CONTINUOUS PERMANGANATE OXIDATION OF 16-DEHYDROSTEROIDS

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16-Dehydroprogesterone, 16-dehydropregnenolone acetate and 16-dehydro-A-norprogesterone have been converted to the corresponding 16α,17α-dihydroxy derivatives in high yield by oxidation with potassium permanganate as a continuous-flow process. The details of the technique and a simple laboratory continuous reactor are described.

A constantly growing literature[1-5] concerned with the preparation of steroids hydroxylated in the 16- and 17-positions attests to the great value and utility of these compounds. In the past, osmium tetroxide[1a-f] or potassium permanganate[2a-c] have been used extensively for hydroxylation of 16-dehydrosteroids when fast, relatively simple conversion to 16α,17α-diols was desired. Oxidation of steroids containing a 17,20-olefinic side chain with selenium dioxide[3a-c] has provided a rather facile means of introducing a 16α-hydroxyl function into these molecules. However, production of hydroxylated steroids on a large scale undoubtedly has been achieved most effectively in the past by microbiological oxidation[4a-c], or by more circuitous chemical routes usually beginning with 16,17-epoxy derivatives[5a-b].

Unfortunately, each of these methods of hydroxylation has inherent limitations. The expense and toxicity associated with oxmium tetroxide and selenium dioxide often preclude their

practical use. Osmium tetroxide frequently may provide higher yields of glycols than permanganate. However, both oxidizing agents yield products contaminated with D-homo isomers[6a-b] and other oxidation products[7] when 16-dehydrosteroids of the corticoid or pregnane series are oxidized. The more complex chemical routes or microbiological oxidations often provide acceptable yields of hydroxylated products, but lack the remarkable simplicity of osmium or permanganate for introduction of hydroxyl groups.

We now wish to describe a novel continuous-flow permanganate oxidation process for the preparation of 16α,17α-hydroxylated steroids which is readily adaptable to any desired scale of preparation. This continuous oxidation process provides either gram or kilogram quantities of hydroxylated steroids in high yield, free of contamination with more highly oxidized and rearranged impurities.

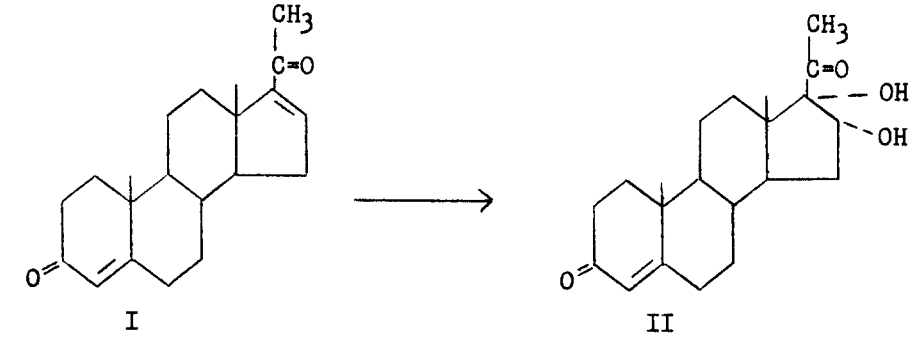
The continuous oxidation process differs from the batch-type preparations previously described in the literature[2a-c,8] essentially in the degree of control which is achievable during oxidation of reactive double bonds. Allen, Bernstein, Feldman, and Weiss recently described[8] an improved batch method for permanganate hydroxylation of 21-acetoxy-11α-p-toluenesulfonyl-oxy-pregna-4,16-diene-3,20-dione which on a five gram scale gave a 78% yield of the 16α,17α-dihydroxy derivative. However, by merely doubling the scale of reaction, a lower-melting material was obtained in only 66% yield.

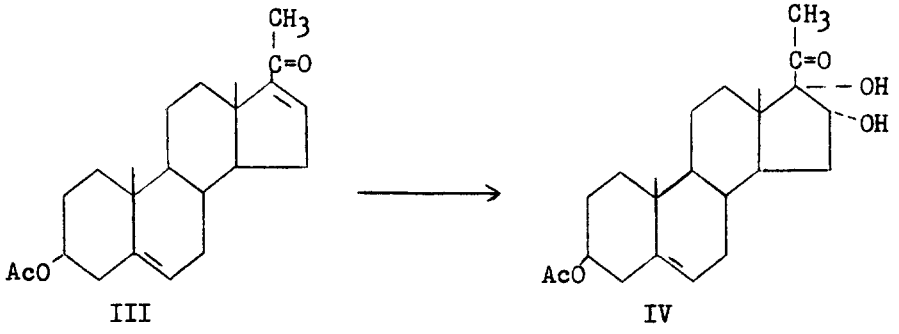
Our experience with permanganate oxidation of 16-dehydro-

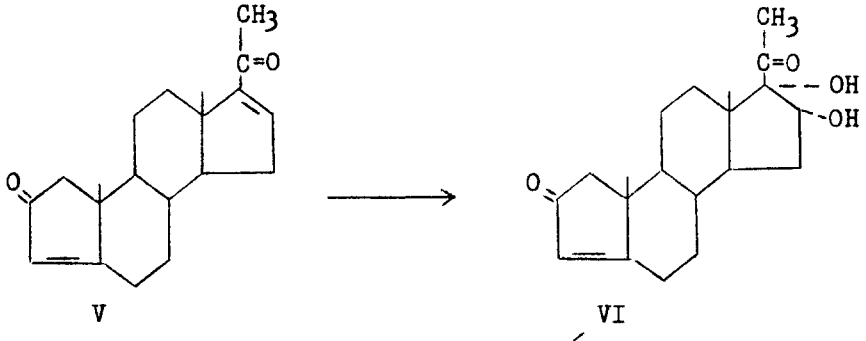
progesterone (I) was similar. On a one gram scale, using the improved method[8], a 65% yield of 16α,17α-dihydroxyprogesterone (II) could be obtained. But by increasing the size of the reaction to twenty grams, the yield dropped to the 30% range previously indicated by Petrow[2b].

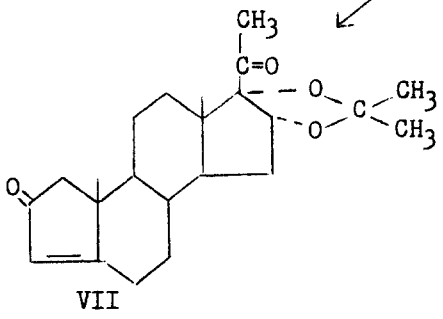
It appeared to be of interest, then, to attempt permanganate oxidation as a continuous process, since this technique would somewhat resemble, at any given moment, a small batch reaction, regardless of the total scale of operation. It was necessary to assume that permanganate oxidation of the 16,17-double bond was reasonably rapid, that this reaction occurred in preference to any other reaction, and that the product mixture could be quenched before other reactions began to occur. Actually this was found to be the case.

The continuous process is described in detail in the Experimental section. Briefly, it was found that when an acetone-water solution of potassium permanganate was allowed to react with an acetone-formic acid solution of 16-dehydroprogesterone (I) in a continuous-flow reactor for about five seconds at 0°C., a remarkably high yield of over 75% of nearly pure 16α,17α-dihydroxyprogesterone (II) could be obtained. Contamination, when present, was usually only a few percent[9] of unreacted 16-dehydroprogesterone, depending on how well the input streams were balanced. Hydroxylation of 16-dehydro-A-norprogesterone (V) under similar conditions gave a 77% yield[11] of 16α,17α-dihydroxy-A-norprogesterone (VI), which was converted to the acetonide VII by treatment of an acetone solution of


CH3
C=O
O
I
CH3
C=O
OH
OH
O
II


CH3
C=O
AcO
III
CH3
C=O
OH
OH
AcO
IV


CH3
C=O
O
V
CH3
C=O
OH
OH
O
VI


CH3
C=O
O
O
C
CH3
CH3
O
VII

the diol with a small amount of 70% perchloric acid. It was found that a slightly increased reaction temperature (10°), and a somewhat longer reaction time (1 minute) was required to completely oxidize 16-dehydropregnenolone acetate (III) to 16α,17α-dihydroxypregnenolone acetate (IV). By this method, using a small, simple continuous reactor, hydroxylated steroids have been produced steadily at a rate of 60 g./min.

The remarkable facility with which the 16,17-double bond in the steroid D-ring can be cleaved permits a wide variety of variously unsaturated steroids to be hydroxylated at the 16 and 17 positions in this manner, since double bonds at the 1, 4, 5, 6, 9(11), 11, and other positions are relatively unaffected by permanganate under the continuous oxidation conditions.

EXPERIMENTAL

Materials. Acetone was purified by distillation from potassium permanganate when required. Usually, however, acetone commercially available was sufficiently free of reducing agents that special purification was unnecessary. The aqueous potassium permanganate solution was boiled for 1 hour, filtered, and assayed for permanganate content prior to dilution with acetone. After preparation, the permanganate-acetone reagent solution was stored at about 5°C., and used within 24 hours. All solutions were filtered prior to use. Melting points of products were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

Description of a Simple Laboratory Continuous Reactor. For small scale continuous permanganate oxidations, two graduated dropping funnels were used to contain the steroid and the permanganate solutions, and to meter their rates of flow. The dropping funnels were mounted above and connected through a Y to a small diameter (10 mm. O.D.) glass tube coiled horizontally so that the coil could be immersed in a dry ice-acetone cooling bath. The outlet end of the glass tubing was bent to extend over the edge of the cooling bath and downward to allow the reaction mixture to flow directly into a container of sodium bisulfite quench solution. The Y-juncture at the inlet end of the coiled glass reactor was bent so that the juncture where

the initial exothermic reaction between the steroid and permanganate occurred was beneath the surface of the cooling bath. About two inches downstream from the Y-juncture, an inlet was provided for a thermometer to measure the reaction temperature. For our purposes, the volume of the reactor measured from the Y-juncture to the point of quenching (in this case the outlet end of the tubing) was designed to be about 10 ml. capacity. A reactor of this volume best accommodated the scale of our preparations.

This extremely simple type of continuous reactor is easily assembled, extremely versatile, and permits much greater control over a rapid chemical reaction than is possible in a batch process. There are several inherent limitations to this reactor which should be mentioned. It is difficult to maintain, manually, a constant flow rate as the levels of reactants in the dropping funnels change. As a result, changes in reaction time, temperature, and mole ratios of reactants occur. Also, it has to be assumed that adequate mixing of reactant streams occurs in the tubing. In spite of these problems, such a simple reactor can provide 70-80% yields of hydroxylated steroids in a simple, rapid fashion. For large scale preparations a reactor employing pumps, flow meters, and thermocouples to accurately control reactant flow rates and temperatures was developed.

16α,17α-Dihydroxypregn-4-ene-3,20-dione. A. Laboratory Scale Process. A solution of 10.0 g. (0.032 mole) of pregna-4,16-diene-3,20-dione in 300 ml. of acetone was acidified with 23 ml. of 10% formic acid and placed in a calibrated dropping funnel. A solution of 6.3 g. (0.04 mole) of potassium permanganate in 90 ml. of water was diluted with 210 ml. of acetone and placed in a second calibrated dropping funnel. The two funnels were then connected with short pieces of gum rubber tubing to the inlet ends of the Y of the glass tube reactor described above. The two reactant solutions were allowed to run at equal rates into the reactor so that the total contact time was about 10 seconds before quenching in 40 ml. of 10% sodium bisulfite solution. The reaction tube was cooled in a dry ice - acetone bath to maintain a reaction temperature of about -5°C. After the run was completed the

quenched mixture was filtered through filter aid to remove precipitated manganese dioxide. The filter cake was washed well with acetone, and the combined filtrate and washings were concentrated to remove acetone. The dihydroxyprogesterone that separated during concentration to an aqueous slurry was filtered, washed well with water and methanol and dried to give 8.0 g. of 16α,17α-dihydroxypregn-4-ene-3,20-dione, m.p. 208-210°. This solid was determined by paper chromatographic analysis[9] to be 94.6% 16α,17α-dihydroxypregn-4-ene-3,20-dione, the remaining steroid being unreacted pregna-4,16-diene-3,20-dione. Crystallization from chloroform gave the analytical sample, m.p. 217-220° (Lit.[2b] 225°); $[\alpha]_D^{26}$ + 91.1° (c 1.0, $CHCl_3$); $\lambda_{max.}^{MeOH}$ 241 mu. (ε 18,400). Anal. Calc'd. for $C_{21}H_{30}O_4$: C, 72.8; H, 8.74. Found: C, 72.8; H, 8.66.

B. Large Scale Process. A solution of 10 kg. of pregna-4,16-diene-3,20-dione in 428 liters of acetone, containing 32.9 liters of 7% formic acid was charged to a feed tank. A solution of 6.5 kg. (41 moles) of potassium permanganate in 130 liters of water was diluted with 448 liters of acetone and charged to a second feed tank. A quench solution of 46 liters of 15% sodium bisulfite was charged to a third feed tank. The steroid and the permanganate solutions were then continuously fed at equal rates into a vertical agitated reactor tube at such a rate that the contact time before quenching was about 5 seconds. The reaction temperature was maintained at about 0°C. The sodium bisulfite quench solution was fed into the reaction stream at a rate equivalent to 3% of the combined reactant rates. The

quenched reaction mixture was treated with filter aid and filtered to remove manganese dioxide. After concentration of the filtrate to remove acetone, the precipitated dihydroxyprogesterone was filtered, washed with water and methanol to give 8.5 kg. of 16α,17α-dihydroxypregn-4-ene-3,20-dione which was found to be about 95% pure by paper chromatography. Yields above 90% of 16α,17α-dihydroxypregn-4-ene-3,20-dione free of contamination have been achieved.

3β-Acetoxy-16α,17α-dihydroxypregn-5-en-20-one. A. Laboratory Scale Process. A solution of 10.0 g. (0.028 mole) of 3β-acetoxypregna-5,16-dien-20-one and 2.4 ml. of formic acid in acetone was diluted to 500 ml. with acetone. A second solution of 6.07 g. (0.038 mole) of potassium permanganate in 110 ml. of water was diluted to 500 ml. with acetone. The two solutions were run at equal rates through the glass reactor described above at 10°C. with a contact time of 60 seconds. The reaction mixture was quenched with 40 ml. of 10% sodium bisulfite solution, filtered, adjusted to pH 7.0 with saturated sodium bicarbonate solution, and concentrated to remove acetone. The resulting mixture was chilled and filtered to give 9.1 g. of 3β-acetoxy-16α,17α-dihydroxypregn-5-en-20-one, m.p. 163-164°. Recrystallization from methanol gave the analytical sample, m.p. 193-195° (Lit.[2b] 210-212°), $[\alpha]_D^{25}$ - 52.7° (c 1.0, $CHCl_3$). Anal. Calc'd for $C_{23}H_{34}O_5$: C, 70.7; H, 8.80. Found: C, 70.6; H, 8.74.

B. Large Scale Process.[10] A solution of 4.94 kg. (13.8 moles) of 3β-acetoxy-pregna-5,16-dien-20-one in 250 liters of acetone was acidified immediately before use with a solution of 1.17

liters of formic acid in 11.5 liters of water. An aqueous solution of 3.03 kg. (19.2 moles) of potassium permanganate in 45 liters of water was added to 250 liters of acetone shortly before beginning the reaction. The steroid and the permanganate solutions were then continuously fed at equal rates into a vertical agitated reactor tube at such a rate that the total contact time was about 70 seconds. The reaction temperature was maintained at about 10°C. After the required reaction time, the mixture was continuously quenched at about 15°C. with 50 liters of 10% sodium bisulfite solution. The reaction mixture was then filtered on a precoated vacuum filter, and the filtrate concentrated to less than one sixth of its original volume. The product was completely precipitated from the concentrate by adding an equal volume of cold water. The product was filtered and washed well with water to give 4.32 kg. of 3β-acetoxy-16α,17α-dihydroxypregn-5-en-20-one.

16α,17α-Dihydroxy-A-norpregn-3-ene-2,20-dione.[11] A solution of 100 g. (0.335 mole) of A-norpregna-3,16-diene-2,20-dione in 3000 ml. of acetone containing 230 ml. of 10% formic acid was allowed to react with a solution of 63 g. (0.4 mole) of potassium permanganate in 900 ml. of water and 2330 ml. of acetone in a glass continuous reactor for 5 secs. as described above. The reaction temperature was maintained at -20°C. The effluent stream was run into a stirred solution of 400 ml. of 10% sodium bisulfite solution. The reaction mixture was filtered, the manganese dioxide cake was washed with acetone, and the combined filtrate and wash concentrated to give 85 g.

of product, m.p. 187-190°. Recrystallization from acetone gave 16α,17α-dihydroxy-A-norpregn-3-ene-2,20-dione, m.p. 189-192°C., $[\alpha]_D^{22}$ - 44° (c 1, $CHCl_3$), $\lambda_{max.}^{EtOH}$ 234 mu. (ε 15,800). Anal. Calc'd for $C_{20}H_{28}O_4$: C, 72.3; H, 8.49. Found: C, 72.1; H, 8.48.

16α,17α-Isopropylidenedioxy-A-norpregn-3-ene-2,20-dione.[11] A solution of 43.8 g. (0.13 mole) of 16α,17α-dihydroxy-A-norpregn-3-ene-2,20-dione in 5 liters of acetone was treated with 9.1 ml. of 70% perchloric acid and stirred at room temperature for one hour. After neutralization with 1000 ml. of saturated sodium bicarbonate solution, the solution was concentrated under vacuum to remove acetone. Dilution with 6 liters of water gave 47 g. of the acetonide, m.p. 187-189°C. Recrystallization from acetone gave the pure acetonide, m.p. 189-190°C.; $[\alpha]_D^{22}$ + 13.5°(c 1, $CHCl_3$); $\lambda_{max.}^{EtOH}$ 234 mu. (ε 15,425). Anal. Calc'd for $C_{23}H_{32}O_4$: C, 74.2; H, 8.66. Found: C, 74.2; H, 8.70.

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9. The oxidation products were inspected for D-homo isomers, unreacted 16-dehydrosteroid, the product glycol, and other oxidation products by paper chromatographic analysis determined by Mr. Henry R. Roberts of the Analytical and Physical Chemistry Division. The quantitative determination of the percent of 16α,17α-dihydroxyprogesterone present in a reaction product was performed chromatographically using Whatman No. 1 filter paper impregnated with propylene glycol, with toluene saturated with propylene glycol as developing solvent. In this system, 16α,17α-dihydroxyprogesterone has an R_f value of 0.25 at 22° compared with an R_f of 0.90 for 16-dehydroprogesterone. The steroid spots were located by ultraviolet absorption, eluted with 95% ethanol, the absorbances of the 16α,17α-dihydroxyprogesterone eluates read at 240 mu. using a Beckman DU Spectrophotometer, and the concentrations determined relative to a standard sample.

10. The large scale procedure was carried out by Dr. E. J. Becker, Mrs. Nancy Kraemer, and Mr. S. J. Lieberman of the Squibb Research Products Laboratory.

11. This synthesis was conducted by Dr. LeRoy B. High of the Squibb Research Products Laboratory.