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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(7) s. = strong, m. = medium.

(8) A. Butenandt, *Z. physiol. Chem.*, **248**, 129 (1937).

(9) L. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **74**, 3309 (1952).

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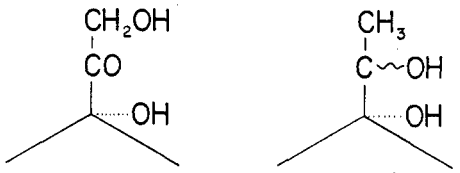
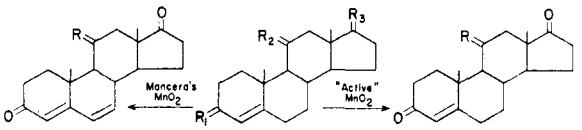
It has been shown previously,[1,2] that cleavage of a steroidal dihydroxyacetone side chain unprotected at C-21 (type I) or a 17,20-glycol (type II) could be effected with manganese dioxide to give a 17-ketone.

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



I II

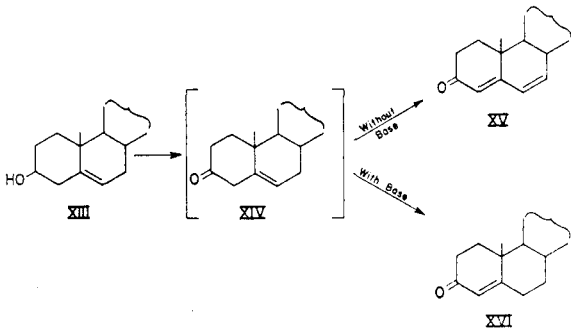
among which the most reliable and highly active forms were the ones introduced by Mancera *et al.*[4] and that of Attenburrow's "active" manganese dioxide.[5] In steroid compounds the most frequently used preparation was that of Mancera *et al.*[4] and consequently was the first one used in our investigations. In all the oxidation studies, the steroid was dissolved in chloroform and five times its weight of manganese dioxide was added and the mixture was refluxed for twenty-five hours. The properties of the isolated reaction products resulting from the oxidation of various steroids are depicted in Table I. The total oxidation product from each experiment exhibited a low intensity maximum at 240 mμ and a very high intensity maximum at 282 mμ indicating that it was a mixture of Δ^4-3-one and $\Delta^{4,6}$-dien-3-one of which the latter predominates. This result was not unexpected, particularly in view of the work carried out by Sondheimer *et al.*[1] In each case the predominently present $\Delta^{4,6}$-dien-3-one was separated from the small amount of Δ^4-3-one by chromatography on alumina. Thus from hydrocortisone (III), $\Delta^{4,6}$-androstadiene-11β-ol-3,17-dione (IV) was



IV. R = α-H, β-OH
VII. R = O
X. R = H_2
III. R_1 = O, R_2 = α-H, β-OH
R_3 = α-OH, β-CO·CH_2OH
VI. R_1 = O, R_2 = O, R_3 = α-OH, β-CO·CH_2OH
IX. R_1 = O, R_2 = H_2, R_3 = α-OH, β-CO-CH_2OH
XII. R_1 = ~OH, ~H, R = H_2, R_3 = α-OH, β-OH-CH
C

V. R = α-H, β-OH
VIII. R = O
XI. R = H_2

obtained and this proved that 11β-hydroxyl group survived the oxidation. Compound IV was previously accessible only through biological hydroxylation and its preparation was reported in a patent.[6] Similarly, hitherto unknown $\Delta^{4,6}$-androstadiene-3,11,17-trione (VII) was obtained from cortisone (VI), and $\Delta^{4,6}$-androstadiene-3,17-dione (X) was obtained from Reichstein's Compound S (IX). To study the cleavage of an α-glycol side chain, 17α-hydroxyprogesterone was reduced with lithium aluminum hydride to Δ^4-pregnene-3ξ,-17α,20,-triol (XII) and was then subjected to oxidation with manganese dioxide. As expected, $\Delta^{4,6}$-androstadiene-3,17-dione (X) was obtained; however, the yield of the 17-ketone from the cleavage of this 17,20-glycol was definitely lower than that resulting from the cleavage of a dihydroxyacetone side chain.



XIII XIV XV XVI

The action of Attenburrow's "active" manganese dioxide was then studied under similar conditions and the results are shown in Table II. In each case a higher yield of the 17-ketosteroid resulted than in the earlier oxidation studies with one marked difference. *The total oxidation product in every case exhibited an exclusive maximum at 240 mμ with no absorption at the 280 mμ region.* The oxidation products could easily be separated from the more polar starting materials by chromatography on alumina. Thus, oxidation of III produced Δ^4-androsten-11β-ol-3,17-dione (V) in a single step in 52% yield. Similarly, compounds VI, IX, and XII upon oxidation with "active" manganese dioxide gave Δ^4-androstene-3,11,17-trione (VIII), and Δ^4-androstene-3,17-dione (XI), respectively. As with the use of Mancera's manganese dioxide the oxidation with "active" manganese dioxide resulted in lower yields of the 17-ketone from the 17,20-glycol than from the dihydroxyacetone.

Furthermore, it has been proposed[3] that in the oxidation of a Δ^5-3β-ol (XIII) with manganese dioxide which gives the $\Delta^{4,6}$-dien-3-one (XV), the primary attack is on the 3β-hydroxyl group to give β,γ-unsaturated ketone of the type (XIV)

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TABLE I

CLEAVAGE OF STEROIDAL DIHYDROXYACETONE AND 17,20-GLYCOL SIDE CHAINS WITH MANGANESE DIOXIDE PREPARED ACCORDING TO MANCERA *et al.*[4]

Compound	Major Oxidation Product	Yield, %	Properties				Reported Properties				Reference
			M.P.	$[\alpha]_D$	λ_{max}	ϵ_{max}	M.P.	$[\alpha]_D$	λ_{max}	ϵ_{max}	
Hydrocortisone III	$\Delta^{4,6}$-Androstadiene-11β-ol-3,17-dione IV	28	239–241°	+247°	283 mμ	25,866	243–245°	+214°	282 mμ	25,800	6
Cortisone VI	$\Delta^{4,6}$-Androstadiene-3,11,17-trione VII	12	242–244°	+400°	281 mμ	20,390	—	—	—	—	8
Reichstein's Compound S IX	$\Delta^{4,6}$-Androstadiene-3,17-dione X	30	169–170°	+130°	283 mμ	23,650	172–173°	+135°	283 mμ	log 4.47	1
Δ^{1}-Pregnene-3ξ,17α,20ξ-triol XII	$\Delta^{4,6}$-Androstadiene-3,17-dione X	15	169–170°	+138°	283 mμ	23,990	172–173°	+135°	283 mμ	log 4.47	1

TABLE II

CLEAVAGE OF STEROIDAL DIHYDROXYACETONE AND 17,20-GLYCOL SIDE CHAINS WITH ATTENBURROW'S "ACTIVE" MANGANESE DIOXIDE[5]

Compound	Oxidation Product	Yield, %	Properties				Properties of Authentic Samples[11]			
			M.P.	$[\alpha]_D$	λ_{max}	ϵ_{max}	M.P.	$[\alpha]_D$	λ_{max}	ϵ_{max}
Hydrocortisone III	Δ^{4}-Androstene-11β-ol-3,17-dione V	52	197–199°	+228°	241 mμ	16,918	195–197°	+222°	241 mμ	17,302
Cortisone VI	Δ^{4}-Androstene-3,11,17-trione VIII	65	221–223°	+350°	238 mμ	15.608	220–222°	+310°	238 mμ	16,628
Reichstein's Compound S IX	Δ^{4}-Androstene-3,17-dione XI	34	169–170°	+205°	240 mμ	16,248	169–170°	+192°	240 mμ	17,433
Δ^{1}-Pregnene-3ξ,17α,20ξ-triol XII	Δ^{4}-Androstene 3,17-dione XI	17	169–170°	+205°	240 mμ	16,140	169–170°	+192°	240 mμ	17,433

followed by a rapid oxidation to the dienone (XV). It would follow from the above hypothesis that when the oxidation was carried out in the presence of alkali in a suitable solvent, the moment the β,γ-unsaturated ketone XIV was formed it would isomerize to the thermodynamically more stable Δ^4-3-ketone (XVI). A good solvent in which to study this reaction appeared to be *t*-butyl alcohol since it has been shown[7] that manganese dioxide does not attack tertiary alcohols. Cholesterol was then oxidized in *t*-butyl alcohol solution in the presence of 0.05*N* potassium hydroxide by refluxing with "active" manganese dioxide.[8] The total reaction product exhibited only one absorption maximum at 240 mμ indicating the exclusive formation of an α,β-unsaturated ketone. However, the conversion was rather low and amounted to approximately 6% based on the ultraviolet data. From this reaction mixture Δ^4-cholesten-3-one was isolated as the 2,4-dinitrophenylhydrazone and was found to be identical to an authentic sample. Oxidation of cholesterol with Mancera's manganese dioxide in *t*-butyl alcohol without alkali gave, as expected, $\Delta^{4,6}$-cholestadien-3-one in about 22% yield.[8]

EXPERIMENTAL[9]

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

Absorption spectra. The ultraviolet absorption spectra were determined in methanol with a Cary Recording Spectrophotometer (Model 11 MS). The infrared absorption spectra were determined in potassium bromide disk on a Perkin-Elmer (Model 21) Spectrophotometer.

Optical rotations. All rotations were measured in chloroform solution.

Alumina. Merck Reagent grade aluminum oxide, labelled as "suitable for chromatographic absorption" was treated with ethyl acetate for 24 hr. at room temperature, filtered, and then activated to give an activity II.

Petroleum ether. Mallinckrodt Analytical Reagent grade, b.p. 30–60°.

Manganese dioxide. Prepared as described by Mancera *et al.*[1]

"Active" manganese dioxide. Prepared as described by Attenburrow *et al.*[2]

Comparison with authentic samples. The oxidation products described in the text were identified by determining their mixture melting points and a comparison of their infrared spectra with that of authentic samples. Analyses are given only in those cases in which authentic samples were not available, and for new compounds.

A typical procedure followed for all oxidations shown in Table I. To a stirred solution of 1.2 g. of hydrocortisone in 120 ml. of chloroform, 6 g. of manganese dioxide was added and the contents boiled under reflux for 25 hr. The chloroform solution was then filtered from manganese dioxide to give 0.6 g. of residue which had absorption peaks at 240 mμ and 282 mμ. The residue was chromatographed on 25 g. of alumina. The fractions eluted with benzene-ether (1:1) and ether gave a total of 0.28 g. of $\Delta^{4,6}$-androstadiene-11β-ol-3,17-dione (IV) (28%), which was crystallized twice from acetone-petroleum ether to give the analytical sample m.p. 239–241°, $[\alpha]_D^{26}$ +247°, $\lambda_{max}^{CH_3OH}$ 283 mμ (ϵ = 25,866) ν_{max}^{KBr} 3440, 1738, 1643, 1615, and 1585 cm.$^{-1}$ Lit.[6] m.p. 243–245°, $[\alpha]_D$ +214°, λ_{max} 282 mμ (ϵ = 25,800).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 76.03; H, 8.12.

Similarly, cortisone (VI) gave $\Delta^{4,6}$-androstadiene-3,11,17-trione (VII), m.p. 242–244°, $[\alpha]_D^{27}$ +400, $\lambda_{max}^{CH_3OH}$ 281 mμ (ϵ = 20,390), ν_{max}^{KBr} 1745, 1710, 1662, 1620, and 1579 cm.$^{-1}$

Anal. Calcd. for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.04; H, 7.66.

A typical procedure followed for all oxidations shown in Table II. To a stirred solution of 2.4 g. of hydrocortisone in 250 ml. of chloroform, 12 g. of "active" manganese dioxide[2] was added and boiled under reflux for 25 hr. The chloroform solution was then filtered from manganese dioxide to give 1.92 g. of residue which exhibited only a single absorption maximum at 240 mμ. The reaction product was then chromatographed on 60 g. of alumina. The fractions eluted with ether-chloroform (8:2), ether-chloroform (1:1) and chloroform gave a total of 1.04 g. (52%) of Δ^4-androstene-11β-ol-3,17-dione m.p. 197–199°, $[\alpha]_D^{26}$ +228°, $\lambda_{max}^{CH_3OH}$ 241 mμ (ϵ = 16,918), ν_{max}^{KBr} 3500, 1743, 1654, and 1620 cm.$^{-1}$

Oxidation of cholesterol with "active" manganese dioxide and 0.05N potassium hydroxide in t-butyl alcohol. To a stirred solution of 15 ml. of 0.05*N* potassium hydroxide in *t*-butyl alcohol, 0.519 g. of cholesterol and 2.5 g. of "active" manganese dioxide were added and the contents boiled under reflux for 11 hr. The reaction mixture was diluted with 100 ml. of ethyl acetate and filtered from the manganese dioxide. The ethyl acetate extract was washed with water until neutral, and then the solvent was evaporated to give 0.442 g. of crystalline residue. The total product exhibited a single absorption maximum at 240 mμ, log ϵ, 3.07, indicating about a 6.7% conversion to the α,β-unsaturated ketone. Δ^4-Cholesten-3-one was separated from the reaction product as the 2,4-dinitrophenylhydrazone m.p. 231–233°, which was found to be identical with an authentic sample.[10,11]

Oxidation of cholesterol with manganese dioxide in t-butyl alcohol. To a stirred solution of 500 mg. of cholesterol in 60 ml. of *t*-butyl alcohol, 2.5 g. of manganese dioxide was added and the contents were refluxed for 12 hr. The solution was then filtered from manganese dioxide and the solvent was evaporated to give 250 mg. of residue. The total product exhibited a single absorption maximum at 285 mμ, log ϵ, 3.84, indicating about a 22% conversion to $\Delta^{4,6}$-cholestadiene-3-one. From the oxidation product $\Delta^{4,6}$-cholestadiene-3-one m.p. 80–81° was isolated by chromatography on 8 g. of alumina, and its identity has been established.

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(8) Present paper. Analysis is given in experimental section.

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(11) The physical properties of authentic samples were determined in these laboratories.