# SYNTHESIS AND SOME REACTIONS OF 6-BROMOANDROGENS: POTENTIAL AFFINITY LIGAND AND INACTIVATOR OF ESTROGEN SYNTHETASE

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## ABSTRACT

The synthesis of epimeric 6-bromo-4-androstene-3,17-dione (1a and 1b), 6-bromotestosterone (2a and 2b) and its acetate (3a and 3b), and 6-bromo-16α-acetoxy-4-androstene-3,17-dione (5a and 5b), and 6β-bromo-16α-hydroxy-4-androstene-3,17-dione (4) is described. The interconversions among compounds 1, 2, and 3 are also studied. The 6β-isomer (1b, 2b, and 3b) was epimerized to the 6α-isomer (1a, 2a and 3a) in carbon tetrachloride or chloroform-methanol (9:1) and the 6α-isomer was isolated by fractional crystallization from the epimeric mixture. 6α-Bromo isomer 1a was also epimerized back to 6β-bromo isomer 1b in chloroform-methanol (9:1). Two polymorphic forms of 6β-bromotestosterone acetate (3b) were isolated (mp. 114-117° and 138-141°). The 6β-bromo isomers were found to be unstable in methanol and decomposed to give 5α-androstane-3,6-dione derivative (6). The results of irreversible inactivation of human placental androgen aromatase with some of these 6-bromoandrogens are discussed.

## INTRODUCTION

Estrogen synthetase (androgen aromatase) catalyzes the conversion of androgens into estrogens. Despite many years of study, it has not been definitively clarified whether or not the sequence of conversion proceeds directly at a single active site [2-5] or whether or not several different aromatases exist for the various androgen substrates [6-9]. Active-site-directed irreversible aromatase inhibitors would play a critical role in further study of purification and characterization of the enzyme system. Schwarzel *et al.* [10], Schubert *et al.* [11], Siiteri and Thompson [12] and Brueggemeier *et al.* [13] examined

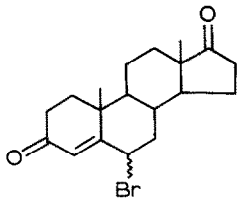
the reversible inhibition of aromatase activity with a large number of steroids. Recently we reported that 6α-bromo-4-androstene-3,17-dione (1a) is an active-site-directed irreversible inhibitor of microsomal aromatase from human placenta [14]. We also found that two distinctive androgen aromatases are separated from human term placenta through solubilization and purification [15,16]. Therefore we planned to synthesize several epimeric steroidal 6-bromo-4-en-3-ones whose structural features may achieve different affinity labeling toward the different aromatases.

The bromination of steroidal 4-en-3-ones at the C-6 position with N-bromosuccinimide (NBS) has been reported to give only the 6β-bromo-isomer [14,17-19], when $CC\ell_4$ dehydrated over $P_2O_5$ is used as solvent. However, Burnett and Kirk [17] reported that the bromination product of 4-androstene-3,17-dione in $CC\ell_4$ dehydrated over $CaC\ell_2$ was a mixture of the 6α- and 6β-isomers (1a and 1b) (6α:6β = 1:3), but that the isomers could not be separated by chromatography or fractional crystallization.

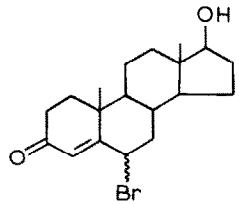
In this paper we describe for the first time the synthesis of 6α- and 6β-bromotestosterone (2a and 2b), 6α-bromotestosterone acetate (3a), 6β-bromo-16α-hydroxy-4-androstene-3,17-dione (4), and 6α- and 6β-bromo-16α-acetoxy-4-androstene-3,17-dione (5a and 5b).

## RESULTS AND DISCUSSION

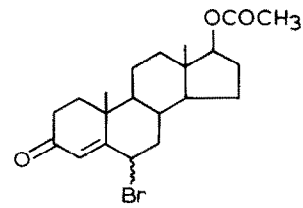
When 4-androstene-3,17-dione, testosterone, its acetate, and 16α-hydroxy-4-androstene-3,17-dione acetate were submitted to the bromination, each showed complete bromination within 20 min in accord with previous reports [14,17-19]. As shown in Table I, however, the relative amount of 6α- to 6β-isomer was significantly different among the



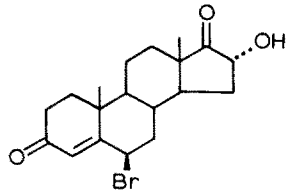
1a : 6α - Br
b : 6β - Br



2a : 6α - Br
b : 6β - Br



3a : 6α - Br
b : 6β - Br



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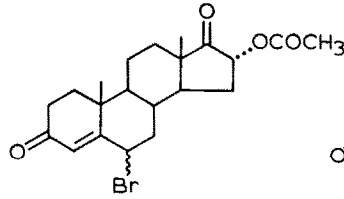
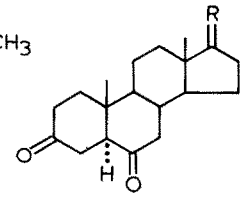
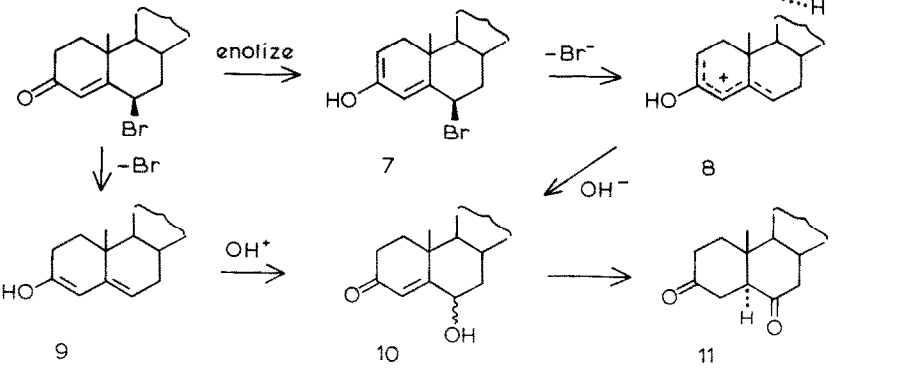


5a : 6α - Br
b : 6β - Br



6a : R = O
b : R = OH, H
c : R = OCOCH3, H




7 8 9 10 11

Table I. Bromination of 4-Androsten-3-ones with N-Bromosuccinimide in $CC\ell_4$

| Steroid | Reaction time (min) | Relative amounts of product[a] 6α-Bromo | 6β-Bromo |
|---|---|---|---|
| 4-Androstene-3,17-dione | 20 | 0 | 100 |
| | 40 | 35 | 65 |
| Testosterone | 20 | 30 | 70 |
| | 40 | 50 | 50 |
| Testosterone acetate | 20 | 0 | 100 |
| | 40 | 0 | 100 |
| | 60 | 0 | 100 |
| 16α-Acetoxy-4-androstene-3,17-dione | 20 | 5 | 95 |
| | 40 | 35 | 65 |

[a]Relative amounts of the 6α- and 6β-isomers were determined by NMR spectra of the reaction mixtures.

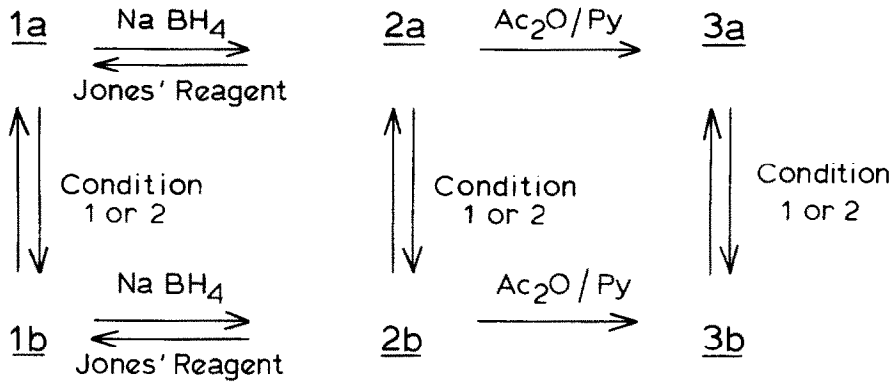
steroidal 4-en-3-ones having different D-ring substituents. This was evident from the characteristic signals of C-4 proton in the NMR spectra of the reaction mixtures which appeared at approximately 5.9 ppm (1H, s, 6β-bromo isomer) and 6.4 ppm (1H, d, J=2Hz, 6α-bromo isomer). Testosterone gave a 3:7 ratio of the 6α- to 6β-bromo isomer at 20 min reaction time. In contrast, double the reaction time was needed to obtain a similar ratio when 4-androstene-3,17-dione or its 16α-acetoxy derivative was brominated. Testosterone acetate did not give any detectable amount of 6α-bromo compound (3a), even after 60 min of heating under reflux. Although detailed conformational analysis by X-ray crystallography of these compounds is not yet available, a conformational transmission of distortion through the B-C-D rings might be in operation. The bromination of 16α-hydroxy-4-androstene-3,17-dione produced only a small amount of the 6β-bromo compound (4) along with unidentified compounds. TLC and NMR (C-4 proton and angular methyl

groups) of the reaction mixture indicated that one of the by-products may be a rearranged ketol.

The 6α-bromo (1a, 2a, and 5a) and 6β-bromo (1b, 2b, and 5b) isomers were readily separated by fractional crystallization of the mixture (6α:6β ≒ 3:7) from EtOH. Successful separation was achieved only when EtOH was used as a crystallizing solvent. Use of other solvents (AcOEt, acetone, n-hexane, $Et_2O$, or MeOH) gave poor results due to the instability of the 6-brominated compounds. As the first 16-hydroxylated 6-bromoandrogen to be synthesized, the 16α-hydroxy-6β-bromo compound 4 was isolated in pure form through recrystallization from EtOH following purification by TLC (AcOEt).

To characterize the brominated compounds and also to synthesize the unavailable 6α-bromotestosterone acetate (3a), we explored the interconversion among these compounds (Scheme 1). Both isomers of 6-bromotestosterone, 2a and 2b, were quantitatively converted to their corresponding acetates (3a and 3b) with acetic anhydride-pyridine. Oxidation of 2a and 2b with Jones' reagent [20] afforded the desired 17-keto compounds (1a and 1b). The yield (5% on isolation) of 1b was less than that (21.5%) of 1a. Reduction of 1a and 1b with $NaBH_4$ in EtOH under cooling gave 2a and 2b in 15.5 and 2.3% yield on isolation, respectively. These differences observed between the 6α- and 6β-bromo isomers might be due to stability differences of these compounds during reaction and isolation.

Epimerization of the 6β-bromo to the 6α-isomer with acid has been reported [14,21], but only in poor yield. Therefore, we examined the epimerization of 3b to 3a using various conditions in an attempt to obtain a higher yield of the 6α-isomer (Table II). Drastic conditions




Condition 1: Refluxing in $CCl_4$.
Condition 2: In $CHCl_3$-MeOH at room temperature.

Scheme 1

Table II. Epimerization of 6β-Bromotestosterone Acetate to 6α-Bromotestosterone Acetate

| | Condition | Relative amounts of product[a] | | |
|---|---|---|---|---|
| | | 6β-Br | 6α-Br | by-product |
| A | MeOH-HCℓ, 50°, 0.5 hr | 0 | 10 | 90 |
| B | MeOH-HCℓ, R.T.[b], 0.25 hr | 85 | 10 | ≈5 |
| C | MeOH-HCℓ, R.T., 0.5 hr | 35 | 30 | 35 |
| D | $CCℓ_4$, R.T., 48 hr | 65 | 30 | ≈5 |
| E | $CHCℓ_3$-MeOH, R.T., 24 hr (9:1) | 68 | 30 | ≈2 |

[a]Relative amounts of the product were determined by NMR spectra of the reaction mixture and TLC.

[b]R.T. = room temperature.

(A, B and C) using HCℓ as catalyst gave undesired by-products in relatively high yield, impeding the crystallization of the 6α-isomer (3a). Using $CCℓ_4$, with prior distillation, or $CHCℓ_3$-MeOH (9:1) (D or E) as solvent, without addition of acid, 3a was isolated in 11 to 13% yield by fractional crystallization. Epimerization of the 6β-bromo to 6α-isomer under conditions D and E might be caused by trace amounts of acid liberated from the 6-bromo compound in the reaction mixture. Recovered 3b from the isomerization mixture showed a different melting point (mp 138-141°) and IR (KBr) spectrum from the starting 3b, mp 114-117°, which was obtained by the bromination of testosterone acetate or acetylation of 2b. On the other hand, Djerassi *et al.* [18], reported mp 140-142° for the product obtained by bromination of testosterone acetate. Both crystalline products showed identical elemental analysis, and NMR, IR ($CHCℓ_3$), and UV (95% EtOH) spectra. The exact reason for this polymorphism is not yet known, and total structure determination of both crystals by X-ray crystallography is under way. Epimerization of 6α-bromo isomer 1a to the 6β-isomer 1b was also observed under condition E resulting in 1:1 mixture of 6α to 6β.

During the course of the epimerization experiments we found that the 6β-isomers (1b, 2b and 3b) were converted to the corresponding 5α-androstane-3,6-diones (6a, b, and c) in good yield by refluxing in MeOH for 10 to 20 min or standing at room temperature for 1 to 2 days. However, the 6α-isomer (1a) was stable under the same conditions. David and Beauloye [21] reported that the 19-nor compounds of both 1a and b were converted to 19-nor-5α-androstane-3,6,17-trione by refluxing in acetone containing 0.5% of 12 N HCℓ and that the 6β- and 6α-hydroxy-4-ene-3,17-diones might be intermediates of the above conversion. The

6β-bromo compound first may be converted to the 3,5-diene (9) [22] or to the mesomeric cation (8) [17]. It would then be followed by an electrophilic or nucleophilic attack of a hydroxyl ion at the 6-position to give rise to the 6-hydroxy compounds (10). Either epimer 10 easily isomerizes to the 5α-3,6-dione (6) [22]. When $CH_3OD$ was used as a solvent for the conversion, there was no observed incorporation of deuterium into the 5α-3,6-dione (6). This result implies that the 4- and 5α-protons of compound 6 are derived from the steroid molecule and not from the solvent. It was also confirmed by TLC that 6 is formed in a minor yield under the epimerization condition D and E (Table II).

It has been shown that both stereoisomers 1a and 1b are competitive inhibitors of 4-androstene-3,17-dione aromatization by placental microsomes and that only the 6α-bromo isomer 1a is effective in the active-site-directed inactivation of microsomal aromatase under nitrogen at 4° in a dithiothreitol (DTT) containing buffer [14]. The 6β-bromo isomer 1b was later found also to be effective when it was incubated with microsomal aromatase without DTT [23]. More recently, using placental tissue slice in a culture medium with NADPH under air at 25°, 1a, 1b and 3a were found to be effective irreversible inactivators of placental estrogen biosynthesis in the inact cell system with the relative effectiveness of 1a<1b ≒ 3a [16]. 6α-Bromotestosterone acetate 3a also showed a marked selectivity of inactivation against aromatase I and II. With 6μM 3a, 80% of estriol formation (aromatase I activity) from 16α-hydroxytestosterone was irreversibly eliminated while only 20% of 4-androstene-3,17-dione aromatization (aromatase II activity) to estrone and estradiol-17β was absolished under the same condition [15], sug-

gesting that the two different androgen aromatase systems may have the major difference in the D-ring contact region of the active site.

## EXPERIMENTAL

Materials and General Methods. 16α-Hydroxy-4-androstene-3,17-dione and its acetate were synthesized in this laboratory according to newly developed methods [24]. Melting points were measured on a Fisher-Jones melting point apparatus and were uncorrected. UV spectra were obtained with a Varian Cary 14 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 267 spectrophotometer in KBr pellets or $CHC\ell_3$. NMR spectra were obtained with a Varian EM-360 spectrometer at 60 MHz using tetramethylsilane ($Me_4Si$) as internal standard.

General Procedure for the Reaction of Steroidal 4-En-3-ones with NBS. To a solution of steroidal 4-en-3-one (34.7 mmol) in 800 ml of $CC\ell_4$, distilled before use, were added NBS (56.2 nmol) and benzoyl peroxide (1.3 mmol). The solution was heated under reflux for an appropriate time. It was then filtered to remove any insoluble material, the filtrate was washed with $H_2O$, 5% $NaHCO_3$ solution and again with $H_2O$. The organic phase was evaporated to dryness under reduced pressure and the residue was triturated with a small amount of 95% EtOH to give 80-95% yield of the crude brominated products.

6α- and 6β-Bromo-4-androstene-3,17-dione (1a and 1b). The crude product (6α:6β ≒ 1:2) obtained from 4-androstene-3,17-dione after 40 min reflux as described under General Procedure was triturated with chilled 95% EtOH (200 ml). The solid portion was collected by filtration, dissolved in 95% EtOH (100 ml) under reflux, and then allowed to stand at room temperature to give the 6α-isomer (1a) as slightly yellow prisms (1.8 g): mp 172-174° (decomp.) (171-173° decomp. [14], 175-177° decomp. [21]). IR (KBr) $\nu_{max}$ 1730, 1660, 1610 $cm^{-1}$. NMR ($CDC\ell_3$) δ 0.92 (3H, s, 18-$CH_3$), 1.25 (3H, s, 19-$CH_3$), 4.85 (1H, m, 6β-H), 6.45 (1H, d, $J_{4,6\beta}$ = 2Hz, 4-H).

The EtOH soluble portion of the product was condensed and repeatedly recrystallized from 95% EtOH to give the 6β-isomer (1b) as slightly yellow needles (7.1 g): mp 156-158° (decomp.) (158-160° decomp. [14], 162-164° decomp. [17], 187-190° decomp. [21]). IR (KBr) $\nu_{max}$ 1735, 1678, 1608 $cm^{-1}$. NMR ($CDC\ell_3$) δ 0.98 (3H, s, 18-$CH_3$), 1.57 (3H, s, 19-$CH_3$), 5.05 (1H, m, 6α-H), 5.95 (1H, s, 4-H).

6α- and 6β-Bromo-17β-hydroxy-4-androsten-3-one (2a and 2b). The crude solid (6α:6β ≒ 1:2) obtained after 20 min reflux was recrystallized from 95% EtOH to give the 6β-isomer rich crystalline material (6α:6β ≒ 1:9). Repeated recrystallization of the mixture from 95% EtOH gave the 6β-isomer 2b as needles (4.8g): mp 128-131°.

Anal. Calcd for $C_{19}H_{27}O_2Br$: C, 62.12; H, 7.40. Found C, 62.01; H, 7.24

UV $\lambda_{max}$ (95% EtOH) 247.5 nm (ε 9040). IR (KBr) $\nu_{max}$ 3480, 1663, 1607 $cm^{-1}$. NMR ($CDC\ell_3$) δ 0.85 (3H, s, 18-$CH_3$), 1.55 (3H, s, 19-$CH_3$), 3.67 (1H, t, $J_{17\alpha,16}$ = 7.6Hz, 17α-H), 4.97 (1H, m, 6α-H), 5.90 (1H, s, 4-H).

The mother liquor of the first recrystallization was condensed to give a crude solid (6α:6β = 3:2). The solid was repeatedly recrystallized from 95% EtOH affording pure 6α-isomer 2a (850 mg) as plates: mp 152-155° (decomp.).

Anal. Calcd for $C_{19}H_{27}O_2Br$: C, 62.12; H, 7.40. Found C, 61.99; H, 7.21.

UV $\lambda_{max}$ (95% EtOH) 237.5 nm (ε 10200). IR (KBr) $\nu_{max}$ 3420, 1676, 1613 $cm^{-1}$. NMR ($CDC\ell_3$) δ 0.79 (3H, s, 18-$CH_3$), 1.23 (3H, s, 19-$CH_3$), 3.67 (1H, t, J = 7.6Hz, 17α-H), 4.87 (1H, m, 6β-H), 6.40 (1H, d, J = 2Hz, 4-H).

17β-Acetoxy-6β-bromo-4-androsten-3-one (3b). The crude solid (6β only) obtained after 40 min reflux was recrystallized from 95% EtOH to give pure 3b (8.9 g) as needles: mp 114-117° (140-142° [18]).

Anal. Calcd for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14. Found C, 61.79; H, 7.23.

UV $\lambda_{max}$ (95% EtOH) 245.5 nm (ε 12800). IR (KBr) $\nu_{max}$ 1730, 1675, 1605 $cm^{-1}$. IR ($CHC\ell_3$) $\nu_{max}$ 1725, 1675, 1605 $cm^{-1}$; NMR ($CDC\ell_3$) δ 0.90 (3H, s, 18-$CH_3$), 1.55 (3H, s, 19-$CH_3$), 2.03 (3H, s, 17β-$OCOCH_3$), 4.60 (1H, t, J = 8Hz, 17α-H), 5.00 (1H, m, 6α-H), 5.87 (1H, s, 4-H).

6β-Bromo-16α-hydroxy-4-androstene-3,17-dione (4). A portion (400 mg) of the crude solid (6β:nonbrominated compound ≒ 1:1) obtained after 7 min reflux was submitted to preparative TLC (Analtech Uniplate Silica Gel GF, 500 μm) using AcOEt as a developing solvent. The lower Rf portion of the chromatogram gave a 6β-bromo-enriched fraction. Recrystallizations of the fraction from 95% EtOH-$CHC\ell_3$ gave 4 (15 mg): mp 158-161° (decomp.).

Anal. Calcd for $C_{19}H_{25}O_3Br$: C, 59.85; H, 6.61. Found C, 59.51; H, 6.80.

UV $\lambda_{max}$ (95% EtOH) 246 nm (ε 10500). IR (KBr) $\nu_{max}$ 3370, 1745, 1652, 1610 $cm^{-1}$. NMR ($CDC\ell_3$-$CD_3OD$, 5:1) δ 1.08 (3H, s, 18-$CH_3$), 1.56 (3H, s, 19-$CH_3$), 4.35 (1H, m, 16α-H), 5.00 (1H, m, 6α-H), 5.90 (1H, s, 4-H).

6α- and 6β-Bromo-16α-acetoxy-4-androstene-3,17-dione (5a and 5b). The crude solid (6α:6β = 1:2) obtained after 30 min reflux was recrystallized from 95% EtOH to give the 6β-isomer 5b (6.9 g) as needles: mp 128-130°.

Anal. Calcd for $C_{21}H_{27}O_4Br$: C, 59.58; H, 6.43. Found C, 59.60; H, 6.23.

UV $\lambda_{max}$ (95% EtOH) 248 nm (ε 12200). IR (KBr) $\nu_{max}$ 1758, 1733, 1676, 1604 $cm^{-1}$. NMR ($CDCl_3$) δ 1.07 (3H, s, 18-$CH_3$), 1.56 (3H, s, 19-$CH_3$), 2.08 (3H, s, 16α-$OCOCH_3$), 4.98 (1H, m, 6α-H), 5.90 (1H, s, 4-H).

The mother liquor was condensed to give a crude solid (6α:6β ≒ 2:3). The solid was removed by filtration, and the filtrate was further condensed to yield a 6α-isomer-enriched product. Recrystallization of the product from 95% EtOH gave pure 6α-isomer 5a (630 mg) as plates: mp 169-172° (decomp.).

Anal. Calcd for $C_{21}H_{27}O_4Br$: C, 59.58; H, 6.43. Found C, 59.60; H, 6.23.

UV $\lambda_{max}$ (95% EtOH) 241 nm (ε 11000). IR (KBr) $\nu_{max}$ 1753, 1732, 1661, 1608 $cm^{-1}$. NMR ($CDCl_3$) δ 1.00 (3H, s, 18-$CH_3$), 1.26 (3H, s, 19-$CH_3$), 2.09 (3H, s, 16α-$OCOCH_3$), 4.80 (1H, m, 6β-H), 6.40 (1H, d, J=2Hz, 4-H).

Acetylation of the 17-Hydroxyl Compounds (2). i) 2a (200 mg) was acetylated in 2 ml of pyridine and 1 ml of $Ac_2O$. Recrystallization of the acetylated product from 95% EtOH gave 6α-bromotestosterone acetate (3a) (120 mg) as colorless plates: mp 129-133° (decomp.).

Anal. Calcd for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14. Found C, 61.81; H, 7.15.

UV $\lambda_{max}$ (95% EtOH) 240 nm (ε 11200). IR (KBr) $\nu_{max}$ 1730, 1675, 1612 $cm^{-1}$. NMR ($CDCl_3$) δ 0.83 (3H, s, 18-$CH_3$), 1.22 (3H, s, 19-$CH_3$), 2.03 (3H, s, 17β-$OCOCH_3$), 4.60 (1H, t, J = 8Hz, 17α-H), 4.81 (1H, m, 6β-H), 6.38 (1H, d, J = 2Hz, 4-H).

ii) 2b was converted quantitatively to 3b by the same procedure described above. Recrystallization of the product from 95% EtOH gave 3b as colorless needles, mp 115-118°. This product was identical to that previously synthesized by bromination of testosterone acetate.

Oxidation of 2 with Jones' Reagent. 2a and 2b (200 mg) were each oxidized with Jones' reagent under cooling. Following the usual procedure, the oily crude product was purified by preparative TLC (n-hexane-AcOEt, 2:1) to give the corresponding 17-oxo compound 1a (21.5% yield) and 1b (5% yield), respectively. The products were identical with those prepared by bromination of 4-androstene-3,17-dione.

Reduction of the 17-Oxo Compounds (1) with $NaBH_4$. 1a and 1b (200 mg) were each dissolved in 95% EtOH (300 ml). To the solution was added $NaBH_4$ (20 mg) and the mixture was allowed to stand at 4° overnight. After the usual procedure of decomposition and extraction, an oily substance was obtained and the product was purified by preparative TLC (n-hexane-AcOEt, 2:1) to give 2a (15.5% yield) and 2b (2.3% yield), respectively. These 17β-hydroxy compounds were identical to those previously synthesized through other routes.

General Procedure for the Epimerization of 6β-Bromo to 6α-Bromo Isomer. i) Conditions A, B and C (Table II): To a solution of 3b (150 mg) in methanol (20 ml) was added 0.1 ml of methanolic HCℓ (25% w/w). The mixture was heated to 50° for 30 min (A), or allowed to stand at room temperature for 15 min (B) or 30 min (C). Epimerization rate was determined by the relative intensity of the C-4 proton signals and the two angular methyl groups in NMR spectra of the reaction mixture.

ii) Conditions D and E (Table II): A solution of 3b (200 mg) in $CCℓ_4$ or $CHCℓ_3$-MeOH (9:1) (5 ml) (D or E) was allowed to stand at room temperature for 48 or 24 hrs in the dark. Epimerization rate was determined by NMR as described above.

A Polymorphic Form of 17β-Acetoxy-6β-bromo-4-androsten-3-one (3b). After the treatment of 3b, mp 114-117°, under Condition D or E described above, the epimeric mixture was separated by fractional crystallization from 95% EtOH. Repeated crystallization of the first crop gave pure 6β-bromo isomer 3b (51 mg) by use of Condition D, and 56 mg by use of Condition E, mp 138-141°, as plates. UV (95% EtOH), NMR and IR ($CDCℓ_3$) spectra of 3b obtained by the epimerization were identical to those synthesized by the bromination of testosterone acetate or acetylation of 2b. Mixed melting point of a 1:1 mixture of the two polymorphic 3b was 126.5-128.5°. They showed different IR in the solid in the range of 1200-1500 $cm^{-1}$, IR (KBr) $\nu_{max}$ 1725, 1672, 1602 $cm^{-1}$.

Anal. Calcd for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14. Found C, 61.59; H, 6.82.

17β-Acetoxy-6α-bromo-4-androsten-3-one (3a). The first mother liquor separated in the fractional crystallization of the epimerized product of 3b described above gave a 6α-bromo isomer-rich crystalline product. Further recrystallizations from 95% EtOH gave pure 3a in 13% yield by use of Condition D and 11% by use of Condition E. The product was identical in all respects with 3a synthesized by acetylation of 2a (see above).

Reverse Epimerization of 6α-Bromo Isomer 1a to 6β-Bromo Isomer 1b. A solution of 1a (50 mg) in 0.5 ml of $CDCℓ_3$-$CD_3OD$ (9:1) was allowed to stand at room temperature in the dark (Condition E in deuterated solvent). NMR spectrum of the solution after 24 hr showed 1:1 mixture of the 6α- and 6β-bromo isomers as analyzed by their characteristic signals of angular methyl groups and C-4 protons. An extensive incorporation (over 80%) of deuterium into C-6 during the epimerization was detected by diminished signals of both 6α- and 6β-hydrogens of the 6-bromo compounds and appearance of the signal due to $CD_3OH$.

General Procedure for the Conversion of 6β-Bromo Isomer to a 5α-Androstane-3,6-dione Derivative. The 6β-isomer (1b, 2b, and 3b, 1.25 mmol) was dissolved in MeOH (50 to 100 ml) and heated under relux for 10 to 20 min. The solvent was evaporated under reduced pressure and the residue was purified by recrystallization to give the corresponding 5α-androstane-3,6-dione derivative in 53-60% yield.

5α-Androstane-3,6,17-trione (6a). Recrystallization from acetone-n-hexane of the crude product of 1b treatment gave 6a, mp 192-194.5° (196-196.5° [21]), as colorless plates. IR (KBr) $\nu_{max}$ 1733, 1722, 1695 $cm^{-1}$. NMR ($CDCl_3$) δ 0.90 (3H, s, 18-$CH_3$), 0.98 (3H, s, 19-$CH_3$).

17β-Hydroxy-5α-androstane-3,6-dione (6b). Recrystallization of the reaction product of 2b from acetone-n-hexane gave 6b, mp 225-227° (232-235° [22]), as colorless needles. IR (KBr) $\nu_{max}$ 3520, 1700 $cm^{-1}$. NMR ($CDCl_3$) δ 0.77 (3H, s, 18-$CH_3$), 0.97 (3H, s, 19-$CH_3$), 3.70 (1H, t, J = 8Hz, 17α-H).

Repetition of this conversion in $CH_3OD$ (100 mg 2b, 8 ml $CH_3OD$ (99.5 atom %) and 10 min reflux) gave 45 mg of 6b, mp 226-227°, as colorles needles. The NMR spectrum was identical with that of 6b obtained from non-labeled medium described above.

17β-Acetoxy-5α-androstane-3,6-dione (6c). Recrystallization from MeOH of the 3b reaction product gave 6c, mp 175-178°, (186.5-188.5° [22]), as colorless leaflets. IR (KBr) $\nu_{max}$ 1724, 1700 $cm^{-1}$. NMR ($CDCl_3$) δ 0.73 (3H, s, 18-$CH_3$), 0.96 (3H, s, 19-$CH_3$), 2.03 (3H, s, 17β-$OCOCH_3$), 4.61 (1H, t, J = 8Hz, 17α-H).

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REFERENCES

The following trivial names have been used in this paper:

Testosterone = 17β-hydroxy-4-androsten-3-one
6α- and 6β-Bromotestosterone = 6α- and 6β-bromo-17β-hydroxy-4-androsten-3-one
6α-Bromotestosterone acetate = 17β-acetoxy-6α-bromo-4-androsten-3-one
6β-Bromotestosterone acetate = 17β-acetoxy-6β-bromo-4-androsten-3-one

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