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A QUANTUM CHEMICAL STUDY ON LEVOMYCETIN INTERACTION WITH SILICA SURFACE

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The biological activity of D(-)-threo-isomer of levomycetin is connected with presence of hydrogen bonding between hydroxyl groups of its alicyclic form. When grafted on silica surface (via impregnation), levomycetin increases its antimicrobial capability. Adsorption-induced changes in the structure and energy parameters of levomycetin molecules probably responsible of its activity have been studied by means of quantum chemistry. It has been found that descriptors of levomycetin bioactivity can be values of the LUMO energy and O-H…O hydrogen bond length within the propanediol fragment of the molecule.

INTRODUCTION

High disperse silica can act as a pronounced detoxicant and promotes bioactivity of a row of remedies [1], so their adsorption grafting on silica surface open a way to create a new generation of composite antibiotic-resistent medical products [2].

The effect was studied in [3] of a silica-levomycetin composite on development of *E. coli* cells and it was shown that in the solution containing levomycetin grafted on silica surface the *E. coli* cells number was 3.75 times lesser than that in the solution of pure antibiotic of the same concentration.

Levomycetin is an antibiotic of a wide spectrum of action concerning both gram-positive and gram-negative microorganisms. Its molecule (LV) (Fig. 1) contains *n*-nitrophenyl radical (I), propanediol group (II), and dichloroacetamide group (III).

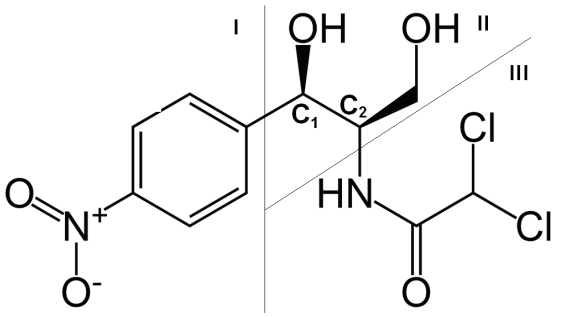

Fig. 1. Structural formula of the bioactive form of levomycetin (*I* – *n*-nitrophenyl radical, *II* – propanediol group, *III* – dichloroacetamide group)

n-Nitrophenyl radical (Fig. 1, *I*) has a considerable effect on the antibiotic activity both due to its electronic nature and strong polarization of the propanediol group, geometric size of this part of the molecule having no key significance. When the nitrogroup of LV molecule is substituted for by less electronegative radicals (CN, Cl, H, CH_3 and so on), an essential decrease in bioactivity of such forms or even its loss takes place [4].

The propanediol part of LV molecule (its has a *D*(-)-*threo*-configuration at C_1 and C_2 carbon atoms) play an essential role in the specific interaction between LV and target (Fig. 1, *II*). This configuration secures a close stationing of hydroxyl groups with hydrogen bonds between them what, according to [5–7], conditions formation of a bioactive closed alicyclic configuration of LV molecule. Such a configuration is not realized for other stereoisomers [4]. The loss of dichloroacetamide fragment of the molecule results in the complete loss in its bioactivity [7].

Thus, the propanediol and dichloroacetamide fragments of LV molecule form active sites providing addition of this molecule to specific sites of some bacterial proteins so exclusing the latter from the normal exchange of microbial cells. According to [4–7], the antibiotic action of LV is controlled by three factors: (1) strong acceptor capability of *n*-nitrophenyl radical; (2) strongly defined geometry size and relative conformation of the propanediol chain; (3) strong polarizing effect of the dichloroacetamide group which

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should simultaneously satisfy definite geometry conditions. The superposition of these effects causes a strong interaction between antibiotic molecule and specific peptide groups of some ensimes what results in the dysbolism in microorganisms.

Unfortunately, there are few articles in the literature concerning studies on the effect of the spatial and electronic structures of levomycetin molecules as well as of their energy characteristics on their antimicrobial activity. As the elucidation of interrelations between structural characteristics, bioactivity, and electron density redistribution due to changes in the electronegativity of substituent of the functional groups of free and adsorbed LV molecules is rather complicated experimentally, quantum chemical studies on these systems are rather relevant.

OBJECTS AND METHODS

The object under study was levomycetin. It is an antibiotic of a wide action spectrum concerning both gram-positive and gram-negative microorganisms. Levomycetin has four spatial isomers but only *D(-)-threo*-isomer in closed alicyclic form has an antibacterial activity. The rest *L(-)-threo*-, *D(-)-erythro*-, and *L(-)-erythro*-isomers have activities of 0.4, 0.4, and 1 to 2% respectively of that of *D(-)-threo*-isomer [5–7]. According to the level of effect on the antimicrobial activity, functional derivatives with substituted nitrogroup can be arranged into a row where their positions are qualitatively relevant to decrease in their electronegativities [4]

$$NO_2>CN>CO_2Me>Cl(Br)>SO_2Me>SO_2NH_2>H>Me.$$

Thus, the bioactivities of *D(-)-threo*-R-isomers (R – functional groups –CN, –Cl, and –H) are of 95, 20, and 0% respectively of that of *D(-)-threo*-nitro-isomer.

In order to clarify the effect of adsorption on the antibiotic properties, quantum chemical calculations have been carried out on spatial and electronic structure as well as on thermodynamic characteristics of levomycetin $C_{11}H_{12}Cl_2N_2O_5$ molecules and its adsorption complexes at the silica–water interface. All the calculations have been carried out by means of PC GAMESS program package (version 7.1 F) within *ab initio* Hatree-Fock method and density functional theory method (B3LYP) with basis set 3-21G**. Separated results were checked with basis set 6-31G**. The models for silica surface were polysilicate acids clusters containing 4, 8, and 13 silicon-oxygen tetrahedra. Taking into account the result obtained experimentally that the LV amount adsorbed on silica surface from alcohol is more than that adsorbed from aqueous medium [3], we also have carried out calculations on the adsorption energy of the complexes of LV molecule–solvent–silica (5 alcohol molecules and/or 15 water molecules) stationed between LV molecule and silica surface. The direct contact has been also examined between levomycetin molecule and silica surface.

RESULTS AND DISCUSSION

Quantum chemical calculations have been carried out on equilibrium spatial structure of four LV isomers (*D(-)-threo, L(-)-threo, D(-)-erythro, D(-)-erythro*). The alicyclic form of *D(-)-threo*-isomer (Fig. 2) appears to be the most stable one where a hydrogen bond is formed between hydroxyl groups of aminopropanediol group (Fig. 1, *II)*. The hydrogen bond length d(OH…O) between hydroxyl groups is of 1.694 Å as compared by density functional theory method (B3LYP, 3-21G**). When nitrogroup in *D(-)-threo*-isomer is substituted for groups –CN, –Cl, –H, or $–CH_3$, this value increases and equals to 1.696, 1.700, 1.706, and 1.707 Å, respectively (see Table) what testifies an effect of the substituent electronegativity on the electronic structure of the models of levomycetin derivatives. The same dependence in d(OH…O) calculations has been found in case of use of DFT method (B3LYP, 6-31G**) with the correlation coefficient between d(3-21G**) and d(6-31G**) values of 0.998. Analogous correlations have been obtained due to comparison of the energies of the frontier orbitals (HOMO and LUMO) found with these basis sets. This fact gives us a reason, in order to reduce the computing time, to use the lesser basis set for studies of the models including large silica clusters.

Characterizing frontier molecular orbitals (HOMO, LUMO) gives us an opportunity to forecast the probable reaction route involving levomycetin and to determine the sites of electrophilic and nucleophilic attacks. Thus, an electrophil should attack the LV atoms with orbitals paying the maximum income into the HOMO whereas the site of the most probable nucleophil attack should be the LV atoms with orbitals paying the maximum income into the

LUMO. A graphic image is presented in Fig. 3 of the frontier orbitals of the *D(-)-threo*-NO_2-isomer of LV molecule in the alicyclic form along with those of relative *D(-)-threo*-R-isomers (R= –CN, –Cl, –H, $–CH_3$).

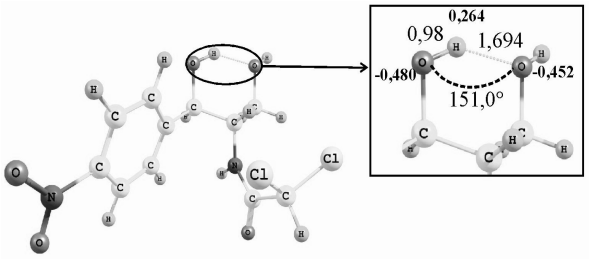

Fig. 2. Equilibrium spatial structure of the alicyclic form of D(-)-threo-isomer of LV molecule as calculated by density functional theory method (B3LYP/3-21G**)

The results of calculations testify that the HOMO is practically equally localized at the atoms of aromatic nucleus (Fig. 1, *I*) as independent on the nature of the substituent functional groups what can point out the absence of the effect of definite group on the specific interaction with a biological target. Nevertheless it should be taken into consideration that for levomycetins with the great biological activity (R = NO_2 or CN, see Table) the HOMO is localized also at the oxygen and nitrogen atoms of dichloroacetamide group (Fig. 1, *III*). At the same time, the oxygen atom of carbonyl group of *D-(-)threo*-NO_2-LV has the largest value of LCAO coefficient in the HOMO (Fig. 3). This fact can testify that the biological activity of levomycetin is conditioned by specific bonding of these atoms with electrophilic areas of biological objects.

It is seen also from Fig. 3 that in biologically active molecules of *D(-)-threo*-NO_2-isomer and its analogue with CN-group, the LUMO is localized at *n*-NO_2- and *n*-CN-aromatic fragments whereas in the rest (biologically inert) molecules with –Cl, –H, and $–CH_3$ groups, the LUMO is localized at dichloroacetamide fragment. So, levomycetin molecule can realize bonding with nucleophilic areas of biological systems via aromatic system. The greater LUMO energy, the lesser both electron acceptor capability of levomycetin analogues and their biological activities (see Table). At the same time, the lesser LUMO energy, the greater LCAO coefficients at the aromatic system of relative levomycetin. So, the LUMO energy can be an electron descriptor of levomycetin molecular structure and biological activity [8].

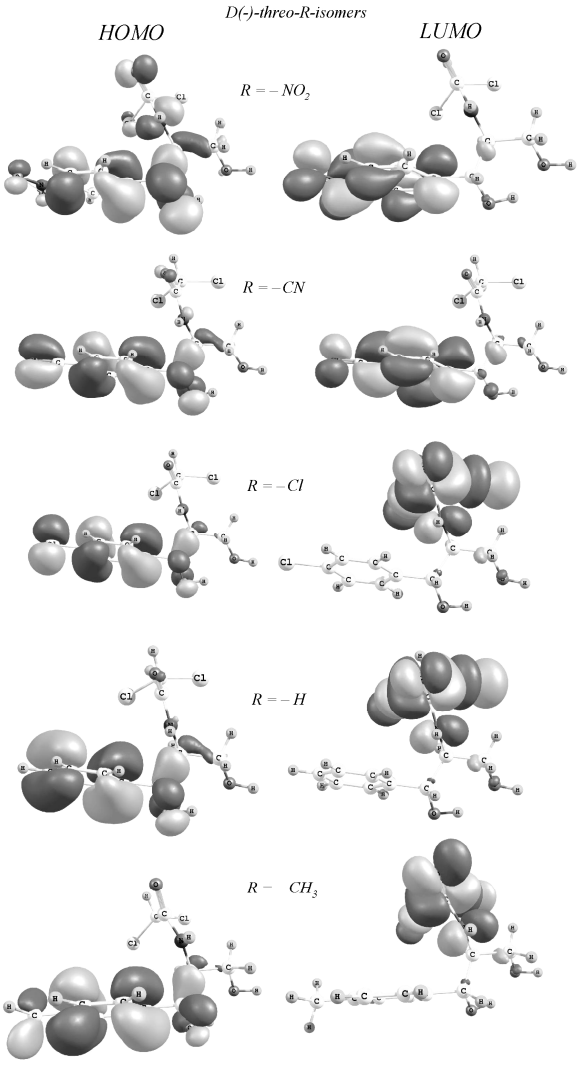

Fig. 3. Graphic image of the HOMO and LUMO of the molecules of *D(-)-threo*-R-isomers of levomycetin (R = – NO_2, –CN,–Cl, –H, $–CH_3$ B3LYP/6-31G**)

An analysis of the values of hydrogen bond lengths in the propanediol fragment of LV molecule (Fig. 1, *II*) with substituted functional group (see Table) performs a clear correlation with the biological activity

biological activity = -8203*(d) +13992,
r=0.945, n=5. (*)

It is remarkable that even little increase in hydrogen bond length results in the practical loss of the biological activity of LV.

The results of calculations on the interaction of isolated hydroxyl group of the tetrahedral cluster containing 4 silicon-oxygen tetrahedra, with groups $–NO_2$, –C=O, –NH, –OH of the alicyclic form of *D(-)-threo*-isomer of levomycetin molecule have shown that the lowest energy relates to the

Table. Calculated data for isolated and adsorbed levomycetin molecule on silica surface

Complex	HOMO energies, eV		LUMO enrgies, eV		d – hydrogen bond length between –OH groups, Å		Bioactivity of compounds, %, (*E.coli* being an example) [6]
	6-31G**	3-21G**	6-31G**	3-21G**	6-31G**	3-21G**	
Isolated LV molecule							
LV D(-)-*threo* (NO_2) (alicyclic form)	-7.246	-7.012	-2.324	-2.54	1.815	1.694	100
LV D(-)-*threo* (CN)	-6.980	-6.838	-1.385	-1.197	1.818	1.696	95
LV D(-)-*threo* (Cl)	-6.525	-6.482	-0.999	-1.039	1.830	1.700	20
LV D(-)-*threo* (H)	-6.523	-6.425	-0.884	-0.920	1.835	1.706	0
LV D(-)-*threo* (CH_3)	-6.275	-6.212	-0.857	-0.893	1.838	1.707	0
Adsorption complexes							
LV D(-)-*threo*-(NO_2) + silica (Fig. 4*b*)		-7.532		-3.494		1.673	375 [7] 270 (calculated)
LV D(-)-*threo*-(NO_2) + silica (Fig. 4*a*)		-6.768		-3.453		1.700	~20 (calculated)

structure including two hydrogen bonds between the oxygen atom of hydroxyl group and the hydrogen atom of the NH group of levomycetine molecule along with that between the hydrogen atom of hydroxyl group and the oxygen atom of levomycetin carbonyl group.

The adsorption complex built of the alicyclic form of *D(-)-threo*-isomer of LV molecule and of the model of silica surface containing 8 silicon-oxygen tetrahedra is for 57 kJ/mol more favorable than the adsorption complex involving the non-cyclic form whereas the energy of free LV molecule in alicyclic form differs from that of non-cyclic one only for 22 kJ/mol. The calculation of the ΔG value as dependent on the temperature within 1 to 450 K shows that the temperature when the alicyclic form of *D(-)-threo*-isomer of levomycetin molecule becomes more favorable than non-cyclic one at about 129 K whereas the interaction with a silica nanoparticle shifts this value to about 136 K. This can testify an effect of silica surface on the favorable form of *D(-)-threo*-isomer and justifies the adsorption of LV on silica surface predominantly in the biologically active form.

Adsorption complexes of LV molecule interacting with a molecular model of silica surface containing 13 silicon-oxygen tetrahedra are shown in Fig. 4. It is seen that a bonding can be realized due to forming hydrogen bonds O–H…O and O–H…N between hydrogen atoms of silanol groups of silica with oxygen atoms of nitro- and/or carbonyl groups as well as with nitrogen atom of –NH group of LV molecule (Fig. 4*a*). In such an adsorption complex, hydrogen bond length (d) is of 1.700 Å what corresponds to the bioactivity of about 20% as calculated according to the equation (*). Nevertheless, more probable (for 38 kJ/mol than previous one) is an adsorption complex where a O–H…O hydrogen bond is formed between silanol groups and oxygen atoms of $–NO_2$ and –OH groups of LV molecule (Fig. 4*b*). The hydrogen bond length between -OH groups in the complex is somewhat lesser (1.673 Å) than that in free levomycetin molecule (1.694 Å). The calculated value of biological activity is close to that found experimentally (375%) and is of 270%.

Fig. 4 shows the isolines of the LCAO coefficients of frontier orbitals of respective adsorption complexes. The distribution of the HOMO and LUMO over levomycetin molecule on silica surface is seen to be similar to that of free LV molecule (Fig. 3) with marked biological activity. At the same time, the LCAO value of the oxygen atom of carboxyl group for the HOMO is greater for the *b* complex as compared with that of the *a* one what can testify the greater biological activity of LV molecule adsorbed on silica surface.

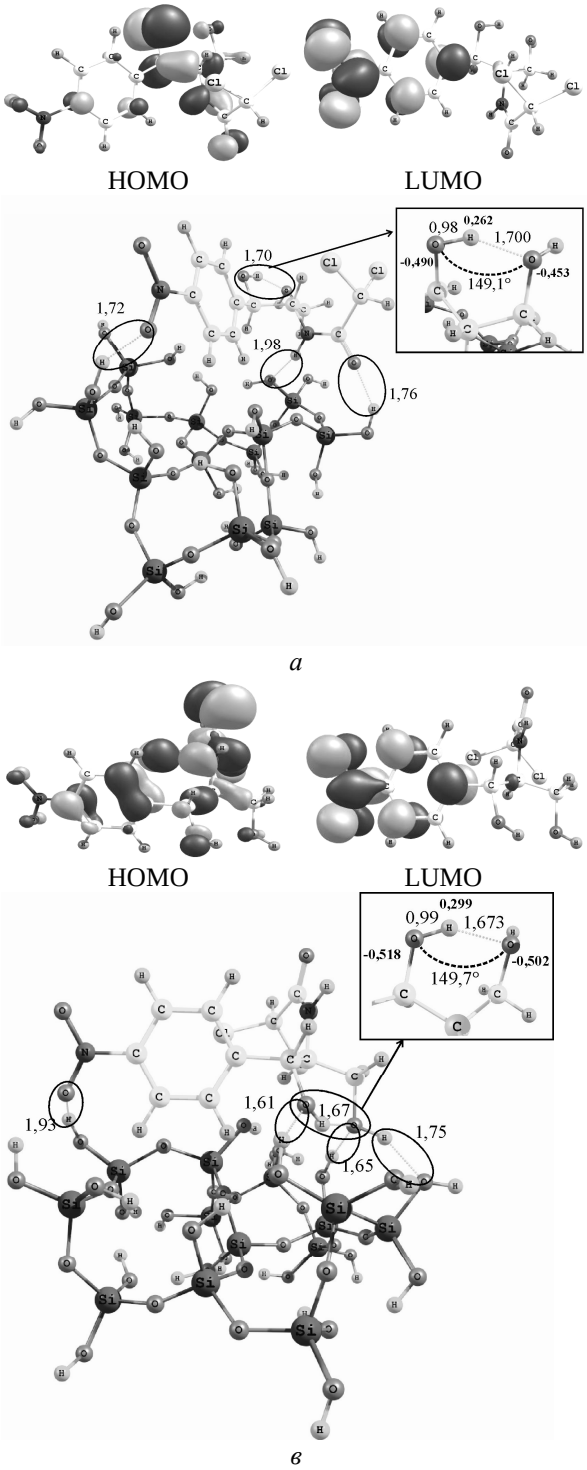

Fig. 4. Equilibrium spatial structures of probable adsorption complexes of levomycetin on silica surface realized due to forming hydrogen bonds O–H…O and O–H…N between hydrogen atoms of silanol groups with oxygen atoms of nitro- and/or carbonyl groups as well as with nitrogen atom of –NH group of LV molecule (*a*), and oxygen atoms of $–NO_2$ and –OH groups of LV molecule (*b*), and the LCAO coefficients distribution over their frontier orbitals

The LUMO for both complexes is stationed at the nitrophenyl fragment. The analysis of the frontier orbitals testifies the greater biological activity of the *b* complex as compared with that of *a* one.

The position of levomycetin molecule on silica surface (simulated by eight silicon-oxygen tetrahedra) surrounded by fifteen water molecules is less favorable energetically if it directly contacts with silanol groups than that where water molecules are stationed between levomycetin molecule and silica surface (the Gibbs adsorption energy is of 296 kJ/mol). Unlike that from water, the adsorption from alcohol should be favorable energetically (the Gibbs adsorption energy is of -39.5 kJ/mol). This result is proved by experimental data [3].

A correlation is seen from the Table between the biological activity of LV and the energies of the frontier orbitals of both free and adsorbed LV on silica surface. A correlation also takes place between the biological activity of LV and the hydrogen bond length between –OH groups.

CONCLUSIONS

It has been found that the descriptors of biological activity of LV molecules can be the hydrogen bond length and the energy and electron density distribution of the frontier molecular orbitals.

When alicyclic form of levomycetin molecule is adsorbed on silica surface, a strengthening takes place of intramolecular hydrogen bond in the propanediol fragment of levomycetin molecule what causes an increase in its bioactivity.

The interaction between levomycetin molecule and silica surface is realized via proton donor groups –OH and nitrogroup what is characterized by somewhat higher value of the formation energy as compared with that in case of interaction via groups >NH, >C=O, and $–NO_2$.

The adsorption of levomycetin from alcohol is more favorable thermodynamically than that from aqueous solution what agrees with experimental data.

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Квантовохімічне дослідження взаємодії левоміцетину з поверхнею кремнезему

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Біологічну активність D(-)-трео-ізомера левоміцетину пов'язують з присутністю в його аліциклічній формі водневого зв'язку між гідроксильними групами. Адсорбційно закріплений (шляхом імпрегнування) левоміцетин на поверхні кремнезему підвищує антимікробну здатність. Методами квантової хімії досліджено зміни структурних та енергетичних параметрів молекул левоміцетину, обумовлені адсорбцією, які можуть відповідати за його активність. Виявлено, що дескрипторами біологічної активності левоміцетину можуть бути енергія НВМО та довжина водневого зв'язку О-Н…О в пропандіольному фрагменті молекули.

Квантовохимическое исследование взаимодействия левомицетина с поверхностью кремнезема

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Биологическую активность D(-)-трео-изомера левомицетина связывают с присутствием в его алициклической форме водородной связи между гидроксильными группами. Адсорбционно закрепленный (путем импрегнирования) левомицетин на поверхности кремнезема повышает антимикробную способность. Методами квантовой химии изучены изменения структурных и энергетических параметров молекул левомицетина, обусловленные адсорбцией, которые могут отвечать за его активность. Обнаружено, что дескрипторами биологической активности левомицетина могут быть энергия НВМО и длина водородной связи О-Н…О в пропандиольном фрагменте молекулы.