Further eluates with 10% ether-benzene gave 195 mg. of 6α-hydroxymethyl-5α-pregnane-3β,20β-diol 3,20-diacetate (I), the starting material.

6α-Fluoromethyl-5α-pregnane-3β,20β-diol (IIc). The foregoing diacetate (IId, 250 mg.) was saponified by refluxing for 3 hr. in 45 ml. of 5% methanolic potassium hydroxide. The solution was neutralized with glacial acetic acid and concentrated to a small volume. Addition of water and filtration gave 193 mg. of the diol. Recrystallization from acetone-hexane gave an analytical sample, m.p. 199–201°.

Anal. Calcd. for $C_{22}H_{37}O_2F$: C, 75.00; H, 10.58. Found: C, 75.08; H, 10.20.

6α-Fluoromethyl-5α-pregnane-3,20-dione (III). The fluorodiol (IIc, 180 mg.) was dissolved in 40 ml. of distilled acetone and titrated to a permanent brown color with 8*N* Kiliani acid. Dilution with 200 ml. of ice water precipitated the steroidal dione (III). Filtration gave 145 mg. of crude product. Recrystallization from acetone-hexane gave an analytical sample, m.p. 136–137°.

Anal. Calcd. for $C_{22}H_{33}O_2F$: C, 75.85; H, 9.54. Found: C, 75.51; H, 9.30.

6α-Chloromethyl-5α-pregnane-3β,20β-diol 3,20-diacetate (IIb). 6α-Hydroxymethyl-5α-pregnane-3β,20β-diol 3,20-diacetate (1.0 g.) was dissolved in 12 ml. of pyridine and treated with 2.1 g. of *p*-toluenesulfonyl chloride. The solution was refluxed for 3 hr., poured into excess ice water, and the resulting solid was filtered and washed with water until free of pyridine. After air-drying, the product had m.p. 180–185° (previous transition in the 170's), weight 383.3 mg. Recrystallization from methanol gave an analytical sample, m.p. 186–187.5°, $[\alpha]_D^{23}$ +45.0 (chloroform).

Anal. Calcd. for $C_{26}H_{41}O_4Cl$: C, 68.92; H, 9.12; Cl, 7.83. Found: C, 69.24; H, 9.00; Cl, 7.99.

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The Epimeric 6-Hydroxyestrones[1]

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There are several references in the biochemical literature to 6-hydroxyestrone,[2–4] but no chemical characterization has been reported. We have prepared one of the epimeric 6-hydroxyestrones to which we assign the *beta* configuration, and have demonstrated a practical route to the *alpha* epimer. Our assignment of configurations is based on those recently reported by Wintersteiner and Moore.[5] Tentatively these authors assigned the *alpha* configuration to the epimer prepared by sodium borohydride reduction of 6-ketoestradiol, and the *beta* configuration to the derivative obtained by catalytic hydrogenation of the 6-keto compound. Similarly, Marrian and Sneddon[6] assigned the *alpha* configuration to the epimer they prepared by sodium borohydride reduction of 6-ketoestriol.

6-β-Hydroxyestrone was obtained by catalytic hydrogenation of 6-ketoestrone, allowing only one mole equivalent of hydrogen to be consumed. The discrepancy between the quantity of hydrogen consumed and yield of 6-β-hydroxyestradiol reported by Wintersteiner and Moore[5] was also observed in the present instance. Analysis of a countercurrent distribution by ultraviolet and infrared spectroscopy and by weight measurements also gave evidence for the presence of 6-ketoestrone (starting material), estrone (hydrogenolysis product), and material that was transparent to ultraviolet light and was, therefore, probably an estrane.

In analogy to the preparation of 6-α-hydroxyestradiol by Wintersteiner and Moore,[5] an attempt to prepare 6-α-hydroxyestrone by sodium borohydride reduction was not successful since the 17-carbonyl function of 6-ketoestrone was reduced first. Although the 17-hydroxyl of 6-α-hydroxyestradiol is blocked from one side by the angular methyl group, it has not been possible to use this triol as starting compound for the preparation of the estrone derivative. In an effort to exploit any possible difference in reaction rates of the 6 and 17 hydroxyl functions, both estradiol-17β as model compound and 6-α-hydroxyestradiol were allowed to react with ethyl chlorocarbonate and with trimethylacetyl chloride. The bulkiness of these two entering groups should emphasize small differences in reactivity due to conformation or steric hindrance.

In practice no appreciable difference was observed. The 17-hydroxyl group of estradiol was completely esterified in eight hours at 37°. Obviously the reactivity of the 17-hydroxyl is so great that it precludes a selective esterification of the 6-hydroxyl.

A synthesis by which 6-α-hydroxyestrone can be obtained was devised by preparing 17-ethylenedioxy-6-β-hydroxyestrone, which by Oppenauer oxidation gave the 6-keto derivative. Although the preparation of 6-α-hydroxyestrone from the latter compound can be readily accomplished by sodium borohydride reduction and hydrolysis, it was not possible to obtain an analytically pure sample because 6-β-hydroxyestrone was available in only very limited quantities. Good evidence was, however, obtained by ultraviolet and infrared analysis that the reaction product was the desired compound. The absorption maxima in the ultraviolet were identical with those of the 6-β-hydroxyestrone, and the molecular extinction coefficients were nearly identical. There was also a very close resemblance of infrared spectra.

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EXPERIMENTAL

6-β-Hydroxyestrone. 6-Ketoestrone was catalytically hydrogenated in 1*M* acetic acid in methanol in the presence of Adam's catalyst. Hydrogen uptake was stopped after 1 mole had been consumed per mole of steroid. The hydrogenation product was distributed between 70% ethanol in water and 5% ethyl acetate in benzene. After 99 transfers, the material with a partition coefficient of 0.74 was crystallized from methanol water and proved to be 6-β-hydroxyestrone. The analytical sample had a m.p. of 265–270°; λ_{max}^{alc} 282 mμ (ε = 2140); λ_{max}^{KBr} 2.94 (m.),[7] 5.87 (s.) μ.

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74. Found: C, 75.22; H, 7.77.

The diacetate had a m.p. of 171–172°; λ_{max}^{alc} 270 mμ (ε = 730) and 276 mμ (ε = 680); λ_{max}^{KBr} 5.76 (s.), 5.87 (s.), 8.14 (s.), 8.28 (s.) μ.

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 71.05; H, 7.39.

Estradiol-3-benzoate,17-cathylate. Estradiol-3-benzoate (prepared from estradiol by the method of Butenandt[8]) was cathylated by the method of Fieser and Fieser.[9] The reaction mixture was allowed to stand at room temperature for a period of time depending upon the conditions of the experiment as outlined below. The analytical sample of estradiol-3-benzoate,17-cathylate had a m.p. of 111–112° (corrected).

Anal. Calcd. for $C_{28}H_{32}O_5$: C, 74.97; H, 7.19. Found: C, 74.96; H, 7.19.

The ultraviolet spectrum was identical to that of estradiol-3-benzoate, while the infrared spectrum was different in the region above 7 μ.

Treatment of estradiol-3-benzoate in the above manner for 24 hr. resulted in 90% conversion to the 17-cathylate derivate, and decreasing the reaction time to 8 hr. gave the same yield. A reaction time of 10 min., however, resulted in 40% recovery of starting material, and a 56% yield of the 17-cathylate in 33.5% conversion.

Attempted preparation of 6-α-hydroxyestradiol-3,6-di(trimethyl)acetate. Trimethylacetyl chloride (prepared from the acid by the method of Meyer[10]) (1 ml.) was added to 6-α-hydroxyestradiol (100 mg.) dissolved in dry pyridine (1 ml.). The reaction was allowed to proceed under the conditions outlined below. In each case, the mixture was neutralized with aqueous sodium bicarbonate and extracted with carbon tetrachloride. Since we were unable to crystallize these derivatives, infrared spectra (in carbon tetrachloride solution) were used to estimate the extent of the reaction [5.65, s. for C=O stretch (phenolic ester), 5.80, s. for C=O stretch (6 and 17 ester), 8.64, s. for C—O stretch (6 and 17 ester), and 8.95 μ, s. for C—O stretch (for phenolic ester)]. When 6-α-hydroxyestradiol and estradiol-17β were allowed to react overnight at room temperature, they were esterified in position 3 only. When estradiol-17β was used as a model compound and allowed to react for 8 hr. at 37°, the major product of the reaction exhibited no hydroxyl bands in the infrared.

17-Ethylenedioxy-6-β-hydroxyestrone. 6-β-Hydroxyestrone-3,6-diacetate (225 mg.) was dissolved in 45 ml. benzene. Ethylene glycol (0.25 ml.) was added and the mixture refluxed with continuous separation of water. After 3 hr., 15 mg. of *p*-toluenesulfonic acid was added, and the reaction allowed to proceed at reflux temperature for a period of 20 hr. After that time the solution was cooled and washed with aqueous sodium carbonate, then with water. Extraction with ether and evaporation of the solvent produced 268 mg. of dry material. This was dissolved in 35 ml. of 5% potassium hydroxide in methanol and left overnight at room temperature. The alkaline solution was poured into ice water and neutralized carefully with acetic acid to *p*H 7. The turbid solution was extracted with ether, and the solvent was evaporated.

The residue, dissolved in 10 ml. of benzene, was adsorbed on 10 g. of neutral alumina. After passing 200 ml. of benzene:ether 4:1, the ketal was eluted with pure ether (500 ml.). The ketal crystallized from methanol water (152 mg.) in 45.7% yield. The analytical sample melted at 224–226° (uncorrected) with decomposition.

Anal. Calcd. for $C_{20}H_{26}O_4$: C, 72.69; H, 7.93. Found: C, 72.39; H, 8.08.

The infrared spectrum showed no 17 ketone C=O stretching band, λ_{max}^{alc} 282 (ε = 2250).

17-Ethylenedioxy-6-ketoestrone. 17-Ethylene-6-β-hydroxyestrone (125 mg.) was oxidized by the Oppenauer method according to Wettstein and Meystre.[11] The ultraviolet spectrum of the crude material (120 mg.) showed a broad peak between 308 and 314 mμ and another peak at 260–266 mμ. The infrared spectrum showed a 6-keto peak (6.0 μ).

The crude material when distributed between 70% ethanol in water and cyclohexane for 99 transfers was separated into three fractions. Fraction 1 (90 mg.) was redistributed in the same system for 400 transfers, whereupon four subfractions were obtained, of which the third (K = 0.11) was the desired product (47.5 mg.). The analytical sample of 17-ethylenedioxy-6-ketoestrone melted at 231–233.5° (corrected).

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.14; H, 7.36. Found: C, 73.37; H, 7.44; λ_{max}^{alc} 326 (ε = 3200) and 256 mμ (ε = 8730); λ_{max}^{KBr} 3.12 (m.) and 6.03 μ (s.).

6-α-Hydroxyestrone-3,6-diacetate. A solution of crude 17-ethylenedioxy-6-ketoestrone (30 mg.) in methyl alcohol (30 ml.) was added dropwise to a solution of sodium borohydride (23 mg.) in methyl alcohol (15 ml.). After the mixture had stood at room temperature for 2 hr., 90% acetic acid in water was added to decompose excess borohydride. The solution was then made about 1*N* with respect to hydrochloric acid and allowed to stand at room temperature for 30 min. The solvents were then evaporated and the material acetylated. Crystallization from methanol water yielded 3.3 mg. of a compound melting at 130–136°; λ_{max}^{alc} 276 (ε = 610) and 270 mμ (ε = 680). The infrared spectrum was similar to that of 6-β-hydroxyestrone-3,6-diacetate and differed only in the region between 9 and 10 μ.

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Manganese Dioxide Oxidation: The Optional Introduction of Δ⁶-Double Bond with Simultaneous Cleavage of Dihydroxyacetone or 17,20-Glycol Side Chains in Δ⁴-3-Ketosteroids

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It has been shown previously,[1,2] that cleavage of a steroidal dihydroxyacetone side chain unprotected at C-21 (type I) or a 17,20-glycol (type II) could be effected with manganese dioxide to give a 17-ketone.

Several types of manganese dioxide have been used for the oxidation of organic compounds[3]