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CONFORMATIONAL STUDIES OF STEROIDS: CORRELATIONS WITH BIOLOGICAL DATA

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SUMMARY

X-Ray crystal structure analysis provides precise and accurate data concerning steroid structure and conformation. The crystal and molecular structure data of over 200 steroids are being collected and analyzed in the *Atlas of Steroid Structure*. This extensive analysis plainly shows that crystal packing forces have little influence upon molecular conformation and that the overall conformation of steroids and the orientation of functional groups are clearly controlled by intramolecular forces. The feasibility of specific interactions with protein molecules is in turn dependent upon overall conformation. Information concerning both of these aspects of steroid behavior is provided by crystal structure studies. The details of molecular mechanisms of conformational transmission and the nature of correlations between molecular structure and function can be proposed on the basis of these data.

The most active naturally occurring steroids are observed to possess the greatest degree of conformational flexibility. Significantly different conformers of a hormone are probably responsible for different aspects of its action in the biological setting. In synthetic steroidal hormones further substitution on the steroid nucleus generally removes conformational flexibility. The less flexible molecule may now be a better competitor than the natural hormone for one type of protein interaction, but be much less competitive in other protein interactions, as is suggested by the highly specific activity enhancement of synthetic steroidal hormones.

INTRODUCTION

Many biological functions have been shown to depend directly upon the presence of a particular steroid. Even in the most thoroughly studied systems, however, the exact nature of all molecular level events involved in this structural–functional dependence is not fully understood. A hypothetical life cycle of an estrogen is depicted in Fig. 1. Any modification of the estrogen structure will result in steric and electronic changes that may alter the interaction potential of the steroid with proteins at any point in this complex system. Similarly the introduction of a synthetic estrogen or a change in the relative concentrations of natural estrogens in the body will affect this cycle in a specific but unknown manner. This highly sensitive correlation between steroid structure and observed function marks the steroid field as one of the most promising in which to pursue a molecular level elucidation of structure–functional relationships.

Precise and accurate X-ray determinations of the crystal and molecular structures of steroids provide a base upon which to build an understanding of intra- and intermolecular interactions of steroids that are significant in determining biological function. The crystal structures of over 200 steroids have been determined since 1945. These crystal and molecular data are being collected and analyzed in the *Atlas of Steroid Structure* [1]*. The first volume of the *Atlas* presents exact structural details and comparative analyses for 118 estrane, androstane, and pregnane structures. The basic molecular data presented in the *Atlas of Steroid Structure* include atomic coordinates, interatomic bonds and angles, and torsional angles. A conformational analysis of each compound is given in terms of ring conformations, ring junction configuration, torsional angles, orientation of functional groups and side chains, and the distances between functional sites. Three views of the molecular packing, selected intermolecular distances and a description of the packing forces comprise the section on packing analysis. This systematic, quantitative analysis of the data facilitates the subsequent detection and classification of conformational patterns. Finally, as a result of comparative analyses of steroid data it is possible to: (1) distinguish intramolecular forces from packing forces, (2) correlate conformational and geometric variations and (3) explain long-range intramolecular effects.

Steroid function is dependent upon molecular composition, constitution, configuration, and conformation. The dependence of function upon composition is exemplified by the effect of the two hydrogen difference between cortisone and cortisol, and the dependence of function upon configuration is exemplified

* Complete references for all X-ray crystal structure data used in the preparation of this manuscript will appear in the *Atlas of Steroid Structure* and are available from one of the authors (WLD).

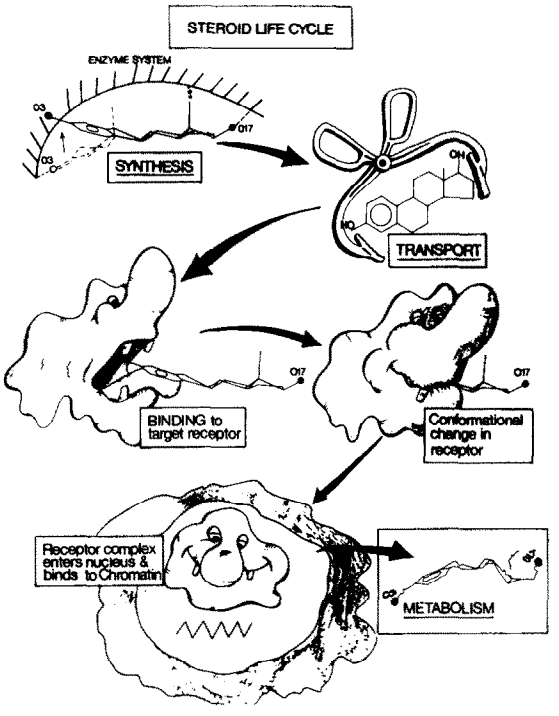


Fig. 1.

by the α- vs β-substitution of the 17-hydroxyl group in estradiol. Although the importance of total conformation to steroid function is not disputed, the exact nature of this dependence is not so easily defined. It is in this area of conformational analysis that crystal structure data are of greatest importance.

Steroid flexibility

From inspection of models it appears that some steroids have greater flexibility than others. This is particularly true in regard to the orientation of side chains and substituent groups. Analysis of crystal structure data indicates that in some cases models may suggest too much or too little flexibility for a particular steroid. In general, a saturated steroid nucleus has very little flexibility and the conformation of such a nucleus in the crystal is not likely to be significantly distorted from the minimum energy form. In addition, that form is probably in a narrow minimum potential well (Fig. 2a) and the structure in solution will oscillate only slightly about this minimum energy form. In contrast to this, most of the naturally occurring biologically active hormones have some degree of unsaturation of the nucleus and have a far more flexible conformation. In these molecules a broad energy minimum (Fig. 2b) may well allow free oscillation of the structure over a continuous range, two or more conformers of nearly equal energy may be separated by a significant barrier (Fig. 2c), or some combination of these conditions may occur (Fig. 2d). Additional substitutions on the steroid nucleus have a marked effect upon this flexibility. Substitution may (1) shift the minimum point, (2) narrow the range, (3) remove double minima, or (4) increase the barrier between existing double minima.

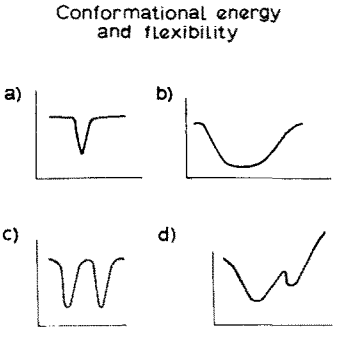


Fig. 2. Graphic representations of the relative energy of various types of steroid nuclei and substituent conformations. (a) Inflexible conformation, narrow minimum; (b) flexible conformation, broad minimum; (c) two conformers of equal energy, large barrier to interconversion, narrow minima; (d) two conformers, small barrier to interconversion, broad minima.

With regard to the side chain and substituent group orientations, the large body of crystal structure data suggests that there is less flexibility in the conformations of these groups than is suggested by examination of models. The following discussions of the conformations of the 4-ene-3-one A-ring and the pregnane 17β-side chain illustrate many of these features.

A-ring conformation

Solvent and crystallization conditions influence the conformational population distribution of flexible steroids resulting in the formation of polymorphic crystal forms. The study of these polymorphic forms provides details concerning the conformational variation in flexible molecules. Six crystallographically independent observations of the unsubstituted testosterone molecule have been made, and the flexibility of the testosterone A-ring is illustrated in Fig. 3 [2–4].

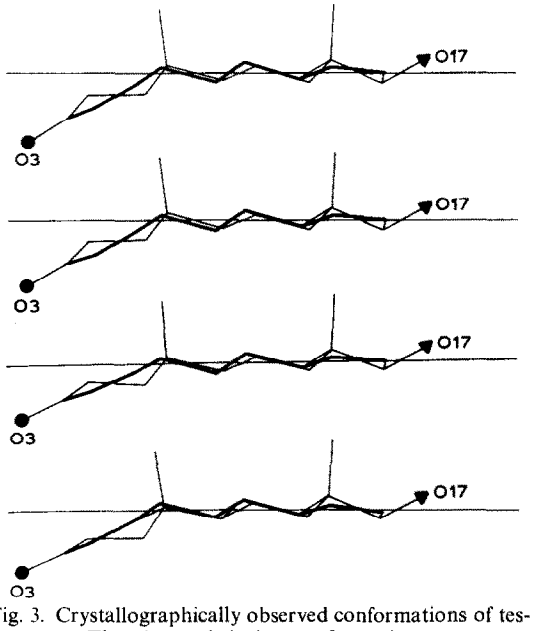
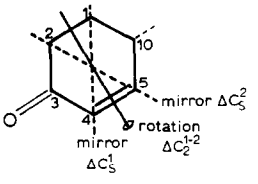


Fig. 3. Crystallographically observed conformations of testosterone. The observed A-ring conformations trace part of the path of least resistance to transformation between 1α,2β-half chair and 1α-sofa conformations.

Including the testosterone structure, 36 steroids having the 4-ene-3-one A-ring have been analyzed for inclusion in the *Atlas*. In the majority of cases the flexible A-ring conformation falls within the range including the ideal forms: 2β-sofa, 1α,2β-half chair, and 1α-sofa. A six-membered ring is described as having a perfect sofa conformation if five atoms of the ring are coplanar and the sixth is out of the plane (α if it is on the α side, etc.). A six-membered ring is described as having a perfect half-chair conformation if four contiguous atoms of the ring are coplanar and the remaining two are equally displaced from the plane and are on opposite sides of the plane. A ring having a sofa conformation possesses a mirror plane of symmetry perpendicular to the plane of the ring which passes through the out-of-plane atom (Fig. 4a). A ring having a half-chair conformation possesses a rotation axis of symmetry bisecting the bond joining the out-of-plane atoms (Fig. 4b). Quantitative evaluation of ring conformation can be established by testing appropriate torsional angles for the presence of these symmetries [5]*. A plot of the deviation from mirror symmetry C(1)—C(4) (ΔC_s^1), which should be near zero for a sofa conformation, vs the deviation from two-fold symmetry through the C(1)—C(2) and C(4)—C(5) bonds, which should be near zero for a half-chair conformation, for all the 4-ene-3-one is given in Fig. 5. Close inspection of the distribution of A-ring conformation reveals that:

1. Fourteen of the 17 structures in range II have unsubstituted A-, B-, and C-rings with configuration identical to those of testosterone.



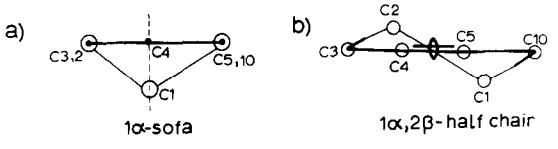


Fig. 4. Symmetric conformations of A-rings having 4-ene-3-one constitution. (a) Mirror symmetry is observed in the sofa conformation, and (b) rotation symmetry is observed in the half chair conformation.

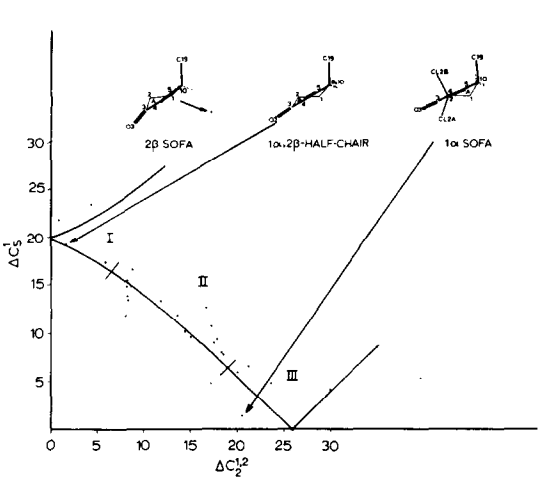


Fig. 5. A graphic display of the relative A-ring conformations of 28 structures having 4-ene-3-one composition in which the goodness of fit to the symmetric models, 1α,2β-half chair and 1α-sofa, are plotted versus one another.

2. The four structures in range I have a C(11) substituent.

3. The seven structures in range III have substituents [on C(11), C(8), or C(6)] in the B- or C-rings.

These observations indicate that B- and C-ring substitutions in 4-ene-3-one structures greatly restrict A-ring flexibility and stabilize the extremes of possible A-ring conformations.

Pregnane 17β-side chain conformation

Although Dreiding models suggest freedom of rotation of the pregnane 17β-side chain, crystallographic findings indicate that rotation is hindered and that deviation from a single minimum energy conformation is highly restricted. Thirty pregnane structures in the *Atlas* have a 20-one substituent and of these 15 have a C(21) oxygen or halogen atoms substituent. In 29 of the 30 20-one structures the O(20) atoms are +clinal [6] with respect to the C(13)—C(17) bond (Fig. 6). The C(13)—C(17)—C(20)—O(20) torsional angle ranges in value from 75 to 115°. The crystal structure data demonstrate that 17β-side chain orientation is influenced by 17α-substitution and 21α-substitution. The correlated variation in side chain orientation and 17α-substitution (Fig. 7a) indicates that the preferred orientation shifts an average of 14°

$$\Delta C_s^1 = \sqrt{\frac{[(\phi_{1-2} + \phi_{1-10})^2 + (\phi_{2-3} + \phi_{10-5})^2 + (\phi_{3-4} + \phi_{5-4})^2]}{3}}$$

$$\Delta C_2^{1-2} = \sqrt{\frac{[(\phi_{2-3} - \phi_{1-10})^2 + (\phi_{3-4} - \phi_{10-5})^2]}{2}}$$

$$\Delta C_s^2 = \sqrt{\frac{[(\phi_{2-3} + \phi_{2-1})^2 + (\phi_{3-4} + \phi_{1-10})^2 + (\phi_{4-5} + \phi_{10-5})^2]}{3}}$$

where ϕ_{i-j} is the intra-ring torsional angle containing atoms i and j as the two central atoms.

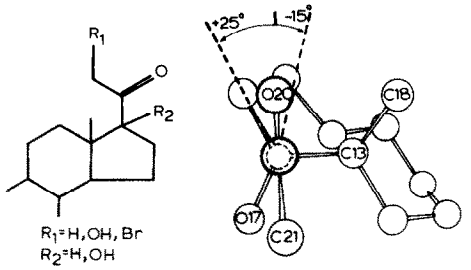


Fig. 6. Observed range of conformational flexibility of the pregnane 20-one-17β-side chain.

with 17α-hydroxyl substitution. The average C(13)—C(17)—C(20)—O(20) torsional angles are 105·1 and 90·6° in structures having 17α(H) and 17α(OH) substitution respectively. C(21) substituents are all observed to be $\pm syn$ periplanar relative to O(20). Substitution at the C(21) position further restricts the rotational freedom of the 17β-side chain decreasing the observed range by about 10° at each extreme (Fig. 7b). Clearly the intramolecular control of side chain orientation is neither an artifact of, nor is it masked by, crystal packing forces. Additional specific examples of substituent influence upon steroid conformation are shown in Fig. 8.

Intermolecular interactions

The hydrogen bonding patterns of corticosteroid molecules and analogues included in the *Atlas* indicate a high degree of directional specificity in the location of hydrogen bond donors and acceptors (Fig. 9). The 11β-hydroxyl consistently hydrogen bonds *trans* to the C(9)—C(11) bond and the 17α-hydroxyl consistently hydrogen bonds *trans* to the C(13)—C(17) bond. There is also a correlation between the orientation of the C(3)—O(3) bond and the location of hydrogen bond donors to that carbonyl. It is apparent that for a 0·5 Å change in the relative position of oxygen atom O(3) the optimum location for a hydrogen bond donor may vary by as much as 3·0 Å. The directionality of these hydrogen bonds is independent of the hydrogen bond acceptor or donor indentity. For example, the O(11) hydrogen bond acceptors in nine structures include O(3), O(17), O(20), O(21) and F(9) atoms of adjacent molecules as well as solvent of crystallization.

Analysis of molecular packing in the crystals has been simplified subsequent to the development of a classification system based upon the orientation of

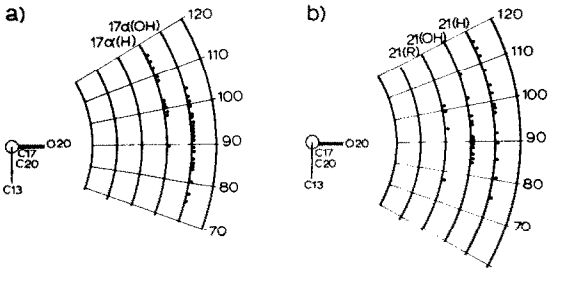


Fig. 7. Dependence of the 17β-side chain orientation upon (a) 17α-substitution, and (b) 21-substitution.

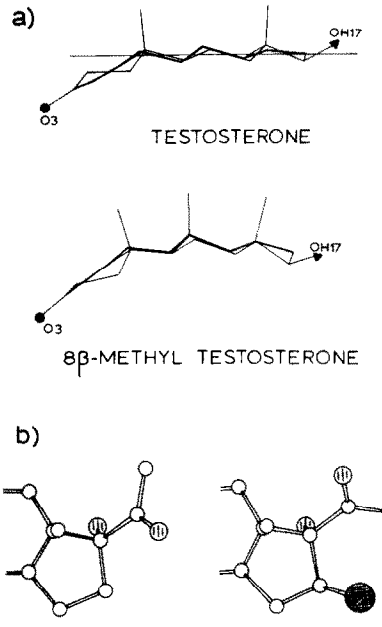


Fig. 8. Specific examples of substituent influence on steroid conformation. (a) 8β-Methyl substitution crowds the β-face and causes a bowing of the entire molecule toward the α-face [7]. (b) 16β-Bromo substitution prevents the 17β-side chain from having its normal conformation in which O(20) is within 20° of eclipsing C(16) [8, 9].

the steroid's length, width and thickness relative to crystallographic cell sides and symmetry operators. Although characteristic patterns in extended structure such as chains, coils, and layers can be found within these classes, no specific correlations between gross molecular packing and steroid conformations or hydrogen bonding patterns have been discovered.

Corticoid conformation and function

The accumulation of protein binding and pharmacological testing data concerning natural and synthetic corticosteroids, coupled with the availability

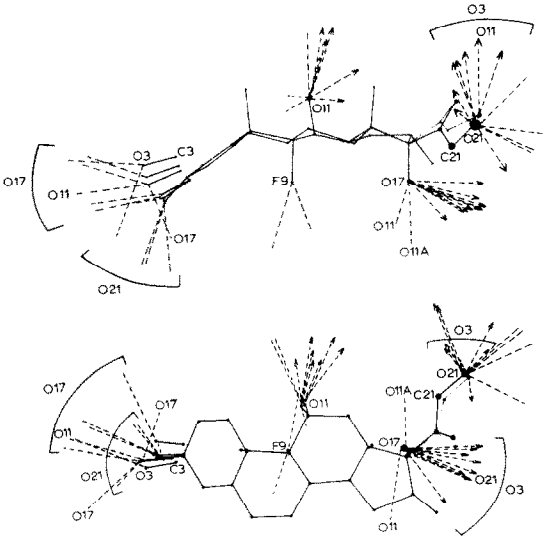


Fig. 9. Hydrogen bonding patterns observed in corticosteroids.

Table 1. Influence of cortisol substituents on pharmacological data [Fried (1960)]

Functional group	Glycogen deposition (rat)*	Anti-inflammatory (rat)*
9α-Fluoro	10	7–10
9α-Chloro	3–5	3
9α-Bromo	0·4	—
1-Dehydro	3–4	3–4

* Cortisol = 1.

of X-ray crystal structure determinations of many of the same compounds, facilitates the establishment of correlations between molecular conformation and function. The pharmacological data on a few of the many synthetic corticoids prepared by Fried prior to 1960 are shown in Table 1 [10]. Some of the data concerning the competition for steroid binding to transcortin as determined by Sandberg *et al.* are given in Table 2 [11]. The crystallographically observed conformations of cortisol [12], 9α-bromocortisol [13], and 6α-methylprednisolone [14] are contrasted with the conformation of 9α-fluorocortisol [15, 16] in Fig. 10. The molecules are viewed parallel to the least-squares plane through atoms C(5) to C(17), thereby emphasizing differences in the orientation of the A-ring relative to the rest of the steroid. The 17β-side chain orientation is largely invariant throughout the corticoid sequence and the most pronounced effect of the 9α-substitutions is their influence on the A-ring orientation. The similarity in location of the O(3) atom relative to the bulk of the nucleus in 9α-fluorocortisol and 6α-methylprednisolone support the contention that this conformation is optimal for glycogen deposition and anti-inflammatory activity. The fact that cortisol is only one-tenth as active as 9α-fluorocortisol could be due to the fact that the A-ring conformation has a broad energy minimum (Fig. 2b) in which only one-tenth of the population is in the 1α,2β-half chair conformation at any time or that the 1α-sofa and the 1α,2β-half chair conformers have local minima (Fig. 2d) in a 9:1 population distribution.

In either case the conformational population distribution could be such that only one-tenth of the cortisol molecules are normally in a conformation similar to that of 9α-fluorocortisol. Two crystallographic determinations of cortisol and one determination of corticosterone have been reported. All three of these molecules are observed in conformations similar to the cortisol example shown in Fig. 10a. This may represent the energetically most favored cortisol conformation and this conformation may be optimal for binding to transcortin. Of the seven strong competitors for cortisol binding, corticosterone is observed to have the cortisol conformation, 2α-methylcortisol and 21-deoxycortisol may be expected to resemble cortisol in conformation, and 11-deoxycortisol, 17α-hydroxyprogesterone, and progesterone have the flexible A-, B-, C-ring configuration of testosterone discussed previously. Therefore the A-rings of all of these strong competitors can adopt conformations identical to that of cortisol. The only anomalous member of the strong competitor class is prednisolone which is constrained to have a bent conformation similar to that of 6α-methylprednisolone (Fig. 10c). The failure of 9α-fluorocortisol to compete for binding would be consistent with the fact that it is restrained from adopting the dominant A-ring conformation of unsubstituted cortisol. Other members of the least com-

Table 2. Competitive binding to transcortin [Sandberg *et al.* (1966)]

Most competitive	
Cortisol	Progesterone
Corticosterone	Prednisolone
Compound S (11-deoxycortisol)	2-Methylcortisol
17α-Hydroxyprogesterone	21-Deoxycortisol
Least competitive	
Aldosterone	Estrone
Compound A (11-dehydrocorticosterone)	Triamcinolone
	Tetrahydro-DOC
Androsterone (?)	5-ene-Pregnenolone
Tetrahydro-F	Pregnane-3α,17α, 20α-triol
Dihydro-F	
Dihydro-E	9α-Fluorocortisol

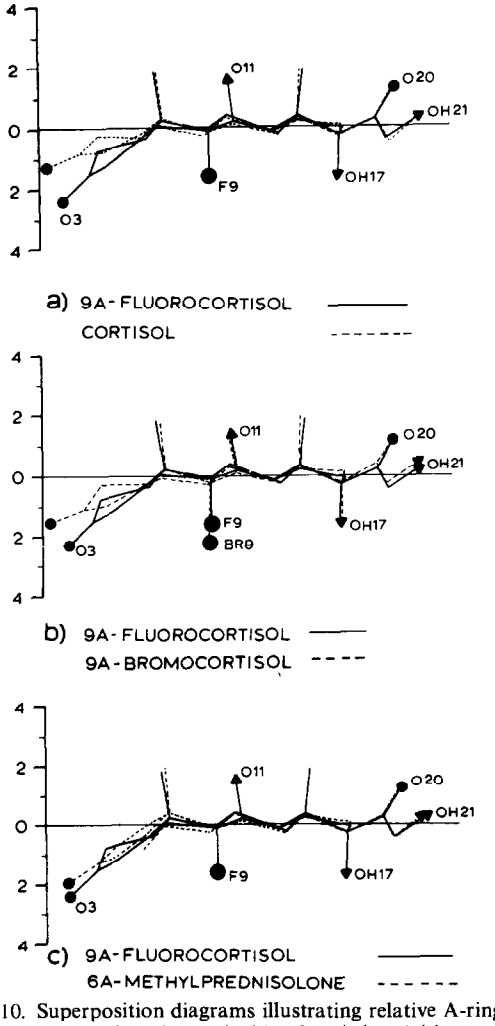


Fig. 10. Superposition diagrams illustrating relative A-ring conformations in corticoids of varied activities.

petitive group may fail to compete for a variety of reasons which in many cases may be more compositional than conformational in nature.

The data suggest that the conformational flexibility possessed by many of the most active steroid hormones (testosterone, estradiol, progesterone and aldosterone, etc.) will facilitate interaction with a number of different proteins and that for a specific interaction a particular conformer may be optimal. Furthermore, many synthetic steroids, such as 9α-fluorocortisol, may have an inflexible conformation that only competes for one of the protein molecules that interact with the natural hormone and consequently will display specific activity enhancement.

Models for enzymic reactions

Crystallographically determined molecular conformations are also being used in the interpretation of the biological data and the development of theories concerning the molecular mechanisms of aromatization in which androgens are transformed to estrogens by human placental, microsomal aromatase. 19-Hydroxy, 19,19-dihydroxy, and the 19-oxo derivatives of androgens have been tentatively identified as intermediates in the aromatization process. Labeled samples of these possible intermediates have been used in enzymic reactions in order to define this process more completely. Since the conformations of these 19-substituents may be flexible, crystallographic studies and estimates of the relative conformational freedom are required for complete understanding of the enzymatic reaction at a molecular level. The crystal and molecular structures of 17β-benzoyloxy-3-oxo-4-androsten-19-al (a) and 19-hydroxy-4-androsten-3,17-dione (b) have been determined. The observed conformations of the 19-substituents are indicated in Fig. 11. The conformations are consistent with expectations based upon considerations of nonbonding and electrostatic interactions. On the basis of labeling experiments, sodium borohydride reduction of (a) is found to be 90% stereoselective and can be regarded as resulting from approach along the less sterically hindered path indicated by the arrow. Enzymatic aromatization of (b) is completely stereospecific and results in first the removal of R_1 to form water followed by further oxidation and removal of C(19) with R_2 attached giving formic acid as a by-product. The stereospecific nature of the aromatization was elucidated by the quantitative analysis of the enzymatic reactions with isotopically labeled substrates. Interpretation of the reaction data on the basis of the crystallographic structure suggests the most probable approach of substrate to the active site of the enzyme is at the out-of-ring tilted β-side orientation indicated by the arrow in Fig. 11b [17].

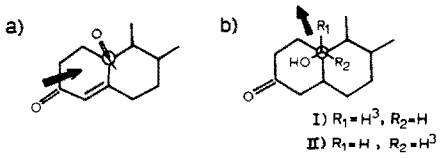


Fig. 11. Observed conformations of 19-oxygenated substituents in tentatively identified intermediates in the aromatization process.

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