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SYNTHESIS OF CANRENONE AND RELATED STEROIDS LABELLED WITH TRITIUM, CARBON-14, AND SULFUR-35

CHARLES S. MARKOS, CLIFFORD R. DORN AND DONALD J. ZITZEWITZ.

Department of Drug Metabolism, G.D. Searle & Co., Skokie, IL 60077

SUMMARY

The synthesis of [1-^{3}H]canrenone, [1-^{3}H]spironolactone, [1-^{3}H] potassium canrenoate, [22-^{14}C]canrenone, [22-^{14}C]spironolactone, [22-^{14}C]potassium canrenoate, and [^{35}S]spironolactone is reported. Tritium labelled compounds were obtained by catalytic reduction of a 3-keto-1,4-diene precursor followed by exchange of enolizable label. Carbon-14 compounds were obtained by reaction of a 17-ethynyl steroid with $^{14}CO_2$. Sulfur-35 spironolactone was synthesized by the in-situ generation of [^{35}S]thiolacetic acid from [^{35}S]sodium sulfide.

Keywords: Spironolactone, Canrenone, Potassium Canrenoate, Sulfur-35, Tritium, Carbon-14

INTRODUCTION

A metabolic study of the aldosterone antagonists potassium canrenoate and spironolactone required dual radioactive labels to compare sulfur containing metabolites with those which had undergone dethiolacetylation. To use dual label liquid scintillation counting, the isotope choices were sulfur-35 and tritium. The targeted specific activities for [^{3}H,^{35}S]spironolactone dose preparation allowed a simple physical mixture of the separately prepared isotopically substituted compounds.

The introduction of isotopic hydrogen by catalytic reduction of steroid 1,4-diene-3-ones has been described by several authors[1,2,3]. Orientation of the addition is dependent on the catalyst used and the

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substitution pattern in the steroid[4,5,6]. Tritium at position-2 is expected to be chemically and biologically labile. Methods for base-catalyzed exchange of 2 to provide the mono tritiated compound 3a were validated with low specific activity tritiated material.

The addition of thiolacetic acid to canrenone (5b) is a highly efficient reaction[7] which produces an easily crystallized product, spironolactone (6). Since the sulfur-35 label was to be our limiting reagent, the stoichiometry was investigated. An equimolar mixture of canrenone and thiolacetic acid on a 0.1 mmole scale afforded a 50% isolated yield of spironolactone. The use of sodium sulfide for in-situ generation of thiolacetic acid was developed in a series of experiments which varied solvents (none, methanol, tetrahydrofuran) and catalysts (HCl, pyridine, acetyl chloride) to afford up to a 28% yield of spironolactone based on 0.13 mmole of $Na_2S \cdot 9H_2O$. Only acetyl chloride and sodium sulfide without acetic anhydride failed to yield any product. Identity of the product was confirmed by mass spectroscopy of HPLC isolated material. The use of acetyl chloride with acetic anhydride to enhance the yield was suggested by a literature preparation of thiolacetic acid from hydrogen sulfide[8].

Carbon-14 labelled potassium canrenoate has been prepared with the isotopic carbon at the C-20,21 positions by Amersham Corp. and also by Daiichi Pure Chemicals Co., Ltd. No literature reports exist for those syntheses which used [^{14}C]acetylene as the label source via the ethisterone route[7]. Since all previous metabolism studies with this compound indicated that the carbon skeleton remains intact[9], the preparation of 17-spironolactone steroids with carbon-14 at position-22 is an attractive alternative. The main advantages are a cheaper starting material and introduction of the label one step later in the sequence. This synthetic route was outlined in a metabolism study[9a] published in 1976. However, details of that synthesis have never been disclosed. Our pilot studies indicated the reaction of the magnesium acetylide from 10 with CO_2 as the limiting reagent gave nearly quantitative yields with no non-acidic by-products. The α-butoxy ethyl ketal is a convienient hydroxyl blocking group[10] which prevents the

formation of magnesium alkoxides and allows the stoichiometry to be adjusted so that the Grignard reagent used to generate the magnesium acetylide does not react with $^{14}CO_2$.

EXPERIMENTAL AND RESULTS

General. Liquid scintillation counting was done either on a Mark II or Mark III LSC using PCS cocktail. Sulfur-35 samples were counted in the carbon-14 channel. Thin layer radiochromatograms were obtained from silica gel plates using a Bioscan System 200 Imaging Scanner. HPLC utilized a Supelco LC-18-DB column (4.6 mm x 25 cm) with a mobile phase of MeOH:H_2O (3:2) at 1.2 and 1.5 mL/min and a FLO-ONE detector. Tritium NMR was obtained on a Bruker WP-200 Spectrometer at E.I. DuPont de Nemours Co., Biomedical Products Department, Boston, MA by Dr. P.R. Srinivasin.

Materials. Sulfur-35 sodium sulfide was obtained from Amersham Corporation as anhydrous Na_2S at a specific activity of 25 mCi/mmole. Carbon-14 barium carbonate was purchased from Pathfinders at 59 mCi/mmole and used with carrier to adjust specific activity to 25 mCi/mmole.

17-Hydroxy-3-oxo-17α-pregna-4-ene-1ξ,2ξ-t_2-21-carboxylic acid, γ-lactone, 2. A solution of 1,4-dien-3-one 1 (20 mg, 0.059 mmole) in 2 mL of toluene was catalytically reduced with tritium gas over 10 mg of 10% Pd/C by DuPont-NEN (Boston, MA). Labile tritium was removed and the product was dissolved in 10.0 mL benzene:EtOH 9:1), packaged in dry ice, and returned to our laboratory. Total tritium as determined from duplicate 0.001 mL aliquots was 2.82 Curies with 7.6% volatile. Analysis by TLRC (EtOAc:CH_2Cl_2, 2:3) indicated 17.9% 2, 65.5% 8a, and 18.4% 9a. The solution was evaporated in a stream of nitrogen and the residue with 32 mg carrier was dissolved in 10 mL EtOAc:CH_2Cl_2 (7:93) for LPLC application. A 6 x 1000 mm silica gel column using a mobile

phase of EtOAc:CH_2Cl_2 (7:93) at 2.3 mL/min and two minutes per fraction gave 2 in fractions 29-74 (159 mCi). After solvent removal, the 26.4 mg of residue was 91.8% 2 by HPLRC.

17-Hydroxy-3-oxo-17α-pregna-4-ene-1ξ-t-21-carboxylic acid, γ-lactone, 3a. A solution of 2 (26.4 mg, 159 mCi, 0.077 mmole) with 84 mg (0.245 mmole) of carrier was dissolved in a mixture of 1.0 mL H_2O, 5.0 mL MeOH, and 0.025 mL of 50% aq NaOH (0.475 mmole) in a screw-capped container and heated at 60°C for 72 h. The yellow solution was made acidic (pH 1) with 5N HCl, warmed for 45 min, and diluted with water. The aqueous suspension was extracted with three portions of benzene (total 25 mL) and the combined organic layers were washed with water. The partition of radioactivity was 48 mCi of tritium in the combined aqueous layers and 96.9 mCi in the combined organic layers.

Solvent was removed from the benzene solution and the residue was again subjected to the exchange conditions (5 mL MeOH, 1.0 mL H_2O, 0.030 mL 50% NaOH) at 60°C overnight. Work-up as before gave 93.7 mCi in the benzene layer and 2.14 mCi in the aqueous layer.

The benzene layer was evaporated and the residue, dissolved in 2 mL EtOAc and 0.5 mL hexane, was treated with charcoal. After filtration, the organic solution was further diluted with hexane until turbid, chilled, and the crystalline precipitate was collected to yield 37.0 mg (32.3 mCi) of 3 with a radiochemical purity of 93.5% by TLRC (EtOAc:hexane, 2:3). The filtrate contained 52.4 mCi, and the charcoal 6.4 mCi. An analytical sample was obtained by preparative TLC of the latter two residues (58.8 mCi) on a 1 mm x 20 cm x 20 cm Analtech silica gel plate with EtOAc: hexane (40:60) to afford 48.5 mCi of 3a (51.3 mg) at > 99% radiochemical purity (TLRC, EtOAc:hexane, 2:3). ^3H NMR ($CDCl_3$ δ:1.66 (s, 35% 1α), 2.00 (s,61% 1β), 2.15 (4% 2α).

17-Hydroxy-3-oxo-17α-pregna-4,6-diene-1ξ-t-21-carboxylic acid, γ-lactone, 5a. A mixture of tritiated compound 3a (88.3 mg, 0.258 mmole, 80.7 mCi) and 160 mg (0.467 mmoles) of unlabeled 3 was slurried

with 1.0 mL of anhydrous ethanol and 0.27 mL (243 mg, 1.62 mmole) of triethylorthoformate. The suspension was stirred and heated to 55°C to obtain a clear solution, to which was added a crystal of TsOH.H_2O. Stirring was continued at 55°C for 10 min then at ambient temperature for 20 min. The reaction mixture was quenched with two drops of pyridine, then diluted with 0.5 mL of hexane and chilled in an ice-bath. The solid was filtered, washed with cold ethanol and dried under high vacuum at 60°C to yield 186 mg of 4a.

A stirred solution of 185 mg (0.497 mmole) of 4a in 15 mL of acetone:H_2O (95:5) was treated dropwise with a solution of DDQ (136 mg, 0.601 mmole) in 5 mL of 95% acetone. The clear red solution was stirred at room temperature for 30 min and then quenched with 10 mL of 10% aqueous sodium thiosulfate. The bulk of the acetone was removed under reduced pressure and the residue was extracted several times with CH_2Cl_2. The combined organic extracts were washed with 5% aqueous sodium thiosulfate, saturated NaCl, dried ($MgSO_4$) and filtered through a pad of celite. Purification was achieved by flash chromatography[11] on silica with EtOAc: hexane (40:60) to afford 158 mg (46.0 mCi) of 5a. Recrystallization of this material from 1.0 mL of EtOAc yielded 91.0 mg of 5a. Radiochemical purity was 99.9% by HPLRC and 101% by TLRC (EtOAc). Specific activity was 90.6 mCi/mmole by comparison of uv absorbance at 282 nm to authentic canrenone.

7α-(Acetylthio)-17-hydroxy-3-oxo-17α-pregna-4-ene-1ξ-t-21-carboxylic acid, γ-lactone, 6a. [^{3}H]Canrenone (5a) (67 mg, 0.197 mmole, 17.1 mCi) was refluxed with 1.0 mL of MeOH and 214 mg (2.80 mmole) of thiolacetic acid for 3 h. Removal of volatile solvents under a stream of nitrogen followed by crystallization of the residue from methanol produced 57.0 mg (0.137 mmole, 12.4 mCi) of 6a. Radiochemical purities were 99.2% (TLRC, EtOAc) and 100% (HPLRC).

17-Hydroxy-3-oxo-pregna-4,6-diene-1ξ-t-21-carboxylic acid, monopotassium salt, 7a. A solution of 5a (70.0 mg, 0.206 mmole, 19.7 mCi) and 2.09 mL of 0.0957 N KOH in 5 mL MeOH was heated at reflux under

nitrogen for 45 min. The solvents were removed on a rotary evaporator. MeOH was added and stripped three times in succession. Trituration of the residue with 4.0 mL of EtOAC was followed by 20 min of boiling. The insoluble product was collected from the cooled EtOAc and vacuum dried to yield 80.5 mg (98.5% based on KOH) of 7a. For HPLRC, 14.2 μCi of 7a were dissolved in 5 mL MeOH/H_2O (4:1) and 5N HCl (0.013 mL) was added to indicate pH < 2. After 20 min at reflux, the solution was dried in a stream of nitrogen and reconstituted in mobile phase at 15.4 μCi/mL. The radiochromatogram indicated 96.3% 5a at 11.5 min. A thin-layer radiochromatogram of [^{3}H]potassium canrenoate using n-butanol:MeOH:H_2O (85:10:5) indicated 97.2% potassium salt 7a and 2.3% lactone 5a. The radiochromatogram obtained with n-butanol:H_2O:pyridine:HOAc (37.5:30:25:7.5) gave a radiochromatogram which indicated 102% radiochemical purity at 12.6 cm.

7α-([^{35}S]Acetylthio)-17-hydroxy-3-oxo-17α-pregna-4-ene-21-carboxylic acid, γ-lactone, 6b. A mixture of [^{35}S]sodium sulfide (17.4 mg, 0.22 mmole, 5.5 mCi), acetic anhydride (540 mg, 5.30 mmole), acetyl chloride (57 mg, 0.72 mmole), and 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylic acid, γ-lactone (5b) (200 mg, 0.59 mmole) was placed in a stoppered scintillation vial and heated on a steam bath for 2 h, then cooled in an ice-bath and quickly diluted with 0.8 mL of MeOH. The stoppered vial was allowed to warm to room temperature, and when the exothermic reaction subsided, was gently warmed on a steam bath (4 h). Unlabelled thiolacetic acid (360 mg, 4.72 mmole) was introduced and the reaction mixture was refluxed for 2.5 h. The dark brown suspension was cooled, diluted with cold water, and extracted twice with CH_2Cl_2. The combined organic layers were washed with 5% aqueous $KHCO_3$ followed by water then dried ($MgSO_4$) and filtered through a pad of celite. Flash chromatography (silica, EtOAc:hexane, 60:40) of the CH_2Cl_2 residue (approx, 2.34 mCi) yielded 825 μCi of [^{35}S]spirono-lactone, which was crystallized from MeOH to give 128 mg (0.31 mmole, 466 mcCi) of 6b. A formula weight of 416 was used to calculate a specific activity of 1.51 mCi/mmole from a 1.46 mg sample. TLRC

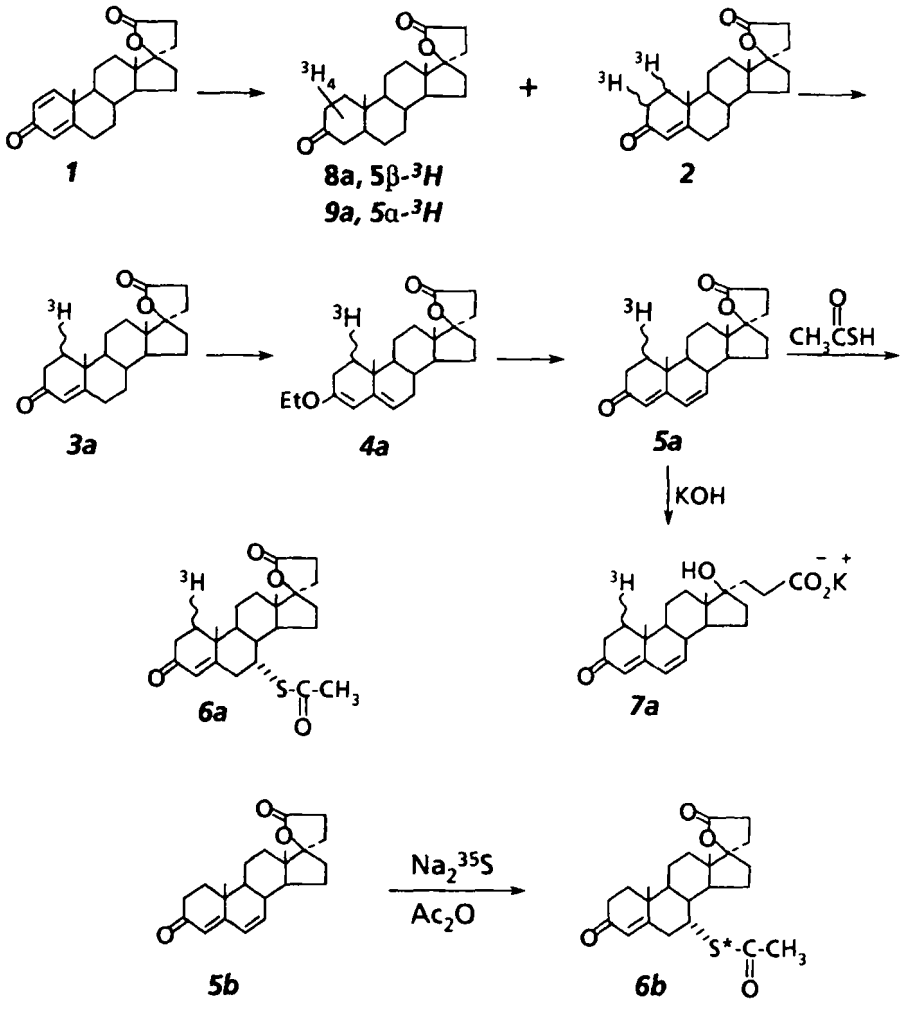
radiochemical purity was 100% (EtOAc). HPLRC radiochemical purity was also 100%.

3β,17-Dihydroxy-17α-pregna-5-ene-20-yne-21-^{14}C-carboxylic acid, 11. A slurry of 17α-ethynylandrost-5-ene-3β,17-diol (3.15 g, 10 mmole) and butylvinylether (2.4 g, 24 mmole) in 10 mL of THF was stirred while 0.1 mL of 10% methanesulfonic acid in THF was added. After 2 h at room temperature, the acid catalyst was neutralized with triethylamine (0.12 mL, 88 mg). The solution was filtered into a 100 mL three-necked flask with a rinse of 2 mL THF and heated to 50°C under a blanket of nitrogen gas. To the warmed and stirred solution 2.1M methylmagnesium chloride in THF (3.8 mL, 8.0 mmole) was added dropwise. The mixture was heated at reflux for 1.5 h, then cooled to room temperature and attached to vacuum transfer line set up for generation of $^{14}CO_2$ from $Ba^{14}CO_3$ (791 mg, 3.99 mmol, 99.4 mCi) and conc H_2SO_4 (25 mL). The generated $^{14}CO_2$ was first trapped at liquid nitrogen temperature, then transferred to the magnesium acetylide which was also frozen by liquid nitrogen. When the transfer was completed, the reaction flask was allowed to reach equilibrium with a dry-ice/isopropanol bath and stirred for 20 min. The cooling bath was then changed to ice-water mixture and stirring was continued for additional 90 min. The reaction was quenched with 1.0 mL of water followed by 4.0 mL 2-methyl-2,4-pentanediol and then a mixture of 4.0 mL of water with 1.6 mL of conc HCl. The mixture was stirred at 30-35°C for 1 h. The acid aqueous layer was separated and the organic layer was washed with saturated NaCl (2 x 5 mL). The THF solution was dried ($MgSO_4$) and filtered. To the stirred filtrate triethylamine (1.8 mL, 12.9 mmol) was added dropwise to produce a precipitate. The solid was collected and washed with THF to yield 1.59 g (3.45 mmol, 72.2 mCi) of compound 11 as the triethylamine salt. The THF filtrate contained 3.6 mCi of carbon-14.

3β,17-Dihydroxy-17α-pregna-5-ene-21-^{14}C-carboxylic acid, γ-lactone, 13. A solution of the triethylamine salt of compound 11 (1.59 g, 3.45

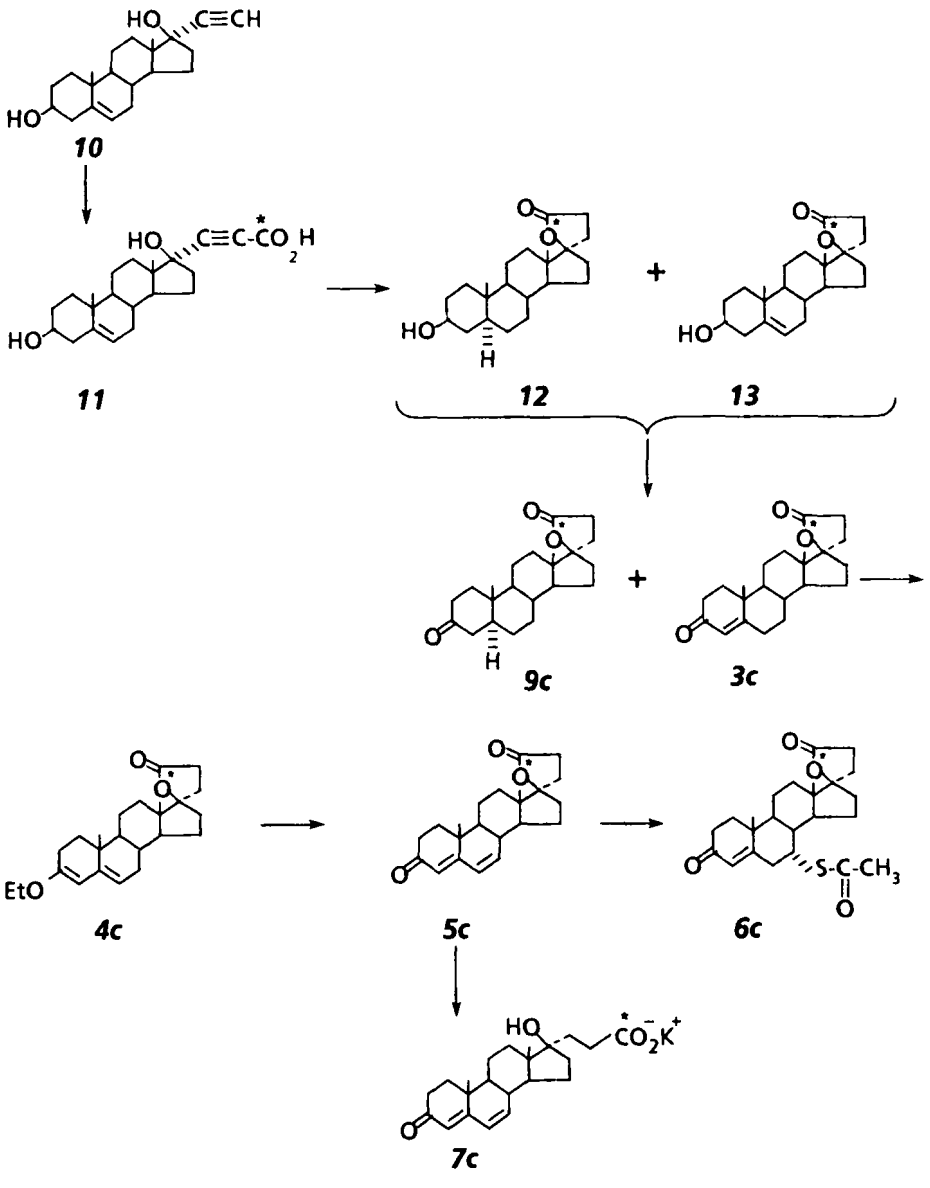
mmol, 72.2 mCi) in 30 mL of MeOH was hydrogenated over a 10% loading of 5% $Pd/CaCO_3$ in a Parr Shaker for 15 min. The catalyst was removed by filtration (celite) and the filtrate volume was adjusted to 25 mL of MeOH. The solution was diluted with 1.0 mL of water, 0.75 mL of conc HCl, and heated at reflux. An additional 16 mL of water was added while MeOH was slowly removed by distillation. A solid precipitate separated on cooling the reaction mixture to room temperature and was collected to yield 957 mg (65.1 mCi) of product. Analysis by TLC indicated poorly resolved [^{14}C]andrenolactone (13) and the 5α-saturated product (12). These two products were 95.8% of the radiolabelled material by TLRC (EtOAc:hexane, 3:1). A 50 mm silica flash column using EtOAc: hexane as the solvent (one liter each of 3:7, 4:6, and 1:1) at 30 mL/fraction gave a mixture of the two products (59.4 mCi) in fractions 24-81 which was 99.4% of the radiolabelled material by TLRC (EtOAc: hexane, 1:1).

17-Hydroxy-3-oxo-17α-pregna-4-ene-21-^{14}C-carboxylic acid, γ-lactone, 3c. A mixture of [^{14}C]andrenolactone and the 5α-saturated compound (59.4 mCi, 874 mg) and N-methyl-4-piperidone (18 mL, 144 mmol) in 90 mL of toluene was dried by distilling 8 mL of the solvent. The solution was cooled slightly, and aluminum isopropoxide (1.14 g, 5.59 mmol) was added in portions. The mixture was heated at reflux for 18 h, cooled in an ice-bath, and treated with 25 mL of conc HCl in 150 mL of water. The layers were separated and the aqueous was extracted twice with 80 mL of toluene. The combined organics were washed with water (3 x 40 mL), dried (Na_2SO_4), and filtered to yield 53.9 mCi of carbon-14 in the extract. Radiochemical purity was 62.6% [^{14}C]3c and 26.1% [^{14}C]9c by TLRC (EtOAc:hexane, 3:1). Purification by LPLC (15 mm x 1000 mm Silica Gel 60, EtOAc:CH_2Cl_2, 7:93 at 8 mL/min, 3 min/fraction) gave 12.8 mCi of [^{14}C]9c in fractions 15-24, and 33.3 mCi of [^{14}C]3c in fractions 27-49. Radiochemical purity was 99.1% by TLRC (EtOAc: CH_2Cl_2, 2:8).



Synthesis of [^{3}H]potassium canrenoate (7a) and [^{3}H,^{35}S]spironolactone (6a, 6b). The asterisk denotes sulfur-35.

17-Hydroxy-3-oxo-17α-pregna-4,6-diene-21-^{14}C-carboxylic acid, γ-lactone, 5c. [^{14}C]Canrenone was prepared similarly to the tritium labelled compound (5a) from [^{14}C]3c (463 mg, 1.35 mmole, 33.3 mCi) via DDQ oxidation of the enol ether to yield 215 mg (0.631 mmole, 9.85 mCi) of recrystallized product. HPLRC showed 99.2% radiochemical purity.



Synthesis of carbon-14 labelled canrenone (5c), potassium canrenoate (7c), and spironolactone (6c).

7α-(Acetylthio)-17-hydroxy-3-oxo-17α-pregna-4-ene-21-^{14}C-carboxylic acid, γ-lactone, 6c. A solution of the filtration liquor residue from 5c (106 mg, 7.34 mCi, RCP = 96.3%) with 0.1 mL of thiolacetic acid in 1.0 mL of MeOH gave 6c at a specific activity of 24 mCi/mmol and a radiochemical purity by HPLRC of 99.3%.

17-Hydroxy-3-oxo-pregna-4,6-diene-21-^{14}C-carboxylic acid, monopotassium salt, 7c. From [^{14}C]canrenone (73.2 mg, 0.215 mmol, 5.09 mCi) and 2.00 mL of 0.102 N KOH, the potassium salt (7c) was prepared similarly to [^{3}H]potassium canrenoate (7a). Yield was 80.3 mg with a specific activity of 22.9 mCi/mmole. HPLRC indicated a radiochemical purity of 97.7% as the lactone 5c after H_3PO_4 treatment.

DISCUSSION

Three separate catalysts were investigated for the reduction of the cross-conjugated dienone 1 with hydrogen. All gave rise to a mixture of the desired product 2 plus the isomeric products of over-reduction, 8a and 9a. The rate of reduction decreased as the catalyst was changed from Pd/C to Pd/Al_2O_3. Use of $(Ph_3P)_3RhCl$ required a 200% loading to complete the reduction in 2.8 hours. This high loading created an extra difficulty in purifying the product. Our experience with tritium catalytic reductions led to the choice of Pd/C; slower acting catalysts often gave poor uptake of the isotope.

The reduction was next studied with a tritium/hydrogen mixture (98 μCi/mmole) at approximately one atmosphere over Pd/C in a Fisher-Porter bottle. The reaction was stopped after five minutes and gave incomplete reduction of 1 to a mixture of 45% 2, 43% 8a, and 12% 9a. A separate reduction, stopped at 40 minutes, gave 38% 2, 36% 8a, and 26% 9a.

The catalytic reduction with high specific activity tritium gas at DuPont-NEN was reported complete after 12 minutes and the product ratios were 30% 2, 55% 8a, and 15% 9a. Product ratios were calculated from TLRC results and are expressed as mole percent. The radiochemical percent of $^{3}H_4$ compounds 8a and 9a was assumed to be twice the molar percent of $^{3}H_2$ compound 2.

Unlabelled carrier was twice added to intermediate 2 during the purification and exchange procedures to aid laboratory manipulations. An approximate specific activity after chromatographic purification of 2 indicated 2 Ci/mmole. This is substantially lower than 9 Ci/mmole

expected from incorporation of tritium at 30 Ci/matom and dilution with carrier. However, catalytic reductions with tritium in toluene may involve tritium exchange with the solvent. This may be even more pronounced under the conditions for this reduction where the catalyst was pre-reduced before adding the dienone 1. An additional lowering of specific activity could have resulted from partial loss of the labile tritium at position 2 during the LPLC purification on silica gel. If the label was equally distributed at C-1 and C-2, the base catalyzed exchange should remove 50% of the tritium. The loss of only 33% of the label suggests that enolizable tritium may have been lost before the sodium hydroxide treatments.

The orientation of the tritium atom in compound 3a was 61% β and 25% α as shown by ^{3}H NMR analysis. Approximately 4% of the tritium remained at position 2. Predominant 1,2β addition of isotopic hydrogen has been reported for catalytic reduction of a cross-conjugated ring-A steroid dienone using palladium on charcoal[5]. However, the α-addition was predominant for similar reductions of both 5α-androst-1-ene-3,17-dione[4] and 17β-hydroxy-19-nor-5α-androst-1-ene-3-one[2].

Oxidation of 3a to the conjugated 4,6-dienone 5a was accomplished via the intermediate enol ether 4a with DDQ [12]. The enol ether 4a underwent decomposition on attempted analyses by TLRC. The product isolated after flash column chromatography was 98.5% canrenone 5a and 1.0% starting enone 3a by HPLRC analysis. Crystallization improved the purity to 99.9% 5a. A literature report describes the loss of tritium[1] from position 2 during an oxidation of a 3-keto-4-ene steroid with chloranil[13] to produce the conjugated dienone. We investigated the DDQ oxidation of unexchanged low specific activity enol ether prepared from 2, but found that the loss of label was incomplete.

For preparation of [^{3}H]potassium canrenoate (7a) the highest purity recrystallized [^{3}H]canrenone (5a) was used in slight excess to potassium hydroxide. The work-up removed unreacted 5a by trituration

with ethyl acetate as the only purification procedure. [^{3}H]Potassium canrenoate was greater than 97.2% pure in all TLRC systems; the only contaminant was 2.3% [^{3}H]canrenone which may be due to silica catalyzed cyclization.

The conversion of canrenone to spironolactone produces a product of higher purity than the starting material by direct crystallization from methanol[7]. Use of 94.5% 5a from its crystallization liquors gave 6a, which after crystallization, was > 99% pure by both TRLC and HPLRC. The yield of crystallized product was 72.5% at a specific activity of 90.6 mCi/mmole.

Evidence that the reaction conditions for preparing [^{35}S]spironolactone did indeed proceed from an in-situ generation of [^{35}S]thiolacetic acid came from HPLC detection of thiolacetic acid as a product from the reaction of sodium sulfide, acetic anhydride, and acetyl chloride. Other identified products were acetic acid and hydrogen sulfide. Similar results were obtained with [^{35}S]sodium sulfide. An Aminex HPX-87H ion exclusion column with a mobile phase of 0.005M sulfuric acid was used.

After the reaction of [^{35}S]thiolacetic acid with excess canrenone, examination of the reaction mixture suggested 44% of the radioactivity as [^{35}S]spironolactone by TLRC. Since both canrenone and spironolactone have similar chromatographic properties, the reaction mixture containing excess canrenone was reacted with unlabelled thiolacetic acid to yield spironolactone for flash column purification.

In the carbon-14 synthesis, catalytic reduction of the acetylenic bond gave substantial quantities of the 5,6-saturated compound 12. A small quantity of this undesired by-product was seen in the pilot studies; literature descriptions indicate a clean product under identical conditions[7]. Chromatographic resolution of the enone 3c from the 3-keto compound 9c was much better than for their 3-hydroxy precursors which made separation of the ketone intermediates the preferred strategy. The reaction sequence from enone 3c followed the route used for the tritium labelled compounds.

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