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A-RING CONFORMATIONAL STABILITY AND PROGESTERONE-RECEPTOR BINDING AFFINITY OF 4-EN-3-ONE STEROIDS

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Summary—The molecular structure and strain energy of 4-en-3-one steroids in two different A-ring conformations are calculated by means of a molecular mechanics technique. The computations for the isolated molecules provide the following order of increasing stability of the inverted A-ring conformers: 10-methyl, 19-nor, 9-ene compound. This tendency is in agreement with X-ray structure data for single crystals. The normal 1α,2β-half chair conformation of 10-methyl steroids is found to be stabilized by bond angles, mainly at C10, and non-bonded interactions from the 10-methyl group. Pitzer strains favour the inverted 1β,2α-half chair conformation in the case of 4,9-diene-3-one compounds. Binding affinities to the progesterone receptor decrease in the series: 19-nor, 9-ene, 10-methyl compound. In view of this ordering, the calculated relative stabilities of A-ring conformers are in conflict with a conformation-controlled receptor binding. Variations of receptor bond strengths are supposed to be more strongly influenced by a steric hindrance of the 10-methyl group and/or steroid-backbone flexibility.

INTRODUCTION

The 4-en-3-one double bond system of progestational steroids is regarded as an essential feature for binding to the progesterone receptor [1–3] and to the progesterone-binding globulin PBG [4]. The carbonyl oxygen is assumed to act as H acceptor in a hydrogen bond. There are indications from quantitative structure–activity relationship (QSAR) studies [5] and binding affinity measurements [4] that the main source of bonding forces both to the receptor and to the binding protein is, however, due to hydrophobic interactions. Furthermore, results of X-ray crystallographic and molecular mechanics investigations into steroid structure combined with extensive pharmacological studies suggest an important influence of the steroid-backbone conformational flexibility [3].

Recently Duax *et al.* [1,6] proposed the inverted 1β,2α-half chair conformers to have an especially high affinity to the progesterone receptor and to be, therefore, the biologically active form of 4-en-3-one steroids. This inverted conformation was observed in crystal structures of some 4-en-3-one compounds, e.g. medroxyprogesterone acetate MPA [7], 2β-acetoxy [8] and 4,9-dien-3-one steroids [1,9]. However, MPA was shown by high field NMR spectroscopy [10] to have the normal 1α,2β-half chair in solution. Consequently, the question arises concerning the relative stability of the different conformations excluding, however, the pharmacologically irrelevant crystal packing forces. To evalute this a comparison of stable and unstable molecular states is necessary. For that reason theoretical methods are generally in a better position to contribute to this problem than experiments. By means of the non-quantum chemical molecular mechanics scheme Bucourt *et al.*[11] found that a loss of the 10-methyl group lowers the energy difference between the normal and inverted A-ring conformation from 11.3 to 4.2 kJ mol^{-1} in the case of testosterone. These values are in good agreement with quantum chemical PCILO results [12]. Molecular mechanics calculations are also used in this paper to examine both A-ring conformations in the cases of 10-methyl and 19-nor 4-en-3-one as well as 4,9-dien-3-one steroids and to explain the energy situation due to structure characteristics. In a second step, relations to progesterone-receptor affinity data are discussed in order to check the role of the inverted A-ring conformation in steroid-receptor interactions.

EXPERIMENTAL

Calculation scheme

The 4-en-3-one steroids employed in the study are shown in Fig. 1. Both the normal 1α-2β-half chair (torsional angle C1–C2–C3–C4 $\vartheta > 0°$) and the inverted 1β,2α-half chair conformer ($\vartheta < 0°$) are calculated for each of the three compounds. A molecular mechanics calculation scheme was chosen because of: (i) the sufficient accuracy of molecular results; (ii) the small amount of computation time compared to molecular orbital methods; and (iii) the direct connection of energetic values and structural data which is not achieved in the case of quantum chemical

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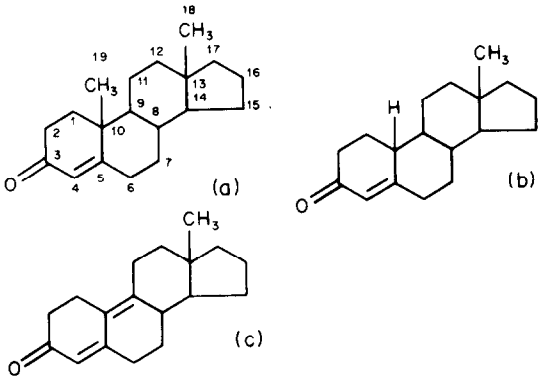


Fig. 1. Investigated steroids; (a) androst-4-en-3-one (10-methyl compound), (b) estr-4-en-3-one (19-nor compound) and (c) estra-4,9-dien-3-one (9-ene compound).

methods even by use of the energy partitioning technique in the semi-empirical SCF–LCAO–MO scheme. All the calculations are executed by means of the GEMO program [13] in Version 804B. Data input was simplified by using the program GEMOS [14] which mainly requires the cartesian or fractional coordinates of atoms. The total strain energy is formed by a stretching, a bending, a torsional and a non-bonded term. The empirical force field parameters were taken from [11, 12, 15]. Full relaxation of all internal coordinates including the angular hydrogen bound to C10 in the 19-nor compound was allowed. A strain-energy convergence limit of 0.04 kJ mol^{-1} was used for the geometry optimization. All calculations were performed on an EC 1040 computer.

Receptor binding experiments

Infantile New Zealand rabbits of 0.8–1 kg were injected daily for 4 days with 5 μg estradiol benzoate, with 10 μg on day 5, and sacrificed on day 8. Excised uteri were minced with scissors and homogenized, with an all-glass homogenizer, in 4 vol of ice-cold Tris–HCl buffer, pH 7.4 (20 mM, with 1.5 mM EDTA, 10% glycerol and 0.25 mM dithioerythritol). After centrifugation at 100,000 *g* for 1 h, the supernatant was used for receptor binding experiments. Three hundred μl of diluted supernatant containing 0.5–1 mg protein/ml were incubated at 0°C for 20 h with 4 nM [^{3}H]progesterone without or with competitors in six suitable concentrations each. Parallel incubations with 1 μM progesterone were run for measuring non-specific binding. Free steroid was removed by addition of 300 μl charcoal–dextran suspension (1%/0.1%) and centrifugation, bound progesterone was measured by ^{3}H-counting. The concentration of each substance required to displace 50% of bound [^{3}H]progesterone was determined (EC_{50}). From this the relative binding affinity was calculated (RBA; standard = progesterone):

$$\text{RBA } [\%] = \frac{EC_{50} \text{ prog.}}{EC_{50} \text{ subst.}} \times 100\%.$$

Values were measured in at least two separate experiments.

RESULTS AND DISCUSSION

Conformation investigations

The androstene and estrene compounds under study can be considered to be also model substances for pregnene or 17α-CH_2R-17β-hydroxy steroids in which the C17 substituents are assumed to have no influence on the A-ring structure. The latter steroids were recently synthesized [16–19], and X-ray crystallographic data of them have been determined [9, 20]. Accordingly, we are in a position to underline the conclusions derived from the calculated energy situation by comparison of experimental and theoretical structure data. On the other hand, a satisfactory agreement between X-ray results found for the crystalline state and molecular mechanics findings for isolated molecules indicates the minor effect of crystal packing forces on our problem concerning the relative conformational stability.

The determined differences in the strain-energy contributions between the inverted and the usual conformations are presented in Table 1. Negative energy values indicate that the inverted conformation is more stable than the normal one.

In the case of the 10-methyl compound, the high positive strain-energy difference is in good agreement with the aforementioned calculation by Bucourt *et al.*

Table 1. Differences in calculated energies (in kJ mol^{-1}) between inverted and normal A-ring conformers

Energy contribution	10-Methyl compound	19-Nor compound	9-Ene compound
Stretching energy (bond length distortion, Hooke's law)	−0.1	−0.3	0.0
Bending energy (valence angle bending, Baeyer strain)	+5.6	+4.3	+4.5
Torsional energy (torsional angle deformation, Pitzer strain)	−0.8	−0.8	−17.2
Non-bonded energy (van der Waals' interaction)	+4.4	+1.7	+0.7
Total strain energy	+9.1	+4.9	−12.0

Table 2. Selected bond angles and nearest distances of van der Waals' interactions (in pm) of 10-methyl 4-en-3-one steroids as well as corresponding energy differences ΔE (in kJ mol^{-1}) between inverted and normal A-ring conformers

	Theoret. ΔE	Normal conf. exp. crystal progesterone [21]	Normal conf. exp. crystal testosterone [22]	Normal conf. theoret. isol. mol.	Inverted conf. theoret. isol. mol.
Bond angle C1–C10–C9	+2.1	108.0°	108.8°; 109.4°	108.5°	115.0°
Bond angle C1–C10–C19	+1.5	111.0°	110.3°; 110.1°	109.7°	105.0°
Bond angle C10–C19–H	+1.1	111°	111° ; 111°	118.5°	120.1°
		114°	113° ; 114°		
		118°	112° ; 107°		
v.d. Waals' C19-H at C1	+2.1	267	258; 271	269	253
v.d. Waals' H at C19-H at C6	+1.0	232	243; 243	237	221
v.d. Waals' H at C19-H at C8	+1.1	240	234; 235	232	218
v.d. Waals' H at C19-H at C1	+0.7	248	239; 239	260	242
v.d. Waals' C19-C6	+0.7	317	318; 318	313	303
v.d. Waals' C19-C8	+0.6	323	317; 322	319	309
v.d. Waals' C19-C2	−1.1	318	313; 313	321	386

[11] and crystallographic findings which show 10-methyl steroids to always adopt the normal A-ring conformation. It can be seen from Table 1 that this stability is due to the bond angle term and to non-bonded interactions. Table 2 records the most important structural features and their energy contributions. The main effects which stabilize the normal conformation are the valence angle situation at C10 and the differences in non-bonded interaction of the 10-methyl group with the hydrogens at C1 as well as with the C6 and C8 regions.

In the 19-nor compounds, the C10 substituent is much smaller. Accordingly, the differences in Van der Waals contacts are diminished and, as can be seen in Table 3, the major influence on the energy characteristics has the remaining Baeyer strain at the C10 carbon atom. The energetically unfavourable opening of the C1–C10–C9 bond angle by 6.1° (X-ray: about 9°) and closure of the C1-C10-H bond angle by 3.8° (X-ray: about 6°) are assigned to be the most important unstabilizing factors for the inverted conformer. However, the strain-energy difference between both conformers is rather small. The value given in Table 1 is in excellent agreement with the result of Bucourt *et al.* [11] and agrees with the fact that both conformations were detected in single-crystal X-ray structure analyses [20]. It is noteworthy that in most cases experimental X-ray data reveal an analogous, but even stronger tendency in structural variations than those calculated by molecular mechanics. Therefore, theoretically determined effects can be considered to give a valid description. In Fig. 2, both stable conformers together with the transition state for conversion between them are shown in stereoscopic view. The transition state is assumed to have a torsional angle C1–C2–C3–C4 of $\vartheta = 0°$. In case of the 19-nor compound, the whole strain-energy curve for the conformational conversion is given in Fig. 3. This curve can be regarded as a pathway on the total potential-energy hypersurface in which all internal coordinates except for the C1–C2–C3–C4 torsional angle are allowed to relax for each point of this curve. Broad minima of the potential curve illustrate the relatively high flexibility of both conformers. The activation energy of the conversion with the most stable conformer as starting point is computed to be 37.7 kJ mol^{-1}. It is somewhat higher than 32.6 kJ mol^{-1} in case of the 10-methyl compound and much higher than that of the 9-ene compound with about 23.6 kJ mol^{-1}. The activation energy barrier is formed by Pitzer strains to a great extent.

For the 9-ene compound, it can be taken from Table 1 that the inverted conformation is more stable than the usual one in contrast to the other steroids under consideration. However, this is not due to bond angles at the C10 atom which even exert an opposite effect. Strong differences in Pitzer strains favour the unusual conformer. Table 4 lists the decisive torsional angles and the corresponding energy portions. The stability of the inverted conformation agrees with the fact that this conformation is predominantly found by X-ray structure analyses for 4,9-dien-3-one steroids [1, 3, 9, 20].

Relations to progesterone-receptor affinities

As noted above, the tendency to form the inverted A-ring conformation is increasing from the energetic

Table 3. Selected bond angles of 19-nor 4-en-3-one compounds and corresponding differences in strain energy ΔE (in kJ mol^{-1}) between inverted and normal A-ring conformers

	Theoret. ΔE	Normal conf. exp. crystal [20][a]	Normal conf. theoret. isol. mol.	Inverted conf. theoret. isol. mol.	Inverted conf. exp. crystal [20][b]
Bond angle C1–C10–C9	+2.7	110.7°; 109.1°	111.1°	117.2°	118.0°
Bond angle C1–C10–H	+0.5	110.4°; 109.8°	108.5°	104.7°	103.9°

[a] 17α-Azidomethyl-17β-hydroxy-estr-4-en-3-one.
[b] 17α-Cyanomethyl-17β-hydroxy-estr-4-en-3-one.

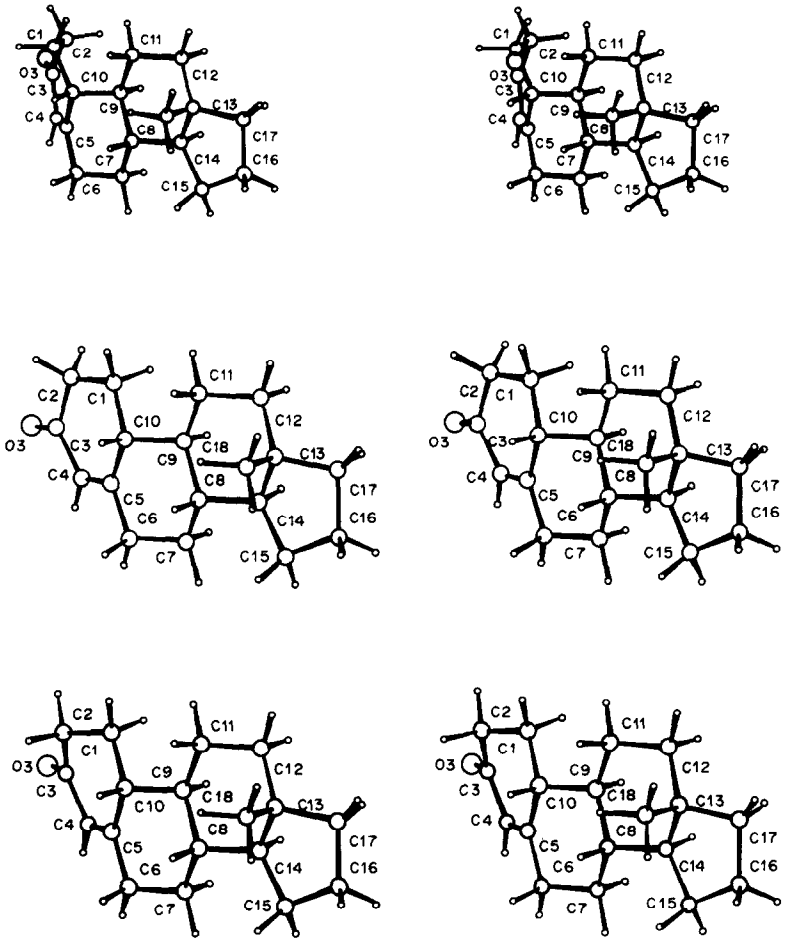


Fig. 2. PLUTO [24] stereoplots of different geometry-optimized conformers for the 19-nor 4-en-3-one steroid; inverted conformation (top), transition state (middle) and normal conformation (bottom).

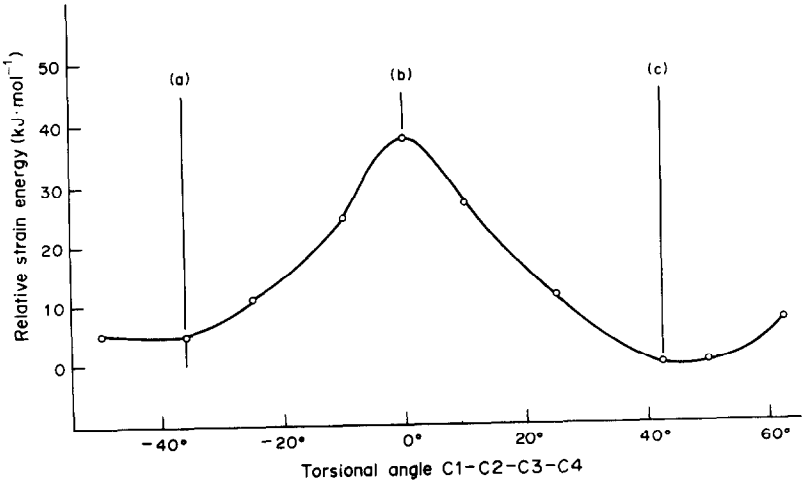


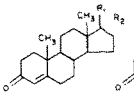
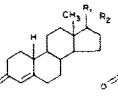
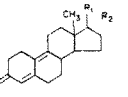
Fig. 3. Strain-energy curve for conformational conversion of the 19-nor compound; (a) inverted conformation, (b) transition state and (c) normal conformation.

Table 4. Selected torsional and bond angles of 4,9-dien-3-one compounds and corresponding differences in strain energy ΔE (in kJ mol^{-1}) between inverted and normal A-ring conformers

	Theoret. ΔE	Normal conf. theoret. isol. mol.	Inverted conf. theoret. isol. mol.	Inverted conf. exp. crystal [9][a]	Inverted conf. exp. crystal [20][b]
Torsional angle C2-C1-C10-C5	−4.1	+25.2°	−41.9°	−32.6°	−43.5°
Torsional angle C2-C1-C10-C9	−2.8	−155.8°	+142.5°	+154.9°	+140.6°
Torsional angle C3-C4-C5-C6	−2.1	+171.8°	−177.9°	−177.7°	−173.2°
Torsional angle C2-C3-C4-C5	−1.9	−20.1°	+6.3°	+11.4°	+1.2°
Torsional angle C4-C5-C6-C7	−1.9	+148.6°	+139.4°	+149.6°	+137.6°
Bond angle C1-C10-C5	+2.8	118.0°	115.8°	115.3°	114.0°
Bond angle C6-C5-C10	+1.5	119.5°	118.1°	117.8°	116.9°

[a] 17α-Cyanomethyl-17β-hydroxy-estra-4,9-dien-3-one.
[b] 17α-Azidomethyl-17β-hydroxy-estra-4,9-dien-3-one.

Table 5. Relative binding affinities to the progesterone receptor of different 4-en-3-one steroids (progesterone = 100%)

		Substituents at C17		Relative binding affinities		
Species and organ	Reference	R_1	R_2			
Human uterus	[4,23]	—$COCH_3$	—H	100%	168%	—
Human uterus	[4,23]	—OH	—H	2%	22%	—
Rabbit uterus	[3]	—$COCH_3$	—H	100%	230%	181%
Rabbit uterus	[3]	—OH	—H	1.0%	20%	17%
Rabbit uterus	This work	—OH	—H	0.5%	7.0%	—
Rabbit uterus	This work	—OH	—CH_2N_3	0.8%	176%	160%
Rabbit uterus	This work	—OH	—CH_2CN	0.3%	24%	12.5%

point of view in the series: 10-methyl < 19-nor < 9-ene compound. By contrast, this order cannot be detected if the progesterone-receptor affinities are considered. In Table 5 the results of the receptor binding measurements are summarized. In all cases 19-nor compounds exhibit an enhanced binding to the progesterone receptor compared to the corresponding 9-ene compounds. Although the steroid conformation preferred in the steroid–receptor complex is unknown, it is obvious that the receptor binding is not mainly controlled by the ability to form the inverted A-ring conformation. This is in accord with the experimental findings that 4,9,11-trien-3-one steroids prefer the normal A-ring conformation [1,3] and show an even stronger affinity to the progesterone receptor than the corresponding 19-nor-4-en-3-one compounds [3].

Furthermore, a strongly diminished affinity can be taken from Table 5 for 10-methyl steroids relative to compounds without the 10-methyl group. Lee *et al.*[5] found in a QSAR study the absence of a hydrophobic receptor pocket in the C10 region. In view of these facts, it should be concluded that a steric hindrance of the 10-methyl group in steroid–receptor interaction and/or the different steroid-backbone flexibility [3] are responsible for variations in the receptor bond strength.

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