Conformational Analysis of 19-Oxygenated Steroids with a 4-Ene or 2,4-Diene Structure, Potential Intermediates of Aromatase Reaction, with Semiempirical Molecular Orbital PM3 Calculations

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Conformational analysis of potent competitive inhibitors of aromatase, androst-4-enes 5, as well as 2,4-diene steroids 3, 4, and 6 was carried out, using theoretical calculations, to determine the stereochemistry of their aromatase-catalyzed oxygenation. In the steroids examined, both the 19-alcohols and the 19-aldehydes favor the above-A ring conformation among the possible three in each. The results suggest that the 3-deoxy steroid 5a as well as the 2,4-diene steroids 4a and 6a would be oxygenated at C-19 by aromatase through the same stereomechanism as that involved in the androstenedione aromatization.

Key words conformational analysis; PM3 calculation; aromatase inhibitor; 19-oxygenation; aromatase reaction; stereochemistry

During the conversion of androstenedione (1a) to estrone by aromatase, a unique P-450 enzyme complex, 1 19-hydroxyandrostenedione (1b) is generated in the first of three NADPHrequiring monooxygenations. Two subsequent oxygenations by the enzyme transform compound 1b into the final product and result in release of the 19-methyl group as formic acid, accompanied with the stereoselective removal of the 1P- and 2P-hydrogens. 2 Aromatase has been thought to deliver oxygen to the C-19 methyl group each time from the out-of-ring position, trans to the C5C10 bond. 3 After the first hydroxyl is added, rotation about the C10C19 bond takes place to generate the stable conformer having the above-A ring conformation of the 19-hydroxyl group. This makes aromatase capable of the second monooxygenation at the identical site to produce 19,19-dihydroxy intermediate, accompanied by stereospecific loss of the 19-pro-R hydrogen. 2,4 This gem-diol is nonenzymatically dehydrated to form 19-aldehyde 1c of which 19-carbon is attacked by the enzyme in the third step to give estrone (Fig. 1). The relative thermodynamical stabilities of the possible three conformers of steroid 1c have not been determined, however.

In the course of our studies on aromatase inhibitors, we have reported that androst-4-en-17-one (5a) and its 19-hydroxy derivative 5b are very potent competitive inhibitors of aromatase. 3 Furthermore, it has been found that 6-oxo steroid 2a, a suicide substrate of aromatase, 6 is oxygenated at C-19 by the enzyme probably through the same stereomechanism as that involved in the andro-
stenedione aromatization.\textsuperscript{7} In addition, 19-oxygenated androst-2,4-dienes 3b and 3c or 6b and 6c with or without an oxygen function at C-3 have been proved to be efficiently aromatized by aromatase.\textsuperscript{8} This strongly suggests that androst-4-ones 5 without an oxygen function at C-3 would be also oxygenated at the C-19 position by the enzyme.

Taken together, we were interested in the stereomechanism of the aromatase-catalyzed oxygenation at C-19 of the 3-deoxy steroids 5 as well as 2,4-diene steroids 3, 4, and 6. Thus, to determine the lowest energy conformations of the 19-oxygenated analogs of steroids 3—6, we carried out their conformational analysis using PM3 calculations.

MATERIALS AND METHODS

**Molecular Modeling Studies** Molecular models were constructed on a Silicon Graphics IRIS 4D workstation starting from molecular dynamics data with the Newton Raphson method using the 3D graphic option of MOLGRAPH software (Daikin, Tokyo, Japan). Each compound discussed in this study was subjected to the Confssearch option using the Monte Carlo analysis to determine all of its minimum-energy conformations.

Finally, some conformations near the minimum-energy ones were selected to further analyze the lowest energy conformations using semiempirical molecular orbital calculations with the PM3 method (MOPAC version 6, Quantum Chemistry Program No. 455). Geometries were considered minimized when the energy change between two subsequent structures was less than 0.001 kcal/mol.

The GIGLIO method was used to calculate rotational profiles for the 19-hydroxy and 19-oxo steroids. For both, the C(5)–C(10)–C(19)–O(19) torsional angle was varied from 0 to 360° in 10° increments.

RESULTS AND DISCUSSION

We initially performed the PM3 molecular orbital calculations on 19-hydroxy-4-ene steroid 5b and 19-hydroxy-2,4-diene steroids 3b, 4b, and 6b. The results of the calculations are shown in Table 1 and, for comparison, the results of steroid 1b are also listed. The 19-hydroxyl group of the lowest energy conformer of all the 19-hydroxy steroids analyzed was oriented above the A-ring irrespective of the A- or B-ring structure, and their torsional angle C(5)–C(10)–C(19)–O(19) ranged between −51° and −65°. The relative preference of the above-A-ring conformation of steroid 1b obtained in this study well corresponds to that previously obtained by MM2 molecular mechanics calculations.\textsuperscript{9} An introduction of a double-bond at C-2 shifted the conformation population thus favoring the conformer having 19-hydroxyl group above the A-ring. This would result from the lack of 1,3-diaxial repulsion between the 2β-proton and the 19-oxygen function in the 2,4-diene steroids.

For the 19-hydroxy steroids, these calculations revealed that barriers to rotation, ranging from about 3 to 13 kcal/mol, are not large enough to preclude the steroids from attaining any of the three minimum-energy con-
Aromatase does not exhibit any stereospecificity if the C-19 group is rotating as long as the enzyme can catalyze oxygenation. Thus, a large part of the stereospecificity could come from hydrogen bonds between the enzyme and the 19-hydroxyl group of the 19-hydroxy steroids. The present results suggest that the 19-position of all the 19-alcohols would be oxygenated by aromatase through the same stereospecificity as that operative in the androstenedione aromatization. It is presumed that the enolization of the C-3 carbonyl function would not change the stereomechanism in the 19-oxygenation.

The most preferred conformer among the three possible ones was also the above-A-ring conformer in all the 19-oxo steroids. Considering aromatase attack on the 19-oxo steroid from the out-of-ring position, this conformer can be easily oxygenated at C-19. In the aromatization process of the 19-oxo steroid 1c, it is assumed that the 19-carbonyl group would be oriented above the A-ring through hydrogen bonding between the 19-oxygen and the enzyme.

The PM3 calculations indicate for the first time the conformations of the 19-hydroxyl group of compounds 3b–6b as well as the 19-oxo group of compounds 1c and 3c–6c, showing that the stereospecific 19-oxygenation may occur, if these compounds can be substrates for aromatase, similar to the aromatization process of androgens.

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