Preparation of Tritium Labelled Estetrol [Estra-1,3,5(10)-Triene-3,15α,16α,17β-Tetrol 6,7-^{3}H]

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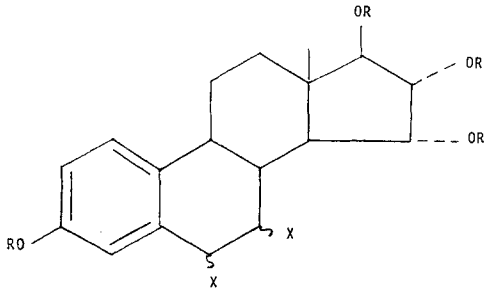
SUMMARY

The preparation of the tritium labelled fetal metabolite estra-1, 3, 5 (10)-3, 15α, 16α, 17β-tetrol 6,7-^{3}H is described. The material was prepared by oxidation of the tetrol tetraacetate Ib to the 6-keto derivative II. Reduction of the 6-ketone to the 6-hydroxy compound III and dehydration with dimethylsulfoxide gave the Δ6 derivative III. Reduction of III with tritium followed by hydrolysis gave the tetrol-^{3}H with a specific activity of 43 curies/mmole.

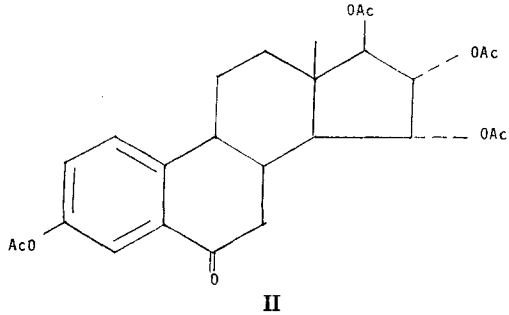
INTRODUCTION

A recently discovered metabolite in pregnancy and neonatal urine [1,2] has been identified as estra-1, 3, 5 (10)-triene-3, 15α, 16α, 17β-tetrol (*Ia*) [3,4,5]. The material is apparently primarily a metabolite of estradiol and is unique in that it is mainly if not exclusively of fetal origin [2,3,5]. In view of its fetal biosynthesis the quantity of the tetrol in pregnancy urine may prove to be a particularly sensitive indicator of fetal health. Such a quantitative assay of reasonable accuracy has heretofore been impossible because of the lack of radioactive tetrol to serve as a measure of procedural losses of this relatively unstable material. The labelled estetrol was also required in order to study its metabolic fate in man since it is possible that its absence in the adult is due to the post natal developement of enzymes for its further transformation to other unknown compounds.

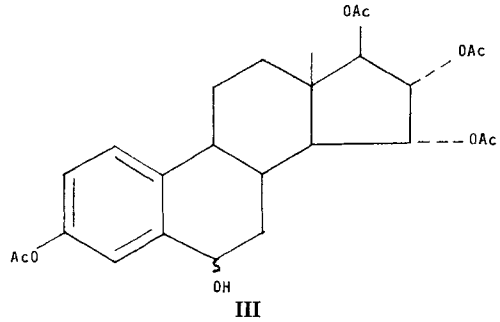
At the outset we wished to use a procedure for the preparation of tritium labelled estetrol that would result in material of sufficiently high specific activity so that it could subsequently be used in any potential radioimmunoassay procedure. Further, it was also clearly desirable to locate the isotope in a chemically and biochemically stable position on the molecule. The latter requirement eliminated from consideration the introduction of tritium into ring



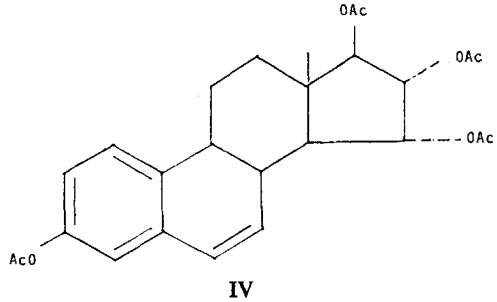
I*a* R = X = H
b R = Ac, X = H
c R = H, X = /H
d R = Ac, X = /H



II



III



IV

D during the elaboration of its trihydroxy structure, and suggested carbons 6 and 7 as the best available sites. The use of the commercially available estrone-6, 7-^{3}H as a starting material was impractical in view of the number of chemical steps required to achieve the transformation to the tetrol. We, therefore, selected a procedure in which the radioisotope is introduced into a compound one chemical step away from the estetrol.

3, 15α, 16α, 17β-tetraacetoxyestra-1, 3, 5 (10)-triene (I*b*) [5] was oxidized with chromic anhydride in acetic acid [7] to give the 6-keto derivative II in reasonable yield. Reduction to the 6-hydroxy structure was best effected by means of catalytic hydrogenation over platinum oxide. The orientation of the 6-hydroxy group in III was not established, but it is presumably β by analogy to the reaction in the estradiol series [8]. The dehydration of 6-hydroxy group with conventional dehydrating reagents gave uniformly poor results. Tosylation and subsequent base elimination also failed to give acceptable yields. The reaction was finally achieved in good yield by heating III in dimethylsulfoxide at 160° C [9], to give the olefin IV. The latter could not be obtained crystalline but it was adequately characterized by its u.v. and nmr spectra and by its hydrogenation to the known tetrol tetraacetate I*b*.

Catalytic reduction of the benzyllic double bond in IV with 10 curies of tritium, gave the tetrol tetraacetate-6,7-^{3}H I*d*. The product was separated from the starting material by preparative thin layer chromatography on silica gel containing 6 % $AgNO_3$. The eluted radioactivity was shown to be 94.3 % homogenous by reverse isotope dilution with inert tetraacetate. Conversion of I*d* to the free tetrol was carried out with K_2CO_3 in methanol under nitrogen. The radioactive tetrol I*c* was isolated by preparative thin layer chromatography and was 95.6 % pure by reverse isotope dilution. The specific activity of the synthesized material was approximately 43 curies per millimole.

Experimental

3, 15α, 16α, 17β-Tetraacetoxyestra-1, 3, 5 (10)-trien-6-one (II). A solution of 455 mg of the tetrol tetraacetate I*b* in 2.6 ml of glacial acetic acid was cooled to 0° C and 1.76 ml of an ice cold solution of CrO_3 (2 g CrO_3 in 1 ml of H_2O and 14 ml of glacial acetic acid) was added dropwise over 30 minutes. The reaction was allowed to stand for 24 hours at room temperature and was then diluted with 50 ml of ice cold water. The mixture was reextracted with chloroform (2 × 50 ml) and the organic layer was washed with 9 % sodium bicarbonate solution and then water. Drying and evaporation gave 400 mg of oil which was chromatographed on 200 grams of silica. Elution with 30 % ethylacetate 70 % cyclohexane gave initially 75 mg of starting material and subsequently 85 mg of the product. The 6-keto derivative II crystallized from petroleum ether-acetone and melted at 224-228° C. The u.v. spectrum in ethanol exhibited maxima at 248mμ (10, 100) and at 297 mμ (2,100).

Anal Calcd for : $C_{26}H_{30}O_9$; C 64.18; H 6.22
Found : C 63.82; H 6.47.

3, 15α, 16α, 17β-Tetraacetoxy estra-1, 3, 5 (10)-triene-6ζ-ol (III). A solution of 70 mg of the 6-ketone II in 5 ml of ethylacetate was reduced with hydrogen at atmospheric pressure over 50 mg of platinum oxide for 20 hours. The filtration of catalyst and removal of solvent gave an oil weighing 55 mg. Purification by preparative thin layer chromatography on silica gel in 7 : 3 ethylacetate-cyclohexane gave 43 mg of the 6 ζ hydroxy compound III, which crystallized from methanol and melted at 188-190° C. The u.v. showed maxima at 266 mμ (590).

Anal Calcd for : $C_{26}H_{32}O_9$; C 63.92; H 6.60
Found : C 63.61; H 6.28.

3, 15α, 16α, 17β-Tetraacetoxyestra-1, 3, 5 (10), 6-pentaene-(IV). A solution of 30 mg of III in 1 ml of freshly distilled dimethylsulfoxide was heated at 165° C for 1 hour in a nitrogen atmosphere. The cooled solution was diluted with 30 ml of water and extracted well with ether which was washed with water, dried and evaporated. The residue was purified by preparative thin layer chromatography on silica gel in 1 : 1 ethyl acetate-cyclohexane to give 12 mg of the olefin IV which resisted crystallization.

The olefin IV gave an nmr spectrum showing the 6 and 7 hydrogens as a pair of doublets centered at 389 and 358 Hz respectively (J = 9 Hz) *. This pattern was superimposable with that obtained from Δ6 estradiol diacetate. The u.v. spectrum showed an intense absorption at 225 mμ with a shoulder at 320 mμ. Hydrogenation of 6 mg of IV gave 5 mg of the tetra-acetate I*b* m.p. 174-176 identical in all respects with the authentic sample.

6, 7-^{3}H Estra-1, 3, 5 (10)-triene-3, 15α, 16α, 17β-tetrol (Ic). A solution of 5 mg of the olefin IV in 1 ml of ethyl acetate containing 4 mg of 10 % palladium on charcoal was reduced with 10 curies of tritium **. The catalyst was removed, the solvent was evaporated and the residue was treated twice with 10 ml of methanol to remove labile tritium. The residue weighing 5 mg and containing 350 mc of radioactivity was purified by preparative thin layer chromatography on silica gel containing 6 % $AgNO_3$ in the system 1 : 1 ethyl acetate-cyclohexane. The ^{3}H tetraacetate I*d* weighed 2.1 mg and contained 220 millicuries. An aliquot containing 14,700 cpm was diluted with 12.8 mg of inert tetraacetate I*b* and recrystallized from methanol. The succes-

* The n.m.r. spectra were obtained in deuteriochloroform on a Varian A60 instrument. The chemical shift values are in hertz downfield for tetramethylsilane as an internal standard. The analyses were by Spang Microanalytical Laboratory.

** This reduction was done commercially by the New England Nuclear Corp., Boston, Mass.

sive specific activities were 1110, 1080, 1084 cpm/mg indicating a radiohomogeneity of 94.5 %.

A portion of the ^{3}H-tetraacetate I*d* containing 90×10^6 cpm in 2 ml of methanol was stirred for two hours at room temperature with 4 mg of anhydrous K_2CO_3. The salt was filtered off and the solution concentrated to 0.1 ml which was applied to the thin layer plate and developed in the system 10 % methanol - 90 % ethyl acetate for 1 hour. The zone coincident with standard tetrol running alongside was eluted and contained 48×10^6cpm. An aliquot containing 22,450 cpm was diluted with 11.3 mg of inert tetrol and recrystallized 3 times from dilute methanol. The successive specific activities were 1914, 1853, 1862 cpm/mg, indicating a 94 % purity.

Another aliquot of the ^{3}H-tetrol was scanned following the above thin layer chromatography and showed a single radioactive peak coincident with the standard tetrol.

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