SYNTHESIS OF [7-^{3}H]-3β,16β-DIHYDROXY-5-ANDROSTEN-17-ONE

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SUMMARY

A method has been described for the synthesis of tritiated [7-^{3}H]-3β,16β-dihydroxy-5-androsten-17-one of high specific activity starting from [7-^{3}H]-5-androsten-17-one. The identity of the radioactive steroid was established by thin layer chromatography, gas chromatography and gas chromatography combined with mass spectroscopy. The radiolabelled steroid may be used to study its disposition and metabolism in experimental animals and also to further investigate the potential role of 3β,16β-dihydroxy-5-androsten-17-one in patients with low renin essential hypertension.

Key Words: [7-^{3}H]-16β-Hydroxydehydroepiandrosterone, Hypertension

INTRODUCTION

The steroid 3β,16β-dihydroxy-5-androsten-17-one (16β-OH-DHEA) was first identified in infant urine (1) and has also been found by incubating dehydroepiandrosterone sulphate with homogenates of human fetal liver (2). Subsequently, this C-19 steroid and its conjugates have been identified and quantified in adult human urine (3) and have been reported to be elevated during pregnancy (4). More recently, levels of this steroid were found to be elevated in the urine (5, 6) of patients with the syndrome of low renin hypertension but not in the plasma (7). We originally embarked upon the synthesis of tritiated 16β-OH-DHEA in order to measure the secretory rate of this steroid as measured by the levels of

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0362-4803/81/081177-08$01.00
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Received June 9, 1980
Revised July 25, 1980

unchanged and conjugated 16β-OH-DHEA in the urine of patients with low renin essential hypertension.

Chemical syntheses of 16β-OH-DHEA have been described in the literature (1, 8, 9). However, one of the major difficulties of the synthesis has been the facile isomerisation of the 16β-ol-17-one compound to the 16-one-17β-ol compound (3β,17β-dihydroxy-5-androsten-16-one; 16-oxo-androstenediol) in the presence of acid or base (10). The penultimate step to the isolation of 16β-OH-DHEA is the deacetylation of the steroid, 3β,16β-dihydroxy-5-androsten-17-one diacetate which may be easily synthesized in high yield (11).

We wish to describe the synthesis of [^{3}H]-16β-OH-DHEA, labelled in the 7-position, starting from commercially purchased [7-^{3}H]-3β,hydroxy-5-androsten-17-

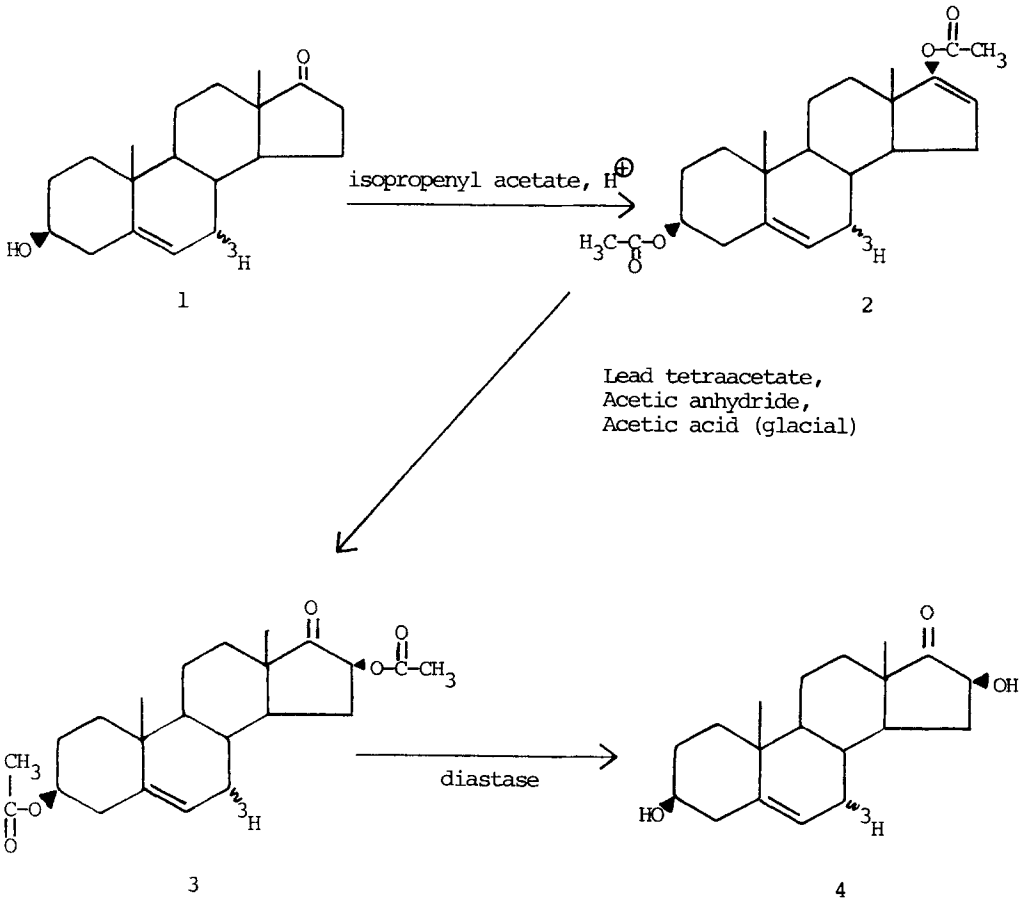

Figure 1. Synthesis of [7-^{3}H]-3β,16β-dihydroxy-5-androsten-17-one

one ([^{3}H]-dehydroepiandrosterone; DHEA) using the reaction scheme outlined in Figure 1 which also involves the ultimate deacetylation of tritiated 3β,16β-dihydroxy-5-androsten-17-one diacetate.

EXPERIMENTAL

Tritiated 3β-hydroxy-5-androsten-17-one, (7-[^{3}H]-DHEA; 10 Ci/mmole) was obtained from New England Nuclear, Boston, MA. Isopropenyl acetate was purchased from Eastman-Kodak, Rochester, NY; acetic anhydride and lead tetraacetate were purchased from Aldrich Chemical Company, Milwaukee, WI and Fisher Scientific Company, Silver Spring, MD, respectively.

Thin layer silica gel plates (Silica gel GF; 5 x 20 cm; 250 μ thickness) were purchased from Analtech, Newark, NJ. Chromatography-grade alumina (aluminum-oxide-Woelm neutral) was obtained from Waters Associates, Frampton, MA.

The diastase preparation was Type V-A (from malt) obtained from Sigma Chemicals, St. Louis, MO. Bovine erythrocyte acetylcholine hydrolase (Type I), human serum acylcholine acylhydrolase (Type VII) and acetylcholine hydrolase (from electric eel; Type III) were also obtained from Sigma.

Authentic 16β-OH-DHEA-diacetate, 16α-OH-DHEA, and 16-oxo-androstenediol and DHEA were bought from Steraloids, Wilton, NH.

The N-methyl oximes of the steroids were prepared by the addition of 500 μl "MOX" (2% methoxylamine hydrochloride in pyridine; Pierce Chemical Company, Rockford, IL) and heated with the steroids at 60°C for 3 hours. Silylation was carried out by the addition of 500 μl of Tri-Sil/BSA (Pierce Chemical Company) or Power Sil-Prep (Applied Science, State College, PA).

All solvents and reagents used were of the highest grade available and were used without further purification.

Gas chromatographic analyses were carried out on a Perkin Elmer 900 Gas Chromatograph utilizing a stationary phase of 3% w/v SE-30 on Chromasorb Q #100/120 (Applied Science) packed in a silanized glass column (2 m in length, 2 mm i.d.). The conditions for the analyses were: oven temperature 209°C, flash heater 286°C, flame detector 285°C and N_2 flow rate 12 mls per minute.

Nuclear magnetic resonance (^{1}H-HMR) spectra were obtained on a Varian 60A apparatus in d_5-pyridine with 1% TMS as internal standard (Silanor P, Merck Sharpe Dohme, Teterboro, NJ).

Gas-chromatography-electron-impact mass spectroscopy was carried out on the derivatised steroids using a VG Micromass 16F mass spectrometer (VG Industries, Winsford, Cheshire, U.K.). Chemical ionisation spectra of the underivatised standards were obtained on the same mass spectrometer using isobutane as the reactant gas at a source pressure of approximately 0.3 torr.

All melting points quoted are uncorrected.

3β,17β-Dihydroxy-5,16-androstadiene diacetate (2)

Into a round bottomed flask (25 ml) was placed DHEA (1; 25 mg) and [^{3}H]-DHEA (25 mCi; 10 Ci/mole) together with isopropenyl acetate (600 µl) and the catalyst solution (25 µl) which was prepared from isopropenyl acetate (2.0 ml) and concentrated H_2SO_4 (40 µl; 12). The flask was fitted with a reflux condenser and heated in an oil bath (80-100°C) for 4 hours with constant stirring after which time a second aliquot of catalyst solution (25 µl) was added and heating continued for an additional 1.5 hours. The reaction vessel was allowed to cool before the addition of hexane (3.8 ml). The hexane solution was passed through a column of alumina (2.1 g) to remove acidic material. The radioactivity was eluted off of the column with hexane (125 ml) and the solvent evaporated to approximately 10 ml; an aliquot was removed from examination by thin-layer chromatography (tlc) developed in cyclohexane:ethylacetate (1:1 v/v). Radiochromatogram scanning (Packard Instruments, Downers Grove, IL) indicated that 95% of the radioactivity was associated with an area of Rf 0.90-0.93 which was identical with the authentic, non-radioactive compound prepared previously [m.p. 168-171°C; lit. 168-170°C (5)]. No starting material was detected. The remainder of the hexane was evaporated to dryness under a slow stream of dry N_2.

3β,16β-Dihydroxy-5-androsten-17-one-diacetate (3)

Into an Erlenmeyer flask (10 ml) was added a solution of tritiated 3β,17β-dihydroxy-5,16-androstadiene diacetate (2) prepared by the above synthesis;

approximately 25 mg in acetic anhydride (40 µl) and glacial acetic acid (1.0 ml). This resulting solution was cooled in ice and to it was added lead tetraacetate (30 mg; 11) in 10 mg aliquots. The temperature of the reaction mixture was allowed to rise to room temperature (21°C) and the slurry was stirred for 18 hours after which time the solution was freeze-dried. The residue was dissolved in anhydrous ether (10 ml) and extracted with $NaHCO_3$ solution (5% w/v) and the organic phase dried over anhydrous Na_2SO_4. The organic solvent was evaporated to a small volume (approximately 20 µl) and a portion examined by tlc developed in cyclohexane:ethanol (9:1 v/v, Rf 0.26). Authentic 16β-OH-DHEA diacetate was chromatographed on the same plate (Rf 0.27). The remainder of the ether was evaporated to dryness under a slow stream of dry N_2 leaving a white residue which was recrystallised from methanol m.p. 173-175°C (authentic 16β-OH-DHEA diacetate, Steraloids: 172-175°C). Yield 18.7 mg (approximately 70%).

3β,16β-Dihydroxy-5-androst-5-ene-17-one (4)

Recognizing the need to utilize mild hydrolytic conditions to prevent the formation of the 16-oxo-isomer, 3β,17β-dihydroxy-5-androsten-16-one (16-oxo-androstenediol), Shackleton and co-workers (1) found that hydrolysis of the diacetate ester could be carried out by the use of aqueous acidic methanol at 30°C for 60 hours. In our laboratory this method, although partially successful, produced material very difficult to purify further. Enzyme hydrolyses were attempted using bovine erythrocyte acetylcholine hydrolase (Type I), human serum acylcholine acylhydrolase (Type VII), acetylcholine hydrolase (from electric eel; Type III) and diastase (Type V-A). Of these, the most efficient was the diastase preparation.

3β,16β-Dihydroxy-5-androsten-17-one diacetate (3; 16 mg) was dissolved in propylene glycol (15.6 ml) with slight warming. To this was added water (77 ml) containing the diastase preparation (1.54 g). The mixture was incubated for 4 days at 37°C and the reaction was halted by extraction with chloroform (6 x 100 mls). The organic phase was dried over anhydrous sodium sulphate before being evaporated to dryness in vacuo. The residue was dissolved in a small volume of

chloroform (approximately 2 ml) and subjected to tlc in a developing solvent of cyclohexane:ethanol (9:1 v/v, Rf 0.11). An authentic sample of 16β-OH-DHEA was chromatographed on the same plate (Rf 0.12). Radiochromatogram scanning of the tlc plate showed only one peak of radioactivity. The radioactive area was scraped from the plate and eluted with chloroform (approximately 100 ml). The organic solvent was dried over anhydrous sodium sulphate, rotary evaporated to dryness in vacuo and the residue redissolved in absolute ethanol (approximately 2 ml). The yield of this reaction was 7%. Specific activity; 103 mCi/mmole. Mass spec. (electron-impact): m/e (rel. intensity) 477(8,M^+), MO-TMSi derivative, 462(13), 466(63), 431(29), 372(8), 356(25), 341(8), 266(70), 239(18), 174(30), 129(48); chemical ionisation: 305(28, MH^+), 289(20), 287(48), 285(23), 273(11), 271(28), 257(6), 255(8).

For clinical purposes the [^{3}H]-16β-OH-DHEA was diluted with ethanol (abs.) and sterile sodium chloride solution (0.9% w/v) to give a solution of specific activity 1 μCi/μl.

RESULTS AND DISCUSSION

The methods described, similar to that reported previously (8) for the synthesis of the non-radioactive product, permit the synthesis of [7-^{3}H]-3β,16β-dihydroxy-5-androsten-17-one ([^{3}H]-16β-OH-DHEA) with high specific activity. In addition to synthesizing the compound we have also examined its purity by the radiochromatogram scanning of thin layer chromatography plates (and subsequent scraping and scintillation counting of 1 cm "strips" of the plate), by gas chromatography (g.c.; as its methyloxime-trimethylsilyl derivative; Table 1) and by gas chromatography-mass spectrometry (g.c.-ms. ei) and chemical ionisation (c.i) mass spectroscopy. Previous batches of unlabeled 16β-OH-DHEA synthesized by the reaction outlined in Figure 1 were also examined by the above methods where applicable as well as by ^{1}H-NMR spectroscopy.

Table 1. Gas chromatography retention times of 16β-OH-DHEA, 16α-OH-DHEA and 16-oxo-androstenediol as their methyloxime-trimethylsilyl-derivatives and methyloxime-derivatives. (For column and conditions, see Experimental section).

Steroid	Retention time (mins.)
16β-OH-DHEA-MO-diTMSi*	46
16α-OH-DHEA-MO-diTMSi*	39
16-oxo-androstenediol-MO-diTMSi*	50
16β-OH-DHEA-MO*	42
16α-OH-DHEA-MO*	35
16-oxo-androstenediol-MO*	53

*Abbreviation. MO = methyloxime derivative. diTMSI = two trimethylsilyl groupings.

Compound 2 (see Figure 1) was not isolated and purified since it was found to be more efficient to continue with the reaction scheme rather than lose radioactivity by isolation procedures. Purity, as assessed by radiochromatogram scanning, was greater than 95% and since no starting material could be detected, the conversion of 1 to 2 was presumed to be very good. The conversion of compound 2 into 3 gave between 50 and 70% yield of compound 2 which compared favorably with that previously reported (11).

The most inefficient step in the reaction sequence involved the conversion of [^3H]-3β,16β-dihydroxy-5-androsten-17-one diacetate into [^3H]-16β,OH-DHEA. Of the enzymic hydrolyses attempted, we found the diastase preparation to be the most successful although the yield of this reaction was only approximately 7%. It is possible that by using a recently reported method involving sulphuric acid in a dioxane-water mixture (9), our yield may have improved; however we have not yet attempted this method.

The use of [^{3}H]-16β,OH-DHEA should undoubtedly contribute to a better understanding of the in vivo distribution and fate of this steroid and may provide a satisfactory means of obtaining accurate urinary secretary rates in a patient population.

ACKNOWLEDGEMENTS

We would like to thank Christopher Connor for his excellent technical help and Mrs. Lynn Binder for the typing of this manuscript.

REFERENCES

1. Shackleton, C.H.L., Kelly, R.W., Adhikary, P.M., Brooks, C.J.W., Harkness, R.A., Sykes, P.J. and Mitchell, F.L. - Steroids 12: 705 (1968).
2. Wynne, K.N. and Renwick, A.G.C. - Biochem. J. 156: 419 (1976).
3. Jänne, O. and Vihko, R. - Steroids 14: 235 (1969).
4. Jänne, O. and Vihko, R. - Acta Endocrinol. 65: 50 (1970).
5. Sennett, J.A., Brown, R.D., Island, D.P., Yarbro, L.R., Watson, J.T., Slaton, P.E., Hollifield, J.W. and Liddle, G.W. - Circ. Res. Suppl. 1 to 36/37: 1-2 (1975).
6. Nowaczynski, W., Messerli, F.H., Kuchel, O., Guthrie, G.P. Jr. and Genest, J. - J. Clin. Endocrinol. Metab. 44: 629 (1977).
7. Sekihara, H., Sennett, J.A., Liddle, G.W., McKenna, T.J. and Yarbro, L.R. - J. Clin. Endocrinol. Metab. 43: 1078 (1976).
8. Wynne, K.N. and Renwick, A.G.C. - Steroids 19: 293 (1972).
9. Kincl, F.A. - J. Steroid Biochem. 7: 419 (1976).
10. Fishman, J. - J. Amer. Chem. Soc. 82: 6143 (1960).
11. Johnson, W.S., Gastambide, B. and Pappo, R. - J. Amer. Chem. Soc. 79: 1991 (1957).
12. Aoki, T., Yamamura, H., Takei, K. and Mori, H. - Chem. Pharm. Bull. (Tokyo) 12: 806 (1964).