Molecular Mechanics Investigation on Conformational Flexibility of 14β Steroids in Drug-Receptor Interactions

Martin Bohl and Manfred Wunderwald

Akademie der Wissenschaften der DDR, Zentralinstitut für Mikrobiologie und experimentelle Therapie, DDR-6900 Jena, Beutenbergstraße 11, Deutsche Demokratische Republik

Z. Naturforsch. **41c,** 297–300 (1986); received October 22/December 10, 1985

Molecular Mechanics, Steroid Geometry, D-Ring Flexibility, Hydrogen Bond, Cardioactivity

The interaction between two 14β steroids being still unsynthesized and a model receptor binding site is theoretically studied by a molecular mechanics scheme. Both compounds containing seven-membered rings in the 17β position are found to form stable hydrogen bonds to the receptor and can be attributed to be potential inhibitors of the Na^+,K^+-ATPase activity. Substantial steroid conformational changes necessary for an efficient receptor binding are calculated to reduce the interaction energy by only 10 kJ mol^{-1}. Therefore, alterations in steroid structure should be generally taken into consideration in the investigation of steroid-receptor interactions.

Introduction

Models using the concept of a mainly rigid steroid geometry are generally applied in quantitative structure-activity relationships (QSAR) studies of cardiotonic steroids. On this basis, linear correlations are found between the ability to inhibit the Na^+,K^+-ATPase activity and several steroid characteristics, *e.g.* the relative spatial position of the lactone-ring carbonyl oxygen [1–4], dipole moments and related quantities [5, 6], or electrostatic potential energies [7].

In contrast with this assumption of rigid drug-molecule geometry, molecular mechanics calculations are used in this paper to study the possibility of drug-molecule geometry alterations as a consequence of the receptor binding and to evaluate their corresponding potential energy requirements. The two 14β steroids under study possess seven-membered rings as 17β substituents. For the choice of these substituents, a concept of a dipolar structure with a localized negative charge and a positive charge delocalized in a π system is used which is usually found in cardiotonic steroids. This charge separation gives rise to a strong dipole moment that is also called for in the model suggested by Repke *et al.* [5, 6]. In addition, the negative charge should be localized in an appropriate region relative to the steroid backbone [1–4, 7]. Both preconditions may be fulfilled, for instance, with tropone having a large dipole moment of 4.3 D.

According to our knowledge, both steroids have neither been synthesized nor pharmacologically tested so far. Thus, the present theoretical investigation may give a first indication to their cardiotonic potency when combined with QSAR findings.

Computational Methods

Fig. 1 presents the steroids employed in this investigation: 4-(3′β,14′-dihydroxy-5′β,14′β-androstan-17′β-yl)-2,4,6-cycloheptatrien-1-one (tropone compound); 5-(3′β,14′-dihydroxy-5′β,14′β-androstan-17′β-yl)-2(7H)-oxepinone (oxepinone compound). In case of the tropone steroid, X-ray crystallographic structure data of the free tropone [8] are used to construct the 17β side chain. The molecular orbital CNDO/2 geometry optimization procedure [9, 10] has been applied without any structural restrictions to generate the coordinates for the oxepinone moiety. The carbonyl bond length is adjusted to the bufa-

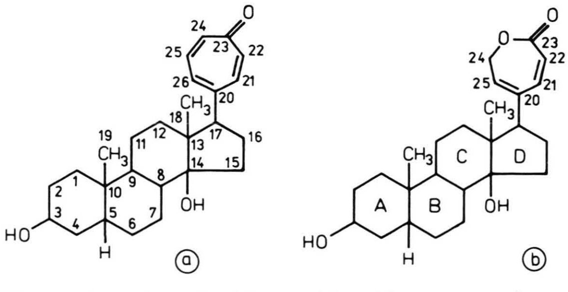

Fig. 1. Investigated 14β steroids with numerotation of carbon atoms: a) tropone compound; b) oxepinone compound.

Reprint requests to Dr. M. Bohl.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0300–0297 $ 01.30/0

lin value because of the known overestimation of this distance by the semiempirical CNDO method.

Molecular mechanics calculations are carried out for the whole steroids with and without being in contact with a model receptor H atom which was mapped and denoted HB in [7]. The coordinates of this receptor hydrogen are kept fixed throughout the geometry optimization. This procedure permits an uneffected description of the steroid flexibility. Especially the D-ring geometrical flexibility is of high interest. Therefore, full relaxation of the atoms C11–C18, C20, O14, and their corresponding hydrogens is allowed. Since molecular mechanics techniques are generally not suitable for conjugated π systems, the internal coordinates of both 17β side chains are constrained to be undistorted during the optimization procedure. No constrains are made on the internal rotations about the C17–C20 bonds and on the exocyclic valence angles at C20.

The total strain energy is minimized up to a convergence limit of 0.04 kJ mol^{-1} and involves the following terms: bond stretching, valence and torsional angle deformations, electrostatic interactions due to the monopole approximation, Van der Waals interactions and hydrogen bonds. All molecular mechanics calculations are executed by means of the GEMO program [11]. Data input was simplified by using the GEMOS program [12] which mainly requires the cartesian or fractional coordinates of atoms. The empirical force field parameters are taken from [13–15] and hydrogen-bond potential constants from [16] are used. Atomic charges of the 17β substituents are determined by CNDO/2 computations. For the receptor hydrogen, a net charge of +0.14 is taken into account corresponding to the CNDO/2 charge value of a hydroxyl hydrogen.

The total interaction energy of a steroid in contact with the model receptor binding site is given by the following equation:

$$U^{\mathrm{theor}} = U_{\mathrm{hb}} + U_{\mathrm{el}} + U_{\mathrm{nb}} + \triangle U_{\mathrm{st}}.$$

The first three contributions are due to direct interactions between the steroid atoms and the receptor H atom: hydrogen bond (U_{hb}), electrostatic (U_{el}) and non-bonded interactions (U_{nb}). $\triangle U_{\mathrm{st}}$ denotes the energy difference with respect to the changes in steroid geometry when the steroid-receptor bond is formed.

All calculations were performed on an EC 1040 computer.

Results and Discussion

In case of the free tropone steroid, two minimum conformations with respect to the 17β side chain orientation are calculated by molecular mechanics. The obtained C13–C17–C20–C21 torsional angles of 88.7° and −97.4° agree well with values determined by X-ray structure analyses or other calculations for cardiotonic steroids [2, 17, 18]. The (C26, O14) conformation is found to be more stable by 2.8 kJ mol^{-1} than the (C21, O14) conformation. Interestingly, this is not accompanied by striking differences in the exocyclic valence angles at C20 as detected in bufalin [17] and in cardenolides [18, 19]. The energy gain is mainly due to a 2.1° smaller valence angle C16–C17–C20, a slightly different torsional angle C14–C15–C16–C17, and a more favourable Van der Waals interaction between the 17β substituent and the C16 region.

However, the strain energy difference calculated is so small that both conformers can be regarded as possibly interacting with the receptor binding site. For the further discussion, only that conformation is considered in which the carbonyl oxygen atom is located close to the model receptor H atom; namely the (C21, O14) conformation.

The various potential energy contributions for the steroid-receptor model interactions are presented in Table I. Similar data are evaluated for both steroids. The values of the total interaction energy indicate the ability to form stable hydrogen bonds. Accordingly, an activity comparable to that of strophanthidin can be theoretically estimated considering the data given in [7].

However, this large binding energy could only be achieved by substantial changes in the steroid geometry induced by the receptor binding. For illustration, a computer-generated PLUTO [20] drawing of the oxepinone compound is shown in Fig. 2. It is interesting and highly important for a sufficient binding energy that all these steroid structural alterations only require an energy of about 10 kJ mol^{-1} as can be taken from Table I. This potential energy effort is mainly due to torsional angle deformations in the D-ring region. The most striking changes are determined in the torsional angles C13–C14–C15–C16 and C14–C15–C16–C17. For both free steroids, a 14β-envelope D-ring conformation is calculated as it is generally observed in cardiotonic steroids by X-ray crystallography [17]. The following

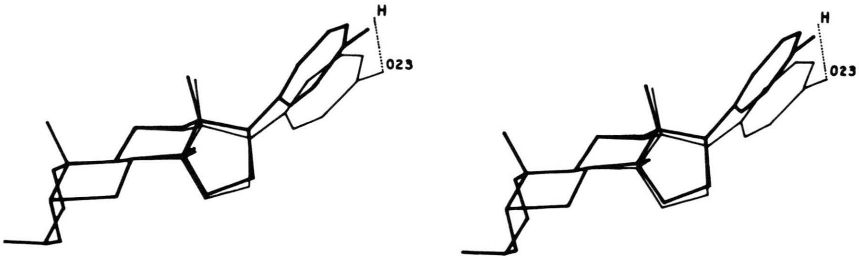

Fig. 2. Superposition of geometry-optimized oxepinone steroid molecules in stereoscopic view: free molecule (thick line), molecule in contact with model receptor H atom (thin solid line), hydrogen bond (dotted line).

Table I. Calculated energies in kJ · mol^{-1} for the interaction of steroid molecules with the model receptor hydrogen atom.

Energy contribution	Tropone compound	Oxepinone compound
Total interaction energy (U^{theor})	−20.8	−18.0
Energy due to hydrogen bond potentials (U_{hb})	−24.8	−25.6
Electrostatic energy due to monopole interactions (U_{el})	−9.3	−7.8
Van der Waals interaction energy (U_{nb})	+3.7	+5.7
Energy of changes in steroid geometry ($\triangle U_{\text{st}}$)	+9.6	+9.7

parameters describing the D-ring conformation are obtained by molecular mechanics: phase angle of pseudorotation [21] $\triangle = -39.7°$, asymmetry parameters [22]: $\triangle C_s(C14) = 1.8°$ and $\triangle C_2(C16) = 23.8°$. In case of the oxepinone steroid, the D-ring conformation is altered into an intermediate between 14β-envelope and 13α,14β-half chair when the steroid-receptor bond is formed ($\triangle = -16.7°$, $\triangle C_s(C14) = 8.3°$, $\triangle C_2(C16) = 9.3°$). Besides conformational changes, deformations of the C14−C15−C16 and C16−C17−C20 valence angles being slightly favourable in energy are computed as well.

As in every geometry optimization method, no information can be obtained if the geometry determined corresponds to the global or a local minimum of the potential-energy hypersurface. Thus, it is possible that other steroid-receptor complex arrangements with even more favourable interaction energies exist. But with regard to the crude receptor model applied, it could not be the aim of the present investigation to evaluate a highly sophisticated description of the receptor-drug complex structure which is, moreover, better imaginable in a mutual adaptation of both the steroid and the receptor. It should rather be demonstrated that conformational changes in the steroid molecule must be taken into account in considering the receptor binding and that structure-activity relationships should be better based on relaxed structures. That is, an "induced fit" approach for steroid structure is recommendable instead of "lock and key" models. In addition, the calculations show both steroids under study to be able to form such hydrogen bonds which suggest, from a theoretical point of view, a considerable activity in Na^+,K^+-ATPase inhibition.

The last finding can be supported by the application of other QSAR correlations. With regard to the model outlined by Fullerton *et al.* [1−3], relative carbonyl-oxygen distances to digitoxigenin of 126 pm and 124 pm are found for the free tropone and oxepinone steroid, respectively. According to the relationships presented by Shamovsky *et al.* [4], the analogous distances relative to bufalin are determined to be 108 pm and 105 pm. All these data are in the range of those for good Na^+,K^+-ATPase inhibitors.

In addition to the fact that the compounds under consideration are novel, possibly cardioactive steroids, a comparative experimental investigation of both substances could clarify the role of the ring oxygen in the receptor binding. According to the theoretical models applied, the influence of this oxygen atom is negligible. However, this is not yet experimentally verified for lactone-ring containing steroids.

[1] D. S. Fullerton, K. Yoshioka, D. C. Rohrer, A. H. L. From, and K. Ahmed, Science **205,** 917 (1979).

[2] D. C. Rohrer, D. S. Fullerton, K. Yoshioka, A. H. L. From, and K. Ahmed, Advances in Chemistry: Computer-Assisted Drug Design **112,** 259 (1979).

[3] D. S. Fullerton, E. Kitatsuji, T. Deffo, D. C. Rohrer, K. Ahmed, and A. H. L. From, Current Topics in Membranes and Transport **19,** 257 (1983).

[4] I. L. Shamovsky, G. M. Barenboim, and A. A. Ovchinnikov, Bioorg. Chim. **9,** 1112 (1983).

[5] F. Dittrich and K. R. H. Repke, in: Proceedings of the Conference on Chemical Structure-Biological Activity Relationships: Quantitative Approaches (M. Tichy, ed.), p. 249. Birkhäuser, Basel 1976.

[6] K. Köpke, F. Dittrich, and P. Berlin, Pharmazie **39,** 173 (1984).

[7] M. Bohl, K. Ponsold, and G. Reck, J. Steroid Biochem. **21,** 373 (1984).

[8] M. J. Barrow, O. S. Mills, and G. Filippini, J. Chem. Soc.: Chem. Comm. **1973,** 66.

[9] J. A. Pople and D. L. Beveridge, Approximate molecular orbital theory, McGraw-Hill, New York 1970.

[10] J. Reinhold and H. Zwanziger, Computer program CNDOCX, Karl Marx University, Leipzig 1973.

[11] N. C. Cohen, Tetrahedron **27,** 789 (1971).

[12] R. G. Kretschmer, Computer program GEMOS, Academy of Sciences of the GDR, Berlin 1984.

[13] R. Bucourt, N. C. Cohen, and G. Lemoine, Bull. Soc. Chim. France **3–4,** 903 (1975).

[14] J.-P. Schmit and G. G. Rousseau, J. Steroid Biochem. **8,** 921 (1977).

[15] C. Romers, C. Altona, H. J. C. Jacobs, and R. A. G. de Graaff, Terpenoids Steroids **4,** 531 (1974).

[16] R. F. McGuire, F. A. Momany, and H. A. Scheraga, J. Phys. Chem. **76,** 375 (1972).

[17] D. C. Rohrer, D. S. Fullerton, E. Kitatsuji, T. Nambara, and E. Yoshii, Acta Cryst. **B 38,** 1865 (1982).

[18] K. Go and G. Kartha, Acta Cryst. **B 36,** 3034 (1980).

[19] E. Höhne and D. Pfeiffer, studia biophys. **97,** 81 (1983).

[20] S. Motherwell and W. Clegg, Computer program PLUTO, University of Cambridge 1976.

[21] C. Altona, H. J. Geise, and C. Romers, Tetrahedron **24,** 13 (1968).

[22] W. L. Duax, C. M. Weeks, and D. C. Rohrer, Top. Stereochem. **9,** 271 (1976).