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MOLECULAR STRUCTURES OF SOME D-HOMO-6-OXA-8α ANALOGS OF STEROIDAL ESTROGENS

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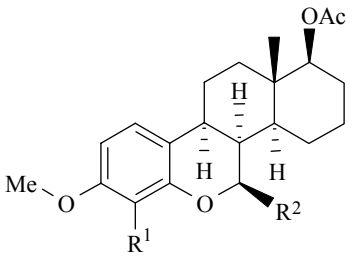
By X-ray crystallographic analysis and NMR spectroscopy it was demonstrated that the conformations of several D-homo-6-oxa-8α analogs of steroidal estrogens are similar in the crystal and in solution. The distances between the hydrogen atoms in these molecules, calculated by the ab initio and MM$^+$ methods, correspond to the experimental data.

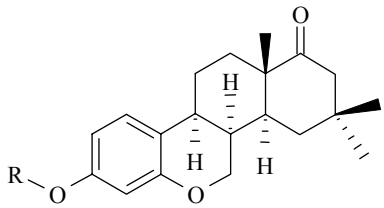
Keywords: steroidal estrogen analogs, molecular structure, molecular modeling.

At the present time intensive searches are being made for specific inhibitors of the enzymes responsible for the metabolism of steroidal hormones [1-7]. Such inhibitors are needed for the treatment of various illnesses caused both by imbalance in the hormone content of the organism and by oncological factors. An important condition for the selection of new steroids for detailed investigation is the absence of hormonal activity.

Steroid-like compounds bonded covalently to other types of biologically active substances could be used to transport the latter into the target organs and, in the case of peptidylsteroids, to protect them against destruction by the action of proteases [8-10]. The D-homo-6-oxa-8α analogs of steroidal estrogens **1-4** are promising for the synthesis of compounds with such properties [11, 12].



1–3



4a,b

1 $R^1 = R^2 = H$; **2** $R^1 = H$, $R^2 = Me$; **3** $R^1 = Me$, $R^2 = H$; **4 a** R = Me, **b** R = H

The biological activity of estrogen receptor modulators is determined to a significant degree by the structure of their complexes with the hormone receptors [13-15]. Ever greater attention is therefore being paid to the analysis of such complexes aimed at revealing criteria that make it possible to evaluate the biological characteristics of new ligands mediated by a given receptor during the planned selection of new substances for synthesis [16].

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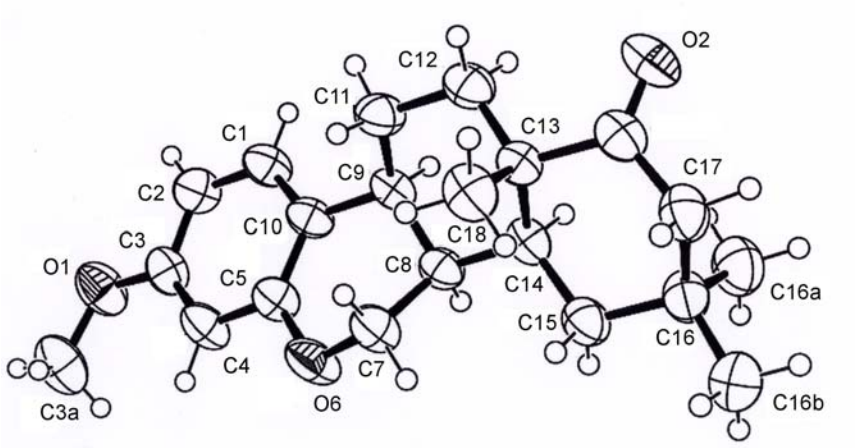

Fig. 1. The three-dimensional structure of the molecule of the steroid **4a** according to data from X-ray crystallographic analysis.

Since a number of computer programs can be used to model the bonding of proteins with ligands [17-20], it is expedient to use one or another method for calculating the conformation of the ligand with respect both to its docking in the hormone that bonds a section of the protein and to comparison of the conformations of the ligand in the solution and in the complex with the proteins. The first stage of the investigations involves comparison of the conformations of the ligands obtained experimentally and calculated by the various methods [21-24].

It seemed necessary to conduct such an investigation in the series of steroids **1**-**4a**. An X-ray crystallographic analysis of compounds **1** and **2** was carried out in [25] and of compound **3** in [26]. We undertook an X-ray crystallographic analysis of compound **4a**, the hydrogen atoms of which were located at the calculated positions (Fig. 1). Absorption was disregarded. The calculations were carried out with SHELX97 software [27].

The conformation of the steroid **4a** is similar to the conformation of the previously studied estrogens of the 6-oxa-8α series: Ring A is planar; ring B is a *distorted half-chair*, the base of which lies in the plane of ring A, while the C(7) and C(8) atoms are deflected from this plane by equal distances (0.41 Å) but to different sides (down for C(7) and up for C(8)). Rings C and D are almost regular *chairs* having *trans* coupling. Their bases C(8)–C(11)–C(12)–C(14) and C(13)–C(15)–C(16)–C(17a) form angles of 141.0 and 138.8° respectively with the plane of ring A. The carbon atom of the methoxy group at C(3) is deflected slightly from plane of ring A and occupies the *trans* position in relation to the C(2)–C(3) bond. The distance between the oxygen atoms at rings A and D (important for bonding with the estrogen receptors) amount to 10.508(5) Å, which is significantly less than for the molecule of the natural hormone estradione (10.93 Å) [17].

The distances between the protons in the steroids **1**-**4a** were calculated by the *ab initio* and unrestricted molecular mechanics (MM^+) methods, and those for compounds **1**-**3** in solution were obtained by NMR spectroscopy as proposed in [24] (Table 1).

It easy to see that the experimental data correspond satisfactorily to the calculated values. This makes it possible to recommend the unrestricted molecular mechanics MM^+ method for calculation of the structure of the ligand-receptor complexes in cases where it is necessary to determine the structure of steroids not having hormonal activity.

We will illustrate the results of modeling for the case of compound **4b**, since estrogens with a hydroxyl group at the C(3) atom must have greater affinity for the respective receptors than the analogs with a methoxyl group [28]. In the complex of an estradiol with an estrogen α-receptor constructed from the data from X-ray

TABLE 1. The Distances (*l*) between the Hydrogen Atoms in Steroids **1-3** and **4a***

Positions of H atoms	*l*, Å															
	1				**2**				**3**					**4a**		
	XCA	NMR	*ab initio*	MM^+	XCA	NMR	*ab initio*	MM^+	XCA Confor-mation 1	XCA Confor-mation 2	NMR	*ab initio*	MM^+	XCA	*ab initio*	MM^+
1–9α	2.60	2.60	2.57	2.56	2.73	2.71	2.70	2.68	2.53	2.52	2.50	2.59	2.55	2.47	2.60	2.51
1–11α	2.55	2.64	2.62	2.44	2.38	2.45	2.35	2.25	2.66	2.61	2.60	2.55	2.44	2.61	2.59	2.49
7α–8α	2.47		2.46	2.51	2.37	2.43	2.32	2.34	2.44	2.44	2.56	2.46	2.51	2.44	2.46	2.51
7α–15α	2.73		2.83	2.82	2.37	2.35	2.51	2.46	2.81	2.85		2.81	2.83	2.78	2.84	2.86
7α–15β	2.19		2.40	2.37	2.40		2.56	2.53	2.27	2.33		2.37	2.37	2.25	2.38	2.34
7β–11β	2.22		2.31	2.38	—	—	—	—	2.19	2.12	2.30	2.39	2.38	2.45	2.40	2.38
7β–15β	3.21		3.23	3.07	—	—	—	—	3.24	3.30		3.21	3.32	3.26	3.22	3.29
8α–H9α	2.41	2.42	2.37	2.37	2.37		2.27	2.26	2.45	2.47	2.40	2.35	2.37	2.42	2.35	2.37
8α–14α	2.24	2.35	2.32	2.31	1.97	2.14	2.21	2.19	2.21	2.27		2.30	2.31	2.24	2.31	2.33
8α–15α	2.41		2.49	2.49	2.43	2.60	2.55	2.57	2.36	2.39	2.38	2.49	2.49	2.37	2.48	2.47
9α–11α	2.39	2.60	2.45	2.47	2.40	2.56	2.43	2.43	2.41	2.44	2.58	2.45	2.47	2.50	2.43	2.46
9α–12α	2.57	2.54	2.56	2.59	2.67	2.50	2.49	2.56	2.51	2.53	2.52	2.58	2.59	2.57	2.61	2.61
9α–14α	2.58	2.56	2.52	2.55	2.53		2.59	2.67	2.44	2.48	2.62	2.54	2.55	2.52	2.50	2.52
11α–12α	2.46		2.46	2.45	2.47	2.62	2.46	2.45	2.43	2.45	2.55	2.44	2.45	2.36	2.45	2.45
12α–17aα	2.36	2.39	2.34	2.36	2.33	2.43	2.36	2.39	2.31	2.36		2.33	2.35	—	—	—
14α–16α	2.52		2.48	2.49	2.45		2.47	2.48	2.55	2.52	2.42	2.52	2.49	—	—	—
14α–17aα	2.18	2.30	2.33	2.35	2.15	2.21	2.27	2.30	2.23	2.21		2.30	2.34	—	—	—
15α–16α	2.43		2.44	2.46	2.36		2.43	2.45	2.43	2.43	2.25	2.42	2.46	—	—	—

* Distance between atoms O(3)–O(17), Å: **1** 10.76 (XCA), 10.64 (*ab initio*), 10.89 (MM^+); **2** 10.89 (XCA), 10.75 (*ab initio*), 10.68 (MM^+); **3** 10.72 (PCA, conf. 1), 10.70 (XCA, conf. 2), 10.72 (*ab initio*), 10.64 (MM^+); **4a** 10.51 (XCA), 10.57 (*ab initio*), 10.49 (MM^+).

crystallographic analysis [29] the analog **4b** was docked manually instead of the natural hormone. It was found that the structure of this compound was poorly compatible with the geometry of the ligand-binding pocket of the receptor. The presence of methyl groups at the C(16) atom forces the ligand to occupy an energetically unfavorable position in the complex with the protein, excluding the formation of hydrogen bonds between the NH group of the His524 and the keto group at the C(17a) atom, which must appreciably reduce the affinity of the steroid for the receptor. In addition, unfavorable interactions arise between the H-4 and H-7α atoms and Met388 and the H-11α and H-11β atoms of the steroid and the methyl group of the Ala350 of the receptor (distance between them 1.9 and 2.2 Å respectively).

The distance between the hydroxyl group at the C(3) atom and the oxygen of the carboxyl group of Glu353 according to the calculated data ~3.8 Å is much greater than in the complex with the natural hormone estradiol (2.4 Å) [28]. Eventually analysis the calculated value of the enthalpy of formation of the ligand in the complex with the receptor is approximately 10 kcal/mole higher than in the free state. It is clear that the given steroid should hardly have any uterotropic activity at all. Even greater differences are found in the enthalpy of formation in the complex with the α-receptor of estrogens and in the free state in the antipode of compound **4b**.

In conclusion it should be mentioned that specific estrogen receptor modulators (e.g., raloxifene) can have serious side effects characteristic of natural hormones [30]. Moreover, it was found that prolonged administration of raloxifene to women in the menopause leads to increase in the content of estradiol and sex-hormone binding globulin in the blood, indicating an increased risk of invasive cancer of the breast [31]. Therefore, compounds of types **2**, **3**, and **4a** not having hormonal characteristics seem more promising for the creation of inhibitors of the metabolism of steroidal hormones and carriers of other classes of biologically active substances into estrogen target organs.

EXPERIMENTAL

Crystals of the steroid **4a** suitable for investigation by X-ray crystallographic analysis were grown from hexane and took the form of colorless tablets. They belong to the triclinic system: space group $P1$, $a = 6.146(2)$, $b = 8.733(2)$, $c = 13.005(2)$ Å, $\alpha = 88.960(5)°$, $\beta = 83.070(5)°$, $\gamma = 70.130(5)°$, $Z = 2$, $D_x = 1.225$ g/cm^3. The structure was interpreted by the direct method and refined to $R = 0.035$ with allowance for the anisotropy of the thermal vibrations of the non-hydrogen atoms using 5182 nonzero unique reflections collected on a SMART CCD autodiffractometer.

The crystal chemical information about this steroid has been deposited in the Cambridge structural data base (CCDC 652864).

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