155–157°. An additional 6.5 mg. of material, m. p. 137–140.5°, was obtained from the filtrate. Further recrystallization of the dibenzoate from alcohol gave colorless needles, m. p. 145–147°, remelting at 157.5–160°. A mixture with an authentic sample of the dibenzoate prepared from natural estradiol (m. p. 146–147°, remelting 158–161°) softened at 146° and melted at 157–160°. Three polymorphic forms for the dibenzoate were reported by David, DeJongh and Laqueur,<sup>31b</sup> m. p. 144.5–145°, 159–160° and 169.5–170.5° (all uncor.).

Other aromatization experiments are described in Table II. When 1,4-dihydronaphthalene (or the 1,2-

(25) Dannenberg, *Abhandl. preuss. Akad. Wiss., Math. naturw. Klasse*, No. 21 (1939); Woodward, *THIS JOURNAL*, **63**, 1123 (1941).

(26) Cook and Hill, *ibid.*, **62**, 1996 (1940).

(27) v. Auwers, *Ber.*, **46**, 2988 (1913).

(28) Straus and Lemmel, *Ber.*, **54**, 25 (1921).

(29) Fieser and Johnson, *THIS JOURNAL*, **61**, 168 (1939).

(30) Durland and Adkins, *ibid.*, **59**, 135 (1937).

(31) (a) Danielli, Marrian and Haslewood, *Biochem. J.*, **27**, 319 (1933); (b) David, De Jongh and Laqueur, *Arch. Intern. Pharmacodyn. Therap.*, **51**, 137 (1935); (c) MacCorquodale, Thayer and Doisy, *J. Biol. Chem.*, **115**, 438 (1936); (d) Dirscherl, *Z. physiol. Chem.*, **239**, 61 (1936); (e) Butenandt and Goergens, *ibid.*, **248**, 136 (1937); (f) Whitman, Wintersteiner and Schwenk, *J. Biol. Chem.*, **118**, 793 (1937).



TABLE II  
 CONVERSION OF 1,4-ANDROSTADIEN-17 $\alpha$ -OL-3-ONE TO  $\alpha$ -ESTRADIOL

Run	Amount of dienolone, mg.	Solvent <sup>a</sup>	Time, min.	Temp., uncor.	Crude phenolic fraction, mg.	Urea <sup>c</sup> complex, mg.	Estradiol (from urea complex), mg.	Estradiol dibenzoate (from urea filtrate) Crude, mg.	Recryst., mg.	Neutral <sup>d</sup> fraction, mg.
1	100	1,4-Dihydronaphthalene (fresh- $n_D$ 1.5570)	30	367-378	23	(Not carried out)		7 139-143° (152-155°)	3 143.5-146° (155-157°)	186
2	100	1,4-Dihydronaphthalene (old- $n_D$ 1.5650)	30	380-390	31 (dark)	0	0	3 <sup>e</sup> 93-129°		
3	100	1,2-Dihydronaphthalene	30	380-384	20	18 (132) 196-215° d.	3, 172-175°	0		
4	100	Tetralin <sup>f</sup>	25	380-392	21	0	0	Gummy		95, after re-cryst. 57 of starting material was recovered m. p. 155-163°
5	100	Dihydrophenanthrene, Sample 1	30	380-393	34	30 (135) 195-216° d.	7 170-173.5°	7.5 125-136°	2 138-143°	40 (used in run 11)
6	100	Same as 5	30	382-390	34	42 (126) 198-213° d.	10 170.5-173°	3 121-133°		(Used in run 12)
7	100	Dihydrophenanthrene, Sample 3	30	382-388	19	20 (142) 209-214° d.	7 169.5-173°	0		(Used in run 12)
8	100	Dihydrophenanthrene, Sample 2	30	386-393	18	70 (133) 189-197° d.	ca. 1 169-173°	4.5 136-140°		(Used in run 11)
9	100	Same as 8	60	384-390	20	15 (132) 198-217° d.	4 170-173.5°	0		
10	100	Same as 8	30	340-350	8	0	0	0		
11	Neutral frac. from 5 and 8	Dihydrophenanthrene, Samples 1, 2	30	382-388	25	65 (130) 185-195° d.	3 168-170°	3 121-135°		<sup>g</sup>
12	Neutral frac. from 6 and 7	Dihydrophenanthrene, Samples 1, 3	30	380-390	38	0	0	6 96-125°		<sup>g</sup>

<sup>a</sup> 1.3 cc. of solvent per 100 mg. of androstadienolone. <sup>b</sup> This is the time at the indicated temperature, not including the time for heating and cooling. <sup>c</sup> The temperature in parentheses denotes sintering. <sup>d</sup> Weight after removal of solvent by steam distillation (runs 1,4) or by evaporative distillation (run 5). <sup>e</sup> This dibenzoate was a dark red solid; the ester from other runs was nearly colorless. <sup>f</sup> Freshly distilled before use, b. p. 98.5-100° at 25 mm. <sup>g</sup> 1-Methylestradiol was isolated from this neutral material (see section on neutral fractions).

isomer) was used, the size of the neutral fraction indicated that some polymerization of the solvent (or reaction with the androstadienolone) had occurred. In the run (no. 4) using tetralin, the neutral fraction (97% recovery after removal of the tetralin by steam distillation) gave unchanged androstadienolone in 57% recovery, m. p. 155-163°, mixed m. p. 155-166°.

**Isolation of 1-Methyl- $\alpha$ -estradiol from the Neutral Fractions.**—Retreatment of the neutral fraction from the runs using 9,10-dihydrophenanthrene gave a small amount of estradiol in one case (run 11) but no pure phenolic material in the second (run 12). The combined neutral fractions from these retreatments (from a total of 400 mg. of androstadienolone) were evaporatively distilled at 165° and 0.05 mm. to remove most of the dihydrophenanthrene. The dark colored residue was dissolved in petroleum ether (b. p. 40-60°), adsorbed on 12 g. of alumina and fractionally eluted, using solvents ranging from benzene, to ether and finally ether-acetone (50/50). The benzene eluates yielded some additional dihydrophenanthrene. The material (55 mg.) eluted by ether and ether-acetone (80/20) crystallized after evaporation of the solvents, and on recrystallization from petroleum ether-acetone gave 18 mg. of 1-methylestradiol (VIb), m. p. 232.5-235° and mixed m. p. 233-236°. Acetylation of the material in the mother liquor by heating on the steam-bath for one hour with acetic anhydride and pyridine gave 15 mg. of 1-methylestradiol diacetate, m. p. 137-138°, mixed m. p. 137-138.5°. The amount isolated corresponded to a yield of 7-14%.

**Aromatization of 1,4-Cholestadienone-3.**—A solution of 250 mg. of 1,4-cholestadienone-3<sup>11</sup> (m. p. 108.5-111°) in 3 cc. of 9,10-dihydrophenanthrene (Sample 3) was heated in an evacuated sealed tube for thirty minutes at 382-388° and worked up as described for the runs with 1,4-androstadienolone. No alkali-soluble material was ob-

tained; this, however, does not necessarily indicate the absence of phenolic material in which the methyl group was eliminated. Most of the dihydrophenanthrene was removed at 165-170° (0.05 mm.) and the residue (400 mg.) was chromatographed on 7.5 g. of alumina, using solvents ranging from petroleum ether (b. p. 40-60°) to acetone. The dihydrophenanthrene was eluted by petroleum ether. From the petroleum ether-benzene (40/60) fraction was obtained 27 mg. (11%) of unchanged 1,4-cholestadienone-3, m. p. 101-107°, mixed m. p. 106.5-111° with softening at 101°. After an intermediate oily fraction (53 mg.) the benzene-ether (90/10, 75/25, 50/50) fractions yielded 25 mg. (10%) of 3-hydroxy-1-methyl-10-norcholestatriene-1,3,5,<sup>11,12</sup> m. p. 138-142°. One recrystallization of the solid from petroleum ether raised the melting point to 145-146°; this was undepressed when the material was mixed with a sample of the phenol prepared by the sulfuric acid-acetic anhydride rearrangement method.<sup>11</sup> Methylation of a sample gave the methyl ether, m. p. and mixed m. p. 104.5-105°.<sup>11</sup>

All succeeding fractions (57 mg.) were oily. Rechromatographing did not lead to crystalline material; treatment of the material with 3,5-dinitrobenzoyl chloride in pyridine also led to oils.

### Summary

The partial synthesis of the female sex hormone  $\alpha$ -estradiol from cholesterol, reported by Inhoffen and Zühlsdorff, has been confirmed.

Improved procedures were developed for preparing the necessary intermediate 1,4-androstadien-17 $\alpha$ -ol-3-one (Vb) from androstenediol 3-acetate 17-benzoate (I).

In the thermal elimination of the C-10 angular



methyl group of Vb to give  $\alpha$ -estradiol, the best yields (10%) were obtained using dihydro-

phenanthrene as a solvent (390°).

MADISON 6, WISCONSIN

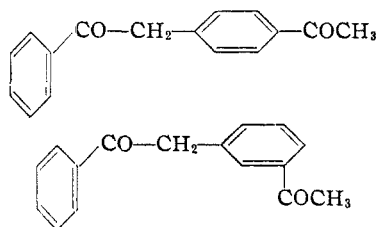
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## Anomalous Orientation in a Friedel-Crafts Ketone Synthesis<sup>1</sup>

BY DOMENICK PAPA, ERWIN SCHIWENK AND ANNA KLINGSBERG

Although the literature records many instances of *m*-dialkylbenzene derivatives prepared by Friedel-Crafts alkylation reactions,<sup>2</sup> only one example of *m*-substitution in a Friedel-Crafts ketone synthesis was found,<sup>3</sup> namely, that desoxybenzoin and acetyl chloride give rise to two isomers.<sup>4</sup>



We have found another example of meta substitution in the Friedel-Crafts reaction of ethyl phenylacetate and acetyl chloride. This reaction was first described by Klewitz<sup>5</sup> and later by Borsche,<sup>4</sup> both of whom apparently obtained only the *p*-isomer. Our results with this synthesis are at variance with those reported in the literature. Instead of obtaining a uniform, solid ethyl *p*-acetylphenylacetate, we obtained a mixture of about equal amounts of a liquid and a solid product. This material would not solidify completely, even after prolonged cooling; and, contrary to the literature, did not crystallize uniformly from petroleum ether. On investigation, it was established that in addition to ethyl *p*-acetylphenylacetate, ethyl *m*-acetylphenylacetate as well as the corresponding *o*-derivative resulted from this reaction. While the *p*- and *m*-isomers were obtained in about equal yield, only small amounts of the *o*-compound were found.<sup>6</sup> In view of these re-

sults, the reaction between ethyl phenylacetate and chloroacetyl chloride which has been reported<sup>7</sup> to yield the *p*-isomer exclusively is worthy of note.

While the *p*-isomer was secured in pure form, it was not possible to free the crude liquid *m*-isomer from small amounts of both the *p*- and *o*-isomers. However, after saponification of the crude *m*-isomer, pure *m*-acetylphenylacetic acid was obtained in good yield. The presence of the *o*-isomer was established by oxidation of the mixture of isomers to the corresponding dicarboxylic acids which gave with resorcinol the characteristic fluorescein test for phthalic acid.

The constitution of the *p*- and *m*-isomers (II and III) was established as follows: The ethyl *p*- and *m*-acetylphenylacetates after saponification to the free acids (IV and VIII) were oxidized with potassium permanganate to terephthalic and isophthalic acids which were identified as the esters (XIII and XII). Oxidation of IV and VIII with alkali and iodine gave the carboxyphenylacetic acids (V and X). In addition, the esters II and III or the acids IV and VIII were converted to the corresponding phenylene diacetic acids VI and IX by the Willgerodt reaction.

### Experimental

All melting points have been corrected for stem exposure. The Friedel-Crafts synthesis is herein described in detail, since Borsche and Sinn<sup>4</sup> give only a brief experimental procedure.

1. **Ethyl Acetylphenylacetate (I).**—In a typical run, 330 g. (2.0 moles) of ethyl phenylacetate was dissolved in three liters of dry carbon disulfide. The mixture was cooled to 0°, and 532 g. (4.0 moles) of finely-powdered anhydrous aluminum chloride<sup>8</sup> was added slowly, with stirring. The temperature was maintained between 0–5° during the addition of the aluminum chloride. To the resulting suspension, at 0°, there was added 250 g. of acetyl chloride in one portion and the reaction mixture stirred for fourteen to sixteen hours at room temperature. The reaction mixture was then placed in a large separatory funnel, the two layers allowed to separate,<sup>9</sup> and the lower layer then slowly added, with stirring, to a mixture of cracked ice and hydrochloric acid. After extraction with ether, the ether extracts were washed with water and dried over sodium sulfate. The solvent was removed under

various types of substituted benzene compounds, the non-identified residues, which in certain cases amounted to a considerable percentage of the total yield, have been tentatively designated as other isomers.

(7) Kunckell, *Ber.*, **38**, 2609 (1905).

(8) The aluminum chloride for these experiments was anhydrous, sublimed, reagent grade, manufactured by the General Chemical Company.

(9) The upper layer of carbon disulfide contained 16 g. of ethyl phenylacetate.

(1) Presented before the Division of Organic Chemistry on April 9, 1946, at the American Chemical Society meeting in Atlantic City.

(2) C. A. Thomas, "Anhydrous Aluminum Chloride in Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1942.

(3) Recently, in searching for examples of Friedel-Crafts reactions on nitriles, we have encountered an indirect and rather interesting confirmation of our present findings. Kunckell (*Ber.*, **39**, 3145 (1906)) obtained from the Friedel-Crafts reaction of benzyl cyanide and acetyl bromide *p*-acetyl benzyl cyanide, as well as the *m*-isomer in unspecified but apparently small yield. He also reported without citing any experimental procedure or evidence that benzyl cyanide and chloroacetyl chloride give rise to two similar isomers. This latter reaction is somewhat surprising in view of his statement that ethyl phenylacetate and chloroacetyl chloride give rise to the ethyl *p*-chloroacetyl phenylacetate exclusively.—(Added during publication.—Editor.)

(4) Borsche and Sinn, *Ann.*, **553**, 261 (1942).

(5) Klewitz, Dissertation, University of Rostock, 1908.

(6) It is of interest to note that in most of the reactions studied by Borsche and others on the use of acetyl and benzoyl chloride with