PREPARATION OF TRITIUM-LABELED COMPOUNDS. V. A GROUP OF STEROIDS BY CATALYTIC REDUCTION OF UNSATURATED PRECURSORS WITH TRITIUM GAS

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SUMMARY

A group of Δ^4-3-keto steroids was prepared by catalytic reduction of suitable conjugated diene-3-keto precursors with tritium gas. Thus, 11α-hydroxy-4-pregnene-3,20-dione-7-^3H (I), 20α-hydroxy-4-pregnen-3-one-7-^3H (II), 6α-methyl-4-pregnene-3,11,20-trione-7-^3H (III), 17α-hydroxy-6α-methyl-4-pregnene-3,20-dione-7-^3H, acetate (IV), and 17α-hydroxy-6-methyl-$^3H_3$-16-methylenepregna-4,6-diene-3,20-dione, acetate (V) were prepared from 11α-hydroxypregna-4,6-diene-3,20-dione (VI), 20α-hydroxypregna-4,6-dien-3-one (VII), 6-methylpregna-4,6-diene-3,11,20-trione (VIII), 17α-hydroxy-6-methylpregna-4,6-diene-3,20-dione, acetate (IX), and 17α-hydroxy-6-dibromomethylene-16-methylene-4-pregnene-3,20-dione, acetate (X), respectively. In addition, 3α-hydroxy-5β-pregnane-11,20-dione-7-^3H, cyclic 20-(trimethylene acetal) (XI) was prepared from I via 11α-hydroxy-5β-pregnane-3,20-dione-7-^3H (XII), 5β-pregnane-3,11,20-trione-7-^3H (XIII) and 3α-hydroxy-5β-pregnane-11,20-dione-7-^3H (XIV).

INTRODUCTION

Over a period of several years a number of tritium-labeled steroids have been prepared in these laboratories for metabolism studies. Among them was a group of Δ^4-3-ketones. This report describes the preparation of these compounds by selective, catalytic reduction with tritium gas of five conjugated diene-3-keto steroids, including two $\Delta^{4,6}$-3-ketones, two 6-methyl-$\Delta^{4,6}$-3-ketones, and a 6-dibromomethylene, 16-methylene-Δ^4-3-ketone. In each case it was necessary to obtain an efficient utilization of tritium while retaining the Δ^4-3-keto struc-

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ture intact. It was felt that the isotope would be most efficiently used by carrying out the reduction in an aprotic solvent and allowing uptake of carrier-free tritium gas first, followed by completion of the reduction with hydrogen. Use of Pd on $SrCO_3$ as a catalyst, together with benzene as the solvent, and careful monitoring of the hydrogen uptake would hopefully favor retention of the Δ^4-3-keto structure.

EXPERIMENTAL

Radioactivity Measurements

All counting was performed with Tri-Carb, Model 314EX2A and 3375, liquid scintillation spectrometers using conditions suitable for measuring tritium. Appropriate aliquots of samples were dissolved in 15 ml of scintillation solvent [toluene-dioxane-methanol (350:350:210 by volume) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.08 g of 1,4-*bis*-2-(5-phenyloxazolyl)-benzene per L.] The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of tritium-labeled toluene and results then expressed as millicuries (mCi).

Paper and thin-layer chromatograms were scanned for radioactivity with Vanguard Models 880 and 885 radiochromatogram scanners, respectively.

Paper and Thin-Layer Chromatography

Paper chromatograms were developed by the descending method using 86-cm lengths of Whatman No. 2 paper in the following systems: (a) Bush A (1,2), sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of Skellysolve C-methanol-water (5:4:1 by volume)-developed in the Skellysolve C phase; (b) Bush B-3 (1,2), sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of benzene-Skellysolve B-methanol-water (333:667:800:200 by volume)-developed in the benzene-Skellysolve B phase; (c) Bush B-5 (1,2), sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of benzene-methanol-water (2:1:1 by volume)-developed in the benzene phase; (d) FBC (2,3), sheet saturated with methanol-formamide (1:1 by volume), dried 15 minutes at 37°, and developed with formamide saturated benzene-chloroform (1:1 by volume); (e) CFS (2,3), sheet saturated with carbitol-formamide-methanol (1:1:1 by volume), dried 15 minutes at 37°, and developed with carbitol-formamide (1:1 by

volume) saturated Skellysolve B; (f) FCF, sheet saturated with methanol-formamide (1:1 by volume), dried 15 minutes at 37°, and developed with formamide saturated cyclohexane; (g) FTCF, sheet saturated with methanol-formamide (1:1 by volume), dried 15 minutes at 37°, and developed with formamide saturated toluene-cyclohexane (1:1 by volume).

Analytical thin-layer chromatograms were developed by the ascending method on 5 x 20 cm glass plates having 0.25-mm layers of silica gel GF. Preparative thin-layer chromatography was carried out with 2-mm layers of silica gel G on 20 x 20 cm plates. Plates were developed in the following systems: (a) EAC, ethyl acetate-cyclohexane (1:1 by volume); (b) EAB, ethyl acetate-benzene (2:3 by volume); (c) BMA, benzene-methanol-acetone (89:10:1 by volume); and (d) EM, ethyl ether-methylene dichloride (1:1 by volume).

Zones absorbing ultraviolet light were located as fluorescence-quenching areas when thin-layer chromatograms were viewed under short-wavelength UV light or when paper chromatograms, irradiated with a similar light source, were viewed through a fluorescent screen.

Apparatus for Catalytic Reduction

The apparatus (Fig. 1), similar to conventional semi-micro reduction equipment except for provision to allow introduction of tritium gas prior to reduction

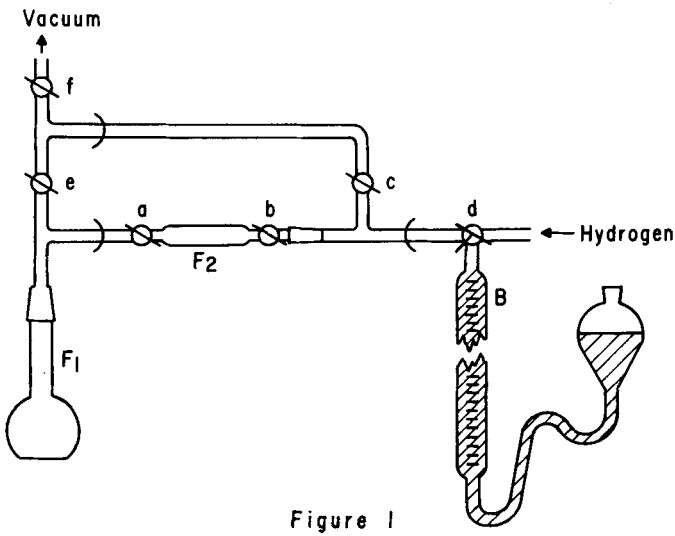


Figure 1

with hydrogen, was patterned after that described by Williams and Ronzio (4). Tritium gas was first transferred into F_2 (removable) with a Toepler pump on a separate high-vacuum line and F_2 was then attached to the apparatus as shown. The sample to be reduced, solvent, catalyst, and magnetic stirring bar were placed in F_1. The system was alternately evacuated (house vacuum) through stopcocks f, e, and c and filled with hydrogen through stopcock d to displace air from the system. It finally was evacuated, stopcocks f, e, c, and d were closed, stirring was started, and stopcock a was opened, to admit tritium into F_1. After about 15 minutes, stopcock a was closed, hydrogen was admitted to point b from the gas buret (B), stopcock b was opened momentarily, then closed, and stopcock a was opened to F_1. This procedure was repeated several more times at 5-minute intervals to flush tritium from F_2. Stopcocks a and b were then opened, the system was brought to atmospheric pressure with hydrogen from the gas buret, and the reduction was completed. Having properly calibrated the system and knowing the amount of tritium gas used, the uptake of hydrogen could be accurately quantified.

Preparation of Tritium-Labeled Steroids

11α-Hydroxypregna-4,6-diene-3,20-dione (VI) - Nonradioactive 11α-hydroxy-4-pregnene-3,20-dione (I) was dehydrogenated with chloranil by the procedure of Agnello and Laubach (5) to give VI: m.p., 165-166°, λ_{max}^{EtOH} = 285 mμ, ε = 26,700; $[\alpha]_D$ = +107° ($CHCl_3$). *Anal.* Calc. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.8; H, 8.8.

11α-Hydroxy-4-pregnene-3,20-dione-7-^3H (I) - A 1.00-g sample of VI in 40 ml of benzene was reduced with 0.1 g of 5% Pd on $SrCO_3$ catalyst using 4 Ci of tritium gas and 73 ml of hydrogen. The catalyst was removed by filtration and the product was crystallized from benzene-heptane and recrystallized twice from ethyl acetate to yield 0.386 g of 11α-hydroxy-4-pregnene-3,20-dione-6,7-$^3H_2$ having a specific activity of 2.40 mCi/mg. This material was refluxed under nitrogen in a mixture of 30 ml ethanol, 4 ml H_2O and 0.2 g KOH for one hour. The reaction mixture was cooled to room temperature, neutralized with HCl, and extracted with methylene dichloride. The extract was applied to a 1.9 x 50 cm Florisil column and the column was eluted using a gradient of 10% acetone in

Skellysolve B to 15% acetone in Skellysolve B while collecting fractions. Fractions showing at least 99% radiochemical purity in the Bush B-5 paper chromatography system were combined (total residue weight, 0.187 g), diluted with 3.23 g of nonradioactive I and recrystallized from methanol-H_2O to yield 3.43 g of tritium-labeled I having a specific activity of 0.109 mCi/mg. The IR and UV spectra and melting point of the product were identical to those of authentic standard. The product was radiochemically pure in the Bush B-5, Bush B-3, and Bush A paper chromatography systems. *Anal.* Calc. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.3; H, 9.5.

A fraction (residue weight, 0.061 g) from the Florisil column, having only 90% radiochemical purity, was separately diluted with 1.14 g of nonradioactive I and crystallized as described above. Thus an additional 1.16 g of tritium-labeled I having a specific activity of 0.088 mCi/mg was obtained.

11α-Hydroxy-5β-pregnane-3,20-dione-7-^3H (XII) - A 1.00 g sample of tritium-labeled I, having a specific activity of 0.109 mCi/mg, in 5 ml of methanol was hydrogenated using 0.15 g of 5% Pd on $SrCO_3$ catalyst. After removing the catalyst by filtration and the methanol by evaporation, the mixture of 5α and 5β isomers in acetone was applied to a 2.5 x 30 cm column composed of one part Darco G60 and two parts Celite and eluted with acetone while collecting fractions. Those fractions containing only 5β isomer, as determined in trial runs, were combined to give 0.75 g of tritium-labeled XII.

5β-Pregnane-3,11,20-trione-7-^3H (XIII) - A cooled and rapidly stirred solution of 0.75 g of tritium-labeled XII in 6 ml of acetone was oxidized by dropwise addition of 0.85 ml of 0.25 *M* CrO_3 in 20% H_2SO_4. After 10 minutes at room temperature, the crude product, precipitated by adding water to the acetone solution, was recrystallized from ethyl acetate-Skellysolve B to yield 0.520 g of tritium-labeled XIII. The product had an IR spectrum and melting point identical to that of authentic XIII.

3α-Hydroxy-5β-pregnane-11,20-dione-7-^3H (XIV) - A solution of 0.40 g of tritium-labeled XIII and 0.40 g of nonradioactive XIII in 7.5 ml of dioxane was reduced by addition of 33 mg of $NaBH_4$ in 4.5 ml of 0.01 *N* NaOH with vigorous stirring. After 15 minutes the product was crystallized by the addition of H_2O.

The crude product was recrystallized from methanol-H_2O to give 0.56 g of tritium-labeled XIV having the same melting point as authentic XIV.

3α-Hydroxy-5β-pregnane-11,20-dione-7-^3H,cyclic 20-(trimethylene acetal) (XI) - A solution of 0.40 g of tritium-labeled XIV, 1 mg of *p*-toluenesulfonic acid monohydrate, and 1 ml of 1,3-propanediol in benzene was refluxed under a Dean-Stark trap overnight. After cooling, the reaction mixture was treated with 0.40 g of sodium methylate and the benzene layer was decanted into a beaker and treated successively with 1 g of Woelm Act. I basic alumina and 50 mg of Darco G60 charcoal. After filtering, the benzene solution was treated with a drop of pyridine and evaporated to dryness *in vacuo*. The residue was recrystallized twice from acetone-water to yield 68 mg of tritium-labeled XI having a specific activity of 0.050 mCi/mg. Paper chromatography in the Bush A system and rotary dispersion analysis showed that the product contained 4% free 20-ketone (XIV). *Anal.* Calc. for $C_{24}H_{38}O_4$: C, 73.8; H, 9.7. Found: C, 73.3; H, 9.7.

20α-Hydroxypregna-4,6-diene-3-one (VII) - Nonradioactive 20α-hydroxy-4-pregnen-3-one (II) was dehydrogenated by the procedure of Agnello and Laubach (5) to give VII: m.p., 156-158°; λ_{max}^{EtOH} = 286 mμ, ε = 25,300; $[\alpha]_D$ = +48° ($CHCl_3$). *Anal.* Calc. for $C_{21}H_{30}O_2$: C, 80.7; H, 9.7. Found: C, 79.7; H, 9.8.

20α-Hydroxy-4-pregnen-3-one-7-^3H (II) - A 0.50 g sample of VII in 15 ml of benzene was reduced with 0.15 g of 5% Pd on $SrCO_3$ catalyst using 3 Ci of tritium gas and 43 ml of hydrogen. After filtering off the catalyst and evaporating the benzene, the product was refluxed under nitrogen in methanol containing 0.50 g of KOH for 1 hour. After cooling to room temperature, the solution was diluted with water, acidified, and extracted with methylene chloride. The residue from the extract was applied to a 4.8 x 72 cm partition column prepared from acid-washed Celite 545 and eluted with the mobile phase of the solvent mixture cyclohexane-methanol-H_2O (20:7:3) while collecting fractions. The fractions containing radiochemically pure II, as determined by paper chromatography in the FTCF and Bush A systems, were combined and the residue was crystallized from acetone-H_2O to yield 0.288 g of product. This material was recrystallized to yield 0.194 g of tritium-labeled II having a specific activity of 1.74 mCi/mg. The IR and UV spectra of the product were identical to those of authentic standard. It was

radiochemically pure in the Bush B-3, Bush A and FTCF paper, and EAC thin-layer, chromatography systems.

6-Methylpregna-4,6-diene-3,11,20-trione (VIII) - Nonradioactive 6α-methyl-4-pregnene-3,11,20-trione (III) was dehydrogenated with chloranil by the procedure of Agnello and Laubach (5) to give VIII: m.p. 169-171°; λ_{max}^{EtOH} = 285 mμ, ε = 23,400; $[\alpha]_D$ = +367° ($CHCl_3$).

6α-Methyl-4-pregnene-3,11,20-trione-7-^3H (III) - A 0.681 g sample of VIII in 30 ml of benzene was reduced with 0.15 g of 5% Pd on $SrCO_3$ catalyst using 4 Ci of tritium gas and 52 ml of hydrogen. The catalyst was removed by filtration, the benzene was evaporated, and the product, 6β-methyl-4-pregnene-3,11,20-trione-6,7-^3H (XV), having a specific activity of 1.67 mCi/mg, was refluxed overnight under nitrogen in a mixture of 0.7 ml methanol and 6.5 ml of 2 *N* HCl. After cooling to room temperature the mixture was extracted into benzene and the extract was washed successively with H_2O, 5% $NaHCO_3$ and brine. The crude, tritium-labeled III having a specific activity of approximately 1.22 mCi/mg was shown by NMR spectrometry to be free of 6β-methyl isomer. The residue from the extract was applied to a 5.8 x 122 cm partition column prepared from acid-washed Celite 545 and eluted with the mobile phase of the solvent mixture cyclohexane-methanol-H_2O (20:7:3) while collecting fractions. The fractions containing radiochemically pure III, as determined by paper chromatography in the Bush B-3 system, were combined and evaporated to dryness to give 0.323 g of product having a specific activity of 1.20 mCi/mg. A 0.270 g sample of this material was diluted with 1.05 g of nonradioactive III and recrystallized once from methanol-water and twice from ethyl acetate to yield 0.752 g of tritium-labeled III having a specific activity of 0.230 mCi/mg. The IR spectrum of the product was identical to that of authentic standard, whereas its UV spectrum indicated the presence of as much as 1.9% of the starting material, VIII. The product was radiochemically pure in the Bush B-3, Bush A, and CFS paper, and EAB thin-layer chromatography systems. *Anal.* Calc. for $C_{22}H_{30}O_3$: C, 77.2; H, 8.8. Found: C, 76.9; H, 8.7.

17α-Hydroxy-6-methylpregna-4,6-diene-3,20-dione, acetate IX - Nonradioactive 17α-hydroxy-6α-methyl-4-pregnene-3,20-dione, acetate (IV) was dehydrogenated

with chloranil by the procedure of Agnello and Laubach (5) to give IX: m.p., 214-219°, λ_{max}^{EtOH} = 288 mμ, ε = 23,150.

17α-Hydroxy-6α-methyl-4-pregnene-3,20-dione-7-^{3}H, acetate (IV A) - A 1.00 g sample of IX in 40 ml of benzene was reduced with 0.30 g of 5% Pd on $SrCO_3$ catalyst using 4 Ci of tritium gas and 64 ml of hydrogen. After removing the catalyst by filtration and the benzene by evaporation, the product was applied to a 2.8 x 45 cm Florisil column and the column was eluted using a gradient of 5% acetone in Skellysolve B to 20% acetone in Skellysolve B while collecting fractions. Fractions containing 17α-hydroxy-6β-methyl-4-pregnene-3,20-dione-6,7-^{3}H, acetate (XVI), as determined by paper chromatography in the Bush A system, were combined to yield 0.57 g of product having a specific activity of 1.06 mCi/mg. A solution of this material in 15 ml of $CHCl_3$ and 0.1 ml of ethanol was cooled in an ice bath and dry HCl gas was passed into the solution for 6 minutes. After washing the solution with H_2O, saturated Na_2CO_3, and H_2O, it was applied to a 1.8 x 50 cm Florisil column and the column was eluted using a gradient of 5% acetone in Skellysolve B to 20% acetone in Skellysolve B. Fractions containing radiochemically pure IV were combined to give 0.38 g of product. This material was diluted with 3.00 g of nonradioactive IV and crystallized from methanol-H_2O to yield 3.20 g of tritium-labeled IV having a specific activity of 0.122 mCi/mg. The UV and IR spectra of the product were identical to those of authentic standard. It was radiochemically pure in the Bush B-3, Bush A, CFS, and FBC paper chromatography systems. *Anal.* Calc. for $C_{24}H_{34}O_4$: C, 74.6; H, 8.9. Found: C, 74.6; H, 9.2.

17α-Hydroxy-6α-methyl-4-pregnene-3,20-dione-7-^{3}H, acetate (IV B) - To a small hydrogenation flask having a free volume of 8 ml was added 100 mg of IX, 30 mg of 5% Pd on $SrCO_3$, 2 ml of benzene, and a small stirring bar. The flask was cooled in liquid nitrogen and evacuated to less than 10^{-5} mm pressure, 4 Ci of tritium gas was added by means of a Toepler pump, and reduction was allowed to proceed at room temperature for 1 hour. The flask was attached directly to the gas buret at stopcock d (Fig. 1) and reduction was completed with uptake of approximately 5 ml of hydrogen. After filtering off the catalyst and evaporating the benzene, the product was equilibrated in methanol several times to remove

labile tritium and taken up in 4 ml of $CHCl_3$ containing 0.05 ml of ethanol. Dry HCl was passed through the solution for 6 minutes while cooling in ice. The solution was washed with H_2O, saturated Na_2CO_3, and H_2O; evaporated to dryness; and the residue was taken up in benzene. The solution was streaked on four 20 x 20 cm plates containing 2mm films of silica gel. The plates were developed in the BMA thin-layer chromatography system. Zones corresponding to IV were removed and eluted with acetone. The residue was recrystallized from ethanol-H_2O to yield 53 mg of IV having a specific activity of 29 mCi/mg. Its radiochemical purity was approximately 90% in the Bush A paper and BMA thin-layer chromatography systems. A stock solution of this material was prepared in benzene for storage. As samples were needed, aliquots of the solution were purified by sequential chromatography in the BMA thin-layer and Bush A paper systems. The material eluted from paper, usually about 50 μg, was radiochemically pure as determined by paper chromatography in the Bush B-3 and Bush A systems, and had a specific activity of 26 mCi/mg based on UV analysis of the eluate.

17α-Hydroxy-6-dibromomethylene-16-methylene-4-pregnene-3,20-dione, acetate (X) - 17α-hydroxy-16-methylene-4-pregnene-3,20-dione, acetate was converted to its 3-enol methyl ether by treatment with methyl orthoformate at 60° in acidified dioxane. The product, recrystallized from methanol-methylene chloride containing a few drops of pyridine, had UV and IR spectra consistent with the expected product. This material was converted to X by treatment with CBr_4, pyridine, and *tert*-butylhydroperoxide in dioxane at room temperature followed by treatment with additional pyridine at 100°. The reaction mixture was worked up by extraction, silica-gel chromatography, and recrystallization from acetone-Skellysolve B to give the expected product: m.p. 190-195°; λ_{max}^{EtOH} = 250 mμ, ε = 10,300, 283 mμ, ε = 6,100; $[\alpha]_D$ = +91° ($CHCl_3$). *Anal.* Calc. for $C_{25}H_{30}O_4Br_2$: C, 53.9; H, 5.8; Br, 28.7. Found: C, 55.1; H, 5.9; Br, 28.2.

17α-Hydroxy-6-methyl-$^3H_3$-16-methylenepregna-4,6-diene-3,20-dione, acetate (V) - A slurry of 0.40 g of 3% Pd on $CaCO_3$, 0.30 ml of triethylamine, and 6 ml of dioxane was prereduced with 6 ml of hydrogen. A solution of 0.40 g of X in 5 ml of dioxane was injected through a rubber septum into the hydrogenation flask and reduction was continued with 4 Ci of tritium gas and 60 ml of hydrogen. After

filtering the catalyst, the dioxane solution was saturated with nitrogen, treated with 6 ml of 2 *N* HCl, and allowed to stand at room temperature for 1.25 hours. The mixture was diluted with water, chilled, and the resulting precipitate was recrystallized from acetone-Skellysolve B to yield 0.220 g of 17α-hydroxy-6α-methyl-$^3H_3$-16-methylene-4-pregnene-3,20-dione, acetate (XVII) having a specific activity of 3.01 mCi/mg. It had the same melting point as authentic standard and was radiochemically pure in the EM thin-layer chromatography system. A solution of 0.210 g of tritium-labeled XVII, 0.220 g of nonradioactive XVII, and 0.430 g of chloranil in t-amyl alcohol was refluxed under nitrogen for 5 hours. After filtering off excess chloranil, the filtrate was diluted with a 1:1 mixture of ether-methylene dichloride and the solution was washed with 5% NaOH, H_2O, and brine. The residue from the organic layer was applied to a 1.4 x 25 cm Florisil column and the column was eluted using a gradient of 5% acetone in Skellysolve B to 15% acetone in Skellysolve B while collecting fractions. Fractions containing radiochemically pure V, as determined by paper chromatography in the Bush A system, were combined and the residue was recrystallized twice from ethyl acetate to yield 0.223 g of tritium-labeled V having a specific activity of 1.54 mCi/mg. The UV and IR spectra of the product were identical to those of authentic V. Its radiochemical purity was greater than 97% in the Bush B-3 and Bush A paper and EM thin-layer chromatography systems. *Anal.* Calc. for $C_{25}H_{32}O_4$: C, 75.7; H, 8.1. Found: C, 75.7; H, 8.5.

RESULTS

Five Δ^4-3-keto steroids were prepared in chemically and radiochemically pure form by catalytic reduction with tritium gas of suitable conjugated diene-3-keto precursors. The compounds prepared included 11α-hydroxy-4-pregnene-3,20-dione-7-^3H (I), 20α-hydroxy-4-pregnen-3-one-7-^3H (II), 6α-methyl-4-pregnene-3,11,20-trione-7-^3H (III), 17α-hydroxy-6α-methyl-4-pregnene-3,20-dione-7-^3H, acetate (IV), and 17α-hydroxy-6-methyl-$^3H_3$-16-methylenepregna-4,6-diene-3,20-dione, acetate (V). In addition, I was converted to 3α-hydroxy-5β-pregnane-11,20-dione-7-^3H, cyclic 20-(trimethylene acetal) (XI). See Fig. 2.

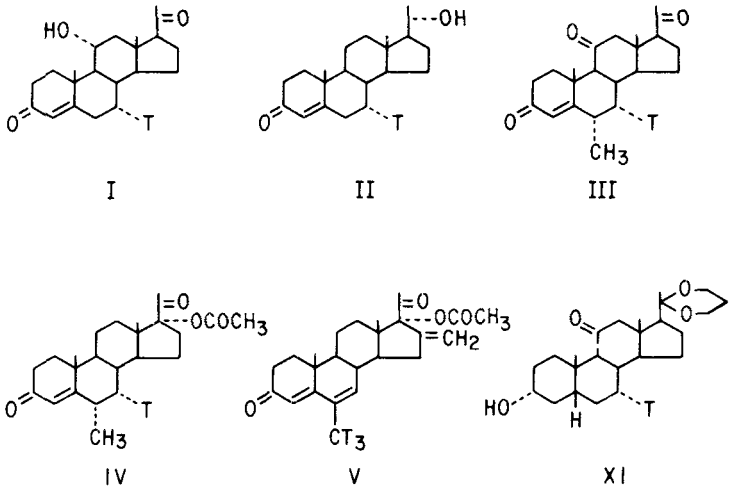


Figure 2

Tritium-labeled I was prepared at a specific activity of 0.109 mCi/mg in 12% radiochemical yield and converted to 3α-hydroxy-5β-pregnane-11,20-dione-7-^{3}H, cyclic 20-(trimethylene acetal), XI, at a specific activity of 0.050 mCi/mg in 6% radiochemical yield by the sequence of reactions shown in Figure 3. Labile tritium in the 6 position of I was removed by treatment with alkali. This resulted in about 20% isomerization of the side chain but the desired isomer was easily separated by Florisil chromatography.

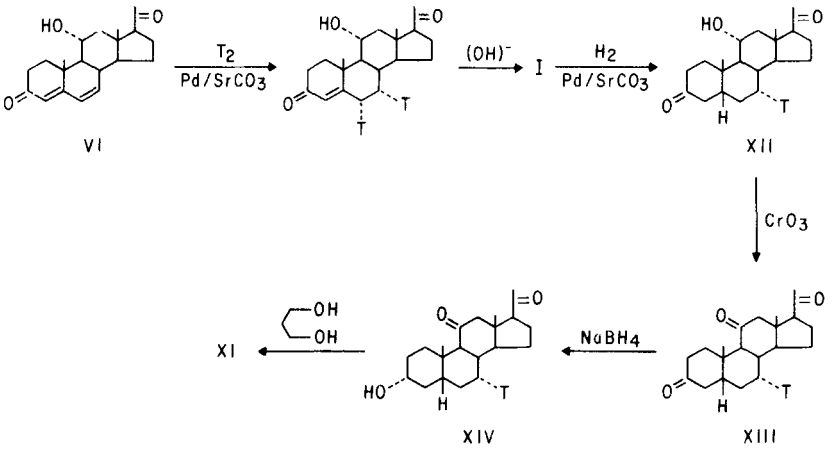
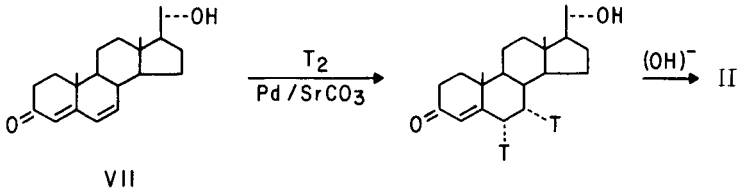


Figure 3

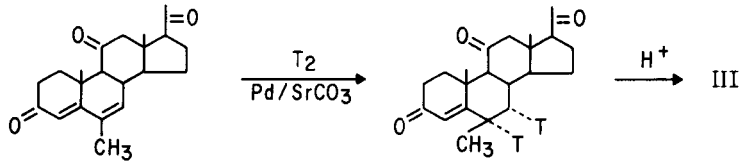
Tritium-labeled II was prepared at a specific activity of 1.74 mCi/mg in 11% radiochemical yield by the sequence of reactions shown in Figure 4. Labile tritium in the 6 position was removed by treatment with alkali.



VII

Figure 4

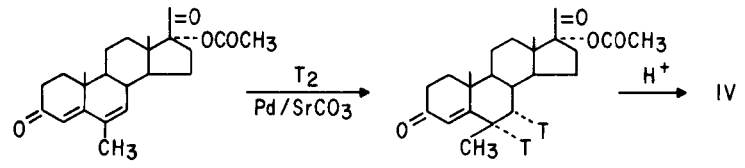
Tritium-labeled III was prepared at a specific activity of 0.230 mCi/mg in 4% radiochemical yield by the sequence of reactions shown in Figure 5. Labile tritium in the 6 position was removed, and the 6-methyl group was isomerized from the β to the desired α configuration of III, by treatment with acid.



VIII XV

Figure 5

Tritium-labeled IV was prepared at a specific activity of 0.122 mCi/mg in 10% radiochemical yield and at a specific activity of 26 mCi/mg in 35% radiochemical yield by the sequence of reactions shown in Figure 6. In both cases possible labile tritium in the 6 position was removed, and the 6-methyl group was isomerized from the β to the α configuration of IV, by treatment with acid.



IX XVI

Figure 6

Tritium-labeled V was prepared at a specific activity of 1.54 mCi/mg in 9% radiochemical yield by the sequence of reactions shown in Figure 7. Labile tritium in the initial reduction product was removed, and the 6-methyl group was isomerized from the β to the α configuration of XVII, by treatment with acid. The 7α hydrogen was then removed during dehydrogenation with chloranil to give V.

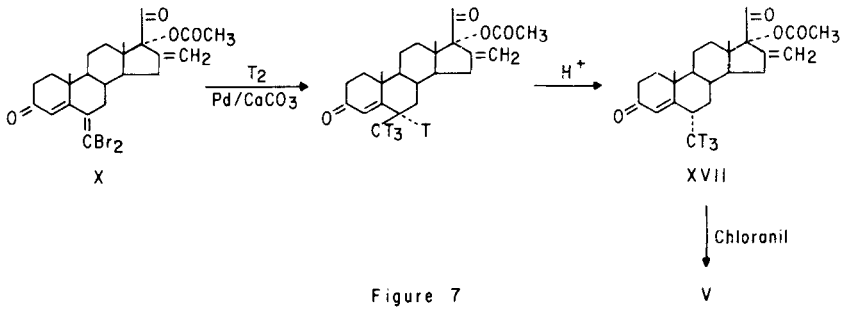


Figure 7

The radiochemical purities of I, II, III, IV, and V were determined by paper chromatography after certain periods of storage. These results are shown in Table I. The G(-M) values (number of molecules of compound destroyed per 100 electron volts of energy absorbed) were calculated taking into account the half life of tritium as well as the rather extensive decomposition of the compounds.

Table I

Compound	Initial Sp. Act. (mCi/mg)	Storage Conditions	Storage Time (months)	Percent Decomposition	G(-M) Value
I	0.109	crystals, -15°C	158	27	8.8
II	1.74	crystals, -15°C	120	67	2.5
III	0.230	crystals, -15°C	72	3	0.7
IV	0.122	crystals, -15°C	154	18	4.3
IV	26	0.01 mg/ml ethanol, -15°C	29	6	0.03
IV	26	0.01 mg/ml ethanol, -15°C	54	12	0.03
IV	26	0.01 mg/cm^2 on paper, -15°C	29	73	0.5
IV	29	5 mg/ml benzene, 5°C	54	79	0.3
V	1.54	7 mg/ml benzene, 5°C	31	71	7.9
V	0.193	crystals, -15°C	93	35	8.4

DISCUSSION

Tritium-labeled Δ^4-3-keto steroids have been prepared by selective, catalytic reduction of $\Delta^{4,6}$-3-keto (6-10), $\Delta^{1,4}$-3-keto (6,11,12), $\Delta^{4,16}$-3-keto (6,13), 16-bromo-Δ^4-3-keto (6,14), and 7- or 16-bromo-Δ^5-3-acetoxy (6,14,16) precursors with tritium gas. Selection of a suitable precursor depends on a number of factors including its availability and the desired location of the tritium label.

The $\Delta^{4,6}$-3-keto precursors of I, II, III, and IV were chosen for reduction, rather than the corresponding $\Delta^{1,4}$-3-ketones or 7-bromo-Δ^5-3-acetoxy intermediates, primarily because of their more ready availability. In the cases of I and II, potentially labile tritium in the 6 position could be removed by alkaline isomerization of the Δ^4-3-ketone structure whereas the necessary isomerization of the 6-methyl group from β to α configuration would remove tritium in the 6 position of III and IV. It was felt that tritium in either the 1 or 7 position would offer a metabolically suitable label for I, II, III, and IV. In the case of compound V, since labeling in the 7 position was not feasible, tritium was introduced into the 6-methyl group, rather than the 1,2 positions, for the reason previously mentioned.

The kinetics of conversion of a $\Delta^{4,6}$-3-keto steroid to the corresponding Δ^4-3-ketone by catalytic reduction have been studied by Garrett, *et al* (17). Conditions for optimal conversion included use of methanol, containing a small but critical amount of alkali, as a solvent, and a prereduced catalyst. These conditions were thought to be not suitable for efficient incorporation of tritium and attainment of sufficiently high specific activities as will be discussed later. However, it was apparent from these kinetic studies that a reasonable yield of Δ^4-3-ketone could be obtained under conditions more suitable for use of tritium. In fact, Woodward, *et al* (18) used a Pd on $SrCO_3$ catalyst with benzene as a solvent to selectively reduce the γ-δ double bond of a conjugated steroid dieneone in good yield. The selectivity has been attributed to this catalyst and the solvent.

Tritium reductions were carried out in benzene using a 5% Pd on $SrCO_3$ catalyst for preparing I, II, III, and IV and in dioxane using a 5% Pd on $CaCO_3$ catalyst for preparing V. Aprotic solvents such as ethyl acetate, dioxane, and

benzene are commonly used in these reductions since, in the presence of a catalyst, tritium entering the product may be substantially diluted by equilibration with the labile hydrogen of a protic solvent. In fact, we once obtained extensive equilibration of tritium with the labile hydrogen of the ethanol solvent when morphine was reduced with tritium gas by the procedure described in this report. The resulting dihydromorphine had a specific activity only about 2% that expected; the missing tritium was found to be associated with the ethanol. Nevertheless, several catalytic reductions using alcoholic solvents have been reported to result in little, if any, dilution of tritium entering a steroid product (8,14). In general, however, efficient utilization of tritium and high specific activity products are favored by use of aprotic solvents. The procedure by which reduction was allowed to proceed initially with carrier-free tritium gas, followed by incremental addition of hydrogen, also appears to have favored these two objectives.

It was decided to slightly over-reduce the starting material since it generally is easier to separate the desired Δ^4-3-ketone from the Δ^4 over-reduction product than from the $\Delta^{4,6}$-3-keto starting material.

Although the tritium in I, II, III, and IV is indicated to be in the 7 position, and with an α configuration, studies were not carried out to ascertain this assignment. Direct addition of tritium to the 6-7 double bond followed by removal of tritium in the 6 position, or conjugate addition of tritium, as suggested by Garrett (17) and Pearlman (7), would have resulted in products labeled in the 7 position. However, scrambling of tritium in I, II, III, and IV could have resulted from both catalytic exchange and migration of the 6-7 double bond on the catalyst surface during reduction. Catalytic exchange would not have been likely because of the mild conditions used: ambient temperature, an aprotic solvent, and a short reaction time. Migration of isolated double bonds during catalytic reduction with deuterium has been shown by Fukushima and Gallagher (19) to result in considerable scrambling of the deuterium entering steroids. However, Chamberlin (20) has reported that isolated 6-7 double bonds and double bonds conjugated with the 3-keto group of steroids undergo catalytic reduction with little scrambling of the entering deuterium. Following similar reasoning,

it is likely that compound V was labeled primarily in the 6-methyl group as shown; this was not proven, however. In this case, if tritium had entered the 7 position by migration of the 6-methylene double bond, it would likely have been removed during dehydrogenation of XVII with chloranil.

Garrett (17) and Pearlman (7) have suggested a mechanism for hydrogenation of the 6-7 double bond of certain $\Delta^{4,6}$-3-keto steroids involving conjugate addition of hydrogen across the 3-keto-$\Delta^{4,6}$ system rather than simply addition to the 6-7 double bond. Introduction of tritium by such a mechanism would result in an initial reduction product containing tritium in the 7, but not the 6, position. Comparing the specific activities of initial reduction products with those following removal of possible tritium in the 6 position suggests that conjugate addition of tritium played an important role in reduction of the $\Delta^{4,6}$-3-keto steroids reported here. Such comparisons indicate that conjugate addition accounted for two thirds, one third, and all of the tritium entering I, III, and IV (A), respectively. The initial reduction products leading to II and IV (B) were not isolated prior to removal of possible labile tritium in the 6 position so the extent of conjugate addition of tritium in these cases could not be estimated.

The assignment of an α configuration to tritium in the 7 position of I and II is based solely on the probability that the $\Delta^{4,6}$-3-keto precursors would be adsorbed to the catalyst on their less hindered rear sides; thus tritiation would be expected to occur by rear attack. The 7α configuration for III and IV, however, is more certain. The intermediates (XV and XVI) resulting from tritium reduction indeed had a 6β-methyl configuration; thus *cis* addition of hydrogen would have given a 7α-tritium configuration. By analogy this also makes a stronger case for the 7α-tritium configuration of I and II.

Decomposition of I, II, III, IV, and V, resulting from self-radiolysis during storage in the crystalline state, corresponded to G(-M) values within the range reported (6) for other tritium-labeled steroids. Storing V as a benzene solution did little to protect it from self-radiolysis. However, a benzene solution of IV was considerably more stable than crystalline IV. Both IV and V would likely have been better stored at considerably lower concentrations in benzene. Storing IV in ethanol at a concentration of 0.26 mCi/ml provided the

best protection. The stability of IV dispersed on filter paper, although superior to that of crystalline IV, was no better than that of the benzene solution.

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