PREPARATION OF 17β-HYDROXY-[1ξ,2ξ-$^3H_2$]4,6-ANDROSTADIEN-3-ONE

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SUMMARY

17β-Hydroxy-[1ξ,2ξ-$^3H_2$]4,6-androstadien-3-one was prepared by the selective reduction of 17β-hydroxy-1,4,6-androstatrien-3-one in benzene with tritium over 5% palladium on carbon. After purification by thin layer chromatography and high pressure liquid chromatography, the specific activity was at least 37 Ci/mmol, and the radiochemical purity was greater than 96%.

INTRODUCTION

Androgen binding protein (ABP) is present in the supernatant (cytosol) of homogenized epididymal tissue of the rat (1,2), rabbit (3,4), and human (5). Studies on the distribution and fate of ABP necessitated the preparation of tritium labeled ABP. Photoaffinity labeling of ABP with a tritium labeled androgen of specific activity greater than 10 Ci/mmol was chosen as the best

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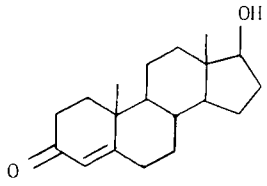
method to achieve this goal. No attempt was made to use tritium labeled 17β-hydroxy-4-androsten-3-one (testosterone) (1) as a photoaffinity reagent due to



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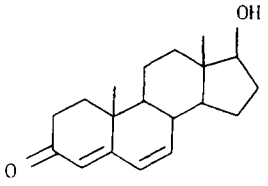

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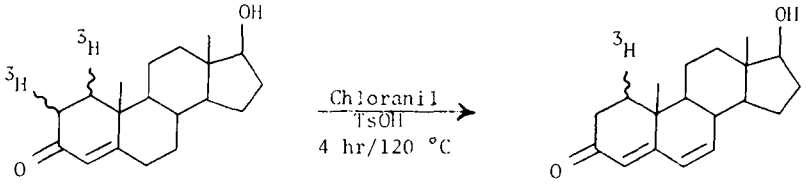
the proximity of its n → π* absorption band (λ_{max} 305 nm) to the cut-off of the Pyrex filter (10% transmission at 305 nm) used to protect the protein chromophores during irradiation. The successful photoinactivation of the binding sites of rat ABP using 17β-hydroxy-4,6-androstadien-3-one (Δ^6-testosterone) (2) (6) prompted us to prepare 2 with a tritium label.

RESULTS AND DISCUSSION

Preliminary experiments with as little as 6 μg of unlabelled testosterone (1) indicated that dehydrogenation with chloranil catalyzed with p-toluenesulfonic acid (TsOH) in o-xylene at 120 °C for 4 hr (7) gave Δ^6-testosterone (2) in a reasonable yield. Thus, 6.4 μg of 17β-hydroxy-[1ξ,2ξ-$^3H_2$]4-androsten-3-one ([$^3H_2$]testosterone) (3) of specific activity 15.4 mCi/mmol (Scheme I) gave

Scheme I



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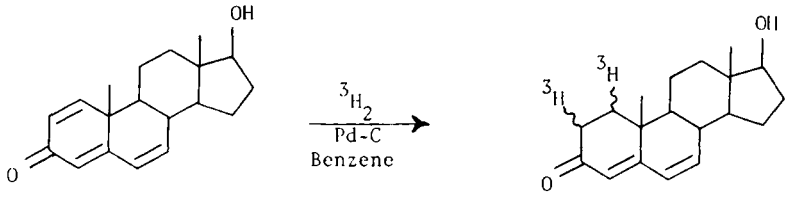
17β-hydroxy-[1ξ-^3H]4,6-androstadien-3-one ([^3H]Δ^6-testosterone) (4) of specific activity 7.7 mCi/mmol in 26% yield, the tritium atom at C-2 being lost during the preparation. When 6.6 μg of 3 of specific activity 44 Ci/mmol was treated

in the same way, no spot corresponding to 4 was visible on a thin layer chromatography (tlc) plate. The combination of high specific activity and drastic conditions promoted radiolytic decay of the steroid (8,9).

The selective catalytic reduction of 17β-hydroxy-1,4,6-androstatrien-3-one ($\Delta^{1,6}$-testosterone) (5) (10) with tritium (Scheme II) was chosen as a milder

Scheme II



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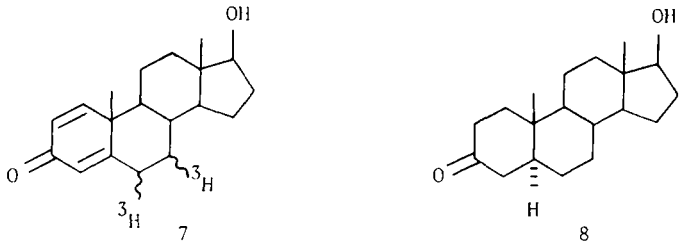
method for the preparation of 17β-hydroxy-[1ξ,2ξ-$^{3}H_2$]4,6-androstadien-3-one ([$^{3}H_2$]Δ^{6}-testosterone) (6). The amount of 6 produced from such a reaction, compared to 17β-hydroxy-[6ξ,7ξ-$^{3}H_2$]1,4-androstadien-3-one ([$^{3}H_2$]Δ^{1}-testosterone) (7),



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depends on the relative rates of reduction of the 1,2- and 6,7-double bonds of 5. Djerassi and Gutzwiller (11) partially reduced 1,4- and 4,6-androstadiene-3,-17-dione to the Δ^{4}-3-ketone but did not report the relative rates of these reductions. No partial reduction of a trienone like 5 has been reported.

Before partial reduction of $\Delta^{1,6}$-testosterone (5) was attempted, a mixture of 5 and unlabeled possible reduction products of 5, testosterone (1), Δ^{6}-testosterone (2), Δ^{1}-testosterone (unlabeled 7), and 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) (8), was subjected to tlc (Table I). As can be seen in Table I, Δ^{6}-testosterone (2) is easily separated from all components except

testosterone (1). However, 1 and 2 are readily separated by high pressure liquid chromatography (hplc).

In preliminary studies, $\Delta^{1,6}$-testosterone (5) in dioxane was reduced with hydrogen at room temperature and atmospheric pressure over 10% palladium on carbon (12) until one equivalent of hydrogen had reacted. Analysis of the mixture of products by hplc showed it to contain only 2% of Δ^{6}-testosterone (2), the

Table I. Thin Layer Chromatographic Data for Unlabeled Androgens

Compound	R_f[a]
Testosterone (1)	0.35
Δ^{6}-Testosterone (2)	0.30
$\Delta^{1,6}$-Testosterone (5)	0.22
Δ^{1}-Testosterone (7[b])	0.22
5α-Dihydrotestosterone (8)	0.47

[a] Tlc on 60F-254 silica gel with hexane-ethyl acetate-acetone (60:35:5) as developing solvent. [b] Unlabeled.

bulk of the material being Δ^{1}-testosterone (unlabeled 7). When the reduction was done in benzene, 2 comprised 14% of the reduction product.

For preparation of [$^{3}H_2$]Δ^{6}-testosterone (6), purified $\Delta^{1,6}$-testosterone (5) was sent to New England Nuclear, Boston, Mass. 02118, for reduction, according to our procedure, in benzene with tritium over palladium on carbon. The resultant crude reaction mixture was returned as a solution in benzene-ethanol. The bulk of the radioactive by-products was removed by tlc, and 6 with specific activity of at least 37 Ci/mmol was isolated by hplc. The identity of 6 was confirmed by co-crystallization with unlabelled Δ^{6}-testosterone (2), and the radiochemical purity of 6 was found to be greater than 96% by tlc.

EXPERIMENTAL

Radioactive samples were counted in a toluene-based scintillation fluid using a Beckman (LS-230) scintillation counter. Silica gel (60F-254) with hexane-ethyl acetate-acetone (60:35:5) as the developing solvent was used for thin layer chromatography (tlc). The compounds were visualized using the long wavelength UV light from a Mineralight UVS·25 lamp. A Waters Associates, Inc. μPorasil column (0.4 x 60 cm) was used in conjunction with a Waters Associates, Inc. high pressure liquid chromatograph (ALC-202 with Valco injector and differential UV detector, λ_{max} 254 nm) for high pressure liquid chromatography (hplc) with chloroform-ethanol as the eluting solvent. The chloroform (Fisher Certified) was fractionally distilled and to 1 L of distillate was added 1 mL of absolute ethanol. At a flow rate of 1.5 mL/min, the hplc retention times for testosterone (1) and Δ^6-testosterone (2) were 18.8 and 20.2 min, respectively. The amount of a particular steroid in the hplc effluent was determined by calibration of the hplc detector peak areas (height x width at half height) by the hplc of known amounts of the respective steroids. Since the amount of a compound eluted from the column during these calibration operations may be slightly less than that charged, the calculated specific activities for the labeled compounds are minimum values. Unless otherwise noted, solvent evaporations to dryness were done with a stream of dry nitrogen.

17β-Hydroxy-[1ξ-^{3}H]4,6-androstadien-3-one (4). A solution of 100 mg of p-toluenesulfonic acid (TsOH) in 150 mL of toluene was boiled until the volume reached 100 mL (13), and 45 μL of this solution containing 45 μg (260 nmol) of TsOH was transfered to a glass tube and evaporated. A solution of 65 μL of chloroform containing 6.4 μg (22 nmol) of [$^3H_2$]testosterone (3) of specific activity 15.4 mCi/mmol in another glass tube was also evaporated. To this latter tube was added 180 μL of o-xylene containing 850 μg (3.46 μmol) of chloranil, freshly recrystallized from toluene. This solution was transfered to the tube containing the TsOH. The tube was sealed, and its contents were heated at 120 °C for 4 hr. After heating, the solution was evaporated, and the residue was dissolved in 1 mL of chloroform. The chloroform solution was spotted on a

tlc plate, and the plate was developed two-dimensionally. The silica gel containing [^{3}H]Δ^6-testosterone (4) was removed from the plate and suspended overnight in 2 mL of chloroform. The mixture was filtered through cotton, and the filtrate was evaporated. The residue was subjected to hplc, and the effluent containing 4 was evaporated. Two repetitions of the hplc procedure gave 1.7 µg (27%) of 4 of specific activity 7.7 mCi/mmol.

17β-Hydroxy-[1ξ,2ξ-^{3}H$_2$]4,6-androstadien-3-one (6). Chromatography of 100 mg of $\Delta^{1,6}$-testosterone (5) on a 2-in diameter column (14) of silica gel (50 g, GF-254) using methylene chloride-acetone (93:7) as eluting solvent gave pure 5, mp 153-154 °C [lit. (10) mp 156-158 °C]. A sample of 5 was sent to New England Nuclear for reduction with tritium.

The procedure used by New England Nuclear was as follows: 56.8 mg (200 µmol) of 3 was dissolved in 4 mL of benzene to which was added 5 mg of 5% palladium on carbon. This mixture was stirred under an atmosphere of tritium during which an uptake of 3.4 cm^3 (140 µmol at 25 °C and 1 atm) of tritium was noted. Labile tritium was removed at reduced pressure using methylene chloride-methanol (1:1) as solvent. After removal of the catalyst by filtration, the reaction mixture was evaporated to dryness at reduced pressure. The residue, 6 Ci, was dissolved in benzene-ethanol (9:1), and 5 mCi portions of this solution were returned for purification.

One 5 mCi portion of the reduction product was evaporated and the residue was subjected to preparative tlc. The tlc plate was developed twice, and the silica gel containing [^{3}H$_2$]Δ^6-testosterone (6) was removed and suspended in 1 mL of chloroform for 1 hr. The mixture was filtered through cotton, and the filtrate was evaporated. The residue was dissolved in 150 µL of hplc solvent and injected into the hplc column. The effluent containing 6 was collected, evaporated, redissolved in hplc solvent and rechromatographed. This second effluent containing 7.2 µg of 6 (15%) was evaporated, and the residue, specific activity 36.9 Ci/mmol, was dissolved in 7 mL (1 µg/mL) of absolute ethanol and stored at -15 °C.

A second 5 mCi portion of the reduction mixture and the same purification scheme gave 9.3 µg of 6 (19%), specific activity 38.5 Ci/mmol.

Co-crystallization of 6 with unlabelled Δ^{6}-testosterone (2) from acetone-water, then from ethanol-water and again from acetone-water gave 6 of unchanged specific activity, 13,815 ± 90 dpm/mg.

A mixture of 2 and 6 was subjected to tlc. After development, the plate was divided into sixteen 10-mm bands with one band centered on the spot corresponding to Δ^{6}-testosterone (2). Each band was removed, and the radioactivity in each band was determined. Greater than 96% of the total radioactivity was found in the band centered on the spot corresponding to 2.

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REFERENCES

1. French, F.S. and Ritzén, E.M. - Endocrinology 93: 88 (1973)
2. Hansson, V., Djøseland, O., Reusch, E., Attramadal, A., and Torgersen, O. - Steroids 21: 457 (1973)
3. Danzo, B.J., Eller, B.C. and Orgebin-Crist, M.-C. - Steroids 24: 107 (1974)
4. Danzo, B.J., Orgebin-Crist, M.-C. and Toft, D.O. - Endocrinology 92: 310 (1973)
5. Hsu, A.-F. and Troen, P. - J. Clin. Invest. 61: 1611 (1978)
6. Taylor, Jr., C.A., Smith, H.E. and Danzo, B.J. - submitted
7. Atwater, N.W., Bible, Jr., R.H., Brown, E.A., Burtner, R.R., Mihina, J.S., Nysted, L.N. and Sollman, P.B. - J. Org. Chem. 26: 3077 (1961)
8. Geller, L.E. and Silberman, N. - J. Label. Compounds 5: 66 (1969)
9. Merill, E.J. and Vernice, G.G. - J. Label. Compounds 9: 769 (1973)
10. Caspi, E., Cullen, E. and Grover, P.K. - J. Chem. Soc.: 212 (1963)
11. Djerassi, C. and Gutzwiller, J. - J. Am. Chem. Soc. 88: 4537 (1966)
12. Ringold, H.J., Hayano, M. and Stefanovic, V. - J. Biol. Chem. 238: 1960 (1963)

13. Perrin, D.D., Armarego, W.L.F. and Perrin, D.R. - Purification of Laboratory Chemicals, Pergamon, New York, N.Y., 1966, p 268

14. Hunt, B.J. and Rigby, W. - Chem. and Ind. (London): 1868 (1967)