Antiarrhythmic agents based on diterpenoid alkaloid lappaconitine. Protonation of *N*-deacethyllappaconitine in methanol solutions

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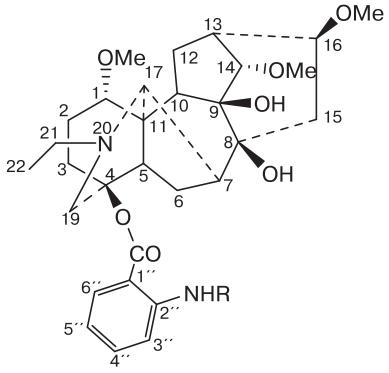
The data on antiarrhythmic drugs based on diterpene alkaloid lappaconitine are summarized. *N*-Deacetyllappaconitine is the main metabolite of allapinin and a potential antiarrhythmics for intravenous use. The basicity of two nitrogen atoms of this compound and the possibility of obtaining mono- and di-salts with the example of hydrochlorides and hydrobromides having good solubility in water have been studied. It was shown using NMR spectroscopy and potentiometric titration that in the methanolic solutions the atom N(20) is protonated at the first step (pK_{b1} = 6.77, 25 °C) in the pH range of 6—7. The nitrogen of the primary aromatic amino group is protonated in the pH range of 2—3 (pK_{b2} = 2.18, 25 °C).

Key words: diterpene alkaloids, lappaconitine, antiarrhythmics, *N*-deacetyllappaconitine, drug, stepwise protonation, salt formation.

Alkaloid lappaconitine (**1**) was isolated from the plant *Aconitum septentrionale* in 1895 by Rosendahl.[1] Its structure was established at the beginning of the 70th of the past century.[2—6] It was established in the course of the study of the pharmaco-toxicological properties of compound **1** that it possesses arrhythmogenic,[7,8] analgesic, and locally anesthetizing properties.[9] Its antiarrhythmic action was revealed in the late 70th.[10]

Hydrobromide of lappaconitine is introduced into medical practice as an antiarrhythmic agent under the name of allapinin in 1987.[10—13] The drug is included in the list of the most vitally important medicines approved by the Ministry of Health of the Russian Federation. It is one of the most effective in the treatment of life-threatening forms of arrhythmias, namely, atrial fibrillation (AF) and ventricle rhythm disorders.[14] The substance of the allapinin drug is fabricated at the experimental production plant of the S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan (Tashkent), and the pharmaceutical form (pills) is manufactured by VILAR Pharmcenter (Moscow). The prolonged pharmaceutical form is also produced by VILAR Pharmcenter under the name of allaforte.[15] The medication aklezin, namely, the mixture of alkaloids from the overground part of the plant *Aconitum Leucostomum* Worosch, is also authorized for use as the antiarrhythmic agent.[16] The main component of aklezin is lappaconitine.



1, **2**

R = COMe (**1**), H (**2**)

Revealed dependence of antiarrhythmic activity on the peculiarities of the structure allowed to carry out a directed synthesis of a series of new active compounds on the basis of diterpene alkaloids.[17,18]

At present, antiarrhythmic drugs amiodarone, disopyramide, flecainide, propafenone, and sotalol are used to control the heart rhythm in patients with AF in Europe. Dofetilide is used for these purposes in the United States. The medications of 1C class, namely, ethacizine, niferidil, and allapinin, are available in Russia in addition to amiodarone, sotalol, and propafenone.[19,20]

The use of allapinin is often complicated by a number of side effects limiting its application. It is established that

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these symptoms are caused by high concentrations of allapinin in the blood plasma.[21]

A new drug based on lappaconitine was developed. Its preclinical investigations were completed in the course of the implementation of the State Contract No. 14.N08.11.0068. Established concentration dependencies of the action (dose-effect) of allapinin and the new preparation on the sodium current and their efficiency in the treatment of the heart arrhythmias testify that the effective dose of the studied agent is in all cases lower by a factor of 3—4 compared to lappaconitine hydrobromide. In addition, the new drug does not possess the side effects inherent to allapinin. Unlike the allapinin, the new agent blocks not only sodium channels, but, in part, also potassium channels.[22—25]

Allapinin is authorized for use in both pills and ampoule (injectable) form. However, intravenous application of this drug did not find wide use because of the late appearance of therapeutic effect and side cardiac effects.[26] *N*-Deacethyllappaconitine (**2**), the main metabolite of the allapinin drug,[27] is not inferior to allapinin by activity in most types of arrhythmias, but it is less toxic, has greater therapeutic breadth, and exceeds allapinin by the rapid development of antiarrhythmic effect. However, it is inferior to allapinin by prolongation of action.[21] These data allow us to suppose that compound **2** can serve as the basis for the creation of a new effective antiarrhythmic agent in an injectable form. The arsenal of such drugs in medical practice is very limited.

N-Deacethyllappaconitine is rarely found in nature, its reserves are very limited and completely insufficient for the development of the drug. The lappaconitine (**1**) can be the main source of compound **2**. The lappaconitine is widespread in nature, and compound **2** can be obtained from compound **1** by acid hydrolysis. At the same time, there are secondary products of the manufacture of the drug allapinin, which can serve as the source of noticeable amounts of compound **2**. The work is already underway in Uzbekistan to prepare compound **2** from the secondary products of the preparation of the drug allapinin, and also to create the intravenous form of this drug.[28]

A stable, water-soluble form of the medication, for example, in the form of a salt, is necessary for the development of a medicament for intravenous administration on the basis of compound **2**.

It should be noted that compound **2** in contrast to lappaconitine contains in its structure two nitrogen atoms having a basic character. One of them is represented by a primary amino group in the anthranilic acid residue, while the second one is a part of the piperidine cycle and alkylated by an ethyl group. Accordingly, compound **2** is capable of forming both mono- and di-salts. The possibility of using salts of *N*-deacethyllappaconitine for the creation of medicines is declared in a number of publications.[29—31] However, the nature of the salt, mono- or di-, which should be used, was not discussed, and physicochemical characterization has not been reported. To solve this problem, we studied the basicity of two nitrogen atoms and the possibility of obtaining mono- and di-salts.

Results and Discussion

The UV spectra of the methanolic solutions of compound **2** at different pH were registered for the study of its protonation. Three absorption maxima in the 220, 250, and 340 nm regions, caused by the π—π^*-transitions[32] in the anthranilic fragment, are observed in the UV spectra at pH 7. The decrease in pH results in a batochromic shift of maxima at 220 and 340 nm by 7—8 nm, which is probably caused by the redistribution of the electronic density at protonation of the primary amino group of the anthranilic fragment of compound **2**.[32,33]

The most informative for the study of the protonation process of compound **2** are the ^{1}H, ^{13}C, and ^{15}N NMR spectra obtained by the addition to the solution of **2** in CD_3OD of one (**3a**) or two (**4a**) moles of HCl. Similar spectra were recorded for the salts with HBr (**3b** and **4b**, respectively).

The most pronounced effect is observed in the ^{13}C, and ^{15}N NMR spectra. Thus, an addition of 1 mole of acid leads to a high-field shift of N(20) atom signal ($\Delta\delta_N$ = +16.97 for **3a** and $\Delta\delta_N$ = +16.93 for **3b**). A high-field shift of the nitrogen atom signal of the aromatic amino group ($\Delta\delta_N$ = −9.30 for **4a** and $\Delta\delta_N$ = −11.84 for **4b**) occurs on the addition of another mole of the protonating agent (HCl or HBr). At that the position of N(20) atom signals is the same as for the salts **3a** and **3b**[34] (Table 1, Fig. 1). The observed change in the spectral pattern indicates a significant difference in the basicity of the two nitrogen atoms in compound **2** and stepwise protonation of nitrogen atoms. Namely, at the first step, N(20) nitrogen is protonated, while at the second step, the nitrogen of aromatic amino group C(2″)—N is protonated.

The stepwise protonation of nitrogen atoms is also confirmed by ^{13}C NMR spectroscopy. At the first step, significant signal shifts are observed for the C(17), C(19), C(21), and C(22) atoms.[34] At the second step of protonation, significant shifts of the atoms of the aromatic cycle C(1″—3″), C(5″) and OCO-group (see Table 1, Figs 1, 2) take place, which indicates the protonation of the primary amino groups of the anthranil fragment.[34]

The constants and thermodynamic characteristics of the protonation of compound **2** in methanol were determined using a standard methodology[35] (Table 2).

According to the results obtained, the protonation constant (pK_{b1}) is 6.77 at 25 °C, *i.e.* in methanol at this value of pH, a half of the molecules of compound **2** are in the protonated form. The proton addition occurs at N(20) atom according to NMR spectroscopy.

Table 1. Values of chemical shifts in ^{13}C and ^{15}N spectra of studied compounds (δ, CD_3OD)*

Atom	**2**	**3a**		**3b**		**4a**		**4b**	
	δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ
N(20)	41.05	58.02	+16.97	57.98	+16.93	58.09	+17.04	57.86	+16.81
C(2″)—N	61.53	62.91	+1.38	63.62	+2.09	52.23	−9.30	49.69	−11.84
C(4)	83.80	83.87	+0.07	83.85	+0.05	83.85	+0.05	83.83	+0.03
C(11)	52.29	51.94	−0.35	52.00	−0.29	51.99	−0.30	52.16	−0.13
C(17)	62.33	63.68	+1.35	63.89	+1.56	63.59	+1.26	63.86	+1.53
C(19)	57.15	58.31	+1.16	58.38	+1.23	57.97	+0.82	58.02	+0.87
C(21)	49.96	50.63	+0.67	50.67	+0.71	50.72	+0.76	50.79	+0.83
C(22)	13.66	10.84	−2.82	10.91	−2.75	10.85	−2.81	10.94	−2.72
OC(O)	168.71	167.99	−0.72	168.23	−0.48	166.30	−2.41	166.15	−2.56
C(1″)	112.37	111.69	−0.68	110.70	−1.67	120.16	+7.79	121.26	+8.89
C(2″)	152.68	151.73	−0.95	153.21	+0.53	139.69	−12.99	138.42	−14.26
C(3″)	117.84	118.51	+0.67	117.87	+0.03	123.55	+5.71	124.05	+6.21
C(4″)	134.87	135.64	+0.77	135.57	+0.70	135.99	+1.12	136.09	+1.22
C(5″)	116.56	117.60	+1.04	116.55	−0.01	126.12	+9.56	127.18	+10.62
C(6″)	132.19	132.14	−0.05	132.08	−0.11	132.83	+0.64	133.07	+0.88

* Δ values correspond to the shifts of atom signals in compounds **3a,b** and **4a,b** relative to compound **2**.

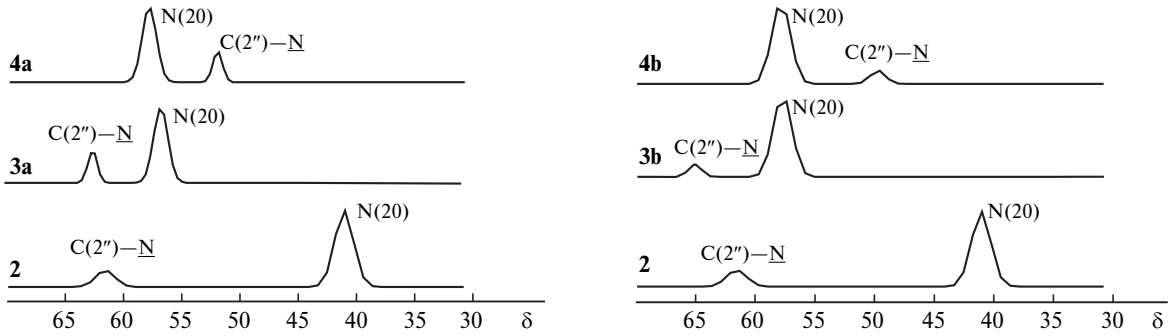

Fig. 1. ^{15}N-Projection of NMR spectra {^1H,^{15}N} in HMBC experiment of compounds **2**, **3a**, **4a**, **3b,** and **4b** in CD_3OD solution.

Figure 3 shows a plot of the change of pH of solution on the ratio of molar concentrations of compound **2** and HCl.

It is evident that the protonation at the first step (at the heterocyclic nitrogen) occurs at pH 6—7, while at the second step (at the primary aromatic amino group) begins at pH below 3.0, pK_{b2} consists of 2.18 at 25 °C. Identical value of pK_{b2} was obtained for aqueous solutions of anthranilic acid by spectrophotometric method.[36]

Thermodynamic characteristics of the acid-base equilibrium (see Table 2) show the energy disadvantage of

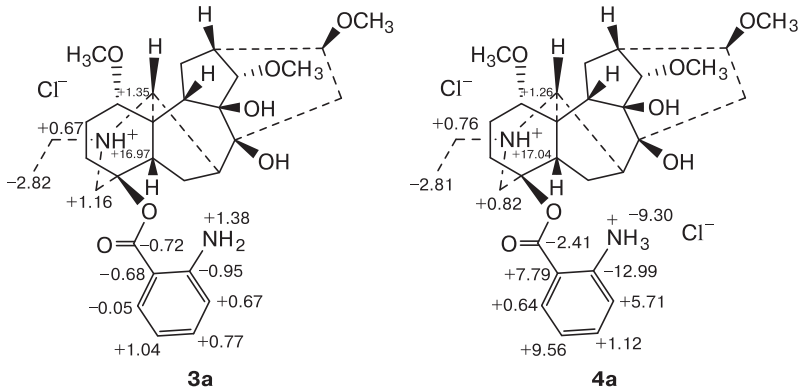

Fig. 2. Change of chemical shift in ^{13}C and ^{15}N spectra of compounds **3a** and **4a** in CD_3OD solution.

Table 2. Constants and thermodynamic characteristics of protonation of compound **2** in CH_3O

Protonation step	T/°C	pK_b	ΔG^{298}/kJ mol^{-1}	ΔH/kJ mol^{-1}	ΔS^{298}/J (mol K)$^{-1}$
I	15	6.92±0.11	38.6±0.8	35.6±0.9	−10.0±1.0
	25	6.77±0.07			
	35	6.59±0.06			
	45	6.30±0.08			
II	15	2.40±0.11	12.4±0.6	23.1±0.7	36.0±2.0
	25	2.18±0.10			
	35	2.13±0.11			
	45	1.46±0.10			

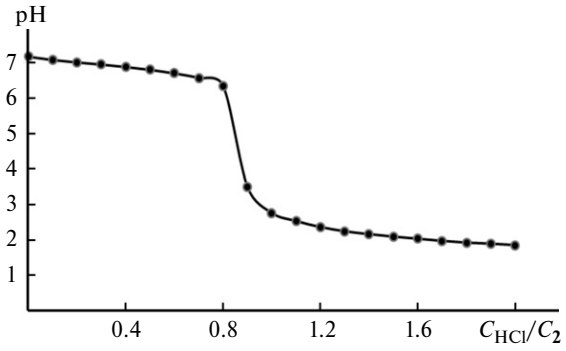

Fig. 3. Dependence of change of pH in methanolic solution of compound **2** on ratio C_{HCl}/C_2.

protonation of compound **2** in methanolic solutions. Therefore, it is necessary to increase H^+ concentration, *i.e.* decrease a pH value for a shift of equilibrium to the protonated form.

Thus, it was shown by NMR spectroscopy and potentiometric titration that a stepwise protonation of nitrogen atoms occurs in the interaction with strong acids (HCl and HBr) in methanolic solutions of *N*-deacethyllappaconitine (**2**). At the first step, N(20) atom (pK_{b1} = 6.77, 25 °C) is protonated in methanolic solutions at pH 6—7, while nitrogen of the aromatic primary amino group (pK_{b2} = 2.18, 25 °C) is protonated at pH 2—3. Consequently, it is possible to obtain both mono- and di-salts by tuning the pH value. Obtained results are undoubtedly important for the creation of a medication on the basis of *N*-deacethyllappaconitine, since the antiarrhythmic action of mono- and di-salts can be rather different.

It is known that the diterpene alkaloid lappaconitine and the compounds obtained on its basis are included in many drug formulations. Considering the availability of lappaconitine and the widespread growth of *Aconitum leucostomum* Worosch, *A. septentrionale* Koelle, and *A. sinomontanum* Nakai plants, the basic sources of its production, it is possible to consider the studies on the creation of new drug agents based on lappaconitine as promising.

Experimental

N-Deacethyllappaconitine (**2**) was prepared by hydrolysis of lappaconitine according to a known procedure.[37] M.p. 213—214 °C, $[\alpha]_D^{20}$ +29.2 (*c* 0.9, $CHCl_3$), physico-chemical characteristics of the sample were identical to those described in the literature.[37,38]

The value of pK_b of compound **2** was determined according to the standard methodology[35] by potentiometric titration in a one-necked thermostated reactor (25 mL volume) with a reflux condenser at four temperatures, namely, 15, 25, 35, and 45 °C. The temperature was maintained to within ±0.1 °C using a LOIP LT-205 thermostate. The titration was carried out using a pH-150MI pH-meter with a combined glass electrode ESK-10307. The calibration of the electrode was carried out with the help of standard buffer solutions. The ionic force was not taken into account at the titrationic in methanol solutions. Methanol for the gradient HPLC (99%, Akvametriya, Russia) was used as a solvent. Due to low solubility of compound **2** in water, its solutions for potentiometric titration were prepared in methanol with a concentration of 0.01 mol L^{-1} as follows. Compound **2** (0.2713 g) was placed in a 500 mL volumetric flask, then methanol (100 mL) was added and the mixture was stirred until complete dissolution. Afterwards, the methanol was added to the label.

UV spectra of methanolic solutions of compound **2** (10^{-4} mol L^{-1}) at different pH were recorded using a Shimadzu UV-1800 spectrophotometer in a wavelength range of 200—400 nm. The pH values were maintained by the small addition of HCl to the initial methanol solution of compound **2**. The dilution was <5%. Pure methanol served as the comparison solution. Quartz cuvettes with optical pathlength of 1 cm were used.

^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance III impulse spectrometer with an operating frequency of 500.13 MHz (^{1}H), 125.47 MHz (^{13}C), and 50.67 MHz (^{15}N) equipped with a Z-axis gradient unit and operated with a 5 mm PABBO probe at a sample constant temperature of 298 K. The chemical shifts in the ^{1}H and ^{13}C NMR spectra are presented relative to the signal of the tetramethylsilane (Me_4Si) internal standard, and in the ^{15}N NMR spectra to the signal of the external standard of liquid ammonia. The delay between impulse sequences was established to achieve complete relaxation. For the sake of increasing the digital resolution, zero-filling and Fourier-image multiplication of the spectrum by an exponential function (0.1 Hz for ^{1}H and 1 Hz for ^{13}C) were used.

The spectra were recorded using the equipment of the Center for Collective Use "Chemistry" of the Ufa Institute of Chemistry of the UFRC RAS and RCCU "Agidel" of the UFRC RAS.

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References

1. H. V. Rosendahl, *Arb. Pharmakol. Inst. Dorpat.*, 1895, **11**, 1.
2. S. W. Pelletier, N. V. Mody, in *The Alkaloids, Chemistry and Pharmacology*, Ed. R. H. F. Manske, R. G. A. Rodrigo, Academic Press, New York, 1979.
3. O. Achmatowicz, Y. Tsuda, Leo Marion, *Can. J. Chem.*, 1965, **43**, 2336.
4. V. A. Tel´nov, M. S. Yunusov, S. Yu. Yunusov, *Chem. Nat. Compd.*, 1970, **6**, 598.
5. G. I. Birnbaum, *Tetrahedron Lett.*, 1969, **26**, 2193.
6. G. I. Birnbaum, *Acta Crystallogr.*, 1970, **26**, 755.
7. M. Shamma, P. Chinnasmy, G. A. Miana, A. Khan, M. Bashir, M. Salazar, A. Patil, J. L. Beal, *J. Nat. Prod.*, 1979, **42**, 615.
8. F. S. Sadritdinov, A. G. Kurmukov, *Farmakologiya rastitelnikh alkaloidov i ikh primenenie v medizine* [*Pharmacology of plant alkaloids and their use in medicine*], Medizina, Tashkent, 1980, 310 pp (in Russian).
9. N. V. Mody, in *The Alkaloids: Chemical and Biological Perspectives*, Ed. S. W. Pelletier, Wiley, New York, 1983, 432 pp.
10. Pat. USSR No. 1335293, *Byul. Izobr.* [*Invention Bull.*], 1987, 33 (in Russian).
11. S. F. Sokolov, F. N. Dzhakhangirov, *Kardiologiya* [*Cardiology*], 2002, 96 (in Russian).
12. F. N. Dzhakhangirov, A. E. Valeev, F. S. Sadritdinov, *Uzb. biol. zhurn.* [*Uzbek Biological Journal*], 1986, 7 (in Russian).
13. F. N. Dzhakhangirov, S. F. Sokolov, A. N. Verkhovsky, *Allapinin — novij protivoaritmicheskij preparat rastitelnogo proiskhozhdeniya [Allapinin — a New Anti-arrhythmic Drug of Plant Origin],* Fan, Tashkent, 1993, 36 pp. (in Russian).
14. S. F. Sokolov, *Kardiologiya i serdechno-sosidistaya khirurgiya* [*Cardiology and Cardiovascular Surgery*], 2011, 73 (in Russian).
15. Pat. RF No. 2513580, *Byul. Izobr.* [*Invention Bull.*], 2014, 11 (in Russian).
16. Sh. Sh. Sagdullaev, A. Z. Sadikov, T. T. Shakirov, R. A. Rafikov, *Pharm. Chem. J.*, 2000, **34**, 310.
17. M. S. Yunusov, *Russ. Chem. Bull.*, 2011, **60**, 633.
18. F. N. Dzhakhangirov, B. Tashkhodzhaev, M. N. Sultankhodzhaev, B. T. Salimov, *Chem. Nat. Compd.*, 1997, **33**, 190.
19. E. B. Maikov, D. Sc. (Med.), Thesis, A. L. Myasnikov Institute of Clinical Cardiology, Moscow, 2014, 204 pp. (in Russian).
20. E. B. Maikov, Yu. A. Yuricheva, N. Yu. Mironov, S. F. Sokolov, S. P. Golizin, L. V. Rosenshtraukh, E. I. Chazov, *Terapevticheskii arkhiv* [*Therapeutic Archive*], 2015, 38 (in Russian).
21. S. F. Sokolov, *Vestn. Aritmologii* [*Bull. Arrhythmology*], 2011, **64**, 60.
22. Pat. RF No. 2664668, *Byul. Izobr.* [*Invention Bull.*], 2018, 24 (in Russian).
23. V. N. Stolyaruk, I. B. Zorin, M. B. Vititnova, T. D. Nikiforova, Yu. I. Murinov, M. S. Yunusov, S. A. Krizhanovsky, *Farmakokinetika i farmakodinamika* [*Pharmacokinetics and Pharmacodynamics*], 2017, **2**, 12 (in Russian).
24. I. B. Zorin, V. P. Zinchenko, I. Yu. Teplov, A. M. Kosenkov, Yu. I. Murinov, M. S. Yunusov, S. A. Krizhanovsky, *Farmakokinetika i farmakodinamika* [*Pharmacokinetics and Pharmacodynamics*], 2017, **2**, 4 (in Russian).
25. V. N. Stolyaruk, I. B. Zorin, M. B. Vititnova, V. P. Gusev, Yu. I. Murinov, M. S. Yunusov, S. A. Krizhanovsky, *Farmakokinetika i farmakodinamika* [*Pharmacokinetics and Pharmacodynamics*], 2017, 2 (in Russian).
26. D. V. Duplyakov, S. I. Dolginina, *Kardiologiya: Novosti, mneniya, obuchenie* [*Cardiology: News, Opinions, Training*], 2016, 25 (in Russian).
27. A. Adnan, V. A. Rulin, N. A. Mazur, S. I. Chikovani, *Farmakol. i toksikol.* [*Farmakol. and Toxicol.*], 1988, 47 (in Russian).
28. A. Z. Sadikov, D. Sc. (Techn.), Thesis, S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, 2015, 137 pp.
29. Pat. US 5290784, 1994.
30. Pat. US 55479956, 1993.
31. T. G. Tolstikova, A. O. Brizgalov, I. V. Sorokina, M. P. Dolgikh, E. E. Shulz, S. A. Osadchy, G. A. Tolstikov, *Dokl. AN*, 2007, **415**, 837 [*Dokl. Chem.* (*Engl. Transl.*), 2007, **415**] (in Russian).
32. T. Stalin, N. Rajendiran, *J. Photochem. Photobiol. A*, 2006, 137.
33. O. K. Abou-Zied, B. Y. Al-Busaidi, J. Husband, *J. Phys. Chem. A*, 2014, 103.
34. M. Witanowski, I. Stefaniak, G. A. Webb, *Annu. Rep. NMR Spectrosc.*, 1978, **7**, 117.
35. A. Albert, E. P. Serjeant, *Ionization Constants of Acids and Bases*, London, Wiley, 1962, 179 pp.
36. Zapała, J. Kalembkiewicz, E. Sitarz-Palczak, *Biophys. Chem.*, 2009, 91.
37. H. Schulze, *Arch. Pharm. Ber. Deutsch. Pharm. Ges.*, 1922, 230.
38. N. V. Anferova, I. Yu. Bagryanskaya, Yu. V. Gatilov, S. A. Osadchii, M. M. Shakirov, E. E. Shults, G. A. Tolstikov, *Russ. Chem. Bull.*, 2003, **52**, 2500.

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