SYNTHESIS OF KHIMKOKTSID — 1,3-BIS-(p-CHLOROBENZYLIDENEAMINO)GUANIDINE, LABELED WITH THE RADIONUCLIDES ^{14}C AND ^{3}H, AND ITS PHARMACOKINETICS

A. D. Shestakov, Yu. L. Kaminskii,
A. A. Mashirova, V. I. Zaionts,
L. A. Korovitskaya, N. A. Zakharova,
A. E. Khovanskikh, and G. A. Mikhailov

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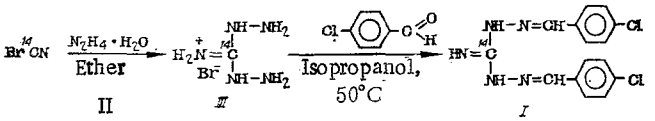
In order to perform pharmacokinetic investigations and to study the mechanism of the action of the anticoccidial and antitoxoplasmic preparation khimkoktsid — 1,3-bis-(p-chlorobenzylideneamino)guanidine (I) [1, 2] — it was considered desirable to effect the synthesis of analogs of it labeled with radionuclides. The existence of preparations with labels introduced in various parts of the structure of (I) could permit reliable results to be obtained on its distribution in the animal organism and also enable separate fragments of the substance to be traced if it underwent cleavage under physiological conditions.

The chemical structure of (I) determined the choice of radionuclides and methods for their introduction: In view of the high lability of the hydrogen atom the guanidine grouping can be labeled only with ^{14}C, while ^{3}H can be introduced comparatively easily into the p-chlorobenzylidene residues.

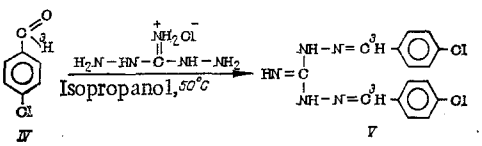
The following scheme was selected for the preparation of [^{14}C]-(I):



As the initial radioactive product we used [^{14}C]cyanogen bromide (II), obtained by a known method [3]. This was treated in ethereal solution with hydrazine hydrate, which gave [^{14}C]-1,3-diaminoguanidine (III) in the form of the hydrobromide. The conditions for performing the reaction in this case were similar to those described for the preparation of unlabeled (III) [4]. Attempts to obtain [^{14}C]-(III) using compound (II) by a method [5, 6], according to which cooled cyanogen chloride was added with stirring to a cooled mixture of 36% sodium chloride and hydrazone hydrate did not give a satisfactory result.

The [^{14}C]-(III) obtained was condensed with p-chlorobenzaldehyde in isopropanol at 50°C with the formation of the hydrobromide of [^{14}C]-(I) [7]. The latter was converted into free [^{14}C]-(I) by treating the reaction mixture with ammonia.

[^{3}H]-(I) was obtained by the following scheme:



A solution of [^{3}H]-p-chlorobenzaldehyde (IV) in isopropanol was treated with an aqueous solution of 1,3-diaminoguanidine hydrochloride under the conditions given in the synthesis of (I), and the resulting condensation gave the desired [^{3}H]-(I).

Experiments performed on the isotope exchange of unlabeled (I) sorbed on 5% palladized carbon with gaseous $^{3}H_2$ for 24-48 h enabled a product to be obtained with a specific activity of 150-300 mCi/mmole and a radiochemical purity of ∿70%, the labeled [^{3}H]-(I) apparently being contaminated with products of hydrogenation of the double bonds. Such samples of [^{3}H]-(I) were not purified further.

V. G. Khlopin Radium Institute. All-Union Scientific-Research Veterinary Institute of Poultry Rearing, Leningrad. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 15, No. 2, pp. 23-28, January, 1981. Original article submitted February 5, 1980.

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TABLE 1. Dynamics of the Absorption of [^{14}C]-(I) in the Intestine of the Chick after a Single Administration of the Preparation

Name of the organ and tissue	Specific activities of tissues, counts/min per 100 mg of dry tissue								
	time after the administration of the preparation, h								
	0.5	1	2	3	4	6	12	24	48
Duodenum	2670±310	6000±480	12 150±740	19 600±320	23 050±1010	13 550±590	6 800±340	1 510±70	35±2
Small intestine	1280±100	5770±510	7 950±430	16 500±810	25 200±910	24 710±1410	7 810±640	2 450±110	30±2
Ileum	1600±140	1800±190	2 250±190	6 100±800	34 780±800	23 680±750	6 320±340	2 230±105	17±2
Mucous membranes of the processes of the intestine	1050±110	1600±140	2 400±170	2 700±120	6 350±220	5 800±290	5 020±280	2 020±90	13±4
Blood plasma	6420±310	9220±570	19 720±2410	34 540±1890	41 910±1300	32 380±1040	20 910±1370	10 430±340	45±3

Note. After 96 h and in later periods the preparation was not detected in any of the organs studied. The specific activity of the blood plasma is given in counts/min per 1 ml.

The electronic absorption spectra and chromatographic characteristics of the [^{3}H]- and [^{14}C]-(I) obtained were identical with the analogous characteristics of repeatedly recrystallized unlabeled product.

The study of the absorption, distribution over the organs and tissues, and times of excretion from the organism of the labeled (I) was carried out on intact chicks. Only 30 min after its peroral administration, [^{14}C]-(I) was detected in the tissues of various sections of the intestine and in the blood (Table 1). Then the concentration of the preparation rose rapidly and 4 h after administration it reached a maximum level both in the intestine and in the blood plasma. The main role in the process of absorption is played by sections of the small intestine: the duodenum, the jejunum and the ileum, the level of (I) in which considerably exceeded that in the following processes of the intestine. Later, 6, 12, and 24 h after the administration of the preparation, its concentration in the intestine and the blood gradually fell, and after 48 h only traces of [^{14}C]-(I) and the products of its metabolism were detected.

Table 2 gives the results of a study of the distribution of [^{14}C]-(I) in the organism of the chick after a single administration of the preparation. A relatively high level of radioactivity, although not so high as in the intestine, was found in the liver, the pancreas, the kidneys, the thymus, and the bursa of Fabricius, and a lower level in the muscles, brain, spleen, and skin. The high level of (I) in the intestine makes possible a high efficiency of its anticoccidial action in the period of intracellular development of the parasites in the intestines, and this relates to those forms of coccidia that parasitize various sections of the small intestine of the chick.

It was established that the maximum concentration of ^{14}C in the intestine was detected at 4 h, and in the other organs 12 h, after the administration of the labeled preparation. The radioactivity in the organs and tissues subsequently decreased, and 48 h after administration it was completely absent from the brain, the pancreas, and the muscles, and was retained in small amounts in the other organs, mainly in the liver, spleen, and skin.

Great practical interest was presented by a study of the distribution, accumulation, and times of excretion of (I) from the organs of birds with the prolonged use of the preparation. In these investigations we used [^{3}H]-(I), which was administered to the chicks perorally every day for 50 days. The radioactivities of the organs and tissues at various times after the last administration of the preparation are shown in Table 3. It is possible to observe a considerably higher level of the preparation in the intestine, kidneys, liver, spleen, and skin, and a lower one in the brain, pancreas, muscle tissue, thymus, and bursa of Fabricius. However, as the results show, the prolonged accumulation of (I) and the products of its metabolism in the organs leads to a smoothing out of the sharp differences observed in the distribution of a single dose of the preparation.

It can be seen from Table 3 that compound (I) and the products of its metabolism after being fed to chicks for 50 days are excreted from the organs of the bird comparatively rapid-

TABLE 2. Dynamics of the Distribution of [^{14}C]-(I) in the Chick Organism with a Single Dose of the Preparation

Name of the organ and tissue	Specific activities of the tissues, counts/min per 100 mg of dry tissue			
	time after administration of the preparation, h			
	4	12	24	48
Duodenum	23 050±1010	6800±70	1510±70	35±2
Small intestine	25 200±810	7810±640	2450±110	30±2
Ileum	34 780±900	6320±430	2230±105	17±2
Mucous membranes of the processes of the intestine	6 350±220	5020±280	2020±90	23±4
Brain	380±20	1150±90	600±17	—
Liver	17 000±110	2330±140	800±30	200±7
Pancreas	660±30	3500±220	700±37	—
Pectoral muscles	120±8	320±20	130±5	—
Kidneys	1 100±120	1500±95	920±50	40±3
Thymus	650±44	1700±115	800±30	67±4
Bursa of Fabricius	750±47	1630±70	280±10	37±2
Spleen	250±17	1600±115	500±23	78±7
Skin	540±37	2250±120	750±50	70±5

Note. After 96 and at later periods the preparation was not detected in any of the organs studied.

TABLE 3. Distribution of [^{3}H]-(I) in the Organism of the Chick and the Rate of Its Excretion after the Use of the Preparation for 50 Days

Name of the organ and tissue	Specific activities of the tissues, counts/min per 100 g; time after administration of the preparation, h		
	6	24	48
Duodenum	4 230±270	1750±105	180±10
Small intestine	10 200±270	3000±190	480±25
Ileum	4 910±190	3150±210	620±28
Mucous membranes of the processes of the intestine	3 190±120	2050±140	420±18
Brain	2 250±120	1600±60	320±10
Liver	4 740±230	6160±410	2430±105
Pancreas	2 340±145	1960±110	510±30
Pectoral muscles	1 380±60	1110±70	270±13
Kidneys	3 310±210	3360±270	740±45
Thymus	2 940±140	2340±80	630±37
Bursa of Fabricius	2 320±140	1820±115	410±15
Spleen	4 280±350	2200±200	780±30
Skin	4 270±310	2300±110	890±30

ly. If we take their level 6 h after the last administration of the preparation as 100, then after 24 h their residual amount in the majority of organs studied was from 40 to 80%. Only in the kidneys had their concentration not changed after this time, and in the liver it had actually increased by 29%. These deviations are obviously connected with the barrier functions of the liver and with the excretion of the preparation through the kidneys. Even 48 h after the cessation of the elimination of [^{3}H]-(I) to the chick, its residual amounts in the liver still reached 50%. In the other organs by this time it was 12-22%, and in the duodenum and the small intestine 4%. Four days after the cessation of its administration, compound (I) had been completely excreted from the chick organism.

EXPERIMENTAL

Radiometry. The activities of solutions of labeled compounds were measured by the liquid scintillation method [8] using a ZhS-8 scintillator and internal standards. The specific activities of the preparations were determined on accurately weighed samples of [^{14}C]-(I) and [^{3}H]-(I). To determine the radiochemical purity of the samples a chromatogram previously sprayed with a benzene solution of 2,5-diphenyloxazole (PPO) and dried was scanned on a chromatographic scintillation counter [8]. The activities of biological samples were determined with the aid of a Mark-3 scintillation counter. For the uniform distribution of the tissue in the measuring cells we used PCS scintillation gel. In the analysis of blood, 0.3 ml of plasma was placed in the cell. Specific activities were calculated in counts per 1 min per 100 mg of dry tissue or per 1 ml of blood plasma.

The spectrophotometry of compound (I) was performed on SF-16 and SF-8 instruments (solvent ethanol, length of the cell 1 cm). For the unlabeled substance, λ_{max} 234 and 355 nm; ε_{355} $4.12 \cdot 10^4$.

1,3-Bis(p-chlorobenzylideneamino)guanidine. The substance was obtained by a multistage synthesis with elaboration of each stage [5-7, 9-18] for the subsequent manufacture of the substance in the chemical industry.

[^{14}C]-1,3-Diaminoguanidine Hydrobromide (III). A mixture of 0.15-ml (3 mmole) of hydrazine hydrate and 0.5 ml of ether in a round-bottomed flask was cooled to 10°C and, with shaking, a solution giving $281 \cdot 10^6$ disintegrations per sec (7.6 mCi) of freshly prepared ^{14}C-(II) with a specific activity of $189 \cdot 10^6$ disintegrations/mmole per sec (5.1 mCi/mole) in ether (2 ml) was added; this led to the appearance of a white crystalline precipitate. The reaction was stopped when the precipitate exhibited a pink coloration. The ether was decanted off and the residue was crystallized twice from ethanol (0.5 ml each time).
The product was dried in a rotary evaporator. The yield of purified (III) was 93 mg, 2.9 mCi, 38%. The specific activity of the preparation was 5.1 mCi/mmole. The radiochemical purity of the labeled (III) determined with the aid of ascending paper chromatography in the solvent system n-butanol—acetic acid—water (4:1:5) on Whatman 3 paper, R_f 0.2, was not less than 95% The activity peak on the scannogram coincided accurately with the spot of the main product revealed on the chromatogram with ninhydrin.

[^{14}C]-1,3-Bis(p-chlorobenzylideneamino)guanidine [^{14}C]-(I). A round-bottomed flask fitted with a reflux condenser was charged with a solution of 170 mg (1.2 mmole) of o-chloro-

benzaldehyde in isopropanol (2.3 ml). The solution was heated in a water bath to 50°C and, with stirring by a magnetic stirrer, a solution of 90 mg (2.8 mCi) of (III) in 2 ml of water was added, whereupon a yellow precipitate rapidly deposited. The reaction mixture was stirred at 50°C for 15 min and then at 20°C for 1 h, after which it was again heated to 40-50°C and, with stirring, a 25% solution of ammonia (0.2 ml) was added. The precipitate acquired a bright yellow coloration. The reaction mixture was stirred at 20°C for 20-30 min. The precipitate was filtered off and was washed with water (3 × 1 ml) and with isopropanol (2 × 0.5 ml) and was dried on a glass filter at 20°C. The [^{14}C]-(I) so obtained was recrystallized from benzene and dried in a vacuum desiccator over calcium chloride and paraffin wax. The yield of purified [^{14}C]-(I) was: by activity, 2 mCi, 71%; by weight 130 mg, 74%. The specific activity of the preparation was 5 mCi/mmole. The radiochemical purity of the [^{14}C]-(I), determined with the aid of thin-layer chromatography on Silufol in the solvent system chloroform (water-saturated)–ethanol (7:1), R_f 0.53, was 99%. The peak of activity on the scannogram accurately coincided with the spot of the [^{14}C]-(I) revealed in UV light. Absorption spectrum (ethanol): λ_{max} 234 and 355 nm; ε_{355} $3.92 \cdot 10^4$. The product was kept in the crystalline form in the refrigerator at 3-5°C.

[^{3}H]-1,3-Bis(chlorobenzylideneamino)guanidine [^{3}H]-(I). The substances was obtained from 103 mg (0.7 mmole) of [^{3}H]-p-chlorobenzaldehyde with a specific activity of 350 mCi/mmole by a method analogous to that described above for [^{14}C]-(I). The yield of purified preparation was: by activity 100 mCi, 33%; by weight 44 mg, 35%. The specific activity of the preparation was 760 mCi/mmole; its radiochemical purity was 99-100%. Absorption spectrum (ethanol): λ_{max} 234 and 355 nm; ε_{355} $4.15 \cdot 10^4$. The product was kept in the crystalline form at 3-5°C.

Study of the Distribution of the Labeled Khimkoktsid and Its Excretion from the Organism. The initial samples of [^{3}H]-(I) and [^{14}C]-(I) were diluted with unlabeled (I) to specific activities of 4 mCi/g and 700 μCi/g, respectively. Ten-day chicks of a broiler strain were used. To study the dynamics of absorption in the intestine and distribution over the organs we used [^{14}C]-(I), which was administered to the chicks in a single peroral therapeutic dose of 12 mg (8.4 μCi) per kg live weight. Compound (I) is insoluble in aqueous solutions, and in the experiments we therefore used a suspension of it in 1.5% carbomethylcellulose. In the study of the accumulation of the preparation in the organs and the rates of its excretion by the chicks, [^{3}H]-(I) was administered in the same daily dose of 12 mg (48 μCi) per kg live weight for 50 days. For each time studied, 10 chicks from the group were sacrificed, their organs were dried at 70°C to constant weight and were ground, and activities were determined at 25-35 mg of dry tissue.

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INVESTIGATION OF THE PHARMACOKINETICS AND MECHANISM OF ACTION OF GLYCOSIDES OF *Eleuterococcus*.

I. INTRODUCTION OF TRITIUM INTO ELEUTEROSIDE B. KINETICS OF ITS ACCUMULATION AND ELIMINATION FROM THE ANIMAL ORGANISM

G. N. Bezdetko, A. V. German, V. P. Shevchenko, Yu. I. Mitrokhin, N. F. Myasoedov, I. V. Dardymov, I. N. Todorov, and G. M. Barenboim

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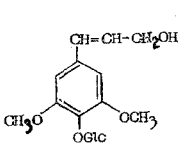
Preparations from the Far Eastern plant *Eleuterococcus senticosus* Maxim, which belongs to the family of Araliaceae, have achieved widespread use in medicine as agents that increase the general nonspecific resistance of the organism [1, 2]. *Eleuterococcus* preparations are widely used in the same role in animal husbandry [3]. In medicine an alcohol extract from *Eleuterococcus* roots (ethanol content 33%) is used. The extract from the roots contains glycosides, with which the basic biological activity of *Eleuterococcus* is associated, along with ascorbic acid, β-carotene, vitamin E, chlorogenic acid, and other compounds [4-9]. Ten different glycosides, which have received the general name of "eleuterosides," have been detected in an extract of *Eleuterococcus* roots the main components among them are eleuteroside A (daucosterol), B (syringin), B_1 (isofraxidin-7-O-α-L-glycoside), D (syringaresinol-di-e-β-D-glycoside), E (a conformer of D), and F [4-9]. In a methanol extract the ratio A:B:B_1:C:D:E:F is equal to 8:30:10:12:24:2:1 [2]. According to other data [4-9], the above-mentioned substances are contained in an extract from the roots in a ratio 2:10:(traces):8:16:2:1.

It is known that at a concentration of $1.0 \cdot 10^{-7}$ g/ml this compound stimulates the hexokinase activity and also inhibits the inhibitory effect of the diabetic β-lipoprotein (obtained from alloxan-treated rabbits) *in vitro* [9]. In male mice eleuteroside B increases the time of complete exhaustion in various stress tests [10]. In a dose of 0.5 mg/kg, eleuteroside B gave an antistress effect on rats with stress due to immobilization, according to such indices as the mass of the adrenals, thymus, spleen, and thyroid gland and the content of ascorbic acid and cholesterol in the adrenals, in comparison with animals with stress that did not receive this preparation [10]. Eleuteroside B increased the level of the androgenic reaction, and also increase the RNA content in the seminal vesicles and prostate in alloxan-treated animals [11]. It is reported that eleuteroside B (5 mg/kg) suppresses the formation of the glucose inhibitor in the blood of rats, in animals that are in a state of stress [12].

It has been shown [2] that eleuteroside B stimulates protein synthesis during early embryogenesis in guinea pigs.

In connection with the high content of eleuteroside B in the extract and its important biological role in the sum of all the eleuteroside, investigations in the field of pharmacokinetics with radioactively labeled preparation and the study of the mechanism of the action of eleuterosides begins with eleuteroside B.



Institute of Marine Biology, Far Eastern Scientific Center, Vladivostok. Scientific-Research Institute for Biological Testing of Chemical Compounds, Moscow Province. Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow. Branch of the Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow Province. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 15, No. 1, pp. 28-33, January, 1981. Original article submitted October 1, 1980.

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