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

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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-(trans-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. HClO₄ in dioxane to produce principally 4β,5β-diol 11 (80%) of which acetylation followed by dehydration with SOC₃ 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 inhibitors8–14 and a recent structural prediction of aromatase by modeling15,16 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 C4,8,9 C610,11 and C712–14 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 418 and their 19-hydroxy analogs 219 and 518 we found that 4β,19-dihydroxy-5-ene steroid 620 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 tolerated 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 4α-acetoxyandrost-5-en-17-one (7) in four steps through hypoiode 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 Discussion

Synthetic sequence employed in this study involves an acid-catalyzed trans-diauxial 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-(trans-butyldimethylsilyloxy)androst-4-en-17-one (8), previously synthesized19 with m-chloroperbenzoic acid (MCPBA) in CH₂Cl₂ 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 1H-NMR spectroscopic data of 4α,5α- and 4β,5β-epoxy steroids having a 19-methyl group20; lower chemical shifts of one of 19-CH₃ (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 HClO₄ 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 SOCl₂ gave the dehydrated product in a trans-diauxial 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 K₂CO₃ 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

Fig. 1. Structures of 3-Deoxy Steroids

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that a carboxyl group of 302Glu and 309Asp, 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 carboxyl 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 1H-NMR spectra were obtained in CDCl3 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-Butyldimethylsilyl)oxyandrost-4-ene-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 CH2Cl2 (22 ml). The reaction mixture was allowed to stir at 4 °C for 15 h, diluted with EtOAc (200 ml), washed with 10% Na2S2O3 solution, NaHCO3 solution, and H2O, sequentially, and dried with Na2SO4. 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-butyldimethylsilyl)oxy) androst-17-one (10) (152 mg, 38%) as an oil. 1H-NMR δ 0.07 and 0.09 (3H each, s, 19-OCMe2), 0.87 (3H, s, 18-Me), 0.91 (9H, s, 19-OSiMe2CMe), 2.87 (1H, d, J = 3.0 Hz, 4-H), 3.51 and 3.90 (1H each, d, J = 10.2 Hz, 19-CH3). 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 C24H41O3Si (M⁻) 418.29030. Found 418.2923.

The more polar product was identified as 4α,5α-epoxy-19-(tert-butyldimethylsilyl)oxy) androst-17-one (9) (212 mg, 52%). mp 125—128 °C. 1H-NMR δ 0.07 and 0.08 (3H each, s, 19-OCMe2), 0.87 (3H, s, 18-Me), 0.89 (9H, s, 19-OSiMe2CMe), 2.97 (1H, d, J = 3.3 Hz, 4-H), 3.82 and 3.93 (1H each, d, J = 10.2 Hz, 19-CH3). 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 C24H41O3Si·C: 71.71; H, 7.11. Found: C 71.83; H, 10.19.

Treatment of 4,5-Epoxy Steroids 9 and 10 with HClO4

4α,5α-Epoxy-9 and its 4β,5β-isomer 10 (150 mg, 0.36 mmol) was separately dissolved in dioxane (9 ml). 0.28 mol/l HClO4 (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 H2O and dried with Na2SO4. 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-butyldimethylsilyl)oxy) androst-17-one (10) (152 mg, 38%) as an oil. 1H-NMR δ 0.07 and 0.09 (3H each, s, 19-OCMe2), 0.87 (3H, s, 18-Me), 0.91 (9H, s, 19-OSiMe2CMe), 2.87 (1H, d, J = 3.0 Hz, 4-H), 3.51 and 3.90 (1H each, d, J = 10.2 Hz, 19-CH3). Fourier transform (FT-IR) (neat): 1740 (C=O) cm⁻¹. MS m/z (relative intensity): 418 (M⁻, 3), 361 (100), 286 (28), 267 (23). Anal. Calcd for C24H41O3Si·C: 71.71; H, 7.11. Found: C 71.83; H, 10.19.

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Structure—activity relationship and the aromatase-catalyzed 19-oxygenation of a series of 3-deoxy C19 steroids23 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 aromatase4,5,13,24—27 indicate that a carboxyl group of 302Glu and 309Asp 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, 302Glu and 309Asp, 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 carboxyl 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

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 Hamiltonian. The C- and D-rings of the steroid backbone as well as the C-17 carbonyl moiety 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.

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(100 mg) obtained was recrystallized from acetonitrile to give 4β,19-diacetate of compound 13 (95 mg, 94%). mp 149—152°C. 1H-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).

**References and Notes**


22) 1H-NMR δ: 1.06 (s, 19-Me) and 2.91 (d, J = 3.6 Hz, 4β-H) for 4α,5α-epoxyandrostan-17β-yl acetate and 0.88 (s, 19-Me) and 2.83 (d, J = 3.8 Hz, 4α-H) for its 4β,5β-epoxy derivative. The configuration of the 4,5-epoxy ring was established, based on their reactivities toward a Grignard reagent, CH3MgBr; treatment of the former epoxide with the reagent followed by dehydration with SOCl2 gave 4β-methyl-5-ene compound, but the latter one did not yield the 4β-methyl compound (reported elsewhere).


