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SYNTHESIS AND ANTITUMOR ACTIVITY OF DERIVATIVES OF 2,3,4,6-TETRAACETYL-1-β-D-GLUCOSAMINOPHOSPHORIC ACID CONTAINING VARIOUS CYTOTOXIC GROUPS

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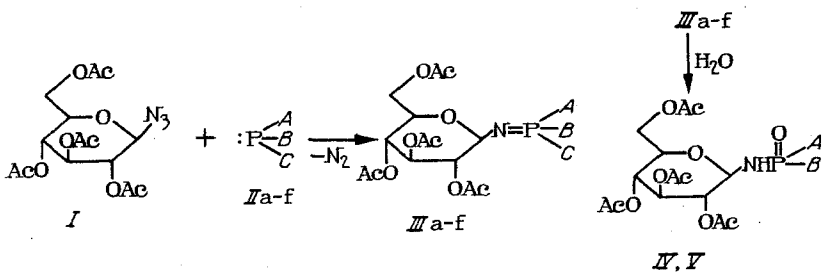
One of the principles lying at the basis of the search for new, more selectively acting antitumor drugs is the introduction of alkylating groups into the carrier molecule; natural physiological active substances, such as amino acids, pyrimidine bases, and sugars, are used as the carrier. It is also known that alkylating drugs that are derivatives of phosphoric acid (TEPA, benzoTEPA, glycifon, imiphos, etc.) are distinguished by high biological activity [6].

In view of this, we were interested in synthesizing, studying, and comparing the antitumor activity of phosphorylated glucose derivatives containing various alkylating groups.

The reaction of 2,3,4,6-tetraacetylglucopyranose-1-β-azide (I) with derivatives of trivalent phosphorus acids, containing bis-chloroethylamine, ethylenimine, and epoxy groups, was used to synthesize the compounds according to the scheme



A=OMe(IIa, IIIa), OEt(IIb, IIIb), $N(CH_2)_2$, (IIc,d IIIc,d IV), $OCH_2CH(-O-)CH_2$ (IIe,f IIIe,f V);
B=OMe(IIa, IIIa), OEt(IIb IIIb), $N(CH_2)_2$ (IIc,d IIIc,d IV), $OCH_2CH(-O-)CH_2$ (IIe,f IIIe,f V);
C=$N(CH_2CH_2Cl)_2$ (IIa,b IIIa,b), $N(CH_2)_2$ (IIc, IIIc), OPh(IId,f IIId,f), $OCH_2CH(-O-)CH_2$ (IIe, IIIe).

The compounds (IIId-f) formed as a result of this reaction are so readily hydrolyzed by moisture of the air that they cannot be isolated and characterized in the form of phosphimino derivatives. After treatment of these substances with access to atmospheric moisture (crystallization, evaporation of the solvent, and recrystallization from organic solvents), derivatives of 2,3,4,6-tetraacetylglucosaminophosphoric acid (IV, V) were isolated. The diglycidyl ester of 2,3,4,6-tetraacetylglucosaminophosphoric acid V was produced on the basis of various trivalent phosphorus derivatives (IIe and IIf), and the constants of the products are in full agreement. Compounds IIIa, b are not hydrolyzed even when heated with water to 80°C, which is apparently associated with the inductive influence of the bis-chloroethylamine groups, and they were investigated in the form of phosphimino derivatives. The triethyleneimide of 1-β-N-phosphimino-2,3,4,6-tetraacetylglucopyranose (IIIc) can be isolated and characterized in the form of phosphimino derivative; however, during longer storage (~1 month) with access to atmospheric moisture, it is also hydrolyzed, giving the diethylenimide of 2,3,4,6-tetraacetylglucosaminophosphoric acid IV. The structure of the compounds obtained was

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TABLE 1. Characteristics of the Compounds Synthesized

Compound	Yield, %	mp, °C	$[\alpha]_D^{20}$	$\delta_{^{31}P}$, ppm	LD_{50}, mg/kg
IIIa	43,2	176,5—8	+46	+22	900
IIIb	39,6	108—11	+14,9	+18	950
IIIc	51,0	127—9	—5,5	+42	—
IV	31,8	178—80	+25,6	+32	86
V	46,0	106,5—7,5	+24,3	+6	600
ThioTEPA		—			18

TABLE 2. Antileukemic Activity of the Compounds Studied

Tumor	Compound	Single dose, mg/kg	Administration of drug, days after transplant	Number of animals that survived more than 60 days	ILT, %	Change in weight of animals by end of experiment
Leukemia La	V	200,0	0—4th	4(6)	266	0
	IV	30,0	0—4th	0(6)	189	—2,0
	IIIa	300,0	0—4th	0(6)	100	0
	IIIb	300,0	0—4th	0(6)	90	0
	ThioTEPA	8,0	0 & 3rd	0(10)	76	—0,5
Leukemia P-388	V	200,0	1,4 & 7th	0(6)	115	—1,9
	IV	30,0	1st & 6th	3(6)	118	0
	IIIa	400,0	1,4 & 7th	0(6)	28	0
	IIIb	400,0	1st & 6th	0(6)	22	0
	ThioTEPA	8,0	1st & 4th	0(8)	125	—2,0
Leukemia L-1210	V	100,0	1, 3, 5 & 7th	0(6)	126	0
	IV	30,0	1,4 & 7-th	1(6)	80	0
	IIIa	400,0	1—5th	0(6)	26	0
	IIIb	400,0	1—5th	0(6)	53	0
	ThioTEPA	9,0	1 & 5-th	0(8)	77	0

Note. Here and in Table 3, the total number of animals in the experiment is given in parentheses.

confirmed by IR and NMR (^1H and ^{31}P) spectroscopy. Absorption bands of the carbonyl groups (four acetyl groups in the glucopyranose molecule) are observed in the IR spectra in the region of 1745 cm^{-1}; for compounds containing a P—O—C bond (IIIa, b, V) there is a broad band in the region of 1045 cm^{-1}. The absorption band of the P=N group in the compounds IIIa, b, c cannot be observed separately since it is superimposed on the absorption bands of the groups of the glucopyranose ring. For compounds containing ethylenimine rings (IIIc, IV), a characteristic low-intensity band is observed in the region of 3070 cm^{-1}. As we go to 2,3,4,6-tetraacetylglucosaminophosphoric acid derivatives (IV and V), the characteristic band of the NH group in the region of ~3200 cm^{-1} appears in the IR spectra; an 8-10 ppm shift of the signal is observed in the ^{31}P spectra (Table 1, compounds IIIc and IV). In the PMR spectra of all the compounds there are signals of protons of the acetyl groups with $\delta 1_H$ 1.8-2 ppm and multiplet weakly resolved signals of the protons of the glucopyranose ring in the region from 5.1 to 3.2 ppm, upon which the signals of the methylene protons and the protons of the epoxide rings are superimposed in the base of bis-chloroethylamine derivatives and diglycidyl esters. The compound IIIa is characterized by a doublet of the methyl protons with $\delta 1_H$ 3.5 ppm (J_{P-O-CH} = 13 Hz). The PMR spectra of compounds IIIc and IV contain a doublet signal of the protons of the ethylenimine rings with $\delta 1_H$ 2 and 2.2 ppm (J_{P-N-CH} = 15 Hz), corresponding in integral intensity to the number of protons of three ethyleneimine rings (compound IIIc) and two ethylenimine rings (compound IV).

EXPERIMENTAL (CHEMICAL)

The IR spectra were recorded on an IR-20 spectrometer in the form of a suspension in liquid petrolatum; the ^{31}P NMR spectra were recorded on a KGU-4 instrument at the working frequency 10.2 MHz relative to 85% H_3PO_4. $[\alpha]_D^{20}$ was measured on a Polamat A polarimeter. The values of elementary analyses correspond to the calculated values.

1-β-Azide of 2,3,4,6-tetraacetyl-D-glucopyranose (I) was produced according to [7].

O,O-Dimethyl-N-bis(2-chloroethyl)amidophosphite (IIa) was produced by the reaction of 3.7 g (0.016 mole) N-bis(2-chloroethyl)amidophosphoryl dichloride [8] with 1.03 g (0.032 mole) CH_3OH in the presence of 3.26 g (0.032 mole) Et_3N in

TABLE 3. Antitumor Activity of the Compounds Studied

Tumor	Compound	Single dose, mg/kg	Administration of drug, days after transplantation	Number of animals that survived more than 60 days	ILT, %	Inhibition of tumor growth, %
WCS	V	100,0	3, 7 & 12-th	0(8)	51	0
	IV	5,0	2, 7 & 12-th	0(8)	23	44
	IV	5,0	7th & 9-th	0(8)	0	24
	Thio-TEPA	2,0	1—12-th	0(10)	0	98
Melanoma B-16	IV	7,5	3—7-th	0(12)	0	0
	Thio-TEPA	2,0	6, 10 & 14-th	0(8)	0	0
Carcinosarcoma Ca-755	V	100,0	3, 5, 7& 9-th	0(10)	48	39
	IV	15,0	3, 5, 7 & 9-th	0(10)	37	54
	Thio-TEPA	2,0	1—10-th	0(10)	0	75

absolute benzene (or diethyl ether) in an atmosphere of argon at —10 to —15°C. After the reaction mixture was mixed for 2 h at 20°C, $Et_3N \cdot HCl$ was filtered off, and the solvent was removed under vacuum. The residue obtained, 3.3 g (92.9%), was redistilled. Yield 2.4 g (68.7%) of a substance with bp 76°C/0.1 mm Hg, n_D^{20} 1.4870, δ_{31P} +150 ppm. The substance can be used unredistilled as well.

O,O-Diethyl-N-bis(2-chloroethyl)amidophosphite (IIb) was produced analogously. Yield after redistillation 57.6%, bp 80-82°C/0.08 mm Hg, n_D^{20} 1.4800, δ_{31P} +148 ppm.

The triethylenimide of phosphorus acid (IIc) was produced according to [3].

The diethylenimide of phenylphosphoric acid (IId) was produced according to [2].

O,O,O-Triglycidylphosphite (IIe) and O,O-diglycidyl-O-phenylphosphite (IIf) were produced according to [4].

2,3,4,6-Tetraacetyl-1-β-D-N-[(O,O-dimethyl-N-bis(2-chloroethylamido)phosphimino]glucopyranose (IIIa). To a solution of 5.4 g (0.014 mole) of the azide (I) in benzene (20 ml) at 20°C, 3.2 g (0.014 mole) of IIa was added in an atmosphere of argon. Vigorous evolution of nitrogen was observed. After the evolution of nitrogen bubbles ended, the solvent was removed under vacuum, and the residue was recrystallized from methanol. Yield 3.5 g (43.2%) IIIa with constants shown in Table 1.

2,3,4,6-Tetraacetyl-1-β-D-N-[(O,O-diethyl-N-bis(2-chloroethyl)amido)phosphimino]glucopyranose (IIId), 2,3,4,6-tetraacetyl-1-β-D-N-phosphiminoglucopyranose triethylenimide (IIIc), 2,3,4,6-tetraacetyl-1-β-D-glucosaminophosphoric acid diethylenimide (IV), and 2,3,4,6-tetraacetyl-1-β-D-glucosaminophosphoric acid diglycidyl ester (V) were produced analogously.

EXPERIMENTAL (BIOLOGICAL)

The toxicity of the compounds presented was studied on noninbred white mice weighing 20-22 g. LD_{50} was calculated by the Burns method [1].

To determine the antitumor activity, experimental models of the following continuous tumor lines were used in the work: leukemia La, L-1210, P-388, melanoma B-16, carcinosarcoma C-755, Walker carcinosarcoma (WCS), and Lewis lung carcinoma. Leukemia P-388 was transplanted intraperitoneally into female BDF_1 mice weighing 21-22 g with a suspension of tumor cells in a concentration of 10^6; leukemia L-1210 was transferred analogously with a suspension of leukemia cells in a concentration of 10^5. Leukemia La was transplanted intraperitoneally with a homogenate of spleen tissue taken from animals with leukemia, in a concentration of 10^8 leukemic cells, into male C57 B1 mice weighing 22-23 g.

Melanoma B-16 was transplanted with a homogenate of tumor tissue diluted 1:1 in a volume of 0.3 ml into female $CBAF_1$ mice weighing 19-21 g, by subcutaneous injection.

Carcinoma Ca-755 was transplanted by subcutaneous injection of a homogenate of tumor tissue diluted 1:1 in a volume of 0.3 ml into female BDF_1 mice weighing 19-21 g.

WCS was transplanted by subcutaneous injection of a homogenate of tumor tissue, diluted 1:1 in a volume of 0.3 ml, into noninbred female white rats weighing 150-200 g.

The criterion for evaluating the effectiveness of the drugs was the lifetime of the treated animals in comparison with the controls (animals that survived for more than 60 days after the therapy were considered as surviving). In he course of the experiment the change in weight of the animals was observed.

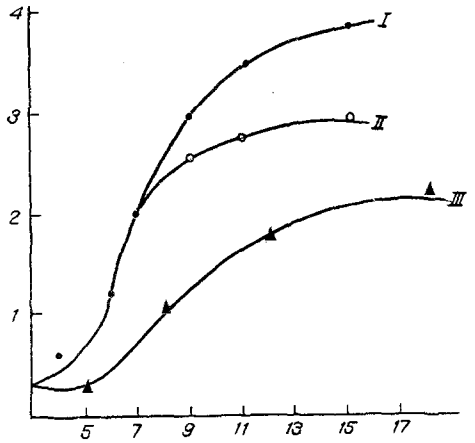

Fig. 1. Kinetic curves of the development of WCS in the control (I) and after administration of the drug IV. II) 5 mg/kg, 7th and 9th days; III) 5 mg/kg, 2nd, 7th, and 12th days. Here and in Fig. 2, along x axis: period of beginning of therapy, days; along y axis: size of tumor node, cm^3.

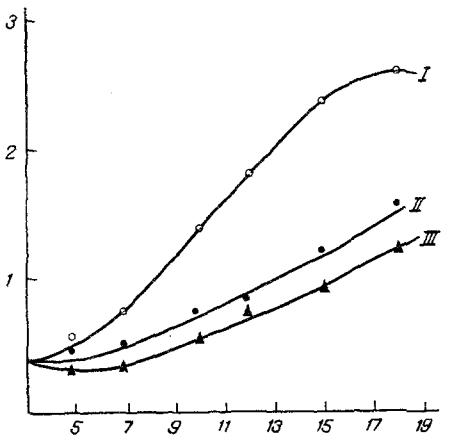

Fig. 2. Kinetic curves of the growth of carcinosarcoma Ca-755 in the control (I) and after administration of drugs. II-V) A dose of 100 mg/kg on the 3rd, 5th, 7th, and 9th days; III-IV) in a dose of 15 mg/kg on the 3rd, 5th, 7th, and 9th days.

All the drugs were dissolved in 10% Tween and injected intraperitoneally. The drug ThioTEPA (thiophosphimide), which was dissolved in physiological saline solution and also injected intraperitoneally, was used for a comparison of the activity of the newly synthesized compound with a known drug from this class [5].

RESULTS AND DISCUSSION

Table 1 presents the LD_{50} values of the drugs studied. A comparison of the structure and toxicity of the compounds presented shows that all the compounds exhibit significantly less toxicity in comparison with ThioTEPA. The most toxic of the newly synthesized substances is IV (LD_{50} 86 mg/kg), which contains two ethylenimine groups. Replacement of the ethylenimine fragment in the molecule by bis-chloroethylenimine leads to a sharp decrease in toxicity (LD_{50} 950 and 900 mg/kg for IIIb and IIIa, respectively).

The antileukemic activity of the compounds, doses, and schemes of administration are presented in Table 2. The greatest activity on leukemia La is exhibited by V. Administration in a dose of 200 mg/kg (1/3 of LD_{50}) daily from day 0 to day 4 leads

to a cure of 67% of the animals and a 266% increase in the lifetime of the remaining animals in comparison with the controls. We should mention that this system of administration of this compound is not toxic for animals, since no decrease in weight was observed during the experiment.

Compounds IV, IIIa, and IIId are less active. They increased the average lifetime of the animals by amounts from 90% (for IIId) up to 189% (for IV). ThioTEPA was less active under these experimental conditions (increase in lifetime 76%).

On leukemia P-388, IV was the most active (50% of the animals survived). The activity of the other compounds was significantly below the activity of ThioTEPA according to the criterion of increase in lifetime (ILT).

Leukemia L-1210 proved most resistant to the action of the compounds studied in this work. But even in this case V exhibited greater activity in comparison with ThioTEPA (ILT for V was 126%, and for ThioTEPA 77%). In the case of treatment with drug IV, one animal out of six survived, although the lifetime of the remaining animals did not exceed the ILT in the case of treatment with ThioTEPA.

Table 3 presents data on the antitumor activity of compounds IV and V on models of solid tumors, WCS, melanoma B-16, and carcinoma C-755, obtained at the optimum doses and conditions of administration. These compounds were selected for study on model solid tumors according to the results of a study of their antileukemic activity.

From Table 3 it is evident that these tumors give a negligible increase in the lifetime of animals with WCS. However, it should be noted that the total dose of ThioTEPA was equal to 24 mg/kg, which exceeds LD_{50} of ThioTEPA by 1/3, whereas the dose of IV, administered a total of three times, was equal to 15 mg/kg, which is 1/6 of LD_{50}. Figure 1 presents the growth of WCS in the control (I) and in the case of treatment with preparation IV with different times of beginning of therapy. When the drug was administered from the 7th day (II) after transplantation of the tumor, when the tumor node was already formed and its size had reached 2 cm^3, a significant inhibition of the growth rate was observed. Administration of the drug from the 2nd day also led to inhibition of tumor growth (III).

Melanoma B-16 proved insensitive to treatment with IV and ThioTEPA. Treatment of carcinoma Ca-755 with the drugs IV and V leads to a slight increase in the lifetime of the animals (by 48 and 37%, respectively) and 39% inhibition of the tumor by the drug V and 54% inhibition by the drug IV (Fig. 2, curves II and III, respectively). Treatment with ThioTEPA inhibits tumor growth by 75%, but under conditions of rigorous therapy with daily administration for 10 days, whereas IV was administered at 2-day intervals, four times beginning with the 3rd day after transplantation of the tumor.

Thus, the data presented are evidence that V and IV, which exhibited a substantial antileukemic effect and also greater therapeutic latitude in comparison with ThioTEPA in experiments on solid tumors, are the most promising of the new compounds studied.

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