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Spectral and proton acceptor properties of chloramphenicol

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The absorption spectra of chloramphenicol aqueous solution were obtained experimentally. The absorption spectra of chloramphenicol isomers and the effect on the spectra of the formation of H-bonded complexes were calculated and interpreted using quantum chemistry methods. Calculation results showed that the absorption spectrum of chloramphenicol by position of bands and their nature is largely determined by the nitrobenzene fragment with little participation of propanol and dichloroacetamide fragments of chloramphenicol. The proton acceptor properties of individual chloramphenicol fragments and the effect of the formation of H-bonded complexes on them have been analyzed.

Keywords: chloramphenicol, enantiomers, electronic absorption spectrum.

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Introduction

Chloramphenicol, a component of laevomycetin antibiotic, falls into the group of antibiotic drugs of aromatic series, which are widely used to treat many infectious diseases, such as enteric fever, dysenteria, pneumonia, pertussis, etc. [1]. Its chemical structure is determined a long time ago, however up to now it is clarifying with the use of state-of-the-art physical research methods, including the application of vibrational spectroscopy (infrared (IR) and Raman scattering spectroscopy (RSS)) in combination with Fourier transforms, NMR-spectra, as well as methods of quantum chemistry [2,3].

The presence of the single bond $C_{10}-C_{12}$ in the structure of chloramphenicol molecule (Fig. 1) formed by asymmetric carbon atoms results in the possibility of existence of two pairs of isomers of $D(-)$- and $L(+)$- types, that are different from each other by spacial arrangement of functional groups in relation to the $C_{10}-C_{12}$ bond. The first pair of isomers is characterized by trans-arrangement of fragments of atoms 10 and 12, these are *tert*-isomers; the second pair has *cis*-arrangement, these are *erythro*-isomers (Fig. 1) [2]. It was found that the conformation of $L(+)$-type in each pair of isomers is a specular reflection of $D(-)$-type isomer. Pairs of $D(-)$ and $L(+)$ isomers are enantiomers, which chemical and physical properties are identical, except for the direction of plane rotation of the electromagnetic vector of plane-polarized light wave: $(+)$ sign corresponds to clockwise rotation, (-) sign corresponds to counterclockwise rotation.

Spatial differences of enantiomers play an important role in pharmaceutics, therefore it is important to take them into consideration. As for isomers of chloramphenicol, it appeared to be that from among the four above-mentioned isomers only *tert*-isomers demonstrate an antimicrobial activity. At the same time the highest activity is demonstrated by the *tert*-isomer $D(-)$, and it is this isomer that has been assigned the name „chloramphenicol". Biological activity of the *tert*-isomer $L(+)$ is twice lower than that of chloramphenicol. As for *erythro*-isomers, they are not applied in pharmacology because of their toxicity.

The study of NMR-spectra [2] has shown that for each pair of enantiomers spectra of *tert*-isomers are identical, but differ from the spectra of *erythro*-isomers. The presence of single C−C-bonds in the structure of chloramphenicol molecule a priori allows for the possibility of existence of a large number of molecular conformations in a solution. Therefore in [2], as a result of analysis of NMR- and RSS-spectra of chloramphenicol, an attempt is made to determine the conformation prevailing in the solution. According to authors of [2], the presence of one conformer should be expected in the solution. The statement is based on the fact that all the observed features of the investigated NMR- and RSS- spectra of chloramphenicol can be only understood if there is a single conformer, and can not be brought in compliance with the obtained experimental spectra if there are two or more conformers.

The analysis of findings of the investigation of intramolecular H-bonds formation by hydroxyl groups of chloramphenicol molecule performed by authors of [2] did not allowed for making a definite statement of the presence of intramolecular H-bonds of this type. The main reason of their absence is, most likely, related to steric features of conformers, creating difficulties for the formation of hydrogen bonds with hydroxyl groups of chloramphenicol. In addition, features of NMR-spectra of chloramphenicol [2] allowed the authors to state that the rotation of propanol structure fragment about single bonds of the side chain is limited, and this can be also an indirect indication of the existence of one conformer in prevailing quantities in the solution.

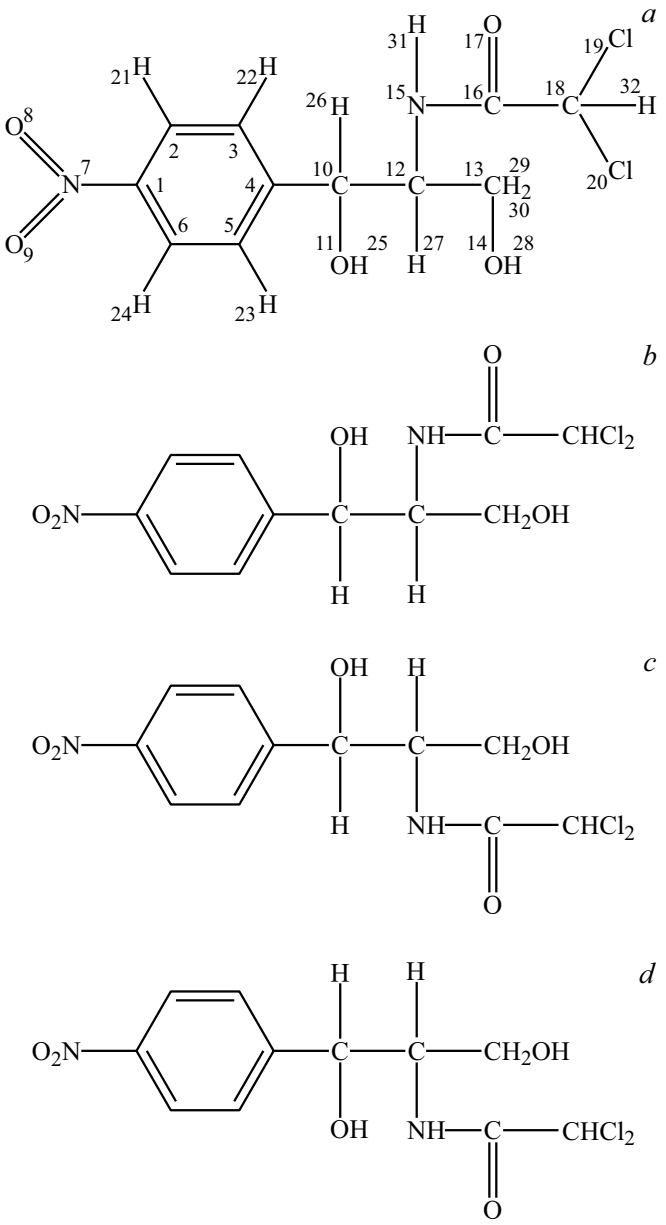

Figure 1. Isomers of chloramphenicol: *a*) *tert*-isomer $D(-)$, *b*) *erythro*-isomer $D(-)$, *c*) *tert*-isomer $L(+)$, (*d*) *erythro*-isomer $L(+)$.

The use of experimental IR- and RSS- spectra together with quantum-chemical calculations [3] allowed optimization of the chloramphenicol structure and obtaining of a number of important findings related to features of its geometry. In addition to the investigated structural features chloramphenicol isomer structures, a great attention is paid to optimization of pharmaceutical properties of the antibiotic and studying of the mechanisms of its action [4]. Thus, for example, authors of [1] have found a strong dependence of antibacterial properties of chloramphenicol on the structure of propanol fragment of the molecule: any changes in this fragment lead to the loss of biological activity of the antibiotic, while changes in other fragments result in a significantly lower effect. At the same time, this main property of the antibiotic is also strongly affected by its symmetry [1,4]. Serious research activities are focused on the mechanisms of the antibiotic interaction with living organisms [1,4]. Thus, for example, investigations of chloramphenicol action mechanism [4] have shown that it manifests its biological activity through the inhibition of bacteria protein synthesis.

Although after 2000 the number of investigations with the use of experimental and quantum-chemical methods has been grown, we do not know any works on calculations, interpreting, and studying of absorption spectra of chloramphenicol and its isomers. That's why the purpose of our study is to interpret absorption spectra of chloramphenicol isomers and their H-bound complexes using methods of quantum chemistry. In addition to this, as it follows from the study of [5], the donor-acceptor properties of certain fragments of the molecule are very important to find out the mechanism of interaction of the antibacterial isomer of chloramphenicol with living cell membranes. Therefore we also have analyzed electron-donor and electron-acceptor properties of certain fragments of isolated isomers of chloramphenicol and the effect of the formation of complexes with H-bond on them.

The technique of investigations

Experimental methods

In the experimental investigation of electronic spectra of chloramphenicol (or laevomycetin, or chloromycetin) we used a compound synthesized by Sigma-Aldrich commercial company (product code Si-Al C0378-5G) with a guaranteed purity > 98%. The structural formulae of the studied object and its isomers are given in Fig. 1. Chloramphenicol is represented in the form of colorless crystals with a bitter taste. It is poorly water-soluble and well soluble in ethanol, pyridine, ethylene glycol, and propylene glycol. In order to produce a matrix solution of chloramphenicol with a concentration of 1 mM a dry weighed quantity was solved in distilled water by means of ultrasonic agitator. Chloramphenicol in the form of powder is resistant to neutral and weakly acid solutions, and at pH> 10 it inactivates quickly.

Absorption and fluorescence spectra of the studied solutions were recorded by means of VARIAN Cary 5000 Scan UV-VIS-NIR spectrophotometer and VARIAN Cary Eclipse spectrofluorometer (AgilentTech., USA–Netherlands–Australia) at room temperature within the spectral band of 200−800 nm. A quartz cell with the pathway of 10 mm was used for the measurement. By the method of derivative spectrophotometry locations of bands were obtained that are manifested only as latent maxima and unclear bends in the absorption spectrum. This method is based on the same principles as a conventional spectrophotometry, however, the analytical response is not absorbance, but its derivative of the order of n (usually,

by the wavelength). The spectrum differentiation allows more clear determination of the location of absorption band maximum, and narrows the bands and allows determining the substances that absorb at similar wavelengths, whose initial spectra are partially overlapping each other. According to this procedure we succeeded in distinguishing electron transitions in the chloramphenicol absorption spectra in water. The error of measurement of the absorption and fluorescence wavelength is ± 1 nm.

Quantum-chemical calculations of absorption spectra of chloramphenicol isomers

Electronic absorption spectra of the studied molecules were calculated by the semiempirical method of Intermediate Neglect of Differential Overlap (INDO) with the use of special parametrization [6] and a software package created in the department of molecule photonics of the Siberian Physical-Technical Institute of the Tomsk State University and successfully applied many times to different classes of chemical compounds to study their spectral-luminescent properties and photochemical processes running in them [7]. The above-mentioned package of quantum-chemical software programs on the basis of wave functions of the INDO method allows calculating of absorption and fluorescence spectra, determining orbital nature of electron excited states, distribution of electron density in the ground and excited electron states of molecules, energy of electrostatic interaction of the molecule with proton (method of molecular electrostatic potential (MEP) [8,9]). The possibility of numerical evaluation of this interaction has made this method effective for the investigation of protonation reaction and for the use at the initial stage of calculation of intermolecular interaction of two molecules in chemical reactions. The package of software programs allows evaluation of transition rate constants for radiative and non-radiative transitions in the molecule and quantum yields of radiative processes.

Molecules of chloramphenicol isomers are nonplanar. It complicates the problem of finding the most precise geometry of molecule, on which the matching between calculated and experimental spectra depends, that serves as the ground for the correctness of conclusions resulted from the calculations. Therefore the issue of chloramphenicol molecule geometry requires a separate consideration.

Selection of geometry of isolated molecules of chloramphenicol isomers and their complexes with water

In recent times a great attention is paid to the issue of chloramphenicol molecule structure. Thus, for example, in [3], on the basis of density functional method the spatial structure of chloramphenicol is optimized and important conclusions are made regarding features of its geometry. Thus, it was shown that the nitrogroup is planar and coupled with the benzene ring of chloramphenicol. At the same time, an increase in $2-1-7$ and $6-1-7$ angles by two degrees in relation to previously assumed 120° takes place, which, in turn, causes „pushing apart“ of the benzene ring and the nitrogroup. The static stress between atoms of O_{11} and H_{23} causes twisting of the $O_{11}-H_{25}$ hydroxyl group. The calculation has confirmed double-bound $C_{16}-O_{17}$ bonds, which length is 1.35 and 1.22 Å respectively. The experiment confirms it: the red shift of vibration frequency of the C=O group may be indicative of formation of the $O_{11}-H_{26}-O_{17}$ hydrogen bond. Our calculation has confirmed the possibility of formation of this H-bond, however taking it into consideration did not result in large changes in characteristics of absorption bands of electron spectra of isomers.

Important conclusions regarding spatial structure of chloramphenicol isomers are made in [10]. The authors used an example of N- and O^+-acylation in 1-phenyl-2-N-acetylamino-1,3-propanediol to show that in *tert*-isomers we should expect *cis*-orientation of $N_{15}-H_{31}$ and $C_{16}=O_{17}$ groups and *trans*-orientation of these groups in *erythro*-isomers in relation to each other. A similar effect is possible in the structure of chloramphenicol isomers as well. The above-mentioned features of isomer geometry have an effect on the energy, forces of oscillators of electron transitions and the effective charge of antibiotic fragments, therefore they were taken into account in the calculation of absorption spectra.

The use of modern methods of molecule geometry optimization does not always allow obtaining the calculated spectrum of molecule matching the experiment. Our calculation of spectra of chloramphenicol isomers with a geometry optimized by the molecular dynamics method of MM2 Chem. Office [11] in AM1 version [12] did not result in a satisfactory match with the experiment. Thus, for example, the energy of $n \to \pi^*$ transitions related to the oxygen atoms of the nitrogroup in the trans-isomer is reduced by $\sim 80\,000\,\mathrm{cm}^{-1}$, which could be caused by the geometry distortion of the nitrobenzene fragment, having a non-planar structure according to our calculation of chloramphenicol geometry optimization, while the nitrogroup and the benzene ring according to X-ray diffraction data of [13] and results of [3], are in the same plane. The distortion of the benzene ring plane during the optimization consisted in that certain carbon atoms appeared to be out of the ring plane. In addition, the optimized molecule geometry did not result in match between the calculated spectrum and the experiment. That's why we only used lengths of chemical bonds from the optimized geometry, and valence and torsion angles for the calculation were taken in accordance with the stereometry of individual groups in the molecule fragments according to [13], with their appropriate correction to achieve the best match with the experiment.

When building up the configuration of complexes with H-bond, the results of our calculations by MEP method were used [8,9]. This method allows determining coordinates of the place of the biggest electrostatic interaction between the molecule and proton of the proton-donor solvent (MEP

Table 1. Minima of molecular electrostatic potential U of chloramphenicol molecule isomers

Atom	U, kJ/M			
	tert-isomers		*erythro*-isomers	
	$D(-)$	$L(+)$	$D(-)$	$L(+)$
O_8	−321	−408	−404	−420
O_9	−357	−332	−326	−341
O_{11}	−71	−114	−126	−108
O_{14}	−154	−43	−186	−206
O_{17}	−384	−344	−274	

minimum) according to [14]. Although the MEP minimum is an integral characteristics of molecule, the minima themselves, as a rule, are located near the atom with excessive electron density, that allows assigning the MEP value to the fragment containing this atom. Thus, minima of O_8 and O_9 atoms are referred to the nitrogroup, minima of O_{11} and O_{14} atoms are referred to hydroxyl groups, the minimum of O_{17} atom is referred to the carbonyl group. MEP minima listed in Table 1 (U, kJ/M) for the molecule of chloramphenicol and its isomers indicate that H-bonds are formed first of all between molecules of water and atoms of oxygen of the nitrogroup and carbonyl group of chloramphenicol.

It follows from Table 1, that for tert-isomers of chloramphenicol the ratios of MEP minima of atoms of hydroxyl groups are different for $D(-)$ and $L(+)$ types of isomers, which is defined by relative locations of atoms O_{11} and O_{14} and positively charged atoms H_{22} and H_{23} of the benzene ring in these isomers, that have an impact on the interaction between them. In chloramphenicol *erythro*-isomer $L(+)$ oxygen atoms of hydroxyl groups are closely located, which results in merging of MEP minima of O_{11} and O_{14} atoms and emergence of a single MEP minimum (Table 1). This makes it difficult to build up an H-complex with these groups and to form a strong hydrogen bond, because it is known that the most strong interaction in the H-bond takes place in the case when all atoms in the H-bond are arranged along one straight line. For the H-bond with O_{11} and O_{14} oxygen atoms this condition is fulfilled. MEP minima of oxygen atoms of hydroxyl groups are considerably weaker.

MEP minima for chlorine atoms are not presented due to their absence: MEP isolines of chlorine atoms have the form of a „bread ring " configuration with its center on the chlorine atom. Thus, MEP calculation of these atoms does not yield coordinates of MEP minima and does not allows bonding of water molecule to chlorine atoms on a reasonable basis. Therefore, for the studied isomers spectra of H-bound complexes only with oxygen atoms are considered. For higher assurance in correctness of the selected configuration of complexes with water, complexes of two compositions 1 : 3 and 1 : 5 are calculated for chloramphenicol (Fig. 2).

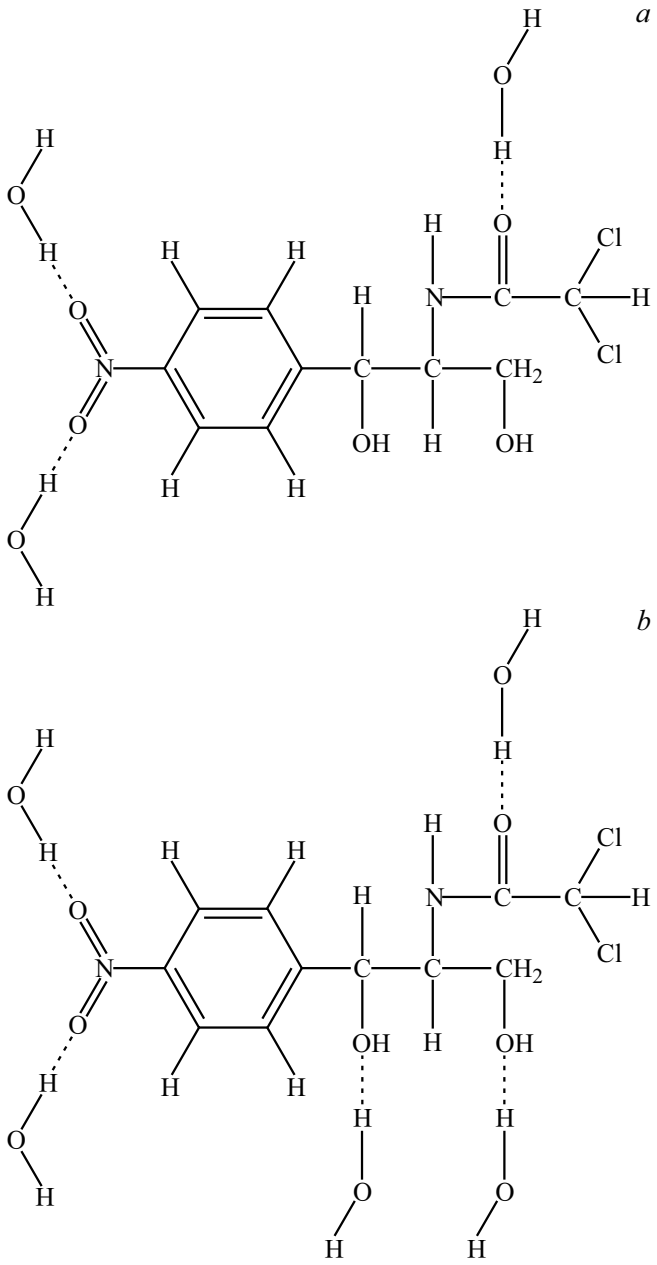

Figure 2. Complexes of chloramphenicol with molecules of water of (*a*) 1 : 3 and (*b*) 1 : 5 compositions.

The complex of 1 : 3 composition took into account the interaction with oxygen atoms of the nitrogroup and the carbonyl group, and the complex of 1 : 5 composition took into account H-bonds with all oxygen atoms of the molecule, i. e. it took into account hydroxyl groups (atoms of O_{11} and O_{14}). Comparison of absorption spectra of complexes with these two compositions of chloramphenicol has shown that taking into account the hydrogen bonds with oxygen atoms of the hydroxyl group has a very little effect on the absorption spectra of isomers, therefore for other isomers of chloramphenicol only complexes with the 1 : 3 composition were considered, i. e. complexes of isomer molecule with three molecules of water.

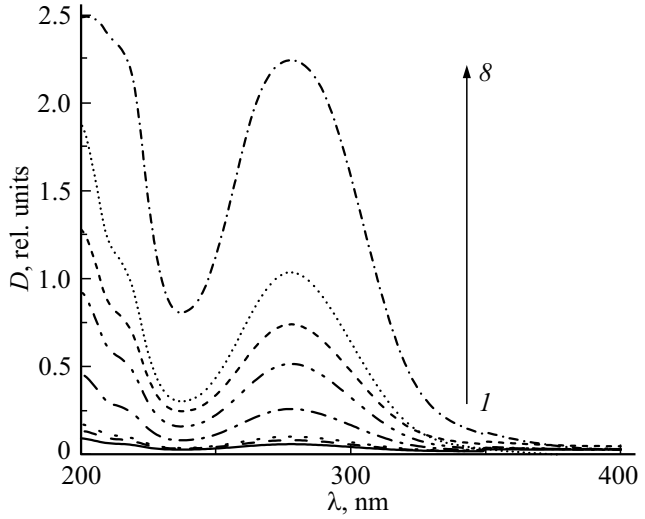

Figure 3. Absorption intensity of chloramphenicol in water as a function of concentration (M): $5 \cdot 10^{-6}$ (*1*), $7.5 \cdot 10^{-6}$ (*2*), 10^{-5} (*3*), $2.5 \cdot 10^{-5}$ (*4*), $5 \cdot 10^{-5}$ (*5*), $7.5 \cdot 10^{-5}$ (*6*), 10^{-4} (*7*), $2.5 \cdot 10^{-4}$ (*8*). Curves are numbered from bottom to top.

Results and discussion

Interpreting lines in absorption spectra of chloramphenicol isomers and their complexes with water

Non-planar structure of molecule of chloramphenicol and its isomers makes it difficult to determine the orbital nature of excited states of the molecule because of mixing of atom orbitals of π- and σ- types. Due to this reason, the characteristics of electron-excited states presented in Table 2 only indicate the predominance of one or another orbital nature or their equal participation in the formation of the electron-excited state.

Absorption spectrum of aqueous solution of chloramphenicol is shown in Fig. 3. According to the experiment, spectrum of aqueous solution of chloramphenicol in the region of 200−400 nm is formed by two absorption bands with maxima at 277 and $\sim$ 200 nm.

With concentrations of chloramphenicol solutions in the range of $5 \cdot 10^{-5}-10^{-4}$ M (Fig. 3) at the long-wave end of the absorption band $\sim$ 200 nm a bend is clearly manifested near $\sim$ 220 nm, indicating of its complex structure. Considering the above, the absorption spectrum of chloramphenicol can be divided into three bands with maxima at 277, $\sim$ 220, and $\sim$ 200 nm. Analysis of the calculation results presented in Table 2 allows distinguishing the electron transitions that form the above-mentioned regions of the absorption spectrum. Taking into account the fact that dispersion of the calculated energies of the electron transitions of chloramphenicol isomers that form the above-mentioned absorption regions is small, in the interpreting the spectral bands we have limited the consideration to the spectra of two isomers that have practical applications, i. e. spectra of *tert*-isomers of chloramphenicol of $D(-)$ and $L(+)$ types. According to the calculation (Table 2), the long-wave absorption band of chloramphenicol includes five electron transitions with different orbital nature and intensity. The first two electron transitions are transitions of $n\pi^*$-type localized on oxygen atoms of the nitrogroup, that can not be identified in the experimental spectra. The intensity of long-wave band id formed by the $S_0 \rightarrow S_5 transition(\pi\pi^*)$ related to the nitrobenzene fragment of chloramphenicol. The absorption in the middle part of the spectrum is defined by two single-electron transitions: $S_0 \rightarrow S_8(\pi\pi^*)$ and $S_0 \rightarrow S_9(\pi\sigma^* + \pi\pi^*)$, which are related to the nitrobenzene fragment of the molecule as well. It's worth noting that the S_9 state of *tert*-enantiomers includes the σ^*-orbital pertaining to the dichloroacetamide fragment.

In the short-wave region of the spectrum there are electron transitions to $S_{11}-S_{16}$ states of $\pi\sigma^*$- and $\sigma\pi^*$-types of the dichloroacetamide fragment with a low intensity ($f = 0.01-0.003$, not shown in Table 2). The intensity of the short-wave band is defined by transitions to $S_{17}-S_{19}$ states of the *tert*-isomer $D(-)$ and to the $S_{20}(\pi\pi^*)$ state of isomer $L(+)$. The most intensive isomer transitions in this region are related to the nitrobenzene fragment as well. This feature of the absorption spectrum of chloramphenicol prompting a suggestion that spectrum of this antibiotic is similar to the spectrum of nitrobenzene to a significant extent.

By analyzing the results of *erythro*-isomers calculation following the scheme used to interpret the absorption spectrum of *tert*-isomers, we get a similar result with some differences in energies and orbital nature of electron transitions, that can be explained by the difference in spatial arrangement of the propanol and the dichloroacetamide fragments in relation to the nitrobenzene part of the molecule.

Comparison of calculated absorption spectra of isolated molecules of *tert*-isomers of chloramphenicol with spectra of their H-bound complexes with water molecules identifies the following regularity. For the electron-excited states with prevailing orbital nature of $\pi\pi^*$-type, the formation of complexes is characterized by a long-wave shift of the appropriate electron transitions. If configurations of $\pi\sigma^*$- and $\sigma\pi^*$-types prevail, including $n\pi^*$-type of any localization, a short-wave shift takes place. Such situation of complex formation with aromatic molecules is not unique: the same regularity is typical for solutions of aromatic molecules in proton-donor solvents [15].

Distribution of effective charges on fragments of chloramphenicol isomers in the ground state

It is known that at the first stage of chemical reactions an important role in the interaction of reacting molecules is played by electrostatic interactions that promote approaching of the reacting structures to each other. The contribution of electrostatic interactions is also important in diffusion processes in complex membrane structures [4,5] when

Table 2. Absorption spectra and nature of electron-excited states of chloramphenicol isomers and their H-bound complexes with water

Calculation						Experiment	
Isolated isomer			Complex of isomer with water				
State	E_i, cm^{-1} (λ, nm)	f	State	E_i, cm^{-1} (λ, nm)	f	E_i, cm^{-1}	λ, nm
tert-isomer $D(-)$			*tert*-isomer $D(-)+3H_2O$				
$S_1(n\pi^*)$	25360 (394)	0.0	$S_1(n\pi^*)$	25760 (388)	0.0		
$S_2(n\pi^*)$	26250 (381)	0.0	$S_2(n\pi^*)$	26720 (374)	0.0		
$S_3(\pi\pi^*+\pi\sigma^*)$	34710 (288)	0.012	$S_3(\pi\pi^*+\pi\sigma^*)$	33930 (295)	0.015	36500	277
$S_5(\pi\pi^*)$	37540 (266)	0.366	$S_5(\pi\pi^*)$	36070 (277)	0.405		
$S_6(\pi\pi^*)$	40240 (248)	0.114	$S_6(\pi\pi^*)$	40530 (247)	0.107		
$S_8(\pi\pi^*)$	44040 (227)	0.496	$S_8(\pi\pi^*)$	43770 (228)	0.293	Bend	
$S_9(\pi\sigma^*)$	44390 (225)	0.395	$S_{10}(\pi\pi^*)$	45560 (249)	0.480	in the region of	220
$S_{17}(\sigma\sigma^*)$	51090 (196)	0.118	$S_{15}(\pi\sigma^*)$	49590 (202)	0.083	45450	
$S_{18}(\pi\pi^*)$	51220 (195)	0.162	$S_{18}(\pi\pi^*)$	50980 (196)	0.073	~ 50000	~ 200
$S_{19}(\sigma\sigma^*+\pi\pi^*)$	51620 (194)	0.477	$S_{20}(\pi\pi^*+\sigma\sigma^*)$	52080 (192)	0.587		
tert-isomer $L(+)$			*tert*-isomer $L(+)+3H_2O$				
$S_1(n\pi^*)$	25140 (397)	0.0	$S_1(n\pi^*)$	25470 (393)	0.0		
$S_2(n\pi^*)$	26140 (382)	0.0	$S_2(n\pi^*)$	26500 (377)	0.0		
$S_4(\pi\pi^*)$	32870 (304)	0.073	$S_4(\pi\pi^*)$	32450 (308)	0.074	36500	277
$S_5(\pi\pi^*)$	36700 (272)	0.362	$S_5(\pi\pi^*)$	36610 (273)	0.383		
$S_6(\pi\pi^*)$	39920 (250)	0.109	$S_6(\pi\pi^*)$	40160 (249)	0.109		
$S_7(\pi\pi^*)$	43030 (232)	0.468	$S_7(\pi\pi^*+\pi\sigma^*)$	43170 (232)	0.344		
$S_{10}(\pi\sigma^*+\pi\pi^*)$	44170 (226)	0.220	$S_{10}(\pi\pi^*+\pi\sigma^*)$	44430 (225)	0.232	Bend	220
$S_{11}(\pi\pi^*)$	45350 (220)	0.127	$S_{11}(\pi\sigma^*)$	45580 (219)	0.090	in the region of	
$S_{12}(\pi\sigma^*)$	46460 (215)	0.144	$S_{12}(\sigma\pi^*+\sigma\sigma^*)$	46820 (214)	0.146	45450	
$S_{14}(\sigma\pi^*)$	47390 (211)	0.162	$S_{14}(\pi\sigma^*+\pi\pi^*)$	48050 (208)	0.179	~ 50000	~ 200
$S_{20}(\pi\pi^*)$	52160 (192)	0.527	$S_{20}(\pi\pi^*)$	52710 (190)	0.600		
erythro-isomer $D(-)$			*erythro*-isomer $D(-)+3H_2O$				
$S_1(n\pi^*)$	25180 (397)	0.0	$S_1(n\pi^*)$	25610 (390)	0.0		
$S_2(n\pi^*)$	26080 (384)	0.0	$S_2(n\pi^*)$	26710 (374)	0.0		
$S_4(\pi\pi^*)$	33830 (296)	0.045	$S_3(\pi\pi^*)$	30940 (323)	0.052		
$S_5(\pi\pi^*)$	36480 (274)	0.450	$S_5(\pi\pi^*)$	36160 (276)	0.248	36500	277
$S_6(\pi\pi^*)$	40000 (250)	0.099	$S_6(\pi\pi^*)$	40500 (247)	0.229		
$S_7(\pi\pi^*+\pi\sigma^*)$	43310 (231)	0.188	$S_7(\pi\sigma^*)$	41300 (242)	0.132		
$S_8(\pi\pi^*)$	44780 (223)	0.307	$S_8(\pi\pi^*+\pi\sigma^*)$	41700 (240)	0.382	Bend	
45450 220 $S_9(\pi\pi^*)$	45000 (222)	0.251	$S_9(\pi\pi^*)$	43040 (232)	0.142	in the region of	220
$S_{10}(\pi\pi^*+\pi\sigma^*)$	45400 (220)	0.286	$S_{15}(\pi\pi^*)$	47630 (210)	0.322	45450	
$S_{16}(\pi\pi^*+\pi\sigma^*)$	50690 (197)	0.173	$S_{17}(\pi\sigma^*)$	50510 (198)	0.150		
$S_{20}(\pi\pi^*)$	52340 (191)	0.202	$S_{18}(\pi\pi^*+\pi\sigma^*)$	51130 (196)	0.214	~ 50000	~ 277
$S_{21}(\pi\pi^*)$	52940 (189)	0.197	$S_{21}(\pi\pi^*)$	52260 (191)	0.234		
erythro-isomer $L(+)$			*erythro*-isomer $L(+)+3H_2O$				
$S_1(n\pi^*)$	25240 (396)	0.0	$S_1(n\pi^*)$	25510 (392)	0.0		
$S_2(n\pi^*)$	26120 (383)	0.0	$S_2(n\pi^*)$	26460 (378)	0.0		
$S_3(\pi\pi^*)$	33540 (298)	0.092	$S_3(\pi\sigma^*)$	29310 (341)	0.142	36590	277
$S_5(\pi\pi^*)$	36130 (277)	0.405	$S_6(\pi\pi^*)$	35150 (284)	0.291		
$S_8(\pi\sigma^*)$	42900 (233)	0.196	$S_7(\pi\sigma^*)$	35630 (281)	0.100		
$S_{10}(\pi\sigma^*+\pi\pi^*)$	44350 (231)	0.237	$S_{13}(\pi\pi^*+\pi\sigma*)$	43750 (228)	0.431	Bend	45450 220
$S_{11}(\pi\sigma^*+\pi\pi^*)$	44480 (225)	0.337	$S_{15}(\pi\pi*+\sigma\pi^*)$	45250 (221)	0.331	in the region of	220
$S_{12}(\pi\sigma^*)$	45100 (222)	0.234	$S_{20}(\pi\sigma^*)$	48550 (206)	0.074	45450	
$S_{23}(\pi\pi^*)$	52120 (192)	0.243	$S_{21}(\pi\pi^*)$	51500 (194)	0.301	~ 50000	~ 200

Note. E_i — energy and wavelength λ of corresponding transition (in brackets), f — oscillator force of the electronic transition.

Table 3. Distribution of effective charge (q, e) over fragments of chloramphenicol isomers and their complexes with water in the ground state

Fragment	q, *tert*-isomers			
	$D(-)$		$L(+)$	
	Isolated molecule	Complex	Isolated molecule	Complex
NO_2	−0.192	−0.128	−0.236	−0.170
benzene ring	0.123	0.130	0.154	0.158
$C_{10}O_{11}H_{25,26}$	0.078	0.081	0.024	0.028
Propanol	0.094	0.095	0.192	0.195
Dichloroacetamide	−0.101	−0.061	−0.133	−0.092
$3H_2O$	–	−0.117	–	−0.119
	q, *erythro*-isomers			
	Isolated molecule	Complex	Isolated molecule	Complex
NO_2	−0.205	−0.135	−0.226	−0.164
benzene ring	0.124	0.121	0.150	0.157
$C_{10}O_{11}H_{25,26}$	0.050	0.074	0.084	0.088
Propanol	0.140	0.147	0.140	0.143
Dichloroacetamide	−0.165	−0.089	−0.146	−0.108
$3H_2O$	–	−0.117	–	−0.117

antibiotic molecules are interacting with the membrane of a living cell, which biological properties are considerably affected by the external electric field as well. Due to these reasons, it appears important to know the distribution of effective charges on different molecule fragments, to know donor-acceptor properties of individual groups and fragments of complex biologically active molecules, that include antibiotics.

Table 3 presents effective charges of fragments of chloramphenicol isomers and their complexes with water in the ground state. The results of effective charge calculations for fragments identify a number of properties, which are common for all investigated isomers. Thus, the electron-acceptor properties in the ground state of isolated structures are manifested by the nutrogroup and the dichloroacetamide fragment. In complexes with water of the 1 : 3 composition, they are added with water molecules participating in the H-bonds. All other molecule fragments, i.e, the benzene ring and the propanol fragment, as well as the $C_{10}O_{11}H_{25,26}$ fragment, which is in para-position in relation to the nitrogroup, manifest electron-donor properties. In this regard, there is no difference between *tert*- and *erythro*-isomers.

The formation of hydrogen bonds results in weakening of electron-acceptor properties of the nitrogroup and the dichloroacetamide fragment and strengthening of electron-donor properties of the rest of fragments. It is worth to note that electron-donor properties in complexes change insignificantly, based on which a conclusion can be made that electron-donor fragments of chloramphenicol isomers in proton-donor solvents keep the effective charge nearly unchanged. On the other hand, such persistence becomes understandable if we take into consideration the fact that these fragments participate in the H-bonds, while the nitrogroup and the dichloroacetamide fragment are the most prone to the formation of H-bonds. On the other hand, the MEP minima near O_{11} and O_{14} atoms (see Table 1) included in the propanol fragment and in the $C_{10}O_{11}H_{25,26}$ fragment, with which the H-bonds can be formed, are much weaker than the MEP minima of oxygen atoms in the nitrogroup and the carbonyl group. In this case the formation of a strong hydrogen bond and, as a consequence, a considerable impact on the effective charges of these fragments should not be expected.

Conclusions

The performed investigations have determined the following.

The formation of the absorption band of chloramphenicol in the range of 240−400 nm (position, intensity, and nature) is participated mainly by the nitrobenzene fragment. The formation of the absorption in the short-wave region of the spectrum, 200−240 nm, is also participated by the dichloroacetamide fragment, although the intensity of absorption in this spectral range is defined by the nitrobenzene fragment as well.

In the ground state, the nitrobenzene and chloroacetamide fragments of all isomers of isolated chloramphenicol molecule manifest electron-acceptor properties, the propanol fragment and the benzene ring manifest electron-

donor properties. The formation of H-bonds considerably reduces electron-acceptor properties of the nitrobenzene and chloroacetamide fragments and have less effect on electron-donor properties of the benzene ring and the $C_{10}O_{11}H_{25,26}$ fragment. The formation of complexes with H-bonds keeps electron-donor properties of the propanol fragment nearly unchanged.

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Conflict of interest

The authors declare that they have no conflict of interest, financial and other conflicts.

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