SYNTHESIS AND ANTIVIRAL ACTIVITY OF PHOSPHONIUM SALTS

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Phosphorus organic compounds constitute a promising group of chemical substances for finding new antiviral preparations [15, 18, 21]. The antiviral properties of quaternary phosphonium and onium bases against the influenza A virus (H2N2) have been identified earlier [4, 5].

As a further step in our investigations [1] in the present work we synthesized and studied the ability of alkyl(aryl)phosphonium salts to suppress the reproduction of virus A/Leningrad 34/72 (H3 No. 2). The phosphonium salts were obtained by reacting triphenyl, tris(2-cyanoethyl)-, diphenylmethylphosphine with halogen alkines (compounds I-XX, XXIV) or by reacting tertiary phosphines with alkylidenemalonodinitriles in the presence of HCl (compounds XXI-XXIII).

$$R^1_2PR^2 + R^3Hal \longrightarrow \underset{\text{I—XX, XXIV}}{(R^1_2P^+R^2R^3)}\ Hal^-$$

R^1=C_6H_5 (I—XIX), CH_3 (XX), CH_2CH_2CN (XXIV); R^2= =C_6H_5 (I—XX), **n**-C_4H_9 (XXIV); R^3=C_2H_5 (I, XI), **n**-C_3H_7 (II, XII), *i*-C_3H_7 (III, XIII), **n**-C_4H_9 (IV, XIV), **iso**-C_4H_9 (V, XV), **iso**-C_5H_{11} (VI), **cyclo**-C_6H_{11} (VII), **n**-C_8H_{17} (VIII), $CH_2CH{=}CH_2$ (IX, XIX), CH_3 (X), **n**-C_5H_{11} (XVI), **iso**-C_5H_{11} (XVII), **n**-C_6H_{13} (XVIII), C_6H_5 (XX), CH_2CH_2CN (XXIV); Hal=Br (I—IX), I (X—XX, XXIV)

$$R^1_2PR^2 + XC_6H_4CH{=}C(CN)_2 \xrightarrow{HCl} [R^1_2P^+(R^2)R^3Hal$$

XXI—XXIII

R^1=**n**-C_4H_9 (XXI), C_6H_5 (XXII, XXIII); R^2=**n**-C_4H_9 (XXI), C_6H_5 (XXII, XXIII); R^3=$CH(C_6H_5)CH(CN)_2$ (XXI, XXIII), CH(**m**-$NO_2C_6H_4CH$)$(CN)_2$ (XXII); Hal=Cl (XXI—XXIII)

The characteristics of the synthesized phosphonium compounds and the values for chemical shifts from the phosphorus rings according to NMR ^{31}P spectrum data are given in Table 1.

EXPERIMENTAL (CHEMICAL)

NMR spectra for ^{31}P were recorded on an NMR KGU-4 instrument at a frequency of 10.2 MHz relative to 85% H_3PO_4 in DMSO or methanol. IR-spectra were recorded on a UR-20 (GDR) instrument (suspension in petroleum jelly). In addition to the absorption bands characteristic, for example, of benzene rings and other molecular fragments, the IR-spectra of compounds XXI-XXIII had two additional absorption bands due to the stretching vibrations of the cyano group bonds in the 2150-2170 cm^{-1} region (the weakest) and in the 2042-2105 cm^{-1} region (the strongest). Melting points were measured on a compact Boetius type heating stand (GDR).

Alkyl(aryl)phosphonium salts I-XX, XXIV were obtained from tertiary phosphines and corresponding halogen alkyls. The tertiary phosphines were alkylated in a medium of diethyl ether, benzene, acetonitrile or in a sealed ampule without a solvent with heating [19]. The constants, yield, NMR ^{31}P spectra, and references to described methods of synthesis are given in Table 1.

(2,2-Dicyano-1-phenylethyl)tributylphosphonium Chloride (XXI). A solution of 1.54 g (0.01 mole) of benzylidenemalonitrile in 20 ml of ether was added to a solution of 2.02 g (0.01 mole) of tributylphosphine in 20 ml of diethyl ether. Dry HCl was slowly passed through

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TABLE 1. Characteristics of Phosphonium Salts $[R^1_2P + R^2R^3_2]Hal^-$

Compound	mp, °C	Yield, %	Empirical formula	NMR ^{31}P, δ, ppm
I	209—210	90 [23]	$C_{20}H_{20}BrP$	26
II	240—241	86 [13]	$C_{21}H_{22}BrP$	24
III	233—236	67 [12, 14]	$C_{21}H_{22}BrP$	30,4
IV	244—246	78 [11, 14]	$C_{22}H_{24}BrP$	24
V	230—232	61 [5]	$C_{22}H_{24}BrP$	22
VI	138—140	62	$C_{23}H_{26}BrP$	25,6
VII	270—278	42 [8, 13]	$C_{24}H_{26}BrP$	26
VIII	Melt	46	$C_{25}H_{32}BrP$	25
IX	220—222	80 [8, 14]	$C_{21}H_{20}BrP$	22
X	187—188	80 [7]	$C_{16}H_{18}IP$	20
XI	169—170	71 [6]	$C_{20}H_{20}IP$	26
XII	208—210	60	$C_{21}H_{22}IP$	23
XIII	200—201	55 [24]	$C_{21}H_{22}IP$	31
XIV	227—228	55 [22]	$C_{22}H_{24}IP$	23
XV	175—177	60 [20]	$C_{22}H_{24}IP$	22
XVI	165—170	90	$C_{23}H_{26}IP$	25
XVII	163—168	82	$C_{23}H_{26}IP$	26
XVIII	136—141	65	$C_{24}H_{28}IP$	25
XIX	177—180	90	$C_{11}H_{20}IP$	22
XX	252	74 [6, 9]	$C_{14}H_{16}IP$	21
XXI	83	54	$C_{22}H_{34}ClN_2P$	38
XXII	77	70	$C_{28}H_{21}ClN_3O_2P$	25
XXIII	97	81	$C_{28}H_{22}ClN_2P$	31
XXIV	153	60 [3]	$C_{13}H_{21}IN_3P$	36

Note: Reaction conditions: VI — 150-160°C, 80 h; VIII — 150-160°C, 100 h; XII — benzene, 7 h; XVI — 120-130°C; XVIII — 130-140°C; 50 h; for the remaining compounds — correspond to those described in the indicates sources [].

the reaction mixture. Part of the solvent was vacuumed off and the crystals were filtered off and recrystallized from a mixture of ethanol and ether to yield 2 g (54%) of XXI, mp 83°C.

(2,2-Dicyano-1-m-nitrophenylethyl)triphenylphosphonium Chloride (XXII). A solution of 2.7 g (0.01 mole) of triphenylphosphine in 50 ml of benzene was added to a solution of 2 g (0.01 mole) of m-nitrophenylmethylenemalonitrile in 20 ml of benzene, and the mixture was heated for 4 h. Dry HCl was then slowly passed through the reaction mixture. The resultant oily substance was crystallized after a part of the solvent was removed. The crystals were recrystallized from a mixture of ethanol and ether. The yield was 3.2 g (70%) XXII, mp 77°C.

(2,2-Dicyano-1-phenylethyl)triphenylphosphonium Chloride (XXIII). In a manner similar to the above-described method, 3.6 g (81%) of XXIII, mp 97°C, was obtained from 1.54 g (0.01 mole) of benzylidenemalonitrile and 2.7 g (0.01 mole) of triphenylphosphine.

EXPERIMENTAL (BIOLOGICAL)

The ability of alkyl(aryl)phosphonium salts to suppress influenza virus A/Leningrad 34/72 (H3N2) reproduction was tested on surviving fragments of chorio-allantoic membrane (CAM) of chick embryos by method [2, 10, 17]. In our study of antiviral activity we employed subtoxic concentrations of the compounds and analyzed their ability to suppress viral reproduction after the CAM cells were inoculated with 1, 10, and 100 infectious doses of the virus. The presence of a virus in the culture was measured by the hemagglutination reaction with a 1% suspension of chick erythrocytes.

The toxicity of the examined phosphonium salts for the CAM cells ranged between 31.2-500.0 μg/ml (Table 2). The compounds containing radicals with an iso-structure (III, VI, XIII, XV, XVII) were 2-4 times more toxic than the corresponding compounds with a normal structure of radicals (compounds II, IV, XII, XIV, XVI). An elongation of the radical on the phosphorus atom resulted in a higher toxicity (compounds VIII, XVIII, and XIX). The minimum toxic dose was 31.2 μg/ml.

All of the investigated compounds exhibited pronounced antiviral activity and retarded influenza virus reproduction when CAM cells were inoculated with infectious doses. The smallest end concentrations of the compounds at which the antiviral effect was manifested were from 32 to 80,000 times smaller than the minimum toxic dose. The activity of most of the examined phosphonium salts (compounds I-IV, VII-XV, XVIII-XX) exceeded the antiviral action of remantadine. The chemotherapeutic indices of the compounds ranged from 512 to 80,645. The presence of cyanoalkyl groups in the examined phosphonium salt compounds resulted in a

TABLE 2. Antiviral Activity of Phosphonium Salts against Influenza Virus A(H3N2), Strain Leningrad 34/72

Compound	Toxicity for the chorion allantoid membrane, μg/ml	Activity				Chemotherapeutic index
		minimal		maximum		
		viral dose	concentration	viral dose	concentration	
I	250	1	0,0062	10 000	0,025	40 322
II	250	1	0,0031	100	0,025	80 645
III	62,5	1	0,06	100	0,97	1024
IV	250	1	0,0031	100	0,025	80 645
V	125	1	0,49	100	1,25	256
VI	125	1	0,97	100	1,95	128
VII	250	1	0,48	100	1,95	512
VIII	31,2	1	0,00381	100	1,95	8192
IX	125	1	0,0062	10 000	0,013	20 161
X	250	1	0,05	10 000	0,11	5000
XI	250	1	0,0062	1 000	0,0125	40 322
XII	250	1	0,025	100	0,05	10 000
XIII	62,5	1	0,12	100	0,48	512
XIV	250	1	0,0062	100	0,025	20 161
XV	125	1	0,49	100	0,97	256
XVI	125	1	0,24	100	0,49	512
XVII	31,2	1	0,97	100	1,95	32
XVIII	31,2	1	0,06	100	0,48	512
XIX	31,2	1	0,06	100	0,48	512
XX	500	1	0,22	100	3,9	2272
XXI	250	1	7,8	100	31,2	32
XXII	250	1	7,8	100	31,2	32
XXIII	125	1	3,4	100	31,2	32
Remantadine	250	1	0,97	100	15,6	256

lowered antiviral effect. The chemotherapeutic indices of compounds XXI-XXIII equaled 32, whereas tris(2-cyanoethyl)butylphosphonium iodide XXIV, which contains three cyanoalkyl groups, turned out to be practically inactive.

The high level of antiviral properties among the phosphonium salt series allowed to assume that they would be able to retard influenza virus reproduction if the viral infectious doses were increased.

We therefore undertook an examination of phosphonium salt activity upon the inoculation of CAM cells with 1000, 10,000, and 100,000 infectious doses of the influenza virus. All three of the tested salts, I, IX, and X, suppressed viral reproduction when the cells were inoculated with 10,000 doses.

This very high level of antiviral properties in the phosphonium salts necessitated additional tests that would eliminate the possibility of non-toxic effects on CAM cells and that would make viral reproduction impossible.

With this purpose in mind, we cultivated CAM cells in a Hank's solution containing the test substances at concentrations that were 45 times greater than the quantity required to produce an antiviral effect. CAM cells cultivated in a Hank's solution served as the control. After 24 h of incubation at 37°C the CAM cells from the experimental and control groups were transferred into new portions of Hank's solution, inoculated with the virus and incubated for 24 h at 37°C. The viral titer was measured in the culture medium in a hemagglutination reaction. The viral titer values experimentally determined were identical to those in the control upon the use of viral dilutions of from 10^1 to 10^6 log LD_{50}.

Data obtained from an earlier study of the antiviral activity of the 2,4,6-trimethoxyphenylphosphonium salts indicated that these compounds were inactive in the cell cultures [4]. This seemed to contradict our results. However, one should note that the examinaton of antiviral properties was made on a cell culture of monkey renal epithelium, but in the tests of antiviral activity of 2,4,6-trimethoxyphenylphosphonium salts on chick embryos those salts were found to exhibit pronounced antiviral properties. These results might be explained by the use of different cell cultures requiring appropriate methodological approaches since the investigations on the CAM cells are methodologially similar to the tests in ovo. This explains the coincidence of the antiviral activity test results.

Our investigations show that the greater part of the tested phosphonium salts exhibit a high degree of antiviral actiity in vitro that exceeds the activity of remantadine which has been used for the prevention and treatment of influenza. The compounds that have radicals with a normal structure on the phosphorus atom exhibit less toxicity and greater antiviral activity than the salts containing the corresponding radicals with an iso-structure. Antiviral activity depends upon the presence of the corresponding halogens in the molecule's structure. Iodine-containing phosphonium salts were less active than the corresponding bromine-containing compounds.

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