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HIGH SPECIFIC ACTIVITY STEROIDS I: 17α-ETHYNYLESTRADIOL-[9,11-^{3}H] and NORETHINDRONE-[9,11-^{3}H]*

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SUMMARY

We report herein the first *high specific activity* tritium labelled synthesis of the widely used oral antifertility agents ethynylestradiol (5) and norethindrone (9). These substances were prepared at a specific activity of 54 Ci/mmol, a figure which is 48 and 377 times greater than any previously described preparations of (5) and (9) respectively. Microscale conditions were developed for all the steps in the sequence, including the Birch reduction (1), so that the initially high level of enrichment usually achieved in the reduction of $\Delta^{9(11)}$-estradiol 3-methyl ether was retained in the final products.

Key Words: tritiated 19-norsteroids, norethindrone-[9,11-^{3}H], EE-[9,11-^{3}H].

INTRODUCTION

The discovery (2) that 19-norprogestagens exhibit greater biological acivity than their natural counterparts led to the synthesis of a variety of such compounds in the hope of finding an orally active and thus, clinically useful antifertility agent. The first progestagen which was highly orally active in man (3) 17α-ethynyl-19-nortestosterone

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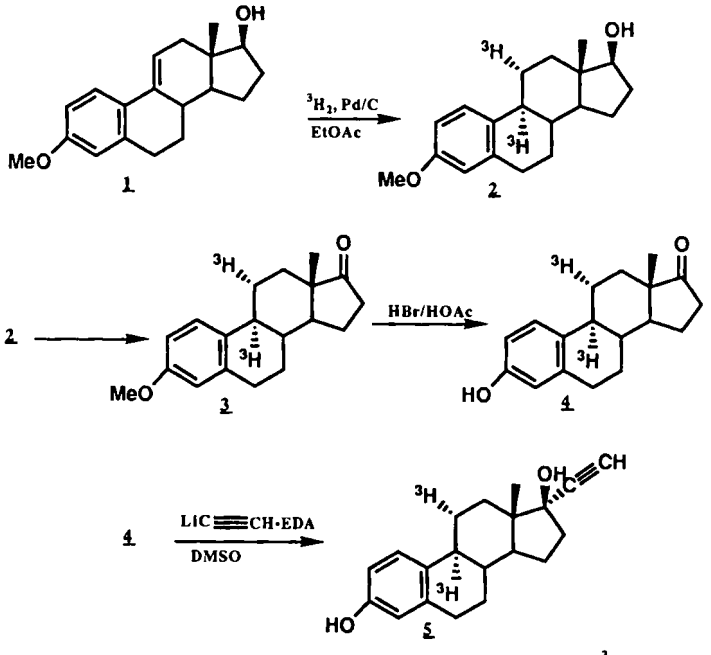
(norethindrone, NET), was synthesized by Djerassi, *et. al.* (4) in 1954. This substance is used to this day in combination with ethynylestradiol (EE) in the Syntex oral contraceptive Norinyl® 1+35 (5). Continuing work with these compounds is concerned primarily with the develoment of enhanced formulations. Such work requires the use of highly sensitive radioimmunoassay methodology in order to evaluate the relative bioavailabilities of various test formulations. To this end we have developed a high specific activity microscale synthesis of metabolically stable 9,11-tritiated EE and NET.

DISCUSSION

Many syntheses of tritiated 19-norsteroids (6,7,9-12) have been described. Initially 6,7-tritiated (7) analogs were prepared. Since tritium in the 6,7-positions is not metabolically stable (8), more recent work has focused on the preparation of 9,11 (9-11) or 14,15-labelled (12) compounds. The common thread among all such previously reported syntheses was that the high specific activity products which were obtained after the initial reduction of either the Δ^6, $\Delta^{9(11)}$, or Δ^{14} substrates were all diluted with a large amount of carrier. Consequently, all the subsequent intermediates and final products were characterized by low specific activities, ranging from less than 143 mCi/mmol (6) to 4.1 Ci/mmol (12). We can only surmise that intermediates were diluted with carrier in these syntheses because of the difficulty of working at the microscale level, especially in the case of the Birch reduction, which must be used in the NET synthesis. Our objective was to prepare 9,11-tritiated EE and NET at a specific activity which would be suitable for radioimmunoassay, i.e., >40 Ci/mmol.

Our synthesis of EE-[9,11-^{3}H] (**5**), shown in **scheme 1**, began with the reduction of 0.1 mmol $\Delta^{9(11)}$-estradiol 3-methyl ether (**1**) (13) with carrier free tritium gas in the presence of 10% Pd/C. Estradiol-[9,11-^{3}H] 3-methyl ether (**2**) was obtained in 70% radiochemical yield (4.2 Ci) at a specific activty of 56.8 Ci/mmol. Oxidation of (**2**) with Jones reagent (14) gave the 17-ketone (**3**) in quantitative yield after chromatographic purification. Use of Collins reagent (15) gave (**3**) in only 70% yield. The 3-ether function was removed with HBr in acetic acid in 59% yield. This method was superior, in our hands, to deprotection with BBr_3 in microscale reactions. Ethynylation with lithium acetylide•EDA complex (16) gave crude EE-[9,11-^{3}H] (**5**).

Scheme 1: SYNTHESIS OF 17α-ETHYNYL ESTRADIOL-[9,11-^{3}H]



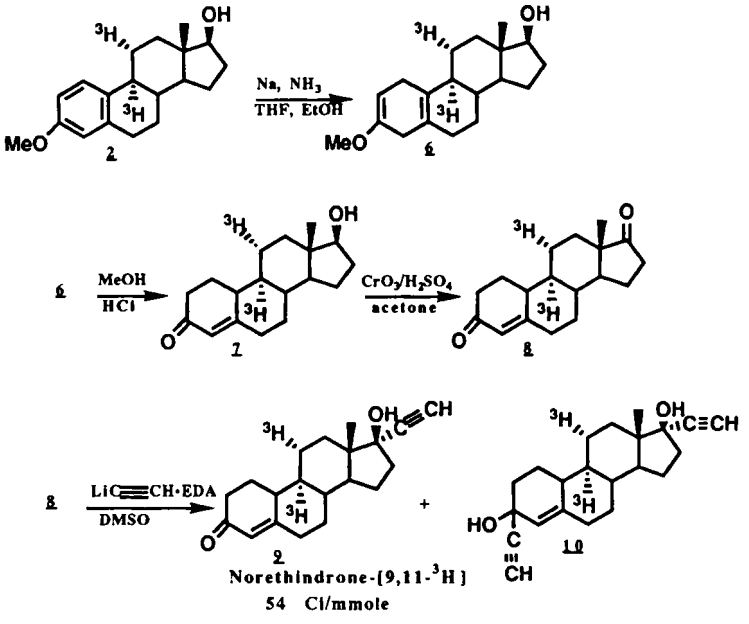
17α-Ethynylestradiol-[9,11-^{3}H]
54 Cis/mmole

Final chromatographic purification required three passes on preparative TLC plates (silica gel, hexane-acetone 3:1) in order to achieve an effective separation of (4) and (5). In this manner, (5) was isolated at a purity of >99%. The specific activity, determined by UV analysis and radioassay, was 54 Ci/mmol.

The synthesis of norethindrone-[9,11-^{3}H] (9) is outlined in Scheme 2.

In previous reports (6,7,9-11) (2) or derivatives of (2) were always diluted with carrier to at least a mmole prior to the Birch reduction step. In one extraordinary case involving the synthesis of norgestrel-[14,15-^{3}H] (17), a carrier free Birch reduction was performed but this was also on a mmole scale. Thus, on a radiochemical scale the Birch reduction was performed, in that instance, on *60.9 Ci of substrate.* This was clearly a potentially very hazardous operation which we were not willing to undertake. We found that the Birch reduction was indeed fickle even on a mmole scale, affording rather variable yields. These problems were magnified in microscale reactions in which we initially obtained yields of 0-30%. Ultimately we were able to increase the yield of (6)

Scheme 2 SYNTHESIS OF 17α-ETHYNYL-19-NORTESTOSTERONE-[9,11-^{3}H] (NORETHINDRONE-[9,11^{3}H])



to 90% *after purification* by carefully controlling various aspects of the reaction. It was essential, for example, to distill the ammonia prior to use in order to remove any traces of iron which are known to interfere with the reduction (18). It was also important to carefully control the temperature at -50° and to quench the reaction by slow addition of methanol and water at -50°. An additional problem was that once the Birch reduction product (6) was isolated it tended to aromatize back to (2) very readily. This process could be slowed down significantly by bubbling all solvents with nitrogen for about one hour prior to use. Even with this precaution we found that the enol ether (6) aromatized back to (2) within two weeks. Hydrolysis of (6) (not more than 24 hrs after isolation) in methanolic HCl gave 19-nortestosterone-[9,11-^{3}H] (7) in 78% yield after chromatographic purification. Jones oxidation afforded a quantitative yield of labelled 19-norandrostenedione (8). Although the 3-enone could be selectively protected in a variety of ways (19) (enol ether, enamine, ketal) in larger scale experiments, we were not able to implement any of these methods in microscale reactions. Since we did not wish to dilute the specific activity of (9), we treated the enedione (8) with lithium acetylide•EDA complex directly. The expected mixture of 3,17-diacetylide (10) and the desired 17-acetylide (9) was separated by column chromatography

(silical gel, eluted with a gradient of hexane to 100% chloroform-heptane (1:1) containing 5% i-propanol). This initial purification was followed by preparative reverse phase TLC using acetone-water (1:1). In this way we obtained norethindrone-[9,11-H^3] (**2**) in modest yield (7.5%) at a purity of >99%. The specific activity, determined by UV analysis and radioassay was 54 Ci/mmol.

EXPERIMENTAL

Carrier free tritium gas was purchased from DuPont NEN Research Products. Cold reagents were purchased from Aldrich Chemical Co. and used without purification. Solvents were HPLC grade. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. UV spectra were obtained using a Hitachi UV-265 spectrophotometer.

Estradiol-[9,11-^{3}H] 3-methyl ether (2)

A 10 mL side-arm septum flask containing a stirring magnet and 10% Pd/C (11.5 mg) was connected to a high vacuum line and evacuated. A solution of $\Delta^{9(11)}$-estradiol 3-methyl ether (**1**) (29 mg; 0.1 mmole), dissolved in ethyl acetate (3 mL), was injected over the catalyst. The stirred reaction mixture was degassed and frozen in liquid nitrogen. Tritium gas (10 Ci; 60 Ci/mmol) was transferred into the reaction flask by means of a Toepler pump. The system was allowed to warm to ambient temperature and then stirred overnight. Excess tritium and most of the solvent was vacuum transferred into a liquid nitrogen cooled waste flask connected to the vacuum line. The residue was taken up in methanol and the catalyst was filtered through a disposable Teflon syringe filter (Millipore). The crude reaction mixture was evaporated to dryness three times from methanol to ensure complete removal of labile radioactivity. The residue, dissolved in ethyl acetate (100 mL), contained 4200 mCi of (**2**) at a purity of 98%. Radio-TLC (silica gel, hexane-ethyl acetate 7:3; toluene-ether 3:1) showed no remaining starting material. The specific activity of (**2**) was determined to be 56.8 Ci/mmole by UV (ethanol, λ_{max} 280 nm, ε=2140) analysis and radioassay.

Estrone-[9,11-^{3}H] 3-methyl ether (3)

A solution of (**2**) (240 mCi; 56.8 Ci/mmol; 4 μmole) in acetone (10 mL) was cooled to 0° and treated with 1 μL of Jones reagent (32 mg CrO_3 dissolved in 27.5 μL of conc. H_2SO_4 and brought to 119 μL with water). Two additional portions of Jones reagent were added (0.5 μL

and 2 μL) over the course of 2 hr at which time radio-TLC (silica gel, hexane-acetone 3:1) showed complete conversion to (3). The reaction mixture was partitioned between water and ethyl acetate, the organic phase was washed sequentially with sodium bicarbonate, water, brine, and the organic phase was dried over sodium sulfate. A quantitative yield of (3) was isolated at a purity of >95% as demonstrated by radio-TLC (silica gel, hexane-actone 3:1; toluene-ethyl acetate 6:4; hexane-ether 3:1).

Estrone-[9,11-^{3}H] (4)

To a solution of (3) (133 mCi, 2.4 μmol) in glacial acetic acid (2 mL) was added 48% HBr (0.5 mL). The reaction, as monitored by TLC (silica gel, hexane-ethyl acetate 7:3), showed complete consumption of starting material after 4 hr. The reaction was partitioned between ethyl acetate and sodium bicarbonate, and the organic phase was washed with water, brine, and dried over sodium sulfate. Purification by column chromatography (silica gel, 0-40% gradient of ethyl acetate-hexane) afforded 78 mCi of pure (4).

17α-Ethynylestradiol-[9,11-^{3}H] (5)

A solution of (4) (90 mCi, 54 Ci/mmole, 1.6 μmole) in DMSO (1 mL) was stirred under nitrogen and treated with a slurry of lithium acetylide•EDA complex (3 mg, 33 μmole). The reaction was stirred overnight at ambient temperature, diluted with water and the product was extracted with ether. Preliminary purification by column chromatography (silica gel, 0-25% gradient of acetone-hexane) was followed by preparative TLC (silica gel, 2000μ, 20x20 cm plates, hexane-acetone 3:1) to yield 5.76 mCi of pure (5) (radio-TLC silica gel; hexane-acetone 3:1, hexane ethyl acetate 7:3). The specific activity was determined by UV analysis (ethanol, λ_{max} 280nm, ε=2040) and radioassay to be 54 Ci/mmole.

1,4-Dihydroestradiol-[9,11-^{3}H] 3-methyl ether (6)

Liquid ammonia, previously collected into a 100 mL three neck flask at -78°, was distilled under a gentle stream of nitrogen into a similar flask cooled to -78° and equipped with a dry ice condenser and rubber septum. When approximately 10 mL was collected, a solution of estradiol-[9,11-^{3}H] 3-methyl ether (2) (891 mCi, 56.8 Ci/mmol, 0.0165 mmol) in THF (1.5 mL) containing 2 drops of ethanol was added by syringe. Small chunks of clean sodium metal were prepared as follows. A large piece of sodium was cut under toluene to expose a clean surface, then transferred to heptane where tiny

pieces <2mm^2 were cut and stored. Three pieces of sodium were added to the reaction mixture at -50° under nitrogen on glass "whiskers" (prepared by drawing out Pasteur pipettes) one at a time, allowing the initial blue color to dissipate before the next piece was added until eventually, the blue color persisted. After 4 hr. methanol (0.2 mL) was added slowly, followed by water (3 mL) and ether (10 mL). Excess ammonia was evaporated overnight under a slow stream of nitrogen. The reaction was partitioned between ether and water which had been previously purged with nitrogen. The organic phase was washed with water, brine, and dried over sodium sulfate. A total of 800 mCi (90%) of (6) was isolated. The purity determined by radio-TLC (hexane-ether 2:1) was >95%.

19-Nortestosterone-[9,11-^{3}H] (7)

To a solution of Birch reduction product (6) (200 mCi, 3.6 μmol) in methanol (4 mL) under nitrogen was added 3N HCl (0.2 mL). After stirring at 60° for 30 min radio-TLC (hexane-ethyl acetate 1:1) showed complete conversion to (7). The reaction was partitioned between ether and water. The organic phase was washed with water, sodium bicarbonate, brine, and dried over sodium sulfate. Purification by column chromatography (silica gel, ethyl acetate-hexane 3:7) afforded 155 mCi of pure (7).

19-Norandrostene-[9,11-^{3}H]-3,17 dione (8)

To an acetone (10 mL) solution of (7) (155 mCi, 2.7 μmol) at 0° was added a solution of Jones reagent (0.5 μL) (32 mg CrO_3 dissolved in 27.5 mL H_2SO_4 and brought to 120 μL with water). An additional 1.5 μL of Jones reagent was added in two portions over 2 hr at which time the oxidation was complete as judged by radio-TLC (hexane-ethyl acetate 7:3). The reaction was partitioned between water and ethyl acetate and the organic phase was washed with water sodium bicarbonate, brine, and dried over sodium sulfate. The product (8) was isolated in quantitative yield.

17α-Ethynyl-19-nortestosterone-[9,11-^{3}], (Norethindrone-[9,11-^{3}H] (9)

To a solution of (8) (155 mCi, 2.7 μmol) in dry THF (1 mL) was added lithium acetylide•EDA complex (1 mg, 11 μmol). The reaction was stirred at ambient temperature and carefully monitored by radio-TLC (silica gel, hexane-ethyl acetate 7:3). Over the course of 4 days an additional 8 mg of lithium acetylide•EDA complex plus DMSO (0.2 mL) (to improve solubility) were added. Formation of (9) reached a

maximum of about 8% and the reaction was quenched by slow addition of water (1 mL), then dilute HCl (1 mL). The reaction was partitioned between water and sodium bicarbonate. The organic phase was washed with water, brine, and dried over sodium sulfate. Initial purification by preparative TLC (silica gel, hexane-ethyl acetate 7:3) gave 12 mCi of 90% pure (**9**) plus the 3-ethynyl compound (**10**) and 100 mCi of unreacted starting material (**8**). The latter was recycled by reaction in THF (1 mL)/DMSO (0.2 mL) with lithium acetylide•EDA complex (8 mg) added over 3 days at ambient temperature. Workup as described above provided an additional 6 mCi of (**9**) at a purity of 60%. The two batches of (**9**) were combined and purified by column chromatography (silica gel, eluted with a gradient of hexane to 5% i-propanol in chloroform-heptane 1:1). Final purification was effected by preparative TLC (RPC-18, acetone-water 1:1) to yield 11.5 mCi of (**9**) at a purity of >99%. The specific activity was determined to be 54 Ci/mmol by UV (ethanol, λ_{max} 239 nm, ε = 18915) and radioassay.

REFERENCES

1. Birch, A.J., and Smith, H. - *Quart. Rev.* 12: 17 (1958)
2. Djerassi, C., Miramontes, L., and Rosenkranz, G. - *J. Am. Chem. Soc.* 75: 4440 (1953)
3. Edgren, R.A., *Clinical Use of Sex Steroids*, Ed. Givens, J.R., Yearbook Medical Publishers, Chicago, 1, (1979)
4. Djerassi, C., Miramontes, L., Rosenkranz, G., and Sondheimer, F. - *J. Am. Chem. Soc.* 76: 4092 (1954)
5. Saperstein, S., Edgren, R.A., Lee, GJ-L., Jung, D., Fratis, A., Kushinsky, S., Mroszczak, E., and Dorr, A. - *Contraception* 40: 581 (1989)
6. Rao, P.N. - *Steroids* 18: 219 (1971)
7. Merrill, E.J., and Vernice, G.G. - *J. Labelled Compds. Radiopharm.* VI: 266 (1970)
8. Knuppen, R., Haupt, O., and Breurer, H. - *Biochem. J.* 101: 397 (1966)
9. Wagner, H., Romer, J., and Kasch, H. - *J. Labelled Compds. Radiopharm.* XVIII: 1611 (1981)
10. Ponsold, K., Romer, J., and Wagner, H. - *J. Labelled Compds. Radiopharm.* X: 533 (1974)
11. Romer, J. and Wagner, H., - *Radiochem. Radioanal. Letters* 25: 255 (1976)
12. Wagner, H., Romer, J., Huber, M., Ponsold, K. - *J. Labelled Compds. Radiopharm.* XVII: 317 (1980)

13. Brown, W., Findley, J.W.A., and Turner, A.B. - *Chem. Comm.*: 10 (1968)
14. Bowden, W., Heilbron, I.M., Jones, E.R.H., and Weedon, B.C.L. - *J. Chem. Soc.*: 39 (1946)
15. Collins, J.C., and Hess, W.W. - *Org. Synth.* 52: 5 (1972)
16. Beumel, O.F., and Harris, R.F. - *J. Org. Chem.* 29: 1872 (1964)
17. Wagner, H., and Romer, J. - *J. Labelled Compds. Radiopharm.* XXV: 645 (1988)
18. Dryden, H.L., Weller, G.M., Burton, R.R., and Cella, J.A. - *J. Org. Chem.* 26: 3237 (1961)
19. Gardi, R., and Ercoli, A. - Organic Reactions in Steroid Chemistry, Vol. 1, Ed. Fried, J. and Edwards, J.A., Van Norstrand Reinhold, New York, 375 (1972)