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Interaction of Topotecan, DNA Topoisomerase I Inhibitor, with Double-Stranded Polydeoxyribonucleotides. 1. Topotecan Dimerization in Solution

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Abstract—Behavior of topotecan, DNA topoisomerase I inhibitor, was studied in aqueous solutions by optical methods. Topotecan absorption spectra were recorded in the pH range 0.5–11.5 and its pKa were determined. Quantum chemical calculations were made for all charge states of the topotecan molecule in lactone and carboxylate form. The calculated absorption maxima agree well with the experimental data. Protonation of the topotecan D ring (pKa ≈ 3.6) was revealed. Comparison of experimental and calculated data showed topotecan structure with a proton at the oxygen atom at C16a rather than N4 to be the most preferable. Topotecan molecules were shown to form dimers at concentrations above 10^{-5} M. Topotecan dimerization is accompanied by an increase in the pKa of hydroxy group of the A ring from 6.5 ([TPT] = 10^{-6} M) to 7.1 ([TPT] = 10^{-4} M), which indicates participation of this group in dimer stabilization, perhaps due to intermolecular hydrogen bonding with N1 of the B ring of a neighboring molecule. Probable dimer structures were proposed. The topotecan dimerization constant was determined, $K = (4.0 \pm 0.7) \cdot 10^3$ M^{-1}.

Key words: DNA topoisomerase I, camptothecin, topotecan, dimerization constant, dimer models, quantum chemical calculation

INTRODUCTION

DNA topoisomerase I is an enzyme that takes part in many important intracellular processes: replication, transcription, and repair [1, 2]. Its inhibitors are camptothecin (CPT) and its derivatives including topotecan (TPT) [3, 4]. They prevent the ligation of DNA termini cleaved by DNA topoisomerase I [5, 6]. This ability seems to predetermine their anticancer activity, and TPT is already used in clinic [7]. CPT and its derivatives were considered for a long time to bind only to the DNA topoisomerase I–DNA complex rather than free DNA [5, 8–10]. However, their ability to directly interact with DNA was shown in the past years [11, 12]. CPT is poorly soluble in water [13], the solubility of TPT is somewhat higher. The compounds consist of a mixture of aggregate forms in solutions [14]. Therefore, before studying the interaction of TPT with DNA, we have studied its aggregate forms in solutions with the use of spectrofluorimetry, circular dichroism (CD), and UV-Vis spectrophotometry. The orientation of the main transition electron dipole moments of TPT molecule was calculated. The values of pKa were determined at different TPT concentrations and assigned to the functional centers of TPT molecule. TPT dimerization constant in solution was found to be $(4.0 \pm 0.7) \cdot 10^3$ M^{-1}. Three possible structures of TPT dimers were proposed on the basis of the performed research.

EXPERIMENTAL

Materials. We used topotecan (hycamtin, NSC609699) from SmithKline Beecham and sodium cacodylate from Sigma. TPT was purified and analyzed for homogeneity by reversed-phase HPLC on a 15×250 mm column with Silasorb C-18 (6 μm) (SKB of the Institute of Organic Chemistry, Russian Academy of Sciences). HPLC was performed on a Gilson chromatograph with UV detector (model 117). We used a linear acetonitrile gradient in 0.1% trifluoroacetic acid (0–80% over 45 min, flow rate 4.8 ml/min). The eluate was monitored at 240 and 380 nm. Retention time for TPT is 35.88 min. The structure of TPT was confirmed by mass spectrometry using a Kratos Analytical Kompact MALDI 4 instrument in the positive ion registration mode with 2-amino-5-nitropyridine as a matrix.

We used a molar extinction coefficient ε_{380} = 20,000 M^{-1} cm^{-1} for dilute aqueous solutions of TPT.

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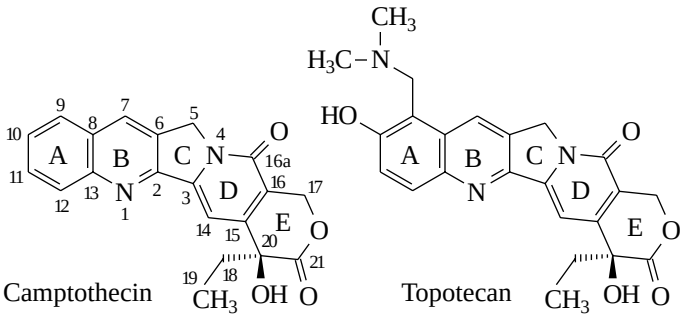

Fig. 1. Chemical structure of camptothecin and topotecan.

UV-Vis, circular dichroism, and fluorescence spectra. Absorption spectra of TPT solutions were recorded on a Cary 118 spectrophotometer, circular dichroism was measured on a CNRS–Roussel–Jouan Jobin-Yvon III dichrograph, fluorescence spectra were obtained on a Hitachi MPF-2A spectrofluorimeter.

Determination of electron transition dipole moments for TPT molecule. Geometry parameters of TPT were calculated using AM1 semiempirical model [15]. Parameters of excited state were determined with the use of INDO/S calculation procedure [16]. At least 19 occupied and at least 19 free molecular orbitals were taken into account in the calculation of configurational interactions (total number of configurations was 723).

RESULTS AND DISCUSSION

Dependence of TPT spectra on its concentration in solution. Figure 1 shows the chemical structures of CPT and TPT, a CPT analog having an additional hydroxy group at the 10th position and a dimethylaminomethylene fragment at the 9th position. On the one hand, these substituents increase TPT solubility in water [17] and, on the other, they slightly alter the spectrum of its therapeutic effect.

All experiments were carried out in 1 mM sodium cacodylate buffer of pH 6.8. We obtained absorption, circular dichroism, fluorescence, and fluorescence excitation spectra of this compound at different concentrations to determine the TPT concentration at which it was in monomeric form. These spectra are shown in Figs. 2a–2c and given for convenience as relative values: apparent molar extinction $\varepsilon_{app} = A/C$, apparent molar dichroism $\Delta\varepsilon_{app} = \Delta D/C$, and apparent specific fluorescence intensity $i_{app} = I/C$, where A, ΔD, and I are the values of absorption, CD, and fluorescence intensity, while C is the corresponding TPT concentration in solution.

The long-wavelength maximum of TPT absorption spectrum at 380–400 nm corresponds to the absorption of its quinoline moiety (rings A–B). This indicates that the chromophore systems of the A–B and the D ring are weakly associated (otherwise the long-wavelength absorption maximum would have been shifted substantially to the visible region). The short-wavelength portion of the spectrum contains absorption of both quinoline fragment and D ring [14].

A hypochromic effect and relative decrease in absorption at 420 nm were revealed when TPT concentration increased. DNA bases are known to show a hypochromic effect. In our case, we consider the effect to be associated with stacking interaction of TPT monomers upon dimerization. The relative decrease in the absorption at 420 nm is due to the decrease in the share of TPT molecules with deprotonated hydroxy group in the A ring when they form the dimer.

Very weak CD is observed in the long-wavelength absorption band of TPT, see Fig. 2b. This is associated with the fact that the quinoline moiety of TPT is flat and its CD is induced by asymmetrical environment. The D ring is not flat owing to the N4 atom. Moreover, there is an asymmetric center, C20 atom in the neighborhood. This results in much stronger CD corresponding to the D ring than that for the quinoline moiety (see, e.g., the band at 228 nm). The specific dichroism of TPT, especially in the long-wavelength band, increases substantially with concentration, which indicates the growth of asymmetry in the environment of the quinoline moiety upon dimerization.

Fluorescence spectra of TPT at low and high concentrations are alike, which indicates that the vibrational sublevels of the ground state of TPT molecule are insensitive to dimerization. Raising the TPT concentration (see Fig. 2c) leads only to the quenching of the specific intensity of fluorescence. In the fluorescence excitation spectrum, the band at 385 nm is quenched more than that at 420 nm, although it is the 420 nm band that decreases more in absorption spectra when TPT concentration increases. The reason of such behavior of fluorescence excitation spectra of TPT is to be established.

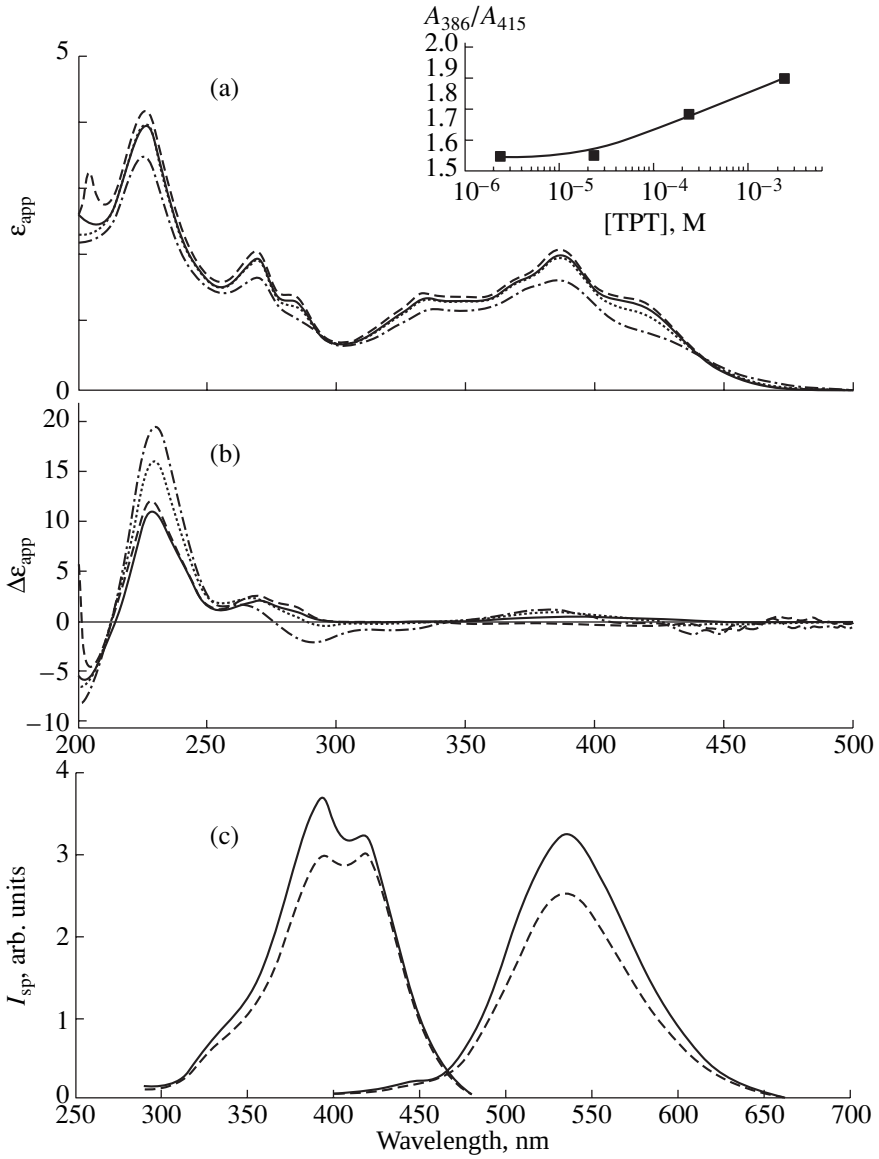

Fig. 2. Spectra of apparent extinction (a) and molar dichroism (b) at different TPT concentrations: (–·–·–) $2.3 \cdot 10^{-6}$ M; (– – –) $2.3 \cdot 10^{-5}$ M; (···) $2.3 \cdot 10^{-4}$ M; (—) $2.3 \cdot 10^{-3}$ M. The corresponding optical path lengths of cuvettes are 10, 1, 0.102, and 0.01 cm. (c) Spectra of apparent specific intensity of fluorescence excitation and fluorescence emission of TPT. (—) $2.2 \cdot 10^{-6}$ M; (– – –) $5.5 \cdot 10^{-5}$ M. Experimental conditions: measurements in a 0.102-cm-path cuvette disposed at the angle of 40° to the exciting beam. The dependence of relative absorption A_{386}/A_{415} at wavelengths 386 and 415 nm on TPT concentration is shown top right. Buffer: 1 mM sodium cacodylate, pH 6.8.

All the figures show both a change in spectrum shape and changes in the above specific values when TPT concentration in solution is varied. These changes evidence the formation of aggregate forms of TPT larger than monomeric one when the concentration rises.

We studied the dependence of A_{386}/A_{415} ratio on TPT concentration to elucidate the aggregate form of TPT at the concentration of $2 \cdot 10^{-6}$ M (insert to Fig. 2a). The values of A_{386}/A_{415} are virtually constant at the TPT concentration below $2 \cdot 10^{-5}$ M, i.e., TPT is in monomeric form at these concentrations.

Determination of TPT dimerization constant in solution. Since TPT is in monomeric form at the concentration of 10^{-6} M, we suppose the observed spectral changes within concentration range from 10^{-6} to 10^{-4} M to be associated with the formation of TPT dimers. To determine the dimerization constant, we measured the fluorescence intensity, since this method is applicable in a wider range of TPT concentrations.

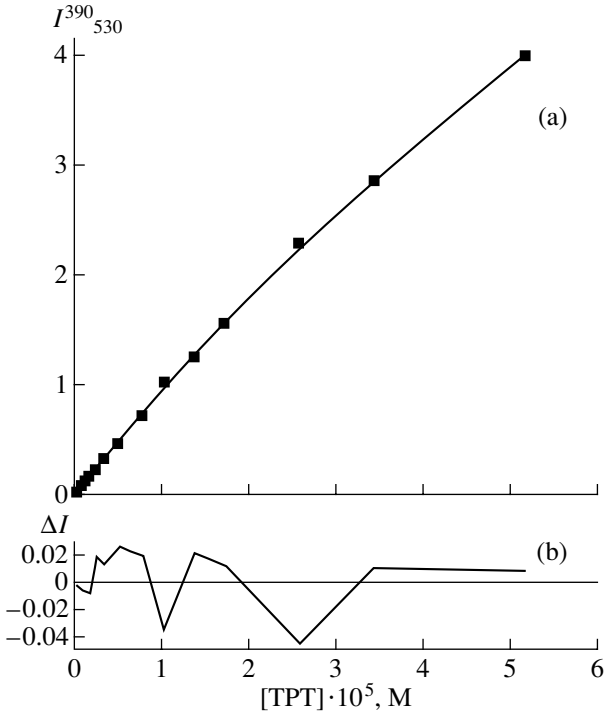

Fig. 3. (a) Concentration dependence of TPT fluorescence intensity. Fluorescence was excited at 390 nm and detected at 530 nm, excitation and emission slits were 20 nm each. Absorbance of TPT on the path length did not exceed 0.1. Squares represent experimental points. (b) Difference between experimental points and theoretical curve corresponding to the minimal sum of rms deviations. Other conditions as in Fig. 2.

Fluorescence intensity was measured at 530 nm, while excitation was accomplished at 330, 360, 390, 400, and 420 nm. One of these dependences is shown in Fig. 3a.

The following simultaneous equations are true for monomer–dimer equilibrium in solution for any TPT concentration (C_j):

$$I_j = i_1 \times C_{1j} + i_2 \times C_{2j},$$

$$K = C_{2j}/C_{1j}^2,$$

$$C_j = C_{1j} + 2 \times C_{2j},$$

where C_{1j}, C_{2j}, and C_j are the concentrations of monomers, dimers and total TPT concentration in solution. K is the dimerization constant. I_j, i_1, and i_2 are the total and specific fluorescence intensities of monomers and dimers of TPT, respectively. Fluorescence intensity (I_j) was measured at 14 different TPT concentrations.

We used MatLab software to minimize the sum of root-mean-square deviations of experimental points from theoretical curve upon the alteration of three variables: K, i_1, and i_2 (initial values of i_1/i_2 were varied from 0.2 to $2 \cdot 10^4$, while K was changed from 20 to $2 \cdot 10^4$). TPT dimerization constant proved to be 4000 ± 700 M^{-1}. Figure 3b shows the deviation of experimental points from the theoretical dependence.

Determination of pKa of TPT was undertaken to reveal charged groups at pH 6.8. We used solutions with TPT concentration of $0.8 \cdot 10^{-6}$ (in monomeric form) and $2.1 \cdot 10^{-4}$ M (~50% of TPT molecules are in dimeric form). Figure 4 presents the results of titration of $0.8 \cdot 10^{-6}$ M TPT solution (spectra are not shown for $2.1 \cdot 10^{-4}$ M). Figures 4a–4d show the series of TPT absorption spectra upon variation in the pH of solution from 0.45 to 12.08. The alteration of absorption spectra of solution indicates the presence of four titratable groups in TPT molecule. Indeed, the spectra shown in Fig. 4a contain isosbestic point in the pH range from 0.45 to 2.64 at 390 nm corresponding to pKa_1 of the first titrated group. New isosbestic point at 384 nm arises when pH increases. It exists at pH from 2.64 to 4.56 and corresponds to pKa_2 of the second titrated group (see Fig. 4b). Spectra alterations in this pH range are much lesser than for other titrated groups. The spectra given in Fig. 4c have isosbestic point at 389 nm. It exists in the pH range from 4.16 to 9.41 and corresponds to pKa_3 of the third titrated group. The spectra shown in Fig. 4d contain isosbestic point at 422 nm. It exists in the pH range from 9.41 to 12.08 and corresponds to pKa_4 of the fourth titrated group. The alterations of absorption spectra are also negligible in this case. Figures 4e–4h show titration curves, the pH dependences of absorbance of TPT solution, measured at 430, 395, 415, and 445 nm, respectively. The pK values were determined as the pH at which the alteration of absorbance is a half of difference between its magnitude at the upper and lower plateau.

We failed to obtain both plateaus for the curves presented in Figs. 4e and 4f. Therefore we only estimate the magnitudes: pKa_1 is lower 0.8 and $pKa_2 \cong 3.6$. Other dependences provide the following values: $pKa_3 = 6.5$ and $pKa_4 = 10.7$. Figures 4e, 4g, 4h also show titration curves for $2.1 \cdot 10^{-4}$ M TPT solution measured at 420, 410, and 410 nm, respectively.

All compounds of CPT family contain additional hydroxy group at the 20 position which belongs to nonaromatic ring E. Furthermore, pH increase to alkali region causes lactone–carboxylate transformation of CPT and its analogs at pH 7.3 [18], which results in hydrolysis of the ring E to form carboxylic group at the 20 position and hydroxy group at the 17 position. Both the carboxylic group at the 20 position and the appeared hydroxy group at the 17 position are aliphatic ones whose pKa are much greater than 12.

To correlate the obtained pKa values with functional centers of TPT molecule, we used data from the work [18] where TPT was studied at intermediate

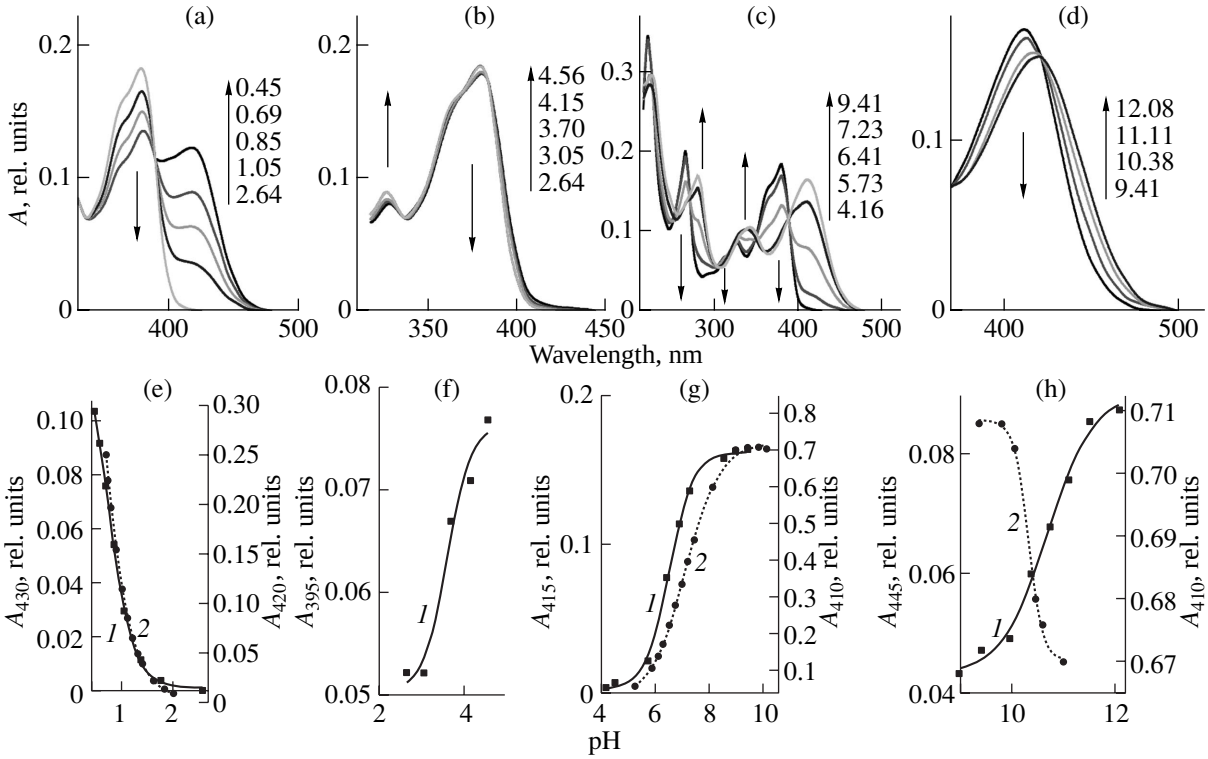

Fig. 4. Absorption spectra of $0.8 \cdot 10^{-6}$ M TPT (monomeric form) in different pH regions: (a) 0.45–2.64; (b) 2.64–4.56; (c) 4.16–9.41; (d) 9.41–12.08. Numeric values show pH at which the spectra were recorded, arrows show spectra alteration upon changing pH. The pH dependence of absorbance measured at wavelengths 430 and 420 (e), 395 (f), 415 and 410 (g), 445 and 410 nm (h). TPT concentration of $0.8 \cdot 10^{-6}$ M corresponds to the left-hand axes and curves *1*, the concentration of $2.1 \cdot 10^{-4}$ M corresponds to the right-hand axes and curves *2* in (e–h). Cuvette path lengths are 10 cm and 0.1 cm for concentrations $0.8 \cdot 10^{-6}$ M and $2.1 \cdot 10^{-4}$ M TPT, respectively. The measurements were performed at 20°C. Other conditions as in Fig. 2.

$(2 \cdot 10^{-5})$ concentration and high (0.5 M NaCl) ionic strength. Thus, we assign pKa_1 to the protonation of N1 atom of the ring B. The obtained assessment, $pKa_1 < 0.8$ at both TPT concentrations, agree well with $pKa_1 = 0.6$ obtained in the work [18].

pKa_3 is assigned to the deprotonation of hydroxy group in the ring A. We obtained value 6.5 at low TPT concentration and 7.1 at high concentration. These values are well consistent with that obtained earlier (pKa = 6.99) in the work [18].

pKa_4 is assigned to the deprotonation of dimethylaminomethylene group. We obtained value 10.7 at low TPT concentration and 10.3 at high concentration which is also in good agreement with the value of 10.5 obtained earlier for TPT [18].

We explain the data obtained as follows. The dimethylamino group in TPT is attached to the ring A through methylene bridge and therefore it very slightly affects the system of conjugated bonds of the rings A–B, and accordingly its titration would have very little effect on spectra, but it is capable of forming a salt bridge with deprotonated hydroxy group of the same A ring in pH range from 6.5 to 10.7. The formation of such a bond facilitates the retention of proton at the dimethylaminomethylene group and thus increasing its pKa. The deprotonation of the dimethylaminomethylene group leads to disappearance of this bond. The alteration of the charge on the dimethylaminomethylene group via interaction with the hydroxy group of the ring A affects the spectra of the quinoline moiety of TPT molecule in such an indirect manner. Indeed, the spectral changes are observed but they are negligible (see Fig. 4d). The fact that we observe the titration of the dimethylaminomethylene group in spectra confirms implicitly the formation of above salt bond.

We refer pKa_2 to the protonation of N4 atom in the ring D. The weak bond of the system of the conjugated rings A–B and the ring D results in very slight but reliable alterations in the spectra of the quinoline moiety of TPT molecule. They are also observed in the absorption band of the ring D, e.g., at 327 and 265 nm, see Fig. 4b (data for 265 nm are not given).

The CPT molecule has neither a hydroxy group in ring A nor a dimethylaminomethylene group. Therefore the pKa_1 (of N1 nitrogen atom) is shifted relative to the pKa_1 of topotecan to the alkaline region and equals 1.2 [18]. Such a shift of this pKa_1 value makes

it difficult to detect weak spectral changes in the absorption of the quinoline moiety of CPT molecule associated with titration of N4 nitrogen atom.

The above variations in the pKa of hydroxy and dimethylaminomethylene groups of the ring A when TPT concentration increases allow us to suppose the participation of these groups in the formation of TPT dimer. We suppose TPT molecules in dimer to be linked via two hydrogen bonds between the hydroxy groups in the rings A and the nitrogen atoms in the rings B of neighboring molecules. The hydrogen bonding upon formation of TPT dimer will lead to additional retaining the proton at the hydroxy group, i.e., to increase in its pKa. In its turn, this decreases the share of TPT molecules with deprotonated hydroxy group and weakens the salt bridge between the oxygen of deprotonated hydroxy group of A ring and the protonated dimethylaminomethylene group that, in turn, reduces pKa of the latter. We observe both the effects upon growth of TPT concentration in solution. This confirms our assumption that the hydroxy group of the ring A takes part in the formation of intermolecular hydrogen bond, which stabilizes TPT dimer.

It should be noted that the formation of TPT dimers at pH 6.8 proceeds in spite of the fact that both the monomers are positively charged due to protonation of the dimethylaminomethylene group under these conditions.

The hydroxy group of the ring A is deprotonated in part at pH 6.8. Therefore it could form intermolecular hydrogen bond only in the TPT molecules where it is neutral. The shift of pH to the higher values, which increases the degree of deprotonation of this hydroxy group, will decrease the content of TPT dimers. And on the contrary, a decrease in pH value reduces the deprotonation degree and will increase initially the concentration of the dimers. Further decrease in pH will result in the protonation of N1 atom that will prevent the existence of these intermolecular hydrogen bonds and hence the dimers of this kind.

Supposed structures of TPT dimers. The above results provide an opportunity to propose a model of dimer formed by TPT molecules. According to X-ray diffraction data [19], CPT monomers having no hydroxy group in the A ring form a dimer with virtually complete overlapping of rings A–D.

We suppose TPT molecules to form another type of dimer which is stabilized by two hydrogen bonds between the hydroxy group of the ring A of one monomer and the N1 atom of the ring B of another monomer as well as by the stacking interaction of the A rings of both the monomers. According to the proposed model of TPT dimer, its formation constant is substantially lower than that of CPT dimer owing to lesser stacking, repulsion of positively charged dimethylaminomethylene groups, and the presence of TPT molecules with deprotonated hydroxy group in the A ring in solution. The lesser dimerization constant corresponds to the better solubility of this compound as compared with CPT [17, 18].

Since the ring E of TPT molecule is not flat, the TPT dimer stabilized by the above hydrogen bonds can exist in three forms shown in Figs. 5a–5c: (1) the rings E of the monomers are directed out of the dimer, (2) the rings E of the monomers are directed into the dimer, (3) the rings E of the monomers are directed to the same side. The structures of dimers presented in Figs. 5a and 5b look the same if observed from the direction perpendicular to the plane of the chromophores A–D (Fig. 5d). Figure 5e shows the view of the structure 5c from the same direction. Qualitative difference for these two dimers has engaged our attention. The dimers shown in Figs. 5a and 5b have twofold symmetry axis passing between the planes of component TPT monomers. The structure shown in Fig. 5c has only helical symmetry axis perpendicular to the plane of ring A and passing through its center. We think all three structures of dimer to coexist in solution under our conditions.

Calculation of electron transition dipole moments of TPT molecule. Quantum chemical calculation was performed for further elucidation of properties of TPT molecule. The comparison of calculated wavelengths of the main electron transition dipole moments with experimental values for TPT molecule in different ionic states is presented in the table. The strength of corresponding oscillator, which is proportional to the intensity of absorption in this band, is given within parentheses. The shift of the positions of calculated absorption maxima to the short-wavelength region relative to the experimental values is due to the effect of polar environment of TPT: all measurements was accomplished in aqueous solutions, whereas the calculations were conducted for TPT molecule in vacuum. Nonetheless, the character of changes in the calculated positions of the bands in absorption spectra conforms to the observed data.

According to the model proposed above, the charge of TPT molecule is +3 at very low pH < pKa_1. The nitrogen atom N1 and the nitrogen atom of dimethylaminomethylene group are protonated in this state. Two structures could exist when N4 atom is protonated: with a proton at the N4 atom or a proton at the oxygen atom that transforms the keto group at C16a into hydroxy group. A double bond between N4 and C16a atoms arises in the latter event that leads to complete aromatization of the ring D. According to calculation, the second structure is more probable. The calculated position of the long-wavelength absorption band for this ionic state of TPT molecule is 391 nm (experimental value is 420 nm).

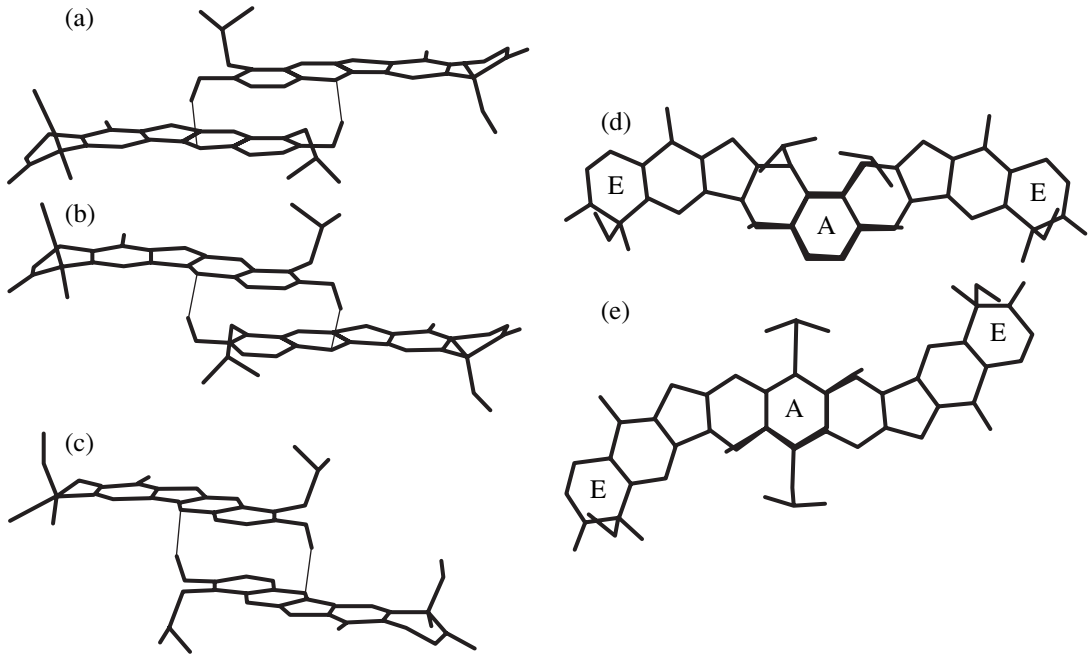

Fig. 5. Supposed structures of TPT dimers: rings E of monomers are turned out of dimer (a) and into dimer (b), the E rings of monomers are turned to the same side (c). Thin lines show possible intermolecular hydrogen bonds. The projections of dimer structures presented in (a) and (b) on the plane of chromophores A–D coincide (d). The same projection of structure (c) is shown in (e). Letters in the rings mark the rings A–E.

The growth of pH leads first of all to the deprotonation of N1 atom. This causes hypsochromic shift of the long-wavelength absorption band to 380 nm (calculated value is 348 nm).

According to calculation, the elimination of one additional proton results in the transformation of hydroxy group at C16a into keto group. In so doing, the double bond between N4 and C16a atoms becomes ordinary one. This is accompanied by a slight bathochromic shift of the long-wavelength band (from 348 to 352 nm) that was detected experimentally. If the proton is located at the N4 atom, the deprotonation would occur with hypsochromic shift of the absorption band from 361 to 352 nm, but it is inconsistent with experimentally observed bathochromic shift from 380 to 382 nm. As compared with previous ionic state of TPT molecule, the experimental magnitude of the shift is negligible but detected reliably. This confirms the results of calculation on the preferable existence of structure where proton is located at the oxygen atom at C16a rather than at the N4 nitrogen atom.

The further raise of solution pH to the pKa_3 increases the content of TPT with deprotonated hydroxy group in the A ring. We have considered two states of TPT molecule with deprotonated hydroxy group in the A ring: lactone form (neutral) and carboxylate form (molecular charge –1). As it was noted above, the transformation from lactone into carboxylate takes place already at pH 7.3, i.e., the presence of both forms is possible at pH ~ pKa_3. Our calculations predict a bathochromic shift of the long-wavelength band to 420 nm (lactone) or to 390 nm (carboxylate). The experimental position of this band at 412 nm seems to be a superposition of these two values, which indicates the presence of both TPT forms under these conditions.

And finally, deprotonation of the nitrogen atom of the dimethylaminomethylene group proceeds at high pH ~ pKa_4. The calculation predicts a further bathochromic shift of the long-wavelength band from 420 to 435 nm, which corresponds to experimental shift from 412 to 421 nm.

It should be also noted that the results of calculations of changes in the position of short-wavelength absorption bands also correlate with experimental data presented in the table.

Thus, the theoretical estimates confirm completely the postulated character of alteration in the ionic state of TPT upon variation of solution pH. As it was noted above, TPT molecules with hydroxy group in ring A in neutral and ionized (deprotonated) states prevail in solution at pH 6.8. Both forms contain a protonated dimethylaminomethylene group. We have calculated the orientation of dipole moments of electron transitions for these two ionic forms of TPT molecule. The projections of vectors of dipole moments for these electron transitions are presented in Figs. 6a and 6b, respectively.

Since TPT has a number of substituents disposed out of the plane of rings A–D, the vectors of dipole moments of electron transitions of TPT form, in the majority of cases, a small angle with the plane of rings A–D. The magnitude of this angle is given in parentheses. The sign "+" signifies that both the vector and the ring E deviate to the same side from the plane of rings A–D.

The analysis of mutual orientation of dipole moments of long-wavelength electron transitions for possible dimer conformations shows that, if rings E of monomers are oriented to the same side (Fig. 5c), the vectors of dipole moments of electron transitions are virtually parallel, whereas the angle between vectors is ~160° for two other conformations (Figs. 5a and 5b). The noticeable CD signal in this spectral region indicates the presence of TPT dimers in solution as the structures shown in Figs. 5a and 5b.

We have determined the main features of TPT behavior depending on environment, conditions of dimer formation, and probable dimer conformations. The latter seems to be very important, since recent Raman spectral studies [20] showed the equilibrium between monomeric and dimeric forms of TPT to be shifted to dimeric form in the presence of DNA. This means that the affinity of TPT dimers to DNA is higher than that of monomers. That is, the dimers play the decisive role in TPT binding to DNA.

Thus, our study is a necessary stage in understanding the molecular mechanisms of TPT–DNA interac-

Main absorption bands at different ionic states of TPT molecule

No.	State of TPT molecule	Absorption bands	
		Calculated[a], λ, nm (oscillator strength)	Experimental[b], λ, nm
1.	The nitrogen atom of dimethylaminomethylene group and the nitrogen atoms at N1 and N4 positions are protonated. Charge of molecule Q = +3.	391 (0.68)	420
		259 (0.52)	267
		214 (0.54)	223
2.	The nitrogen atom of dimethylaminomethylene group and the oxygen atom at C16a are protonated. Charge of molecule Q = +2.	348 (0.78)	380
		313 (0.03)	327
		250 (0.62)	267
		218 (0.43)	223
3.	The nitrogen atom of dimethylaminomethylene group is protonated. Charge of molecule Q = +1.	352 (0.80)	382
		301 (0.09)	327
		249 (0.31)	267
		220 (0.17)	223
4.	The nitrogen atom of dimethylaminomethylene group is protonated, the hydroxy group in ring A is deprotonated. Q = 0.	420 (0.84)	412
		351 (0.24)	340
		272 (0.21)	282
		262 (0.25)	No
		223 (0.51)	227
5.	The nitrogen atom of dimethylaminomethylene group is protonated, the hydroxy group in the ring A is deprotonated, the lactone ring is hydrolyzed (carboxylate form). Q = –1.	390 (0.68)	412
		335 (0.44)	340
		271 (0.15)	282
		242 (0.56)	No
		220 (0.41)	227
6.	The hydroxy group in ring A is deprotonated, the lactone ring is hydrolyzed (carboxylate form). Q = –2.	435 (0.67)	421
		352 (0.30)	345
		276 (0.59)	282
		259 (0.26)	No
		222 (0.52)	227

[a] Calculations were made by the method INDO/S without regard for solvent effect. Nineteen occupied and nineteen free orbitals were taken into account in the calculation of configurational interactions (the total number of configurations was 723).

[b] Experimental values were determined at concentration [TPT] = 10^{-6} M.

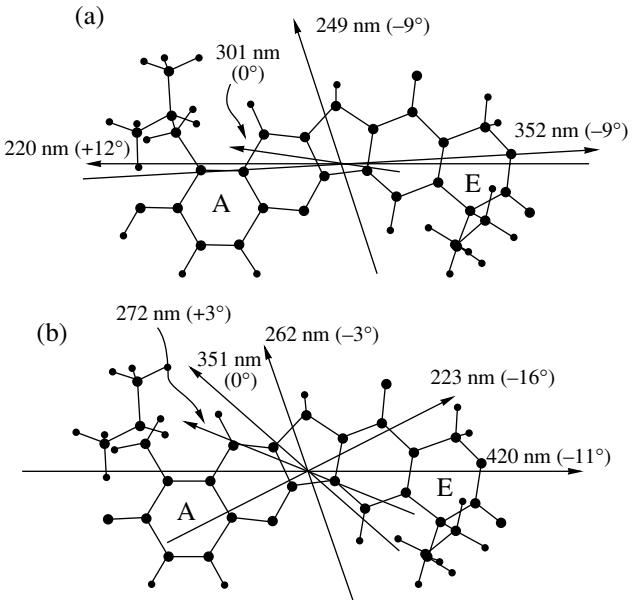

Fig. 6. The projections of vectors of transition dipole moments on the plane of the ring system A–D of TPT molecule with protonated nitrogen atom of dimethylaminomethylene group: (a) with hydroxy group in the ring A; (b) with deprotonated hydroxy group in the ring A. The numeric values show wavelengths corresponding to electron transition and angles formed by the vectors with the plane of the ring system A–D of TPT molecule. Sign "+" corresponds to deviation of the vector to the same direction as the lactone ring E.

tion, formation of ternary complexes TPT–DNA–topoisomerase I, and TPT-induced inhibition of topoisomerase I interaction with DNA.

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