ment of the dark brown reaction mixture in a manner similar to that described for IV afforded 24.7 g. of a dark red-brown oil. Saponification of this oil with alcoholic sodium hydroxide followed by acidification and decarboxylation afforded 3.7 g. of a yellow oil. On treatment with benzene there was obtained 0.18 g. (2% based on amine) of DL-β-phenyllactic acid, m.p. 91–94°, undepressed on admixture with authentic DL-β-phenyllactic acid.

Procedure B. (Condensation of II with benzyl chloride.) From a mixture of 22.70 g. (0.104 mole) of II, 12.64 g. (0.100 mole) of benzyl chloride, 2.30 g. (0.100 g.-atom) of sodium, and 50 ml. of absolute ethanol heated at reflux temperature for 67 hr. was obtained 24.8 g. of a yellow oil. Saponification of this oil with alcoholic sodium hydroxide followed by acidification and decarboxylation of the dicarboxylic hydroxy acid with quinoline (at 105°) afforded 1.65 g. (10% based on benzyl chloride) of DL-β-phenyllactic acid, m.p. 93–95°.

SALT LAKE CITY, UTAH

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

3,16β-Dihydroxy-$\Delta^{1,3,5}$-estratrien-17-one and Related Compounds[1]

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The preparation of 3,16β-dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIb) and the diacetate (IIa) are described. Reduction with lithium aluminum hydride yielded $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb). The factors involved in the reduction of ring D ketols are briefly discussed.

In view of the fact that ring D ketols appear to be potential intermediates in the biochemical transformation of the estrogenic hormone as well as end products of its metabolism, satisfactory syntheses for these compounds were desired. In a prior report from these laboratories a ready synthesis for 3,16α-diacetoxy-$\Delta^{1,3,5}$-estratriene-17-one (IIIa) was described and this compound was employed as an intermediate in the preparation of estriol.[2] In the present communication, synthesis of the epimeric 3,16β-dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIb) and a means for preparation of the naturally occurring metabolite, $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb) are described. At the same time it was possible to characterize IIIb more completely and, as an extension of the investigation, to identify a previously reported minor reaction product obtained from the lithium aluminum hydride reduction of IIIa.

The preparation of IIa is a direct application of the investigations of Johnson, Gastambide and Pappo[3] who described a stereoselective oxidation of the enol acetate of isoandrosterone to yield 3β,16β-diacetoxyandrostane-17-one. When the reaction conditions described by these authors were employed with estrone-enol diacetate (I) a 42% yield of IIa was obtained. The compound crystallized in at least two polymorphic modifications but the physical constants, and especially the infrared spectrum clearly distinguished the product from IIIa. The compound was readily rearranged by means of either alkali or acid to the stable isomer 3,17β- dihydroxy- $\Delta^{1,3,5}$ -estratriene- 16 -one (VIb). Reduction of IIa by means of lithium aluminum hydride yielded only the known $\Delta^{1,3,5}$-estriene-3,16β,17β-triol (IVb).[4] Despite intensive search no evidence was obtained for the presence of the as yet undescribed fourth isomer of estriol, *i.e.* $\Delta^{1,3,5}$-estratriene-3,16β,17α-triol.

The virtual stereospecificity observed in the metal hydride reduction of IIa may be ascribed to the interposition of the C-18 methyl group and the complex of the reagent with the C-16β-oriented oxygen to the approach of the hydride toward C-17. On the other hand, when IIIa was reduced with lithium aluminum hydride, in addition to the major product, estriol, about 10% of the epimeric $\Delta^{1,3,5}$-estratriene-3,16α,17α-triol (Vb) was formed. The latter was reported earlier[2] as an unidentified component in the reduction of IIIa. The lesser stereoselectivity toward lithium aluminum hydride in the case of IIIa is explicable in the same terms except that the C-16 hydroxyl is on the opposite side of the molecule. The transposition of this group may facilitate formation of the 17α-hydroxy isomer both by removal of a shield over the β face of C-17 and through a cyclic complex of the metal with the two oxygen atoms at C-16 and C-17. The C-18 methyl group appears to be an important factor in obstructing the β attack of C-17 by the metal hydride since reduction of estrone by

(1) This investigation was supported in part by a grant from the American Cancer Society, and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(2) N. S. Leeds, D. K. Fukushima, and T. F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954).

(3) W. S. Johnson, B. Gastambide, and R. Pappo, *J. Am. Chem. Soc.*, **79**, 1991 (1957).

(4) G. F. Marrian, E. J. D. Watson, and M. Panattoni, *Biochem. J.*, **65**, 12 (1957); M. N. Huffman and H. H. Darby, *J. Am. Chem. Soc.*, **66**, 150 (1944).

the same reagents affords a very high yield of estradiol-17β.[5]

The stereoselectivity of the reaction of lead tetraacetate with the enol diester of estrone was investigated. The total product resulting from the oxidation by lead tetraacetate without isolation or further manipulation was treated immediately with an excess of lithium aluminum hydride in order to "freeze" the hydroxyl group at C-16 in the orientation resultant from the action of the reagent. Isolation of the reaction product by direct crystallization and countercurrent distribution of the residual material revealed that $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb) was the major product of the reaction. There was, however, a relatively small amount of $\Delta^{1,3,5}$-estratriene-3,16α,17β-triol present. It is thus established that while the introduction of an acetoxy group at C-16 in this way is not stereospecific in the strict sense, it is highly stereoselective, a conclusion in accord with that reached by Johnson and his colleagues.

EXPERIMENTAL[6]

3,16β-Diacetoxy-$\Delta^{1,3,5}$-estratriene-17-one (IIa). To a solution of 2.00 g. of estrone–enol diacetate, m.p. 143–148.5°,[7] in 40 ml. of glacial acetic acid and 2 ml. of acetic anhydride was added 2.50 g. of freshly recrystallized lead tetraacetate. Solution was complete at the end of 2 hr. After 18 hr. at room temperature the starch-iodide test was negative; the solvent was then evaporated under reduced pressure at 30–35°, benzene was added and the solution was washed successively with water, 5% sodium bicarbonate solution, and water. The solution, after drying over anhydrous sodium sulfate was concentrated and the product was chromatographed on alumina. The chromatogram yielded 0.151 g. of estrone–enol diacetate, m.p. 148–152°, 0.812 g. (42% based on unrecovered enol diacetate) of 3,16β-diacetoxy-$\Delta^{1,3,5}$-estratriene-17-one, m.p. 140–148°, and 0.187 g. of less pure material, m.p. 119–135°; the infrared spectrum showed that this also was principally IIa.

An analytical sample was recrystallized from petroleum ether as clusters of blunt prisms, m.p. 148–149°,[8] $[\alpha]_D^{24}$ +130.4° (ethanol). The infrared spectrum in carbon tetrachloride solution exhibited bands at 1764 (carbonyl stretching vibrations of phenolic acetate and the C-17 ketone, displaced from its normal position at 1745), at 1750 (carbonyl stretching vibrations of ketol acetate displaced from normal acetoxy band at 1742–1737) and at 1608 and 1492 cm.$^{-1}$ (carbon:carbon stretching vibrations of aromatic ring). The absence of absorption between 1425 and 1400 cm.$^{-1}$ is indicative that there was no unsubstituted methylene group adjacent to the ketone function. In carbon disulfide solution there were bands at 1228 (ketol acetate, displaced from normal acetoxy bands at 1245) and 1206 cm.$^{-1}$ (phenolic acetate).

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.57; H, 7.10.

3-Acetoxy-17β-hydroxy-$\Delta^{1,3,5}$-estratriene-16-one. Further elution of the column with methanol gave 0.230 g. of oily material which partially crystallized from acetone–petroleum ether. Repeated recrystallization gave 10 mg. of a compound, m.p. 166–177° (appearance of melt suggested polymorphism); $[\alpha]_D^{28.5}$ −68.7° ($CHCl_3$). The infrared spectrum supported the structure of the rearrangement product, 3-acetoxy-17β-hydroxy-$\Delta^{1,3,5}$-estratriene-16-one. The diacetate, prepared with acetic anhydride and pyridine gave an infrared spectrum identical with that of authentic 3,17β-diacetoxy-$\Delta^{1,3,5}$-estratriene-16-one (VIa).

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 73.29; H, 7.51.

3,16β-Dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIb). Hydrolysis of IIa yielded IIb which was recrystallized from ethanol. The melting point was not sharp although some crystals melted at 213–216°. Heating under reduced pressure to remove solvent of crystallization did not improve the melting point. An additional recrystallization from alcohol, however, produced the analytical sample in the form of fine needles, m.p. 219–221°, $[\alpha]_D^{26}$ +173.7° (ethanol) after drying at 0.1 mm. and 70° for 1 hr.

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.40; H, 7.66.

The product exhibits polymorphism as evidenced by the melting behavior and infrared spectrum in potassium bromide dispersion. Major differences in spectra were observed in four regions: 1800–1700, 1500–1425, 1300–1100 and 900–650 cm.$^{-1}$ A further description of these spectral alterations will be published elsewhere.

The material moved as a single spot on paper in the system chloroform:formamide. In this system IIb is the most polar (*i.e.* nearest to the origin), VIb is intermediate, and IIIb moves nearest to the solvent front.

$\Delta^{1,3,5}$-Estratriene-3,16β,17β-triol (IVb). A solution of 100 mg. of IIa, m.p. 143–146°, in 25 ml. of dry ether was slowly added to a suspension of 100 mg. of powdered lithium aluminum hydride in 100 ml. of dry ether. A white fluffy precipitate formed and when addition was complete, the suspension was refluxed for 2 hr. The excess reagent was destroyed with ethyl acetate. After washing with cold water, the ether solution was dried and the ether was evaporated leaving a white solid which was recrystallized from ethanol. Two crops (60 mg.; 78%) of $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol, m.p. 281–289° (285–289° in an evacuated capillary) were obtained. The same triol was prepared by lithium aluminum hydride reduction of VIa. There was no depression of the melting point when the triols from the two sources were mixed; infrared spectra of the triacetates were identical; paper chromatograms in the system chloroform:formamide showed the same rate of migration and no contamination with other products.

Stereochemistry of the reaction of $\Delta^{1,3,5,16}$-estratetraene-3,17-diol diacetate (I) *with lead tetraacetate.* Two grams of estrone–enol diacetate was oxidized with 2.80 g. of lead tetraacetate according to the above procedure. The reaction product, without isolation, was dissolved in 300 ml. of dry ether and 1.00 g. of powdered lithium aluminum hydride was carefully added. After heating under reflux for 2 hr., the excess reagent was destroyed with ethyl acetate followed by water and dilute hydrochloric acid. Ethyl acetate was added and the organic layer was separated, washed with saturated salt solution, and dried over anhydrous sodium sulfate. Evaporation of the solvent left a semisolid residue which was recrystallized from ethanol to give 0.669 g. (42% based upon the enol diacetate) of $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb), m.p. 265–275°. Paper chromatography indicated that the compound was essentially pure. The material which remained in the filtrate, 0.954 g., was separated in a 99-tube countercurrent distribution in the system cyclohexane-ethyl acetate (1:1) upper layer, and ethanol-water (1:1) lower layer. The separation is shown in Figure 1. There were four definite but overlapping areas which were identified from the K values of known compounds. These were estriol (peak tube 28), $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (peak tube

(5) A. C. Ott and M. F. Murray, Abstracts of the 113th Meeting, AMERICAN CHEMICAL SOCIETY, Chicago, 1948.

(6) Melting points were taken on a Kofler-type hot stage melting point apparatus unless otherwise indicated, and are corrected.

(7) One sample resolidified and then melted 150–152°.

(8) In subsequent preparations a second polymorphic form was obtained, m.p. 140–141.5°.

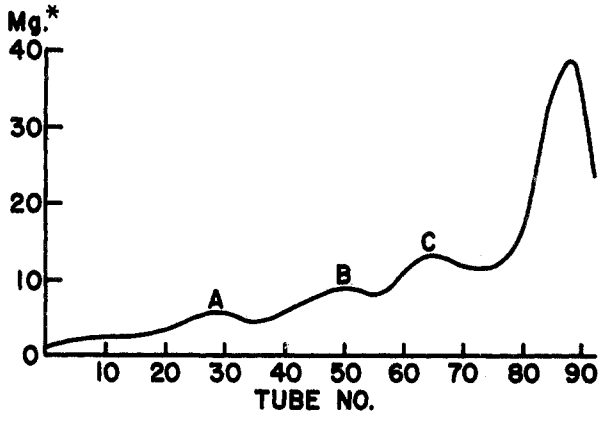


FIG. 1. COUNTERCURRENT DISTRIBUTION OF 954 MG. OF REDUCTION MIXTURE REMAINING AFTER REMOVAL OF A FIRST CROP OF IVb IN: upper layer, cyclohexane–ethyl acetate (1:1); lower layer, ethanol–water (1:1); *, based on ϵ_{2800} = 2100. A = estriol-16α,17β; B = estriol-16β,17β; C = estradiol-17β.

49), and estradiol-17β (peak tube 65); the major portion of relatively nonpolar material was present in tubes 80–94.

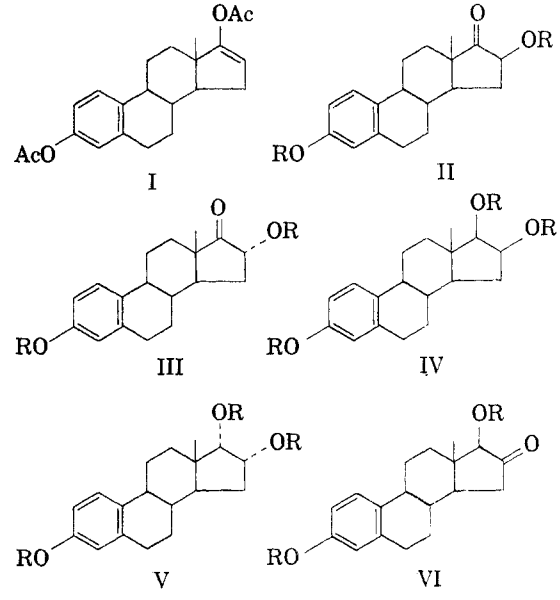
The combined material in tubes 20–36 (estriol fraction), weighed 86 mg. This was acetylated and chromatographed on alumina to yield 50 mg. of estriol triacetate identified from the infrared spectrum. An additional 100 mg. of $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol was obtained from the combined contents of tubes 40–53. The remainder of the material was discarded.

3,16α-Dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIIb). A solution of 1.00 g. of 16α,17α-epoxy-$\Delta^{1,3,5}$-estratriene-3,17β-diol diacetate, m.p. 150–153°, in 150 ml. of methanol and 25 ml. of 6*N* sulfuric acid was allowed to stand at room temperature for 3 days. The solution was concentrated under vacuum at room temperature to one third of its original volume. The suspension was cooled in an ice bath and filtered to give 0.703 g. of IIIb (91%), m.p. 205–228° after drying to constant weight in a desiccator. Recrystallization from acetone–petroleum ether gave a first crop of 0.520 g., m.p. 210–235° and a second crop of 0.128 g., m.p. 190–232°. The compound crystallized with a molecule of acetone of crystallization as shown by analysis and melting behavior. Repeated recrystallization from acetone–petroleum ether, followed by drying at 0.1 mm. and 100° for 8 hr. to remove acetone of crystallization, gave the analytical sample, m.p. 205–206.5°, $[\alpha]_D^{28.5}$ +168.8° (ethanol).

Anal. Calcd. for $C_{19}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.38; H, 7.73.

In spite of the fact that two types of crystals were observed,[9] clusters of needles and hexagonal plates, the compound was chromatographically pure in the system chloroform:formamide. The infrared spectrum in potassium bromide dispersion exhibited bands at 3540 and 3340 (O—H stretching vibrations), 1717 (carbonyl stretching), 1615, 1577, and 1495 (aromatic ring) cm.$^{-1}$ The absence of absorption between 1425 and 1400 cm.$^{-1}$ indicated that there was no unsubstituted methylene group adjacent to the ketone function.

$\Delta^{1,3,5}$-Estratriene-3,16α,17α-triol (Vb). A previously reported synthesis of estriol[2] also produced a side product having a K value of 0.98 in the system cyclohexane–ethyl acetate (1:1), upper layer; ethanol-water (1:1), lower layer. The compound isolated from tubes 40–55 of the countercurrent distribution melted at 230–235°; the melting point was not depressed upon admixture with authentic $\Delta^{1,3,5}$-estratriene-3,16α,17α-triol[10] and the infrared spectra of the compounds from the two sources were identical.



II,III,IV,V,VI a R = Ac
II,III,IV,V,VI b R = H

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(9) This compound was previously prepared in these laboratories by Dr. D. K. Fukushima as a higher-melting modification, m.p. 222–223.5°, by crystallization from ethyl acetate.

(10) Prepared in these laboratories by Dr. Stephen Kraychy according to the Procedure of V. Prelog, L. Ruzicka, and P. Wieland, *Helv. Chim. Acta*, 27, 250 (1944).