SEARCH FOR NEW DRUGS

CYTOTOXIC AND ANTITUMOR ACTIVITY OF ANTIMONY(III) NITRILOTRIACETATE COMPLEXES $M_2SB(Nta)(HNta) \cdot nH_2O$ ($M = NH_4$, Na; $n = 1, 2$)

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Antimony(III) nitrilotriacetate complexes with mixed Nta^{3-} and $HNta^{2-}$ ligands of composition $(NH_4)_2Sb(Nta)(HNta) \cdot nH_2O$ (I) and $Na_2Sb(Nta)(HNta) \cdot 2H_2O$ (II) were synthesized. The proposed structures were confirmed by chemical analysis, x-ray diffraction, IR spectroscopy, and thermogravimetry. The cytotoxic properties of antimony chelates *in vitro* and their antitumor activity with respect to Ehrlich adenocarcinoma (EAC) in mice were studied. Compounds I and II produced a significant (60 – 90%) increase in the survival rate of test mice with ascitic EAC, at an optimum therapeutic dose of 25 – 50 mg/kg, in the absence of significant toxicity in this dose range. The results show good prospects in the search for new antitumor agents among Sb(III) complexes with aminopolycarboxy ligands.

In recent years, considerable progress was observed in the search for new antitumor agents among both natural and synthetic compounds [1 – 4]. Among the latter compounds, antimony(III) and bismuth(III) complexes are of special interest from the standpoint of oncology [5]. In the middle of the 20th century, Chinese researchers established that some antimony(III) complexes with aminopolycarboxy ligands possess antitumor properties [6, 7].

Recently, we obtained for the first time a nitrilotriacetate complex compound of antimony(III) with mixed Nta^{3-} and $HNta^{2-}$ ligands of composition $(NH_4)_2Sb(Nta)(HNta) \cdot H_2O$ (I) and described the synthesis and crystal structure of the new compound [8]. This paper describes the synthesis of nitrilotriacetate complexes with mixed Nta^{3-} and $HNta^{2-}$ ligands of compositions $M_2Sb(Nta)(HNta) \cdot nH_2O$ ($M = NH_4$, Na; $n = 1, 2$) and presents data on the cytotoxicity and antitumor activity of these compounds.

EXPERIMENTAL CHEMICAL PART

The nitrilotriacetate complex I was synthesized as described in [8]. An analogous procedure was used to obtain a new complex compound with the composition $Na_2Sb(Nta)(HNta) \cdot 2H_2O$ (II): a solution of Sb_2O_3 (0.01 mole) in H_3Nta (0.04 mole) was filtered, adjusted at pH 4 – 5 with a NaOH solution, and evaporated to dryness. The residue was dried in vacuum, washed on a filter with a small amount of acetone, and dried in air (for several hours).

The proposed structure of complex II was confirmed by chemical analysis, x-ray diffraction, IR spectroscopy, and thermogravimetry. The IR spectrum of compound II was close to that of compound I. Previously, it was established [8] that the crystal structure of complex I consists of $[Sb(Nta)(HNta)]^{2-}$ complex anions, NH_4^+ cations, and crystallization water (H_2O) molecules. The $[Sb(Nta)(HNta)]^{2-}$ anion contains ligands of two types: completely deprotonated nitrilotriacetic acid (Nta^{3-}) anions and protonated ($HNta^{2-}$) anions. In complex I, the nitrilotriacetate fragment Nta^{3-} is a tertadentate chelate ligand coordinated to Sb via the nitrile N(1) atom and three deprotonated oxygen atoms O(1), O(3), and O(5) of carboxy groups (Fig. 1). The protonated anion $HNta^{2-}$ is a monodentate ligand coordinated to Sb via a single carboxy oxygen atom O(7).

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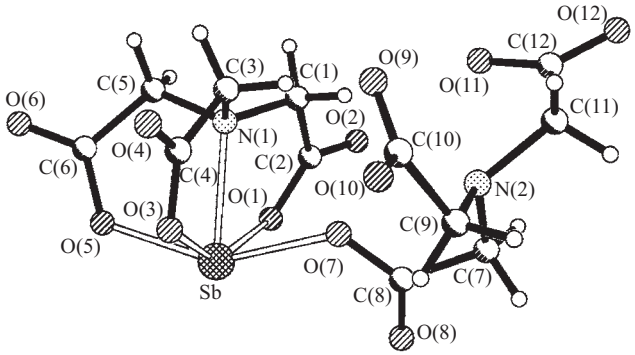

Fig. 1. Schematic diagram of the structure of $[Sb(Nta)(HNta)]^{2-}$ anion in compound I.

The coordination number of Sb in these complexes is 5, and the coordination polyhedron represents a tetragonal bipyramid with a vacant axial vertex. The equatorial plane of this bipyramid is formed by four oxygen atoms, while the axial vertices are occupied by the nitrogen atom N(1) and the unshared electron pair of Sb^{3+} ion. In the polyhedron of complex I, the Sb–O bond lengths range within 2.107(1) – 2.258(2) Å and the Sb–N(1) distance is 2.304(1) Å.

EXPERIMENTAL BIOLOGICAL PART

The cytotoxicity of the compounds studied was determined using the method of supravital staining with Trypan Blue [9]. The experiments were performed on Ehrlich adenocarcinoma (EAC) taken on the 8th day from inoculated mice. To 90 μl of cell suspension (3×10^6 cells/ml) in Hanks' solution was added 10 μl of an aqueous solution containing a preset amount of complex I or II and the mixture was incubated for 1 h at 37°C. Then, the cell suspension was mixed (1 : 1, v/v) with an 0.2% Trypan Blue solution and the amounts of stained (lost) and unstained (live) tumor cells was determined by examination under microscope. The cytotoxic effect (*CE*) was evaluated in terms of the percentage loss of tumor cells calculated as

$$CE = [(C_{lost}/C_{tot})] \times 100\%,$$

where C_{lost} is the number of lost cells and C_{tot} is the total number of cells in a sample.

The cytotoxic activity of antimony(III) complexes I an II with respect to an EAC cell suspension was also studied using a radiometric technique sensitive to inhibition of the inclusion of a labeled precursor of the DNA synthesis *in vitro*. The EAC cells taken from allogenic mice on the 7th day after tumor explantation were washed in a centrifuge and the residue was resuspended in medium 199 or RPMI-1640 containing 5% of calf thymus serum. To this cell suspension (containing 2×10^6 cells/ml) was added methyl-[^{3}H]-thymidine with a specific activity of 1.85×10^6 Bq/mg so as to obtain a label concentration of 1×10^5 Bq/ml in the inoculation medium. The labeled suspension aliquots were distributed into microplate wells containing compounds I and II in preset amounts, and the samples were incubated for 4 h at 37°C. Then, the samples were transferred onto paper filters (Whatman, 3 mm) and triply treated with a 5% trichloroacetic acid solution (5 ml per filter). Finally, the filters with samples were washed with ethanol, dried, and placed into flasks with a scintillator. The radioactivity was measured on a β-counter (Mark-III, USA). Nonspecific (zero-time) binding was determined immediately upon adding DNA synthesis precursors to a nutrient medium with tumor cells. The results of radioactivity measurements were plotted as the amount of included precursor versus the concentration of the test substance. Using this plot, the degree of cytotoxicity was evaluated and expressed as the drug dose (ED_{50}) producing a 50% inhibition of [^{3}H]-thymidine inclusion.

The general toxicity of antimony(III) complexes was determined with respect to allogenic mice weighing 22 – 24 g bearing ascitic and solid forms of EAC. The ascitic tumor was inoculated and maintained using a standard method [11] developed at the Blokhin Oncological Research Center (Moscow). The test mice were kept under standard vivarium conditions and received water and meals *ad libitum*. The test drugs were suspended in 200 μl of distilled water and introduced either *per os* or by intraperitoneal injections. Animals in the untreated control group received an equivalent volume of distilled water. The treatment was started 24 h after tumor inoculation.

The results of experiments with EAC in a solid form were evaluated by comparing the average tumor weights in mice of the test and control groups. The tumors were transplanted by means of subcutaneous inoculation of 3×10^6 ascitic tumor cells in 0.2 ml of physiological solution. The test compounds were introduced by intraperitoneal injections on the next day upon inoculation. The animals were killed on the 12th day and the antitumor activity was evaluated and expressed in terms of the degree of tumor growth inhibition (*TGI*) calculated as

$$TGI = [(M_{contr} - M_{test})/M_{contr}] \times 100\%,$$

where M_{contr} and M_{test} are the average tumor volume in the control and test groups of animals, respectively.

The antitumor activity with respect to EAC in the ascitic form was determined upon intraperitoneal inoculation of 5×10^6 ascitic tumor cells in 0.5 ml of isotonic aqueous NaCl solution. The test compounds were introduced by intraperitoneal injections on the next day upon inoculation. The antitumor effect was evaluated and expressed in terms of survival rate increase (*SRI*) calculated as

$$SRI = [(T - C)/C] \times 100\%,$$

where *T* and *C* are the average lifetimes of animals in the control and test, respectively.

The hemolytic action of drugs was evaluated using a test system of murine erythrocytes. Samples of heparinized blood were doubly washed with isotonic aqueous NaCl solution in a centrifuge at 2500 rpm. Compounds I and I were dissolved in distilled water to a stock solution concentration of 2 mg/ml and then distributed by double serial dilutions in 10 μl aliquots over microplate wells (with 10 μl of pure solvent in the control wells). Then 90 μl of 0.5% erythrocyte suspension was added to each well and the samples were incubated for 1 h in a thermostat set at 37°C. The results were evaluated by visual examination and expressed in terms of the minimum effective dose producing a 100% hemolysis of the test cells (ED_{100}).

RESULTS AND DISCUSSION

It was found that compounds I and II inhibit [^{3}H]-thymidine inclusion into acid-insoluble fraction of tumor cells at a concentration of 50 and 75 μg/ml, respectively. It should be noted that the hemolytic activity was also manifested, but at higher doses (ED_{100} = 250 μg/ml). The acute toxicity of compounds I and II was characterized approximately by LD_{50} = 150 m g/kg.

Compounds I and II produced a reliable increase in the survival rate of mice with Ehrlich tumor in the ascitic form (Table 1). The maximum *SLI* value was observed for mice treated with compound I in a dose of 50 mg/kg. At the same time, the activity of antimony(III) complexes injected once per day in a dose of 25 ort 50 mg/kg (i.p.) in mice with solid tumors showed a relatively low antitumor activity: a tumor growth inhibition by 23% was observed only for mice treated with compound II in a dose of 25 mg/kg.

Thus, the results of our investigation showed evidence of a moderate toxicity and a sufficiently high antitumor activity of antimony(III) nitrilotriacetate complexes $(NH_4)_2Sb(Nta)(HNta) \cdot H_2O$ (I) and $Na_2Sb(Nta)(HNta) \cdot 2H_2O$ (II) in mice bearing ascitic Ehrlich carcinoma. With respect to the tumor growth inhibition compounds I and II exceeded previously studied imidazole (pilocarpine and is derivatives) and thiazole (thiacarpine and its derivatives) alkaloids [1], minor ginseng glycosides (Rh_2, M_1) and their analogs [2], and phenolic compounds of plant origin such as genisteine (isoflavone) and resveratrol (stilbene) [3].

In conclusion, complex compounds of antimony(III) with mixed nitrilotriacetic acid anions possess antitumor activity at a relatively low toxicity, which show promise in the search for new effective antitumor agents in the class of Sb(III) complexonates with aminopolycarboxy ligands.

TABLE 1. Antitumor Activity of Antimony(III) Complexes with Respect to Ascitic Ehrlich Carcinoma in Mice (n = 8)

Compound	Dose (mg/kg)/Interval (h) × Number of treatments	Average lifetime, days
Control	–	23.0 ± 1.1
I (n = 8)	25/24 × 5	38.40 ± 7.4
I (n = 8)	50/24 × 5	44.20 ± 6.3
I (n = 8)	100/24 × 5	34.20 ± 5.1
II (n = 8)	25/24 × 5	38.20 ± 4.7
II (n = 8)	50/24 × 5	37.90 ± 5.3
II (n = 8)	100/24 × 5	36.00 ± 2.7

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