Optical spectroscopy methods have been used to study the interaction between the plant alkaloids – berberine, sanguinarine, and chelidonine – of the antitumor drug amitozine and the DNA in the aqueous solution. The corresponding electron spectra of absorption, fluorescence excitation, and fluorescence have been obtained and analyzed for free alkaloids and in the mixture with DNA. The hypochromic effect has been observed in the absorption spectra of berberine bound with DNA. The addition of DNA molecules to the solution of alkaloids was found to enhance the fluorescence intensity of berberine and to decrease that of sanguinarine and chelidonine. The fluorescence polarization is nonzero for all the three alkaloid–DNA mixtures. Those facts are the spectral manifestations of the alkaloid–DNA interaction. Possible mechanisms of DNA–alkaloid complex formation in aqueous solutions have been discussed.

1. Introduction

Interaction between small natural molecules and DNA finds a wide application spectrum, such as medical preparations (in particular, antineoplastic and immune-response modulating ones), fluorescent probes, and so forth. The creation of low-toxic drugs with a wide therapeutic effect is a challenging task for modern medicine. It is difficult enough to understand the mechanism of therapeutic functioning of preparations without studying the interaction between these compounds and DNA at the molecular level. At the way to achieve this purpose, more and more important become the researches of macromolecule–DNA interaction making use of low-molecular-ligand methods in the framework of optical spectroscopy. In this work, our investigations were directed at elucidating the origin of phenomena that occur, when DNA interacts with main alkaloids of amitozine–berberine, sanguinarine, and chelidonine. Amitozine is an antineoplastic and immune-response modulating preparation obtained at the Institute of Molecular Biology and Genetics (IMBG) of the National Academy of Sciences of Ukraine by alkalizing the celandine alkaloids with triethylenethiophosphoramide [1]. The majority of celandine alkaloids belong to the isoquinoline group. The isoquinoline backbone is a base structural block for structures of various types, such as benzophenantridines (sanguinarine, chelidonine, and others), protoberberines (berberine), protopines, and many others. In Fig. 1, the structural formulas of studied molecules are depicted. The reactivity of alkaloids of this type is explained by the sensitivity of imine bond C=N⁺ to nucleophilic reagents [2].

Berberine has been used in medicine long ago to treat a wide spectrum of diseases. Its preparations attract the enhanced interest of researchers, because a wide spectrum of effective antineoplastic actions through influencing the metabolism of cancer cells and their unrecoverable destruction has been detected. At the same time, berberine preparations have rather a low toxicity, and the cured organism develops the corresponding immunity [3, 4]. As to sanguinarine, the bactericidal, anti-inflammatory, and antineoplastic effect [5] of this biologically active alkaloid has been proved. A lot of physical experiments to study the interaction between berberine and sanguinarine, on the one hand, and DNA, on the other hand, have been carried out (see review [6]). Chelidonine is also included into antineoplastic preparations [7] on the basis of alkaloids of this type; however, detailed researches of the interaction between chelidonine and DNA by physical methods have not been carried out.

Three types of noncovalent binding of low-molecular ligands with nucleic acids (NAs) are known [2]:

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Three types of noncovalent binding of low-molecular ligands with nucleic acids (NAs) are known [2]:
Fig. 1. Structural schemes of molecules under investigation: berberine (a), sanguinarine (b), and chelidonine (c)

1. External binding, i.e. the formation of electrostatic contacts with the sugar-phosphate backbone of DNA. The mechanism of electrostatic binding with the external part of DNA is mainly realized for cation molecules which do not contain \( \pi \)-electron systems. This mechanism is active at the first stage of the molecule binding to DNA, and they interact via the groove or the intercalation mechanism.

2. Arrangement in the DNA minor groove. The binding of this type is the most selective with respect to the sequence of DNA base pairs, owing to specific hydrogen bonds with the latter and with the DNA backbone. The main stabilization factor for the complexes of such a type is hydrogen bonds between the ligand amide groups and the acceptor groups of nucleotide bases, in particular, between atoms N3 of adenine and atoms O2 of thymine.

3. Intercalation, i.e. an establishment of long and somewhat specific van der Waals bonds between the ligand molecules and DNA. The interaction between ligands and DNA through the intercalation mechanism is characteristic of flat aromatic molecules which are located between neighbor DNA base pairs, being stabilized due to the van der Waals interaction between the \( \pi \)-electron clouds of aromatic rings of the ligand and nucleotide bases.

Protoberberines are known to bind with DNA, but the physical mechanisms of complex formation have not been ultimately determined. According to the results of NMR researches [8], berberine partially intercalates into DNA. This conclusion was supported – in particular, by computer simulation – in other works (see review [6]) as a phenomenon that is common for protoberberines. However, the analysis of fluorescence spectra in work [9] testifies that it more probably occupies a DNA groove rather than intercalates into DNA. On the contrary, it was shown in work [10] that protoberberines are intercalators. Recent NMR researches [11] of the interaction between desoxoyoligonucleotides and berberine exclude the intercalation at all as a probable type of binding with DNA: it was demonstrated that berberine is located in the minor groove of the DNA double helix at the level of A4-T7 and A5-T6 base pairs. As a consequence, no final conclusions on the mechanism of interaction between berberine and DNA were made in review [6].

In general, alkaloids of the isoquinoline type interact with DNA either as intercalators, or they are located in its minor groove. In so doing, sanguinarine interacts more effectively with pairs G–C, whereas berberine with pairs A–T [11–17].

Nevertheless, the physical mechanisms of interaction between amitozine alkaloids and DNA still remain undetermined in general. Our work continues researches of the interaction between isoquinoline alkaloids and DNA. We present the results obtained by optical spectroscopy methods. The spectra of optical absorption, fluorescence excitation, and fluorescence are analyzed both for the aqueous solutions of alkaloids and for their mixtures with DNA. Conclusions on the interaction between main amitozine alkaloids and DNA are drawn, and an assumption on the binding type is made.

2. Experimental Results and Their Analysis

2.1. Specimens and experimental technique

We used the following alkaloids in powder form supplied by the IMBG: berberine hydrochloride (BeCl), berberine sulfate (BeS), sanguinarine dihydrochloride (Sa), and chelidonine hydrochloride (Che). Since these alkaloids dissolve badly in water at room temperature, their aqueous solutions were prepared at a temperature of about 60–70 °C (at this temperature, the alkaloids concerned are not destroyed). Their concentration was
12.5–100 μM for photoluminescence measurements and 12.5–50 μM for absorption measurements. The concentrations were rather low, so that the reabsorption or concentration effects were insignificant. We used DNA from chicken erythrocytes (product of “Serva”, Germany), the length was more than 550000 nucleotide pairs, and the molar mass was about 40000 kDa). The average molar mass of two base pairs is about 1300 Da. For the majority of measurements, the mixed solutions were prepared with the relative concentration of one alkaloid molecule per two DNA base pairs (such a concentration was selected to make the number of binding sites maximal). For the better registration of long-wave absorption bands of berberine, a solution with the relative concentration \( N = 1 : 8 \) (1 molecule of alkaloid per 8 base pairs) was taken additionally. Measurements were carried on at room temperature.

Absorption spectra were registered on a “Specord UV VIS” spectrophotometer in the range 200–700 nm. The spectral resolution was 1 nm. The fluorescence spectra were obtained with the help of a “Cary Eclipse” fluorometer. The slit width for fluorescence measurements was 5 nm, which provided a sufficient resolution for given experiments. Standard quartz cuvettes 1 cm in thickness were used in experiments. The fluorescence spectra for registration were excited either by far ultraviolet (\( \lambda \sim 300 \) nm) or by light with the wavelengths corresponding to the positions of maxima in the fluorescence excitation (FE) spectra recorded previously.

### 2.2. Absorption and photoluminescence

#### 2.2.1. Berberine

**Absorption.** The optical absorption spectra of isoquinoline alkaloids are known to be caused by the absorption of alkaloid \( \pi \)-electron systems associated with available auxochromous (OCH\(_3\), OH, NH\(_2\)) groups.

Fig. 2, (a) Absorption spectra of berberine (BeCl) and DNA+berberine mixture, their difference spectrum (for \( N = 1 : 2 \)), and the absorption spectrum of DNA normalized to the difference one. (b) Fluorescence excitation and fluorescence spectra of berberine and DNA+berberine mixture. The alkaloid concentration is 12.5 μM. \( N=1:2 \) and 1:8 (one alkaloid molecule per two or eight base pairs).

![Absorption spectrum of berberine and DNA+berberine mixture](image1)

Table 1. Positions of maxima (in nm units) in the optical absorption spectra of studied alkaloids in the free state and in the mixture with DNA

<table>
<thead>
<tr>
<th></th>
<th>BeCl</th>
<th>BeCl+DNA</th>
<th>Sa</th>
<th>Sa+DNA</th>
<th>Che</th>
<th>Che+DNA</th>
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</thead>
<tbody>
<tr>
<td>( \lambda_{max} )</td>
<td>426</td>
<td>*440</td>
<td>475.406</td>
<td>485.414</td>
<td>291</td>
<td>286</td>
</tr>
<tr>
<td>( \lambda_{max} )</td>
<td>348.340(s)</td>
<td>*351.337(s)</td>
<td>329.350(s)</td>
<td>335.355(s)</td>
<td>241</td>
<td>245</td>
</tr>
<tr>
<td>( \lambda_{max} )</td>
<td>265.276(s)</td>
<td>264.277(s)</td>
<td>275.286(s)</td>
<td>260.272(s)</td>
<td>229.230(s)</td>
<td>228.239(s)</td>
</tr>
</tbody>
</table>

Footnote: “s” means band “shoulder”. \( N=1:2 \), \( \ast N=1:8 \)
molecules are added to the alkaloid solution, the optical
density decreases in the range 300–500 nm, and the
positions of first (long-wave) absorption maxima become
shifted toward the long-wave side (from 3 to 20 nm,
as the relative concentration of DNA grows). In the
difference spectrum, the maximum of DNA absorption
manifests itself pronouncedly in the UV-region at
260 nm.

Noticeable dips of the intensity in the difference
spectrum at 350 and 425 nm testify to hypochromism
which arises, in our opinion, as a result of the
dipole-dipole interaction between π-electron systems of
berberine molecules which are built into the DNA
matrix. Such an effect can be observed, if DNA interacts
with flat molecules, whose aromatic ring planes are
coplanar to one another. Such an arrangement is
possible, if alkaloid molecules are built in between DNA
base pairs, i.e. if the interaction is of the intercalation
type.

One of the intensive absorption bands of berberine
(at λ ~ 348 nm) is located in the range of DNA
fluorescence (the maximum at λ ~ 350 nm). This
means that the overlap integral of those bands can
be rather large, which, following Förster, evidences
for an opportunity of dipole-dipole resonance energy
transfer (here, from DNA to berberine) at the interaction
between π-electron systems of molecules. In addition,
the positions of the absorption UV-bands of berberine
(at about 265 nm) and DNA (about 260 nm) practically
coincide. A possibility of effective energy transfer also
testifies in favor of the intercalation model.

Hence, the absorption spectra evidence for the
intercalation of berberine into DNA. But, since the
berberine molecule is bent [25,26] and its van der Waals
thickness is larger than 3.5 Å, the complete intercalation
of a berberine molecule into DNA is very complicated.
We believe that only a partial intercalation of the flat
section of a berberine molecule (rings A and B) is
realized.

Fl uorescence. In Fig. 2b, the fluorescence
excitation and fluorescence spectra of berberine and
its mixture with DNA are depicted. The fluorescence
excitation and absorption spectra are rather similar,
which evidences for a common nature of radiation
absorption and emission centers.

The fluorescence spectrum of berberine extends over
the interval 300–800 nm. It consists of two adjacent,
practically unresolved intense bands with the maximum
at about 555 nm. From the comparison between the
position and width of this band and the relevant
parameters of the first absorption band, it follows that it
corresponds to the first electron level. A weak short-wave
band is also observed at 315–325 nm. The excitation
spectra of the short- and long-wave fluorescence bands of
berberine differ substantially from each other, which can
testify to different origins of luminescence centers. The
band shapes and the maximum positions are somewhat
different for BeCl and BeS (Table 2,a).

The fluorescence spectra of the alkaloid solution,
when DNA is added, are characterized by an
enhancement of the radiation emission intensity by a
factor of 10–20, depending on the exciting radiation
wavelength, solution concentration, and specimen
type in comparison with that of free berberine
(Table 2,a). The maxima in the excitation spectra of the
berberine+DNA mixture are shifted toward long waves
by 5–20 nm for various bands (except for the band at
about 260 nm) in comparison with the maxima for pure
berberine, which agrees with modifications in absorption
spectra. The growth of the fluorescence intensity, when
DNA is added to the berberine solution, evidences for its
binding with DNA. The intensity growth is associated
with the fact that the number of freedom degrees and,
respectively, the number of ways for the excitation to

<table>
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<tr>
<th><strong>Table 2.a.</strong> Fluorescence intensities and intensity ratios for berberine and its mixture with DNA (the berberine concentration of 12.5 μM) for fluorescence maxima at various excitations (the numbers in brackets are the excitation wavelengths in nm units)</th>
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<tr>
<td>alkaloid</td>
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<tr>
<td>alkaloid+DNA</td>
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<tr>
<td>(k(I_{alk+DNA}/I_{alk}))</td>
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<tr>
<th><strong>Table 2.b.</strong> Fluorescence intensities and intensity ratios for sanguinarine, chelidonine, and their mixtures with DNA for fluorescence maxima at various excitations (the numbers in brackets are the excitation wavelengths in nm units)</th>
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<tr>
<td></td>
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<tr>
<td>alkaloid</td>
</tr>
<tr>
<td>alkaloid+DNA</td>
</tr>
<tr>
<td>(k(I_{alk+DNA}/I_{alk}))</td>
</tr>
</tbody>
</table>
relax without radiation emission diminish at the complex formation, so that the quantum yield of fluorescence increases [17, 18]. Moreover, the maximum of the fluorescence band for the berberine+DNA mixture is shifted by 20–25 nm toward the short-wave region with respect to the fluorescence band maximum of free alkaloid, which also testifies to the interaction between berberine and DNA.

The general behaviors of BeCl and BeS have little differences in the described experiments.

2.2.2. Sanguinarine

A b s o r p t i o n. The optical absorption spectrum of sanguinarine consists of five complex bands (Fig. 3, a). If DNA is added to the sanguinarine solution, the intensity of absorption grows, and the bands shift substantially. The long-wave maxima at 475 and 406 nm and the doublet at 329 and 350 nm move toward the long-wave side of the spectrum (by up to 10 nm). The bands in the short-wave region (at 275–286 and 208 nm) are difficult to analyze owing to their complicated structure and the overlapping with the DNA absorption band. In the difference spectrum – as it was for berberine – the DNA absorption band reveals itself at 260 nm; in addition, an extra band at 292 nm and a doublet at 340 and 360 nm appear, which is a consequence of long-wave shifts of absorption maxima. Hence, the absorption spectrum of the mixture of sanguinarine with DNA is not a sum of two spectra, which evidences for the formation of the sanguinarine–DNA complex.

In general, the sanguinarine absorption is caused by the $\pi$-electron system of the molecule as a whole, rather than the $\pi$-systems of separate rings, as it takes place, e.g., for chelidonine (see below).

F l u o r e s c e n c e. The spectrum of sanguinarine fluorescence excitation (Fig. 3, b) consists of four bands and corresponds to the absorption spectrum. If DNA is added to the sanguinarine solution, the spectrum shifts toward long-waves. Two bands manifest themselves – at 420 and 587 nm – in the fluorescence spectrum of sanguinarine. The excitation spectra of those bands are different, which testifies to different origins of fluorescence centers. The 587-nm band is associated with the first electron level. In work [19], the existence of sanguinarine in two forms – imine (I) and alkanolamine (II) – was discussed. The key factor for that or another form to exist is the medium (solvent) acidity. Sanguinarine I (pH = 1 ÷ 6) possesses an effective positive charge; it binds with DNA, with selectivity to G–C pairs. Sanguinarine-II form (pH = 8.5 ÷ 11) is neutral, and it is asserted that this form does not interact with B-DNA at all. Form I has a fluorescence maximum at 577 nm observable at any excitation, whereas form II at 418 nm, and this maximum manifests itself only at the 327-nm excitation. Comparing these data with ours (taking the absorption spectra into account as well), we may assert that both forms of sanguinarine are present in our experiments (our pH $\approx$ 7).

It follows from our and literature [5] data that the growth of alkaloid concentration – from 1 to 3 mg/l in
work [5], and from 25 to 100 μM (from 10 to 40 mg/l) in our experiments – is accompanied by a reduction of the intensity of the band at 420 nm and by an increase of the intensity of the band at 587 nm (in work [5], the ratio between those intensities is inverse to ours). Most probably, this intensity redistribution between fluorescence bands that correspond to two forms of sanguinarine is a simple consequence of the reabsorption (the 420-nm band is in the range of the appreciable absorption), so that it is difficult to talk about a transformation from one form into another.

If DNA molecules are added, the fluorescence band at 587 nm shifts by 7 nm to the short-wave side, and the radiation emission intensity becomes 2.9–3.1 times lower (Table 2). The peak at 420 nm observable for the mixture of alkaloid with DNA shifts more considerably, by about 25 nm, but toward the long-wave range. One can hardly say anything on the intensity variation of this band, because it falls within the sanguinarine absorption range. Such a significant shift of the short-wave band contradicts the data of work [17]. Probably, sanguinarine II does interact with DNA.

The reduction of the sanguinarine fluorescence intensity in the presence of DNA may also be due to the formation of exciplexes, the quantum yield of which is relatively low (of about 0.1%).

2.2.3. Chelidonine

Absorption. The absorption spectrum of chelidonine falls within the range λ ≤ 300 nm, which also contains the absorption spectrum of DNA. It consists of two wide bands with the maxima at 241 and 291 nm (Fig. 4,a). The fact that chelidonine absorbs in a shorter-wave range than berberine and sanguinarine is caused by peculiarities of the chelidonine structure. Namely, since rings B and C are nonsaturated, the nonconjugate π-systems of rings A and D absorb as individual centers, giving rise to the appearance of the bands at 241 and 291 nm. If DNA molecules are added to the alkaloid solution, the absorption bands shift. The difference spectrum (Fig. 4,a) gives a wide band with a maximum at 260 nm, which coincides with the normalized absorption spectrum of DNA almost within the whole absorption range under investigation except for the interval of 280–310 nm, where the absorption band of chelidonine is located. That is, the band shift effect cannot be considered in this case as a result of the interaction with DNA. Only the indicated region of 280–310 nm may serve as an evidence for a weak interaction between chelidonine and DNA. The spectral indications of energy transfer are also absent. Hence, the absorption spectra allow us to say that the interaction between chelidonine and DNA in the aqueous solution is very weak.

Fluorescence. The fluorescence excitation spectra of chelidonine (Fig. 4,b) include three complicated bands with the maxima of comparable intensity at about 212, 237, and 289 nm. The excitation spectrum agrees with the absorption one, which evidences for a common nature of absorption and
radiation centers. The fluorescence excitation spectra of the chelidonine+DNA mixture demonstrate the same bands, but slightly shifted; however, their intensity is 1.4 to 1.8 times lower (Table 2).

The fluorescence spectrum of chelidonine reveals a band at 326 nm, the fluorescence spectrum of the chelidonine+DNA mixture is the same, differing only in intensity (1.7 times lower). The Stokes shift is very small (≤1 nm), which may evidence for a weak interaction.

From common reasons and on the basis of the fluorescence spectrum analysis, a conclusion can be drawn that chelidonine weakly interacts with DNA. A reduction of the fluorescence intensity can be a result of this weak interaction.

2.2.4. Analysis of the results

Following [20], we found the intersection of the fluorescence band and the first absorption band (expressed in terms of $I/\nu^4$ and $\alpha/\nu$ units, respectively) to determine the energy of the first electron transition $0$–$0$ and the next electron-vibrational transitions for all alkaloids concerned and their mixtures with DNA (Table 3).

We also measured the polarized fluorescence of the aqueous solutions of alkaloids and their mixtures with DNA. The degree of fluorescence anisotropy $A = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$ was determined taking the correction $G$-factor into account. The degree of anisotropy for pure alkaloid was found to be practically zero. For the alkaloid+DNA mixtures, it equals about 0.12 for berberine, about 0.1 for sanguinarine, and about 0.02 for chelidonine. This result testifies that the alkaloids bind with DNA, because the viscosity of the solutions remained practically unchanged. Note that the results obtained for chelidonine are less reliable, owing to the low intensity of light after passing through polarizers (it is the far ultraviolet range). Polarization studies of the interaction between alkaloids and DNA will be described in details elsewhere.

Note that we have not registered new radiation emission bands in the fluorescence spectra of the alkaloid+DNA mixture. This means that we dealt only with ligand monomers, whereas dimers and other complexes were not formed at the concentrations used.

For the analysis of the results obtained to be more complete, we should consider – in addition to the absorption and fluorescence spectra – the Raman spectra as well. The manifestations of the interaction between berberine and DNA in Raman spectra were described in our works [21, 22] in detail. A considerable growth of the intensity was found for lines that corresponded to vibrations of both DNA and berberine in the berberine+DNA mixture. It looks like a manifestation of the resonant interaction between DNA and berberine molecules just in the range, where the spectra of those molecules overlap (1100–1700 cm$^{-1}$). According to the data of work [29], the resonant interaction between intramolecular vibrations of neighbor optical centers is possible at distances up to about 6 Å, i.e. when those centers are located close enough to one another. We think that the resonant amplification of Raman spectra evidences for the intercalation type of the binding of berberine and DNA.

3. Final Remarks and Conclusions

An enhancement or a reduction of the alkaloid fluorescence intensity, which accompanies the addition of DNA to the solution, is governed, first of all, by a variation of the number of radiationless relaxation channels and by a capability to transfer energy (see, e.g., our [18, 21] and literature [23, 24] data). A conclusion can be also drawn that the fluorescence of isoquinoline alkaloids is inhibited (quenched) for those of them which

| Table 3. Energies of electron transition 0–0 and higher electron-vibrational transitions in alkaloids and their mixtures with DNA (E × 10$^{-3}$, cm$^{-1}$) |
|------------------------------------------|--------------|---------|---------|---------|---------|
| Alkaloid | E(0–0) | E | E | E |
| Be | 20.76 | Cl | 23.48 | 28.67, 29.4(s) | 36.1(s), 37.72 | 42.1(s), 43.58 |
| Be | 20.78 | S | 23.46 | 28.60, 29.3(s) | 36.0(s), 37.70 | 42.2(s), 43.58 |
| Be+DNA | 20.70 | Cl | 23.52 | 28.76, 29.5(s) | 36.2(s), 37.85 | 42.2(s), 43.72 |
| Be+DNA | 20.49 | S | 23.22 | 28.50, 29.4(s) | 35.9(s), 37.58 | 42.4(s), 43.52 |
| Sa | 18.78 | 20.8, 24.5 | 28.5(s), 30.35 | 35.1(s), 36.1 | 47.9 |
| Sa+DNA | 18.74 | 20.5, 23.8 | 28.3(s), 29.75 | 36.0(s), 37.9 | 47.7 |
| Che | 32.84 | 34.34 | 41.28 |
| Che+DNA | 32.82 | 34.89 | 40.59 |

Note: “s” means band “shoulder”.


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interact mainly with GC-pairs of DNA and enhanced for those interacting with AT-pairs. Such a behavior may testify in favor of different mechanisms of binding of alkaloids possessing the structure of protoberberine or phenanthridine with different DNA base pairs. The DNA sequences in the G-C sections have a smaller minor groove, which complicates their groove binding with ligands. The minor groove of DNA sections enriched with A-T pairs has a small negative charge, which strengthens the binding of those sites with positively charged molecules of some alkaloids. Since the sanguinarine molecule is flat and the molecules of berberine and chelidonine are bent a little – it is so, because they include one and two unsaturated rings, respectively [25,26] – the intercalation of the latter two is complicated in comparison with that of sanguinarine. However, new opportunities for other types of binding with DNA emerge in this case. These opportunities are evidently realized for berberine, the molecule of which, being flat enough, has a crescent shape, which promotes the groove binding. This agrees with our results as well, which demonstrate more pronounced manifestations of the binding of berberine with DNA in the optical spectra in comparison with those for sanguinarine and chelidonine, as well as the manifestations of different types of the binding of berberine with DNA. In particular, manifestations of the hypochromism and the resonant vibration interaction in the Raman spectra evidence for the intercalation mechanism. Our previous computer simulations showed that berberine, while binding with DNA, is located in its minor groove, though the binding with phosphatic groups (external stacking) cannot be excluded.

It is worth to recall one of the first works on the study of mechanisms of the binding of berberine with DNA [27], where berberine was shown to intercalate into DNA partially and not following the classical mechanism. Later on, the mechanism of partial intercalation as a type of protobberine binding was studied in works of the Indian group (Maiti, Kumar, Das, and others (see work [6])). However, in recent works, the intercalation or groove binding is either denied or proved. In our opinion, berberine binds with DNA in two ways, and which mechanism dominates depends on a number of factors still unknown. Probably, the type of the binding of berberine with DNA depends on the relative concentration P/D (nucleotide Phosphate/Drug ratio) [28]. A detailed study of how different mechanisms of the binding of berberine reveal themselves spectrally and how the P/D ratio and other concentration dependences affect those manifestations is our next task. In general, a conclusion can be drawn that berberine is the main amitoxine alkaloid that binds with DNA most effectively.

Note that no new radiation emission bands were registered in the fluorescence spectra of the alkaloid+DNA mixture. This means that we dealt only with ligand monomers, and that dimers and other complexes were not formed at the concentrations used.

As was indicated above, the absorption and fluorescence spectra of chelidonine testify that it interacts weakly with DNA. It is difficult to make a conclusion on the mechanism of interaction. We can only mark a certain similarity between the spectral behaviors of chelidonine and sanguinarine. As the component of amitoxine, chelidonine effectively affects the whole cell rather than DNA only. The apoptotic effect of chelidonine has been recently proved by biological methods [28].

Thus, in this work, making use of optical spectroscopy methods, we showed the following.

– Isoquinoline alkaloids interact with DNA. The mechanisms of binding with DNA can be different for protoberberine (berberine) and benzophenantridine (sanguinarine, chelidonine) structural types. According to optical manifestations, berberine binds with DNA most effectively.

– Berberine reveals itself as an intercalator (may be, partial) at the interaction with DNA. However, the groove binding is also possible.

– Spectral manifestations evidence for the intercalation type of the interaction between sanguinarine and DNA.

– Spectral manifestations of the interaction between chelidonine and DNA are insignificant. The alkaloid interacts with DNA very weakly.