Redox reactions of natural alkaloid lappaconitine

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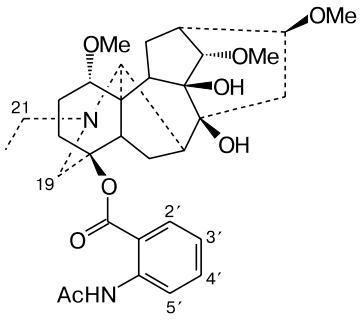
A review on redox reactions of natural alkaloid lappaconitine, a known sodium channel blocker, is presented. The NMR and CIDNP data on the mechanism of phototransformation of lappaconitine, in particular, of its paramagnetic species formed by both the direct photolysis and photoinitiated interaction with electron donors and acceptors, are analyzed. Special attention is given to the interaction of lappaconitine with amino acids, which are present in the active site of the sodium channel. Hypotheses about a relationship between this process and the mechanism of therapeutic activity of lappaconitine are discussed.

Key words: lappaconitine, electron transfer, radicals, photodecomposition, phototoxicity, chemically induced dynamic nuclear polarization, supramolecular complexes, glycyrrhizic acid, amino acids.

Introduction

Lappaconitine (**1**), *viz.*, alkaloid isolated from aconite (*Aconitum septentrionale* Koelle), is used in medical practice as a hypotensive drug and for treating arrhythmia (its commercial name is alapinin).[1,2] Interest of researchers in photoinduced transformations of biomolecules and reactions of their paramagnetic species is due to the high reactivity of free radicals formed under irradiation and the related toxic effect of many drugs.[3] It is known that many popular hypotensive preparations induce photodermatitis of patients subjected to solar radiation because paramagnetic species of these preparations are formed in the epidermis and deeper cutaneous layers.[4] According to modern concepts,[5] secondary active oxygen-containing free radicals formed upon the interaction with radical forms of the drugs can induce many serious diseases, including cancer and atherosclerosis, and aging of the organism.



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Administration of the alapinin preparation[1] is restricted by its toxicity and side effects[2,6] mainly related to its high photochemical lability.[3] Synthetic analogs of lappaconitine, namely, anthranilic acid esters, are also used in practice but as components of sunblocks. *In vitro* study of compounds that are components of sunblocks made it possible to find that some of them form long-lived triplet states upon photolysis in various solvents. Oxygen capture by these triplet states generates singlet oxygen in solution.[7]

Another urgent problem in biochemistry and pharmacology is elucidation of mechanisms of the action of drugs at the molecular level and their chemical transformations when reacting with cell receptors. Some data indicate that charge-transfer complexes can participate in processes of ligand—receptor binding of drugs that block ion channels.[8] According to the results of calculations,[8] the formation of a complex of the active site of the calcium receptor with the common hypotensive preparation nifedipine and its structural analogs is accompanied by the electron transfer between the HOMO of amino acid (tyrosine) and the LUMO of nifedipine.

The interaction of nifedipine with tyrosine was experimentally studied by chemically induced dynamic nuclear polarization (CIDNP) using the model photoinitiated process in solution as an example.[9] It was assumed that the properties of the formed paramagnetic particles in the first approximation are independent of the method of their generation. This study confirmed the possibility of electron transfer from tyrosine to nifedipine and also allowed one to obtain another result important for understanding the mechanism of ligand—receptor interaction.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 608—619, April, 2007.
1066-5285/07/5604-0631

The radical anion of nifedipine formed due to the electron transfer was found to be unstable and transform within microseconds into nitrosopyridine, which, as shown by molecular modeling,[9] provides no binding with the receptor. Thus, the hypothesis on the electron transfer in the ligand—receptor complex explains both its formation and dissociation.

The next system for verifying a possibility of the chemical interaction of the drug with the amino acid residues of the active site in the corresponding receptor was lappaconitine (LC).[10] According to the data of pharmacological studies,[11] the therapeutical effect of LC is associated with the irreversible blocking of the sodium channels. It is known that LC is bound to site 2 of the sodium channel consisting of the known amino acid sequence.[12]

The study of the interaction of lappaconitine with the amino acids tyrosine and tryptophan revealed the formation of the lappaconitine radical anion, which is unstable as the nifedipine radical anion and transforms into compounds that are not bound with this receptor.[10] The reaction mechanism was studied by the CIDNP method.[13—20]

The reactivity of supramolecular lappaconitine complexes with glycyrrhizic acid (GA) is of independent interest, because the therapeutical effect of lappaconitine is strongly enhanced in the presence of GA with a simultaneous decrease in its toxicity.[21] Similar effects were detected for several other drugs.[22,23] This influence is suggested to be due to the formation of GA complexes with the drug molecules. However, the nature of the GA influence on the therapeutical effect of the drugs remains unclear. The observed decrease in the rate of the model reactions that involve the complexes[24] compared to the reactions in a homogeneous solution made it possible to advance some hypotheses on possible sequences of complex formation for processes that occur in living systems. A decrease in the reactivity due to complex formation can affect the medical properties of the complexes. First, the inhibition of LC metabolism in the organism (or binding by serum proteins) increases the effective LC concentration in the organism. Second, the decrease in the rate of LC transformation into the product increases the effective retention time of the drug on the receptor. In principle, both these processes can enhance the therapeutical effect of the drug. An alternative explanation of the observed therapeutical effect could be some specific interaction between the active site of the receptor and the complex. Similar binding could also increase the retention time of the drug on the receptor but only if this binding would be reversible and the ligand—receptor complex dissociation would not be limited by its chemical transformation on the receptor. From this point of view it is important that the therapeutical effect of LC is related to the irreversible blocking of the sodium channels.[11]

1. Photodecomposition of alkaloid lappaconitine and other anthranilic acid esters in solutions

In discussions of possible directions of LC phototransformation it should be taken into account that a lappaconitine molecule is bifunctional. On the one hand, the presence of the amide and ester carbonyl groups in the aromatic ring (anthranyl fragment) imparts the electron-withdrawing properties to the LC molecule. On the other hand, the N(20) nitrogen atom rigidly bound to the ester oxygen atom through the hydrocarbon bridge provides the electron-releasing properties for LC. For this reason, the intra- or intermolecular electron transfer from the amino group just to this fragment can be expected in photoinitiated processes. Examples for intramolecular electron transfer in similar systems are published.[25,26] The authors of these studies observed the formation of a strongly bound intermediate radical ion pair and 1,4-biradical upon the photolysis of dialkylamino ketones and esters of acylbenzoate. In the both cases, the presence of the amine nitrogen in the molecule resulted in the fast quenching of the excited triplet state with the rate constant about $5 \cdot 10^8$ s^{-1}. In the case of α-amino ketone, the biradical was formed due to the hydrogen atom transfer from the nearest to the nitrogen atom CH_2 group to the carbonyl group of the ketone. The final reaction product was the corresponding acetophenone formed upon biradical decomposition.[25] However, the authors assumed the formation of the biradical directly from the excited triplet state of the initial molecule in parallel with the formation of a charge-transfer complex. At the same time, the use of high time resolution techniques (ESR, CIDNP) suggests that the interaction of tertiary amines with carbonyl compounds in the triplet-excited state proceeds through the sequential electron and proton transfers.[27,28] The rates of amine radical cation deprotonation measured in these studies are 10^8—10^{10} mol^{-1} s^{-1} for media of different polarity and acidity.

We assumed that lappaconitine photolysis can proceed *via* similar mechanism; however, the efficiency of the reaction can substantially be lowered in this case because of steric hindrance caused by rigidity of an LC molecule. Of course, a route alternative to intramolecular electron transfer is possible: deacylation with acyl radical rejection. Just the deacylated form of LC is its major metabolite in the organism[29] and, therefore, it is of certain practical interest to reveal a possibility of deacylation during photolysis, because LC possesses medical properties.

1.1. Analysis of CIDNP effects and photolysis products in neutral media. Only one product containing aromatic protons is observed in the ^{1}H NMR spectrum detected after photolysis (Fig. 1). The position of lines of the aromatic protons of this product coincides with that of signals in the ^{1}H NMR spectrum of *N*-acetylanthranilic

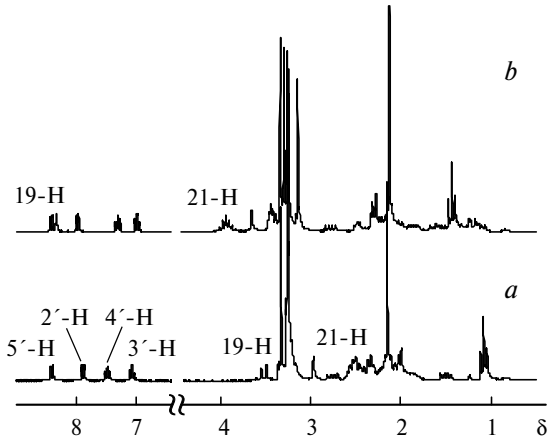
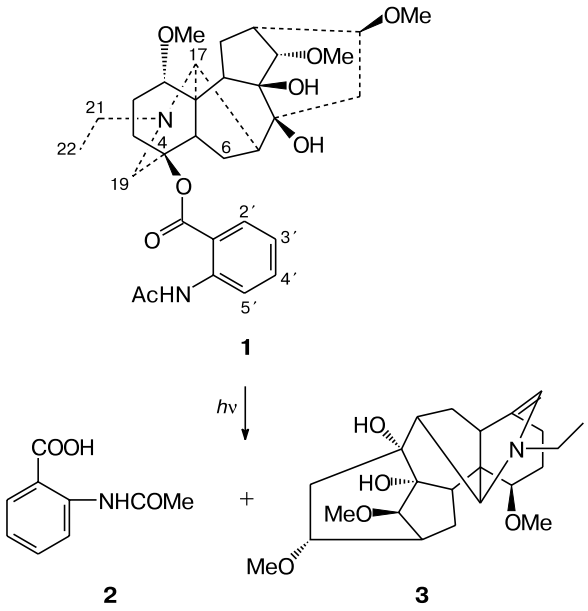


Fig. 1. ^{1}H NMR spectra (200 MHz) of lappaconitine before (*a*) and after (*b*) photolysis in CD_3OD.[30]

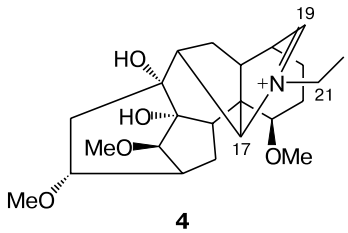
acid (**2**).[10] An analysis of the ^{1}H NMR and mass spectra made it possible to suggest the structure of the photolysis products (Scheme 1).[30]

Scheme 1



However, the observed chemical shifts in the ^{1}H NMR spectra of the photolysis products are not characteristic of compound **3**. It was assumed that in the presence of *N*-acetylanthranilic acid this compound can be protonated to form imine cation **4**.

To study the reaction mechanism, we used the CIDNP method with time resolution.[30] The fact that the polarization is observed indicates the formation of polarized products *via* the radical mechanism. The CIDNP spectrum contains information on the structure of paramagnetic



intermediates involved in the reaction.[13–21] This is why the CIDNP method is a powerful vehicle for studying mechanisms of radical reactions.

Effects of chemical nuclear polarization in the reaction under study were observed only in polar solvents, methanol and acetonitrile. The CIDNP spectrum detected for LC photolysis in methanol (Fig. 2) demonstrates both the polarization of protons of the N—CH_2 groups of the starting LC, namely, adsorption on the 21-H (two protons at 2.5 ppm) and 19-H protons (one proton at 2.5 ppm, one proton at 3.5 ppm), and the polarization on the corresponding protons of product **4**: adsorption on the 19-H proton (one proton at 8.3 ppm) and emission on the 21-H proton (two protons at 3.9 ppm). In addition, the polarization of the protons of water (4.8 ppm) is observed. The described CIDNP effects indicate that the first act of photolysis is electron transfer. The polarization on the CH_2 groups of the ethyl fragments of LC and **4** and its absence on the CH_3 group are characteristic of radical cations of amines and reflect the distribution of the HFI constants in these paramagnetic particles (for instance, for triethylamine $A(CH_2) \approx 3$ mT, $A(CH_3) \approx 0$).[28] In addition, the formation of CIDNP in the electron transfer act is indicated by the appearance of CIDNP only in polar solvents. Based on the above mentioned data on the photophysical properties of LC and found CIDNP effects, we assumed[30] that the positive charge is localized on the N(20) nitrogen atom, whereas the negative charge is concentrated on the anthranyl fragment (Scheme 2).

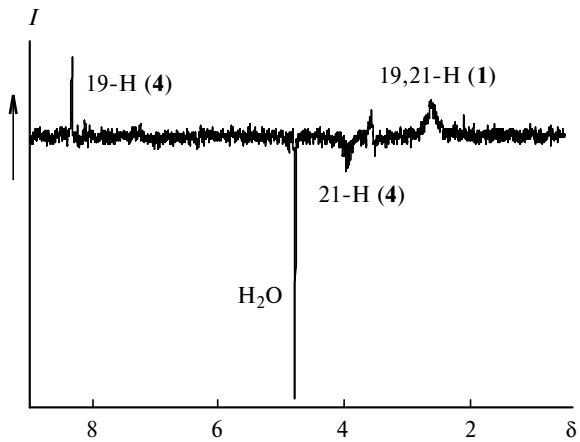
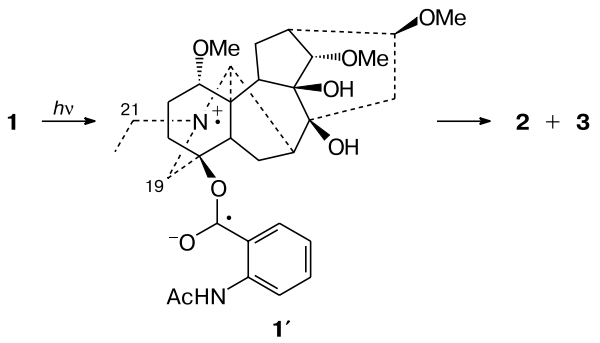


Fig. 2. Time resolved CIDNP spectrum for the photolysis of LC (10 mmol) in CD_3OD[30] (*I* is the CIDNP intensity).

Scheme 2



According to the concepts of the CIDNP theory,[13] the chemical polarization similar to the observed one can be formed in this biradical only if the biradical formed is rather "rigid," *i.e.*, has low conformational mobility of the "donor" and "acceptor" fragments relative to each other. The formation of *N*-acetylanthranilic acid **2** during LC photodecomposition assumes the proton transfer from position 19 to the carboxylic oxygen atom followed by the ester bond cleavage. We observed this step of formation of **2** during LC photodecomposition in the presence of electron donors, where the LC radical anion was formed due to the intermolecular electron transfer to the anthranyl fragment of LC.[10] In the present study, we also cannot ignore the intermolecular electron transfer to CIDNP. However, in our opinion, the bimolecular reaction between two lappaconitine molecules in the used concentration range (~10^{-3} *M*) even at diffusional quenching rates cannot compete with the intramolecular quenching of the LC triplet that occurs in the nanosecond time scale.[25,26]

The contribution of intermolecular electron transfer to CIDNP of LC follows from the analysis of the time dependence presented in Fig. 3. The appearance of the

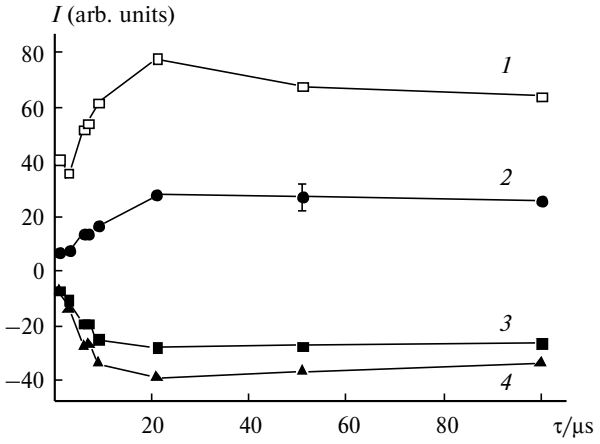
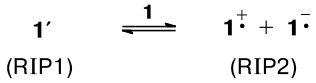


Fig. 3. Plots of the integral CIDNP intensity (*I*) of the $H_2C(21)$ protons in lappaconitine (**1**) (*1*), HC(19) (*2*) and $H_2C(21)$ (*3*) protons in product **4**, and H_2O protons (*4*) *vs.* time delay (τ) between the laser pulse and detecting pulse P1 = 1 μs.[30]

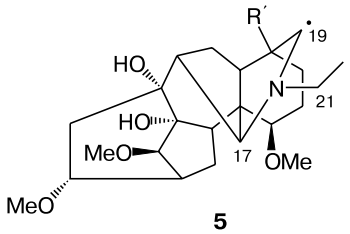
polarization only on the starting LC at the initial time moment indicates that LC is the product of cage recombination of the primary radical ion pair (RIP) (reverse electron transfer). The further smooth increase in the LC polarization is usually attributed to the contribution of diffusion F-pairs.[31,32] It should be mentioned, however, that the time within which the CIDNP time dependence reaches a plateau for LC (20 μs) exceeds substantially the characteristic times of diffusion processes (1—5 μs) observed in the reactions of LC with amino acids.[10] We would like to relate the time dependence detected for LC to the appearance of recharging between the bipolar radical and neutral LC molecules in the bulk (Scheme 3).

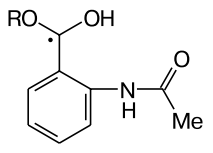
Scheme 3



The reaction rate of degenerate electron exchange between the radical anion and neutral LC molecule resulting in recharging is $1.5 \cdot 10^8$ mol^{-1} s^{-1}.

Analysis of the observed CIDNP effects allows one to monitor all stages of formation of the final reaction products. Based on the assumed structure of the primary radical ion pair in which considerable HFI constants are observed only for the protons bound to the nitrogen atom (17-H, 19-H, and 21-H),[28] one could expect that all the three groups of the corresponding protons in compound **4** would also be polarized similarly, as it is observed for the protons of LC. However, the H-19 and H-21 protons in compound **4** have different polarization signs: adsorption at 8.3 ppm and emission at 3.9 ppm. The most probable reason for this divergence can be the formation of CIDNP effects of these protons in different radical pairs. The observed difference between the CIDNP signs of H-19 and H-21 well satisfies the hypothesis of formation of the sequential neutral pair of radicals **5** and **6** due to the proton transfer from position 19 of the radical cation to the carboxylic oxygen atom of the LC radical anion.



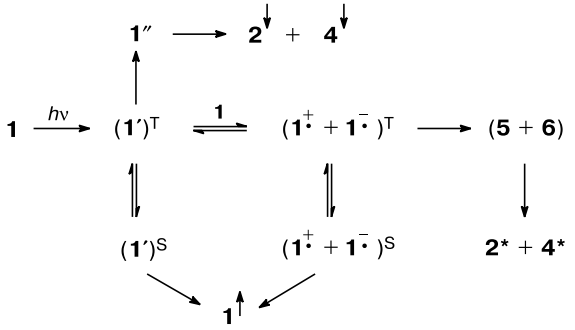


Indeed, in radical **5** the highest HFI constant will be on the α-protons 19-H ($A \approx -2.3$ mT) and its sign should be negative. By analogy to the neutral triethylamine radical, $A \approx 0.4$ mT and $g \approx 2.0033$ (see Ref. 28) can be expected on the γ-protons (17-H and 21-H). Thus, the

observed polarization of the protons in **4** corresponds to a superposition of the CIDNP effects formed in the RIP and the pair of neutral radicals. For the H-21 and H-17 protons the contribution from the RIP will be prevailing, whereas for H-19 the contribution of the neutral radical pair is prevailing.

The existence of two sources of CIDNP of the protons in positions 19 and 21 is reflected in the run of the time dependences of CIDNP: the rate of polarization accumulation is different for these protons (12 and 5 μs, respectively), and the course of the time dependences coincides for H-19 and the protons of water. The latter observation indicates that the polarization of these protons originates from the same source (RIP2). Indeed, the most natural source for the appearance of the polarization on the water molecules is the exchange of the protons of water with the carboxyl group of *N*-acetylanthranilic acid. The polarized carboxyl group can be formed only due to the postulated above proton transfer from the 19-CH_2 group of the radical cation to the ester fragment of the radical anion. Radical **6** undergoes fragmentation to form *N*-acetylanthranilic acid.[10] The rate constant of fragmentation of this radical is $k_{fr} = 4 \cdot 10^5$ s^{-1}. Thus, the polarization on the water molecule evidences for the proposed scheme of LC photolysis (Scheme 4).

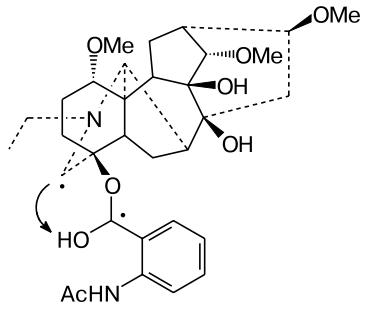
Scheme 4



Note: signs ↑ and ↓ indicate the cage and escape polarization, respectively, formed in the RIP, and sign * designates the polarization from the neutral radical pair.

Polarized product **4** can be formed only due to the fragmentation of radical **5** during the time shorter than the spin-lattice relaxation time in the radical ($T_1 \approx 10^{-5}$ s). Note that the same products can be formed upon the decomposition of neutral biradical **1″**. However, according to the existing concepts,[13] it hardly be expected that the chemical nuclear polarization would be formed in the short 1,4-biradical under our experimental conditions (magnetic field of an NMR spectrometer).

Thus, in polar media the first step of the process is the intramolecular electron transfer in the triplet-excited state of LC from the N(20) nitrogen atom to the anthranyl



1″

fragment to form biradical **1′**. The subsequent recharging of this biradical with a lappaconitine molecule in the bulk affords the RIP2 radical ion pair in which the proton transfer occurs to form a neutral radical pair. The final reaction products are *N*-acetylanthranilic acid **2** and compound **3** formed due to the fragmentation of the neutral radicals.

1.2. Reaction mechanism in acidic media. We additionally confirmed the above conclusions on the reaction mechanism by comparison of the CIDNP effects in neutral and acidic media. The idea was to diminish the donor ability of the amine group by the protonation of the nitrogen atom in an acidic medium. As expected, the polarization on the products formed from the primary RIP and the neutral radical pair decreased considerably (Fig. 4, lines at 8.3 and 3.9). In addition, the polarization of **1** and its deacylated form **8** (Scheme 5) was observed. The signals at 6.4—6.6 ppm correspond to the 3′-H and 5′-H protons of the deacylated LC form.

As already said, the CIDNP intensity on the product is proportional to the HFI constant on the precursor radical. The stronger polarization on the 3′-H and 5′-H protons compared to that on the 2′-H and 4′-H protons allows us to preliminarily suggest the structure of the radical, which is a precursor of the polarized product. *N*-Centered radical **7** seems to be the most probable struc-

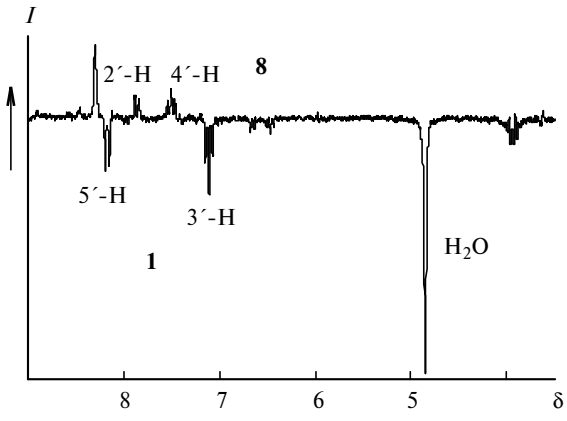
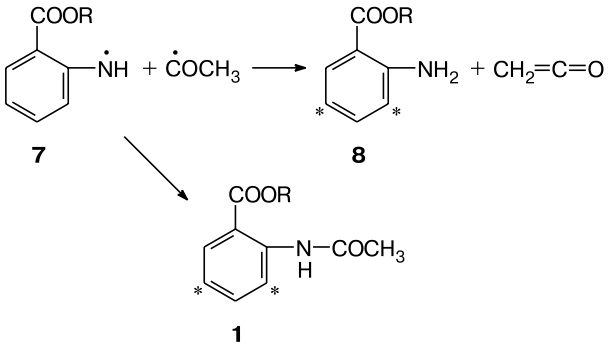


Fig. 4. CIDNP spectrum detected after laser irradiation of LC (10 mmol) in CD_3OD in an acidic medium (in the presence of 0.2 mole of acetic acid).[30]

Scheme 5



Sign * designates the aromatic protons on which the maximum polarization is observed.

ture. This radical can be formed in the monomolecular reaction of decomposition of the LC excited state to form the acyl radical as a partner (see Scheme 5). No protons of the NH and NH_2 groups are observed under these experimental conditions because of fast exchange with water. The whole polarization from these groups is transferred to the protons of water, which is observed experimentally (emission at 4.8 ppm).

1.3. Photolysis of other esters of *N*-acetylanthranilic acid. It seems reasonable to assume that deacylation will be the main channel of photolysis of simpler aliphatic esters of anthranilic acid containing no donor groups. The hypothesis was verified by the photolysis of methyl *N*-acetylanthranilate.[33] In this case, the CIDNP spectrum is similar to that for LC photolysis in an acidic medium. The maximum polarization was observed on the 3′- and 5′-protons of the initial ester and its deacylated form **8** (Fig. 5). The polarized lines at 2—2.5 ppm belong to the reaction products of the acyl radical in solution. The existence of the free acyl radical in solution agrees with the hypothesis on the formation of radical pairs of the acyl and *N*-centered radicals.

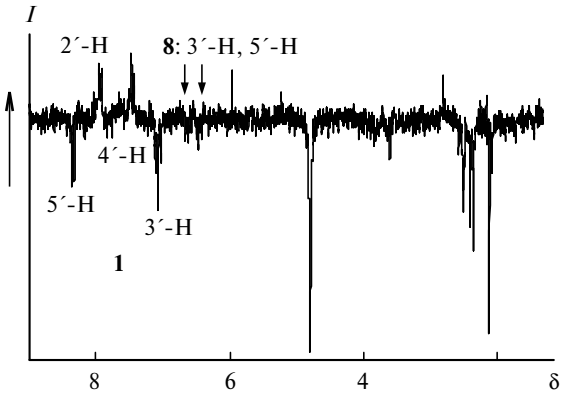


Fig. 5. CIDNP spectrum detected after laser irradiation of methyl *N*-acetylanthranylate (10 mmol) in CD_3OD.

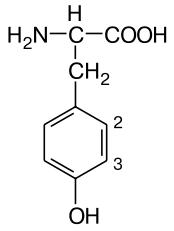
Note that the quantum yield of formation of the deacylated product is substantially (more than an order of magnitude) lower than the yield of products **2** and **3** during photolysis of LC.

Summarizing the results, we can conclude that two routes of LC transformation from the triplet-excited state exist: (1) intramolecular electron transfer from the N(20) nitrogen atom to the anthranyl fragment followed by recharging and proton transfer to form a neutral radical pair; the final products of neutral radical fragmentation are *N*-acetylanthranilic acid and compound **4**; (2) deacylation with the C—N bond cleavage in the anthranyl fragment and formation of a neutral radical pair; the final product of this reaction is *N*-deacetyllappaconitine. In a neutral medium the second route is minor for LC; however, it is the major route for aliphatic esters of *N*-acetylanthranilic acid containing no donor fragments in the aliphatic part. The probability of LC deacylation increases substantially in an acidic medium due to the protonation of the N(20) nitrogen atom of lappaconitine or upon its chemical modification (for example, for its medicinal form hydrobromide).

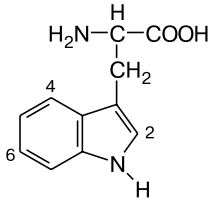
It should be emphasized that the high photosensitivity of lappaconitine compared to other esters of anthranilic acid is associated precisely with the presence of the electron-releasing N(20) group, which creates prerequisites for the intramolecular electron transfer. Both radical ions and neutral free radicals (carbon-, nitrogen-, and oxygen-centered) are formed as a result of light absorption by an LC molecule. This can be a reason for the high phototoxicity of lappaconitine.

2. Electron transfer in the reactions of lappaconitine with amino acids

We found no published works on the reactions of LC with amino acids, although the electron transfer reactions between tyrosine, tryptophan, and some biologically significant molecules have already been studied earlier using CIDNP and laser flash photolysis techniques.[34,35] It was found in these studies that the indicated amino acids can act as electron and proton donors if an appropriate acceptor is present in the solution. The magnetic resonance parameters of the tyrosine and tryptophan radical cations and neutral radicals were characterized.[34,35]



Tyrosine

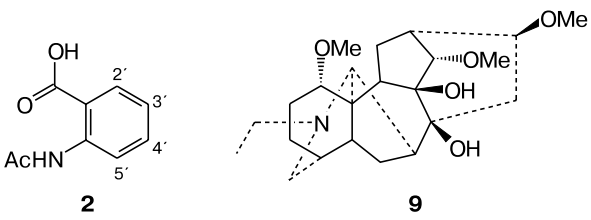


Tryptophan

To investigate the interaction between LC and these amino acids, we used CIDNP with photogeneration.[10] This was made in the framework of the earlier developed procedure based on the use of model processes for studying the mechanisms of interaction of drugs with cell receptors.[9] Photoinitiation of electron transfer was used in terms of this approach, and the further destiny of the radical particles was monitored by the CIDNP method. The obtained results are discussed from the viewpoint of a possible influence of LC transformations on the ligand—receptor binding.

2.1. Analysis of reaction products. When discussing possible routes of LC transformation due to the photoinitiated interaction with electron donors, one should take into account that a lappaconitine molecule is an aromatic ester with the electron-withdrawing properties.[36] Photolysis of aromatic esters in the presence of electron donors is characterized by the cleavage of the C—O ester bond to form the corresponding acid.[36] It is known that the photophysical properties of LC are completely determined by the anthranyl fragment[37,38] and, therefore, electron transfer from amino acids to just this fragment can be expected in the photoinitiated processes. As already said, the presence of the amido group does not exclude an alternative reaction route: deacylation. This assumption is confirmed by the fact that the deacylated form of LC is its major metabolite in the organism.[29]

The obtained results[10] made it possible to unambiguously choose between these mechanisms and identify the reaction products. The analysis of the NMR spectra of the products of LC photolysis in polar solvents (acetonitrile, methanol, water) and chromatographic analysis of the products indicate the formation of *N*-acetylanthranilic acid (**2**) and compound **9** and the absence of any traces of the deacylated product.



2 **9**

2.2. Analysis of CIDNP effects. The effects of chemical nuclear polarization on both the initial compounds and product **2** were detected[10] for the photoinitiated interaction of LC with *N*-acetyltyrosine (AcTyr) and *N*-acetyltryptophan (AcTrp). Significantly, although in the both cases the light is absorbed predominantly by lappaconitine, the reaction with the amino acids occurs more efficiently than the photolysis itself. This is manifested in the CIDNP spectrum as a substantial decrease (down to the complete disappearance at high concentrations of amino acids) in the CIDNP intensity of the products of LC self-decomposition.

The observed CIDNP effects unambiguously indicate that paramagnetic species are involved in the reaction.[13] To determine the structures of these particles, the signs of polarized lines and their intensities are compared with the magnetic resonance parameters (HFI constants and *g*-factors) of the assumed paramagnetic precursors of the products.[14—20] First, it should be mentioned that the CIDNP effects were detected for the photoinitiated interaction of LC with the amino acids only in polar solvents: acetonitrile, dimethyl sulfoxide, methanol, and water.[10] The presence of CIDNP in polar solvents along with the absence of polarization in a nonpolar medium (in our case, benzene) is one of the tests for the electron transfer as the primary step of the process.[13]

The CIDNP spectrum detected during the photolysis of LC in the presence of *N*-acetyltryptophan in a water—methanol solution is shown in Fig. 6 as an example. The CIDNP spectrum presented in Fig. 6 demonstrates the polarization on the aromatic and acyl protons of lappaconitine and *N*-acetylanthranilic acid and on the protons of water molecules. An analysis of the signs and intensities of the polarized lines according to the modern rules[13] with account for the magnetic resonance parameters of the assumed paramagnetic particles[31,39] suggested the formation of a radical ion pair consisting of the LC radical anion and AcTrp radical cation in this reaction. In this case, lappaconitine reacts in the triplet-excited state. The opposite sign of polarization of LC and *N*-acetylanthranilic acid indicates that the latter is formed due to the fragmentation of the LC radical anion in solution. The polarization on the water protons detected in experiments with *N*-acetyltryptophan[10] (see Fig. 6) should specially be considered. Its most natural source seems to be the deprotonation of the AcTrp radical cation in the bulk occurring *via* Scheme 6. Published data indicate that the deprotonation rate of the *N*-acetyltryptophan radical cat-

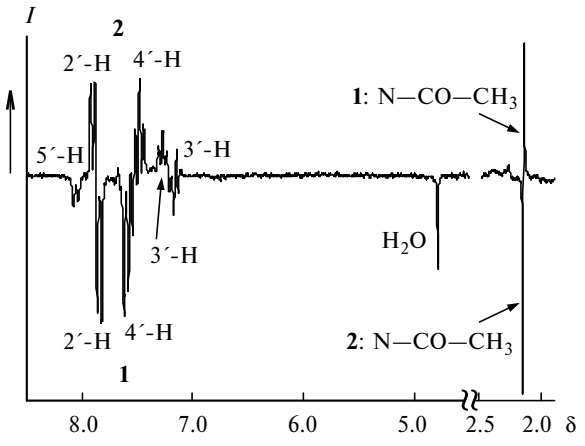
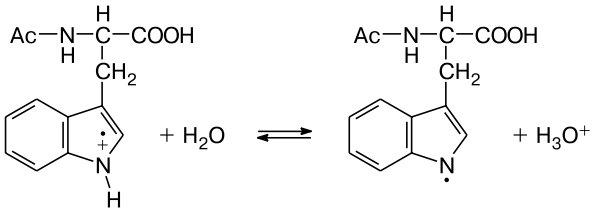


Fig. 6. ^{1}H CIDNP spectrum detected after irradiation of lappaconitine (3 mmol) with the laser light (λ = 308 nm) in the presence of the *N*-acetyltryptophan amino acid (10 mmol) in an aqueous-methanol solution.[10]

ion in aqueous solutions is about 10^6 s^{-1}, which substantially exceeds the spin-lattice relaxation rates in radicals.[31] This fact gives additional evidence in favor of RIP formation, because no polarization on the NH group can be formed in a neutral radical pair: this proton is absent in the *N*-acetyltryptophan neutral radical.

Scheme 6



An analysis of the time evolution of the CIDNP signals of the reaction products (Fig. 7) indicates high rates of lappaconitine radical anion transformation into *N*-acetylanthranilic acid. In addition, the decrease in the CIDNP intensity of lappaconitine reflects the contribution of the electron exchange of the LC radical anion with its diamagnetic precursor compared in rate with its protonation affording **2** ($k_e \approx 1.5 \cdot 10^8$ mol^{-1} s^{-1}). Based on the time dependence, *N*-acetyltryptophan is involved in the slower reaction of proton exchange with the neutral radical ($k_p \approx 10^7$ mol^{-1} s^{-1}).

Similar effects were observed for LC photolysis in the presence of *N*-acetyltyrosine.[10] The CIDNP effects on water detected in experiments with AcTrp are absent in the case of AcTyr. The absence of polarized water can be due to both the known fact that the AcTyr radical cation loses proton already at times characteristic of geminal processes and the absence of functional groups that can exchange with water with noticeable HFI constants.[32] In fact, the CIDNP effects of *N*-acetyltyrosine correspond to the spin density distribution in the free radical rather than in the radical cation as in the case of *N*-acetyltryptophan, because the ratio of CIDNP intensities observed for the *ortho*- and *meta*-protons is 3.5 instead of 7.0 expected for the radical cation.[10,32] It should be noted that the earlier observed and described CIDNP effects in redox photoprocesses involving tyrosine were also ascribed to the pair of the neutral tyrosine and substrate radicals.[34] Presumably, the AcTyr neutral radical was formed due to the fast deprotonation of the radical cation with proton leaving to the medium. An analysis of the time dependence of LC (fast reaching a plateau) also indicates the acceleration of protonation of the LC radical anion in the bulk in the presence of AcTyr compared to the reaction with AcTrp. This agrees with the difference in the pK_a values for the deprotonation of *N*-acetyltryptophan (4.7)[31] and *N*-acetyltyrosine (2.2).[40] The presence of polarization on the methyl protons of the amido group in LC and **2**, which could not be formed in the LC radical ion, indicates that in the LC radical anion the protonation can proceed to the both carbonyl groups (ester and amide) and the fast exchange occurs between these structures (Scheme 7).[10]

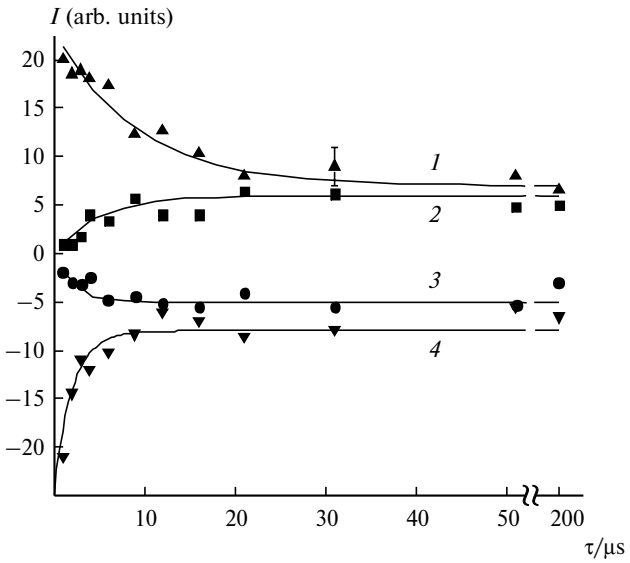
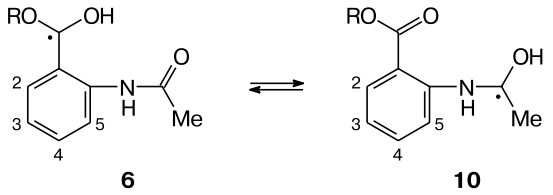


Fig. 7. Plots of the integral CIDNP intensity (*I*) of the aromatic protons of *N*-acetyltryptophan (*1*), *N*-acetylanthranilic acid (*2*), H_2O (*3*), and lappaconitine (**1**) (*4*) *vs.* time delay between the laser pulse and detecting pulse P1 = 1 μs (see Ref. 10).

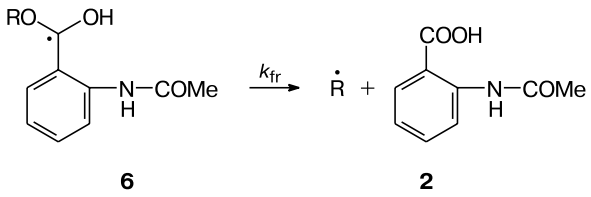
Scheme 7



The polarization of the CH_3 protons is a result, in fact, of the processes that occur in the bulk, which is indicated by its decrease with an increase in the pH of the medium down to the complete disappearance at pH 14. The fragmentation rate constant k_{fr} of radical **6** (Scheme 8) formed due to the protonation of the LC radical anion was estimated from the time dependence of the CIDNP effects of compound **2**. The estimate was made in the framework of assumptions that three processes contribute to the kinetics of the polarization increase on the escape product: fragmentation of polarized radical **6** to form **2**, CIDNP relaxation in this radical, and finally, decay of the polarized radical in other processes that are not related to fragmentation. However, taking into account that the intensity of polarization of the aromatic protons of

N-acetylanthranilic acid almost coincides with that in LC, we can conclude that the fragmentation rate exceeds the rates of all other indicated processes and the one-exponential approximation can be used to estimate the fragmentation rate constant. Thus obtained rate constant of fragmentation of radical **6** is $k_{fr} \approx 4 \cdot 10^5$ s^{-1}.

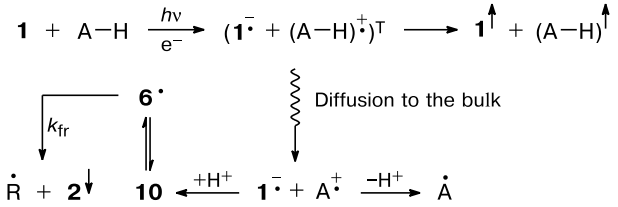
Scheme 8



6 **2**

2.3. Reaction mechanism. Thus, based on the analysis of the CIDNP effects and composition of the products, we concluded that the mechanism of photoinduced interaction of LC with the amino acids is similar to the described[36] mechanism of photolysis of esters, which are characterized by the cleavage of the C(O)O—R ester bond to form the corresponding acid. The difference in the photoinduced reactions of LC with *N*-acetyltyrosine and *N*-acetyltryptophan is reduced only to the difference in structures of the paramagnetic particles of the amino acids. Since the same products are formed in the reactions of LC with these amino acids, we proposed the general mechanism for the transformation of LC in the presence of AcTyr and AcTrp (Scheme 9).

Scheme 9



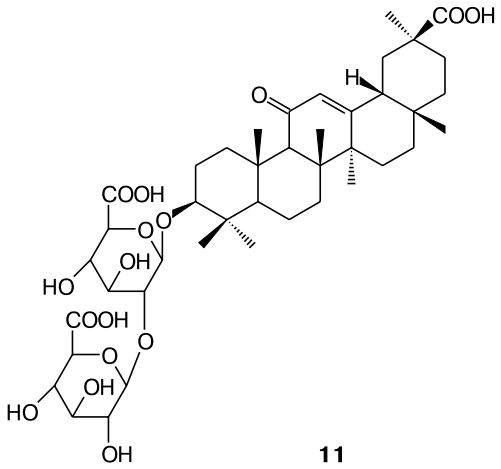
Note: A—H is AcTyr or AcTrp amino acid, A is the deprotonated form of amino acids; signs ↑ and ↓ mark the polarized products formed by cage recombination and escape processes, respectively.

Thus, the analysis of the CIDNP effects and composition of the products suggested the three-step mechanism of the photoinduced interaction of LC with amino acids. The first step of the process is the electron transfer from the amino acid to the anthranyl fragment of LC. The second step is the protonation of the LC radical anion that occurs in the bulk to form a neutral radical with two mutually transformed structures. The third step is the fragmentation of neutral radical **6** affording final product **2** and radical **R**·. Diamagnetic product **9** can be formed through hydrogen atom abstraction by radical **R** from the solvent.

2.4. Biological significance of the result. Thus, electron transfer from the AcTyr or AcTrp amino acids to lappaconitine affords the LC radical anion, which undergoes fast decomposition to form compounds **2** and **9**. It seems reasonable to assume that these compounds, which are not medicinal preparations, will not be blockers of sodium channels. As a result, the ligand—receptor binding of alapinin with site 2 of the sodium channel can be violated. Instability of the radical anion of another drug (nifedipine — blocker of calcium receptor) was demonstrated in our previous study.[9] It was shown that the product formed is not already bound with the receptor. Thus, these two examples demonstrate a possible role of the simplest chemical reaction (electron transfer) in processes of ligand—receptor interaction. The chemical transformation of a drug can provide reversibility of binding of the drug with the receptor.

3. Reactivity of lappaconitine in complex with glycyrrhizic acid

The main reason for interest of physicochemists in investigation of the influence of glycyrrhizic acid (GA, **11**) on the properties of lappaconitine was the experimentally found substantial change in its therapeutical activity in the presence of GA.



11

The addition of GA to a solution of lappaconitine was found[21] to decrease the therapeutical dose by an order of magnitude and diminish the toxic effect of the drug. Since the observed effects were not explained in the cited publications, we studied the complex formation of LC with GA and possibilities of the effect of this process on the reactivity of LC in model processes. The results of the studies confirmed the fact of complex formation of lappaconitine with GA and allowed us to advance a

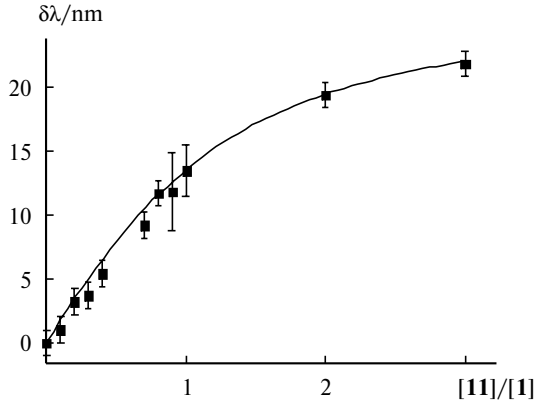


Fig. 8. Experimental plot and computer simulation of the shift of the absorption maximum of LC (**1**) *vs.* concentration of glycyrrhizic acid (**11**). Measurements were carried out in a 20% aqueous-methanol solution at an LC concentration of 0.01 mmol L^{-1} (see Ref. 24), $K = (2\pm0.13)\cdot10^5$ mol^{-1}.

hypothesis describing how GA can affect the reactivity of LC.[24] This reaction is above described in detail (see item 2 of the present review).

3.1. Investigation of stability of the complex. Figure 8 shows an example of estimation of the stability constant of the lappaconitine complex with GA from the change in the optical spectrum of an LC solution at different concentrations of glycyrrhizic acid. The stability constant of the complex K is the ratio of rates of the forward and backward reactions and is equal (for the 1 : 1 complex) to the ratio of the concentrations of the complexed and free lappaconitine.

$$A + B \underset{}{\overset{K}{\rightleftharpoons}} AB$$

$$K = k_+/k^- = [AB]/([A][B])$$

The computer simulation[24] of the experimental data shown in Fig. 8 made it possible to estimate the stability constant of the complex in an aqueous medium: $K = (2.0\pm0.13)\cdot10^5$ M^{-1}. The stoichiometry of the complex was concluded from analysis of Job´s plot.[41,42] The obtained value indicates the extreme stability of the complex. It should be mentioned for comparison that the stability constants of the cyclodextrin complexes[41,43] widely used in pharmacology are 10^3 M^{-1} on the average. The high stability constants were also obtained by several authors for the GA complexes with a series of other drugs.[22,44] This makes glycyrrhizic acid to be a very promising object for pharmacology because allows one to use complex formation when low concentrations of drugs are applied. It can be assumed that such a high stability of the GA complexes is related to the poor solubility of both GA and LC in water. Hydrophobic interaction can stimulate the formation of supramolecular associates of complicated structure.

The stability constants of this complex in pure methanol and in an aqueous solution of DMSO were measured to reveal the solvent effect on the stability of the glycyrrhizic acid complexes in solution.[24] It was shown that the complex in a 20% aqueous solution of DMSO has the same stability as that in a 20% solution of methanol. At the same time, in pure methanol the stability constant decreased by an order of magnitude.

Since LC is poorly soluble in water, its hydrobromide is used in pharmacological practice. From the viewpoint of practical use of the complexes in pharmacology, it was important to elucidate whether the hydrobromide complex is such highly stable as the complex of the initial LC or not. The obtained stability constant for the complex of lappaconitine hydrobromide ($K = 2.6\cdot10^3$ M^{-1}) is by two orders of magnitude lower than the corresponding value for the LC itself. We believe that this is due to the high solubility of the salt in water. Thus, the found effect of the solvent on the stability of the complex and the decrease in the stability for the water-soluble lappaconitine salt indicate a substantial role of the hydrophobic interaction in the complexation ability of GA.

3.2. Photoinduced interaction of LC with tyrosine in complex. To reveal the influence of GA on the reactivity of lappaconitine, we studied the electron phototransfer between lappaconitine and tyrosine, *viz.*, amino acid present in the active site of the sodium channel.

The effect of complex formation on the reactivity of LC was estimated from both the intensity of the CIDNP effects, which reflect the number of radical pairs formed in the reaction, and the yield of the reaction products with tyrosine. The NMR spectra of the final photolysis products and the CIDNP spectra in the presence and absence of GA are shown in Fig. 9 for comparison. The polarized lines of both the starting compounds (LC and GA) and the product (*N*-acetylanthranilic acid) are seen in the CIDNP spectrum. Two additional lines at 8.1 and 8.3 ppm observed in the absence of glycyrrhizic acid belong to the product of lappaconitine self-decomposition. At the used reactant concentrations the intramolecular electron transfer occurs in parallel to the intermolecular reaction with the amino acids and affords another set of products.[24]

The first conclusion that follows from a comparison of the CIDNP spectra (see Fig. 9, spectra *2* and *4*) is that GA almost completely blocks the radical pathway of the reaction. Experiments showed that this effect is not due to a change in the acidity of the medium. The polarization intensity on tyrosine decreases substantially (by ~4 times) with the same change in the yield of the reaction product (anthranilic acid) (*cf.* spectra *1* and *3*). Thus, the presence of GA substantially decreases the rate of LC phototransformation. In addition to the obvious reason, namely, the appearance of steric hindrance for bimolecular reactions, the results of investigation of the GA complexes

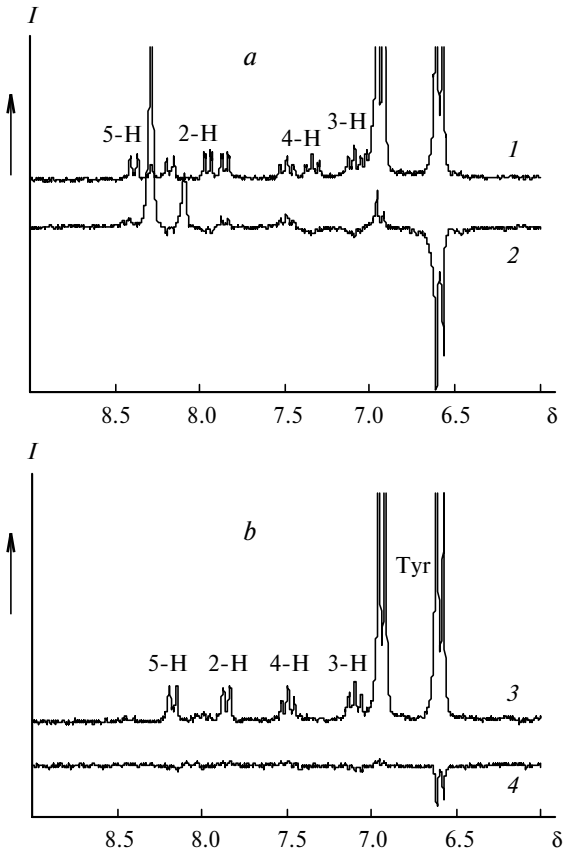


Fig. 9. ^{1}H NMR spectrum (200 MHz) of the photolysis products in the absence (*1*) and presence of GA (*3*).[24] The CIDNP spectra (aromatic part) detected after laser irradiation of lappaconitine (3 mmol L^{-1}) in the presence of the *N*-acetyltyrosine amino acid in CD_3OD in the absence (*2*) and presence (*4*) of glycyrrhizic acid.

with carotenoids[42,45] can be mentioned when discussing about the mechanism of influence of the complex formation on the reactivity of lappaconitine. It was shown[45] that the influence of complex formation on the oxidation potential of carotenoids is a reason for the substantial inhibition of the electron transfer in the reactions with different acceptors. This result can be of practical significance for understanding the mechanism of the effect of GA on the therapeutical activity of drugs.

It should be noted in conclusion that the lappaconitine alkaloid in redox processes can manifest both electron-releasing and withdrawing properties. In the presence of electron donors, lappaconitine forms radical anions, while radical cations are formed in the presence of electron acceptors. In addition, lappaconitine in the triplet excited state undergoes intramolecular electron transfer to form a biradical. An important point is the instability of the lappaconitine radical ions. They are rapidly protonated/deprotonated in solution to form neutral radicals. The latter undergo fragmentation at the C—O ester bond to form *N*-acetylanthranilic acid. The deacylation of lappaconitine in a neutral medium is the minor process; however, it can play a noticeable role in an acidic medium. Complex formation with glycyrrhizic acid decreases the rate of lappaconitine decomposition and the yield of free radicals.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 04-03-32449), the Russian Academy of Sciences (Integration Project of the Siberian Branch of the Russian Academy of Sciences No. 146 "Scientific Foundations for Production of Medical Preparations. Challenges of Using Biological Raw Materials" and the Program of the Presidium of the Russian Academy of Sciences No. 18.2 "Origin and Evolution of Biosphere"), and the Ministry of Education and Science of the Russian Federation (Program "Development of Scientific Potential of the Higher School," grant RNP 2.1.1.1969).

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Received December 12, 2006;
in revised form April 5, 2007