Synthesis of 17α-Ethynylestradiol-6,7-^{3}H and 17α-Ethynylestradiol-6,7-^{3}H, 3-Cyclopentyl-l-^{14}C Ether

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SUMMARY

Several authors have reported on the physiological differences between 17α-ethynylestradiol and its 3-cyclopentyl ether. The radiolabeled steroids were required to compare their relative absorptions, excretions, and metabolic products. Tritium was introduced into the steroid nucleus by catalytic tritiation of 6-dehydroestrone. Etherification with either cyclopentyl bromide or cyclopentyl-^{14}C p-bromobenzenesulfonate gave the cyclopentyl ether of estrone. Ethynylation of either estrone-6, 7-^{3}H or its cyclopentyl-^{14}C ether gave 17α-ethynylestradiol-6, 7-^{3}H and 17α-ethynylestradiol-6, 7-^{3}H, 3-cyclopentyl-^{14}C ether. It was also possible to etherify 17α-ethynylestradiol directly although in poorer yields.

INTRODUCTION

Falconi and Ercoli [1] and Meli *et al.* [2] have described the strikingly different physiological properties of various alkyl ethers of 17α-ethynylestradiol when compared to the parent 17α-ethynylestradiol (17α-ethynylestra-1, 3, 5 (10)-trien-3, 17β-diol) (III*a*). In particular, the 3-cyclopentyl ether of 17α-ethynylestradiol (3-cyclopentyloxy-17α-ethynylestra-1, 3, 5 (10) trien-3, 17β-diol) (Va) [3] was chosen for in-depth study. In a series of papers, Steinetz and Meli [4,5,6,7] have compared the absorption, excretion, distribution, and metabolism in rats and rabbits using the radioisotopically labeled III*b*, V*b*, and 17β-estradiol-6, 7-^{3}H. Layne and Williams [8,9,10] also used these compounds to study the urinary metabolites in rabbits and humans.

This report will describe the procedures used to synthesize these radioisotopically labeled compounds.

DISCUSSION.

The synthetic paths used to introduce tritium into these steroids are shown in Figure 1.

O'Donnell and Pearlman [11] have described the catalytic reduction of 6-dehydroestrone acetate (3-acetoxyestra-1, 3, 5 (10), 6-tetraen-17-one) (I*a*) with tritium followed by basic hydrolysis to give estrone-6, 7-^{3}H (II*b*) in 16-34 % radiochemical yield having a specific activity of 5.5 Ci/mM. Our work confirms their results as we obtained a 40 % radiochemical yield. The direct catalytic reduction of 6-dehydroestrone (estra-1, 3, 5 (10), 6-tetraen-3-ol-17-one) (I*b*) gave II*b* in 64 % radiochemical yield. The extent of this reduction can readily be followed by subjecting the crude reduction product to ascending thin-layer chromatography (T.L.C.) using System B (see Experimental). This system separates I*b* from the faster moving II.

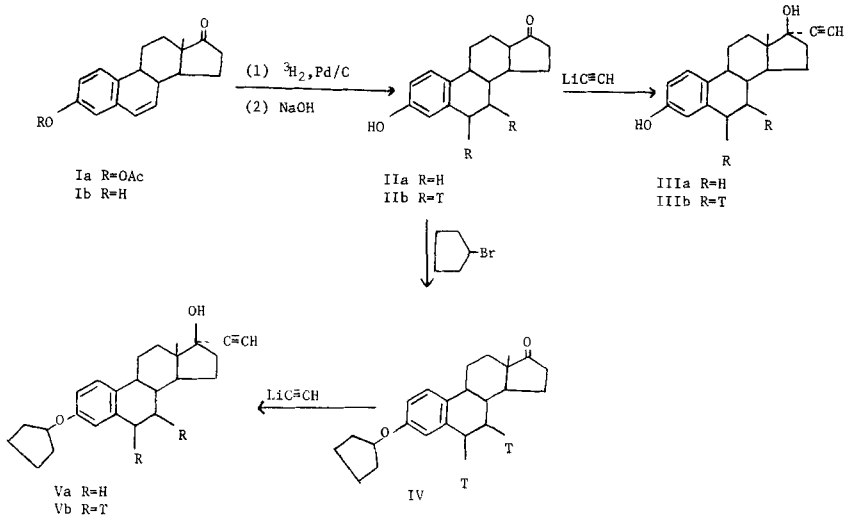

Figure 1. Synthetic scheme for preparation of the tritium labeled steroids.

Inhoffe, *et al.* [12] prepared III*a* by allowing potassium acetylide to add to II*a* in a mixture of dioxane and liquid ammonia. Despite the attractive yield of 90 % reported for this reaction, the small quantities of high specific activity steroids precluded the use of this procedure. Beumel and Harris [13] described the addition of the solid ethylene diamine complex of lithium acetylide [14] to a number of ketones under mild conditions. Using this reagent in a 1 to 20 molar ratio in dimethyl acetamide, the synthesis of III*b* was accomplished in 57 % yield [15].

The introduction of the non-radioactive cyclopentyl group to give IV was readily accomplished by allowing cyclopentyl bromide to react with the sodium salt of the steroid using the procedure described by Ercoli [16]. Ethynylation of IV gave the desired V*b* using the same conditions as previously described in a yield of 54 %.

Figure 2 shows the routes used to incorporate ^{14}C into the molecule.

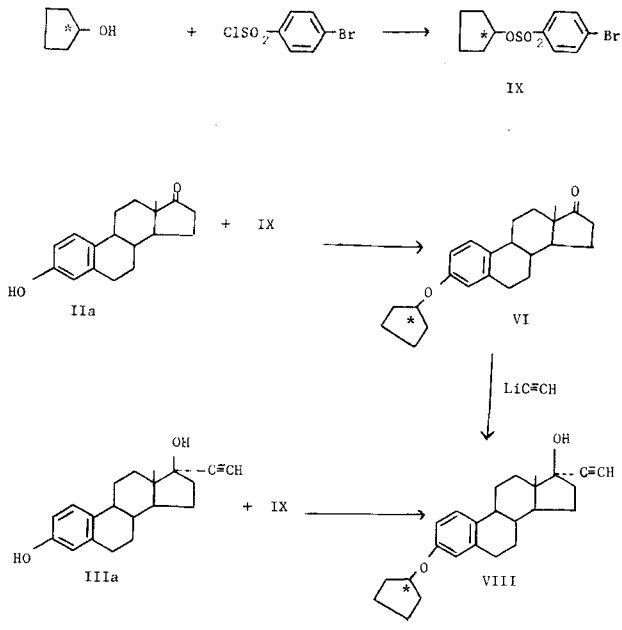

Figure 2. Synthetic scheme used to prepare ^{14}C labeled steroids.

Loftfield [17] described the preparation of cyclopentyl-1-^{14}C iodide. His procedure utilized the base catalyzed cyclization of adipic-1, 6-^{14}C acid with decarboxylation to give cyclopentanone-1-^{14}C. This ketone was reduced with lithium aluminum hydride to the alcohol. Loftfield then prepared cyclopentyl p-bromobenzene sulfonate which was converted into cyclopentyl-1-^{14}C iodide. The latter reaction was unsatisfactory since purification was a serious problem.

Since the brosylate group is an excellent leaving group, we reasoned that this compound should form the desired cyclopentyl ether VI when allowed to react with the sodium salt of II*a*. This indeed was the case when pyridine was used at the solvent. Yields of between 30-50 % were obtained in preliminary reactions, but the reaction with the labeled steroid gave a disappointing 14 % yield.

Ethynylation with the ethylene diamine complex of lithium acetylide as previously described gave the desired VIII. Again, the yield with labeled material was 36 %, whereas a yield between 47-53 % was anticipated from non-labeled experiments.

In the initial work, the cyclopentanol-1-^{14}C was purchased. We later synthesized this alcohol using the procedure of Korte and Rechmeier [18] modified slightly by using 10 % molar excess of barium hydroxide to effect the cyclization. The ketone was isolated as was its bisulfite adduct. When the bisulfite adduct was reduced with sodium borohydride to cyclopentanol, a purer product was obtained than when the ketone was reduced. The latter method gave a slightly higher yield and was chosen for the radioactive synthesis.

When the original sample of VIII was exhausted, we investigated the direct etherification of III*a* with IX. Although more by-products were formed using this approach, the desired product was readily isolated and purified by preparative T.L.C. in Systems A and C (see Experimental). The expected yield was 23-35 %, but only 11 % of VIII was obtained from the experiment using labeled reactants.

Several authors have cautioned about the decomposition of steroids when placed in contact with unpurified solvents [19,20]. In all of this work, the solvents were purified by the methods of Frankel and Nalbandov [21], and Fieser [22]. In addition, silicone grease was not used to lubricate any of the ground glass joints as we have found that significant contamination from the grease can result.

For radiochemical stability considerations, all products were stored under nitrogen at 5° C in dry benzene containing 10 % ethanol.

Experimental.

Thin-Layer Chromatography Systems.

System A Silica gel GF; Skellysolve B/Ethyl acetate (2 : 1)
System B Silica gel GF impregnated with 10 % $AgNO_3$; Chloroform/Ethyl acetate (4 : 1)
System C Silica gel GF; Cyclohexane/acetone (4 : 1)

Estrone-6, 7-^{3}H (IIb).

Thirty-two mg (0.1 mM) 6-dehydroestrone acetate [23] was reduced with 7 Ci $^3H_2$ [24] in 1 ml ethyl acetate containing 25 mg 10 % Pd/C. The crude product was hydrolyzed, diluted with 2.7 g (10 mM) estrone and recrystallized from methanol to give 2.39 g (88.5 %) II*b* which melted at 261-263° C (authentic 261-263° C). A T.L.C. of this material using System A showed 99.7 % radiochemical purity [25]. The specific activity was 281 mCi/mM as determined by liquid scintillation spectrometry [26]. This represents a 40.5 % radiochemical yield.

When 64.5 mg (0.25 mM) of 6-dehydroestrone [27] was reduced as above with 16 Ci $^3H_2$ [24], 64.5 mg (99 %) of II*b* were obtained. In this experiment, a T.L.C. in System B demonstrated the presence of unreduced 6-dehydroestrone. The reduction was completed with hydrogen. The specific activity was 41 Ci/mM and represents a 64.4 % radiochemical yield.

Ethynylestradiol-6, 7-^3H (IIIb).

To a suspension of 2.0 g (20 mM) lithium acetylide as the ethylene diamine complex in 6 ml dimethylacetamide was added a solution of 270 mg (1 mM) estrone-6, 7-^{3}H (280 mCi, S.A. = 280 mCi/mM) dissolved in 5 ml dimethylacetamide and acetylene gas allowed to bubble through the reaction for 3.5 hours. The flow of acetylene was stopped, but the reaction stirred overnight.

The reaction was poured over 25 g of ice containing 5 ml conc. hydrochloric acid, and extracted 5 × 10 ml diethyl ether. The organic phases were combined, dried with $MgSO_4$, filtered, and the solvent removed. The residual oil was recrystallized twice, each time from 3 ml hot methanol to which was added an equal volume of warm water.

The resulting impure solid was dissolved in 6 ml ethyl acetate and percolated through an 8 g column of Florisil. The product was eluted with ethyl acetate. The solvent was removed and the residue was recrystallized by dissolving in 3 ml hot methanol and adding 3 ml warm water. On cooling, filtering, and washing with 25 % aqueous methanol, 174 mg (59 %) of crystalline III*b* was obtained. After drying in vacuo at 100° C for 3 hours, the solid melted at 178-180° C. The specific activity was 275 mCi/mM. A T.L.C. in System A gave a single red spot at an R_f of about 0.45 when sprayed with 2 % sulfuric acid in 50 % aqueous methanol and heated for 30 minutes at 100 °C [28]. This spot contained 99+ % of the radioactivity on the plate. The infrared absorption spectra, when measured as a KBr pellet, exhibited the same maxima and minima as an authentic sample. The molecular extinction coefficient measured in ethanol at 281 nm was 2,050 which was 98.7 % of an authentic sample.

Estrone-6, 7-^3H, 3-Cyclopentyl Ether (IV).

A solution of 810 mg estrone -6, 7-^{3}H (840 mCi, S.A. = 280 mCi/mM) 0.99 ml cyclopentyl bromide, and 84 mg sodium previously dissolved in 8.4 ml abs. ethanol was refluxed for 3 hr, cooled and 50 ml H_2O added.

The reaction was extracted 2 × 25 ml and 2 × 10 ml methylene chloride. The organic phases were combined, dried with $MgSO_4$, filtered, and the solvent removed. The residue was dissolved in excess methylene chloride and percolated through an 8 g Florisil column. The eluate was collected, and the solvent removed. This residue was recrystallized from methylene chloride and 3 ml methanol to give, on cooling, 627 mg (62 %) of IV melting at 150-152° C.

17α-Ethynylestradiol-6, 7-^{3}H, 3-Cyclopentyl Ether (Vb).

To a suspension of 3.9 g (37 mM) lithium acetylide as the ethylene diamine complex in 9.25 ml dimethylacetamide was added a solution of 627 mg (1.85 mM) IV dissolved in 7.4 ml dimethylacetamide and acetylene gas allowed to bubble through the reaction for 3.5 hours. The flow of acetylene was stopped but the reaction stirred overnight.

The reaction was poured over 46 g ice containing 9.25 ml conc. hydrochloric acid, and extracted 4 × 25 ml diethyl ether. The organic phases were combined, dried with $MgSO_4$, filtered, and the solvent removed. The residual oil dissolved in 6.7 ml methanol and 0.48 ml glacial acetic acid. To this solution was added 200 mg Girard T reagent dissolved in 3.7 ml methanol and refluxed for 1 hour. The reaction was poured over 18.5 g ice containing 18.5 ml 1N sodium carbonate and extracted 4× 25 ml diethyl ether. The organic phases combined, washed 2 × 15 ml H_2O, dried with $MgSO_4$, filtered, and the solvent removed. The oily residue was transferred to the top of a 15 g Florisil column with petroleum ether and the column washed with petroleum ether, and then cyclohexane. The product was eluted with benzene. On removal of the solvent, the residue was recrystallized by dissolving in 7.4 ml hot methanol and adding 3.7 ml H_2O. On cooling, fine crystals formed, which were filtered and washed with cold 50 % aqueous methanol to give 361 mg (54 %) of V*b*, melting at 102-105° C after drying in vacuo at 61° C for at least 10 hrs.

The specific activity was 293 mCi/mM. A T.L.C. in System A gave a single red spot at an R_f of about 0.75 when sprayed with the 2 % sulfuric acid in aqueous methanol reagent previously mentioned. This spot contained 99+ % of the radioactivity on the plate. The infrared absorption spectra, when measured as a KBr pellet exhibited the same maxima and minima as an authentic sample. The molecular extinction coefficient measured in ethanol at 279 nm was 1,940. This value is 100.1 % of the authentic sample.

Adipic-1, 6-^{14}C Acid.

The procedures described by Korte and Rechmeier [18] and Loftfield [17] were used to convert 7.5 mM of KCN-^{14}C [150 mCi, specific activity of 20 mCi/mM] [29] into the nitrile and then into adipic-1, 6-^{14}C acid. The resulting 392 mg of acid represented a 72 % yield and was used directly in the preparation of the cyclopentanone-1-^{14}C.

Cyclopentanone-1-^{14}C.

To a solution of 392 mg (2.68 mM) adipic-1, 6-^{14}C acid in 5 ml water was added 933 mg (2.96 mM) barium hydroxide octahydrate. The suspension was heated on a steam bath for about 5 minutes, cooled and lyophylized. The solid was packed into an 11 mm pyrex tube and held in place with plugs of quartz wool. The tube was placed into a ceramic electrically heated combustion furnace and a slow stream of nitrogen allowed to flow through the

system. The internal temperature was raised to 250° C for 20 minutes to remove water from the solid. The temperature was increased to 550° C and held at that temperature for 2 hr, manually rotaring the tube a quarter turn every 15 minutes. The crude distillate was collected in a test tube cooled to −78° C. The weight was 186 mg (82.6 %) and was used directly to prepare cyclopentanol-1-^{14}C.

Alternatively, on this scale, the distillate would be allowed to bubble through 1.4 ml of a 40 % aqueous sodium bisulfite solution. The adduct would be cooled, filtered, and washed with anhydrous ether to give about 280 mg (55 %) of a white solid which would be used to prepare the cyclopentanol-1-^{14}C.

Cyclopentanol-1-^{14}C.

The previously reported [18] reduction with sodium borohydride in water was complete after 15 minutes, and the alcohol was isolated by ether extraction of the reaction after saturating with sodium chloride and adjusting the pH to 2. After drying, the ether was allowed to evaporate, to give 82.2 mg. This represents a 43.5 % yield and was used directly to prepare IX. Gas chromatography on a 20 % carbowax column operated at 115° C of several unlabeled samples of cyclopentanol prepared in the same manner demonstrated purities between 97-99 %.

Cyclopentyl-1-^{14}C p-Bromobenzenesulfonate (IX).

To a magnetically stirred solution of 236 mg (2.74 mM) cyclopentanol-1-^{14}C (40 mCi; specific activity of 14.6 mCi/mM) [24] in 2 ml dry pyridine cooled in an icebath was added a solution of 1.85 g (7.22 mM) *p*-bromobenzenesulfonyl chloride in 1.5 ml dry pyridine dropwise over 20 minutes. The reaction was stirred in the cold for 20 hours, poured into 50 ml 3 N hydrochloric acid, and extracted with four 25 ml portions of carbontetrachloride. The organic phases were combined, washed with three 15 ml portions of 1 N hydrochloric acid and two 15 ml portions of water, dried with K_2CO_3, filtered, and the solvent removed. The resulting orange oil weighed 460 mg (67 %) and was used directly to prepare VI.

Estrone, 3-Cyclopentyl-1-^{14}C Ether (VI).

To a magnetically stirred solution of 493 mg (1.82 mM) II*a* in 5.84 ml absolute ethanol containing 58.4 mg (2.19 millatoms) sodium metal was added a solution of 460 mg (about 1.82 mM) IX dissolved in 4 ml absolute ethanol over five minutes. The reaction was allowed to stir at room temperature overnight and was then poured into 25 ml water. This solution was saturated by adding about 15 g ammonium sulfate and extracted with four 25 ml portions of methylene chloride. The organic phases were combined, dried with K_2CO_3, filtered, and the solvent removed.

The oily residue normally obtained at this point with non-radioactive materials usually was purified by elution from an 8 g Florisil column followed by recrystallization from a mixture of methylene chloride and methanol. The "hot" run, by this procedure, gave a product with a low melting point. Repeated chromatography over Florisil followed by repeated recrystallization did not improve this material.

The crude material was chromatographed using preparative T.L.C. in System A by distributing a solution of this solid over two 20 × 20 cm plates coated with 250 microns of silica gel G, previously washed with methanol and reactivated by heating at 105° C for 30 minutes. The radioactive portion of the plate was removed by scraping, packed into a column, and the desired material eluted with ethyl acetate. On removal of the solvent, the resulting solid was recrystallized from methylene chloride and methanol to give 89 mg (14 %) of VI melting at 149-150° C.

17α-Ethynylestradiol, 3-Cyclopentyl-1-^{14}C Ether (VIII).

To a suspension of 0.5 g (5 mM) lithium acetylide as the ethylene diamine complex in 2 ml dimethylacetamide was added a solution of 80 mg (0.26 mM) VI dissolved in 1 ml dimethylacetamide and acetylene gas allowed to bubble through the reaction for 3.5 hours. The flow of acetylene was stopped but the reaction allowed to stir overnight. The workup of the reaction was similar to that described for V*b*. Recrystallization gave 34.2 mg (36 %) of VIII, melting at 102-105° C after drying in vacuo at 61° C for at least 10 hrs.

The specific activity was 14.3 mCi/mM. A T.L.C. in System A gave a single red spot at an R_f of about 0.75 when sprayed with the 2 % sulfuric acid solution in aqueous methanol reagent previously mentioned. This spot contained 99+ % of the radioactivity on the plate. The infrared absorption spectra, when measured as a KBr pellet, exhibited the same maxima and minima as an authentic sample. The molecular extinction coefficient measured in ethanol at 279 nm was 1,910. This value is 99.6 % of an authentic sample.

VIII from 17α-Ethynylestradiol.

To a magnetically stirred solution of 252 mg (0.85 mM) ethynylestradiol in 3 ml absolute ethanol containing 1.96 ml of a solution prepared by allowing 100 mg sodium metal to dissolve in 10 ml absolute ethanol, was added a solution of 251 mg (about 0.85 mM) of crude IX in 2 ml absolute ethanol, dropwise over about 10 minutes. The reaction was allowed to stir at room temperature overnight. The slurry was poured into 12.2 ml water, 7.1 g ammonium sulfate added, and extracted with four 10 ml portions of methylene chloride. The organic phases were combined, dried with K_2CO_3, filtered, and the solvent removed. A brown oil which weighed 300 mg was obtained. This was purified using four 20 × 20 cm silica gel G plates using System A by the preparative T.L.C. procedure described for the purification of VI. The product was eluted from the scrapings with 40 ml methanol. This purification was repeated and

then twice more using System C. A T.L.C. in System C demonstrated that more than 2 % of a radioactive unknown was still present which moved with an R_f just slightly less than VIII. This contaminant was removed by recrystallization twice by dissolving in 1 ml hot methanol and adding 2 × 0.25 ml portions of hot water. This yielded 41.85 mg (10.7 %) of VIII after drying in vacuo at 61° C for at least 10 hours.

The specific activity was 14.3 mCi/mM. A T.L.C. in System C gave a single red spot at an R_f of about 0.75 when sprayed wyth the 2 % sulfuric acid reagent previously mentioned. This spot contained 99+ % of the radioactivity on the plate. The infrared absorption spectra when measured as a KBr pellet exhibited the same maxima and minima as an authentic sample. The molecular extinction coefficient measured in ethanol at 279 nm was 1,890 which is 98.6 % of an authentic sample.

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24. Purchased from New England Nuclear Corp., Boston, Mass. 02118.
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26. Either a Packard Tri Carb Liquid Scintillation Spectrometer Model 3310 equipped with automatic external standardization or a Model 314 X quench corrected via internal standardization was used.
27. Purchased from Mann Research Laboratories, New York, New York 10006.
28. The use of the more sensitive Kober reagent (2 g hydroquinone in 100 ml 76 % sulfuric acid) will give a blue spot and is now preferred.
29. Purchased from Mallinckrodt/Nuclear, St. Louis, Missouri.