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STRUCTURE–ACTIVITY RELATIONSHIPS FOR GLUCOCORTICOIDS—III. STRUCTURAL AND CONFORMATIONAL STUDY OF THE RINGS AND SIDE-CHAIN OF STEROIDS WHICH BIND TO THE GLUCOCORTICOID RECEPTOR

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SUMMARY

The structure of fifteen steroids which bind to the glucocorticoid receptor has been determined by complete geometry optimisation. This method calculates the spatial atomic coordinates for each molecule in the conformation with the minimum internal constraint (lowest-energy state). In addition, the relative energy changes associated with a complete rotation of the side-chain have been calculated for nine of these steroids.

The molecules in this series differ by their substituents at C11, C16, C17 and C21, and by a C1-C2 double bond. First we find that, except for the deformable A-ring and side-chain, the optimized structures are in good agreement with the available crystallographic ones.

As to the structural influence of substituents, it is of course most evident in their immediate vicinity. More subtle effects which, however, become apparent when one considers the overall shape of the steroid, are discussed in the accompanying paper. In all optimized molecules except prednisolone (Δ1-cortisol), the A-ring is a 1α, 2β-half-chair. The different conformations seen by crystallography are consistent with the distortability of this ring. The rigid B- and C-rings all have similar chair conformations. The D-rings range from a 13β-envelope to a 13β, 14α-half-chair, depending on the substitution. Concerning the side-chain, all optimized molecules exhibit a C13-C17-C20-O20 torsion angle between 70° and 120°. The 17-H-substituted steroids have a mean angle of 79°. This value increases to 101° in the presence of a 17-hydroxyl group.

Finally, the energetical curves corresponding to the complete rotation about the C17-C20 bond imposed to the side-chain are all different for the steroids studied. In each molecule, the range of energy changes is relatively small indicating the ability of the molecules to minimize the introduced perturbation. The hypothesis is formulated that receptor binding involves a specific conformation of the side-chain, different from the lowest energy state.

1. INTRODUCTION

We are currently studying the relationships between steroid structure and glucocorticoid activity at the cell level. In the first paper of this series [1] we have determined the hormonal properties of a number of steroid analogues using cultured rat hepatoma (HTC) cells as a model system. Glucocorticoid activity was assessed quantitatively in terms of induction of a glucocorticoid-sensitive enzyme, tyrosine aminotransferase. On this basis, four classes of steroids could be distinguished, agonist, antagonist, partial agonist and inactive glucocorticoids. In addition, the potency of each compound was determined based on the affinity (equilibrium dissociation constant) for the intracellular glucocorticoid receptor.

A thorough description of the structural features and internal mobilities of these compounds is in order before one attempts to evaluate which of those parameters could be involved in determining the glucocorticoid properties. Unfortunately, X-ray data are not available for many of the molecules in our series. We have therefore determined their structure by geometry optimisation, a method which we have shown, in a second paper [2], to be in good agreement with crystallography. Since this approach describes the molecules in their lowest energy state, free of external constraints, it has the additional advantage of providing structures which, unlike in crystals, are comparable to each other. Yet, one must keep in mind that in the intracellular environment and especially when interacting with the receptor, a steroid might assume a conformation which is distinct from that corresponding to the lowest energy (fundamental) state. Given the great number of steroid analogues capable of interacting with the glucocorticoid receptor [1], it is obvious that conformational modifications of the steroid, and very likely of the receptor, are involved in this interaction. These changes presumably take place in the two most mobile parts of the steroid

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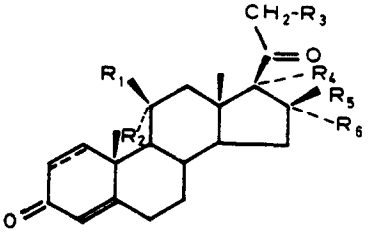
molecule, the side-chain and the A-ring. Moreover, depending on the nature of the substituents, slight conformational modifications in the D-ring can also be expected. On the other hand, the presence of a Δ4-3-one A-ring, of a 11β-hydroxyl, and of a 21-hydroxy-20-one pregnane side-chain seems a prerequisite for maximum glucocorticoid activity, but these features are not necessarily required for binding to the glucocorticoid receptor [1]. Furthermore, both the affinity and the activity seem very much influenced by the nature of the side-chain neighbouring substituents. As we shall see, several of them are able to modify the fundamental side-chain conformation. Therefore, any comprehensive study of the glucocorticoid-receptor interaction implies knowledge of the conformational possibilities of the A-ring and the side-chain, including the associated perturbations in the steroid skeleton. The optimisation method seems especially attractive for such a study since it allows one to compute the relative energy changes associated with conformational modifications of selected regions of the steroid molecule.

We have now determined the complete structure of twenty-four steroids. Because the method is computer-time consuming, we had to restrict ourselves to molecules in which substitutions had a distinct influence on biological activity [1] such as those at position 11 (α and β), 16 (α and β), 17 and 21. We have also studied steroids with Δ1, Δ6, and Δ7 double bonds. For six steroids, X-ray coordinates were available. Although it is not the main objective of this paper, a comparison of our optimized structures with these crystallographic data was of interest since differences between results by both methods give some idea of the potential conformations diverging from the lowest energy state and which can be assumed by the steroid molecules. More important, we wished to provide as thorough a description as possible of the structural features of the individual steroids in their lowest energy state, namely in a comparable situation. When possible, we also tried to evaluate the internal mobilities of some molecules i.e. their ability to depart from this "fundamental" state.

In this paper, for a family of fifteen steroids

Table 1. The series of steroids selected for structural and conformational study



No	Molecule	Formula	R_1	R_2	R_3	R_4	R_5	R_6
I	11β,17,21-trihydroxypregn-4-ene-3,20-dione *cortisol, hydrocortisone*	C21 H30 O5	OH	H	OH	OH	H	H
II	11β,17,21-trihydroxypregna-1,4-diene-3,20-dione *prednisolone, 1-dehydrocortisol*	C21 H28 O5	OH	H	OH	OH	H	H
III	17,21-dihydroxypregn-4-ene-3,20-dione *cortexolone, Reichstein "S"*	C21 H30 O4	H	H	OH	OH	H	H
IV	17-hydroxypregn-4-ene-3,20-dione *17-hydroxyprogesterone*	C21 H30 O3	H	H	H	OH	H	H
V	11β,21-dihydroxypregn-4-ene-3,20-dione *corticosterone*	C21 H30 O4	OH	H	OH	H	H	H
VI	21-hydroxypregn-4-ene-3,20-dione *11-deoxycorticosterone, DOC*	C21 H30 O3	H	H	OH	H	H	H
VII	16α-methylpregn-4-ene-3,20-dione *16α-methylprogesterone*	C22 H32 O2	H	H	H	H	H	CH_3
VIII	16α-methyl-17,21-dihydroxypregn-4-ene-3,20-dione *16α-methylcortexolone*	C22 H32 O4	H	H	OH	OH	H	CH_3
IX	pregn-4-ene-3,20-dione *progesterone*	C21 H30 O2	H	H	H	H	H	H
X	16β-methylpregn-4-ene-3,20-dione *16β-methylprogesterone*	C22 H32 O2	H	H	H	H	CH_3	H
XI	16α-methyl-17-hydroxypregn-4-ene-3,20-dione *16α-methyl-17-hydroxyprogesterone*	C22 H32 O3	H	H	H	OH	H	CH_3
XII	11β-hydroxypregn-4-ene-3,20-dione *11β-hydroxyprogesterone*	C21 H30 O3	OH	H	H	H	H	H
XIII	11α-hydroxypregn-4-ene-3,20-dione *11α-hydroxyprogesterone*	C21 H30 O3	H	OH	H	H	H	H
XIV	16β-methyl-17,21-dihydroxypregn-4-ene-3,20-dione *16β-methylcortexolone*	C22 H32 O4	H	H	OH	OH	CH_3	H
XV	16β-methyl-17-hydroxypregn-4-ene-3,20-dione *16β-methyl-17-hydroxyprogesterone*	C22 H32 O3	H	H	H	OH	CH_3	H

(Table 1) we discuss some characteristic influences of the various substituents on the different parts of the steroid molecules in their "fundamental" state, together with a specific analysis of the conformational potentialities of the side-chain. To be consistent with published literature and to facilitate comparison with crystallographic data, we have followed the description method of Duax and Norton[3]. In the accompanying paper [4] we will describe the effects of these substituents on the general shape of the complete family of 24 molecules. Only then should we be in a position to appreciate to what extent biological properties such as glucocorticoid activity and affinity for the receptor can be ascribed to specific structural and conformational characteristics of the steroid molecule.

II. METHOD

The steroids were optimized in terms of their molecular energy by relaxation of the internal strains [2]. The energy was computed by the GEMO program [5] based on a Westheimer-type equation involving the sum of the stretching (Hooke's law), bending (Baeyer's strain), torsion (Pitzer's strain) and non-bonded atoms interactions and internal hydrogen bonds energies. The starting geometries [2] were optimized down to a convergence limit of 0.01 kcal·mole^{-1}. The initial perturbation increments were 0.001 Å and 0.5° for the bond lengths and the valence and torsion angles, respectively, and decreased until four successive perturbation trials were without further significant effect on the molecular energy. Due to the large number of non-bonded atoms interactions, only those within 6 Å were taken into account with the exception of the side-chain where the limit was extended to about 7 Å, depending on the circumstances.

For studying the rotation of the side-chain with simultaneous relaxation of the internal molecular strains, selected values of the C16-C17-C20-O20 torsion angle were imposed by stepwise increments until a complete rotation (360°) of the side-chain was achieved. The 0° reference corresponds to superimposition of bonds C16-C17 and C20-O20 in a Newman projection along the C17-C20 axis (Fig. 1). The torsion angle values give the magnitude of rotation required for the upper bond to eclipse the lower one. The clockwise rotation of the torsion angle C16-C17-C20-O20 is taken as positive. For each imposed torsion angle, the molecule was fully optimized by relaxing the internal strains as described above.

The optimized structures were described with the help of the program Mean Plane [6]. The computations were carried out on a Xerox CP/V computer. All coordinates of the optimized structures are available from the authors upon request. A complete description of the individual steroids will be presented elsewhere.

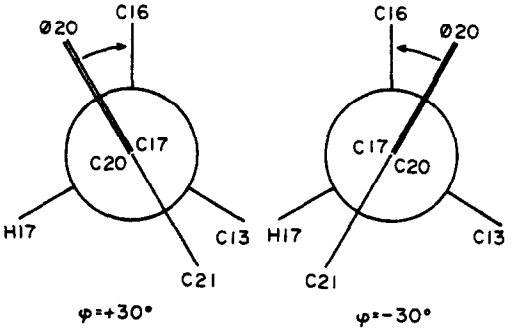


Fig. 1. C16-C17-C20-O20 torsion angle. Newman projection about the C20-C17 bond.

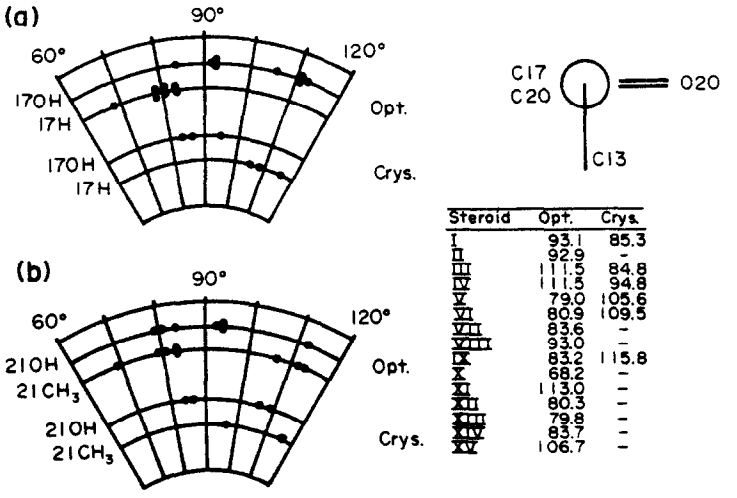
III. RESULTS AND DISCUSSION

A. Side-chain

1. *Influence of substituents on the fundamental structure.* From a structural standpoint, the mechanical Dreiding model shows that the pregnane keto side-chain is theoretically free to rotate about the C17-C20 axis. Numerous investigators have studied this aspect by chemical [7], physico-chemical [8, 9], quantum chemical [10], and crystallographic [11, 12] methods.

Results obtained by geometry optimisation are presented in Fig. 2. In this figure we compare the influence of the C17-(Fig. 2a) and C21-(Fig. 2b) substituents on the position of the side-chain relative to the D-ring. This position is defined by the value of the C13-C17-C20-O20 torsion angle. The fundamental side-chain conformations range between about 70° and 120°. As a rule, the substitution on C21 does not appear to have a characteristic influence on this angle, either in optimized molecules (the mean value is 90.6° ± 4.2 S.E. for 21OH- and 90.8° ± 6.0 S.E. for 21H-substituted steroids) or in crystallographic structures (mean values are 96.3° ± 6.5 S.E. and 105.3° ± 10.5 S.E., respectively). In the 16-methyl substituted optimized compounds **XI** and **XV**, however, introduction of an hydroxyl group at C21 decreases the side-chain angle by 20.0° and 23.0°, respectively. In contrast to the C21 position, the nature of the substituent on C17 allows one to classify the steroids into two groups with respect to the side-chain angle. By crystallography ($n = 6$) the mean angle is 110.3° ± 3.0 S.E. for H-substituted molecules; it drops down to 88.3° ± 3.3 S.E. in OH-substituted compounds, a significant ($P < 0.01$) difference of −22°. By optimisation ($n = 15$), however, the mean angle is 79.3° ± 2.0 S.E. for H-substituted molecules; it increases up to 100.7° ± 4.0 S.E. in OH-substituted compounds, a significant ($P < 0.001$) difference of +21.4°. This is illustrated in Fig. 3 where the average position of the side-chain, depending on the nature of the C17-substituent, is shown relative to the D-ring with superimposition of the C20 and C17 atoms.

One reason for the discrepancy between optimized and X-ray diffraction data could be the influence in the crystal of the intermolecular hydrogen bonds and of the packing forces which perturb the side-chain



Steroid	Opt.	Crys.
I	93.1	85.3
II	92.9	-
III	111.5	84.8
IV	111.5	94.8
V	79.0	105.6
VI	80.9	109.5
VII	83.6	-
VIII	93.0	-
IX	83.2	115.8
X	68.2	-
XI	113.0	-
XII	80.3	-
XIII	79.8	-
XIV	83.7	-
XV	106.7	-

Fig. 2. Side-chain conformation. The side-chain conformation is defined by the torsion angle C13-C17-C20-O20. Both parts of the figure show the values (degrees) of this angle corresponding to the fundamental state, for the optimized (opt.) and, when available, crystallographic (crys.) structures. Each point on the graph corresponds to one molecule, classified according to the nature of the substituent, as indicated. The inset gives the actual value (degrees) of the angle for the fifteen molecules.

conformation. This is supported by two experimental observations. The first example is 5-bromo-6β,19-epoxy-3β-hydroxy-5α-pregnan-20-one [13] which shows two symmetry-independent conformations by crystallography. While conformational ring differences are very subtle, the C17 side-chain orientation is different in the two crystal structures. This difference is expressed by the following parameters: the torsion angle C13-C17-C20-O20 (99.9° *vs* 90.3°), the dihedral angle between the side-chain and the C5 to C17 reference mean plane (39.8° *vs* 51.7°), and the twist of the steroid molecule defined as the value of the dihedral angle C19, C10, C13, C18 (−15.2° *vs* −19.8°). Another example is 9α-fluoro-6α-methyl-prednisolone [14] which also displays differences (85.8° *vs* 99.2°) of the C13-C17-C20-O20 torsion angle in two symmetry-independent crystallographic structures. Therefore, the crystallographic data do not necessarily give an unambiguous picture of the side-chain conformation.

To further clarify the issue, we have computed the individual contribution of the main Van der Waals interactions (energetic contribution of interactions between non bonded atoms [2]) to the energy differences in the side-chain conformations between crystallographic and optimized structures. We found that the strongest of these interactions involve the O20 with the C18 and C16 groups and, when present, the 17-hydroxyl with the C21 substituent. For molecules **V**, **VI** and **IX** which bear no hydroxyl group at C17, the sum of these interactions has a mean value of 0.722 and 0.935 kcal·mole^{-1} by optimisation and crystallography, respectively, a difference of 0.213 kcal · mole^{-1}. For molecules **I**, **III**, and **IV** which bear a 17-hydroxy substituent, these values are 1.628 by optimisation and 2.126 kcal · mole^{-1} by crystallography, a difference of 0.498 kcal · mole^{-1}. Thus, not only are the Van der Waals energies of the side-chain lower in optimized than in crystallographic conformations, as expected: the energy differences between both methods are also greater when the molecules bear a 17-hydroxy substituent. Since the latter can form in the crystal an intermolecular hydrogen bond, our results could illustrate the perturbing influence of such a bond on the D-ring conformation.

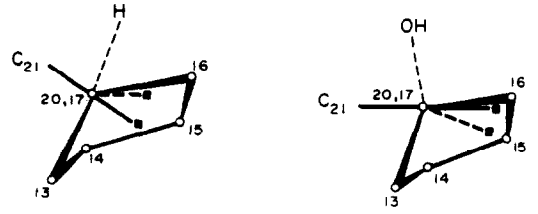


Fig. 3. Average position of the side-chain relative to the D-ring. Newman projection of the average position of the 20-keto (■) group onto the D-ring, depending on the nature of the 17-substituent. Optimized and crystallographic keto groups are represented by solid and broken lines, respectively (mean from data in Fig. 2).

Another factor which cannot be neglected in the study of the side-chain conformation is the presence of intramolecular hydrogen bonds. The 21-hydroxy substituent introduces the possibility of such a bond with the 20-keto group. Also, one cannot *a priori* discard the existence of a similar but probably less strong interaction between 17-hydroxyl and 20-keto groups. Thus, we have calculated (Table 2) the distances between these substituents, as well as the associated McGuire "10–12 energetic potential" [15]. We find, first, that neither the optimized nor the crystallographic structures are compatible with the presence of a 17-hydroxy,20-keto intramolecular hydrogen bond as indicated by the very weak McGuire potential. In contrast, a hydrogen bond can occur between OH21 and O20. In addition, the energetic

Table 2. Length (R) and energy (U partial) of possible intramolecular hydrogen bonds*

Molecule	OH17—O20		OH21—O20	
	OPT	CRYS	OPT	CRYS
I	3.511	3.553	2.894	2.032
	(−0.017)	(−0.015)	(−0.102)	(−2.125)
II	3.514	—	2.892	—
	(−0.016)		(−0.102)	
III	3.312	3.721	2.889	2.180
	(−0.029)	(−0.009)	(−0.103)	(−1.227)
IV	3.323	3.571	—	—
	(−0.028)	(−0.014)		
V	—	—	2.739	2.244
			(−0.168)	(−0.967)
VI	—	—	2.741	2.082
			(−0.167)	(−1.767)
VIII	3.492	—	2.602	—
	(−0.017)		(−0.268)	
XI	3.270	—	—	—
	(−0.032)			
XIV	3.625	—	2.613	—
	(−0.012)		(−0.258)	
XV	3.399	—	—	—
	(−0.022)			

* The length R (in Å) is the distance between the hydrogen and the corresponding oxygen. The energy (data in parentheses) refers to the "10-12 general hydrogen bond potential" defined [15] as U partial (in $kcal \cdot mol^{-1}$) = $A/R^{12} - B/R^{10}$, where A = 13340.1 $Å^{12}$ $kcal \cdot mol^{-1}$ and B = 5781.2 $Å^{10}$ $kcal \cdot mol^{-1}$. OPT: optimized structures: CRYS: crystallographic structures.

potentials associated with this bond are much stronger in the crystal than in the optimized structures. This could result from the greater easiness of the optimized molecules to deal with the perturbation introduced by surrounding substituents in the absence of external constraints, the side-chain being able to slightly modify its orientation and shape accordingly.

In conclusion, the conformation of the side-chain corresponding to the lowest energy state of the molecule clearly depends mainly on the nature of the C17 substituent. In all the molecules studied by optimisation, the presence of a hydroxyl on carbon 17 makes the 20 carbonyl almost eclipse the C16-C17 bond. Although it is premature to draw a correlation with the glucocorticoid properties of these molecules, it is noteworthy that the 17-hydroxy substitution consistently reduces their affinity for the receptor [1]. Finally, our analysis of the differences between the crystallographic and optimized conformations of the side-chain emphasizes the influence of hydrogen bond formation in this region. If such bonds are involved in the interaction of the steroid with the receptor, then these differences give an idea of the perturbation in the fundamental structure which could be expected in the course of this interaction.

2. *Conformational side-chain potentialities for individual steroids.* Rakhit and Engel[7] have attempted to determine by a chemical approach the preferred conformation of the side-chain of 20-keto steroids. Their method is based mainly on the rates and yields of reduction of the 20-keto group by lithium aluminum hydride and the nature of the products of the reaction. These parameters could depend on the ability of the side-chain to deal with the constraints inherent to the necessary transition state of the reaction. Indeed, one can expect not only some direct influence of the surrounding substituents through their inductive effect on the electronic molecular properties, but also steric hindrance in the course of the reaction. Thus, the yields of reduction could reflect the relative easiness of the molecules to accomodate themselves with the perturbation imposed by the transition state, rather than depend on the "preferred" conformation. Unfortunately, such valuable data are not available for large series of substituted molecules.

Using a different approach, Kier[10, 16] applied the Extended Huckel Theory (EHT) to determine the "preferred" side-chain conformation of progesterone, corticosterone and cortisol. On the basis of standard bond lengths and angles, this investigator computed the energy differences in the D-ring and side-chain part of the molecule resulting from a complete rotation of the C13-C17-C20-C21 torsion angle (60° stepwise increments). The conformation of the D-ring was assumed to be a 14α-envelope (i.e. atom C14 below the plane of the other D-ring atoms). This approach is debatable, since the use of standard bond lengths and angles does not necessarily describe the actual intramolecular interactions of the side-chain with the concerned parts of the molecule, especially the 18 methyl and the D-ring substituents. In addi-

tion, for each molecular model, Kier found the same two distinct energy minima corresponding to values of the C13-C17-C20-C21 torsion angle of 120° and 240°. This is somewhat surprising, since these molecules differ, among other things, in their C21 and C17 substitutions which, as we have seen, are among the most perturbing for the side-chain conformation. Finally, these computations do not take into account the inherent potentialities of the molecule to react to the artificial perturbation introduced. Thus it was worth studying these conformational potentialities by geometry optimisation.

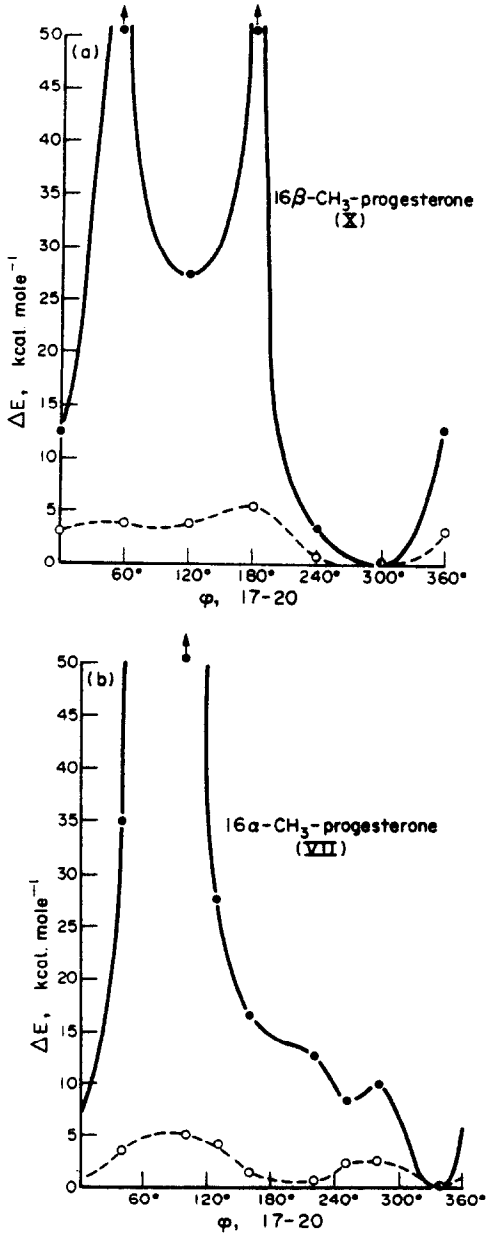


Fig. 4. Difference between optimized and unoptimized structures in the energetic variations associated with side-chain rotation. In both parts of the Figure, the abscissa gives the value of the torsion angle C16-C17-C20-O20. On the ordinate is shown the molecular energy variation from the fundamental state with (broken lines) or without (solid lines) optimisation.

We have compared the energy changes associated with the complete rotation of the side-chain without and with full optimisation of the conformers involved in this rotation. This is illustrated in Fig. 4a and b which show the energy changes associated with a 360° rotation of the C16-C17-C20-O20 torsion angle (ϕ_{17-20}) for 16β-methylprogesterone (**X**) and 16α-methylprogesterone (**VII**), respectively. In both pictures the broken line was obtained by optimisation of the whole molecule for each value assigned to this torsion angle. The solid one corresponds to data obtained by the empirical GEMO method, starting from the lowest energy structure, but without further optimisation, and which are therefore comparable to those from Kier's approach. The deepest optimized minimum of each molecule was obtained by total minimization and therefore is actual, while the location of the energy barriers is only obtained by extrapolation of the neighbouring energies. One weakness of the optimisation process must be pointed out. The methyls are considered as composite entities in which the atoms keep their identity. The consequence is the possibility for the methyls to easily reorganize themselves to minimize the interaction with neighbouring atoms during the optimisation process. This can be seen as a gearing interaction and does not take into account the actual spin of the methyls. One solution would be to consider the methyls as unique bulky substituents with the appropriate Van der Waals radius and the corresponding energetic constants. The main result would probably be some increase in the potential barriers height associated with the methyl groups.

It can be seen in Fig. 4, first that the range of energy changes is much more limited after optimisation, suggesting that the whole molecule can "absorb" most of the energy variations introduced by the perturbation. Thus it seems that, at least in the absence of external constraints, the side-chain could rotate relatively easily about the C17-C20 bond.

Second, for each molecule, both energy curves present the same two minima locations, although unoptimized 16α-methylprogesterone (**VII**) has a shoulder instead of minimum around $\phi_{17-20} = 210°$. Finally, in both molecules, optimized and unoptimized energy barrier positions are in good agreement. Examination of detailed energies (Table 3) of both molecules shows that without optimisation the highest interactions occur between hydrogens 21 and methyl 18 in molecule **VII** and between hydrogens 21 and methyls 18 and 16β for molecule **X**. In striking contrast, the optimized structures as well as the Dreiding model show that the involved methyls and side-chain can easily accomodate themselves and reduce the energy of the interaction accordingly. Therefore, it is plausible that, in the cell, the molecule is able to orient its side-chain depending on the perturbation introduced by the solvent or by the receptor.

Table 3. Comparison of non-bonded interactions of highest energy in two unoptimized and optimized molecules*

Molecule	Interactions	Energies (kcal·mol^{-1}) unoptimized	optimized
VII	$H18_{ii}$–$H21_{i}$	19.2	0.5
$\phi_{17-20} = 80°$	$H18_{ii}$–$H21_{ii}$	9.2	0.2
	$H18_{iii}$–$H21_{ii}$	19.0	0.2
	C18–$H21_{ii}$	12.5	0.4
	C21–$H18_{ii}$	8.9	0.1
VII	$H18_{ii}$–$H21_{i}$	14.1	0.4
$\phi_{17-20} = 200$	H16–$H21_{ii}$	7.5	0.2
	C18–$H21_{i}$	2.3	0.7
X	$H18_{ii}$–$H21_{ii}$	77.9	0.2
$\phi_{17-20} = 60$	C18–$H21_{ii}$	6.2	0.2
	C21–$H18_{ii}$	6.5	0.2
X	$H21_{i}$–H22	23.1	0.4
$\phi_{17-20} = 180$	$H21_{iii}$–H22	15.7	0.4
	C21–H22	13.9	0.4

* ϕ_{17-20} refers to the C16-C17-C20-O20 imposed torsion angles values (see Fig. 5). Subscripts $_{i}$, $_{ii}$ and $_{iii}$ are introduced to distinguish the H-atoms of the methyl groups. H22 is one of the H-atoms in the 16β-methyl groups. Energies are calculated as described in ref. [2].

Several steroids in our series possess the same reactive functions and have very similar skeletons. Yet, inside such groups, various molecules may exhibit considerable differences in their affinity for the receptor [1]. It is not unlikely that this could result, at least in part, from differences in the conformational potentialites of deformable parts of the steroid such as the side-chain. This would imply that the interaction with the receptor involves a specific conformation of the side-chain.

This hypothesis can be explored by comparing the relative molecular energy differences between various steroids associated with a complete rotation of the side-chain about the C17-C20 axis. For a valid comparison, at least three main requirements must be met. First, the molecules examined must have similar structural skeleton characteristics (i.e. A, B, and C rings conformation). In addition, during the rotation each individual steroid skeleton cannot become significantly different from its starting (lowest energy) conformation. This is the case for all steroids studied. For instance, for a given molecule, the maximum perturbation observed during the side-chain rotation is not more than 3° in the dihedral angle between A- and D- rings mean planes, or between the D- and B—C-rings mean planes. The maximum variation of the distance between methyls C18 and C19 is only 0.02 Å. Second, the molecules must be chosen in such a way that a lower affinity cannot be *a priori* ascribed to steric hindrance during receptor binding. The 16-methyl substituents fulfill this criterion. The very low affinity of compound X is not necessarily a matter of steric hindrance due to the 16β-methyl group since, in other steroids, a 16β-methyl substitution does not necessarily decrease the affinity. For example, 9α-fluoro-16β-methyl-11β, 17,21-trihydroxy-1,4-pregnadiene-3,20-dione (betamethasone) has a high affinity very similar to that of its 16 unsubstituted analogue 9α-fluoro-prednisolone (unpublished). Third, due to its exceptional importance for receptor binding, the functional characteristics of the 20-ketone must be the same in the molecules under comparison, whatever the inductive effect of the neighbouring substituents. This requirement can be confirmed by determination of the ^{13}C NMR spectroscopy chemical shift of carbon atom C20. It was found (Saunders, Easton, and Schmit, unpublished) that the ^{13}C NMR shift of C20 is the same (208.03 ppm ± 0.05 S.E.) for all the molecules bearing no hydroxy substituent at C17 and C21 positions. The inductive effect of a α-hydroxyl group is apparently the same whether in position 17 or 21 (chemical shift of 209.86 ppm ± 0.11 S.E.) and finally, in the presence of both a 17- and 21-hydroxy substituents the chemical shift is 211.06 ppm ± 0.19 S.E. All these data are statistically different at the $P < 0.01$ level or less. All chemical shifts were taken in $DMSO_{d_6}$ as a solvent.

We have determined by full optimisation the energetical rotation curves of the side-chain for nine molecules in our series which fit these criteria (Fig. 5–7). Three of them bear no hydroxyl group (Fig. 5), three others have either a 17- or a 21-hydroxy substituent (Fig. 6) and three molecules have both a 17- and a 21-hydroxy substituent (Fig. 7). Thus, inside each group, the steroids are structurally and functionally comparable. As shown in Figs 5–7 all energetical profiles differ. As expected they exhibit a minimum around 310° except for 17-hydroxy substituted steroids which show a minimum around 360°C. This is consistent with results presented earlier concerning the angle C13-C17-C20-O20. Secondly, all energetical profiles show a peak around 100° due to the Van

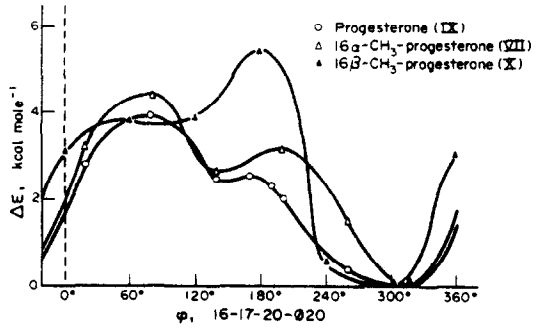


Fig. 5. Energetic profiles associated with side-chain rotation for three steroids without hydroxy-substituent in position 17 and 21.

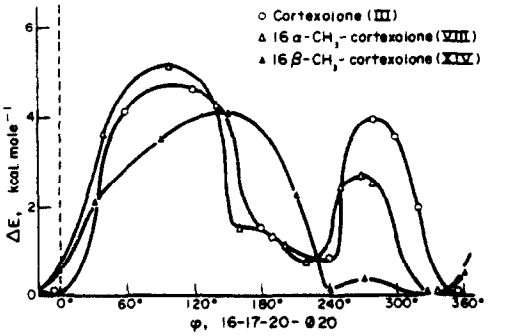


Fig. 7. Energetic profiles associated with side-chain rotation for three steroids with both a 17- and 21-hydroxy substituent.

der Waals interactions between the 18-methyl and 21 group. In addition, all the steroids bearing a 17-hydroxy substituent exhibit a smaller but characteristic peak around 270°, except for 16β-methylcortexolone (**XIV**) where the presence of the 16β-methyl substituent drastically reduces the 17-hydroxyl peak. This specific influence of the 16β-methyl group could result from the changes introduced by this substituent into the D-ring conformation (see table 4). As seen above, it is noteworthy that a 17-hydroxy group consistently reduces the affinity for the receptor. Thus, it is plausible that the "preferred" side-chain conformation expected by the receptor is close to a C16-C17-C20-O20 torsion angle value of 270°. Two distinct observations could corroborate this hypothesis. First, 16α-methylcortexolone (**VIII**) exhibits a 17-hydroxy peak height between the values of cortexolone (**III**) and 16β-methylcortexolone (**XIV**) (Fig. 7), and has a higher affinity for the receptor than cortexolone [1]. Secondly, betamethasone has a higher affinity (unpublished) than its 16α-methyl epimer, dexamethasone. These two molecules are comparable in their D-ring substitution, hence in their side-chain potentialities, to 16β and 16α-methylcortexolone, respectively. Thus one could expect that 16β-methylprogesterone (**X**) has a higher affinity than its 16α-methyl analogue (Fig. 5). This is not the case [1]. In fact, comparison of the position of the 16β-methyl group relative to the D-ring shows striking differences between compounds **X** and **XIV**. The torsion angle C20-C17-C16-C22 (C22 is the 16β-methyl carbon atom) increases from 15.3° in 16β-methylcortexolone up to 23.7° in 16β-methylprogesterone, while the distance between carbon atoms of methyls 18 and 16β extends from 3.649 Å up to 3.731 Å. Thus, in contrast to 16β-methylcortexolone and its analogue betamethasone, one can wonder whether the very weak affinity of 16β-methylprogesterone is not, in this case only, a matter of steric hindrance.

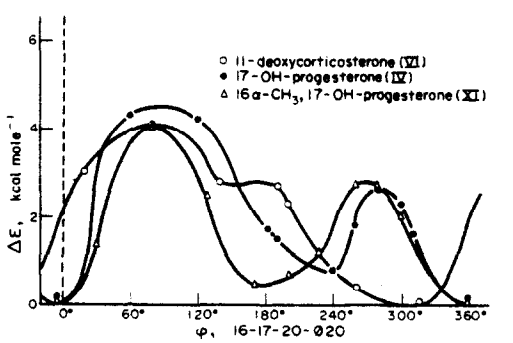


Fig. 6. Energetic profiles associated with side-chain rotation for three steroids with either a 17- or a 21-hydroxy substituent.

In conclusion, the fact that, in each group, the energetical rotation curves are different may have interesting implications for structure–activity relationships. Indeed, glucocorticoid receptor binding might actually involve a specific side-chain conformation, similar for all steroids. This would imply that at equilibrium, the proportion of rotamers in the "preferred" side-chain conformation would be different for different steroids. Such differences could account, at least in part, for differences in affinity for the receptor.

B. *Conformation of the D-ring*

There are several ways to quantitatively express the structure of this ring. The phase angle of pseudorotation Δ [17] is a good measure of the conformation of the ring

$$\tan\frac{\Delta}{2} = \frac{(\phi_2 + \phi_4) - (\phi_1 + \phi_3)}{3.0777\,\phi_0}$$

where ϕ_0 to ϕ_4 are torsion angles C17-C13-C14-C15, C14-C13-C17-C16, C15-C16-C17-C13, C14-C15-C16-C17, and C13-C14-C15-C16, respectively, inside the D-ring. A Δ value of −36° corresponds to a 14α-envelope, a value of 0° to a 13β, 14α-half-chair and a value of +36° corresponds to a 13β-envelope. The maximum amplitude of the deformation is measured by the torsion angle $\phi_m = \phi_0/\cos\Delta/2$. The puckering of the ring can also be expressed by the average value of the absolute magnitude (Φ_D) of the torsion angles inside the D-ring.

We have calculated the value of these parameters for the molecules under study (Table 4). The pseudorotation angles Δ of the optimized structures and those derived from crystallography are somewhat different. However, they vary in the same direction and ampli-

Table 4. Conformational parameters of the D-ring*

Molecule	Δ	ϕ_m	Φ_D	Conformation
I	22.7	48.7	30.2	distorted 13β-envelope
	(26.3)	(48.2)	(30.2)	13β-envelope
II	23.9	48.5	30.0	distorted 13β-envelope
III	31.4	47.9	29.2	13β-envelope
	(37.7)	(47.0)	(29.2)	distorted 13β-envelope
IV	13.5	48.2	30.5	distorted 13β,14α-half-chair
	(4.8)	(45.4)	(29.3)	13β,14α-half-chair
V	14.4	48.1	30.8	distorted 13β,14α-half-chair
	(10.9)	(45.0)	(28.9)	distorted 13β,14α-half-chair
VI	16.3	47.8	30.7	distorted 13β,14α-half-chair
	(13.3)	(44.8)	(28.9)	distorted 13β,14α-half-chair
VII	6.1	48.7	31.5	distorted 13β,14α-half-chair
VIII	33.4	48.5	29.5	13β-envelope
IX	6.1	48.6	31.3	13β,14α-half-chair
	(1.3)	(46.2)	(29.9)	13β,14α-half-chair
X	0.6	47.0	30.5	13β,14α-half-chair
XI	20.8	48.4	30.3	distorted 13β-envelope
XII	9.1	49.0	31.4	distorted 13β,14α-half-chair
XIII	9.5	48.8	31.3	distorted 13β,14α-half-chair
XIV	19.7	45.7	29.0	distorted 13β-envelope
XV	12.2	45.7	29.2	distorted 13β-14α-half-chair

* Data in parentheses refer to crystallography. The parameters are defined in the text.

tude, depending on the substituent. Table 4 shows that for all molecules the conformation of the D-ring is between a 13β-envelope and a 13β,14α-half-chair. By optimisation, a 11β-hydroxy substituent decreases the Δ value strongly in the presence of both a 17- and a 21-hydroxyl groups (III *vs* I) and slightly with the 21-hydroxyl alone (VI *vs* V). On the contrary, the same 11β-hydroxy substituent leads to an increase of Δ in the absence of both 17- and 21-hydroxyl groups (IX *vs* XII). A 16α-methyl substitution does not modify Δ in the absence of other groups (IX *vs* VII) but yields a higher Δ in the presence of a 17-hydroxy substituent (IV *vs* XI and III *vs* VIII). In contrast, a 16β-methyl group decreases Δ down to a very small value (IX *vs* X) in the absence of other substituents, but does not modify Δ in the presence of a 17-hydroxyl group (IV *vs* XV). As to the 17-hydroxy substituent, it increases Δ but the magnitude of this effect seems highly dependent on the nature of the neighbouring groups. In order of increasing importance one finds the following cases: no other substitution (IX *vs* IV), a 11β together with a 21-hydroxyl (V *vs* I and II), a 16α-methyl group (VII *vs* XI) and a 21-hydroxyl (VI *vs* III). Finally, the 21-hydroxyl substitution markedly increases the pseudorotation parameter Δ whatever the substitution (XII *vs* V; IX *vs* VI; XI *vs* VIII and IV *vs* III).

On the other hand, conversion from α to β substitution leads to interesting observations on the Δ value. For instance, epimerisation of 11α-hydroxyl (XIII) into 11β-hydroxyl (XII) does not modify the Δ value significantly (9.5° to 9.1°). In contrast, epimerisation of the 16-methyl substituent consistently decreases the Δ value. This decrease is of 5.5° for 16-methylprogesterone (VII *vs* X), of 8.6° for 16-methyl, 17-hydroxyprogesterone (XI *vs* XV) and of 13.7° for 16-methylcortexolone (VIII *vs* XIV). Thus, the magnitude of the effect on the D-ring of the epimerisation of the 16-methyl group depends on the nature of the C17 and C21 substituents. This might pertain to the glucocorticoid properties of the steroids since a 16-methyl substitution may have opposite effects on the affinity for the receptor, depending on the neighbouring substituents [1 and unpublished observations].

In summary, some substitutions such as a 17- or a 21-hydroxyl consistently shift the D-ring conformation towards the 13β-envelope. Others shift the D-ring conformation towards a 13β,14α-half-chair. It is the case for the 16α- to 16β-methyl epimerisation and for a 11β-hydroxy substitution on 17 and 21 hydroxylated steroids. Finally, the D-ring conformation may not change, such as upon addition of a 16α-methyl group on an otherwise unsubstituted steroid skeleton or upon conversion of a 11α-hydroxyl into its 11β-epimer. Contrary to the phase angles of pseudorotation, the ϕ_m and Φ_D parameters do not appear sensitive enough to describe the influence of a particular substitution.

C. *Conformation of the A-ring*

In Δ4-3-one steroids, different conformations of the A-ring can be expected from its unsaturated character. From a survey of the crystallographic literature Duax *et al.*[3] have shown that, for most of these steroids, the conformation of the A-ring usually falls between a 1α-sofa in which atoms C2, C3, C4, C5 and C10 are coplanar while C1 is below the plane, and a 1α,2β-half-chair in which atoms C3, C4, C5 and C10 are coplanar while C1 is below the plane and C2 is above the plane (Fig. 8). The sofa conformation possesses a mirror plane of symmetry which is perpendicular to the plane of the ring and which passes through the out-of-plane atom C1. A deviation

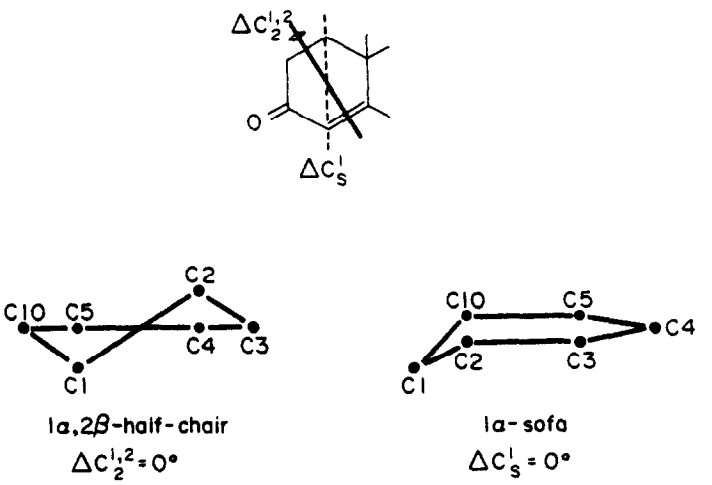


Fig. 8. A- ring conformations. $\Delta C_2^{1,2}$: rotational symmetry bisecting bonds C1-C2 and C4-C5. ΔC_s^1: mirror symmetry passing through C1 and C4 atoms.

from this symmetry is defined by the mirror asymmetry parameter [3]

$$\Delta C_s^x = \left[\frac{\sum_{i=1}^{m} (\phi_i + \phi_i')^2}{m}\right]^{1/2}$$

where x is the out-of-plane atom, ϕ_i and ϕ_i' are the symmetry-related paired torsion angles (in this case angles ϕ_{1-2} and ϕ_{1-10}, ϕ_{2-3} and ϕ_{10-5}, ϕ_{3-4} and ϕ_{4-5}) and m is the number (3 in this case) of pairs of torsion angles. When the mirror symmetry is perfect, the paired torsion angles are of equal magnitude and opposite signs and therefore ΔC_s^x equals zero. The ring is a perfect sofa.

A perfect half-chair has a two-fold axis of symmetry bisecting the bond joining the out-of-plane atoms (C1 and C2 in this case). Deviation from this symmetry is expressed by the rotational asymmetry parameter [3]

$$\Delta C_2^{y,z} = \left[\frac{\sum_{i=1}^{m} (\phi_i - \phi_i')^2}{m}\right]^{1/2}$$

where y and z design the atoms of the bisected bond, and the other symbols are identical to those in the mirror symmetry formula.

The conformation of the A-ring of a steroid can thus be evaluated from its position on a plot ΔC_s^x *vs* $\Delta C_2^{y,z}$. We have calculated these parameters for our optimized steroids and have compared them to the available crystallographic data. Figure 9 shows the correlation between the two asymmetry parameters for the optimized ($y_1 = 20.3 - 0.8\,x$) and crystallographic ($y_2 = 20.2 - 0.6\,x$) structures. The asymmetry parameters of prednisolone (**II**) obtained by optimisation are only shown for comparison since this steroid has a Δ_1 double bond.

From Table 5, which summarizes the conformational parameters of the A-ring, it can be seen that optimized structures all have a similar 1α,2β-half-chair conformation, while the crystallographic data appear somewhat discrepant. Indeed, the asymmetry parameters indicate that by X-ray diffraction (data in parentheses in the table), the A-ring of 11-deoxycorticosterone (**VI**) is a 1α,2β-half-chair and that of cortexolone (**III**) is a 1α-sofa, while it is the converse for progesterone (**IX**) and 17-hydroxyprogesterone (**IV**). The only difference between both pairs of steroids is the C21-substituent but it could hardly account for these striking differences in the A-ring conformation. Such differences cannot be immediately ascribed to particular intermolecular hydrogen bond formation in the crystal, since neither 11-deoxycorticosterone nor progesterone establish such bonds, while 17-hydroxyprogesterone has one (2.79 Å) between O-3 and corresponding OH-17 and cortexolone has two of them, one (3.07 Å) between O-3 and OH-17 and one (2.84 Å) between O-3 and OH-21. The unexpected crystallographic conformation of the progesterone A-ring, a distorted 1α-sofa, cannot therefore be easily interpreted.

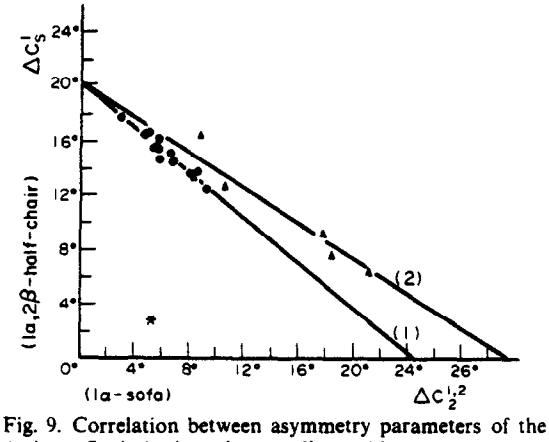


Fig. 9. Correlation between asymmetry parameters of the A-ring. Optimized and crystallographic structures are represented by dots and triangles, respectively. The optimized structure of prednisolone (star) is shown for comparison. The corresponding regression lines are shown for the optimized (1) and the crystallographic (2) parameters. The asymmetry parameters and regression equations are given in the text.

Table 5. Conformational parameters of the A-ring

Molecule	$\Delta C_2^{1,2}$	ΔC_s^1	C1-C2	C2-C3	C3-C4	C4-C5	C5-C10	C10-C1	Φ_A
I	5.7	16.3	−55.8	37.1	−9.9	−2.0	−14.6	43.7	27.2
	(21.2)	(6.5)	(−53.9)	(27.6)	(+2.7)	(−7.1)	(−18.7)	(48.5)	(26.4)
III	8.0	13.6	−53.2	34.4	−8.3	−1.5	−15.6	43.0	26.0
	(17.9)	(9.3)	(−53.0)	(28.0)	(+3.0)	(−9.3)	(−15.2)	(45.6)	(25.7)
IV	5.1	16.7	−55.9	37.6	−10.2	−1.9	−14.6	43.3	27.3
	(8.8)	(16.6)	(−56.1)	(35.1)	(−3.4)	(−8.7)	(−12.1)	(44.0)	(26.6)
V	5.7	14.6	−51.8	34.4	−9.3	−1.4	−14.2	40.8	25.3
	(10.5)	(12.7)	(−55.6)	(35.6)	(−7.6)	(−1.7)	(−18.0)	(46.1)	(27.4)
VI	5.6	15.4	−53.3	35.6	−9.3	−1.8	−14.3	41.8	26.0
	(8.4)	(13.4)	(−52.2)	(33.1)	(−6.8)	(−2.9)	(−14.6)	(42.0)	(25.3)
VII	8.5	13.7	−54.4	34.9	−8.2	−1.6	−16.1	43.9	26.5
VIII	9.2	12.5	−52.5	33.4	−7.3	−1.5	−16.2	42.9	25.6
IX	6.8	14.6	−53.6	35.4	−8.9	−1.7	−15.2	42.6	26.2
	(18.5)	(7.8)	(−53.5)	(28.7)	(+0.5)	(−6.0)	(−18.1)	(47.2)	(25.7)
X	6.6	15.1	−54.5	35.9	−9.1	−1.7	−15.1	43.0	26.6
XI	5.6	15.6	−54.1	36.0	−9.5	−1.7	−14.5	42.2	26.3
XII	4.7	16.5	−54.5	36.6	−9.9	−1.9	−13.9	41.9	26.5
XIII	3.0	17.8	−54.7	37.5	−10.8	−2.0	−12.9	41.2	26.5
XIV	6.7	14.4	−53.0	34.9	−8.9	−1.5	−15.1	42.1	25.9
XV	5.4	15.7	−53.7	35.9	−9.6	−1.8	−14.2	42.0	26.2
Mean value for a 1α,2β-half-chair†			(−55.3)	(36.4)	(−7.0)	(−5.2)	(−13.5)	(43.3)	(26.8)
Mean value for a 1α-sofa			(−53.1)	(29.8)	(−1.8)	(−4.4)	(−18.5)	(46.8)	(25.7)

* All data are in degrees. C1-C2... C10-C1 refer to torsion angles inside the A-ring. Data in parentheses refer to crystallography. Compound II was omitted because of the particular perturbation introduced by the Δ1 double bond. The other parameters are defined in the text. † Taken from crystallographic data available in the literature [see p. 32 of ref. 3].

These unexpected crystallographic structures could be due to the influence of crystal packing forces as illustrated by the six symmetry-independent crystallographic structures of 17β-hydroxy-4-androstene-3-one (testosterone). These structures only differ in the A-ring conformations which range from a 1α,2β-half-chair ($\Delta C_2^{1,2} = 8.2°$; $\Delta C_s^1 = 15.5°$) to a 1α-sofa ($\Delta C_2^{1,2} = 18.2°$; $\Delta C_s^1 = 7.9°$) [see p. 257 of ref. 3]. Thus, it appears that the A-ring conformation can be influenced by the intermolecular interactions. In contrast with these inconsistencies between the crystallographic structures of the steroids studied, the optimisation method leads to the more coherent picture of a 1α,2β-half-chair.

On the other hand, examination of the Dreiding model shows that when two rings (e.g. rings A and B) are fused along a common bond involving a sp^2 carbon atom, two distinct conformations can be observed, the quasi-*cis* and the quasi-*trans* conformations. By optimisation of testosterone, Bucourt *et al.* [18] have found that in the quasi-*trans* conformation, the C1-C2-C3-C4 torsion angle can be varied from 22° up to 53° without more than 1 kcal · mole^{-1} change in the molecular energy.

In conclusion, the "fundamental" structure of the A-ring seems relatively insensitive to the presence of substituents on the other rings. However, this is not a consequence of an intrinsic rigidity of this ring. On the contrary, the A-ring appears to be particularly deformable and this might facilitate the interaction with the receptor binding site.

D. *Conformations of the B- and C-rings*

We have calculated for both rings the mirror (ΔC_s^x) and rotational ($\Delta C_2^{y,z}$) asymmetry parameters as well as the puckering parameters Φ_B and Φ_C. These data are in good agreement with the available crystallographic ones. Both rings have quite similar chair conformations and are negligibly influenced by the various substituents, as expected from the rigidity of these rings. For the B-ring, four molecules (I, III, VIII, XIV) have a dominant rotational symmetry bisecting the bond C5-C10 ($\Delta C_2^{5,10}$ close to 0°); four molecules (V, VI, XII, XIII) have a dominant mirror symmetry related to atom C7 (ΔC_s^7 close to 0°), whereas two other molecules have both a dominant rotational and mirror symmetry with the same references except for molecule II (prednisolone) which exhibits a rotational $\Delta C_2^{5,6}$ and a mirror ΔC_s^5 symmetries.

For the C-ring, three molecules (I, II, V) have a dominant rotational symmetry bisecting the bond C9-C11; seven molecules (III, IV, VIII, XI, XII, XIII, XV) have a dominant mirror symmetry related to atom C9; and the other molecules have both a dominant rotational and mirror symmetry with the same references.

As to the rings puckering, except for the Δ1-unsaturated prednisolone, the B-ring is a little more flattened (mean $\Phi_B = 52.1° \pm 0.14$ S.E.) than the C-ring (mean $\Phi_C = 54.4° \pm 0.23$ S.E.) ($P < 0.001$). This is consistent with the presence in all molecules under study of a sp^2 carbon atom C5. Thus, because of the

constancy of their conformation, whatever the substitutions in the steroid, the B- and C-rings are an excellent frame of reference for describing variations in other parts of the molecule [4].

In conclusion, we have examined the influence of certain substituents on two aspects of the structure of glucocorticoids, the lowest energy "fundamental" conformation and the possibility for the individual molecules to depart from that particular conformation. As to the first aspect, there was a good agreement between the optimized structures and the available crystallographic ones. When discrepancies were found, they involved deformable parts of the molecule, thus particularly susceptible to the influence, in the crystal, of hydrogen bonds and packing forces. Since the molecules studied differ mostly by substitutions on the D-ring and side-chain, it is not surprising that they have very similar A-ring structures in the fundamental state. These substitutions also have a negligible effect on the rigid B- and C-rings. As to the D-ring and side-chain, this study has provided a quantitative and precise estimation of the influence of substituents known to affect the glucocorticoid activity of the analogues. Actually, when compared in the "fundamental" state, the corresponding atomic coordinates of the molecules in this series appear very similar. However, the additive effect of minor differences in the hundred-odd coordinates of each steroid molecule may lead to marked differences in the overall shape of the various steroids. This will be considered in the accompanying paper [4].

Concerning the second aspect of this study we conclude that each steroid may exhibit several distinct conformations, at least at the level of the A-ring and side-chain. The probability for the latter to assume some of these conformations depends very much on the nature and the position of the substituents studied. Work is in progress to integrate this information into a comprehensive analysis of the relations between the data presented here and elsewhere [4] and the glucocorticoid properties of steroids.

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