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SOME HYPOTHESESE ABOUT INTERACTIONS BETWEEN ESTROGEN AND ANDROGEN AND THEIR POSSIBLE RECEPTORS*

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SUMMARY

A 17β hydroxy function on the D ring is a common part of natural steriods which have estrogenic or androgenic activity. Crystal structures of such steroids show that there are two possible directions for hydrogen bonds to the 17β hydroxy function whatever the activity of the molecule. If similar interactions occur on binding to the biological 'receptors' it is proposed that the specificity of activity (estrogen or androgen) might result from the particular geometry of the A ring area of the steroids relative to this part of the molecule.

INTRODUCTION

The main natural steroids hormones with estrogenic (estradiol) and androgenic (testosterone, androstanolone) activity have a 17β hydroxy function on their pentagonal D ring. The principal conformational differences occur in the A ring of the steroid which is aromatic for estrogens and satured or has a C_4–C_5 double bond for androgens.

The 17β hydroxy function of these molecules seems to play an important role in the binding to specific carrier proteins such as sex Steroid Binding Protein (SBP) [1] or Testosterone Binding Globulin (TeBG) [2]. Even in the case of 'receptor' proteins the pentagonal D ring of an estrogen or androgen steroid cannot be modified in any way without an important decrease of the affinity. However the binding of hormones to its receptors seems rather more complicated than their binding to carrier proteins. Thus the prostatic 'cytosol receptors' allow the binding of progesterone and cyproterone [3] and the uterine 'cytosol receptors' bind non steroid molecules like diethylstilboestrol, benzestrol, cyclofenyl, etc. [4].

In previous papers we suggested an explanation for the structure–affinity relationship which may occur between estradiol and other non steroid estrogens [5, 6]. In the present paper, we examine the geometry of hydrogen bonding involving the 17β hydroxy function in the hope of elucidating conformational preferences.

EXPERIMENTAL

The crystal structures of a great number of estrogen and androgen steroids have been studied and the molecules fall into two groups:

the first with a 17β hydroxy function (group I)

1,3,5(10)-estratriene-3-17-diol (Estradiol), [7], [8], [9];

17β-hydroxy-4-androsten-3-one (testosterone), [10], [11], [12];

17β-hydroxy-5-androstan-3-one (androstanolone), [13], [14].

5α-androstane-3β 17β-diol [15]; 5α-androstane-3α, 17β-diol [16];

17β-hydroxy-19-nor-androst-4-en-3-one(19-nortestosterone) [17];

18 methyl-1,3,5(10)-estratriene-3-17β-diol (18-methylestradiol) [18]

The second (group II)

without such a function

3-hydroxy-1,3,5 (10)-Estratrien-17-one (estrone) [19] for which we have determined 3 different crystal forms

1,3,5 (10)-estratriene-3-17α-diol (isoestradiol) [20]

or where the environment of the 17β hydroxy group is modified

1,3,5 (10)-estratriene-3-16α-17β-triol (estriol) [21]

All structures were determined by X-ray analysis using data collected on automatic diffractometers using the CuKα radiation. Except for references [12] and [21] which correspond to structures determined in other laboratories all the determinations and refinements were performed on a CII-IRIS 80 computer. The main crystallographic results were reported elsewhere, and we discuss below only directly relevant crystallographic results.

RESULTS

Nearly all the 17β-hydroxy steroids, have at least one hydrated crystalline form. Anhydrous crystal

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forms were also described for testosterone [12] and androstanolone [14]. It was not possible to obtain hydrated crystal forms for 5α-androstane-3β, 17β-diol. The association between steroid and water molecule seems very stable and it is sometimes very difficult to avoid the hydrated crystal forms. For example the hydrated monoclinic forms of androstanolone [13] and testosterone [10] may be obtained from a benzene solution, and in order to crystallize the estradiol–propanol complex [8] we had to de-hydrate the commercial estradiol for several days in a drying oven. Although the observed hydrated forms correspond to quite different space groups (monoclinic $P2_1$ for androstanolone, testosterone, 5α-androstane-3β,17β-diol; orthorhombic $P2_12_12$ for estradiol and $P2_12_12_1$ for testosterone) and crystal packing arrangements, the position of the water molecule is always the same in relation to the steroid hormone. The hydrogen bond with the 17β hydroxy group forms in such a way that the water molecule lies between two hydrogen atoms of the C(18) angular methyl group of the steroid. For the complex estradiol–propanol the position of the water molecule is occupied by the oxygen of propanol. In the anhydrous crystal forms obtained for testosterone and 19 nortestosterone the 17β hydroxy function of the steroid is linked to the 3-one function of another steroid by a hydrogen bond and the position of the O(3)

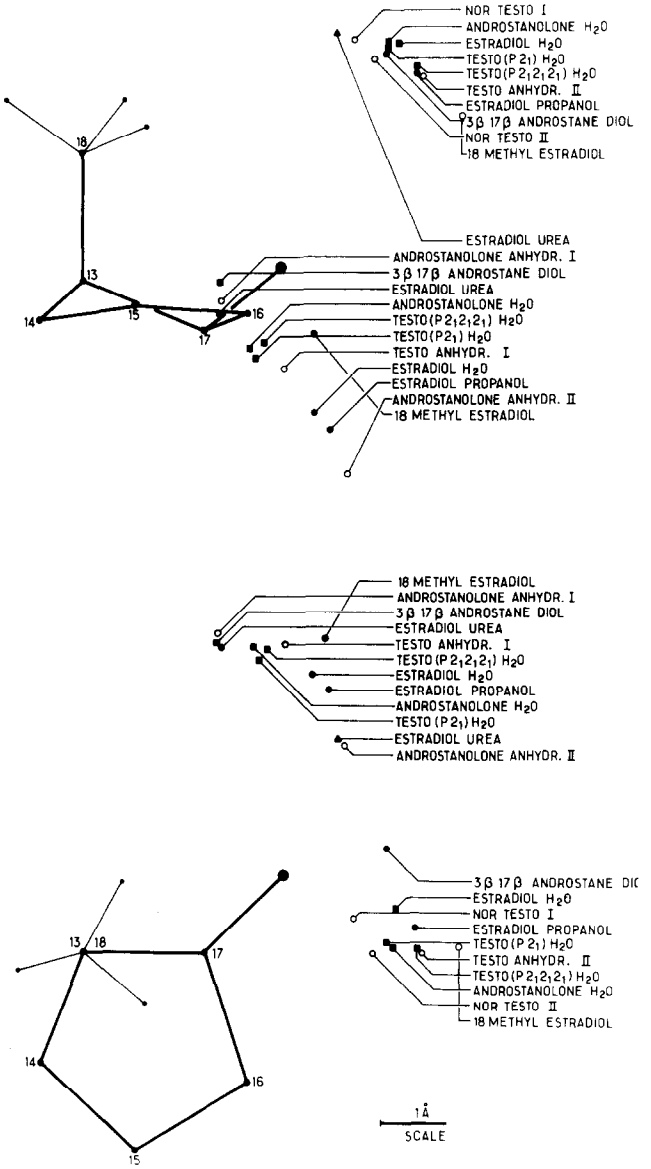

Fig. 1. Orthogonal projections of D ring on the plane C(18-C(13)-C(17). The different external points are the spatial positions occupied by the atoms linked to O(17) by hydrogen bonds in steroid crystal structures. Full square (■) is for water molecules. Full circle (●) for hydroxy groups, light circle (○) for carbonyl groups and full triangle (▲) for amino groups. Distortions are observed from the ideal water position, when strong constraints are endured by the bound molecules as is the case of the urea molecule involved in 5 different hydrogen bonds.

oxygen is close to the position of the water in the hydrated crystal forms. The only exceptions are for molecule I of testosterone ($P2_12_12_1$) and androstanolone I and II. Even if the environment of the D ring is modified e.g. by changing the C(18) angular methyl group into an ethyl group the oxygen of the solvent molecule is located at the same place [18]. We may define such a position by its distances to the oxygen of the 17β hydroxy function (hydrogen bonds between 2.70 and 2.90 Å) and to the angular methyl group (Van der Waals between 3.60 and 3.80 Å); the dihedral angle round the C(13)–C(18) bond C(17)-C(13)-C(18)-W) lies between 0 and 8°.

When a second hydrogen bond is formed with the 17β hydroxy function it occurs in a less well defined area without extra Van der Waals contacts with the C(18) methyl group of the steroid. These observations are illustrated in Fig. 1 which shows two orthogonal projections of the D ring and of the bound atoms linked to the 17β hydroxy function.

Conversely we have never observed such an arrangement in the different crystal structures of group II. In Fig. 2 all the positions of the atoms bound by hydrogen bonds to the O(17) atom are shown. Most hydrogen bonds are established on the α side of the steroid molecule in a similar way to the second

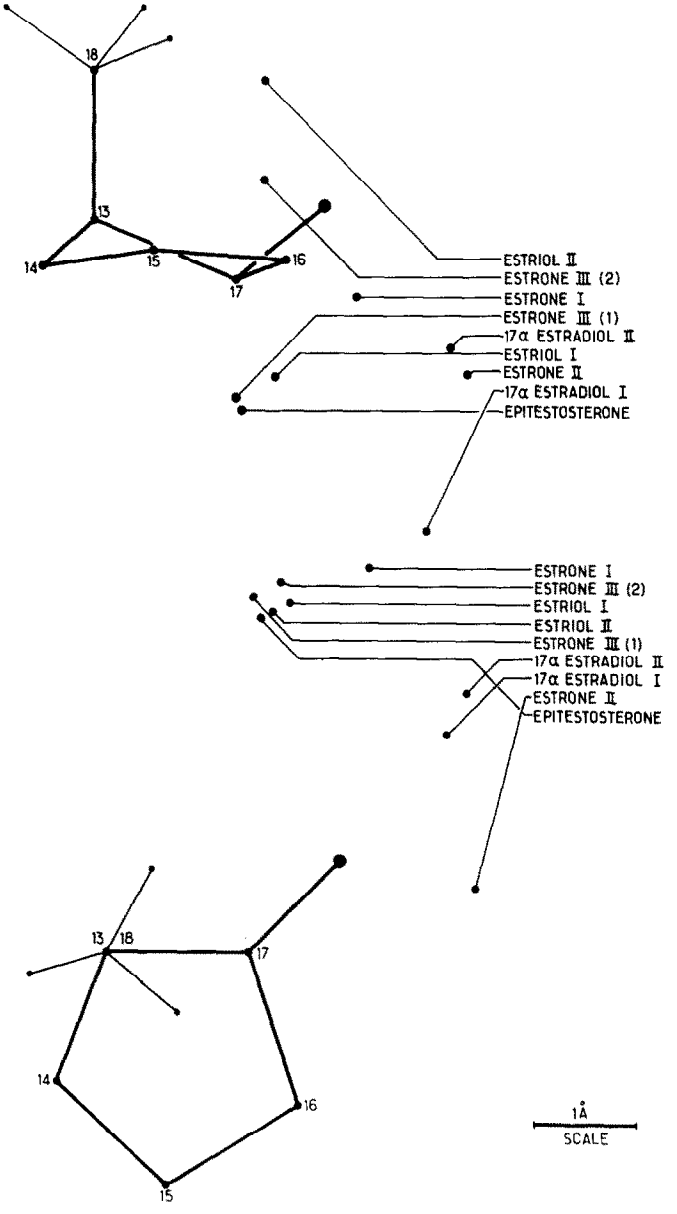

Fig. 2. The same as Fig. 1 for group I. We carry on using a D ring with a 17β hydroxy function as in Fig. 1 although the O(17) function may be a 17α hydroxy or a 17 oxo one. Note that for estriol the presence of another attractive hydroxy group in 16α completely destroyed the normal approach of the 17β hydroxy function as in estradiol. This difference might be an explanation of the weak affinity (about 1/10 of the estradiol one) of estriol for the estrogen receptor.

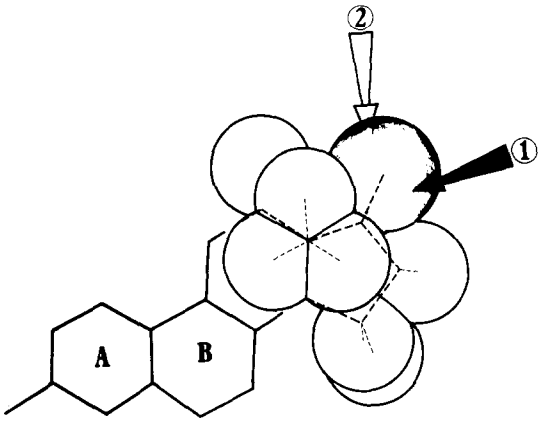

Fig. 3. Possible directions of interaction for the 17β hydroxy function.

'undefined' bond observed for the unaltered 17β hydroxy function. Thus, in replacing the 17β hydroxy function by a 17α hydroxy or a 17-one function, or in adding a 16α hydroxy function to the steroid we disturb the hydrogen bonds and change the role of the C(18) angular methyl group.

Figure 3 summarises the possible interactions occurring with the 17β hydroxy function: the black arrow shows the specific hydrogen bond, whereas the white one gives the mean direction of the unspecific bond.

DISCUSSION

The best description of steroid–receptor interactions would be given by the three dimensional structures of the receptors with the steroids in the active site; but this is not possible in the near future due to difficulties in extraction of pure receptor molecules. With this lack of information we are obliged to propose an hypothetical model on the basis of observed crystal interactions. The relevance of such a model must be checked by further biochemical or biological experiments.

The presence of similar hydrogen bonds in many different crystal environments makes it reasonable to suggest that corresponding interactions exist on binding of steroid hormones to their protein receptors. These results are consistent with two hypotheses for the binding process.

On the one hand, for an estrogen receptor a water molecule may be necessary for the activity of a steroid molecule as suggested by the similarity between the oxygen O(3)–O(W) distance observed for estradiol (12.24 Å) and the oxygen–oxygen distance in the potent synthetic estrogen diethylstilbestrol (12.13 Å) [22]. The active estrogenic hormone would be the complex estradiol–water instead of estradiol alone. An increase of binding with a longer 17β function that occupies the water site would confirm such an hypothesis.

A more interesting hypothesis is suggested by the analysis of the crystal structure of the estradiol–propanol complex. The alcohol molecule has exactly the same location with regard to the 17β hydroxy function as the water molecule in the hydrated crystal forms. The alcoholic oxygen linked by hydrogen to the 17β hydroxy function lies in the groove of two hydrogen atoms of the angular methyl group.

It is not too speculative to assume that, when the steroid hormones are bound to the estrogen or androgen receptors, the 17β hydroxy function forms 2 hydrogen bonds in the directions shown by arrows in Fig. 3 The chemical group of the receptor involved in the hydrogen bond corresponding to the black arrow will prevent the steroid molecule from rotating around the C(17)–O(17) bond, by steric constraints onto the 18 methyl group.

A steroid with a 17β hydroxy function bound to a protein in such a way has no more degrees of freedom*. Its other extremity lies in a quite definite position. It is therefore interesting to study the position of the other extremity of the steroid hormone when the D ring is in a fixed position. The projection of the O(3) oxygen atoms and the atoms linked to them by hydrogen bonds in the plane C(13), C(17), C(18) of the different steroids are shown on Fig. 4 and it is apparent that the mean position of the O(3) oxygen atoms is very different for estrogens and androgens (about 3 Å). Indeed the hormonal specificity is usually assigned to the nature of the A ring and the location of the O(3) oxygen atom relative to this; but in our view when the D ring is fixed the specificity would only result from the position of the O(3) oxygen, and not from the electronic state of ring A. A molecule with a D ring and the same thickness as a steroid, would have the corresponding activity of an estrogen or androgen depending on the location of the terminal atom of the A ring. Our proposal seems in good agreement with the estrogenic activity of 7α-methyl 19-nor-androstene diol compared to that of 19-nor-androstene diol [23]. The presence of 7α-methyl is responsible for the moving of the O(3) oxygen in the direction of the β face of the steroid by changing the ring B conformation.

As, for a steroid hormone the chemical nature of the O(3) oxygen atoms is generally different (an hydroxy group for estrogens and an OXO group for androgens), a different system of hydrogen bonds may be expected. Thus, Fig. 4 shows that hydroxy groups are implicated in two hydrogen bonds and OXO groups only in one, but it also clearly appears that hydrogen bonds are settled on the β face of estrogens and on the α face of androgens.

Such an arrangement suggests that the amino acid of the androgen (or estrogen) receptor, which links to the O(3) oxygen, would have to move at least 8

* In such an hypothesis, if the binding sites for DES and estradiol on receptors are identical we have to admit different binding schemes for the two molecules.

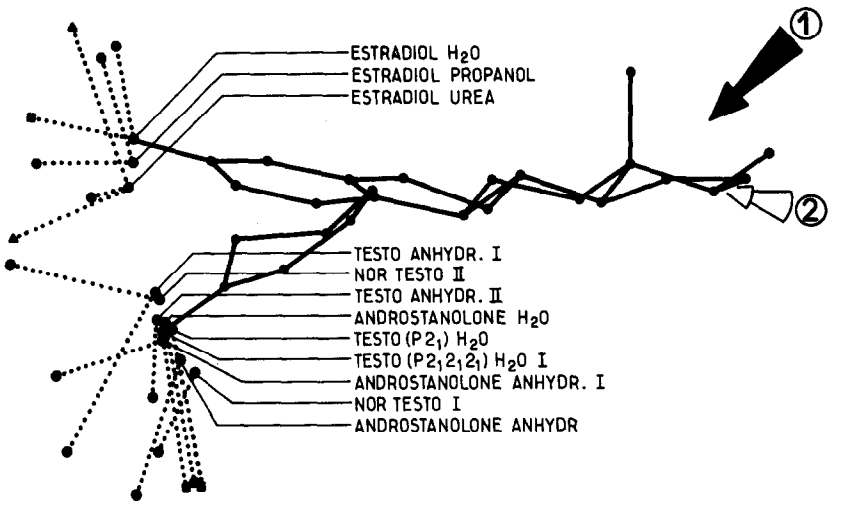

Fig. 4. Projection of the atom O(3) on the plane C(18)-C(13)-C(17) for different steroids and hydrogen bonds involved by it.

Å to link the corresponding oxygen of the estrogen (or androgen) hormone, and then involve such a distortion of the receptor that the response of the receptor would be quite different.

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