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Dedicated to Professor Dr. Cozar Onuc on His 70th Anniversary

CONFORMATIONAL LANDSCAPE AND UV-VIS SPECTRUM OF S-BISOPROLOL

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ABSTRACT. The conformational changes of bisoprolol **(BISO)** are crucial for understanding its interaction with the receptor and the mechanism of action. Therefore, here we investigated the free energy conformational landscape of the free bisoprolol base, aiming at describing the 3D structures and energetic stability of its conformers. Twenty-three unique conformers, within an energy window of 2.44 kcal·mol^{-1} were identified by conformational search in gas-phase, at B3LYP/6-31+G theoretical level of theory. Among these, the 10 most stable were further refined in water at the same level of theory. The most stable conformers in gas-phase exhibit an S-shape structure. The most stable conformer was used to compute the absorption spectrum of bisoprolol.

Keywords: *bisoprolol; conformational landscape.*

1. INTRODUCTION

Bisoprolol with the IUPAC name (RS)-1-{4-[(2-isopropoxyethoxy)methyl] phenoxy}-3-(isopropylamino)propan-2-ol (BISO, see Fig. 1), is a cardio-selective beta1-adrenergic blocking agent, mainly used in the treatment of hypertension and heart failure [1-2]. Bisoprolol is a second generation agent, having higher beta-1 selectivity with respect to first generation agents like propranolol and timolol (that are non-selective to beta-1 and beta-2 adrenergic receptors) and to other second generations agents as metoprolol, atenolol and betaxolol [3-4]. The beta-blocker agents are usually administrated as racemic mixtures of S- and R-enantiomers and the affinity of these enantiomers to beta-1 and beta-2 adrenoceptors can be markedly different [5-7]. Bisoprolol is also provided as a racemic mixture where the S-enantiomer is

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responsible for most of the beta-1 blocking activity [8-9]. Due to higher beta-1 selectivity, [3H]-bisoprolol was used as radioligand to count the density of beta-1 adrenoceptors in different organs, especially in heart [9]. Having different affinities to beta-1 and beta-2 adrenoreceptors, [^{11}C]S-bisoprolol and its mirror form were used as radioligands in lung, heart and brain PET studies [10].

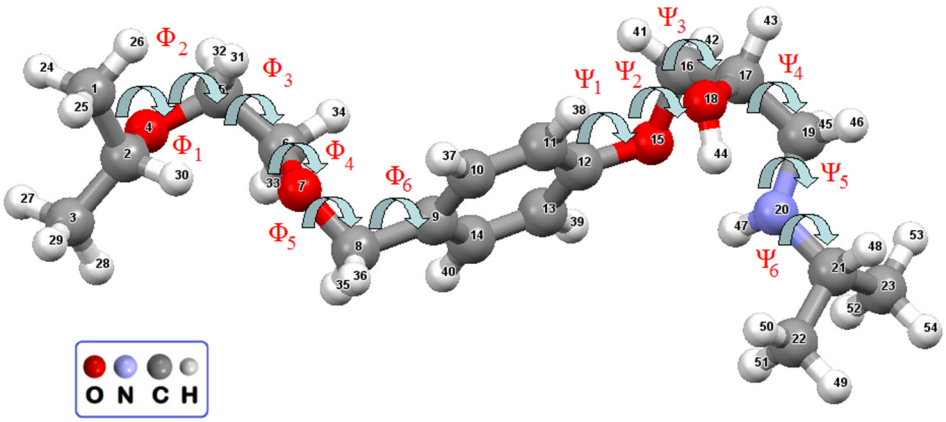


Fig. 1. Optimized molecular structures of the most stable conformer of S-bisoprolol in gas-phase at B3LYP/6-31+G level of theory, with the atom numbering scheme and the twelve dihedral angles defining the possible conformers.

The UV-Vis spectrum of bisoprolol in water and hydrochloric acid solution was obtained in work [11], in methanol with water [12] and with other drugs [13-14]. The absorption spectrum is influenced by Boltzmann populations of different conformers of bisoprolol. In this work we study the conformers in gas-phase and water and use the most stable conformer in water to compute the absorption spectrum of bisoprolol.

2. COMPUTATIONAL DETAILS

The conformational space of S-bisoprolol was initially explored with the Tinker software, using MMFF94 molecular mechanics force field [15] that has been developed based on quantum mechanics methods with the specific aims of being used in pharmaceutical science for predicting molecular geometries, conformational energies and energetic of drug-receptor interactions [16-19].

A systematic conformation search was performed using the MMFF94 molecular mechanics force field via Tinker software (convergence criterion was chosen to the default value of 10^{-4} kcal·mol^{-1}·Å^{-1}). This way, we identified 2x136x737=200464 conformations of BISO (136 conformers for fragment A and 737 conformers for fragment B within an energy window of 64 kcal·mol^{-1}, see fig. 1). The factor of two appears because there are two ways of gluing together fragments A and B to build the bisoprolol molecule. The fragment A contains the atoms 1-14 (see fig.1), while fragment B contains the atoms 9-23. They have in common the benzene group containing the atoms 9-14.

For the next part of the study we used the hybrid B3LYP exchange-correlation functional [20-23] in conjunction with Pople's 6-31+G basis set [24]. From the first most stable 23 unique conformers, whose relative free energies in gas-phase are lower than 2.44 kcal·mol^{-1} were selected the first 10 conformers and re-optimized in water at B3LYP/6-31+G level of theory. In Tables 1 and Fig. 2 are presented these first 10 most stable conformers whose relative free energies are less or comparable with the room temperature energy (0.592 kcal·mol^{-1}).

Frequency calculations confirmed that all the optimized geometries correspond to minima on the potential energy surface. The optimization of bisoprolol geometry and calculations of vibrational frequencies were performed with the Gaussian09 software package [25]. Boltzmann weighting factors for each conformer are derived at room temperature (T=298 K) by using the relative free energies (ΔG). The latter values are obtained from the frequency calculations including thermal corrections to energies [26]. Figures representing the structures of BISO have been created using the Mercury 3.3 [27] and Molegro Molecular Viewer [28] program packages.

3. RESULTS AND DISCUSSION

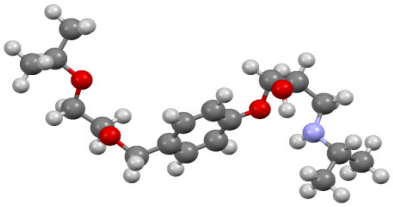
3.1. Conformation landscape

Bisoprolol conformers can be generated by varying the torsion angle around twelve rotatable bonds (see Fig. 1): C2-O4, O4-C5, C5-C6, C6-O7, O7-C8, C8-C9 and C12-O15, O15-C16, C16-C17, C17-C19, C19-N20, N20-C21. Table 1 summarizes the dihedral angles Φ_1-Φ_6 and Ψ_1- Ψ_6 that characterize the ten most stable conformers whose structures are shown in Fig. 2. Their names, relative Gibbs energies and populations are summarized in Table 2. Besides the ten most stable conformers, Fig. 2 includes also the optimized conformations of S- and R-bisoprolol obtained by using as starting geometries these ligands coupled with the beta-1 receptor ligand pocket structures found in two states (2VT4) [31] and (2Y03) [34].

Table 1. B3LYP/6-31+G calculated dihedral angles (degrees) characterizing the ten most stable *S*-bisoprolol conformers in water

Conformer	Φ_1	Φ_2	Φ_3	Φ_4	Φ_5	Φ_6
A1-B1-S (1)	76.4	177.8	74.4	175.2	69.1	68.7
A1’-B1-S (2)	-77.5	-176.9	-76.1	-179.5	-70.6	-69.7
A2-B1-S (3)	156.8	-92.7	73.6	-175.4	-67.9	-66.8
A3’-B1-S-(4)	-74.8	-174.0	-73.6	88.7	71.5	65.3
A2’-B1-S (5)	-157.3	92.8	-72.4	176.9	69.1	68.8
A3-B1-S (6)	76.4	174.9	74.9	-86.8	-72.1	-68.3
A2-B1-U (7)	156.7	-93.6	72.8	-174.4	-68.4	--69.9
A2-B2-S (8)	156.3	-93.2	72.3	-174.6	-69.1	-70.4
A4-B1-S (9)	74.2	172.8	-72.0	90.3	71.3	65.8
A3-B2-S (10)	75.2	174.1	74.0	-85.8	-71.9	-71.1
	Ψ_1	Ψ_2	Ψ_3	Ψ_4	Ψ_5	Ψ_6
A1-B1-S (1)	-26.4	105.4	47.8	-84.8	-152.6	170.7
A1’-B1-S (2)	-26.8	106.6	48.1	-85.0	-152.4	170.2
A2-B1-S (3)	-24.1	105.5	47.9	-84.9	-153.0	170.5
A3’-B1-S-(4)	-29.0	106.8	48.1	-85.2	-151.6	170.5
A2’-B1-S (5)	-26.7	106.3	48.0	-85.1	-152.5	170.2
A3-B1-S (6)	-26.3	106.1	48.4	-84.9	-153.7	170.4
A2-B1-U (7)	153.9	106.0	47.9	-85.5	-151.6	170.0
A2-B2-S (8)	-24.7	105.4	48.4	-84.1	-156.3	76.4
A4-B1-S (9)	-28.8	107.2	48.5	-85.3	-152.3	170.4
A3-B2-S (10)	-27.6	106.3	48.5	-83.7	-156.5	76.6

(1)



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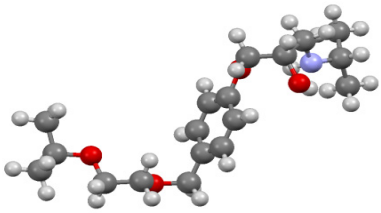

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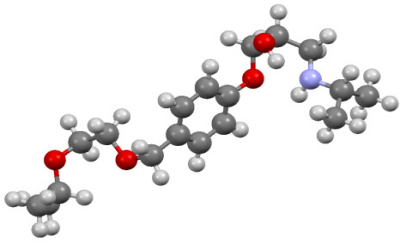

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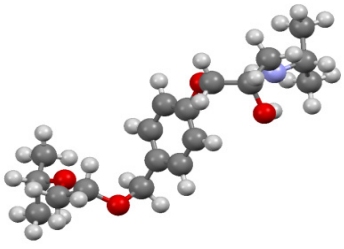

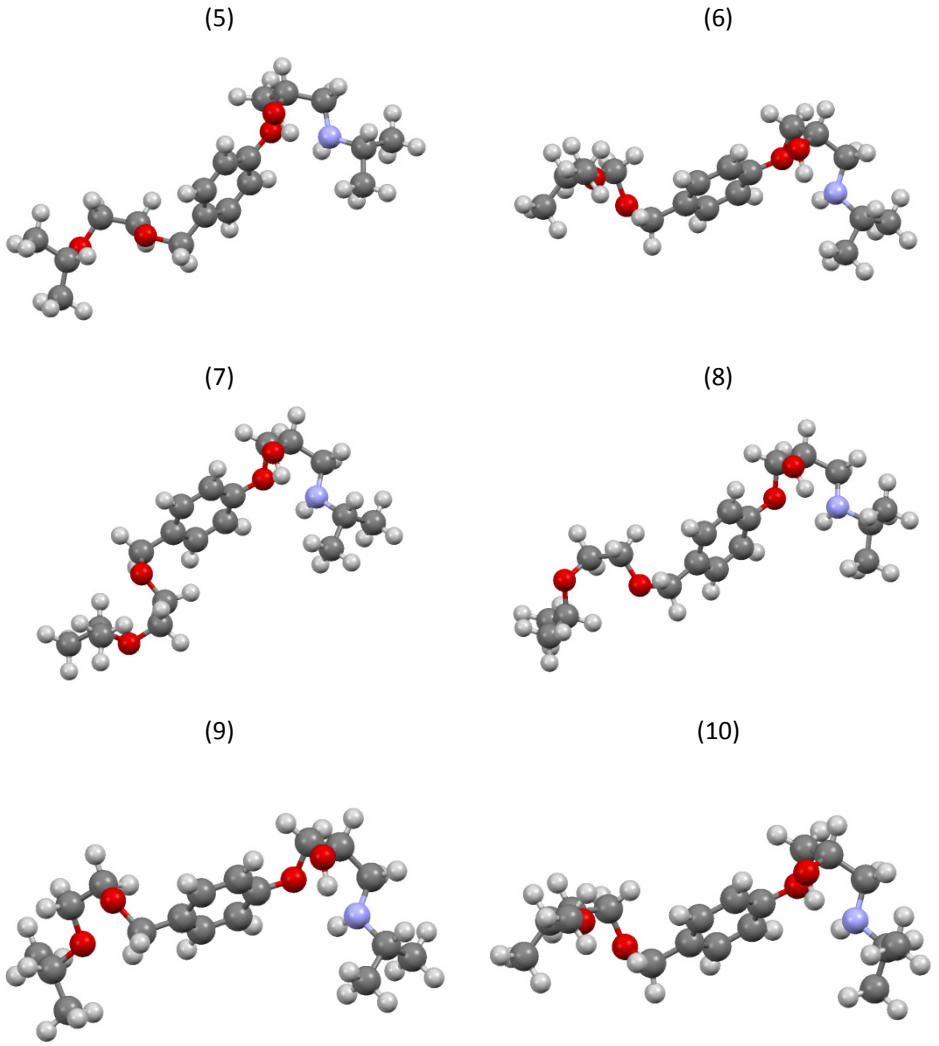

Fig. 2. B3LYP/6-31+G optimized structures of the ten most stable S-bisoprolol conformers in water.

It is important to note here that for bisoprolol's conformers there are two kind of structural transformations that do not affect their energies: the first one is related to the rotation of benzene ring along the C9-C12 axis, while keeping unchanged all the other dihedrals. Such transformations involve changes of the dihedrals Φ_6 (O7-C8-C9-C10) $\rightarrow$ O7-C8-C9-C14 and Ψ_1 (C11-C12-O15-C16) $\rightarrow$ C13-C12-O15-C16.

Another transformation that changes the structure, but not the energy, is the mirror symmetry related to transformation of dihedral angle $\Phi_6 \rightarrow 180 - \Phi_6$ and $\Psi_1 \rightarrow 180 - \Psi_1$ and the change of sign of the other five dihedral angles Φ and Ψ. To avoid counting the symmetry-related conformations, only those conformers are considered in Table 2 that have the sixth dihedral angle Φ_6 between -70° and 70° and the first dihedral angle Ψ_1 negative.

Table 2. Relative free energies and Boltzmann populations of the ten most stable conformers of S-bisoprolol in gas-phase and water (B3LYP/6-31+G level of theory) at room temperature

Conformer	Gas-phase B3LYP/6-31+G		Water B3LYP/6-31+G	
	ΔG (kcal·mol^{-1})	Population (%)	ΔG (kcal·mol^{-1})	Population (%)
A1-B1-S (1)	0.45	8.13	0.00	33.29
A1'-B1-S (2)	0.51	7.42	0.27	21.07
A2-B1-S (3)	0.01	17.08	0.68	10.73
A3'-B1-S-(4)	0.37	9.41	0.86	7.94
A2'-B1-S (5)	0.00	17.35	1.05	5.77
A3-B1-S (6)	0.19	12.53	1.10	5.26
A2-B1-U (7)	0.60	6.35	1.11	5.17
A2-B2-S (8)	0.48	7.81	1.17	4.68
A4-B1-S (9)	0.46	8.05	1.36	3.43
A3-B2-S (10)	0.65	5.87	1.51	2.67

The conformational analysis shows that for each dihedral angle there are three different conformers (when the third atom involved in dihedral angle is carbon) and two different conformers (when the third atom involved in dihedral angle is oxygen or nitrogen). The number of different conformers of fragment A is $2^2 \cdot 3^4 = 324$, the theoretical maximum value being larger than 136, the value obtained with Tinker software and MMFF94 force field, due to steric interactions. The number of different conformers of fragment B is $2^4 \cdot 3^4 = 1296$, where the number of conformers is doubled two times, one time due to the chiral center and the second time due to two orientations of hydroxyl group. The theoretical maximum value for fragment B is larger than 737, the value obtained with Tinker software and MMFF94 force field, due to steric interactions. The fragments A and B have benzene group in common that act as space separator and the conformers with low energy of the two fragments are practically independent in bisoprolol. The fragments A and B can be glued together in bisoprolol on the same side (U-like shape) or on different sides (S-like shape) relative to benzene plane giving a total number 2x136x737=200464 of conformations of bisoprolol molecule.

In Table 1 it can be seen that the ten most stable conformers contain only two unique fragments B and seven unique fragments A with minor changes of dihedral angles that glue together in nine S-like shapes and one U-like shape. The fragments B1 and B2 differ only in the sixth dihedral angle Ψ_6, but for fragments A is a greater variety with all dihedral angles being different. However this variety is only apparent, because the pairs A1 and A1', A2 and A2' and also fragments A3 and A3' are mirror forms with respect to a plane perpendicular to benzene plane. The energy difference between conformers **(3)** and **(5)** having fragments A2 and A2' respectively is only 0.01 kcal·mol^{-1} in gas-phase, but is a significant 0.37 kcal·mol^{-1} in water (see Table 2). The conformers **(6)** and **(9)** having fragments A3 and A3' have large energy differences in gas-phase as well as in water.

The most stable conformer of S-bisoprolol in gas-phase is conformer **(5)** and in water is conformer **(1)** (see Table 2). The energy difference 0.43 kcal·mol^{-1} of conformer **(3)** and **(7)** is determined by the different gluing of fragments A2 and B1 in S-shape and U-shape respectively.

3.2. UV-VIS spectrum of bisoprolol

The experimental absorption spectrum of bisoprolol fumarate in water [11] (see Fig. 3) is characterized by absorption maxima at wavelengths of 229 nm, 271 nm and a shoulder at 276 nm. The simulated spectrum of S-bisoprolol monomer (see Fig. 3) presents an absorption maximum at wavelength of 229.1 nm. The simulated spectrum of fumaric acid alone presents an absorption maximum at wavelength of 231.5 nm and has the same intensity with that bisoprolol monomer. The absorption of fumaric acid is obscured by bisoprolol due to the stochiometry of 2:1 in bisoprolol fumarate and cannot be responsible for the peak at wavelength of 271 nm. In Table 3 it is observed another mild absorption band of bisoprolol monomer at wavelength of 249.4 nm. This band could be enhanced and shifted to larger wavelengths by the interaction of bisoprolol monomer with fumaric acid as happens with other salts dissolved in water. In work [11] was obtained also the absorption spectrum for bisoprolol in hydrocloric acid solution indicating a decrease in the intensity of peak at wavelength of 229 nm, but not of that at 271 nm. The ionization state of the weak fumaric acid is influenced by modification of pH having two acid dissociation constants of 3.03 and 4.44. The increase of pH decreases the amount of fumaric acid and the intensity of peak at 229 nm. The fact that the peak at wavelength of 271 nm is not influenced by modification of pH indicates that it is not due to interaction bisoprolol-fumaric acid, another possibility being the formation of bisoprolol dimmers. We conclude that the absorption maximum at wavelength of 271 nm might be produced by the absorption band at 249.4 nm of bisoprolol molecule that is shifted and enhanced by the interaction with another bisoprolol monomer.

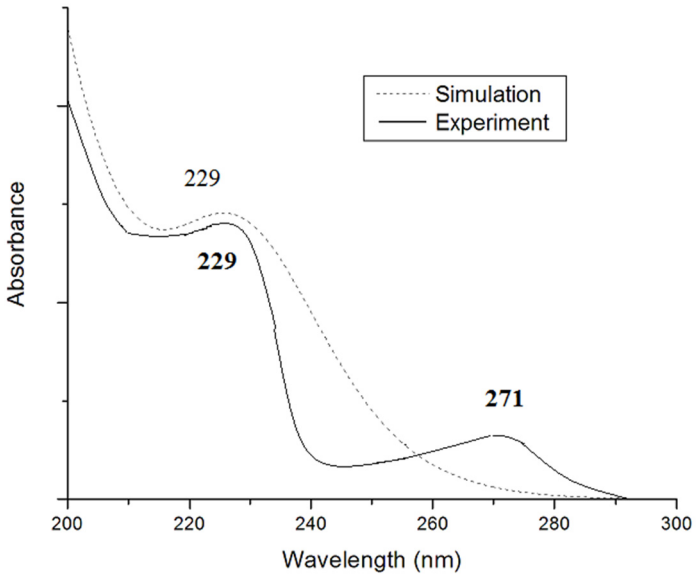

Fig. 3. The experimental absorption spectrum ob bisoprolol fumarate [11] and simulated absorption spectrum of the most stable conformer of S-bisoprolol, at B3LYP/**6-31+G** level of theory, in water at room temperature.

Table 3. TD-DFT B3LYP/6-31+G calculated electronic transitions of the most stable conformer (1) of bisoprolol in water at room temperature

λ(nm)	f[a)]	Transitions	Contributions %
249.4	0.0262	H→L	54.34
		H→L+1	22.19
230.1	0.0557	H-1→L+1	78.78
229.1	0.2373	H→L+1	39.15
		H→L	20.37
218.8	0.0251	H→L+2	69.79
214.0	0.0274	H-5→L+2	39.07
		H-2→L+1	34.38
206.9	0.0292	H-2→L+1	26.89
		H-3→L	21.61
203.8	0.0552	H→L+4	75.42
201.1	0.0341	H→L+5	30.21
		H→L+6	25.81

[a)]only transitions with f>0.025 are included

In Table 3 are shown the simulated absorption bands of the most stable conformer (1) of bisoprolol with the main band around 229 nm, due mainly to HOMO → LUMO + 1 transition and HOMO→LUMO transition. The experimental spectrum (see Fig. 3) shows the increase of absorbance for wavelengths near 200 nm indicating the presence of another important peak for wavelengths lower than 200 nm. Indeed, the simulated absorption spectrum indicates the existence of a peak at wavelength of 186 nm (not listed in Table 3) with the intensity three times larger than that of the peak at wavelength of 229 nm.

4. CONCLUSIONS

Using molecular mechanics and DFT quantum chemistry methods we characterized the ten most stable conformers of S-BISO with relative free energies in water within 1.51 kcal·mol^{-1}. The simulated absorption peak of the most stable conformer of S-bisoprolol presents a peak at wavelength of 229 nm in good agreement with the experimental data [11]. However the peak at wavelength of 271 nm requires a further investigation and most probable is determined by bisoprolol dimmers.

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