PREPARATION OF [2α-$^{14}CH_3$]-DIHYDROTESTOSTERONE 17-PROPIONATE*

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SUMMARY

An efficient preparation of a very large quantity (650 mCi) of [2α-$^{14}CH_3$]-dihydrotestosterone 17-propionate by methylation of the N,N-dimethylhydrazone derivative of dihydrotestosterone is described. A variety of "classical" methylation methods failed to produce the desired product in satisfactory yields unless an unacceptably large excess of $^{14}CH_3I$ was employed.

Key Words: Dimethylhydrazone, Alkylation, Carbon-14

INTRODUCTION

Metabolic and tissue residue studies in cattle involving the anabolic agent 2α-methyldihydrotestosterone 17-propionate required the preparation of at least 500 mCi of the C-14 analogue of this material. In view of the very large quantity of labeled product needed, it was essential to devise a cost-effective synthesis.

Methylation of dihydrotestosterone 17-THP (2) with methyl-^{14}C iodide was deemed to be the only practical approach to this problem.** Several of the usual methylation methods were tried; none gave satisfactory results unless a large excess of alkylating agent was used. [2α-$^{14}CH_3$]-dihydrotestosterone (2α-$^{14}CH_3$-DHT) (6) was finally obtained in good radiochemical yield by methylation of the N,N-dimethylhydrazone (DMH) derivative 3 to 4.

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**Other groups could, of course, have been used to protect the 17-hydroxy function.

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DISCUSSION

Reactions which are ordinarily taken for granted in organic chemistry sometimes present formidable problems when applied to radiochemical synthesis. Often the reasons for such problems are not obvious, but in the present case the cause of our difficulties soon became apparent.

In non-radioactive alkylations, the alkylating agent is normally the inexpensive component of the reaction and is thus used in large excess. Indeed, various reported preparations of 2α-methyl DHT, whether by methylation of DHT or its derivatives[1,2,4] or by formylation followed by catalytic hydrogenation,[3,4] utilize large excesses of the appropriate electrophile. But our attempts to either methylate or formylate DHT using 1 equivalent of methyl iodide or ethyl formate, respectively, under the conditions listed below, resulted in very complex reaction mixtures and/or low yields (< 20%):

a) lithium diisopropylamide (LDA) in THF, with or without hexamethylphosphoramide/DHT/CH_3I;[5]

b) (2)/NaH-CH_3OH/CH_3I;[1,2,4]

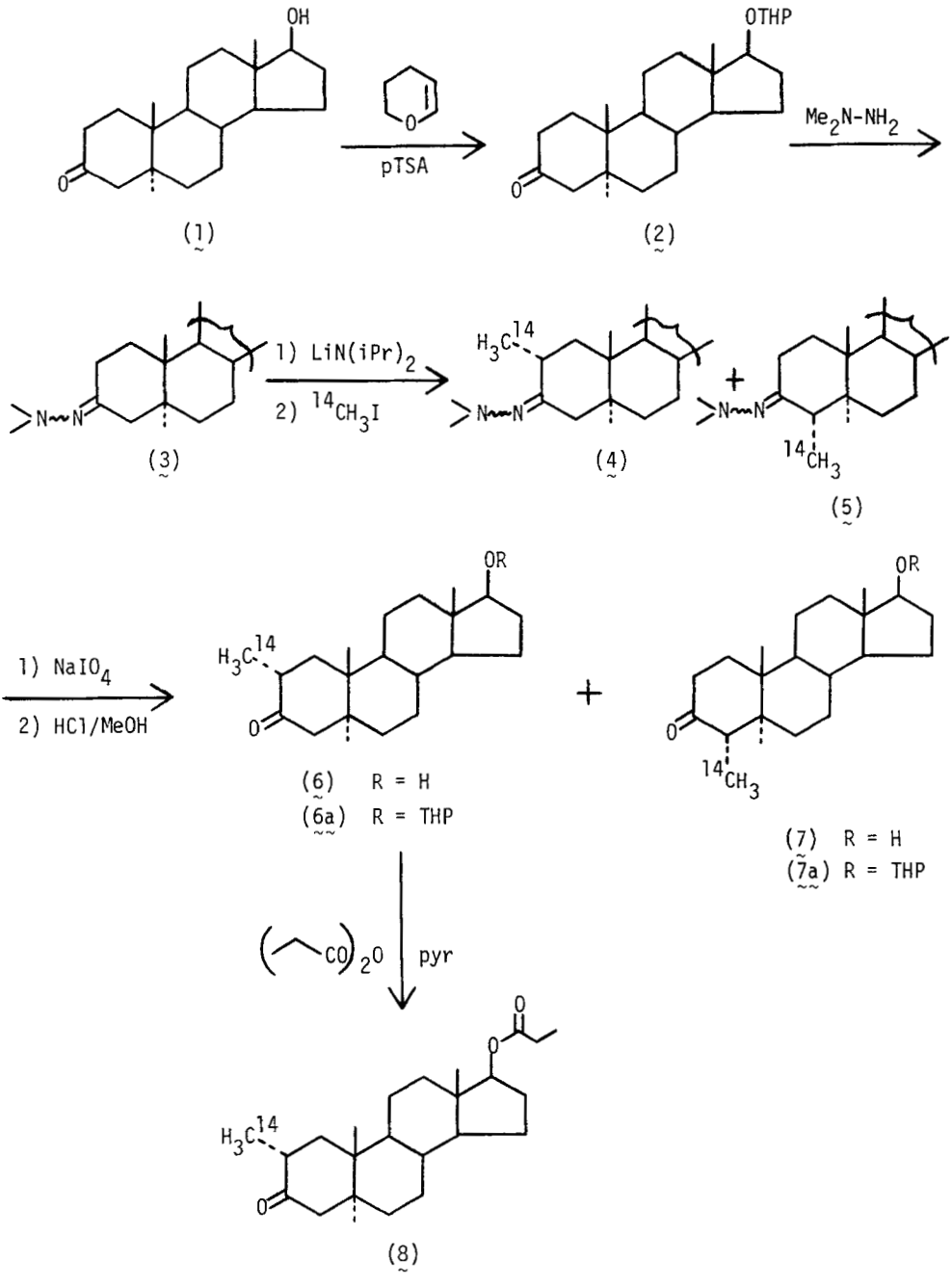
c) (2)/$(EtO_2C)_2$/K_2CO_3 or NaH or LDA/CH_3I;

d) (2)/methylmagnesium carbonate/CH_3I;[6]

e) (2)/ethyl formate/NaH-φH, then H_2/Pd-C.[3]

The last reaction afforded 6 in nearly quantitative yields when a four-fold excess of ethyl formate was used. When the excess was reduced, the yields became progressively worse. In an attempt to limit the amount of alkylating agent, many of the above reactions were tried using an excess of 2. We again obtained low yields and/or very complex reaction mixtures.

The desired methylation, with methyl-^{14}C iodide as the limiting reagent, was finally achieved by using the N,N-dimethylhydrazone[7] derivative 3 as the substrate (Scheme 1).


OH
pTSA
OTHP
Me_2N-NH_2
(1)
(2)
1) $LiN(iPr)_2$
2) $^{14}CH_3I$
(3)
(4)
(5)
1) $NaIO_4$
2) HCl/MeOH
OR
(6) R = H
(6a) R = THP
(7) R = H
(7a) R = THP
pyr
(8)

Scheme 1

The 17-hydroxyl of DHT 1 was first protected as the tetrahydropyranyl ether 2. Treatment of 2 with N,N-dimethylhydrazine in refluxing ethanol afforded 3 in 93% yield. Metallation of 3 with LDA in THF at -78° C followed by addition of one half equivalent* of methyl-^{14}C iodide afforded a product in 98.5% radiochemical yield whose chromatographic mobility was equal to that of 4. Reaction of this product with $NaIO_4$ in THF/phosphate buffer[7] served to regenerate the 3-keto function 6a. When the 17-THP was removed with dilute methanolic HCl, the major product seemed indeed to be the desired 2α-$^{14}CH_3$-DHT 6 However, very careful chromatography (SiO_2, ethyl acetate-benzene, 1:9, run 3 times) revealed a slightly more polar radiochemical impurity. Separation of this mixture by column chromatography (SiO_2, ethyl acetate-benzene, 2:8) afforded pure 6 and the impurity in radiochemical yields of 65% and 15%, respectively. The impurity, isolated from a parallel cold reaction, was identified by nmr and mass spectrometry as the isomeric product, 4α-methyl-DHT 7.

Conversion of 6 to the 17β-propionate 8 was accomplished by acylation with propionic anhydride/pyridine. After aqueous workup and chromatographic purification the product was shown to be free of any contaminants due to 7 by reverse isotope dilution analysis.

We found our results somewhat surprising, since recent reports indicated that alkylations of ketone dimethylhydrazone anions were completely regiospecific (although there was some disagreement regarding the stereochemistry of the intermediate anion) even in symmetrical substrates.[7,8,9] In unsymmetrical substrates, Corey[7] has shown that the product resulting from alkylation of the more stable anion is formed exclusively. Thus, methylalkylketones alkylate exclusively on the methyl group, but benzylmethyl ketone alkylates only on the benzylic carbon. In any case, none of the reports cited indicate that mixtures were obtained during alkylation of either symmetrical (deuterium labeled)[8] or unsymmetrical substrates.

Since it is well known that 3-ketosteroids preferentially form the Δ-2(3) enolate, we naturally expected that treatment of 3 with $^{14}CH_3I$ would furnish

*Only one half equivalent was used in order to maximize incorporation of $^{14}CH_3I$.

the 2α-methyl product exclusively. A very recent report[10] demonstrated that alkylation of (Z)-[1-^{13}C]-3-pentanone DMH afforded a mixture of the 2- and 4-alkylated products in a ratio of (3:1). We were, however, unable to find precedent for our results in which a mixture of products was obtained from a DMH substrate in which formation of one anion was clearly preferable to another.

In spite of the lack of regiospecificity we encountered with our particular substrate, alkylation of the DMH derivative still afforded the desired product in good yield using less than one equivalent of labeled electrophile. The combined radiochemical yield of both isomers was, in fact, nearly quantitative. Thus, we feel that this is clearly the method of choice for the alkylation of ketones with labeled electrophiles.

EXPERIMENTAL

Methyl-^{14}C iodide (59.4 mCi/mmol) was purchased from Amersham Corp. All solvents were reagent grade and, except as noted, were used without purification. Radiochemical purity was determined by radio-tlc using a Packard Model 7201 Radiochromatography Scanner and/or reverse isotope dilution analysis. Radioactivity was determined using a Packard Tricarb Model 574 Liquid Scintillation Counter. Proton nmr spectra and mass spectra (of cold compounds from parallel reactions) were obtained using Varian HA 100 and Varian 112S spectrometers, respectively. Compounds for which spectral data is not given were identified by comparison of their chromatographic mobility on tlc against authentic standards.

17β-Hydroxyandrostan-3-one 17-THP (2)

To an ice cold solution of dihydrotestosterone (10 g; 34 mmol) in 30 ml of dry THF was added dihydropyran (15 g; 14 ml; 178 mmol) and 25 ml of a solution of p-toluenesulfonic acid in THF (1.75 mg/ml). The reaction was stirred overnight at ambient temperature. The solvent was removed by evaporation and the residue was dissolved in ether. After aqueous workup, the product was crystallized from methanol to afford the desired product 2 in 76% yield and greater than 98% purity [tlc: ethyl acetate-hexane (1:2)].

17β-Hydroxyandrostan-3-one 3-N,N-dimethylhydrazone 17-THP (3)

A solution of 2 (8.7 g; 23.2 mmol) and N,N-dimethylhydrazine (19.78 g; 25 ml; 329 mmol) in 30 ml of EtOH was stirred at reflux for 4 h. The solvent was evaporated at reduced pressure. The residue was stirred with toluene and taken to dryness again. The glassy residue was dissolved in ether and the solvent was slowly evaporated to afford a white crystalline solid 3 (9 g; 22 mmol) in 93% yield.

[2α-$^{14}CH_3$]-17β-hydroxyandrostan-3-one 17-propionate (8)

A 50 ml side-arm flask containing a stirring magnet was connected to a vacuum system and evacuated. Ten to fifteen milliliters of THF (over $LiAlH_4$) were distilled into the reaction vessel, which had been pre-cooled to -78° C. Lithium diisopropylamide was formed by injection of diisopropylamine (1.12 ml; 8 mmol) and n-BuLi (5 ml of 1.6 M solution; 8 mmol) through the rubber septum in the side arm and stirring at 0° C for 10 min. A solution of the dimethylhydrazone 3 (3.3 g, 7.9 mmol) in THF was injected and stirring was continued overnight at ambient temperature. The deep yellow solution was cooled to -78° C and methyl-^{14}C iodide (250 mCi; 59.4 mCi/mmol; 4.2 mmol), contained in a breakseal vial and connected to the vacuum manifold, was vacuum distilled into the reaction vessel. The reaction mixture was allowed to slowly warm to room temperature, and stirring was continued for an additional 3 h. Labile radioactivity was removed and collected in a cold trap and the reaction mixture was taken to dryness. The residual oils, 4 and 5, were dissolved in ethanol-benzene and assayed at 246 mCi (98.4% incorporation).

The solution was evaporated to dryness, redissolved in THF-H_2O (60 ml; 4:1) and pH 7 phosphate buffer (4 ml). Upon addition of 12 ml of a saturated sodium periodate solution the reaction mixture became dark red-brown in color. After stirring for 18 h, the reaction mixture was filtered and the yellow filtrate was extracted two times with methylene chloride. The solution containing crude 6a and 7a [tlc: ethyl acetate-toluene (1:9)] was evaporated to dryness. Treatment of the residue with methanol-10% HCl (3 h) effected removal of the 17-THP group [tlc: MeOH-toluene (5:95)]. Three additional alkylations were performed

as described above, yielding a total of 962 mCi (96.2% from $^{14}CH_3I$) of a mixture of crude 6 and 7.

Purification by column chromatography (5 x 143 cm; approximately 1.3 kg SiO_2; 20% EtOAc-hexane) afforded 699 mCi and 155 mCi of pure 6 and 7, respectively. NMR ($CDCl_3$), compound 6: δ 0.9 (3H, s, 18-CH_3), δ 1.0 (3H, d, J = 6 Hz, 2α-CH_3), δ 1.12 (3H, s, 19-CH_3); compound 7: δ 0.75 (3H, s, 18-CH_3), δ 0.92 (3H, d, J = 6 Hz, 4α-CH_3), δ 1.14 (3H, s, 19-CH_3). Finally, acylation of 6 with propionic anhydride in pyridine (25° C, 24 h) followed by chromatographic purification furnished 8 (650 mCi; 59.4 mCi/mmol) in 65% overall yield. The purity of the product was unequivocally demonstrated to be > 99% by reverse isotope dilution analysis.

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