PREPARATION AND PURIFICATION OF 1,2-^{3}H-11ß-HYDROXYTESTOSTERONE*

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In vitro studies of the metabolism of 4-^{14}C-testosterone (17ß-hydroxy-androsta-4-ene-3-one) with normal human adrenal homogenate from this laboratory have demonstrated the efficient conversion of testosterone to 11ß-hydroxytestosterone (11ß,17ß-dihydroxy-androsta-4-ene-3-one).[1] Since 11ß-hydroxytestosterone is the major metabolite of testosterone in the adrenal tissue, it warrants further study. Thus, a need arises for radioactive 11ß-hydroxy-testosterone for use as a tracer in metabolic experiments.

11ß-Hydroxytestosterone has been prepared previously by Mancera et al.,[2] using adrenosterone (androsta-4-ene-3,11,17-trione) as the starting compound, and by Herr and Heyl,[3] from 11α-hydroxy-androsta-4-ene-3,17-dione.

In the present communication, we wish to report a rapid and convenient method for the preparation and purification of 1,2-^{3}H-11ß-hydroxytestosterone from commercially available 1,2-^{3}H-11ß-hydroxyandrostenedione** (1,2-^{3}H-11ß-hydroxy-androsta-4-ene-3,17-dione). The 1,2-^{3}H-11ß-hydroxytestosterone contains 75% stable tritium and 25% labile tritium as shown by alkaline equilibration.

Experimental

(A) Preparation of non-radioactive 11ß-hydroxytestosterone

Androsta-4-ene-3,11ß,17ß-triol: 11ß-Hydroxy-androstenedione (I)[+] (1.123 g.) was dissolved in 50 ml. of methanol. The methanol solution was chilled to 0-5°, and sodium borohydride (0.5 g.) was added. The mixture was agitated by means of a magnetic stirrer for one hour, and then gradually warmed to room temperature. The reaction was allowed to continue at room temperature for an additional two hours. At the end of the reaction period, the mixture was poured into one liter of ice-cold water containing 10 ml. of acetone. The mixture was allowed to stand at 0° overnight, when the androsta-4-ene-3,11,17-triol (II) formed needle-shaped crystals. It was collected, and recrystallized once from ethanol. The crystalline product weighed 0.63 g. The analytical sample had a m.p. of 206-210°. Partition coefficient[4] (K) = 3.9.

Anal. Calculated for $C_{19}H_{30}O_3$: C,74.47; H,9.87. Found: C,73.65; H,9.22.

11ß-Hydroxy-3,17-diacetoxy-androsta-4-ene: Compound II (0.199 g.) was dissolved in 2-3 ml. of freshly distilled pyridine and treated with 1.5 ml. of acetic anhydride at room temperature for 24 hours. The reaction mixture was then poured into 25 ml. of ice-cold water. The white solid product was collected and washed with water. After the residue was thoroughly dried, it was recrystallized from ether-hexane. The crystalline material weighed 0.220 g. and had a m.p. of 154-155°.

Anal. Calculated for $C_{23}H_{34}O_5$: C,70.74; H,8.78. Found: C,71.03; H,9.26.

11ß-Hydroxytestosterone: Compound II (0.079 g.) was dissolved in 50 ml. of chloroform, and freshly prepared manganese dioxide[5] (2 g.) was added. The mixture was stirred, and the reaction was allowed to continue for 16 hours at room temperature. The solid material was then removed by filtration, and the clear filtrate was evaporated to dryness under reduced pressure. The crystalline residue, 11ß-hydroxytestosterone (III), was recrystallized from acetone-hexane. The flat needle-shaped crystals had a m.p. of 230-233°. λ_{max}^{EtOH} 242mμ, log ε 4.19, K = 1.13.

Anal. Calculated for $C_{19}H_{28}O_3$: C,74.97; H,9.27. Found: C,74.65; H,9.22.

Literature: m.p. 232-234°, λ_{max} = 242mμ, ε = 16,600[2]; m.p. 233-235°, λ_{max}^{EtOH} = 242mμ, log ε = 4.19[3].

(B) Preparation of 1,2-^{3}H-11ß-hydroxytestosterone:

Carrier-free 1,2-^{3}H-11ß-hydroxy-androstenedione (20 μc) was dissolved in 10 ml. of methanol. After cooling to 0°, 100 mg. of sodium borohydride was added. The reaction was carried out as described previously. After pouring the reaction mixture into 100 ml. of water, the product was recovered by several extractions with diethyl ether. The combined extracts were evaporated to dryness under a gentle stream of nitrogen. The dried residue was purified by counter-current distribution in a 70% aqueous methanol/40% chloroform, 60% carbon tetrachloride solvent system with 50 transfers. The distribution curve (Fig. 1, · - ·) showed a minor peak at tubes #4-20 (K = 0.32), which was due to unchanged 11ß-hydroxy-androstenedione. The major peak was located between tubes #30-46, and showed a partition coefficient (K = 3.9) which was identical to that of androsta-4-ene-3,11ß,17ß-triol (II).

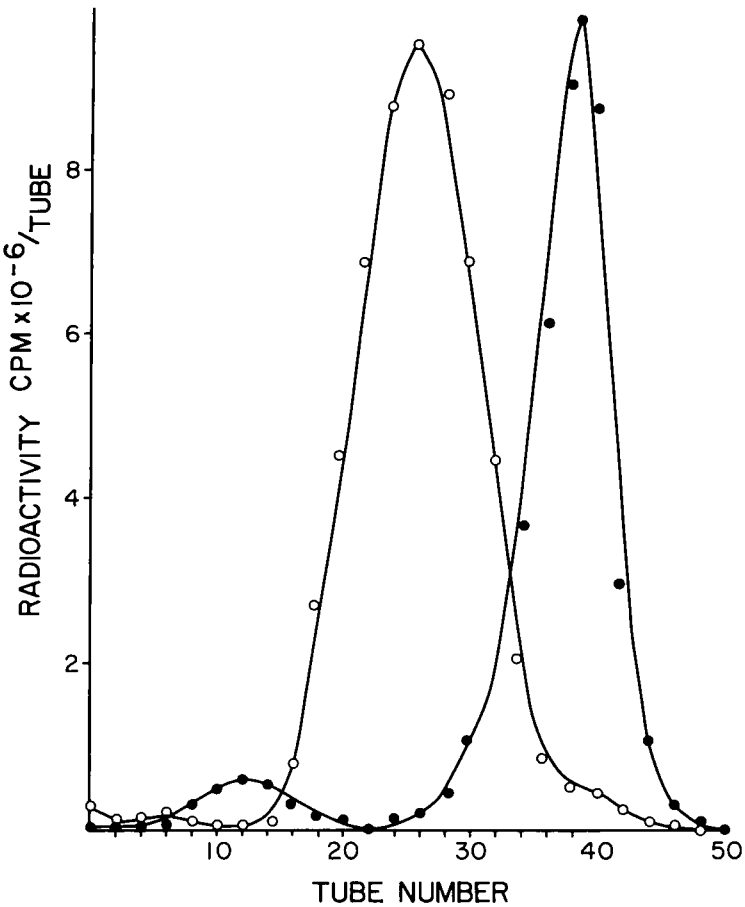

Fig. 1 Counter-current distributions. · - ·, Androsta-4-ene-3,11ß,17ß-triol; ∘ - ∘, 11ß-Hydroxytestosterone.

The material from tubes #30-46 was pooled. Then the residue was dissolved in 10 ml. of chloroform, and oxidized with 20 mg. of manganese dioxide at room temperature for 3 hours. After the workup, the product was purified by counter-current distribution in the same manner as described above. The distribution curve (Fig. 1, ∘ - ∘) showed that the majority of the radiochemical was located in tubes #18-34. The partition coefficient of the radiochemical (K = 1.13) was identical to that of 11ß-hydroxytestosterone (III). Successive recrystallizations of an aliquot of the

radiochemical with added 11ß-hydroxytestosterone as carrier showed no decrease in specific activity ($^{CPM}/_{mg.}$:7,661; 7,961; 7,736.)

(C) Chemical stability of the label:

It is assumed that 1,2-tritiated Δ^4-3-keto steroids were prepared by partial catalytic hydrogenation of $\Delta^{1,4}$-3-keto steroids with tritium gas over a suitable catalyst. The stereochemical course of the reaction usually involves the cis addition,[6] thus reduction of steroid olefinic bonds in ring A occurs predominately on the alpha side of the molecule. Consequently, tritiation of Δ^1 bond of $\Delta^{1,4}$-steroid resulted in the labelling at 1α and 2α positions.[7] Recently, Brodie et al.[8] reported that the attack at C-1 is predominately beta when 17ß-hydroxy-androsta-1,4-dien-3-one was reduced with tritium gas over palladium. The 1,2-tritiated steroid of this type was shown to be chemically stable by Osinski.[9] A sample of purified 1,2-^{3}H-11ß-hydroxytestosterone (4.25×10^6 cpm) was refluxed with 50 ml. of 1N KOH in 75% aqueous methanol for 1 hour under nitrogen. After cooling to room temperature, 250 ml. of diethyl ether was added to the alkali solution. The mixture was washed with water till the final washing was neutral to red litmus paper. The solvent was then evaporated under nitrogen and the residue was submitted to counter-current distribution. The tubes contained 11ß-hydroxytestosterone were pooled and the total radioactivity recovered was 3.21×10^6 cpm (75% recovery).

Footnotes

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