Improved Synthesis and Molecular Modeling of 4β,19-Dihydroxyandrost-5-en-17-one, an Excellent Inhibitor of Aromatase

Mitsuteru NUMAZAWA,* Keiko YAMADA, Yoko WATARI, and Momoko ANDO

Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan.
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4β,19-Dihydroxyandrost-5-en-17-one (6) is an excellent competitive inhibitor of estrogen synthetase (aromatase). Alternate, improved synthesis of this inhibitor was established. Treatment of 19-(*tert*-butyldimethylsilyloxy)androst-4-en-17-one (8) with *m*-chloroperbenzoic acid gave a 1.4 : 1 mixture of 4α,5α-epoxide 9 and its 4β,5β-isomer 10. The mixture was reacted with dil. $HClO_4$ in dioxane to produce principally 4β,5α-diol 11 (80%) of which acetylation followed by dehydration with $SOCl_2$ yielded 4β,19-diacetoxy-5-ene compound 14 in good yield. Alkaline hydrolysis of diacetate 14 gave 4β,19-diol 6. The minimum energy conformation of the powerfull aromatase inhibitor 6 was obtained with the PM3 method and compared with that of the structurally related diol steroid, 4-ene-5β,19-diol 3, a weak competitive inhibitor.

Key words aromatase inhibitor; 4β,19-dihydroxyandrost-5-en-17-one; synthesis; molecular modeling; PM3 method

Aromatase is a cytochrome P-450 enzyme complex responsible for the conversion of the 4-en-3-one androgens, androst-4-ene-3,17-dione (AD) and testosterone, into estrogens, estrone and estradiol.[1—3] Aromatization of the androgens is thought to proceed through three sequential oxygenations at the C-19 position, respectively.[4—6] In the third step, the angular methyl group at C-19 and 1β,2β-hydrogens are eliminated to result in the aromatization of the A-ring of the androgen molecule to form estrogen. Inhibitors of aromatase are valuable as therapeutic agents in the treatment of the advance breast cancer.[7]

Structure–activity studies on aromatase inhibitors[8—14] and a recent structural prediction of aromatase by modeling[15,16] indicated the existence of a hydrophobic binding pocket extending roughly in the plane of substrate AD from the position that would be occupied by its C_4,[8,9] C_6[10,11] and C_7[12—14] atoms. On the other hand, in the course of studies on structure-activity relationships of series of 3-deoxy androgens, androst-4-en-17-one (**1**)[17] and its 5-ene isomer **4**,[18] and their 19-hydroxy analogs **2**[19] and **5**,[18] we found that 4β,19-dihydroxy-5-ene steroid **6**[20] is one of the most powerful competitive inhibitor of aromatase among the steroidal compounds reported so far (Fig. 1). This finding indicates that the polar 4β,19-diol moiety of 3-deoxy compound **6** can be torelated in the pocket of the active site of aromatase. Thus, diol **6** would play an important role in not only the development of a powerful aromatase inhibitor but also understanding the spacial and electronical nature of the active site.

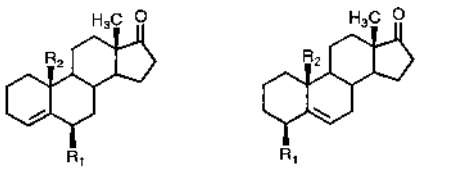
Compound **6** was previously synthesized from 4β-acetoxyandrost-5-en-17-one (**7**) in four steps through hypoiodite oxidation of 5α-bromo-6β-ol intermediate, giving 5α-bromo-6β,19-epoxide, as a key reaction.[21] We report here the alternate, improved synthesis of diol **6** suitable for a gram-order scale synthesis along with molecular modeling of this compound with the PM3 method for understanding its three dimensional structure.

Results and Desucussion

Synthetic sequence employed in this study involves an acid-catalyzed *trans*-diaxial cleavage of an epoxy moiety of 4,5-epoxy steroids **9** and **10** as a key reaction for the introduction of a 4β-hydroxy group to a steroid nucleus (Chart 1). Treatment of 19-(*tert*-butyldimethylsilyloxy)androst-4-en-17-one (**8**), previously synthesized,[19] with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 gave a 1.4 : 1 mixture of 4α,5α-epoxide **9** and its 4β,5β-isomer **10** in 90% yield. The configuration of the epoxy ring was established according to the ^{1}H-NMR spectroscopic data of 4α,5α- and 4β,5β-epoxy steroids having a 19-methyl group[22]; lower chemical shifts of one of 19-CH_2 (δ 3.76 ppm) and 4α-H (δ 2.97 ppm) of 4α,5α-epoxide **9**, compared to the corresponding C-19 and C-4 protons (δ 3.51 and 2.87 ppm) of the 4β,5β-epoxide **10**. Irradiation of the 4-H signal of compound **9** or **10** produced no significant nuclear overhauser effect (NOE) enhancement of the 19-methylene protons in each case.

4α,5α-Epoxide **9** and the β-isomer **10** were separately treated with diluted $HClO_4$ in dioxane produced the kinetic controlled product *trans*-axial diol 4β,5α-diol **11**, in 75% and 79% yields where the *trans*-equatorial diol product, 4α,5β-diol **12**, was also obtained as a minor product in 3 and 7% yields, respectively. Reaction of 4β-acetoxy-5α-ol **13**, obtained by acetylation of steroid **11** with acetic anhydride and pyridine, with $SOCl_2$ gave the dehydrated product in a *trans*-diaxial manner, 4β-acetoxy-5-ene steroid **14**, in a good yield. An axial acetoxy group at C-4β prevented production of 4-ene analog of compound **14**. Hydrolysis of diacetate **14** with K_2CO_3 in aqueous MeOH gave 4β,19-diol **6**.

In a sequence developed in this study, chromatographic separation of the intermediate is not essential in each step. This fact along with a higher yield of diol **6** from the starting material **8** indicate that this synthesis would be suitable for a



1 ; R_1=H, R_2=CH_3
2 ; R_1=H, R_2=CH_2OH
3 ; R_1=OH, R_2=CH_2OH
4 ; R_1=H, R_2=CH_3
5 ; R_1=H, R_2=CH_2OH
6 ; R_1=OH, R_2=CH_2OH
7 ; R_1=$OCOCH_3$, R_2=CH_3

Fig. 1. Structures of 3-Deoxy Steroids

* To whom correspondence should be addressed. e-mail: numazawa@tohoku-pharm.ac.jp

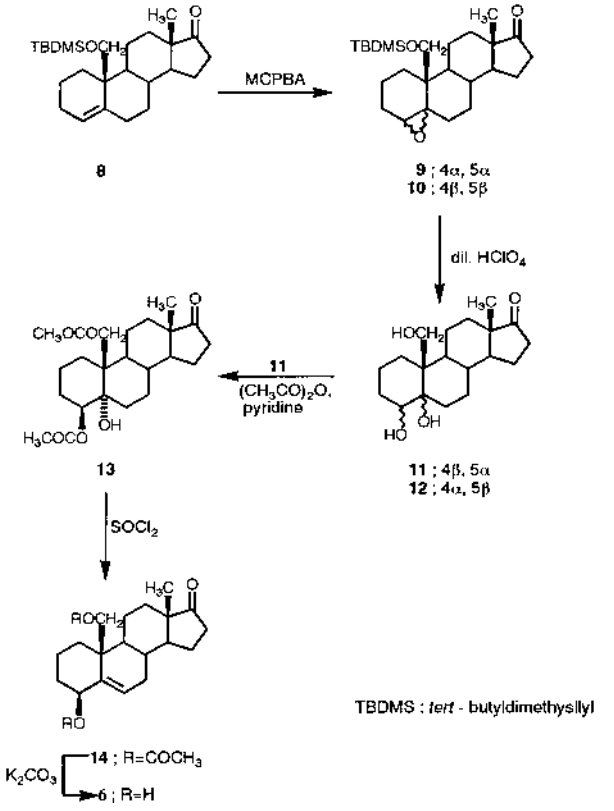


Chart 1

Fig. 2. Overlay of 6β,19-Diol **3** (Light Line) and 4β,19-Diol **6** (Dark Line) by Superimposing Their Respective Steroid Nucleus

gram-order synthesis of the powerful aromatase inhibitor **6**.

The minimum-energy conformations of inhibitor **6** and its structurally related analog 4-ene-6β,19-diol **3**, a weak aromatase inhibitor,[20] were determined by the MOPAC package using PM3 Hamitonian. The C- and D-rings of the steroid backbone as well as the C-17 carbonyl miety and the C-19 angular methyl group of the two diols were excellently superimposed each other (Fig. 2). There was observed a marked difference of the orientation of the C-3, C-4, C-5, and C-6 positions between the two compounds.

Structure–activity relationship and the aromatase-catalyzed 19-oxygenation of a series of 3-deoxy C_{19} steroids[23] previously revealed that there is a marked difference in the binding manner between the two parent 3-deoxy-4-ene and 5-ene steroids, **1** and **4**, in the active site, and that the binding aspect of the latter is comparable to that of the natural substrate AD. The mechanistic, site-directed mutagenesis, and molecular modeling studies of aromatase[4,5,15,24—27] indicate that a carboxyl group of ^{302}Glu and ^{309}Asp would play a critical role in the catalytic mechanism of aromatase reaction. On the basis of these previous reports, it is likely that 4β,19-diol **6** would be anchored by hydrogen bonds between two hydroxy groups of the inhibitor and two carboxyl groups of the polar amino acid residues, ^{302}Glu and ^{309}Asp, in the active site, indicating that the orientation of a 4β,19-diol function of inhibitor **6**, rather than that of the C-3, C-4, C-5, and C-6 positions, would be essential for producing thermodynamically stable enzyme-inhibitor complex in the active site. The remaining polar function of this inhibitor, the C-17 carbonyl group, also would be very important in anchoring this in the active site, as seen in the binding of the other 3-deoxy steroids.[17,18]

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Parkin-Elmer FT-IR 1725 spectrophotometer and ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with a JEOL EX 270 (270 MHz) spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) was obtained with a JEOL JMS-DX 303 spectrometer. TLC was performed on E. Merk pre-coated with silica gel (E. Merk, 70—230 mesh). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Tokyo Kasei Kogyo Co.

Epoxydation of 19-(*tert*-Butyldimethylsilyloxy)androst-4-en-17-one (8) with MCPBA MCPBA (222 mg, 1.29 mmol) was added to a solution of 4-ene steroid **8** (390 mg, 0.97 mmol) in CH_2Cl_2 (22 ml). The reaction mixture was allowed to stirred at 4 °C for 15 h, diluted with EtOAc (200 ml), washed with 10% $Na_2S_2O_3$ solution, 5% $NaHCO_3$ solution, and H_2O, sequentially, and dried with Na_2SO_4. Evaporation of the solvent gave an oil (440 mg) which was purified by column chromatography (hexane/EtOAc) to yield two products. The less polar product was identified as 4β,5β-epoxy-19-(*tert*-butyldimethylsilyloxy)androstan-17-one (**10**) (152 mg, 38%) as an oil. ^{1}H-NMR δ: 0.07 and 0.09 (3H each, s, 19-OCMe$_2$), 0.89 (3H, s, 18-Me), 0.91 (9H, s, 19-OSiMe$_2$C<u>Me</u>$_3$), 2.87 (1H, d, J=3.0 Hz, 4-H), 3.51 and 3.90 (1H each, d, J=10.2 Hz, 19-CH$_2$). Fourier transform (FT)-IR (neat): 1740 (C=O) cm^{-1}. MS *m/z* (relative intensity): 418 (M$^+$, 3), 361 (100), 269 (40), 255 (63). High resolution (HR)-MS Calcd for $C_{25}H_{42}O_3Si$ (M$^+$) 418.29030. Found 418.2923.

The more polar product was identified as 4α,5α-epoxy-19-(*tert*-butyldimethylsilyloxy)androstan-17-one (**9**) (212 mg, 52%). mp 125—128 °C. ^{1}H-NMR δ: 0.07 and 0.08 (3H each, s, 19-OCMe$_2$), 0.87 (3H, s, 18-Me), 0.89 (9H, s, 19-OSiMe$_2$C<u>Me</u>$_3$), 2.97 (1H, d, J=2.3 Hz, 4-H), 3.76 and 3.89 (1H each, d, J=10.2 Hz, 19-CH$_2$). FT-IR (KBr): 1740 (C=O) cm^{-1}. MS *m/z* (relative intensity): 418 (M$^+$, 30), 361 (100), 286 (28), 267 (23). *Anal.* Calcd for $C_{25}H_{42}O_3Si$: C, 71.71; H, 10.11. Found: C, 71.83; H, 10.19.

Treatment of 4,5-Epoxy Steroids 9 and 10 with $HClO_4$ 4α,5α-Epoxide **9** and its 4β,5β-isomer **10** (150 mg, 0.36 mmol) was separately dissolved in dioxane (9 ml). 0.28 mol/l $HClO_4$ (1.6 ml, 0.45 mmol) was added to this solution and the mixture was stirred at room temperature for 10 h. After dilution with EtOAC (200 ml), the mixture was washed with H_2O and dried with Na_2SO_4. Evaporation of the solvent gave a solid product (120 mg from **9** and 160 mg from **10**). Column chromatography of the product (hexane/EtOAc) yielded two products in each experiment. The less polar product was identified as 4β,5α,19-trihydroxyandrostan-17-one (**11**) (87 mg, 75% from **9** and 92 mg, 79% from **10**). mp 233—235 °C (from acetone). ^{1}H-NMR δ: 0.87 (3H, s, 18-Me), 3.58 (1H, s, 4-H), 3.93 and 4.25 (1H each, d, J=12.2 Hz, 19-CH$_2$). FT-IR (KBr): 3380 (OH), 1731 (C=O) cm^{-1}. MS *m/z* (relative intensity): 322 (M$^+$, 86), 304 (82), 286 (53), 274 (93), 250 (100), 232 (54), 219 (42), 199 (40). *Anal.* Calcd for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.88; H, 9.41.

The more polar product was identified as 4α,5β,19-trihydroxyandrostan-17-one (**12**) (3 mg, 2.6% from **9** and 10 mg, 6.8% from **10**). mp 259—261 °C (from acetone). ^{1}H-NMR δ: 0.87 (3H, s, 18-Me), 3.71 (1H, d, J=4.3 Hz, 4-H), 3.82 and 3.98 (1H each, d, J=8.2 Hz, 19-CH$_2$). FT-IR (KBr): 3260 (OH), 1722 (C=O) cm^{-1}. MS *m/z* (relative intensity): 304 (M$^+$−18, 100), 286 (27), 260 (11), 233 (17), 215 (10). *Anal.* Calcd for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.70; H, 9.50.

4β,19-Diacetoxy-5α-hydroxyandrostan-17-one (13) A solution of 4β-ol **11** (80 mg, 0.25 mmol) in pyridine (1.3 ml) and acetic anhydride (0.6 ml) was allowed to stand at room temperature for 40 h, diluted with EtOAc (50 ml), washed with 5% HCl, 5% $NaHCO_3$ solution, and H_2O, sequentially, and dried with Na_2SO_4. After evaporation of the solvent, a solid product

(100 mg) obtained was recrystallized from acetone to give 4β,19-diacetate **13** (95 mg, 94%). mp 149—152 °C. ^{1}H-NMR δ: 0.85 (3H, s, 18-Me), 2.09 and 2.23 (3H each, s, 3- and 19-OCOMe), 4.48 and 4.84 (1H each, d, J=12.5 Hz), 4.72 (1H dd, J=2.1 and 3.5 Hz, 4-H). FT-IR (KBr): 3446 (OH), 1737 (C=O), 1709 (C=O) cm^{-1}. MS m/z (relative intensity): 406 (M^+, 41), 346 (100), 328 (13), 304 (21), 286 (76), 273 (47), 255 (35), 232 (72). *Anal.* Calcd for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 68.22; H, 8.55.

4β,19-Diacetoxyandrost-5-en-17-one (14) $SOCl_2$ (0.11 ml, 1.51 mmol) was added to a chilled solution of 5α-ol **13** (100 mg, 0.25 mmol) in pyridine (1.1 ml) and the mixture was stirred for 10 min at 0 °C. After this time, water was added to the mixture and the product was extracted with EtOAc (100 ml). The organic layer was washed with 5% HCl, 5% $NaHCO_3$ solution, and water, sequentially, and dried with Na_2SO_4. Evaporation of the solvent yielded an oil which was purified by column chromatography (hexane/EtOAc) to give compound **14** (192 mg, 96%) as an oil. ^{1}H-NMR δ: 0.88 (3H, s, 18-Me), 1.97 and 2.03 (3H each, s, 4β- and 19-OCOMe), 4.16 and 4.58 (1H each, d, J=11.7 Hz, 19-CH_2), 5.38 (1H, s, 4-H), 6.01 (1H dd, J=2.1 and 5.1 Hz, 6-H). FT-IR (neat): 1732 (C=O). MS m/z (relative intensity): 388 (M^+, 3), 364 (12), 328 (21), 286 (11), 268 (68), 255 (100), 237 (18), 226 (15). HR-MS Calcd for $C_{23}H_{32}O_5$ (M^+) 388.22500. Found 388.2249.

4β,19-Dihydroxyandrost-5-en-17-one (6) A mixture of diacetate **14** (80 mg, 0.19 mmol), K_2CO_3 (100 mg, 0.72 mmol), MeOH (7 ml), and H_2O (1 ml) was stirred at room temperature for 40 h, diluted with EtOAc (200 ml), washed with water, and dried with Na_2SO_4. Evaporation of the solvent afforded an oil which was subjected to column chromatography. Product eluted with hexane–EtOAc (4 : 1) was recrystallized from EtOAc gave diol **6** (48 mg, 70%). mp 153—156 °C (lit.[21] mp 151—154 °C). ^{1}H-NMR δ: 0.93 (3H, s, 18-Me), 3.71 and 3.76 (1H each, d, J=11.0 Hz, 19-CH_2), 4.30 (1H s, 4-H), 5.90 (1H dd, J=2.6 and 4.7 Hz, 6-H).

Molecular Modeling Studies Molecular models were constructed on a Silicon Graphics IRIS 4D workstation starting from data of semiempirical molecular orbital calculations with the PM3 method (MOPAC version 6, Quantum Chemistry Program No. 455) using Insight II, version 95.0 software (BiosystemTechnologies, San Diego, CA, U.S.A.). Each compound discussed in this study was subjected to a systematic conformational analysis using CVFF force field to determine all of its minimum-energy conformations. Geometries were considered minimized when the energy change between two subsequent structures was less than 0.001 kcal/mol. Low energy conformations, of which geometries were optimized with the PM3 method, were overlapped within Insight II which uses a least squares fitting algorithm to minimize the displacement between matching atoms in the structures that are superimposed.

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