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Catalytic reduction of 3β-hydroxyandrost-4-ene-17-one with tritium.

I. The distribution and configuration of label in 3β-hydroxy-5α-androstane-17-one (1)

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SUMMARY

*Catalytic reduction of 3β-hydroxyandrost-4-ene-17-one (I) with tritium gas gave a mixture of 3β-hydroxy-5α-androstane-17-one (II) and 3β-hydroxy-5α-androstane-17-one (III). Oxidation of compound II followed by alkaline equilibration resulted in 37 % loss of label from position 4. 5α-Androstane-3,17-dione (IV*a*) was converted via the 2,4-dibromide to androst-4-ene-3,17-dione (VIII*a*) with 43 % loss of radioactivity from position 5. Chloranil dehydrogenation and alkaline equilibration experiments have shown that the additional label was at C-6α. The distribution and configuration of label in compound II was found to be 37 % 4α, 43 % 5α and 20 % 6α.*

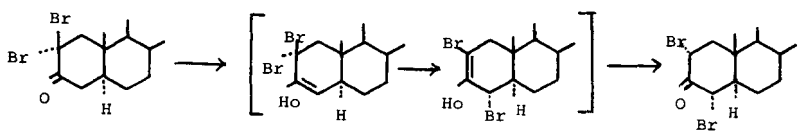
In order to investigate the stereochemistry of Δ^4-dehydrogenation of 5α- and 5β-saturated-3-ketosteroids by microbial Δ^4-dehydrogenases, we wished to prepare labelled 5α-androstane-3,17-dione and 5β-androstane-3,17-dione at the 4 and 5 positions. Catalytic reduction of 3β-hydroxyandrost-4-ene-17-one with tritium gas was carried out by New England Nuclear hoping to obtain 3β-hydroxy-5α-androstane-17-one-4α, 5α-^{3}H and 3β-hydroxy-5β-androstane-17-one-4β, 5β-^{3}H which could be converted to their corresponding 5α- and 5β-diones. This paper provides proof for the distribution and orientation of tritium in 5α-androstane-3,17-dione.

Catalytic reduction of 3β-hydroxyandrost-4-ene-17-one (I) with carrier free tritium using platinium oxide in ethyl acetate was performed by the New England Nuclear Corporation under the conditions described by Shoppee *et al.* [2]. The reaction mixture was resolved by paper chromatography using

the ligroin-propylene glycol system. A radioscan of the chromatogram showed a peak corresponding in mobility to 3β-hydroxy-5α-androstane-17-one (II) containing about 32 % of the radioactivity, while about 50 % of the remaining radioactivity occurred in the area corresponding to 3β-hydroxy-5β-androstane-17-one (III).

The radioactive area corresponding to II was diluted with carrier 3β-hydroxy-5α-androstane-17-one and crystallized to constant specific activity. Equilibration with base resulted in no loss of tritium indicating no tritium incorporation occurred at C-16 during catalytic reduction. Oxidation of compound II with chromium trioxide in pyridine [3] gave 5α-androstane-3,17-dione (IV*a*) virtually without loss of tritium. Alkaline equilibration of compound IV*a* resulted in 37 % loss of radioactivity probably from C-2 and C-4 (Compound IV*b*).

Bromination of IV*a* [4] to 2,2-dibromo-5α-androstane-3,17-dione (V) followed by isomerization with warm acetic acid [4] gave 2,4-dibromo-5α-androstane-3,17-dione (VI), possibly by allylic rearrangement in the enolic form :



V VI

There was no loss of tritium in the dibromination and isomerization steps showing that no tritium was incorporated into position 2 during catalytic reduction. The fact that there was an appreciable loss of tritium (from C-4) after alkaline equilibration of 5α-androstane-3,17-dione (IV*a*) indicates that the isomerization step proceeded stereospecifically, resulting in no loss of tritium from carbon 4. Since catalytic reduction usually takes place by *cis* addition, it was expected that 5α-androstane-3,17-dione would be labelled in the 4α-position. Thus, the lack of tritium loss during the isomerization of 2,2-dibromo compound (V) to the 2,4-dibromo compound (VI) is not inconceivable since the loss of the 4β-hydrogen (axial) rather than the 4α-hydrogen (equatorial) is favored by more efficient orbital overlap in the transition state.

Treatment of compound VI with sodium iodide in acetone [5] gave a mixture of 2-iodoandrost-4-ene-3,17-dione (VII) and androst-4-ene-3,17-dione (VIII*a*), both having the same radioactivity (57 % of IV*a*). In addition to compounds VII and VIII*a*, a small amount (10 %) of 5α-androstane-3,17-dione (IV*c*) was obtained having a specific activity 28 % higher than starting material IV*a*. This isotope enrichment is probably due to a primary isotope effect, whereby the untritiated 2α, 4α-dibromo compound reacted faster than the tritiated dibromide causing isotope enrichment of unreacted dibromide

which then reacted at a slower rate to form, by debromination rather than dehydrobromination, 5α-androstane-3,17-dione IV*c*.

Deiodination of compound VII with zinc and acetic acid [5] gave androst-4-ene-3,17-dione (VIII*b*) virtually with no loss of radioactivity.

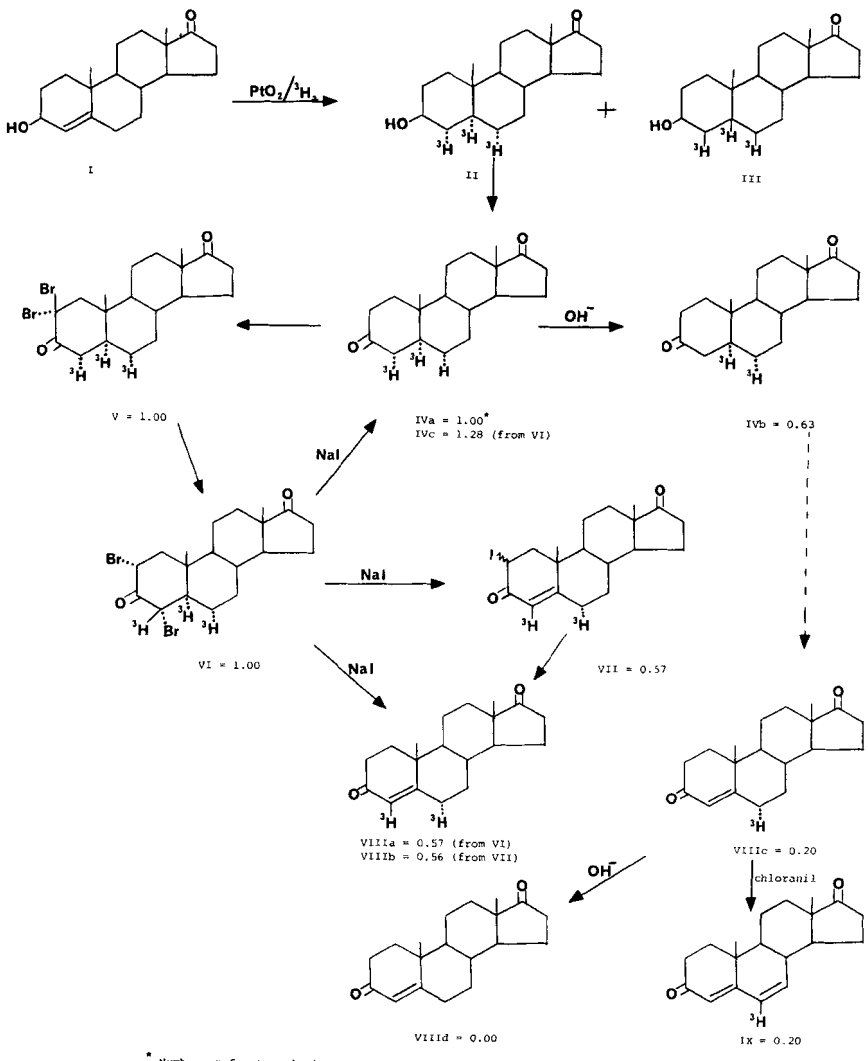
To establish the amount of tritium at position 6, compound IV*b* was converted via the 2,4-dibromide and 2-iodoandrostenedione to androst-4-ene-3,17-dione (VIII*c*) having a specific activity 20 % that of starting material IV*a*. Alkaline equilibration of compound VIII*c* resulted in complete loss of radioactivity (Compound VIII*d*). The orientation of tritium at position six was verified using chloranil (2,3,5,6-tetrachloroquinone) which has been shown [6] to introduce a C-6,7-double bond into Δ^4-3-ketosteroids by a stereospecific *trans* diaxial elimination of the 6β and 7α hydrogens. When compound VIII*c* was dehydrogenated to androsta-4,6-diene-3,17-dione (IX), there was no loss of activity indicating that the label at position 6 was only at the 6α position.

It is clear from the above findings that the catalytic reduction of 3β-hydroxyandrost-4-ene-17-one to 3β-hydroxy-5α-androstane-17-one proceeds by *cis* saturation of the double bond. The introduction of label at C-6 during the course of platinium-catalyzed hydrogenation appears to come preferentially from the α-side, either by an exchange mechanism mediated by the catalyst analogous to the mechanism proposed by Fukushima and Gallagher [7] for the catalytic reduction of cholesterol acetate, or by a mechanism involving isomerization of the 4,5-double bond to the 5,6-double bond which may then undergo saturation from the α-side, thus introducing a label at the 6α-position.

It is therefore evident from this series of experiments that catalytic reduction of 3β-hydroxyandrost-4-ene-17-one using platinium catalyst over tritium gas gives 3β-hydroxy-5α-androstane-17-one in which the distribution of label is 37 % at 4α, 43 % at 5α and 20 % at 6α.

EXPERIMENTAL.

Silica gel G was obtained from Brinkman Instruments, Inc. All solvents and inorganic chemicals were reagent grade. Radioactivities were determined on a Packard Model 3375 liquid scintillation counter on weighed amounts of steroids (5-15 mg) in 10 ml of scintillation fluid, which consisted of 4 g of PPO (2,5-diphenyloxazole) and 50 mg POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] in 1 kg of toluene. Product and reactant were counted in the same counting period in triplicate using varying weights of material. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer infracord in nujol unless otherwise stated. Ultraviolet spectra were determined in 95 % ethanol on a Cary Model 14 recording spectrophotometer. Abbreviations : mp = melting point; sp. act. = specific activity; ir = infrared spectrum; uv = ultraviolet spectrum.



* Numbers refer to relative specific activities of reactants and products.

1. — *3β-Hydroxy-5α-androstane-17-one-4α, 5α, 6α-^3H (II).*

3β-Hydroxyandrost-4-ene-17-one was reduced with carrier-free tritium by the New England Nuclear Corporation under the conditions described by Shoppee *et al.* [1]. A portion of the reaction mixture was chromatographed on paper in ligroin-propylene glycol for 12 hours. A radioscan showed a peak corresponding in mobility to 3β-hydroxy-5α-androstane-17-one containing about 32 % of the radioactivity. This zone was eluted with acetone, diluted with 1.62 g of authentic material and was recrystallized to constant specific activity (3.36×10^5 cpm/μmole).

2. — *5α-Androstane-3,17-dione-4α, 5α, 6α-^3H (IVa) and 5α-androstane-3,17-dione-5α-6α-^3H (IVb).*

A solution of 1.62 g of compound II in 15 ml of pyridine was combined with 1.55 g of chromium trioxide in 15 ml of pyridine and allowed to stand at room temperature overnight. The reaction mixture was poured into water and extracted three times with ethyl ether. The combined organic solution was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. Recrystallization of the residue from ethyl acetate-petroleum ether gave 1.31 g of 5α-androstane-3,17-dione (IV*a*, mp = 132-133°, sp. act. 3.35×10^5 cpm/μmole).

A solution of compound IV*a* (0.3 g) was refluxed with 2 % potassium hydroxide in 75 % methanol-water for 12 hours. The reaction mixture was diluted with water, neutralized with hydrochloric acid, extracted several times in ether, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. Recrystallization of the residue from ethyl acetate-petroleum ether gave pure 5α-androstane-3,17-dione-5α, 6α-^3H (IV*b*, 0.12 g, mp 133-134°, sp. act. = 2.12×10^5 cpm/μmole).

3. — *2,2-Dibromo-5α-androstane-3,17-dione-4α; 5α, 6α-^3H* (V) and *2α, 4α-dibromo-5α-androstane-3,17-dione-4β, 5α, 6α-^3H (VI).*

To 30 ml of C.P. glacial acetic acid was added 1.0 g of 5α-androstane-3,17-dione (IV*a*, sp. act. = 3.35×10^5 cpm/μmole) and 13 ml of bromine solution (0.833 g of bromine in 13 ml of acetic acid). At the end of the bromine solution addition, 0.63 g of anhydrous sodium acetate was added immediately. The crystalline product formed within a few minutes was filtered and recrystallized from ethanol-chloroform to give pure 2,2-dibromo-5α-androstane-3,17-dione-4α, 5α, 6α-^3H (V, mp 146-148°, sp. act. = 3.37×10^5 cpm/μmole).

A solution of 1.00 g of 2,2-dibromo-5α-androstane-3,17-dione in 20 ml of acetic acid was warmed for five minutes and the solution cooled for several hours at 10°. The crystalline material formed was filtered and recrystallized from ethanol-chloroform to give pure 2α, 4α-dibromo-5α-androstane-3,17-dione-4β, 5α, 6α-^3H (VI, mp 211-213°, sp. act. = 3.32×10^5 cpm/μmole).

4. — *2-Iodoandrost-4-ene-3,17-dione-4, 6α-^{3}H (VII).*

To a solution of 1.02 g of 2α, 4α-dibromo-5α-androstane-3,17-dione (sp. act. = 3.32 × 10^5 cpm/μmole) in 50 ml of dry acetone, was added 1.1 g of sodium iodide. The mixture was refluxed for 15 hours, poured into 200 ml of cold water, and extracted three times with peroxide-free ether. The combined ethereal extracts were washed with sodium thiosulfate solution, then water, dried over anhydrous sodium sulfate and evaporated to dryness in vacuum. The residue was dissolved in benzene and applied to a column of silica gel packed in benzene. Elution with 5 % ethyl acetate in benzene gave 5α-androstane-3,17-dione (IV*c*, 0.12 g, mp 132-134°, sp. act. = 4.29 × 10^5 cpm/μmole). Elution with 15 % ethyl acetate in benzene yielded a white solid (0.30 g) composed of VII. Crystallization from acetone-petroleum ether afforded pure 2-iodoandrost-4-ene-3,17-dione-4, 6α-^{3}H (VII, mp 128-129°, sp. act. = 1.91 × 10^5 cpm/μmole). Further elution with 25 % ethyl acetate in benzene gave androst-4-ene-3,17-dione-4, 6α-^{3}H (VIII*a*, 1.90 × 10^5 cpm/μmole) having the same specific activity as compound VII.

5. — *Androst-4-ene-3,17-dione-4, 6α-^{3}H (VIIIb).*

A suspension of 0.5 g of 2-iodoandrost-4-ene-3,17-dione (sp. act. = 1.91 × 10^5 cpm/μmole) in 20 ml of anhydrous ethyl ether was stirred for 5 hours at room temperature with 2 g of zinc dust. The solution was filtered and evaporated to give 0.3 g of solid material which was chromatographed on a silica gel column as described in Section 4 of the Experimental. Androst-4-ene-3,17-dione obtained (VIII*b*, 1.89 × 10^5 cpm/μmole) had essentially the same specific activity as that obtained in Section 4.

6. — *Androst-4-ene-3,17-dione-6α-^{3}H (VIIIc) and Androst-4-ene-3,17-dione (VIIId).*

5α-Androstane-3,17-dione-5α, 6α-^{3}H (IV*b*), sp. act. = 2.12 × 10^5 cpm/μmole) was converted via the dibromide and iodide to androst-4-ene-3,17-dione-6α-^{3}H (VIII*c*, sp. act. = 6.72 × 10^4 cpm/μmole) as described in Sections 3, 4 and 5 in the Experimental. Alkaline equilibration of compound VIII*c* followed by isolation and purification of the product gave androst-4-ene-3,17-dione (VIII*d*, sp. act. = 1.12 × 10^2 cpm/μmole) essentially with complete loss of radioactivity.

7. — *Androsta-4, 6-diene-3,17-dione-6-^{3}H (IX).*

To a solution of 0.25 g of androst-4-ene-3,17-dione (VIII*c*, sp. act. = 6.72 × 10^4 cpm/μmole) in 20 ml of dry benzene, was added 0.8 g or 2, 3, 5, 6-tetrachloroquinone (chloranil). The mixture was refluxed for 6 hours, poured into water and extracted with methylene chloride. The combined

methylene chloride extracts were filtered, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed on a silica gel column and eluted with 20 % ethyl acetate in benzene. The fractions containing the product were combined, evaporated, and the residue subjected to preparative thin layer chromatography on silica gel HF (1 mm thick). The ultraviolet zone corresponding to the product was eluted with acetone and crystallized to constant specific activity from ethylacetate-petroleum ether to give pure androsta-4, 6-diene-3,17-dione-6-^{3}H (IX, mp 166-167°, sp. act. = 6.69×10^4 cpm/μmole) identical in mp, ir and uv to an authentic sample of androsta-4,6-diene-3,17-dione.

ACKNOWLEDGEMENT.

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1. The following trivial names were used in this paper : 2, 2-dibromo compound (2, 2-dibromo-5α-androstane-3,17-dione); 2, 4-dibromo compound (2α, 4α-dibromo-5α-androstane-3, 17-dione); 2-iodoandrostene-dione (2-iodoandrost-4-ene-3, 17-dione).
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