A FACILE CHEMICAL SYNTHESIS OF 2 - HYDROXYESTRONE - 6,7 - ^{3}H.

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2-Hydroxyestrone is a major metabolite of estradiol-17β or estrone in several species (1-4). As part of an effort to investigate its fate in vivo, a simple chemical method of synthesis of the radiolabelled compound has been developed. Enzymatic methods have previously been described (5, 6).

The several large scale chemical syntheses that have been described either involve lengthy reaction sequences or result in poor overall yields (7-12). The relatively simple method as described by Nambara et al (13) for the synthesis of 2-methoxyestrogens was therefore adapted for the present purpose. The reaction sequence followed is shown in the chart.

Friedel-Crafts acylation of estrone 3-methyl ether (II) gave the 2-acetyl compound (III) which was subjected to Baeyer-Villiger oxidation with 3-chloroperbenzoic acid for 3 hours to afford the acetate IV. Treatment of IV with hydrobromic acid in acetic acid cleaved both the acetate and the methyl ether groups in one step to generate 2-hydroxyestrone. Since this compound is relatively unstable in small quantities the synthesis was carried out as far as the penultimate step and the reaction sequence completed immediately prior to use. A minimum of approximately 25 µg of the acetate IV was required for this final step.

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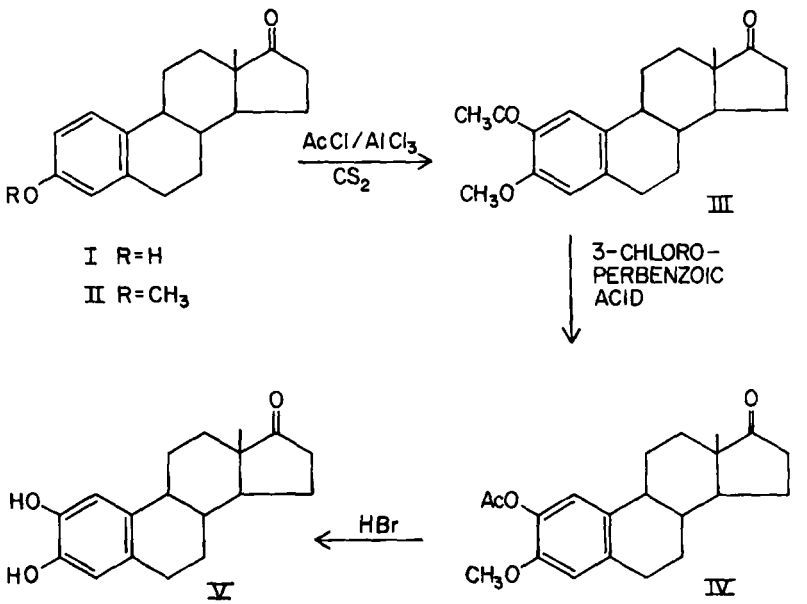

EXPERIMENTAL

Estrone-6,7-^{3}H 3-methyl ether (II)

Estrone-6,7-^{3}H (I, 873 μCi, obtained from Amersham-Searle and diluted to a sp. activity of 23.4 mC/mM) in methanol (0.5 ml) was treated with dimethyl sulfate (0.1 ml) and aqueous potassium hydroxide (30%, 0.1 ml) and the solution was stirred at 43° for 30 min. A further quantity of dimethyl sulfate (0.1 ml) and potassium hydroxide solution (0.1 ml) was then added and the mixture was stirred for a further 30 min. This was repeated a third time with addition of excess potassium hydroxide. Water (2 ml) was added and the product isolated with chloroform. T.l.c.* of an aliquot with cold standards indicated that 96% conversion into the 3-methyl ether II had occurred. 834 μCi of the 3-methyl ether were recovered. This product was used directly without further purification.

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* Solvent system - chloroform, ethanol 19:1

2-Acetyl-3-methoxyestra-1,3,5(10)-trien-17-one-6,7-^3H (III)

Estrone-6,7-^3H 3-methyl ether (II, 834 μCi) was drawn up into the bulb of a lambda pipette and the solvent evaporated. The acetylating mixture (3 μl of aluminum chloride 0.4 g, acetyl chloride 0.6 ml, and carbon disulfide 2 ml) was added and the pipette closed off at both ends to prevent evaporation. The reaction was allowed to proceed for 45 min. at room temperature, and then quenched with 1N hydrochloric acid. The extracted product was purified on t.l.c.* . The 2-acetyl compound, III, was obtained in a yield of 78% (647 μCi).

2-Acetoxy-3-methoxyestra-1,3,5(10)-trien-17-one-6,7-^3H (IV)

2-Acetyl-3-methoxyestra-1,3,5(10)-trien-17-one-6,7-^3H (III, 647 μCi) was treated with 3-chloroperbenzoic acid in chloroform (0.4 M, 0.1 ml) at room temperature for 4 hr. Following dilution with chloroform, the solution was washed with 5% aqueous sodium hydroxide and water, and dried (Na_2SO_4). The product was isolated by t.l.c. in a yield of 47%. During synthesis of unlabelled material, the following characteristics were found for this compound: mp 138-140.5°; $[\alpha]_D$; ν_{max}(KBr) 1770 (acetate), 1740 (ketone) and 1230 $cm.^{-1}$ (acetate); (molecular wt. by mass spectrometry 342); nmr δ 0.90 (3H, s, $C_{(18)}H_3$), 2.29 (3H, s, CH_3COO-), 3.80 (3H, s, CH_3O-), 6.70 (1H, s, $C_{(4)}$-H), and 6.96 (1H, s, $C_{(1)}$-H).
Found: C, 73.22; H, 7.37%. Calc. for $C_{21}H_{26}O_4$:C, 73.66; H, 7.65%

2-Hydroxyestrone-6,7-^3H (V)

Compound IV was treated with hydrobromic acid (20% in acetic acid, 10 μl) at 90° for 1 hr in a tube fashioned from a Pasteur pipette. The reaction product was isolated by t.l.c.*, and was found to be pure by reverse isotope dilution analysis. The overall yield of 2-hydroxyestrone-6,7-^3H was 14%.

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* Solvent system - acetic acid, cyclohexane, chloroform I:2:2

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