IMPROVED SYNTHESIS OF 16α-HYDROXYLATED ANDROGENS: INTERMEDIATES OF ESTRIOL FORMATION IN PREGNANCY

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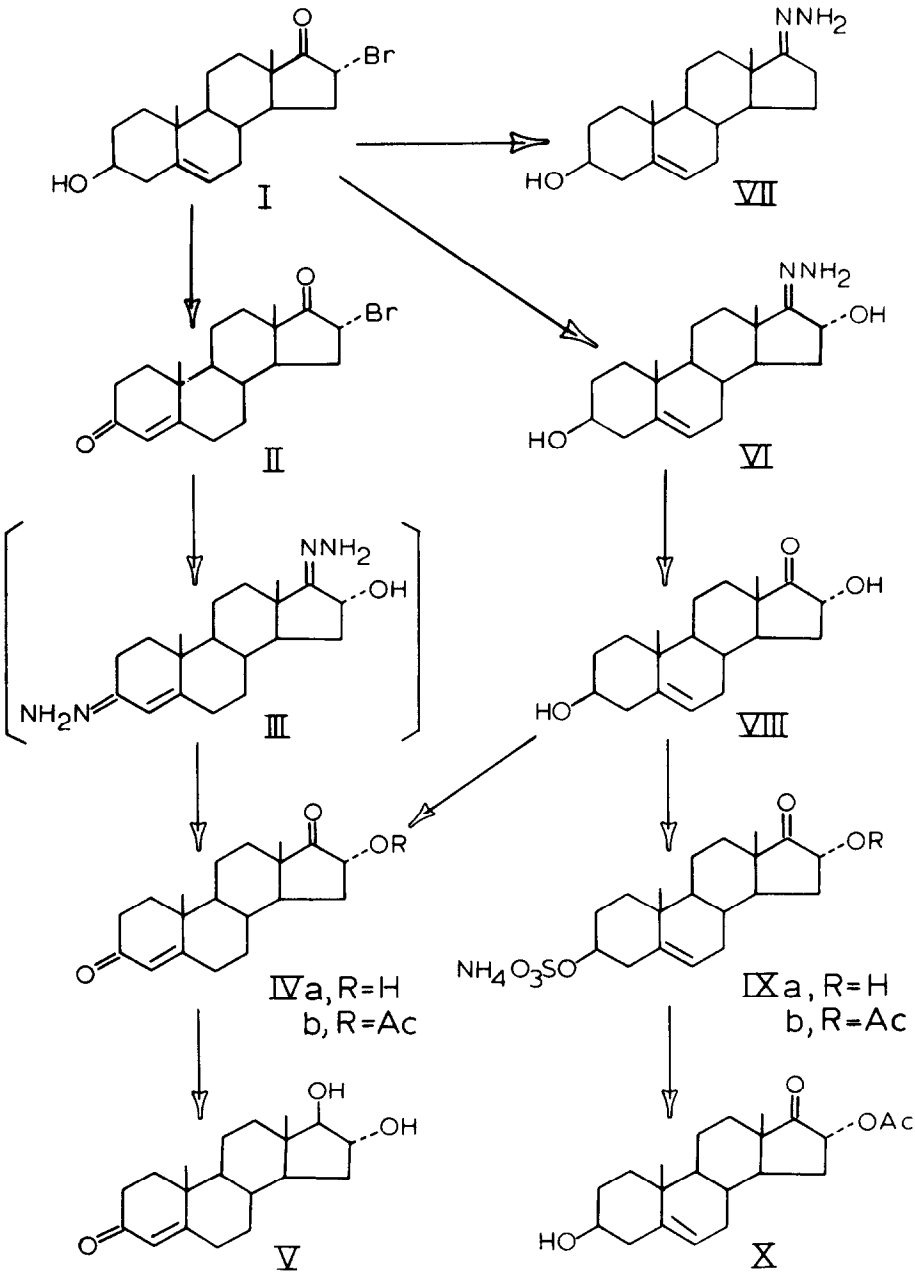
ABSTRACT

16α-Hydroxyandrostenedione (16α-hydroxyandrost-4-ene-3,17-dione), 16α-hydroxytestosterone (16α,17β-dihydroxyandrost-4-en-3-one) and 16α-hydroxydehydroepiandrosterone 3-sulfate (3β,16α-dihydroxyandrost-5-en-17-one 3-monosulfate) were synthesized by a new chemical approach with much improved yield. 16α-Bromoandrostenedione was converted to the hydrazone of 16α-hydroxyandrostenedione which gave 16α-hydroxyandrostenedione on acid hydrolysis in total 63% yield. Oxidation of 16α-hydroxydehydroepiandrosterone with Jones' reagent also selectively afforded 16α-hydroxyandrostenedione. 16α-Hydroxytestosterone was observed by selective reduction of 16α-hydroxyandrostenedione with sodium borohydride. Reaction of 16α-hydroxydehydroepiandrosterone with chlorosulfonic acid in pyridine selectively gave the 3-monosulfate. The structure of the sulfate was deduced from its solvolysis to the starting material, and its acetylation and subsequent solvolysis to 16α-hydroxydehydroepiandrosterone 16-acetate. All procedures are suitable for large scale synthesis without the use of microorganisms.

INTRODUCTION

The formation of estriol from 16α-hydroxylated C_{19} steroids precursors by human placenta has been well known (2-5). 16α-Hydroxydehydroepiandrosterone 3-sulfate (IXa) (6), which is a major C_{19} steroid in the umbilical cord blood (3,7,8), is transported from the fetus to the placenta, where it is hydrolyzed and aromatized to form estriol. However, such intermediates of estriol formation are not readily available, primarily because of the difficulties of their synthesis. Sanda and Fajkos (9) and Gardi and Gandolfi (10), previously reported the chemical


HO
Br
O
I
NNH2
VII
II
OH
VI
NH2N
III
VIII
OR
IVa, R=H
b, R=Ac
NH4O3SO
IXa, R=H
b, R=Ac
V
OAc
X

synthesis of 16α-hydroxyandrostenedione. The former method involves seven steps from dehydroepiandrosterone with the selective epoxidation of the 17-enol acetate of dehydroepiandrosterone 3-formate as a key process. The latter uses seven steps from 16-dehydroprogesterone, having the oxidation with potassium permanganate and Beckmann rearrangement as key reactions. Both methods have total yield of approximately 10%. 16α-Hydroxylation of androstenedione by a microorganism was also reported (11). 16α-Hydroxydehydroepiandrosterone 3-sulfate has only been reported by biochemical synthesis using a microorganism and dehydroepiandrosterone sulfate as the substrate (12). However, microbiological hydroxylation is not easily applicable for large scale synthesis.

This paper describes a short-step chemical synthesis of 16α-hydroxyandrostenedione (IVa), 16α-hydroxytestosterone (V) and 16α-hydroxydehydroepiandrosterone 3-sulfate (IXa) starting from readily available 16α-bromodehydroepiandrosterone (I).

RESULTS AND DISCUSSION

16α-Bromodehydroepiandrosterone (I) and 16α-bromoandrostenedione (II) were synthesized according to known methods using cupric bromide as the brominating reagent (13) and Jones' reagent as oxidizing reagent (14). The structures of the two brominated compounds were confirmed by melting point, IR and NMR spectra. Catsoulacos and Hassner (15) reported that the reaction of 16α-bromo-17-ketones with hydrazine constitutes a useful synthesis of 16α-hydroxy-17-keto compounds. They reported a quantitative yield in obtaining 16α-hydroxydehydroepiandro-

sterone hydrazone (VI) from I by refluxing a solution of I in ethanol containing hydrazine hydrate. On repeating their procedure, however, we obtained dehydroepiandrosterone hydrazone (VII) as an unexpected major product in an amount of 2:1 for VII:VI. On further study, we found that when hydrazine hydrate was added to a boiled solution of I in ethanol and refluxed for 1 hr, the reductively debrominated product VII was quantitatively formed, as opposed to the desired VI formed as a sole product when hydrazine hydrate was added to a chilled solution and allowed to stand at room temperature for 4 hr. Wharton *et al* (16) reported the Kishner eliminative reduction of 2α-halo-5α-cholestane-3-one with hydrazine to form 5α-cholest-2-ene. However, to the best of our knowledge, this is the first example of reductive dehalogenation without the loss of the carbonyl function of the steroidal α-haloketone with hydrazine. We then used the chilled condition to obtain the 16α-hydroxy-17-hydrazone in the following experiments.

Treatment of 16α-bromoandrostenedione (II) with an excess amount of hydrazine hydrate gave 16α-hydroxyandrostenedione hydrazone (III). Compound III was characterized by the IR spectrum [ν_{max} 3200-3450 cm^{-1} (OH and NH), 1625 cm^{-1} (C=N)]. Acid hydrolysis of III without further purification afforded 16α-hydroxyandrostenedione (IVa). By this route IVa was obtained from II in 63% and the total yield of IVa from dehydroepiandrosterone was 38%. The NMR spectrum of IVa in deuteriochloroform showed two angular methyl groups at δ 1.02 (18-CH_3) and 1.22 (19-CH_3), the 16β-H at 3.37 (multiplet), and the 4-H at 5.73 (singlet). The IR spectrum of IVa showed characteristic absorptions

of the conjugated ketone at 1650 cm^{-1} and five-membered ring ketone at 1743 cm^{-1}. The expected NMR and IR spectra were also obtained from the acetate (IVb). Alternatively, short time oxidation of 16α-hydroxydehydroepiandrosterone (VIII), with Jones' reagent afforded crude IVa. The crude product was purified by column chromatography to give pure IVa in 24% yield by this route from dehydroepiandrosterone.

Selective reduction of 16α-hydroxyandrostenedione (IVa) with a stoichiometric quantity of sodium borohydride in cold methanol gave a good yield of 16α-hydroxytestosterone (V) as indicated by the IR [ν_{max} 3250-3500 cm^{-1} (OH), 1645 (conjugated ketone)] and NMR [δ 0.81 (18-CH_3), 1.23 (19-CH_3), 3.39 (doublet, J = 5.8 Hz, 17α-H), 4.03 (multiplet, 16β-H) and 5.71 (singlet, 4-H).

Recently Parmentier and Eyssen (17) reported the selective sulfation of the equatorial 3-hydroxyl group of bile acids with chlorosulfonic acid in pyridine. We applied their method to prepare 16α-hydroxydehydroepiandrosterone 3-sulfate (IXa) from 16α-hydroxydehydroepiandrosterone (VIII). After reaction of VIII with 1.5 equivalent of chlorosulfonic acid in pyridine at -15° for 15 min, the reaction mixture was poured into a chilled 0.1N ammonium hydroxide solution. The precipitate (free steroid) was removed by filtration. Small amounts of the disulfate and of unreacted starting material in the filtrate were effectively removed by chromatography on Amberlite XAD-2 resin eluting stepwise with 10%, 50% and 90% aqueous methanol. A small amount of the other isomer (16-sulfate) was removed by repeated recrystallization from methanol-ether. The yield of IXa from VIII

was 47%. The structure of IXa was deduced from its elemental analysis, IR [ν_{max} 3100-3500 cm^{-1} (OH, NH), 1742 cm^{-1} (17-ketone), 1200-1250 cm^{-1} sulfate)] and NMR spectra [δ 0.93 (18-CH_3), 0.98 (19-CH_3), 4.1-4.7 (broad multiplet, 3α-H, 16β-H)], and derivatization to 16α-acetoxy-dehydroepiandrosterone (X) by acetylation and subsequent solvolysis. The results show that the steroidal equatorial homoallylic 3-hydroxyl group with the presence of ketol type hydroxyl group can be selectively sulfonated by the chlorosulfonate method. This simple symthesis of IXa avoids the use of microorganisms and is suitable for large scale preparation due also to the ease of purification of the product.

EXPERIMENTAL

General methods. Melting points were measured on a Fisher-Jones melting point apparatus and were uncorrected. IR specra were recorded on a Perkin-Elmer 267 spectrophotometer in KBr pellets. NMR spectra were obtained with a Varian EM-360 spectrometer at 60 MHz using tetramethylsilane as an internal standard.

3β-Hydroxy-16α-bromoandrost-5-en-17-one (I). I was synthesized according to Glazier (13). Longer reaction time (72 hrs) than the reported improved the yield to 78%.

16α-Bromoandrost-4-ene-3,17-dione (II). II was synthesized according to Bellino *et al* (14) from I.

Reaction of the bromo ketone I with hydrazine hydrate. To 1g of I in 20ml of EtOH was added 6ml of aqueous 85% solution of hydrazine hydrate at room temperature and refluxed for 90 min (condition A), at 78° and refluxed for 30 min (condition B), or at 0° and then allowed to stand at room temperature for 4 hrs (condition C). Each of the above reaction mixtures was poured into ice water and the precipitate was collected by filtration. The solid was washed with water and dried to give 85-90% yield of the hydrazone (VI and/or VII), IR (KBr); ν_{max} 3200-3450 cm^{-1} (OH, NH), 1660-1672 cm^{-1} (C=N).

Condition A. The NMR spectra of the product showed that it was a mixture of VI and VII in 1:2 ratio. NMR [($CDC\ell_3$-CD_3OD (10:1)]: δ 0.88 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.37 (1H, m, 3α-H), 4.82 (1/3 H, m, 16β-H), 5.30 (1H, d, 6-H).

Condition B. Recrystallization from EtOH yielded 3β-hydroxyandrost-5-en-17-one hydrazone (VII) (805 mg) as colorless leaflets, mp 211-214° (decomp.) (lit. (18) mp 287°, sintering 210°). NMR [($CDC\ell_3$-CD_3OD (10:1)]: δ 0.88 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.37 (1H, m, 3α-H), 5.30

(1H, d, 6-H).

Condition C. Recrystallization from EtOH yielded 3β,16α-dihydroxyandrost-5-en-17-one hydrazone (VI) (785 mg) as colorless leaflets, mp 218-221° (lit. (15) mp 217-220°). NMR [$CDCl_3$-CD_3OD (10:1)]: δ 0.88 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.37 (1H, m, 3α-H), 4.82 (1H, m, 16β-H), 5.30 (1H, d, 6-H).

General procedures for hydrolysis of hydrazone. Hydrazone (1g) was dissolved in 100ml of MeOH. To this solution was added 6N H_2SO_4 (10ml) and the mixture was heated under reflux for 1 hr. The precipitated salt was removed by filtration and the filtrate was poured into water. After extraction with AcOEt the organic layer was washed with water and dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure to give a crude product.

3β-Hydroxyandrost-5-en-17-one. Recrystallization from MeOH gave 525mg of dehydroepiandrosterone as colorless needles, mp 140-141°, identical by IR and NMR spectra with the authentic sample.

3β,16α-Dihydroxyandrost-5-en-17-one (VII). Recrystallization from MeOH gave 495mg of VII as colorless needles, mp 188-190° (lit. (15) mp 187-189°). IR (KBr): ν_{max} 3150-3400 (OH), 1748 (C=O). NMR ($CDCl_3$): δ 0.94 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 3.40 (1H, m, 3α-H), 4.31 (1H, m, 16β-H), 5.32 (1H, d, 5-H).

16α-Hydroxyandrost-4-ene-3,17-dione (IVa). (A) II (5g) was first converted to the hydrazone (III) under the condition C and then III was hydrolyzed without further purification. The product was submitted to silica gel column chromatography. The fractions eluted with n-hexane-AcOEt (2:1) was combined and then recrystallized from acetone to give IVa (2.6g) as colorless prisms, mp 188-191° (lit. (9) 185-186°). IR (KBr): ν_{max} 3350 (OH), 1743 and 1648 (C=O). NMR ($CDCl_3$): δ 1.00 (3H, s, 18-CH_3), 1.21 (3H, s, 19-CH_3), 4.36 (1H, m, 16β-H), 5.72 (1H, s, 4-H).

(B) To a solution of VIII (1g) in acetone (100ml) was added 1.3ml of Jones' reagent (19) and allowed to stand in an ice bath for 3 min. The mixture was poured into ice water and then extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ solution and water and dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure to give an oily substance. The residue was treated with 50mg of p-toluenesulfonic acid monohydrate in 50ml of acetone for 15 hr at room temperature. AcOEt was added and the reaction mixture was washed with 5% $NaHCO_3$ and water. After usual work up an oily substance was obtained. The oily compound was purified by silica gel column chromatography. The fractions eluted by n-hexane-AcOEt (2:1) were combined and recrystallized from acetone to give VIa (327mg) as colorless prisms, mp 187-189°.

16α-Acetoxyandrost-4-ene-3,17-dione (IVb). IVa was acetylated in usual manner using acetic anhydride and pyridine. Recrystallization from MeOH gave IVb as colorless needles, mp 171-172° (lit. (9) 172-173°). NMR ($CDCl_3$): δ 0.99 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 2.07 (3H, s, 16α-OAc), 5.35 (1H, m, 16β-H), 5.68 (1H, s, 4-H).

16α,17β-Dihydroxyandrost-4-ene-3,17-dione (V). To a solution of IVa (120mg) in MeOH (5ml) was added $NaBH_4$ (16mg) and stirred at -10° for 15 min. After this time, 0.2ml of acetic acid was added to the reaction mixture and after usual work up a crude solid (105mg) was obtained. Two recrystallizations of the solid from MeOH afforded a pure 16α,17β-diol (V) (48mg) as colorless plates, mp 190-192° (lit. (10), mp 192-193°). IR (KBr): ν_{max} 3180-3520 (OH), 1640 (C=O). NMR (CD_3OD): δ 0.81 (3H, s, 18-CH_3), 1.23 (3H, s, 19-CH_3), 3.39 (1H, d, J=5.8Hz, 17α-H), 4.03 (1H, m, 16β-H), 5.71 (1H, s, 4-H).

Ammonium 3β,16α-dihydroxy-17-oxoandrost-5-en-3β-yl sulfate (IXa). A pyridine-SO_3 complex was prepared by adding dropwise 650mg of chlorosulfonic acid to 10ml of dry pyridine cooled at -10°. VIII (1.2g) in 10ml of dry pyridine was added to this complex. After 15 min, the reaction mixture was poured into a chilled 0.1N NH_4OH solution (1ℓ). The precipitate was removed by filtration. The filtrate was passed through a column of Amberlite XAD-2 (4 × 100cm). After washing with water (500 ml), the absorbed steroids were eluted stepwise with aqueous 10% MeOH (300ml), 50% MeOH (500ml) and MeOH (300ml). The fraction eluted with 50% MeOH contained mainly 3-monosulfate of VIII, and small amounts of VIII and its 16-sulfate. This fraction was evaporated to dryness under reduced pressure at 45° to give colorless solid. The solid was dissolved in 0.1N NH_4OH solution (300ml) and extracted with ether (100ml) to remove unreacted steroid. The aqueous layer was lyophilized and recrystallized from MeOH-ether to give IXa (740mg) as colorless amorphous powder, mp>280°. Anal. Cacd. for $C_{19}H_{31}O_6NS \cdot H_2O$: C, 54.40; H, 7.93; N, 3.34. Found C, 54.31; H, 7.87; N, 3.67. IR (KBr): ν_{max} 3100-3150 (OH, NH), 1742 (C=O), 1225 (SO_4). NMR [pyridine-d_5-CD_3OD (1:3)]: δ 0.88 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 4.17-4.67 (2H, m, 3α-H and 16β-H).

Characterization of the sulfate IXa. IXa (125mg) was acetylated with 3.5ml of pyridine-acetic anhydride (6:1) for 15 hr at room temperature. The solvent was removed under N_2 gas and the residue was dissolved in 10ml of water. To the solution was added 50% H_2SO_4 to adjust to pH 1 and added NaCℓ to saturation. The solution was extracted with AcOEt (10ml) and the organic layer was allowed to stand at 37° for 8 hr. The organic layer was washed with 5% $NaHCO_3$ and water and dried over anhyd. Na_2SO_4. The solvent was evaporated to dryness to yield a solid. The residue was recrystallized from MeOH to give 3β-hydroxy-16α-acetoxyandrost-5-en-17-one (X) as colorless plates, mp 164-165° (lit. (9) mp 162-163°). NMR ($CDCl_3$): δ 0.94 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 2.07 (3H, s, 16α-OAc), 3.38 (1H, m, 3α-H), 5.27-5.53 (2H, m, 5-H and 16β-H)

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