Synthesis of Dexamethasone Labeled with Carbon-14 and Tritium

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SUMMARY

The syntheses of dexamethasone-16α-(methyl)-^{14}C and dexamethasone-16β-^{3}H are described. Tritium labeling was achieved by catalytic addition of tritium to 3-acetoxypregn-16-ene-11,20-dione, followed by bromination and dehydrobromination to give 3α-acetoxy-16-^{3}H-pregn-16-ene-11, 20-dione. The stereochemistry of this sequence of reactions has been studied in detail. Carbon-14 was introduced into the steroid nucleus by reaction of methyl-^{14}C-magnesium iodide with 3α-acetoxypregn-16-ene-11,20-dione. These tritium and carbon-14 labeled intermediates were then carried through to the corresponding labeled dexamethasone by parallel reaction sequences.

INTRODUCTION

Dexamethasone labeled with tritium at positions 1, 2, and 4 [1], and with general tritium labeling [2] has been reported previously, but labeling of this substance with carbon-14 has not been described. Many requests have come to us for samples of the labeled materials, to permit studies of absorption, metabolism, etc.; and we now wish to report the synthesis of dexamethasone labeled with carbon-14 in the 16α-methyl carbon, and separately with tritium in the 16β-hydrogen. Our studies with tritium provide information on the stereochemical specificity of the hydrogenation of the substituted pregn-16-ene (I), as well as for related bromination and dehydrobromination reactions.

Tritium was introduced by hydrogenation of 3α-acetoxypregn-16-ene-11,20-dione (I) with high specific activity tritium/hydrogen over palladium on charcoal catalyst to form 3α-acetoxy-16α, 17α-^{3}H-pregnane-11, 20-dione (II-^{3}H) [3]. Benzene was chosen as a medium for this reduction to minimize exchange of tritium with the solvent, and thus to avoid complications of the

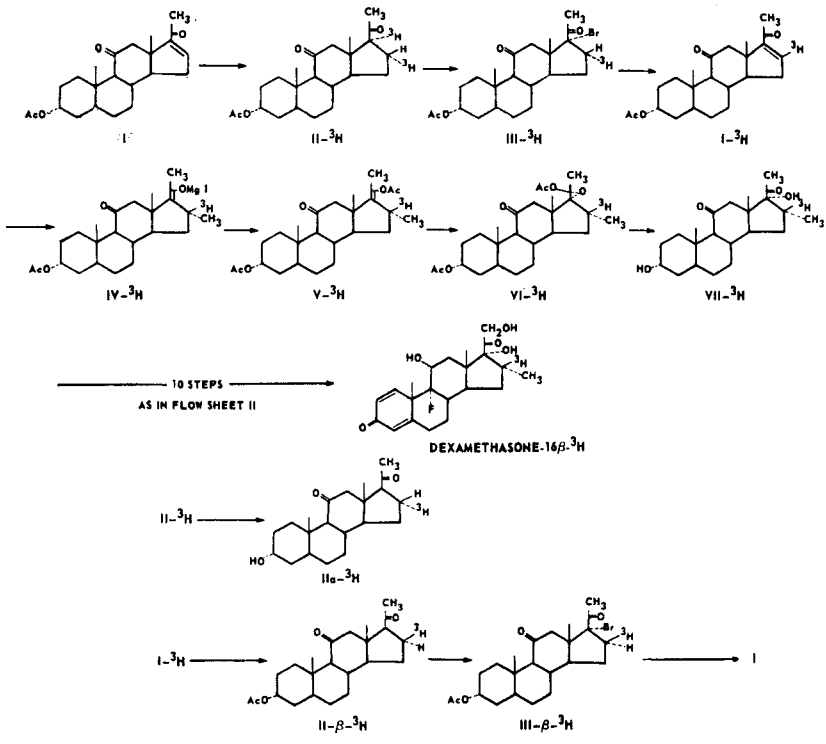
sort reported with platinum in acetic acid [4a] or alcohol [4b]. After removal of labile tritium by repeated treatment with methanol, a small sample of the high specific activity reduction product was diluted with purified carrier for a detailed study of the bromination and dehydrobromination steps. The diluted II-^{3}H was recrystallized from 95 % ethanol to constant specific activity and single spot thin layer chromatography (T.L.C.) purity. Subsequent intermediates (III-^{3}H, I-^{3}H, II-β-^{3}H, III-β-^{3}H, IIa-^{3}H) in the bromination, dehydrobromination, rehydrogenation, and equilibration studies were similarly purified from suitable solvents.

Light catalyzed bromination of II-^{3}H with 1,3-dibromo-5,5-dimethylhydantoin in dry chloroform gave 3α-acetoxy-17α-bromo-16α-^{3}H-pregnane-11,20-dione (III-^{3}H). This transformation resulted in a loss of 21.7 % of the tritium from the molecule, and represents that tritium which had been at 17α. Dehydrobromination of III-^{3}H by refluxing with pyridine gave 3α-acetoxy-16-^{3}H-pregn-16-ene-11,20-dione (I-^{3}H) with a further drop of only 3.5 % in the molar specific activity. This tritium must have been removed from locations in the molecule other than 17α (where bromine had already replaced the tritium) and 16α (if the dehydrobromination is exclusively trans), and most likely represents loss of tritium from enolizable positions. In the liquid distilled from the acidified aqueous side stream from this dehydrobromination was found radioactivity corresponding to 2.6 % of the starting III-^{3}H radioactivity. For comparison with the bromination-dehydrobromination sequence, a sample of II-^{3}H was subjected to hydrolysis and equilibration with alcoholic sodium methoxide. This treatment, which removed the acetyl from the 3α-oxygen and equilibrated all enolizable hydrogens, gave 16α-^{3}H-pregnan-3α-ol-11,20-dione (II*a*-^{3}H) with a molar specific activity 27.4 % less than that of the acetate II-^{3}H. This loss of tritium upon hydrolysis-equilibration is comparable to that noted in the bromination-dehydrobromination sequence. In each of these cases the 17α-hydrogen and all other enolizable hydrogens are equilibrated or removed. The main point of difference is in the removal of hydrogen at C-16. In the bromination-dehydrobromination sequence the 16β-hydrogen is removed, while in the other study neither of the hydrogens at C-16 is disturbed. The good agreement between the two separate treatments thus indicates that little or no tritium was at 16β.

Discussion

A commonly accepted mechanism for catalytic hydrogenation of olefins is that postulated by Horiuti and Polanyi [5]. Exchange of hydrogen on carbon atoms near as well as on those comprising the double bond during, or as part of, a stepwise hydrogen transfer process is included in the scope of this mechanism [6]. Accordingly, it seemed necessary to examine the possibility that tritium had also entered the steroid molecule at the allylic position,

FLOW SHEET I



C-15. Purified I-^{3}H from the aforedescribed dehydrobromination was again hydrogenated under conditions approximately the same as those used for the initial introduction of tritium, but with ordinary hydrogen. The product from this hydrogenation, 3α-acetoxy-16β-^{3}H-pregnane-11,20-dione (II-β-^{3}H), had a molar specific activity almost the same as that of its precursor (I-^{3}H). This finding indicates that the extent of exchange of hydrogen adjacent to or near the double bond is small relative to the incorporation of hydrogen at C-16.

A further series of experiments was carried out to demonstrate the extent of permanent introduction of tritium at positions other than C-16. A sample of high specific activity I-^{3}H was diluted with carrier and purified. This material was then subjected to hydrogenation, as before, with ordinary hydrogen. The resulting product was carried through the sequence II-β-^{3}H to I (i.e. to de-16-^{3}H-I). The radioactivity was decreased by about 70 % by this series of treatments. If it is assumed that labeled hydrogen is exclusively at C-16 and in the β-configuration after rehydrogenation, that bromine enters exclusively at 17α, and that dehydrobromination is exclusively a *trans* elimination,

this sequence should have removed all the radioactivity from the molecule. The finding that all the radioactivity was not so removed in conjunction with the rehydrogenation experiments already discussed, allows the conclusion that tritium may have been introduced to some extent at non-labile positions other than at C-16, and/or that the reactions of the sequence I to I-^{3}H are less than completely stereospecific. A definitive answer as to which of these alternatives predominates is being sought in work designed to selectively remove the hydrogen at C-15.

The specific activity of tritium in the high specific activity initial hydrogenation product (II-^{3}H) was 480 mCi/mmole. The tritium enriched hydrogen

TABLE I. Dexamethasone and Intermediates

Material	mCi/mmole [a]
II-^{3}H[b]	533
III-^{3}H[b]	408
I-^{3}H[b]	385
VII-3H[b]	375
XVII-^{3}H[b]	351[c]
XVII-^{3}H[f]	222
Dexamethasone-16 β-^{3}H[g]	69.9
II-^{3}H[d,e]	0.207
II-β-^{3}H[e]	0.153
IIa-^{3}H[e]	0.151
III-^{3}H[e]	0.165
I-^{3}H[e]	0.156
I-^{3}H[h]	0.774
II-β-^{3}H[h]	0.867
III-β-^{3}H[h]	0.593
I[h,i]	0.262

[a] The tritium specific activities have been corrected for radioactive decay.

[b] Main sequence. These specific activities are comparable to the tritium-hydrogen mixture used for double bond saturation.

[c] The deviation of this specific activity from that of VII-^{3}H is due to known presence of inert impurity.

[d] Diluted with carrier, then carefully purified.

[e] These intermediates comprise a series used to study the stereochemistry of hydrogenation, and dehydrobromination. They were each carefully purified, and are inter-related as to specific activity.

[f] Main sequence. After dilution with carrier at this stage.

[g] Main sequence. A further dilution with carrier was made before this, the final product was given its final recrystallization, to reduce radiochemical decomposition on storage.

[h] These four entries comprise a second, independent series of diluted carefully purified compounds, derived from main stream I-^{3}H.

[i] Although some tritium remains, this compound is considered as "unlabeled" since it has been through a sequence designed to remove the specific 16 β-^{3}H label.

used to prepare this product had a specific activity (see experimental) of 595 mCi/mmole (297.5 mCi/mg atom). When this value is compared to those found for intermediates VII-^{3}H and XVII-^{3}H (see Table 1), it may be seen that about 1.2 enriched atoms per molecule have been permanently introduced. This finding is compatible with the conclusions (based on the diluted, carefully purified intermediates) already discussed. Changes in specific activity through intermediates II-^{3}H, III-^{3}H, and I-^{3}H in the high specific activity series where the material was carried through without rigorous purification were comparable to those observed in the diluted, purified series (see Table I).

The results of the aforedescribed experiments permit the conclusion that the predominating stereochemical course for the reaction sequence I to I-^{3}H is : Addition of hydrogen to the less hindered side of the molecule to give the 16α,17α-di-^{3}H derivative II-^{3}H (*); displacement of the 17α-hydrogen (some ^{3}H; see footnote * by bromine, with retention of configuration of the side chain **; and *trans* elimination of hydrogen and bromine from C-16 and C-17, leaving a marked hydrogen at C-16.

Treatment of 3α-acetoxy-16-^{3}H-pregn-16-ene-11,20-dione (I-^{3}H) with methylmagnesium iodide, followed by acetylation, using the procedure described by Cutler *et al.* [8], epoxidation, and hydrolysis [9,10], gave 16α-methyl-16β-^{3}H-pregnane-3α,17-diol-11,20-dione (VII-^{3}H). The tritium which entered the molecule at C-16 in the α-configuration was thus shifted by these transformations to the 16β-position, where it remained fixed throughout the remainder of the synthesis.

Treatment of I with methyl-^{14}C-magnesium iodide, using a sequence exactly as described for the tritium marked series, gave 16α-^{14}C-methyl-pregnane-3α,17-diol-11,20-dione (VII-^{14}C).

16α-Methyl-16β-^{3}H-pregnane-3α,17-diol-11,20-dione (VII-^{3}H) and 16α-^{14}C-methylpregnane-3α,17-diol-11,20-dione (VII-^{14}C) were carried through to the respective dexamethasone-16β-^{3}H and dexamethasone-16α-(methyl)-^{14}C via the indicated sequence, using previously reported procedures for these transformations [11,12,13].

To limit radiochemical decomposition of the tritium labeled intermediates and product (see [14]), dilutions with carrier were made at the 16α-methyl-16β-^{3}H-prednisolone 21-acetate (XVII-^{3}H) stage, and again with the final product, dexamethasone-16β-^{3}H, before its final recrystallization. Constancy of the specific activities of the intermediates of both the ^{3}H- and ^{14}C- labeled series (see Table I and II) were well within the limits set by the known or estimated purity of the intermediates and the standard deviation of the radio-

* The incorporation of labeled hydrogen at C-17, remaining after neutral methanol equilibration, but quantitatively removed by bromination in the II-^{3}H to III-^{3}H conversion or by the alkoxide treatment, was about one fourth the permanently bound amount.

** Orientation of entering bromine in this series (at C-17) has been well established as "α". See [7].

TABLE II. Dexamethasone-16α-(methyl)-^{14}C and Intermediates

Material	mCi/mmole	Δ %[a]
VII-^{14}C	4.03	3.6
XII-^{14}C	4.27	2.2
XVII-^{14}C	4.10	1.9
Dexamethasone-16α-(methyl)-^{14}C	4.33	3.6
Dexamethasone-16α-(methyl)-^{14}C 21-acetate	4.19	0.2

[a] Deviation, expressed as per cent, for the mCi/mmole of the materials listed versus average of the specific activities for these five compounds.

activity measurements. The specific activity of the dexamenthasone-16β-^{3}H was 69.9 mCi-mmole, while that of the dexamethasone-16α-(methyl)-^{14}C was 433. mCi-mmole.

EXPERIMENTAL

Melting points were measured in open capillaries in a modified Hershberg apparatus, with totally immersed thermometers (calibrated), and are not corrected. Thin layer chromatography was done on silica gel G plates with adsorbent 250 microns in thickness. Radioactivity measurements were made with a Packard Instrument Compagny "Tri-Carb" liquid scintillation spectrometer, Series 3000, using a phosphor system comprised of 3 g of 2.5-diphenyloxazole (PPO) and 100 mg of 1,4-*bis*-[2-(4-methyl-5-phenyloxazolyl)]-benzene (dimethyl POPOP) in a mixture of 700 ml of toluene and 300 ml of ethanol (20 ml per measurement). All tritium counts are corrected for radioactive decay. Nitrogen was bubbled through the counting solutions to eliminate oxygen quenching; and the internal standard method was applied to correct for chemical quenching.

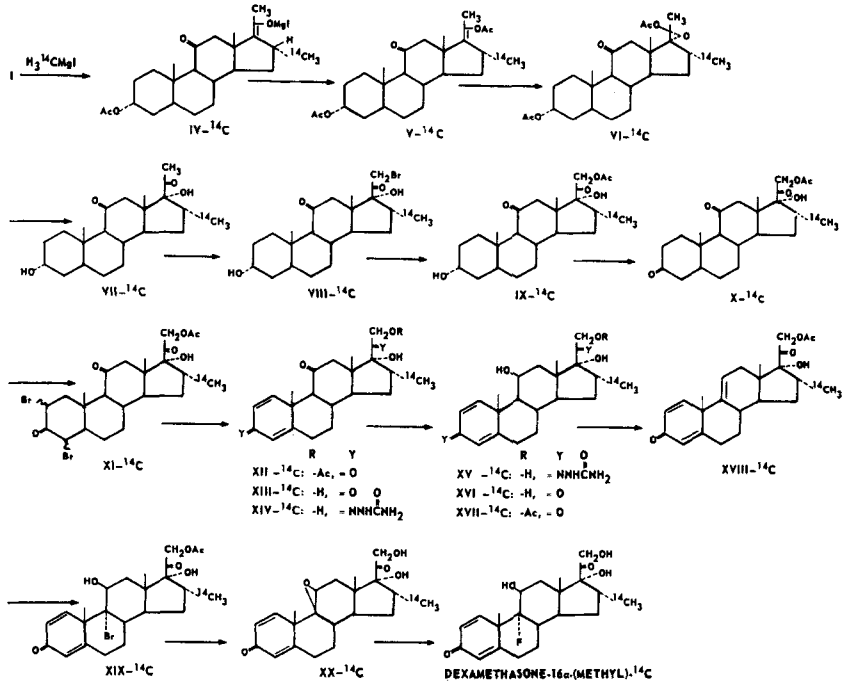
Specific Activity of Tritium Hydrogen Mixture. — Three replicate samples of the gas used for the labeling hydrogenation were combusted to water over hot copper oxide ***. The water was collected and aliquots were measured by liquid scintillation counting. The average value so determined was 595 mCi/mmole, 297.5 mCi/mg atom ± 4 %.

3α-Acetoxy-16α,17α-$^{3}H_2$-pregnane-11,20-dione (II-^{3}H).

A solution of 7.45 g (20 mmoles) of 3α-acetoxy-pregn-16-ene-11,20-dione (I) in 30 ml of benzene with 1.5 g of 5 % palladium on charcoal catalyst was shaken under tritium/hydrogen (specific activity 595 mCi/mmole) at

*** We express our appreciation to Mr. Andrew Reich of these laboratories for assistance in the combustion experiments.

FLOW SHEET II



760 mm and 22° C for two hours. Total uptake as measured by burette and corrected to S.T.P. was 494 ml (22 mmoles). The solution was filtered from the catalyst. Solvent was removed under reduced pressure. The crude product was dissolved in 125 ml of methanol and allowed to stand for eighteen hours. The methanol was removed under vacuum and the dissolution, standing, evaporation cycle, was repeated twice more with fresh methanol. After the final methanol was removed three 50 ml portions of hexane were added and distilled off under reduced pressure. A final 50 ml of hexane was distilled (atm.) down to a volume of 20 ml, giving a thick slurry of the product II-^{3}H which was collected and air dried. So obtained was 6.50 g (17.3 mmoles) (86.8 %), m.p. 125-127.5° C. Specific radioactivity 533 mCi/mmole.

Approximately 1 mg of this high specific activity product was added to 2 g of carrier 3α-acetoxypregnane-11,20-dione and the combined material was recrystallized six times from 95 % ethanol. Material so prepared had a m.p. of 133.5-134° C (lit. [15] m.p. 132-133° C), and gave a single spot on T.L.C. (developed with a mixture of methylene chloride and 2-propanol, 6 : 1; visualized with iodine vapor and radioactivity scan). The specific activity of this material was 0.197 mCi/mmole.

16α-^{3}H-PREGNANE-3α-OL-11,20-DIONE (IIA-^{3}H).

To a solution of 34.7 mg (0.1 mmole) of II-^{3}H (specific activity 0.197 mCi/mmole) in 10 ml of methanol (purified by refluxing with and distilling from sodium hydroxide pellets) and under an atmosphere of nitrogen was added a solution of 10.8 mg (0.2 mmole) of sodium methoxide in 1 ml of methanol. The resulting solution was stirred under nitrogen for three hours, during which time it was warmed to reflux for one and one half hours. With ice cooling, a solution of 0.34 ml (0.357 g, 0.59 mmole) of glacial acetic acid in 2 ml of water was added. The solvent was removed to near dryness under reduced pressure, and the crude product was isolated by ether extraction. Recrystallization from hexane to constant m.p. (175-176° C; lit. [3,15] 175-177° C and 172-174° C) and constant specific activity, 0.151 mCi/mmole, and single spot TLC purity, gave 9.6 mg of IIa-^{3}H (0.03 mmole; 15 %).

3α-ACETOXY-17α-BROMO-16α-^{3}H-PREGNANE-11,20-DIONE (III-^{3}H).

A solution of 1.00 g (2.67 mmoles) of II-^{3}H specific activity 0.207 mCi/mmole, and 0.386 g (1.32 mmole, 2.64 m eq.) of 4,4-dimethyl-1,3-dibromohydantoin in 15 ml of chloroform was irradiated with a photoflood lamp (R-34) while stirring in a cold bath to hold the temperature at 0° ± 2° C. In about fifty minutes all the active bromine was consumed. The mixture was washed well with water, and the chloroform was removed under reduced pressure. Trituration of the resulting oil with methanol gave a slurry of III-^{3}H which was collected and dried. The crude product (1.059 g, 2.34 mmoles, 87.7 %) was recrystallized four times from chloroform-methanol to yield material with m.p. 170.5-171° C (lit. [7,15] m.p. 168-171° C, and 168-170° C), and specific activity 0.161-0.165 mCi/mmole, single spot TLC, with specific activity constant over the last two recrystallizations.

A sample of II-β-^{3}H with a specific of 0.867 mCi/mmole was brominated in a similar manner to provide 3α-acetoxy-17α-bromo-16β-^{3}H-pregnane-11,20-dione (III-β-^{3}H) with specific activity 0.593 mCi/mmole.

In another experiment 6.50 g (17.3 mmoles) of II-^{3}H with specific activity 533 mCi/mmole (main sequence) was transformed to 6.29 g (13.9 mmoles, 80.3 %, of II-^{3}H, with m.p. 168.5-170° C, and specific activity 408 mCi/mmole.

3α-ACETOXY-16-^{3}H-PREGN-16-ENE-11,20-DIONE (I-^{3}H).

A mixture of 815 mg (1.8 mmole) of III-^{3}H, specific activity 0.165 mCi/mmole, gave upon refluxing for three hours with 2 ml of dry pyridine, followed by precipitation with water, 657.5 mg (1.76 mmole, 98.1 %) of I-^{3}H. After five recrystallizations from ethanol, the specific activity was constant (at 0.156 mCi/mmole), m.p. 170-171.5° C (lit. m.p. : [3b] 197-198° C, and 205-206° C [17]; 170-171° C [18]; 163-165° C and [19] 167-169° C).

The aqueous mother liquor from the precipitation of crude product was made strongly acidic with conc. sulfuric acid, and was allowed to stand at 20-22° C for eighteen hours. Some of this liquid was then vacuum distilled two times, with precaution against mechanical carry over. The specific radioactivity of the final distillate was 0.241 μCi/ml. The volume after acidification before distillation was 32 ml. Total radioactivity in this aqueous stream is thus 7.72 μCi, 2.6 % of that in the III-^{3}H charged to this reaction.

In a manner similar to that described for the low specific activity material, but with a single recrystallization of the product, 6.5 g (15.1 mmoles) of III-^{3}H with specific activity 408 mCi/mmole was transformed to I-^{3}H specific activity 385 mCi/mmole, m.p. 166.5-169.5° C, 5.31 g (14.2 mmoles), 94.3 %.

A separate, low specific activity, series employing 3α-acetoxy-17α-bromo-16β-^{3}H-pregnane-11,20-dione (III-β-^{3}H) with specific activity 0.593 mCi/mM gave, after four recrystallizations of the crude product, I which still contained some tritium as evidenced by the specific activity, 0.262 mCi/mmole.

3α-Acetoxy-16β-^{3}H-pregnane-11,20-dione (II-β-^{3}H).

A solution of 400 mg (1.07 mmole) of I-^{3}H, specific activity 0.156 mCi/mmole, in 30 ml of benzene with 0.08 g of 5 % palladium on charcoal catalyst was shaken with hydrogen at 760 mm ± 100 mm at 22° C for four hours. Hydrogen uptake was not measured. After work-up in the usual manner, the resulting II-β-^{3}H was recrystallized four times from 95 % ethanol to give pure material, m.p. 133-134° C, specific activity 0.153 mCi/mmole, constant across all four crystallizations, and single spot TLC.

In a separate run, using a sample of I-^{3}H with specific activity of 9.774 mCi/mmole which had been prepared by carrier dilution of the high specific activity I-^{3}H, the derived II-β-^{3}H had a specific activity of 9.867 mCi/mmole.

16α-Methyl-16β-^{3}H-pregnane-3α,17α-diol-11,20-dione.

To a suspension of 31.5 mmoles of methylmagnesium iodide in tetrahydrofuran (THF) was added 0.43 g (2.17 mfw.) of anhydrous cuprous chloride as a suspension in THF. To this mixture was added 5.13 g (14.2 mmoles) of I-^{3}H, specific activity 385 mCi/mmole, as a solution in THF. The resulting mixture was stirred for thirty minutes in the ice bath, then 17.0 g (16.6 mmoles) of acetic anhydride was added and the resulting slurry was again aged for thirty minutes. The mixture was then quenched with water, benzene was added, and the two-phase mixture was filtered through filter aid. The benzene layer was separated and was dried over magnesium sulfate.

The benzene solution of V-^{3}H was filtered and concentrated to one third volume. To it was added 75 ml of 0.62 N monoperphthalic acid in ethyl acetate. After one hour at 60° C, the solution was cooled, and the excess peracid was quenched with sodium bisulfite. The organic layer from the

quenched mixture was separated and washed with water. Solvent was removed at reduced pressure, and the resulting crude VI-^{3}H was dissolved in purified methanol. Hydrolysis with sodium hydroxide (2.5 g in 10 ml of water; one hour at 30-34° C, under nitrogen) followed by water to precipitate the product gave 4.06 g (11.2 mmoles) (78.8 % from I-^{3}H) of 16α-methyl-16β-^{3}H-pregnane-3α,17-diol-11,20-dione (VII-^{3}H), m.p. 185.5-187° C (lit. [11] m.p. 185-187° C), specific activity 375 mCi/mmole.

16α-^{14}C-METHYLPREGNANE-3α,17α-DIOL-11,20-DIONE (VII-^{14}C).

To a suspension of 620 mg (25.5 m.a.w.) of magnesium turnings in 5 ml of dry (< 0.1 mg water/ml) ether under an atmosphere of dry argon was added 186 mg (c.a. 1 mmole) of 1,2-dibromoethane. Evidence of reaction (bubbling due to liberation of ethylene, etc.) indicated that the system was dry and reactive and ready for the introduction of methyl iodide. Methyl-^{14}C iodide was purified by distillation from copper, followed by distillation from sodium hydride. To the suspension of activated magnesium in ether, with stirring was then added 3.125 g (22 mmoles) of methyl-^{14}C iodide with 92 mCi of carbon-14, specific radioactivity 4.2 mCi/mmole, as a solution in 1-2 ml of ether. Reaction began promptly, and proceeded smoothly to furnish methyl-^{14}C-magnesium iodide. When the Grignard formation was complete, the ether was displaced with tetrahydrofuran, and after the addition of a THF slurry of 0.30 g of cuprous chloride, a solution of 5.85 g (15.7 mmoles) of 3α-acetoxy-pregn-16-ene-11,20-dione (I) was introduced. Reaction of the magnesium enolate so prepared with acetic anhydride (18.7 g, 18.3 mmoles), followed in turn by epoxidation with 52.2 ml (64.5 m eq.) of 1.23 N monoperphthalic acid in ethyl acetate. After hydrolysis, the mixture was worked up, as described for the tritium labeled series, to give 4.65 g (12.8 mmoles), 81.8 % (from the steroid (I) precursor), 58.3 % (from the methyl-^{14}C iodide) of 16α-^{14}C-methyl-pregnane-3α,17α-diol-11,20-dione (VIII-^{14}C), specific activity 4.03 mCi/mmole.

The specific activity of the final product dexamethasone-16α-(methyl)-^{14}C derived from this intermediate was 4.3 mCi/mmole. The average specific activity of intermediates, including the VII-^{14}C, was 4.23 mCi/mmole. The specific activity of the starting methyl-^{14}C iodide, a combination of several samples of material, was estimated from this average value. Variations from the series average as indicated in Table II, while so small as to be near the standard deviation of the specific activity measurements, can easily be equated with the estimated bulk purity of the intermediates. Only the final product was carefully purified.

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