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INVESTIGATION OF BIOTRANSFORMATION PROCESSES AND PHARMACOLOGICAL ACTIVITY OF VIOLURIC ACIDS AND THEIR METABOLITES IN *IN VIVO* EXPERIMENTS

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Introduction. 5-Hydroxyiminobarbituric (violuric) acid and its derivatives exhibit pronounced anti-hypoxic, hepatoprotective, cytoprotective, actoprotective, and other properties, making this group of compounds a promising field for pharmaceutical research. Data on the metabolism of violuric acids (VAs) are of significant practical and theoretical importance for the tracking of pharmacokinetics and substance distribution within the organism, in which process metabolites serve as markers of biochemical processes involving endogenous substrates.

Objective. To determine the structure of violuric acid metabolites and perform their quantitative assessment in an *in vivo* experiment.

Materials and methods. The studied substances (VAs) and their metabolites (purpuric acids) were synthesized at the Golikov Research Center of Toxicology. Their structure and purity were confirmed by nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), and spectrophotometry (SP). Metabolism and anti-hypoxic activity were studied using a model of hemic hypoxia induced by a lethal dose of sodium nitrite in outbred male white rats. Solutions of violuric acids for *in vitro* studies and administration to animals were prepared in distilled water with the addition of tris(oxyethyl)aminomethane (TRIS), while purpurates in the form of salts were dissolved in distilled water. The dosages of the test substances for rats ranged from 50–75 mg/kg for intraperitoneal administration and 50–100 mg/kg for intragastric administration. The reference substance (amitizole succinate) was administered to animals at a dose of 100 mg/kg. A 0.9% sodium chloride solution, administered at a volume of 1 mL per animal, was used as a control. Quantitative analysis of the substances and their metabolites in biological media was performed by HPLC with SP detection.

Results. It was established that the studied substances (violuric acid, 2-thiovioluric acid, 1-butylvioluric acid, and 1-(4-bromophenyl)violuric acid) are metabolized in the animal organism to form the corresponding derivatives of purpuric acid (purpurates), whose structure was confirmed by counter synthesis. Violuric acids and their metabolites are primarily excreted in the urine. It was demonstrated that 1-butylvioluric acid and its metabolite *N,N'*-dibutylpurpuric acid exhibit pronounced anti-hypoxic activity in the model of acute sodium nitrite poisoning, preventing mortality in 100% of animals, whereas the reference antihypoxant amitizole only prolongs survival time (by 23%).

Conclusions. The formation of purpurates is a characteristic pathway of metabolic transformation for the violuric acid scaffold. These metabolites exhibit pronounced activity and, in all likelihood, can contribute to the overall biological effect of violuric acids.

Keywords: violuric acid; derivatives; metabolism; purpuric acids; rats; antihypoxants; HPLC; amitizole

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ИЗУЧЕНИЕ ПРОЦЕССОВ БИОТРАНСФОРМАЦИИ И ФАРМАКОЛОГИЧЕСКОЙ АКТИВНОСТИ ВИОЛУРОВЫХ КИСЛОТ И ИХ МЕТАБОЛИТОВ В ЭКСПЕРИМЕНТАХ *IN VIVO*

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Введение. 5-Оксииминобарбитуровая (виолуровая) кислота и ее производные проявляют выраженные антигипоксические, гепатопротекторные, цитопротекторные, актопротекторные и другие свойства, что делает эту группу веществ перспективным полем для фармацевтических изысканий. Данные о метаболизме виолуровых кислот (ВК) представляют большую практическую и теоретическую значимость, позволяя отслеживать фармакокинетику и распределение вещества в организме, причем метаболиты служат маркерами биохимических процессов, протекающих при участии эндогенных субстратов.

Цель. Установление структуры продуктов метаболизма виолуровых кислот и их количественная оценка в эксперименте *in vivo*.

Материалы и методы. Исследуемые вещества (ВК) и продукты их метаболизма (пурпуровые кислоты) были синтезированы на базе Научно-клинического центра токсикологии им. С.Н. Голикова Федерального медико-биологического агентства, их структура и чистота подтверждены методами ядерного магнитного резонанса (ЯМР), высокоэффективной жидкостной хроматографии (ВЭЖХ)

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и спектрофотометрии (СФ). Метаболизм и антигипоксическую активность изучали на модели гемической гипоксии, вызванной смертельной дозой нитрита натрия, на беспородных белых крысах-самцах. Растворы виолуровых кислот для исследований *in vitro* и введения животным готовили в дистиллированной воде с добавкой трис(оксиметил)аминометана (ТРИС), а пурпураты, используемые в форме солей, растворяли в дистиллированной воде. Дозировки исследуемых веществ для крыс составляли 50–75 мг/кг при внутривенном и 50–100 мг/кг при внутрижелудочном введении. Эталонное вещество (амтизол сукцинат) вводили животным в дозе 100 мг/кг. В качестве контроля использовали раствор хлорида натрия 0,9%, который вводили в объеме 1 мл на животное. Количественный анализ веществ и их метаболитов в биосредах осуществлен методом ВЭЖХ с СФ-детектированием.

Результаты. Установлено, что исследуемые вещества (виолуровая, 2-тиовиолуровая, 1-бутилвиолуровая и 1-(4-бромфенил)виолуровая кислоты) в организме животных метаболизируются с образованием соответствующих производных пурпуровой кислоты (пурпуратов), структура которых была доказана встречным синтезом. ВК и продукты их метаболизма выводятся преимущественно с мочой. Показано, что 1-бутилвиолуровая кислота и ее метаболит *N,N'*-дибутилпурпуровая кислота на модели острого отравления NaNO_2 обладают выраженной антигипоксической активностью, предотвращая гибель 100% животных, тогда как эталонный антигипоксикант Амтизол лишь продлевает время жизни (на 23%).

Выводы. Образование пурпуратов является характерным направлением метаболической трансформации скаффолда виолуровых кислот. Данные метаболиты обладают выраженной активностью и, по всей видимости, могут участвовать в формировании общего биологического эффекта виолуровых кислот.

Ключевые слова: виолуровая кислота; производные; метаболизм; пурпуровые кислоты; крысы; антигипоксиканты; ВЭЖХ; амтизол

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Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ «Научно-клинический центр токсикологии имени академика С.Н. Голикова» Федерального медико-биологического агентства (№ 1/08-25 от 13.08.2025).

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INTRODUCTION

Antihypoxants, antioxidants, adaptogens, actoprotectors, and other pharmacological agents that enhance the body's adaptive capabilities play a significant role in modern pharmacology. Drugs from these pharmaceutical classes — primarily antihypoxants — are widely used in various medical fields, such as surgical practice [1], cardiology [2], neurology [3, 4], sports and extreme medicine [5], psychiatry [6], in cases of traumatic brain injury and cerebrovascular disorders [7, 8], treatment of liver intoxications [9], and many other areas [10]. Consequently, the development of new anti-hypoxic drugs and the search for novel approaches in the therapy of hypoxic conditions remain highly relevant [11].

Priority directions in the search for antihypoxants focus on original groups of synthetic heterocyclic compounds, among which are found substances capable of engaging additional mechanisms to enhance the body's adaptive reserve. In this regard, 5-hydroxyiminobarbituric acids, also referred to as violuric acids (VAs), represent a group of heterocyclic oximes offering promising pharmacological properties [12].

The simplest derivative of this series is the unsubstituted violuric acid (VA1), whose structure is presented in

Figure 1. This substance was investigated in a previous study,¹ in which its high antidotal efficacy in models of hemic hypoxia induced by carbon monoxide and methemoglobin-forming poisons (sodium nitrite, aniline, etc.) was established, surpassing standards such as unithiol, amtizole, gutimine, and methylene blue. VA1 also demonstrated good results in models of hypobaric and hypercapnic hypoxia. Another derivative of this series, 2-thiovioluric acid (VA2), similarly exhibited antidotal activity in nitrite-induced hemic hypoxia. Furthermore, VA2 showed a pronounced protective effect in a model of nitric oxide-induced pulmonary edema.² 1-Butylvioluric acid (VA3), used in the form of a zinc complex, demonstrated a protective effect in carbon monoxide poisoning, surpassing the known drug acizol, as well as showing a therapeutic effect in animals poisoned with neurotoxic agents [12].

For VA1 and its analogs, certain antioxidant and membrane-stabilizing properties, as well as the ability to increase hemoglobin stability *in vitro*, have been noted at the biochemical level [12, 13].³ In addition to the aforementioned types of activity, various derivatives within the VA series have demonstrated hepatoprotective, antimicrobial, antiviral, analgesic, tranquilizing, and other properties [14]. It is worth noting separately that

¹ Burbello AT. Barbituric Acid Derivatives — A New Class of Compounds for the Prevention and Treatment of Nitrocompound Poisonings. Abstract of Doctoral Dissertation (Dr. Med. Sci.). St. Petersburg, 1991 (In Russ.).

² Ibid.

³ Ibid.

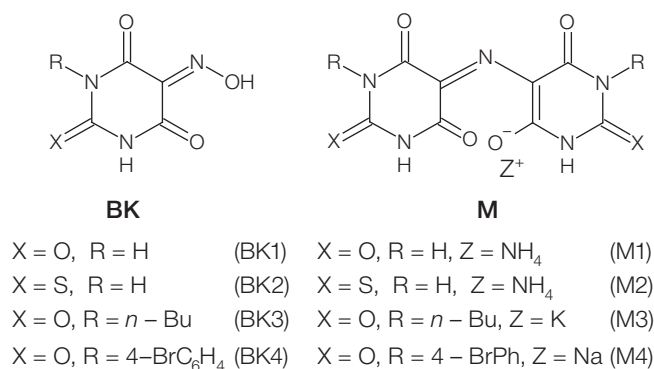


Figure prepared by the authors based on original data

Fig. 1. Structural formulas of violuric acids and salt derivatives of purpuric acid.

1-(4-bromophenyl)violuric acid (VA4) was officially registered as a hepatoprotective drug⁴.

This collectively positions violuric acids as a pharmacologically promising group, serving as a basis for the development of new drugs, particularly antihypoxants, actoprotectors, and antidotal agents, which are highly relevant for modern medicine. However, the biological targets and mechanisms of action of violuric acids remain unknown to date. Consequently, given the crucial role of drug metabolism data for screening and pharmaceutical development [15], investigating the patterns of distribution and biotransformation of violuric acids *in vivo* is of significant practical importance.

The aim of this study is to determine the structure of violuric acid metabolites and perform their quantitative assessment in an *in vivo* experiment.

MATERIALS AND METHODS

Physicochemical analysis

To confirm the molecular structure of the synthesized substances, nuclear magnetic resonance (NMR) spectroscopy was used. ¹H and ¹³C NMR spectra were recorded in DMSO-*d*₆ on a Bruker Avance 400WB spectrometer operating at 400 MHz and 100 MHz, respectively. Electronic spectra of the synthesized substances in the UV and visible regions were recorded on a Shimadzu UV-1800 scanning spectrophotometer using standard quartz cuvettes. Analysis of the purity of the synthesized substances, along with their identification and an assessment of their quantitative content in the biological media of experimental animals, was performed by high-performance liquid chromatography (HPLC) on a Shimadzu LC-20 Prominence chromatograph equipped with an autosampler and a diode array spectrophotometric detector. Separation was carried out on a Chromolith Performance-RP reversed-phase monolithic column having a length of 10 cm and at a

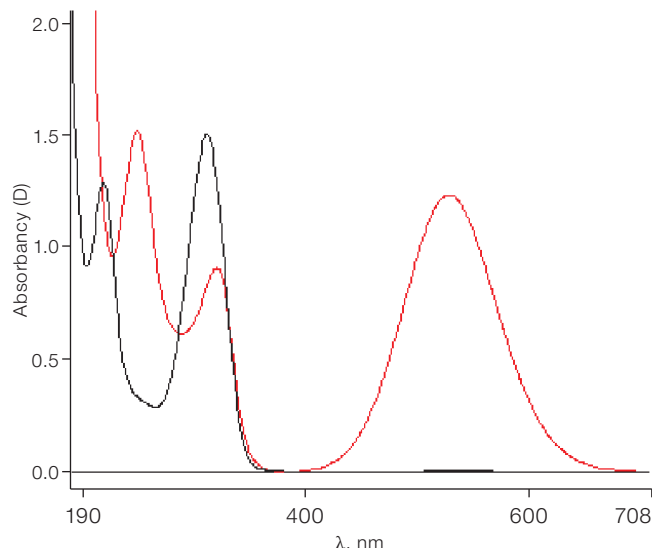


Figure prepared by the authors based on original data

Fig. 2. Absorption spectra of 1-butylvioluric acid (black) and its metabolite (red) at a concentration of 9×10^{-5} mol/L for each substance (water, pH 7.0)

column temperature of 40°C. The eluent flow rate was 5 mL/min, using a mobile phase of acetonitrile (phase A) and a 0.1% aqueous ammonium acetate solution (phase B) in a gradient elution mode. The gradient conditions were: A 1–30% (0–3 min), A 30% (3–5 min).

The analytical characteristics of the investigated substances and their metabolites, used for their identification and quantitative analysis in biological media, are presented in Table 1.

As an illustration, Figure 2 presents the electronic spectra of 1-butylvioluric acid (VA3) and its metabolite, *N,N'*-dibutylpurpurate (M3).

Synthesis of test and reference substances

The synthesis of all investigated substances was performed at the Golikov Scientific and Clinical Center of Toxicology. Violuric acids (VAs) were synthesized according to the general method described in patent [14]. The VA synthesis scheme consisted of two stages. In the first stage, the corresponding barbituric acid derivatives were obtained from substituted ureas and malonic ester. In the second stage, the target VAs were produced from these derivatives via nitrosation. The purity of the synthesized substances was not less than 98%. Purpuric acid in the form of its ammonium salt (M1, murexide) was a commercial product of AR (analytical reagent) grade (LenReaktiv, Russia). All other substances used in the work were commercially available products. The metabolites of violuric acid derivatives — 2,2'-dithiopurpuric acid (M2) and *N,N'*-dibutylpurpuric acid (M3) — were obtained in the form of ammonium and potassium salts,

⁴ State Register of Medicines. Moscow: Ministry of Health of Russia, Pharmaceutical Information Foundation; Marketing Authorization No. LP-00444 dated September 1, 2017 (In Russ.). https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=89b99d38-99b5-45b1-a65d-565936e3ded0

Table 1. Spectral and chromatographic characteristics of violuric and purpuric acids

Substance	$\lambda_{\max}$, nm	$E_{\max}$, $M^{-1} \times cm^{-1}$	RT, min
VA1	311.0	24,800	0.289
VA2	349.0	22,300	0.300
VA3	312.0	18,700	0.617
VA4	314.0	14,900	0.910
M1	521.0	10,400	0.300
M2	568.5	19,500	0.340
M3	528.5	12,900	1.832
M4	526.5	10,000	1.920

Table prepared by the authors based on original data

Note: $\lambda_{\max}$ — absorption maximum wavelength; $E_{\max}$ — molar extinction coefficient at the absorption maximum; RT — retention time.

respectively, according to the method described in reference [16], at a purity of not less than 95%.

Synthesis protocol for the sodium salt of N,N'-di-(4-bromophenyl)purpuric acid (M4). 0.005 mol (1.56 g) of 1-(4-bromophenyl)violuric acid (VA4) and 0.005 mol (1.41 g) of 1-(4-bromophenyl)barbituric acid [14] were dissolved in 10 mL of DMSO (dimethyl sulfoxide) at 50°C. 0.01 mol of ammonium acetate dissolved in 3 mL of methanol was added to this solution with stirring. The resulting mixture was stirred for 1 h at 50°C, then diluted with 25 mL of a 3% aqueous sodium bicarbonate solution and left for 1 h at room temperature for precipitate formation. The resulting precipitate was isolated using a Schott filter, washed with 10 mL of a 1% aqueous sodium bicarbonate solution, followed by 5 mL of distilled water, and then air-dried to constant weight. 1.35 g of a dark red powder of M4 with a decomposition temperature above 250°C was obtained. The yield of purpurate M4 was 78% of the theoretical at a purity of not less than 95%.

¹H NMR spectrum (DMSO-*d*₆), chemical shift (δ), multiplicity in parts per million (ppm): 7.23 (4H, Ar), 7.66 (4H, Ar), 10.5–11.5 broad singlet or br.s (2H, 2NH). ¹³C NMR spectrum (DMSO-*d*₆), δC, ppm: 121.1 (C⁵ + C^{5a}), 131.5 (4C_{Ar}), 131.6 (4C_{Ar}), 135.2 (4C_{Ar}), 149.9 (2C²O), 155–165 br.s (4CO).

The drug amtizole, used as a reference antihypoxant, was synthesized in the form of its succinate according to the method described in patent [17]; the purity of the preparation was not less than 98%.

The structure and purity of all synthesized substances were confirmed by NMR and HPLC methods.

Animals and housing

Experiments were conducted on outbred male white rats aged three months, weighing 200–240 g, procured from

the Rappolovo Laboratory Animal Nursery (Kurchatov Institute). The animals were housed under standard conditions with a 12-h light/dark cycle with ad libitum provision of standard rat chow and drinking water. Animals were randomized by weight when assigned to experimental groups.

Determination of violuric acids and their metabolites in rat urine

The study was conducted on 16 animals, which were divided into 4 groups of 4 individuals for each test substance. Administration was performed using an atraumatic gastric tube. Derivatives VA1 and VA2 were administered to animals at a dose of 80 mg/kg body weight as 5% aqueous suspensions with the addition of 2% Tween-80, while derivatives VA3 and VA4 were administered at a dose of 100 mg/kg body weight as 1% aqueous solutions with the addition of tris(oxyethyl) aminomethane (TRIS).

Following substance administration, the animals were placed in metabolic cages for urine collection, where they were housed for 24 h without access to food, while access to water was not restricted. Urine was collected at specified time intervals (4, 8 and 24 h) with volume recording. The obtained urine samples were stored in a freezer at –10°C for subsequent HPLC analysis. The methods for quantitative analysis of VAs and their metabolites in urine were developed and validated in accordance with domestic and international guidelines.⁵

For quantitative analysis, urine was diluted twofold with distilled water and analyzed by HPLC at an injection volume of 10 µL. Calibration solutions of the corresponding standards were chromatographed in parallel. A linear relationship between the chromatographic peak area and the concentration of the analyte was established in the range of 0.1–50 µg/mL. The quantitative content of

⁵ Beregovykh VV. Validation of analytical methods for drug manufacturers. Standard enterprise guideline for the production of medicinal products. Moscow; 2008 (In Russ.).

the compound in urine samples was calculated based on the chromatographic peak area and corresponding analytical characteristics as listed in Table 1.

Investigation of the metabolism of 1-butylvioluric acid (VA3) based on analysis of rat blood plasma and organs

The study was conducted on 20 animals, with 2 individuals per time point (15, 30, 60, 120, 180 min). To investigate the biotransformation and distribution of VA3 *in vivo*, the animals were divided into 2 groups of 10 individuals each, based on the route of administration: intraperitoneal (Group 1) and intragastric (Group 2). The dosage of VA3 in both cases was 50 mg/kg body weight; the test substance was administered to the animals as a 1% aqueous solution with the addition of 0.7% TRIS. Following the specified time intervals, the animals were euthanized by decapitation. After collecting whole blood (4–6 mL) from the rats, plasma was separated from erythrocytes by centrifuging the blood at 6000 rpm. Internal organs (liver and kidneys) were also collected. Plasma samples and organs were frozen immediately after collection and stored at -10°C for subsequent HPLC analysis.

Preparation of Blood Plasma Samples for HPLC Analysis. 0.5 mL of rat blood plasma was placed into a centrifuge tube with the addition of 1.0 mL of methanol, mixed, and the protein precipitate was separated by centrifugation for 5 min at 16,000 rpm. The supernatant was separated and analyzed by HPLC at an injection volume of 30 μL . The content of substances VA3 and M3 in the blood plasma samples was determined from the chromatographic peak area using the corresponding analytical characteristics provided in Table 1. The method for calculating the concentration of VA3 and its metabolite M3 in blood plasma was validated in accordance with domestic and international guidelines.⁶

Preparation of liver tissue samples for HPLC analysis. 0.5 g of rat liver sample was ground in an agate mortar to produce a homogeneous paste. The ground material was mixed with 1 mL of methanol, transferred to a centrifuge tube, and centrifuged for 5 min at 16,000 rpm. The supernatant was separated and analyzed by HPLC, with an injection volume of 30 μL . Preparation of kidney tissue samples for HPLC analysis was performed in the same manner as for the liver.

The content of substance VA3 and its metabolite M3 in the liver and kidney samples was determined from the chromatographic peak area using the corresponding analytical characteristics provided in Table 1.

To study the anti-hypoxic activity of 1-butylvioluric acid (VA3) and its metabolite, potassium *N,N'*-dibutylpurpurate (M3), four experimental groups ($n = 6$) were formed: No. 1 — control; No. 2 — reference

standard; Nos. 3 and 4 — test groups. The study was conducted using a model of hemic hypoxia induced by a lethal dose of sodium nitrite, in accordance with methodological guidelines for the investigation of anti-hypoxic agents.⁷ The protective activity was assessed under conditions of prophylactic intraperitoneal administration of the substances. The drug amtirole, recognized as one of the most universal antihypoxants [18], was used as the reference substance.

Control animals in group No. 1 were administered 1 mL of a 0.9% sodium chloride solution. Animals in group No. 2 were administered amtirole succinate at a dose of 100 mg/kg as a 10% aqueous solution. Animals in groups No. 3 and No. 4 were administered with the test substances VA3 and M3, respectively, as 7.5% aqueous solutions, at substance dosages of 75 mg/kg.

Sodium nitrite (AR grade, LenReaktiv, Russia) was administered to rats subcutaneously as a 2% aqueous solution 30 min after the administration of the test substances. The dose of sodium nitrite was 100 mg/kg, which is 25% higher than the lethal dose for rats ($\text{LD}_{100} = 80 \text{ mg/kg}$). During the experiment, the animals were housed in enclosures of 6 individuals each for free observation of the clinical picture.

The clinical manifestations of the action of sodium nitrite and the test substances were recorded during the experiment. Animal death was recorded at the moment of cardiac arrest. Observations of surviving animals were conducted for 3 days. The criteria for the efficacy of the test substances were the increase in animal survival time and the survival percentage compared to the control. Statistical processing (calculation of mean value and absolute error) of the data was performed using Microsoft Office Excel software.

RESULTS AND DISCUSSION

The design of the *in vivo* study was primarily aimed at identifying the qualitative composition of violuric acid metabolites while minimizing the number of animals used in the experiments.

The determination of metabolites in animal urine was conducted following intragastric administration of the corresponding substances VA1–VA4. The urine collection time points were chosen to assess the initial, intermediate, and final stages of excretion. When analyzing the urine composition of the experimental animals by HPLC, corresponding derivatives of purpuric acid M1–M4 were detected in all cases, alongside the unchanged compounds VA1–VA4. The corresponding structures are presented in Figure 1.

Identification of purpurates in urine was performed in each case by comparing the retention time on the chromatogram and the diode-array spectrum with synthesized reference substances M1–M4. As noted earlier,

⁶ Ibid

⁷ Lukyanova LD, ed. Methodological recommendations for the experimental study of drugs proposed for clinical investigation as anti-hypoxic agents. Moscow: USSR Ministry of Health, 1990.

purpurates possess characteristic absorption spectra in the UV and visible regions (Table 1 and Fig. 2), which allowed for their unambiguous identification in biological media using the synthesized standards M1–M4.

Quantitative indicators of the excretion of violuric acids and their metabolites in rat urine over the corresponding time intervals are presented in Table 2 and Figure 3.

The obtained results indicate that the formation of purpurates is a general pattern of biotransformation for violuric acids in the rat organism. Among the substances considered, 1-butylvioluric acid (VA3) undergoes the most extensive metabolism, with 47% excreted in urine as *N,N'*-dibutylpurpurate (M3) and 53% excreted unchanged. The substrate least prone to metabolism is 1-(4-bromophenyl)violuric acid (VA4), for which the conversion rate to its metabolite is only 15%.

The observed metabolic transformation of violuric acids is quite unusual and can even be considered unique. Judging by the structure of the formed purpurates, the biotransformation of violuric acids involves the condensation and cross-linking of two fragments of the original molecule. This cannot be classified among the well-known pathways of xenobiotic metabolism, such as oxidation, hydrolysis, conjugation, and other common transformations [19]. Since, given the low substrate concentration in the organism, the random cross-linking of two VA3 molecules seems unlikely, this transformation apparently requires a specific biochemical mechanism. This is particularly relevant since such a reaction is not characteristic for violuric acids in *in vitro* solutions, even at significantly higher concentrations.

To explain the ongoing process, we propose a mechanism schematically depicted in Figure 4. From a chemical standpoint, it is evident that one of the reaction stages involves the reduction of the oxime group in VA3. However, it is more challenging to explain how the reduced intermediate couples with the original molecule under physiological conditions. Here, the complexing properties known to be possessed by violurates [20] may play a key role. As a result of complex 1 formation with a multivalent metal cation (e.g., iron, zinc, calcium, or another cation), two VA molecules are grouped around a single coordinating center. Subsequent enzymatic reduction of the oxyimino group, forming intermediate 2, and its condensation via a nucleophilic substitution mechanism appears to be a logical continuation of the process, leading to the formation of a new chelating system — the purpurate molecule M.

According to preliminary data,⁸ substance VA3 was of the greatest interest among the VA1–VA4 derivatives in terms of its anti-hypoxic activity and was therefore selected for in-depth investigation. In the course of the present work, we assessed the concentration kinetics of 1-butylvioluric acid (VA3) and its metabolite (M3) in rat blood plasma following intragastric and intraperitoneal administration of VA3. The results are presented in Table 3.

Metabolite M3 appeared in the blood plasma as early as 15 min at a concentration of 0.10 mg/L after intragastric- and 0.38 mg/L after intraperitoneal administration of 1-butylvioluric acid, indicating rapid metabolism (Fig. 5). Furthermore, judging by the fact that the maximum

Table 2. Average amounts of parent substances and their metabolites excreted in urine following intragastric administration of violuric acids

Parent substance (dose, mg/kg)	Products in urine	Excreted in urine, mg/kg			Total excreted over 24 h	
		Time period, h			mg/kg	% of administered dose
		0–4	4–8	8–24		
VA1 (80)	VA1	4.27	4.37	0.30	8.94	11.2 ± 1.8
	M1	1.57	1.76	0.37	3.70	4.63 ± 0.91
VA2 (80)	VA2	1.25	0.30	0.28	1.83	2.29 ± 0.35
	M2	0.27	0.071	0.023	0.36	0.45 ± 0.12
VA3 (100)	VA3	8.35	3.24	1.83	13.42	13.42 ± 2.26
	M3	4.94	4.99	2.32	12.25	12.25 ± 1.92
VA4 (100)	VA4	5.12	0.91	0.18	6.21	6.21 ± 1.45
	M4	0.95	0.12	0.012	1.08	1.08 ± 0.34

Table prepared by the authors based on original data

⁸ Search for anti-hypoxants and actoprotectors in the series of violuric acid derivatives: research report / Head: Krasnov KA; Executors: Krasnova AA, Lapina NV, Ivnitsky YuYu, et al.; Golikov Scientific and Clinical Center of Toxicology. St. Petersburg, 2024. Reg. No. SRW 124022400178-1.

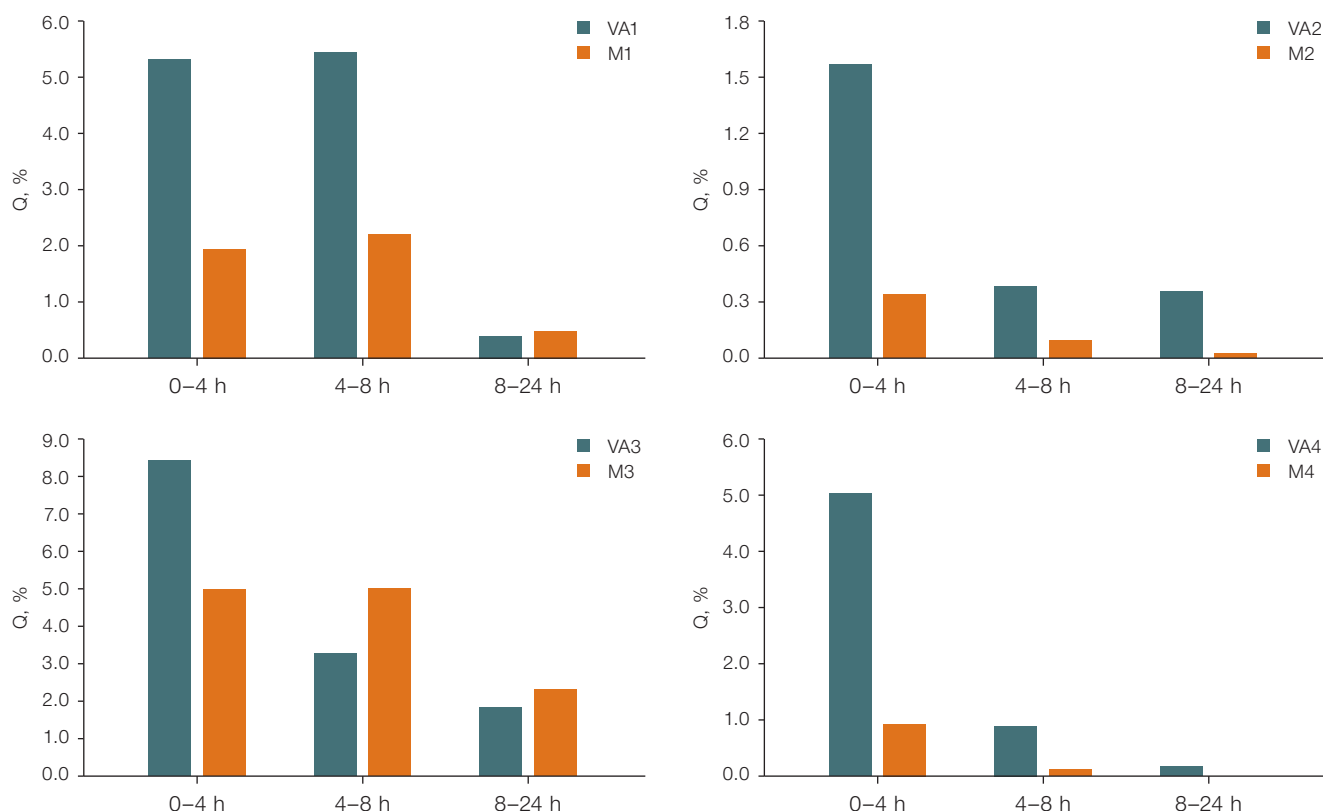


Figure prepared by the authors based on original data

Fig. 3. Excretion kinetics of violuric acids and their metabolites in rat urine following intragastric administration: data are presented as mean values; $Q\%$ — mass amount of excreted substance as a percentage of the administered dose

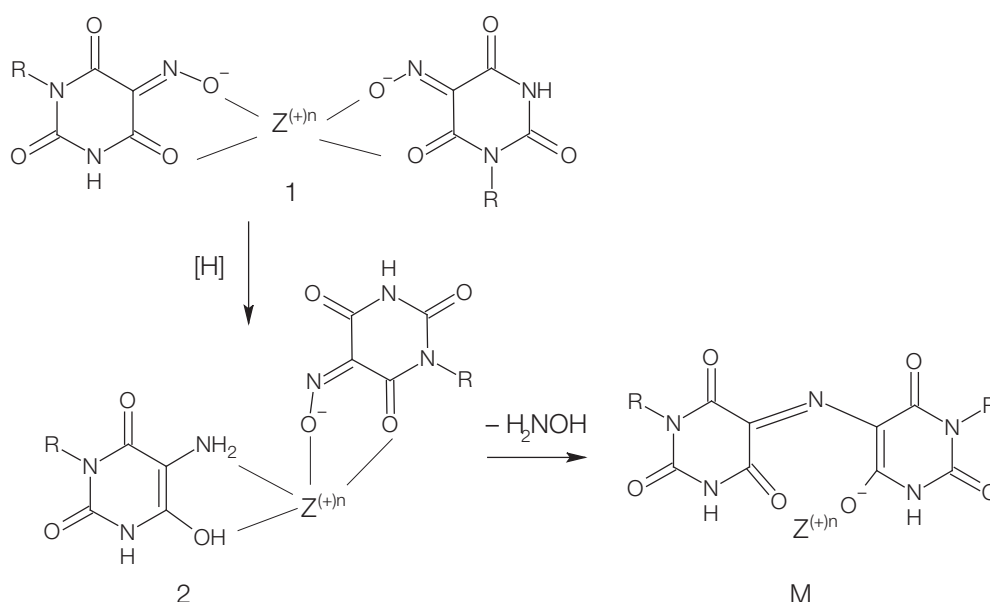


Figure prepared by the authors based on original data

Fig. 4. Hypothetical mechanism of violuric acid transformation into purpurate *in vivo*

concentration of M3 did not exceed 0.75 mg/L, no significant accumulation of the metabolite in the blood was observed, which was associated with its faster elimination via the kidneys into urine compared to the parent

substance. The purpurate was also detected in the livers and kidneys of rats. The maximum concentration after intraperitoneal administration was reached after 1 h, i.e., later than in the blood. The maximum purpurate

Table 3. Concentration of substances in blood plasma following intragastric and intraperitoneal administration of 1-butylvioluric acid

Time, min	Substance	Concentration, mg/L	
		Intragastric administration	Intraperitoneal administration
15	VA3	16.6 ± 2.5	70.8 ± 19.5
	M3	0.10 ± 0.05	0.38 ± 0.40
30	VA3	27.4 ± 3.9	29.3 ± 5.3
	M3	0.13 ± 0.03	0.74 ± 0.24
60	VA3	16.9 ± 2.7	8.7 ± 2.3
	M3	0.43 ± 0.11	0.75 ± 0.19
120	VA3	5.5 ± 1.2	1.9 ± 0.5
	M3	0.35 ± 0.14	< 0.05
180	VA3	3.9 ± 1.2	1.8 ± 0.5
	M3	0.09 ± 0.02	< 0.005

Table prepared by the authors based on original data

Note: VA3 — 1-Butylvioluric acid; M3 — *N,N'*-Dibutylpurpuric acid.

content in the liver homogenate was 0.46 mg/kg, which is 1.6 times lower than in the blood, while in the kidneys it was 1.23 mg/kg, which is 1.6 times higher than in the blood.

To address the question of the biological activity of metabolite M3, we conducted an experimental study, which demonstrated that this product possesses pronounced anti-hypoxic activity in the hemic hypoxia model in rats. Prophylactic administration of potassium *N,N'*-dibutylpurpurate (M3) completely prevented the death of animals that received a lethal dose of sodium nitrite. A similar protective effect was demonstrated by 1-butylvioluric acid (VA3) (Table 4).

The clinical picture of poisoning in rats developed 25–30 min after the subcutaneous administration of nitrite. Ataxia and dyspnea, followed by convulsions and

death of the animals were observed in the control group. In rats pre-treated with substances VA3 and M3, symptoms of poisoning were also noted; however, these were less pronounced and practically disappeared 2 h following nitrite injection. Starting from the 4th h, all animals in these groups appeared completely healthy. Under the same conditions, the reference drug amtizole did not prevent animal death, only prolonging their survival time by 23%.

Thus, it is established that M3 is an active metabolite of acid VA3 and likely plays a role in the development of the latter's pharmacological effect. This result also indicates that purpurates, whose biological activity has not been previously investigated, may be of interest as potential agents for the treatment of poisoning by methemoglobin-forming agents.

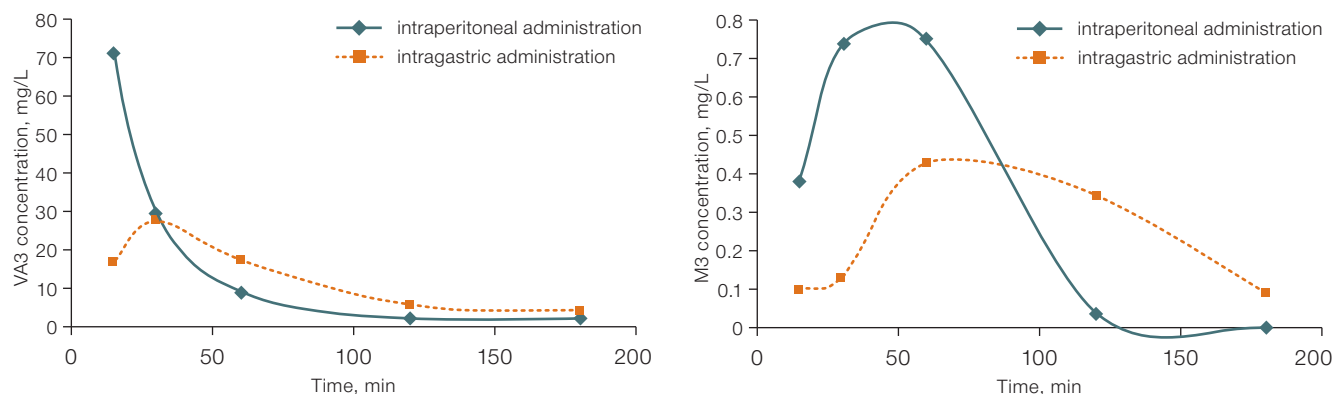


Figure prepared by the authors based on original data

Fig. 5. Concentration kinetics of 1-butylvioluric acid (left) and its metabolite (right) in rat blood plasma following its intraperitoneal and intragastric administration

Table 4. Protective efficacy of substances in rats under conditions of lethal sodium nitrite poisoning

Substance	Dose, mg/kg	Survival rate, %	Mean survival time	
			min (± ΔX)	% of control
Control (water)	–	0	46.1 (± 4.1)	100
Amtizole succinate	100	0	56.8 (± 4.5)	123
VA3	75	100	No mortality	
M3	75	100	No mortality	

Table prepared by the authors based on original data

Note: ΔX — absolute error of values; «—» — administered 1 mL of water without the active substance.

CONCLUSION

- 1. Violuric acids are metabolized in animals’ organisms, yielding corresponding derivatives of purpuric acid.
- 2. The biotransformation mechanism of violuric acids into purpurates involves reduction of the oximino group and cross-condensation with a molecule of the original substrate.

- 3. Violuric acids and their metabolites are largely excreted from the body via the kidneys in urine.
- 4. 1-butylvioluric acid (VK3) and its metabolite N,N'-dibutylpurpuric acid (M3) exhibit pronounced protective activity against sodium nitrite poisoning.
- 5. Purpuric acids, along with violuric acids, are of interest for further study as potential agents for controlling poisoning with methemoglobin-forming toxins.

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