Molecular Mechanics Investigation on Side-Chain Conformations of a 17α-Ethyl-17β-hydroxy Steroid with Regard to Receptor Binding

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Energetically favourable conformations for simultaneous intramolecular rotations of both the 17α ethyl side chain and the 17β hydroxyl group of a model steroid are calculated by MM2 molecular mechanics. In accordance with recent IR and NMR interpretations, the 17α substituent is found to preferably adopt conformations which may sterically hinder the formation of hydrogen bonds between the steroidal 17β oxygen atom and the receptor protein. Furthermore, the 17α ethyl substitution is computed to influence the D-ring conformation and to alter the location of the 17β oxygen function by 28 pm in space.

Introduction

The 17β hydroxyl group is a common feature of several steroid hormones, *e.g.* estrogenes, androgens, or progestins of the ethisterone type. Two properties of this OH group are discussed to have a significant influence upon receptor binding and biological response: (i) the spatial location of the oxygen atom as outlined, *e.g.*, in the A-ring binding and D-ring acting model [1, 2] and (ii) the capabilities of hydrogen bond formation including directional specificity [3–8].

Both properties might be affected by appropriate cosubstitution at the 17α position.

Actually, 17α substituents in addition to the 17β hydroxyl group are found to produce substantial changes in biological functions. Introduction of the 17α ethinyl group, for instance, drastically reduces the binding of testosterone to the androgen receptor but simultaneously induces some binding to the progestin receptor [9]. In the case of estradiol derivatives, the 17α ethinyl substituent enhances the affinity to the estrogen receptor [9] whereas the 17α ethyl group induces a strong reduction in receptor binding and estrogenic activity [10, 11].

There are two possible ways in which 17α substituents can influence receptor binding: (i) direct substituent-receptor interactions and (ii) indirect effects on steroid characteristics as, *e.g.*, on the abovementioned properties of the 17β oxygen function. The former must be taken into consideration for progestins since quantitative structure-activity relationship (QSAR) studies reveal that the hydrophobicity in the 16α,17α region is an important factor in determining progestational potency [12]. On the basis of molecular mechanics calculations on androgens, some shift in the O17 oxygen atomic spatial position and a slight effect on D-ring conformational features caused by the introduction of a 17α methyl group are found [8]. From recent interpretations of IR and NMR spectra [10, 11] it is concluded that the 17α ethyl substituent modifies the geometrical abilities of the 17β-OH group to form hydrogen bonds to the receptor and, thus, strongly reduces the receptor binding affinity in contrast with other, more linearly shaped 17α substituents.

In the present paper, molecular mechanics calculations are used to investigate the energetically favoured conformations of the 17α-ethyl-17β-hydroxy model steroid shown in Fig. 1 in order to study intramolecular influences due to double substitution at the C17 carbon atom.

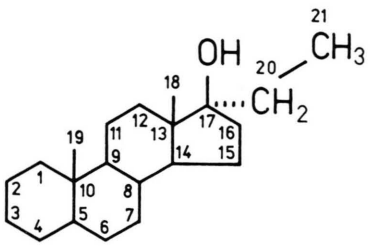

Fig. 1. Investigated 17α-ethyl-17β-hydroxy-androstane and carbon atom numerotation.

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Theory

All calculations are executed by means of the MM2 program [13]. The total strain energy is formed by a stretching, a bending, a stretch-bend, a torsional, and a nonbonded term using the standard MM2 force field including lone pairs on the hydroxyl oxygen. Torsional angle increments of 30° are used in the computation of the potential energy surface for rotations about the C17–O17 and C17–C20 bonds (Fig. 2). For each point of this surface, the energy value is obtained by full relaxation of all internal coordinates except the torsional angles C16–C17–C20–C21 and C16–C17–O17–H (driving coordinates). The energy-minimum conformations listed in Table I are calculated by full-scale geometry optimization without any constraints. In Fig. 3, the energetically most preferred conformation is illustrated in stereoscopic view generated by the PLUTO program [14]. All calculations were performed on EC 1040 and EC 1056 computers.

Results and Discussion

Fig. 2 gives an impression of the potential energy surface for rotations of 17α and 17β substituents about the C17–C20 and C17–O17 bonds, respectively. This surface exhibits nine local minima which are determined by further geometry optimization each. The corresponding energy-minimum conformations A–I are recorded in Table I together with their energy characteristics compared to the most stable conformation A. As can be taken from Table I, the most favoured conformers adopt a torsional angle C16–C17–C20–C21 of about −65° regardless of the actual hydroxyl hydrogen orientation (conformations A–C). The other *syn*-clinal location of the 17α side chain with respect to the O17 oxygen is next favoured (conformations D–F). The most unstable conformers (G–I) are found to be those which bring the terminal methyl group in proximity to the steroid-skeleton ring D (*anti*-periplanar to O17). To avoid close contacts to steroid-skeleton atoms mainly of the D ring, the valence angles C17–C20–C21 and C13–C17–C20 are expanded by 4.4° and 3.2°, respectively, when going from the A to the H energy-minimum conformation. Accordingly, 75% of the total strain-energy difference between these two conformers are due to bond-angle distortions (Baeyer strains).

As can be seen from the variability of minimum torsional-angle values C16–C17–O17–H of Table I, the orientations of the oxygen lone pairs and the

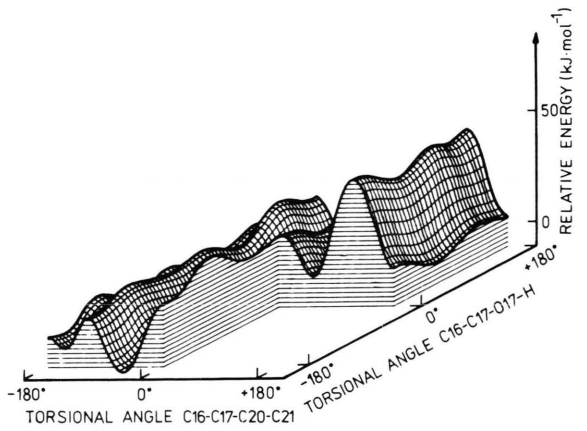

Fig. 2. Representation of the potential energy surface for rotations of 17α and 17β substituents calculated by MM2 molecular mechanics.

Table I. Minima of the potential energy surface (*cf.* Fig. 2).

Minimum designation	Relative strain energy [kJ mol^{-1}]	Torsional angle C16–C17–C20–C21	Torsional angle C16–C17–O17–H
A	0.0	− 65°	−174°
B	0.2	− 65°	+ 57°
C	1.6	− 66°	− 47°
D	4.6	−161°	+ 70°
E	5.2	−161°	−167°
F	6.5	−160°	− 45°
G	7.7	+ 56°	+ 66°
H	8.1	+ 56°	−171°
I	10.1	+ 55°	− 45°

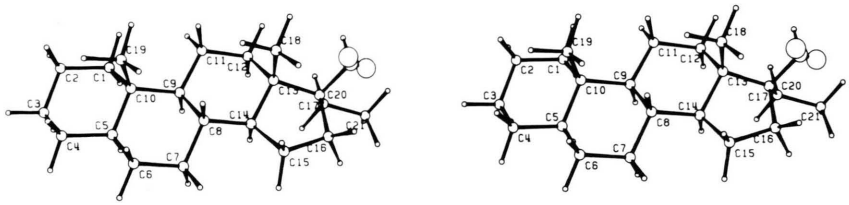

Fig. 3. Stereoplot of the global energy-minimum conformation A calculated by full-scale geometry optimization including O17 oxygen lone pairs.

hydroxyl hydrogen have a greater geometrical flexibility as compared to the ethyl side chain. For the corresponding unsubstituted 17α−H steroid, MM2 calculations show minima in terms of C16−C17−O17−H at 180°, −50°, and 69°. In view of these values and the high degree of flexibility, it cannot be concluded that the additional 17α ethyl substituent significantly alters the energetically preferred positions of the 17β hydroxyl group. However, the ethyl locations *syn*-clinal to O17 might affect the receptor hydrogen-bond formation by steric hindrance as it can, for instance, be derived from Fig. 3. This explanation and the fact that these *syn*-clinal orientations represent the energetically preferred conformations are in excellent agreement with recent IR and NMR interpretations [11] which assign the IR band at 3633 cm^{-1} to a β-side directed rotamer. Other evidence that the ethyl conformation shown in Fig. 3 is the most stable one comes from crystallographic data of similar compounds with the C20 carbon atom having sp^3 hybridization. Values of the torsional angle C16−C17−C20−R distributed near −60° are predominantly found by X-ray single crystal analyses of 17α−CH_2X substituted 17β-hydroxy-estr-4-en-3-ones ([15]: X = CN: −59.6°; [16]: X = N_3: −56.7° and −55.2°; [17]: X = Cl: −64.2° and −60.8°) and 17β-hydroxy-estra-4,9-dien-3-ones ([18]: X = CN: −60.5°; [19]: X = N_3: −58.6°). Calculations of the potential energy profile for 17α side-chain rotation of this type of compounds keeping, however, bond lengths and valence angles fixed [19] give the same relative-stability order of the three 17α side-chain conformations as found in this paper.

From examinations of hydrogen bonding patterns in crystal packing arrangements, two hydrogen bonding zones preferred in receptor binding are proposed [3−8]. These zones are described to be approximately located in directions characterized by torsional angles with respect to C16 of roughly 180° and 60°. Also, in a steroid-DNA intercalation model [20] hydrogen bonds to phosphate oxygens are stereospecifically formed with this torsional angle being about 60°. Thus, these H bonding zones are placed in those regions which are blocked up to a great extent by the two mostly preferred locations of the terminal methyl group in the 17α side chain. Hence, the present molecular mechanics calculations support the explanation suggested by Kaspar and Witzel [11] that binding of 17α-ethyl-estradiol and related compounds to the estrogen receptor is disturbed by the ethyl group directionality. The total binding energy of steroid-receptor interactions should be reduced by the energy contribution due to intramolecular conformational conversion as a precondition for selective receptor-steroid hydrogen bond formation. Also in the case of progestins, it is experimentally found that a change from the linearly arranged 17α ethinyl group to 17α−CH_2X substituents diminishes both the progestational activity of 19-nor derivatives ([21]: X = CN, N_3, Cl, Br, OCH_3) and their receptor binding: relative binding affinity to the rabbit-uterine progesterone receptor of norethisterone: 263% [22]; of 17α-cyanomethyl-17β-hydroxy-estr-4-en-3-one: 24% [23]; of the 17α-azidomethyl-17β-hydroxy-estr-4-en-3-one even with additional azido group binding: 176% [23].

Further sources of receptor binding energy are Van der Waal's interactions and hydrophobic bonding from the steroid backbone. Therefore, it is interesting to see which influence the 17α side chain exerts, *e.g.*, on the D-ring conformation. The 17α−H analogue of the steroid under study is calculated to adopt an intermediate between 13β,14α-half chair and 13β-envelope (phase angle of pseudorotation $\Delta = 14.1°$; asymmetry parameters: $\Delta C_2(C16) = 9.4°$, $\Delta C_s(C13) = 11.1°$). This D-ring conformation is changed by the 17α ethyl group in the most favoured side-chain conformation A into a 13β,14α-half chair ($\Delta = -3.7°$; $\Delta C_2(C16) = 2.3°$, $\Delta C_s(C13) = 19.2°$). However, such D-ring conformational alterations cannot be used to explain substantial variations in biological functions since, already in

the case of 17α unsubstituted 17β-hydroxy steroids, a variability of D-ring conformations ranging from 13β-envelope to 13β,14α-half chair is crystallographically observed [24]. On the other hand, the conformational change induced by the 17α ethyl group is much more striking than that calculated for a 17α methyl group [8].

In connection with the reorientation of D-ring atoms, the MM2 computations reveal that the O17 oxygen atom is slightly displaced when the 17α ethyl substituent is introduced. A least-squares fit of all carbon atoms of the A, B, and C rings for the 17α−CH_2CH_3 versus the 17α-H steroid yields a geometrical separation between both O17 oxygens of 28 pm in space.

A similar displacement has been found in the case of the 17α methyl substitution by means of molecular mechanics calculations [8]. An appropriate spacing of this oxygen atom is claimed, *e.g.*, by Duax *et al.* [1, 2] for expression of steroid hormone activity.

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[1] W. L. Duax, J. F. Griffin, D. C. Rohrer, and C. M. Weeks, in: Hormone antagonists (M. K. Agarwal, ed.), p. 3, Walter de Gruyter & Co., Berlin 1982.
[2] W. L. Duax, J. F. Griffin, D. C. Rohrer, C. M. Weeks, and R. H. Ebright, in: Biochemical actions of hormones, **Vol. XI,** p. 187, Academic Press, New York 1984.
[3] B. Busetta, C. Courseille, G. Precigoux, and M. Hospital, J. Steroid Biochem. **8,** 63 (1977).
[4] J. Delettré, J. P. Mornon, T. Ojasoo, and J. P. Raynaud, in: Perspectives in steroid receptor research (F. Bresciani, ed.), p. 1, Raven Press, New York 1980.
[5] W. L. Duax, C. M. Weeks, and D. C. Rohrer, in: Recent progress in hormone research, **Vol. 32,** p. 81, Academic Press, New York 1976.
[6] J. P. Raynaud, J. Delettré, T. Ojasoo, G. Lepicard, and J. P. Mornon, in: Physiopathology of endocrine diseases and mechanisms of hormone action, p. 461, Alan R. Liss, New York 1981.
[7] J. P. Raynaud and T. Ojasoo, in: Steroid hormone receptors (H. Eriksson and J. A. Gustafsson, eds.), p. 141, Elsevier, Amsterdam 1983.
[8] J. P. Schmit, J. I. Quivy, and G. G. Rousseau, J. Steroid Biochem. **13,** 1387 (1980).
[9] J. P. Raynaud, T. Ojasoo, E. Bignon, J. C. Doré, and E. Surcouf, in: Innovative approaches in drug research (A. F. Harms, ed.), p. 47, Elsevier, Amsterdam 1986.
[10] P. Kaspar and H. Witzel, J. Steroid Biochem. **23,** 259 (1985).
[11] P. Kaspar and H. Witzel, J. Steroid Biochem. **23,** 611 (1985).
[12] F. J. Zeelen, in: QSAR and strategies in the design of bioactive compounds (J. K. Seydel, ed.), p. 135, Verlag Chemie, Weinheim 1985.
[13] N. L. Allinger and Y. H. Yuh, Computer program MM2, University of Georgia, Athens (USA) 1980.
[14] S. Motherwell and W. Clegg, Computer program PLUTO, University of Cambridge (UK) 1976.
[15] G. Reck, M. Hübner, M. Bohl, G. Bannier, and R.-G. Kretschmer, Cryst. Res. Technol. **21,** 1035 (1986).
[16] G. Reck, M. Hübner, M. Bohl, and P. Leibnitz, Cryst. Res. Technol. **21,** 1041 (1986).
[17] G. Reck, M. Hübner, and D. Pfeiffer, Cryst. Res. Technol. **21,** 1185 (1986).
[18] G. Reck and M. Hübner, Cryst. Struct. Comm. **11,** 1815 (1982).
[19] G. Reck, M. Hübner, G. Bannier, and P. Scharfenberg, Cryst. Res. Technol. **21,** 1191 (1986).
[20] L. B. Hendry, E. D. Bransome jr., A. F. Lehner, T. G. Muldoon, M. S. Hutson, and V. B. Mahesh, J. Steroid Biochem. **24,** 843 (1986).
[21] M. Hübner and K. Ponsold, Exper. Clin. Endocrinol. **81,** 109 (1983).
[22] J. P. Raynaud, M. M. Bouton, M. Moguilewsky, T. Ojasoo, D. Philibert, G. Beck, F. Labrie, and J. P. Mornon, J. Steroid Biochem. **12,** 143 (1980).
[23] M. Bohl, G. Kaufmann, M. Hübner, G. Reck, and R.-G. Kretschmer, J. Steroid Biochem. **23,** 895 (1985).
[24] W. L. Duax and D. A. Norton, Atlas of steroid structure, **Vol. I,** Plenum, New York 1975.