SYNTHESIS OF A PRECURSOR FOR THE PREPARATION OF 9α,11α-TRITIATED 5α-ANDROSTANE-3α,17β-DIOL 17-GLUCURONIDE

P. Narasimha Rao and K. M. Damodaran
Department of Organic Chemistry
Southwest Foundation for Biomedical Research
P. O. Box 28147, San Antonio, Texas 78284

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ABSTRACT

Starting from 11β-hydroxytestosterone, we achieved the synthesis of a strategic precursor, C-9(11) unsaturated 5α-androstane-3α,17β-diol 17-glucuronide (9a), for the preparation of 9α,11α-tritiated 5α-androstane-3α,17β-diol 17-glucuronide. We optimized the reaction conditions for catalytic reduction employing hydrogen and subsequent base hydrolysis followed by purification on Amberlite XAD-2 resin to obtain the saturated 5α-androstane-3α,17β-diol 17-glucuronide.

INTRODUCTION

Recent studies suggest that 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one), 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3α,17β-diol 17-glucuronide present in human plasma are mostly derived from extrasplanchnic metabolism of testosterone [1,2]. It was noted that peripheral plasma levels of 3α-diol glucuronide were ten times larger than the free steroid in young men, and also the glucuronide concentration was higher in young men compared with elderly males. It was also observed that in idiopathic hirsuitism the level of 3α-diol glucuronide was markedly elevated. In light of these observations it is suggested that 3α-diol glucuronide might serve as an excellent marker for events occurring in sexual target tissues [2]. In order to study the role of 3α-diol glucuronide in androgen metabolism and for radioimmunoassay, we needed the tritiated glucuronide with high specific activity. In the present communication we describe the synthesis of a strategic precursor which, after catalytic reduction with tritium gas and subsequent base hydrolysis, will readily yield the labeled glucuronide with high specific activity.

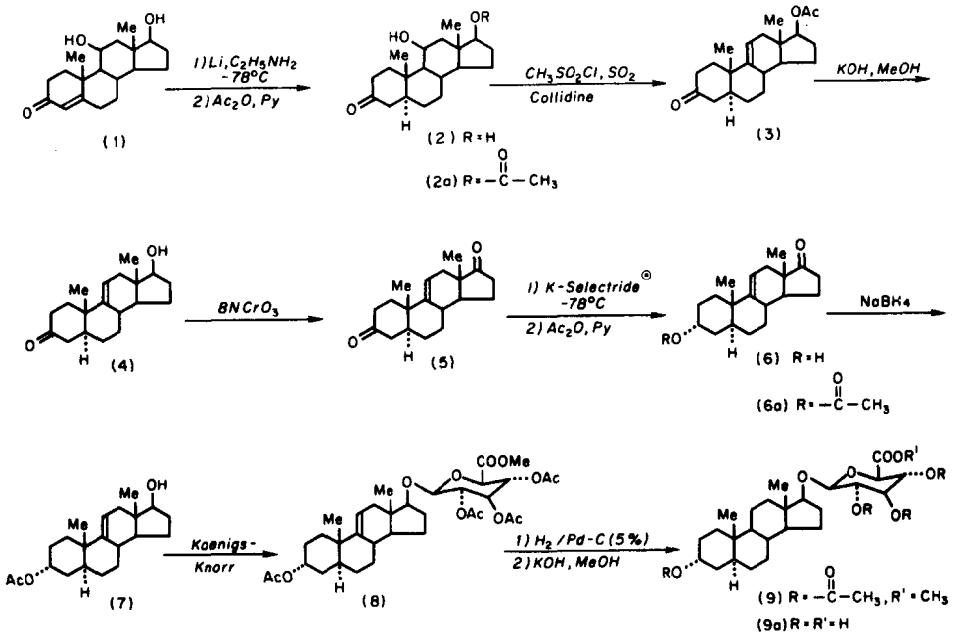
RESULTS AND DISCUSSION

We have earlier described a procedure for tritiation of steroids at the biochemically stable C-9(11)-position [3]. The precursor in these studies is a C-9(11) unsaturated steroid. Similarly, for the ultimate synthesis of labeled 3α-diol glucuronide we prepared the C-9(11) unsaturated 3α-diol glucuronide derivative (8). Scheme I illustrates our current synthetic approach.

11β-Hydroxytestosterone (1) reported in the literature [4] served as the starting material. Stereoselective reduction of the Δ^4-double bond present in compound (1) with lithium in ethylamine at -78°C [5] followed by acetylation with acetic anhydride and pyridine at room temperature gave the 17β-acetoxy-5α-dihydro compound (2a) in excellent yield. Conversion of the alcohol (2a) to the 9(11)-dehydro steroid (3) was readily accomplished by facile dehydration with methanesulfonyl chloride-sulfur dioxide reagent developed by Hazen and Rosenburg [6]. Hydrolysis of the keto acetate (3) with aqueous potassium hydroxide in methanol gave the hydroxy ketone (4). The hydroxy ketone (4) was oxidized with Jones reagent to the diketone (5) which, upon selective reduction with K-selectride® [7] followed by acetylation with acetic anhydride and pyridine, gave the 3α-acetoxy derivative (6a). Sodium borohydride reduction of 6a gave the 17β-hydroxy-3α-acetate (7). Koenigs-Knorr reaction of the hydroxy acetate (7) [8,9] with methyl-2,3,4-tri-O-acetyl-1α-bromo-1-deoxy-α-D-glucuronate in the presence of silver carbonate and molecular sieve (3Å) gave the 17-glucosiduronate (8).

The reaction conditions were optimized for catalytic tritiation and subsequent base hydrolysis to obtain the 9α,11α-tritiated 3α-diol 17-glucuronide by carrying out model experiments with the unsaturated

Scheme 1



(1)

(2) R = H

(2a) R = $-C(=O)-CH_3$

(3)

(4)

(5)

(6) R = H

(6a) R = $-C(=O)-CH_3$

(7)

(8)

(9) R = $-C(=O)-CH_3$, R' = CH_3

(9a) R = R' = H

glucuronide (8) and hydrogen. Accordingly, compound (8) was smoothly hydrogenated in the presence of 5% palladium-carbon catalyst and subsequent hydrolysis with potassium hydroxide in aqueous methanol followed by purification of the glucuronide on Amberlite XAD-2 resin gave the pure 3α-diol 17-glucuronide (9a).

EXPERIMENTAL

Melting point determinations were made on a Thomas-Hoover Model 6406-H apparatus and are not corrected. Infrared spectra were recorded as potassium bromide pellets with a Perkin-Elmer Model 467 spectrophotometer. NMR spectra were measured in deuteriochloroform, unless otherwise stated, using tetramethylsilane as internal standard, with a Varian EM-390 90 MHz spectrometer. Mass spectra were determined on a Finigan quadrupole mass spectrometer. Flash chromatography was performed as described by Still et al. [10]. Microanalyses were obtained by Midwest Microlab, Ltd., Indianapolis, Indiana.

11β,17β-Dihydroxy-5α-androstan-3-one (2)

To mechanically stirred dry ethylamine (200 ml, distilled over sodium) were added small pieces of lithium (2.1 g). When lithium started to dissolve, the reaction flask was cooled in a dry ice-acetone bath (-78°C), and the stirring continued for 45 min to insure complete dissolution of lithium. A solution of 11β-hydroxytestosterone (1, 3.4 g) in a mixture of tetrahydrofuran (125 ml) and dry t-butanol (10.8 ml) was added dropwise to the lithium-ethylamine solution. After stirring for an additional 30 min at -78°C, water (41 ml) was added (disappearance of blue color), and the mixture allowed to warm to room temperature. Ethylamine and most of the tetrahydrofuran were evaporated under a stream of nitrogen. The mixture was cooled in ice and acidified with 4N hydrochloric acid. The organic material was isolated with ethyl acetate. The ethyl acetate extract was washed with water, saturated sodium bicarbonate, brine, and then dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a crystalline solid (3.4 g); mp 256-260°C. δ (DMSO-d_6) 0.85(s, 3H, C-18-CH_3), 1.18(s, 3H, C-19-CH_3), 4.01(m, 1H, C-17α-H) and 4.32(m, 1H, C-11α-H)ppm. ν_{max} 3450 and 1690 cm^{-1}. Anal. calc'd for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.26; H, 9.46.

17β-Acetoxy-11β-hydroxy-5α-androstan-3-one (2a)

To a cold solution of the dihydroxyandrostanone (2, 3.7 g) in pyridine (30 ml) was added acetic anhydride (30 ml) and the mixture let stand at room temperature overnight. Acetic anhydride and pyridine were removed in vacuo under a stream of nitrogen to give a crystalline residue. Recrystallization of the product from acetone:hexane gave a white crystalline solid; mp 197-201°C. Lit. [11] mp 192-196°C. δ 1.03(s, 3H, C-18-CH_3), 1.26(s, 3H, C-19-CH_3), 2.05(s, 3H, c-17β-$OCOCH_3$),

4.36(m, 1H, C-11α-H), and 4.58(m, 1H, C-17α-H)ppm. ν_{max} 3400, 1740, 1690, 1255 and 1240 cm^{-1}.

17β-Acetoxy-5α-androst-9(11)-en-3-one (3)

A solution of the hydroxy steroid (2a, 2.6 g) in dry N-dimethylformamide (16 ml) and collidine (5.3 ml) was cooled to 10°C. The cooling bath was removed and during the course of 1-2 min a reagent containing methanesulfonyl chloride (2 ml) and sulfur dioxide (0.1 g) was added to the clear solution. The temperature rose quickly and the reaction was allowed to proceed for a period of 15 min during which time a pale yellow precipitate separated and the solution turned bright red. At the end of the reaction period, the cooling bath was replaced and the excess mesyl chloride was decomposed by the slow addition of water (3.4 ml). The clear red solution was added dropwise with stirring to water (200 ml). After stirring the resulting slurry for 1 h at 20-25°C, the product was filtered, washed with water and dried. The dry solid was taken up in dichloromethane, and the solution washed with cold dilute sulfuric acid, water, brine, and then dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a crystalline solid (2.2 g). Purification of the product by flash chromatography over silica gel using ethyl acetate:dichloromethane (1:19) as solvent, followed by crystallization from acetone:hexane afforded a white, crystalline solid; mp 137-138°C. δ 0.76(s, 3H, C-18-CH_3), 1.16(s, 3H, C-19-CH_3), 2.07(s, 3H, C-17-$OCOCH_3$), 4.72(m, C-17α-H) and 5.41(m, 1H, C-11-H)ppm. ν_{max} 1730, 1715, 1250, and 1240 cm^{-1}. Anal. calc'd for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 75.94; H, 8.79.

17β-Hydroxy-5α-androst-9(11)-en-3-one (4)

A mixture of the steroid acetate (3, 2 g), methanol (120 ml), 1.7N potassium hydroxide (18 ml) and water (7 ml) was refluxed in an atmosphere of nitrogen for 20 minutes. After cooling the mixture in an ice bath, acetic acid (2 ml) was added. Most of the solvent was removed in vacuo under a stream of nitrigen. The residue was dissolved in ethyl acetate and the ethyl acetate solution washed with saturated sodium bicarbonate solution, water and brine, then dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a pale yellow residue. Purification of the product by flash chromatography over silica gel using ethyl acetate:dichloromethane (1:19) as solvent, followed by crystallization from acetone:hexane afforded 1.7 g of a white crystalline solid; mp 168-170°C. δ 0.72(s, 3H, C-18-CH_3), 1.17(s, 3H, C-19-CH_3), 3.77(m, 1H, C-17α-H) and 5.42(m, 1H, C-11-H)ppm. ν_{max} 3390 and 1690 cm^{-1}. Anal. calc'd for $C_{19}H_{28}O_2$: C, 79.12; H, 9.78. Found: C, 78.96; H, 9.90.

5α-Androst-9(11)-ene-3,17-dione (5)

A solution of the hydroxy ketone (4, 1.06 g) in acetone was degassed, filled with nitrogen, and cooled to 0°C in an ice bath. Jones reagent was added dropwise to the solution with vigorous stirring until a light orange color persisted. Excess reagent was decomposed with methanol, solid sodium bicarbonate was added, and stirring continued. The mixture was then filtered through Celite and washed with acetone.

Acetone and methanol were removed in vacuo under a stream of nitrogen. Water was added to the residue and the organic material isolated with ethyl acetate. The ethyl acetate extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave 1 g of a crystalline residue. Purification of the product by flash chromatography over silica gel using ethyl acetate:hexane (1:5) solvent system, followed by crystallization of the pure fraction from ethyl acetate:hexane afforded a white crystalline solid, mp 150-151.5°C. Lit. [12] mp 154°C. δ 0.87(s, C-18-CH_3), 1.19(s, 3H, C-19-CH_3), and 5.48(m, 1H, C-11-H)ppm. ν_{max} 1730 and 1715 cm^{-1}. Anal. calc'd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.35; H, 8.94.

3α-Hydroxy-5α-androst-9(11)-en-17-one (6)

To a solution of the steroid dione (5, 1.1 g) in dry tetrahydrofuran (75 ml) at -75°C was added, under argon, a 0.5M solution of K-selectride® in tetrahydrofuran (9.2 ml) and the mixture was stirred for 2 h. Water (1 ml) was added and the solution stirred for 5 min. More water was added to the mixture and stirring continued for an additional 30 min. The organic material was isolated with ethyl acetate. Evaporation of the solvent in vacuo gave a white foam which was purified by flash chromatography over silica gel using ethyl acetate:dichloromethane (1:9) solvent system to afford unreacted dione (5, 0.35 g) and hydroxy ketone (6, 0.75 g). Recrystallization of the hydroxy ketone from acetone:hexane gave a white crystalline solid; mp 188-189.5°C. Lit. [13] mp 187-189°C. δ 0.81(s, 3H, C-18-CH_3), 0.92(s, 3H, C-19-CH_3), 4.03(m, 1H, C-3β-H) and 5.40(m, 1H, C-11-H)ppm. ν_{max} 3560 and 1720 cm^{-1}. Anal. calc'd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.78. Found: 79.21; H, 9.88.

3α-Acetoxy-5α-androst-9(11)-en-17-one (6a)

A mixture of the hydroxy ketone (6, 1.0 g), pyridine (10 ml) and acetic anhydride (6 ml) was stirred at room temperature for 18 h. Acetic anhydride and pyridine were removed in vacuo under a stream of nitrogen. Purification of the product by flash chromatography over silica gel using ethyl acetate:dichloromethane (1:19) as solvent system gave pure acetoxy ketone (6a, 0.79 g) which was recrystallized from ethyl acetate to afford a white crystalline solid; mp 188-190°C. Lit. [13] mp 191-192°C. δ 0.82(s, 3H, C-18-CH_3), 0.94(s, 3H, C-19-CH_3), 2.03(s, 3H, C-3α-$OCOCH_3$), 5.03(m, 1H, C-3β-H), 5.40(m, 1H, C-11-H)ppm. ν_{max} 1742, 1720, 1250, and 1240 cm^{-1}. Anal. calc'd. for $C_{21}H_{30}O_3$: C, 76.33; H, 9.13. Found: C, 75.91; H, 8.87.

5α-Androst-9(11)-ene-3α,17β-diol 3-acetate (7)

To a solution of the acetoxy ketone (6a, 0.79 g) in 95% ethanol (55 ml) was added a solution of sodium borohydride (0.95 g) in 95% ethanol (30 ml) and the mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo in a stream of nitrogen and the product isolated with ethyl acetate. Evaporation of ethyl acetate in vacuo gave a white foam (0.80 g). Purification of the material by flash chromatography over silica gel, followed by crystallization of the pure fraction from ethyl acetate:petroleum ether afforded the hydroxy

acetate (7) as a white crystalline solid; mp 135-136.5°C. δ 0.72(s, 3H, C-18-CH_3), 0.96(s, 3H, C-19-CH_3), 2.05(s, 3H, C-3α-$OCOCH_3$), 3.76(m, 1H, C-17α-H), 5.05(m, 1H, C-3β-H) and 5.40(m, 1H, C-11-H)ppm. ν_{max} 3440, 1735, 1700, 1270 and 1240 cm^{-1}. Anal. calc'd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.50; H, 9.46.

Methyl 3α-acetoxy-5α-androst-9(11)-en-17β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (8)

A solution of methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosuronate (2.4 g) in dry benzene (60 ml) was added dropwise under argon to a stirred solution of the hydroxy acetate (7, 0.89 g) in benzene (30 ml) containing anhydrous silver carbonate (3.0 g) and molecular sieve (Aldrich Linde 3Å). The mixture was stirred at room temperature in the dark for 48 h, with additional bromoglucuronate (1.3 g) and silver carbonate (1.2 g) being added to the mixture after a period of 24 h. The solids were filtered and washed with benzene, and the combined filtrate evaporated in vacuo to give a pale yellow gum. Purification of the product by flash chromatography over silica gel using ethyl acetate:hexane (2:5) solvent system gave two fractions. The first fraction (0.56 g) was apparently an ortho ester which exhibited peaks at δ 0.72(s, 3H, C-18-CH_3), 0.98(s, 3H, C-19-CH_3), 1.74(s, 3H, ortho acetate-CH_3), 2.05(s, 3H, C-3α-$OCOCH_3$), 2.13(s, 6H, pyranose acetate methyls), 3.64(m, 1H, C-17α-H), 3.78(s, 3H, -$COOCH_3$), 5.05(m, 1H, C-3β-H), 5.39(m, 1H, C-11-H), and 4.25-5.90(m, 3H, pyranose methines)ppm. The second fraction (0.7 g), when crystallized from acetone:hexane, afforded the steroid glucopyranosiduronate (8) as a white crystalline solid, mp 193-196°C. δ 0.70(s, 3H, C-18-CH_3), 0.95(s, 3H, C-19-CH_3), 2.05-2.10(m, 12H, acetate methyls), 3.72(m, 1H, C-17α-H), 3.80(s, 3H, $OCOOCH_3$), 5.08(m, 1H, C-3β-H), 5.35(m, 1H, C-11-H), and 4.00-5.40(m, 5H, pyranose methines)ppm. ν_{max} 1760, 1735, 1240 and 1220 cm^{-1}. m/e = 331 (steroid fragment), 317 (glucopyranose fragment) and 155 (base peak). Anal. calc'd. for $C_{34}H_{48}O_{12}$: C, 62.94; H, 7.46. Found: C, 62.83; H, 7.50.

Methyl 3α-acetoxy-5α-androstan-17β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate (9)

Catalytic hydrogenation:

To a solution of the unsaturated steroid glucopyranosuronate (8, 0.20 g) in ethyl acetate (20 ml) was added 5% palladium-carbon catalyst (0.20 g), and the mixture hydrogenated at 20 p.s.i. in a Parr hydrogenator at room temperature for 2 h. The catalyst was filtered off over Celite in a fritted funnel and the solvent evaporated in vacuo to give the androstanyl glucopyranosiduronate (9) as a white crystalline solid (0.20 g) which was recrystallized from acetone:hexane; mp 149-150°C. δ 0.69(s, 3H, C-18-CH_3), 0.78(s, 3H, C-19-CH_3), 2.05(m, 12H, acetate methyls), 3.60(m, 1H, C-17α-H), 3.75(s, 3H, -$COCH_3$), 5.03(m, 1H, C-3β-H) and 3.45-5.40(m, 3H, pyranose methines)ppm. ν_{max} 1760, 1735, 1240 and 1220 cm^{-1}. Anal. calc'd. for $C_{34}H_{30}O_{12}$: C, 62.75; H, 7.75. Found: C, 62.74; H, 7.81.

3α-Hydroxy-5α-androstan-17β-yl β-D-glucopyranosiduronic acid (9a)

Base hydrolysis:

A solution of the androstanyl glucopyranosiduronate (9, 0.2 g) in methanol (30 ml) was stirred with 2N sodium hydroxide solution (4 ml) in an atmosphere of nitrogen at room temperature for 18 h. Methanol was distilled off in vacuo at 40°C (bath temperature). Water (50 ml) was added to the residue and the solution cooled in an ice bath. The pH of the solution was adjusted to 2.5 by the addition of cold 1N hydrochloric acid to give a turbid solution. The solution was diluted with water to 100 ml and passed through a column (3.2 cm x 60 cm) of Amberlite XAD-2 resin, and washed with 0.01N sulfuric acid (500 ml) followed by water (ca. 2 liter). The adsorbed steroid glucuronic acid was then eluted with methanol. The first 200 ml of eluate contained most of the glucuronic acid (130 mg). The next 300 ml of the eluate gave, on evaporation, 20 mg of white solid. TLC of the two fractions on silica gel plates using the solvent system of chloroform:isopropanol:formic acid (15:5:3) showed single spots having an R_f of 0.5. Recrystallization of the combined material from methanol:acetone:ethyl acetate gave a white crystalline solid; mp 233-235°C (dec.). δ (CD_3OD) 0.80(s, 6H, C-18 and C-19 methyls) and 3.20-4.50(m, 7H, C-17α-H, C-3β-H and pyranose methines)ppm. ν_{max} 3390, 1725 and 1650 cm^{-1}. Anal. calc'd. for $C_{25}H_{40}O_8$: C, 64.08; H, 8.60. Found: C, 63.47; H, 8.10.

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