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Composition on the Basis of Phosphonoacetic Acid. Synthesis and Antiviral Activity

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Abstract—A manufacturable technology for the production of an antiviral composition containing phosphonoacetic acid, involing PH-alkylation of dimethyl phosphite with methyl chloroacetate under phase-transfer conditions and subsequent in situ hydrolysis of the intermediate product, was developed. The composition showed expressed antiviral effect against herpesviral infections caused by herpes simplex virus 1 and 2 and cytomegalovirus. The composition in the effective concentration range was found to exhibit no cytotoxic effect on VERO green monkey kidney and M-19 human diploid cell cultures. The resulting data suggest potential uses of the composition as an antiviral agent in practical medicine and as an antiviral additive to synthetic detergents for sanitation in health care and food production facilities.

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INTRODUCTION

The problem of herpesviral infections (HIs) has not lost urgency. Even though great progress has been reached in the understanding their etiolog y, pathogenesis, and therapy, HIs still cause some the common infectious diseases [1–5]. The main causative agents of HIs are representatives of the *Herpesviridae* family, specifically herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), as well as cytomegalovirus (CMV), which, depending on conditions, can cause patholog ical processes with different localization and degree of expression, capable of retaining activity in body cells for a long time, and tending to recur. From 60% to 95% of the world population is infected with HSV, and the prevalence of CMV in the total population of different countries makes reaches 20–75% [1, 3]. The clinical course of diseases caused be His features asymptomatic forms in 70–80% cases, and clinical symptoms of herpesviral diseases are observed in 20–30% of infected patients [1].

Pathogens are best destroyed by means of disinfection [6, 7]. The hospital environment is one of the main sources of infections, because most pathogens are transmitted via direct contact with patients (for example, via hand contact), even though a certain role in this process also belongs to contaminated equipment and materials [8]. Therefore, the use of disinfectants in hand hygiene of medical staff, as well as in sanitary treatment of surfaces plays an important role in the prevention of hospital-acquired infections.

The antiviral activity of phosphonoacetic acid $(HO)_2P(O)CH_2COOH$ (**1**, FAA) was discovered in 1973 [9], about 50 years later than it had been first synthesized [10]. This discovery prompted intense research into the biolog ical activity of FAA [11–17], which showed that the acid and a number of its derivatives strongly inhibit replication of many viruses, including hepatitis, immunodeficiency, and human herpes viruses. The inhibitory activity of PAA and its derivatives against herpes viruses, in particular, HSV, CMV, and Epstein–Barr virus, is associated with the fact that these compounds, being analog antimetabolites of pyrophosphates, are capable of inhibiting DNA polymerase responsible for replication of herpes viruses [1, 3]. Furthermore, PAA is a respresentaive of phosphonic acids which are widely used as a component of synthetic detergents. Therefore, aiming at developing technolog ies for provoding safety for humans and animals (including design of antiviral agents for medicine and veterinary), we have developed a method of synthesis of a PAA-containing composition to be

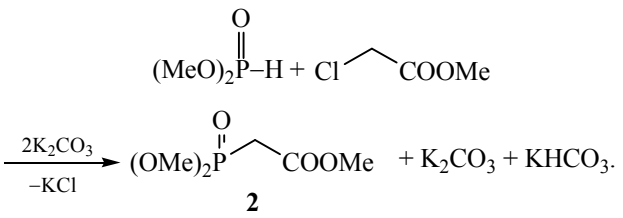
Table 1. Chemical shifts of signals in the $^{31}P\{^1H\}$ solutions of phosphonoacetic acid **1** and its derivatives **2–8** prepared by independent synthesis

Comp. no.	Formula	Solvent	δ_P, ppm
1	$(HO)_2P(O)CH_2COOH$	CD_3OD	17.7
		H_2O	15.4
		D_2O	16.2
2	$(MeO)_2P(O)CH_2COOMe$	$CDCl_3$	22.3
		D_2O	26.0
		H_2O	25.0
3	$(MeO)_2P(O)CH_2COOK$	D_2O	29.0
4	$(MeO)(KO)P(O)CH_2COOK$	D_2O	20.6
5	$(MeO)(HO)P(O)CH_2COOH$	D_2O	18.1
6	$(HO)_2P(O)CH_2COOK$	D_2O	13.2
7	$(KO)(HO)P(O)CH_2COOK$	D_2O	16.2
8	$(KO)_2P(O)CH_2COOK$	D_2O	13.8

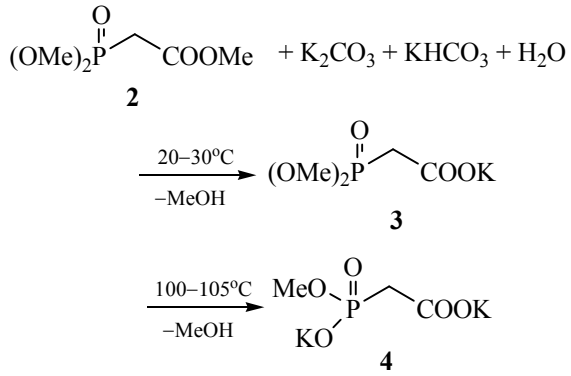
used as an antiviral additive to detergents for sanitation in health care and food production facilities.

Synthesis of the Composition

The composition is synthesized in two stages. The first stage involves the synthesis of trimethyl phosphonoacetate **2** by the previously developed facile and convenient PH-alkylation of dimethyl phosphite with methyl chloroacetate in the presence of potassium carbonate in the heterogeneous system "organic liquid phase–inorganic solid phase" [18] without using phase-transfer catalysts. The role of the latter is played to success by the starting and final phosphoryl-containing compounds [19–21].

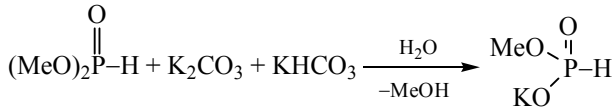


At the second stage trimethyl ester **2** is hydrolyzed in situ under the action of potassium carbonate and bicarbonate, which are present in the reaction mixture. As shown previously, the reaction proceeds via consecutive dealkylation of the methoxycarbonyl group [22–24] and one of the methoxy groups on phosphorus [10, 23, 25] to form mono- and dipotassium salts of phosphonoacetic acid **3** and **4**.



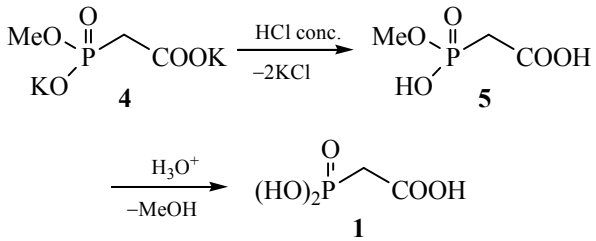
The progress of the conversion of trimethyl ester **2** into phosphonoacetic acid **1** was monitored by $^{31}P\{^1H\}$ NMR spectroscopy of solutions of the reaction mixtures in deuterated solvents.

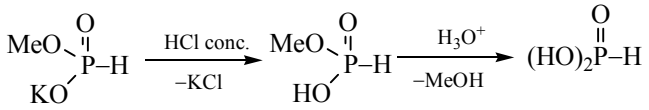
The reaction mixture obtained at the first stage was diluted with water and left to stand for 15 h at room temperature. Comparative analysis of the $^{31}P\{^1H\}$ NMR data for the solution of the reaction mixture in D_2O and independently synthesized derivatives of phosphonoacetic acid (Table 1) showed that under these conditions trimethyl ester **2** converts into potassium (dimethoxyphosphinoyl)methylacetate **3** (δ_P = 29.0 ppm, 92.1%), whereas dimethyl phosphite converts into potassium methyl phosphite (δ_P = 8.6 ppm, 7.9%).



The subsequent 4-h boiling of the reaction mixtures gives dipotassium salt of (hydroxymethoxyphosphinoyl)acetic acid **4** (δ_P = 20.6 ppm, 89.2%), implying hydrolysis of only one methoxy groups on phosphorus in salt **3**. Potassium methyl phosphite remains unchanged.

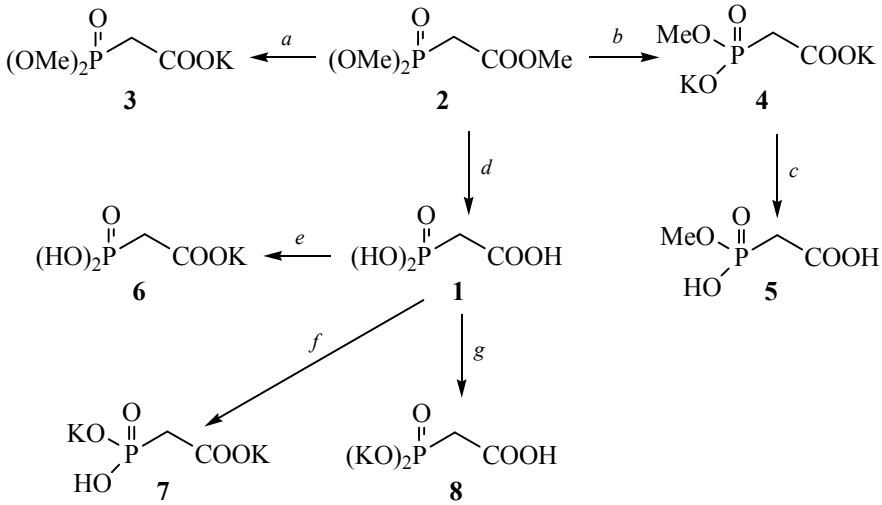
The last methoxy group in dipotassium salt **4** could only be hydrolyzed under acid catalysis. The $^{31}P\{^1H\}$ spectrum of a D_2O solution of the mixture of monoester **4** and potassium methyl phosphite acidified with conc. HCl at room temperature no longer showed the signals of the components at δ_P = 20.6 and 8.2 ppm, respectively; instead, a signal of acid **5** (δ_P = 18.1 ppm) appeared.





The subsequent 12-h boiling of the mixture with distillation of the water–methanol fraction at 90°C led to dealkylation of the methoxy groups in acid **5** and methyl dihydrogen phosphite to form phosphonoacetic acid **1** and phosphorous acid. According to $^{31}P\{^1H\}$ NMR data, the resulting mixture contained 58.1% of acid **1** (δ_P = 16.4 ppm), 29.5% of unhydrolyzed monoester **5** (δ_P = 20.5 ppm), and 12.4% of phosphorous acid (δ_P = 3.0 ppm).

The identification of the intermediate products formed in the course of hydrolysis of trimethyl ester **2** was confirmed by comparative analysis of the chemical shifts of signals in the ^{31}P NMR spectra of the reaction mixtures with those in the spectra of $CDCl_3$, CD_3OD, H_2O, and D_2O solutions of phosphonoacetic acid derivatives **3**–**8** (Table 1) prepared by independent synthesis (see scheme below). As the composition was intended to be used as an antiviral additive to detergents which are basic in nature, the reaction mixture was neutralized with 40% aqueous KOH to pH = 8 and then filtered to remove the precipitate of KCl. The resulting antiviral composition (PAAC) is an aqueous solution of a mixture of di- and trisalts of [hydroxy(methoxy)phosphinoyl]acetic (**4**) and phosphonoacetic (**7**, **8**) acids (21% per phosphonoacetic acid, ratio 1 : 2), as well as phosphoric acid (1.6% per phosphoric acid) and KCl.



a: KOH/MeOH, 50°C, 1 h [22]; *b*: aq. KOH, 100°C, 10 h [23]; *c*: HCl conc., 20°C; *d*: H_2O, 150°C, 10 h [18]; *e, f, g*: 1 equiv., 2 equaiv., 3 equiv. 1 N aq. NaOH, respectively.

Assessment of the Antiviral Activity of the PAA Composition

Procedure of Antiviral Activity Assessment

The antiviral activity of PAAC was assessed in vitro against experimental herpesviral infections by standard procedure [26–29] using freshly prepared serial samples obtained by successive half-dilutions of the starting sample (from 1 : 2 to 1 : 512) in a serum-free Eagle's nutrient medium. The test viruses were human herpes viruses HSV-1 (strains K1 and L2), HSV-2 (strain BH), and CMV (strain AD-169), obtained from the State Collection of Viruses, Ivanovo Research Institute of Virolog y, Ministry of Health of the Russian Federation.

HSV-1 and HSV-2 were cultured and titered in a multilayer VERO green monkey kidney cell culture and CMV in an M-19 human diploid cell culture. The infectious virus titers were determined by standard by standard Reed–Muench assay and expressed in log TCD_{50}/0.1 mL.

The cytotoxicity of PAAC was tested against to the cell cultures used for infectious activity testing: VERO (Ivanovo Research Institute of Virology) and M-19 (Chumakov Institute of Poliomyelitis and Viral Encephalitis, Russian Academy of Medical Sciences).

Cells were cultured in Eagle's growth medium containing 10% inactivated fetal bovine serum (FBS, PanEko, Russia), 2 mM of L-glutamine (Sigma, USA),

and antibiotics (100 units/mL of penicillin, 100 μg/mL of streptomycin, and 80 μg/mL of gentamycin). The maintenance medium contained all the above-mentioned ingredients plus 2% of FBS. Cells were grown in plastic flasks or plates (Costar, USA) and incubated in a thermostat at +37°C in a humid atmosphere containing 5% of CO_2. A 24-h monolayer of cell cultures was used.

According to methodical recommendations on preclinical specific activity testing of medicines, at the first stage we assessed the cytotoxicity of PAAC with respect to intact cell cultures in terms of its effect on the morpholog y and viability of cells [26]. To this end, 1 : 2–1 : 512 composition samples in Eagle's nutrient medium were introduced into 24-h VERO or M-19 cell cultures preliminarily freed from the growth medium and washed with three portions of Hanks' solution. For each dilution we used four wells. The nutrient medium without PAAC was used as control. Culturing was performed in a thermostat for 96 h, as described above. Cytodestructive and morpholog ical changes in the cell monolayer were monitored every day by means of optical microscopy.

Viable cell counting was performed daily using the vital dye exclusion assay with Trypan Blue which colors nonviable cells blue. To this end, cells were removed with a trypsin–versene mixture and stained vitally with a 0.4% solution with Trypan Blue for 5 min at +37°C. Cell viability was assessed by the percentage of uncolored cells in the total cell population. For statistically reliable results each experimental series was repeated in triplicate.

The concentration of the composition, which caused microscopically visible changes in morpholog y in 50% of cells in the monolayer or death of 50% of cells in the population was taken as 50% cytotoxic concentration (CTC_{50}) [26, 28]. Along with this parameter we also determined the maximum tolerable concentration (MTC), specifically, the highest concentration of the composition that did not cause visible cytodestructive changes in the cell monolayer or cell death. In further research, the composition was used in concentrations not higher than the MTC.

The antiviral activity of the composition was determined by commonly accepted criteria, i.e. ability to prevent virus-induced cytopathic effect (CPE) and inhibit virus replication in the cell culture [26–29].

Activity assessment was performed using a treatment scheme. Treatment was initiated 1 h after test cultures (VERO or M-19) had been inoculated with test viruses (HSV-1, HSV-2, CMV). The infection procedure was as follows: cells were cultured in 96-well plates, the growth medium was removed, and cells were washed with 3 portions of Hanks's solution, inoculated with test viruses in the dose 100 TCD_{50}, and incubated for 1 h in a thermostat at 37°C. The inoculate was removed, cell cultures were washed with 3 portions of Hanks's solution, after which serial PAAC solutions were added. The positive controls were cell cultures inoculated with test viruses, to which the maintenance medium containing no PAAC was added. The negative controls were intact cell cultures with added pure maintenance medium. The cytotoxicity controls were intact cell cultures with added maintenance medium and PAAC . Cells were incubated for 4 (with HSV-1 and HSV 2) or 14 days (with CMV); the virus-specific CPE in the test and control cell cultures was recorded daily by optical microscopy.

The antiviral activity study of PAAC in cell cultures also included assessment of the pathogenicity of the virus formed in the presence of the composition. To this end, at the 4th (with HSV-1 and HSV-2) or 14th day (with CMV) of incubation of infected cell cultures and PAAC samples the virus-containing fluid (VCF) from the test and control wells was taken and tested for virus pathogenicity using subculture cell lines. Before measurements the samples were stored at –70°C. Immediately before measurements 10-fold serial dilutions of the VCF samples (10^{-1}–10^{-8}) were prepared on Eagle's nutrient medium and used to infect the 24-h monolayer of cell cultures after removal of the growth medium and washing with Hanks's solution. Cell cultures inoculated with HSV or CMV (positive control) and intact cell cultures (negative control) were used as reference. Incubation was performed at 37°C for 4 (with HSV-1 and HSV-2) or 14 days (with CMV). During this time the accumulation of test viruses in control samples reached a maximum and a 100% CPE was observed.

The antiviral effect was evaluated by the inhibition of virus accumulation under the action of the composition compared to the control samples ($\Delta\log TCD_{50}$), as well as by the inhibition coefficient (IC) [26, 28]. At $\Delta\log TCD_{50}$ 1.0–1.5 the sample was considered to possess a low antiviral activity with respect to HSV or CMV and at $\Delta\log TCD_{50}$ 1.5 – 2.0 and >2.0, moderate and expressed antiviral activity, respectively.

The lowest concentration of PAAC, which prevented death of 50% of cells in the infected

population or decreased the virus-induced CPE by 50% compared to 100% in the control infected culture 4–14 days after infection was taken as the minimum inhibitory concentration (MIC_{50}) of the composition.

We also calculated the chemotherapeutic index (CTI) as the ratio of CTC_{50} (which relates to the effect on the cell morphology) to MTC_{50} (relates to the antiviral effect)

Statistical treatment was performed using Microsoft Excel-50 and Statistika 5.0 programs. Statistical significance was evaluated by Student's *t*-test at $p < 0.05$.

Results of Antiviral Activity Assessment of the Composition

First of all we studied the cytotoxic effect of the composition on the morpholog y and viability of intact cells of the subculture VERO cell line and M-19 human diploid cell culture. The resulting data are presented in Table 2.

In was found that 1 : 2 to 1 : 4 dilutions of the composition after 24-h incubation caused visible destructive changes in 100% and 75% of VERO cells and 100% of M-19 cells, respectively, and a 1 : 8 dilution changed morpholog y in 50% in both VERO and M-19 cells on the third day of observations. The 1 : 8 dilution of the composition was taken as CTC_{50}. The PAAC samples diluted from 1 : 16 to 1 : 512 showed no cytotoxic effect on VERO and M-19 cells over the entire observation period (by optical microscopy and Trypan Blue assay). The viability characteristics of VERO and M-19 cells incubated for 96 h both in the presence of PAAC at the maximum tolerable concentration or at higher dilutions, scarcely differed from those of control cells incubated in the absence of the composition.

Table 2. Cytotoxic effect of the composition on intact subculture Vero and M-19 cell lines

Dilution of the starting sample	Cytotoxic effect, %	
	Vero	M-19
1 : 2	100.0	100.0
1 : 4	75.0	100.0
1 : 8	50.0	50.0
from 1 : 16 to 1 : 256	0.0	0.0

The 1 : 16 dilution was considered as a dilution corresponding to the MTC of the composition, which is nontoxic for 100% of cells in the monolayer. In further experiments we used PACC samples in dilutions not lower than 1 : 16.

The results of the assessment of the antiviral activity of PAAC with respect to the His caused by HSV-1, HSV-2, and CMV are summarized in Tables 3–5.

As follows from Table 3, PAAC efficiently inhibited HSV-1 replication when added 1 h after the cell monolayer had been infected.

Thus, PAAC diluted 1 : 8–1 : 32 completely inhibited replication of HSV-1 (strain K1), and its 1 : 8–1 : 16 inhibited replication of HSV-1 (strain L2); the IC was 100%.

The effect of 1 : 64 and higher dilutions on the HSV-1 strains was statistically significant. The

Table 3. Antiviral activity of the composition against the experimental herpesviral infection caused by herpes simplex virus 1

Scheme of study	Dilution	Infectious titers of HSV-1					
		strain K1			strain L2		
		virus accumulation, log TCD_{50}/0.1 mL	inhibition of virus replication, Δlog TCD_{50}	IC, %	virus accumulation, log TCD_{50}/0.1 mL	inhibition of virus replication, Δlog TCD_{50}	IC, %
1 h post inoculation	1 : 8	0±0	6.0	100.0	0±0	6.0	100.0
	1 : 16	0±0	6.0	100.0	0±0	6.0	100.0
	1 : 32	0±0	6.0	100.0	0.5± 0.25	5.5	91.6
	1 : 64	0.5± 0.25	5.5	91.6	1.0±0.1	5.0	83.3
	1 : 128	1.5±0.2	4.5	75.0	1.5±0.3	4.5	75.0
	1 : 256	2.0±0.2	4.0	66.7	2.5±0.1	3.5	58.3
	1 : 512	3.0±0.2	3.0	50.0	3.0±0.1	3.0	50.0
Control	0	6.0±0.5	–	–	6.0±0.5	–	–

Table 4. Antiviral activity of the composition against the experimental herpesviral infection caused by herpes simplex virus 1 and cytomegalovirus

Scheme of study	Dilution	Infectious titers of viruses					
		HSV-2, strain BH			CMV, strain AD-169		
		virus accumulation, log TCD_{50}/0.1 mL	inhibition of virus replication, Δlog TCD_{50}	IC, %	virus accumulation, log TCD_{50}/0.1 mL	inhibition of virus replication, Δlog TCD_{50}	IC, %
1 h post inoculation	1 : 8	0.25 ± 0.15	5.75	95.8	–	–	–
	1 : 16	1.0 ± 0.1	5.00	83.3	1.25 ± 0.15	6.25	83.3
	1 : 32	1.0 ± 0.3	5.00	83.3	1.5 ± 0.2	6.00	80.0
	1 : 64	1.0 ± 0.2	5.00	83.3	3.0 ± 0.2	4.50	60.0
	1 : 128	2.25 ±0 .1	3.50	62.5	4.75 ± 0.3	2.75	36.7
	1 : 256	3.25 ± 0.4	3.75	45.8	6.25 ± 0.1	1.50	16.7
	1 : 512	4.5 ± 0.25	1.75	25.0	7.0 ± 0.4	0.50	6.7
Control	0	6.0 ± 0.5	–	–	7.5 ± 1.0	–	–

composition reduced the pathogenicity of HSV-1 by 3.0–5.5 log TCD_{50} compared to control. The infectious titers of HSV-1 (strain K1) in the inoculated but not PAAC -treated cell culture in 24, 48, 72, and 96 h were 3.5, 4.5, 5.25, and 6.0 log TCD_{50}/0.1 mL, respectively. The same trend was observed with HSV-1 (strain L2). With the 1 : 8 to 1 : 64 dilutions, the specific CPE in the cell culture inoculated cwith HSV-1 was only observed 96 h post inoculation, whereas in the control cultures, already 24 h post inoculation. The inhibition coefficient of the HSV-1 strains under the action of PAAC diluted 1 : 8–1 : 512 was 50–100%.

Thus, PAAC in the dilution range from 1 : 8 to 1 : 512, applied by the treatment scheme 1 h after inoculation, shows a statistically significant antiviral effect with respect to certain HSV-1 strains (K1 and L2), the strength of which depends on the dose of the composition.

It was found that PAAC in the dilution range from 1 : 8 to 1 : 64 strongly inhibited HSV-2 replication in cell cultures (Table 4).

In these cases the virus-induced CPE in the cell culture inoculated with HSV-2 was observed only 96 h post inoculation, whereas in the control cultures, already in 24 h. The highest IC (95.8%) was observed with the 1 : 8 dilution.

The infectious titers of HSV-2 in the absence of the composition were 2.0log TCD_{50}/0.1 mL in 24 h after inoculation, 4.0log TCD_{50}/0.1 mL in 48 h, 50log TCD_{50}/0.1 mL in 72 h, and 6.0log TCD_{50}/0.1 mL in 96 h. The infectious activity of HSV-2 in the presence of PAAC diluted 1 : 16 – 1 : 64 in 96 h after inoculation was lower by 5.0log TCD_{50} compared to the respective characteristic in control ($p < 0.05$). The infectious titers of HSV-2 were not higher than 1.0log TCD_{50}/0.1 mL. It should be noted that the 1 : 256–1 : 512 dilutions of the composition statistically significantly inhibited HSV-2 replication (by 1.75–3.75log TCD_{50}).

The infectious activity of HSV-2 under the action of PAAC in the dilution range 1 : 8–1 : 512 decreased by 1.75–5.75log TCD_{50}, the IC was 25.0–95.8%.

Similar results were obtained with CMV. The experimental results in Table 3 show that PAAC diluted from 1 : 8 to 1 : 256 exhibits a high antiviral activity with respect to CMV, significantly inhibits CMV reproduction in the M-19 cell culture. The infectious titers of CMV in the presence of PAAC were 1.5–6.25log TCD_{50} lower than the respective values in control. The IC was 16.7–83.3%.

Comparative data on the antiviral activity of the composition on the basis of phosphonoacetic acid against the experimental herpesviral infection caused by HSV-1, HSV-2, and CMV are presented in Table 5. Analysis of these data shows that PAAC exhibits expressed chemotherapeutic effect on the test viruses. The CTI of the composition with respect to HSV-1/HSV-2 and CMV was 64 and 32, respectively, which

Table 5. Comparison of the antiviral activity of the composition against the experimental herpesviral infections 1 h after tripling

Herpes virus	Cytotoxic concentration (CTC_{50})	Minimum inhibitory concentration (MIC)	Chemotherapeutic index (CTI)
HSV-1 (strains K1 and L2)	1 : 8	1 : 512	64
HSV-2 (strain BH)	–	1 : 512	64
CMV (strain AD-169)	1 : 8	1 : 256	32

allows us to conclude that it holds promise both as a specific antiviral agent and as antiviral additive to preparations used in sanitary practice.

EXPERIMENTAL

Synthesis of the composition. Methyl chloroacetate, 35.8 g (29.0 mL, 330 mmol), was added dropwise with vigorous stirring at 20–30°C to a mixture of 36.6 g (30.4 mL, 330 mmol) of dimethyl phosphite and 91.0 g (660 mmol) of powdered K_2CO_3. The reaction mixture was stirred at 20–30°C for 18 h, diluted with 50 mL of water, heated under reflux for 4 h, and let to cool down to room temperature. Concentrated HCl, 85 mL (990 mmol) , was then added with stirring, and the mixture was boiled for 6 h with distilletion of the water–metanol fraction up to bp 90°C. The still residue at 20–30°C was neutralized with 40% aqueous solution of 35.3 g (630 mmol) of KOH to pH 8, the mixture was stirred for 1 h at room temperature, and the precipitate of KCl that formed was filtered off to obtain 215.8 g of aqueous solution of di- and trisalts of [hydroxy(methoxy)phosphinoyl]-acetic (**4**) and phosphonoacetic (**7**, **8**) acids (21% per phosphonoacetic acid, ratio 1 : 2), as well as phosphoric acid (1.6% per phosphoric acid) and KCl.

Phosphonoacetic acid (**1**) [18], its trimethyl ester **2** [18], monopotassium salt of (dimethoxyphosphinoyl)-acetatic acid **3** [22], and dipotassium salt of [hydroxyl-(methoxy)phosphinoyl]acetic acid **4** [23] were synthesized by previously described procedures (Table 1).

[Hydroxy(methoxy)phosphinoyl]acetic acid (**5**). The aqueous solution of salt **4**, prepared from 1.0 g of triester **2** [23], was neutralized with 1 mL of conc. HCl (Table 1).

Mono-, di-, and tripotassium salts of phosphonoacetic acid (**6**–**8**). Three 0.0144 g portions of acid **1** [18] were placed in NMR tubes, and 1 N aquoues solution of NaOH was added to each tube (0.1, 0.2, and 0.3 mL to the first, second, and third tube, respectively). The solutions were diluted with D_2O to standard volumes (Table 1).

The ^{31}P NMR spectra in $CDCl_3$ and D_2O were measured on a Bruker Avance 300 spectrometer at 121.495 MHz, external reference 85% H_3PO_4.

CONCLUSIONS

A manufacturable technolog y for the production of an antiviral composition containing phosphonoacetic acid, involing PH-alkylation of dimethyl phosphite with methyl chloroacetate under phase-transfer conditions and subsequent in situ hydrolysis of the intermediate product, was developed.

The composition showed expressed antiviral effect against HSV-1 and HSV-2, as well as CMV. In vitro experiments revealed the ability of the composition to inhibit replication of test viruses in different cell lines, leading to a statistically significant reduction of their infectious activity. The effect is the stronger the higher is the concentration of the composition. Furthermore, the composition in the effective concentration range was found to exhibit no cytotoxic effect on VERO green monkey kidney and M-19 human diploid cell cultures.

The resulting data suggest potential use of the composition in the development of human and animal safety technolog ies, including its uses as an antiviral agent in practical medicine and as an antiviral additive to synthetic detergents for sanitation in health care and food production facilities.

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