Synthesis of Some Radiolabeled 17α-Ethynyl-19-nor Steroids

Received October 23, 1970

During the course of study of the metabolism of some anti-fertility drugs it was necessary to prepare several 17α-ethynyl-19-nor steroids. Although the use of some of the specifically labeled drugs reported here have been cited in the literature [1] there has been no report of their synthesis.

The synthetic scheme employed for the preparation of these steroids is outlined in Chart I. Estradiol labeled either in the 6,7-positions with tritium or the 4-position with carbon-14 was used as the starting material for the preparation of the correspondingly labeled steroids.

The 17α-ethynyl-17-hydroxy-5(10)-estren-3-one (**6**) and 17α-ethynyl-19-nortestosterone (**7**) were prepared by a method based on the reaction scheme described by Colton [2]. The most significant modification of Colton's procedure was in the oxidation of 1,4-dihydroestradiol-3-methyl ether (**3**) to 1,4-dihydroestrone-3-methyl ether (**4**). Although acceptable yields of 4 were obtained by the reported Oppenauer oxidation of **3**, it was found that the oxidation could be performed more efficiently and conveniently using the pyridine-sulfur trioxide-dimethyl sulfoxide oxidizing reagent of Parikh [3].

17α-Ethynylestr-4-en-3β,17β-diol (**8**) was prepared in good yield by the reduction of **7** with lithium tri-t-butoxyaluminum hydride [4]. The diol **8** was converted to the diacetate **9** in satisfactory yield by a reported procedure [4].

Experimental

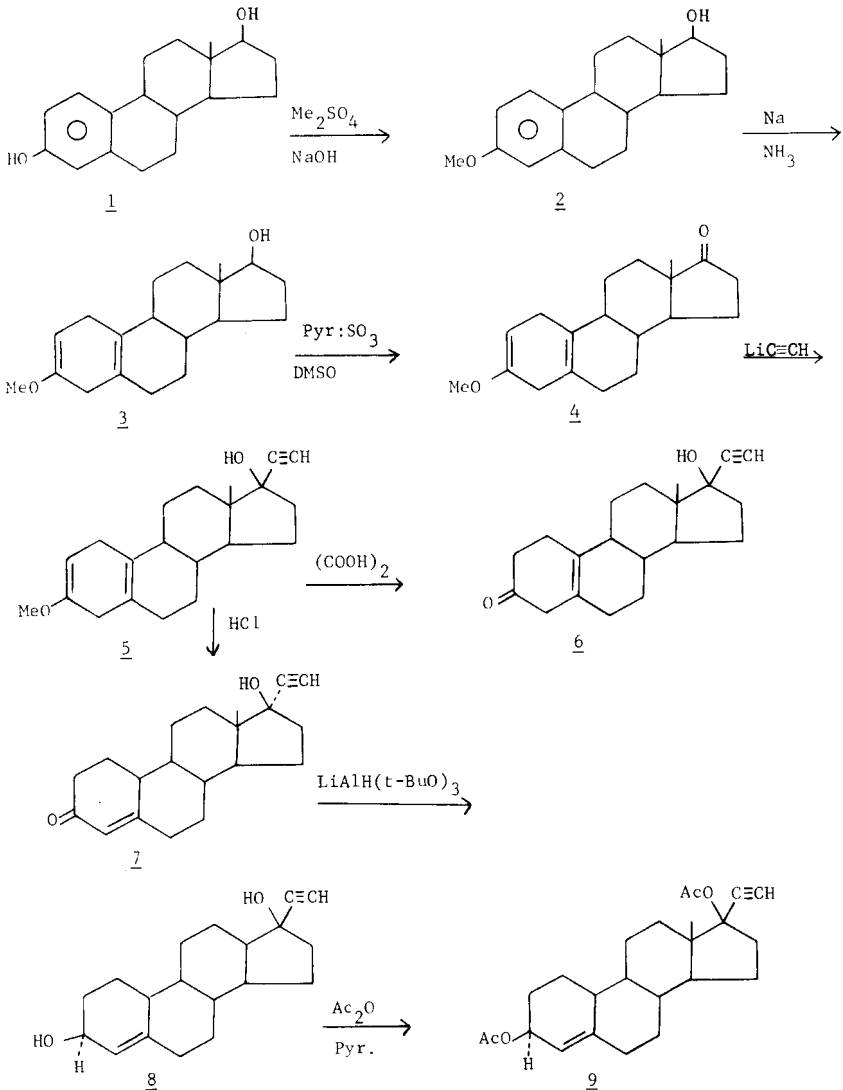
Estradiol-3-methyl ether-6,7-^3H [5].

Estradiol-6,7-^{3}H (50 mg 0.19 mmole; SA 56.8 μCi/mg) (Amersham/Searle) was etherified with dimethyl sulfate [6]. Radiochromatography (silica gel; benzene-acetone 9 : 1) showed the product (92 % yield) to be 94 % radiochemically pure.

1,4-Dihydroestradiol-3-methyl ether-6,7-^3H [5].

The crude estradiol-3-methyl ether-6,7-^{3}H obtained from the above reaction was reduced by the method of Dryden *et al.* [7]. Radiochromatography (alumina; ethyl acetate-cyclohexane 1 : 1) showed the product (99 % yield) to be 75 % radiochemically pure.

CHART I
Synthesis of some radiolabeled 17α-Ethynyl-19-nor steroids



1,4-Dihydroestrone-3-methyl ether-6,7-^3H.

The crude 1,4-dihydroestradiol-3-methyl ether-6,7-^{3}H from the preceeding reaction was dissolved in 1.0 ml of freshly distilled dimethyl sulfoxide (calcium hydride) and 0.5 ml of dry triethyl amine (KOH). Freshly prepared pyridine-sulfur trioxide complex (150 mg) dissolved in 0.75 ml of dimethyl sulfoxide was added dropwise with stirring. Additional 50 mg portions of the pyridine-sulfur trioxide complex in 0.25 ml of dimethyl sulfoxide were added at 20 min

intervals until thin-layer chromatography (silica gel; benzene-acetone 9 : 1) showed no starting material remained. In all, 250 mg of pyridine-sulfur trioxide complex (of unknown purity) was added; total reaction time was 60 min. Water (3 ml) was added and the mixture was stirred for 10 min, poured into 20 ml of water, and extracted with chloroform. The chloroform extract was washed with water, saturated sodium chloride, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed on two 20 × 20 cm ChromAR 1000 sheets with benzene containing 3 % triethyl amine. The product was eluted from the ChromAR with acetone containing 5 % triethyl amine to give a yield of 40.0 mg 82 % of yellow crystals that showed a single peak on a radiochromatogram (benzene-acetone 9 : 1; silica gel).

17α-Ethynyl-1,4-dihydroestradiol-3-methyl ether-6,7-^{3}H.

Freshly distilled dioxane (10 ml) was saturated with purified acetylene, and 500 mg (5.4 mmole) of lithium acetylide-ethylene diamine complex was added in one portion. A solution of the 1,4-dihydroestrone-3-methyl ether-6,7-^{3}H (40.0 mg; 0.141 mmole) from the preceeding reaction in 3 ml of dioxane was added dropwise, and the reaction mixture was stirred at room temperature for 3 hrs. Saturated aqueous ammonium chloride (3 ml) was added dropwise with vigorous stirring, and the mixture was partitioned between ether and water. The ether solution was washed with saturated sodium chloride, dried (Na_2SO_4) and evaporated under reduced pressure to yield 40.9 mg (93 %) of yellow crystals.

A small portion of the crude product was chromatographed (silica gel; benzene/acetone 9 : 1) with various known compounds, and the resulting spots (visualized with 5 % phosphomolybdic acid in ethanol) were scraped from the plate and counted in a Packard model 3375 liquid scintillation counter. The composition of the crude reaction product was as follows; 1,4-dihydroestrone-3-methylether-6,7-^{3}H — 12.2 %; 17α-ethynyl-1,4-dihydroestradiol-3-methyl ether-6,7-^{3}H — 63.0 %; 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one-6,7-^{3}H — 3.6 %; 17α-ethynyl-19-nortestosterone-6,7-^{3}H — 8.6 %; unidentified compounds — 12.5 %.

17α-Ethynyl-17β-hydroxy-5(10)-estren-3-one-6,7-^{3}H (Norethynodrel-6,7-^{3}H) [5].

The 17α-ethynyl-1,4-dihydroestradiol-3-methyl ether-6,7-^{3}H from the previous reaction was hydrolyzed to norethynodrel-6,7-^{3}H by the method of Colton [2]. Radiochromatography (silica gel; chloroform-ethyl acetate 7 : 3) showed the product (31 % yield based on estradiol; (SA 51.8 μCi/mg) was better than 95 % radiochemically pure.

17α-Ethynyl-19-nortestosterone-6,7-^{3}H (Norethyndrone-6,7-^{3}H) [5].

Crude 17α-ethynyl-1,4-dihydroestradiol-3-methyl ether-6,7-^{3}H (50 mg, 34 μCi/mg) prepared by the above procedure was hydrolyzed to norethyn-

drone-6,7-^{3}H by the method of Colton [2]. Radiochromatography (silica gel; chloroform-ethyl acetate 7 : 3) showed the product (30 % yield based on estradiol; SA 35 μCi/mg) to be better than 95 % radiochemically pure.

17α-Ethynylestr-4-ene-3β,17β-diol-4-^{14}C (Ethynodiol-4-^{14}C) [5].

Norethyndrone-4-^{14}C (100 mg; 0.33 mmole; SA 0.39 μCi/mg) was reduced to Ethynodiol-4-^{14}C by the method of Klimstra [4]. Radiochromatography (alumina; chloroform-ethyl acetate 7 : 3) showed the product (94 % yield; SA 0.39 μCi/mg) to be better than 95 % pure.

Ethynodiol-6,7-^{3}H diacetate [5].

Ethynodiol-6,7-^{3}H (50 mg; 0.167 mmole; SA 21 μCi/mg) was acetylated by the method of Klimstra [4] with care being taken not to allow the temperature of the reaction mixture to rise above 115° C. Radiochromatography (silica gel; benzene-acetone 9 : 1) showed the product (69 % yield) to be better than 98 % radiochemically pure.

17α-Ethynylestr-4-ene-3β,17β-diol-3α-^{3}H (Ethynodiol-3α-^{3}H).

Five millicuries of lithium aluminium tritide (2.7 mg, 0.071 mmole) was transferred into a flask under dry, oxygen-free nitrogen in a dry bag. The flask was fitted with a rubber septum, a positive pressure of nitrogen was applied to the flask through a syringe needle, and the dry bag was removed. Freshly distilled tetrahydrofuran (lithium aluminum hydride; 0.75 ml) was added to the flask, followed by the dropwise addition of 0.25 ml of dry t-butanol (distilled from sodium onto 3A molecular sieve). The mixture was stirred for 5 min, and 17α-ethynyl-17β-hydroxyestr-4-ene-3-one (30 mg, 0.10 mmole) in 1.0 ml of tetrahydrofuran added. Stirring was continued at room temperature (25° C) for 2 hrs and water (0.25 ml) was added slowly. The mixture was transferred to a separatory funnel with 25 ml of 10 % acetic acid, extracted with chloroform, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by preparative thin layer chromatography (20 × 20 cm alumina HF; chloroform-ethyl acetate 7 : 3), and crystallized from acetone-water to yield 16.0 mg of ethynodiol-3-^{3}H (75 % based on lithium aluminum hydride) with specific activity of 236 μCi/mg. Radiochromatography showed the ethynodiol-3-^{3}H to be better than 97 % pure.

ACKNOWLEDGEMENTS

The authors thank Dr. F. B. Colton for making available the experimental details for a small scale preparation of norethyndrone and norethynodrel which had been developed in his laboratory and Dr. M. E. Wall, the director

of this laboratory, for helpful discussions. This work was supported by the National Institute of General Medical Sciences, Contract No. PH43-65-1057.

J. A. KEPLER and G. F. TAYLOR
Chemistry and Life Sciences Laboratories
Research Triangle Institute
Research Triangle Park, North Carolina 27709

REFERENCES

1. (*a*) FOTHERBY, K., KAMYAB, S., LITTLETON, P. and KLOPPER, A. I. — *Biochem. J.*, **99** : 14 (1965).
   (*b*) FOTHERBY, K. — *J. Endocrin.*, **40** : R15 (1968).
   (*c*) KAMYAB, S. — *J. Endocrin.*, **41** : 263 (1968).
2. (*a*) COLTON, F. B. — U. S. 2,655,518, Oct. 1953.
   (*b*) COLTON, F. B. — U. S. 2,691,028, Oct. 1954.
   (*c*) COLTON, F. B. — U. S. 2,725,389, Nov. 1955.
   (*d*) COLTON, F. B. — Private Communication.
3. PARIKH, J. R. and VON E. DOERING, W. — *J. Ann. Chem. Soc.*, **89** : 5505 (1967).
4. KLIMSTRA, P. D. and COLTON, F. B. — *Steroids*, **10** : 411 (1967).
5. Experimental details will be supplied upon request.
6. VYAS, G. N. and SHAH, N. M. — *Org. Syn.*, Coll. Vol. **4**, 837 (1963).
7. DRYDEN, H. L., Jr., WEBBER, G. M., BUTNER, R. R. and CELLA, J. A. — *J. Org. Chem.* **26** : 3237 (1961).