ANTIMICROBIAL ACTIVITY OF CERTAIN DERIVATIVES OF PHOSPHINE AND PHENYLPHOSPHINE

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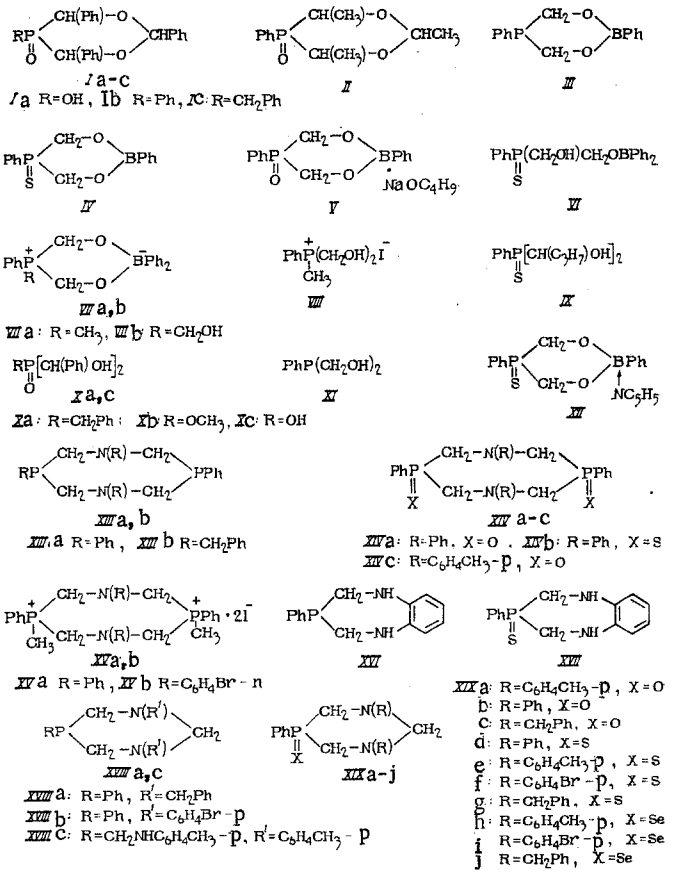
A prospective field in the search for new antimicrobial compounds is hydroxyalkyl- and aminoalkyl-substituted organophosphorus compounds. Compounds were discovered among them with fungicidal, bactericidal, and herbicidal activity [1-3]. Recently, research has been devoted to compounds based on phosphines. Hydroxymethyl and aminomethyl derivatives of phosphines comprise an interesting, but little studied, field of organophosphorus chemistry.

In the present article, the results are reported of a study of antimicrobial activity and toxicity of a series of compounds belonging to the class of hydroxymethyl and aminomethyl derivatives of phenylphosphine and phosphine. These are the representatives of acyclic di-(α-hydroxyalkyl)phenylphosphines; 1,3,5-dioxaphosphorinanes and their derivatives; phosphorus-boron-containing heterocycles; aminomethyl derivatives of phosphines; 1,3,5-diazaphosphorinanes and their derivatives, and 1,5,3,7-diazadiphosphacyclooctanes and their derivatives [4]. With this series of compounds we can trace the dependence of the physiological activity on the structure of the compounds, on the hetero atom, the O, N, B included in the molecule, the size of the ring, coordination of the phosphorus atom, and on certain chemical properties of the compounds.



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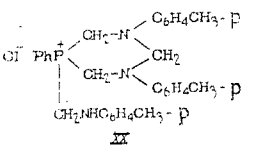

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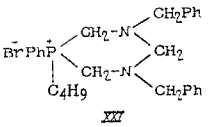

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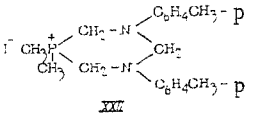

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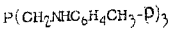

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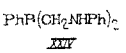

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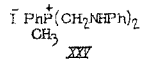

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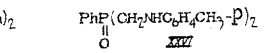

XXVI

In the study of the spectrum of activity of the compounds, we also included the inducers of surfacial and deep-rooted mycoses, taking into account that various types of fungi have different, and sometimes very fine selective responsiveness to the same chemical compounds. The importance of mixed bacterial and fungal infections becomes increasingly evident in modern pathology, and this necessitates the production of preparations with simultaneously antifungal and antibacterial activity. The antimicrobial activity of the compounds was studied with respect to the following types of fungi and bacteria: *Trichophyton rubrum* (strain 695), *Trichophyton mentagrophytes* (strain 1773), *Microsporum canis* (strain 84), *Candida albicans* (strain 624), *Escherichia coli* (strain 60), *Staphylococcus aureus* (strain 40499). The cultures of the dermaphytia and candidosis inducers and bacteria were obtained from the deep-rooted mycoses section of the Leningrad State Advanced Training Institute for Doctors and from the Microbiology Department of the Kazan State Advanced Training Institute for Doctors. The fungicidal action of compounds I-XXVI (Tables 1-3) appears at concentrations of 0.18-3.9 mmole/liter. The bacteriostatic concentration of the compounds studied with respect to bacteria is within 0.7-3.9 mmole/liter (Table 4).

The fungicidal activity of the compounds is shown in Tables 1-3. Table 1 shows that the fungicidal activity is higher in acyclic α-hydroxyalkyl derivatives of phenylphosphine (VIII, IX, XI) than in derivatives of 1,3,5-dioxaphosphorinanes (I, II) with respect to *T. rubrum* and *M. canis* ($P = 0.05$). The introduction of the boron-containing fragment into the 1,3,5-dioxaphosphorinane ring leads to a pronounced fungicidal activity of compounds III-VI, VIIIa, b, XII ($P < 0.05$) toward *T. rubrum* and *M. canis*. This increase in activity is independent of the coordination of phosphorus and boron atoms, and is high for both 4-coordinated boron and phosphorus atoms (VI, VIIa, b, XII) and compounds with 3-coordinated boron and 4-coordinated phosphorus atom (IV, VI), and also for compound III, where phosphorus and boron are 3-coordinated. A change in the fungicidal properties of compounds I-XII is related to their ability to enter into aminolysis reactions. It is known that 1,3,5-dioxaphosphorinane rings do not undergo aminolysis, even under rigid conditions. The reaction of acylic α-hydroxymethyl derivatives of phenylphosphine with 3-coordinated phosphorus atom (in the case of compound XI) and phosphonium salts (compound VIII) with primary and secondary amines proceeds at room temperature with evolution of heat, and leads to aminomethyl derivatives of phenylphosphine [5]. The boron-containing heterocycles also readily enter reactions with amines with displacement of the hydroxyboryl grouping and formation of the corresponding derivatives of phenylphosphine [6].

The fungicidal activity of aminomethyl derivatives of phosphines is shown in Tables 2 and 3. A comparison of the fungicidal concentrations of compounds XIIIa-XVII shows that in this series, compound XVb belonging to the class of cyclic phosphonium salts is active. No increase in the fungicidal activity is observed with increase in the ring size from 7 to 8-membered one, nor on changing the radical at the nitrogen atom, or on change in the coordination of the phosphorus atom. It should be noted that all the compounds listed in Table 2 are characterized by poor solubility in water and in organic solvents.

In the series of 1,3,5-diazaphosphorinanes and their derivatives (XVIIa-XXII) and acyclic aminomethyl derivatives of phosphines (XXIII-XXVI) (see Table 3), no increase in activity toward fungi is observed when the radicals at the nitrogen atom or the coordination of phosphorus atom are changed. Only in the case of phosphonium salts, a pronounced fungicidal activity is observed for compounds XXI, XXII, and XXV ($P < 0.05$).

TABLE 1. Fungicidal Activity of Hydroxymethyl Derivatives of Phosphines and Boron-Containing Heterocycles (in mmole/liter)

Compound	T. rubrum (strain 695)	T. mentag (strain 1773)	M. canis (strain 34)	C. albicans (strain 624)
Ia	2,7	2,7	2,7	>2,7
Ib	>2,3	>2,3	>2,3	>2,3
Ic	>2,3	>2,3	>2,3	>2,3
II	3,3	1,65	3,3	3,3
III	1,95	1,95	0,78	>3,9
IV	1,7	1,7	0,34	1,7
V	0,27	0,8	0,27	1,4
VI	1,4	2,7	1,4	1,4
VIIa	1,4	1,4	0,9	1,4
VIII	1,0	1,0	0,3	1,6
IX	0,9	1,5	0,6	1,5
Xa	>2,8	>2,8	>2,8	>2,8
Xb	>3,4	>3,4	>3,4	>3,4
Xc	>3,6	>3,6	>3,6	>3,6
XI	1,8	1,8	0,6	>2,4
XIIb	0,8	1,0	0,5	2,7
XII	1,4	2,8	1,4	2,8

TABLE 2. Fungicidal Activity of 1,5-Diaza-3,7-diphosphacyclooctanes, 1,5-Diaza-3-phospha-6,7-benzocycloheptane, and Their Derivatives (in mmole/liter)

Compound	T. rubrum	T. mentag	M. canis	C. albicans
XIIIa	>2,2	>2,2	>2,2	>2,2
XIIIb	>2,1	>2,1	>2,1	>2,1
XIVa	>2,1	>2,1	>2,1	>2,1
XIVb	>1,9	>1,9	>1,9	>1,9
XIVc	>0,7	>0,7	>0,7	>0,7
XVa	>1,4	>1,4	0,7	>1,4
XVb	0,6	0,6	0,6	1,1
XVI	>4,1	>4,1	>4,1	>4,1
XVII	>3,6	>3,6	3,6	>3,6

TABLE 3. Fungicidal Activity of 1,3,5-Diazaphosphorinanes and Their Derivatives (in mmole/liter)

Compound	T. rubrum	T. mentag	M. canis	C. albicans
XVIIIa	1,4	>2,8	1,4	>2,8
XVIIIb	>2,0	>2,0	>2,0	>2,0
XIXa	>2,7	>2,7	>2,7	>2,7
XIXb	0,9	1,1	0,9	>2,9
XIXc	>2,7	>2,7	>2,7	>2,7
XIXd	>2,7	>2,7	>2,7	>2,7
XIXe	>2,6	>2,6	1,3	>2,6
XIXf	>1,9	>1,9	>1,9	>1,9
XIXg	>2,6	>2,6	>2,6	>2,6
XIXh	>2,3	>2,3	>2,3	>2,3
XIXi	>1,8	>1,8	>1,8	>1,8
XIXj	>2,3	>2,3	>2,3	>2,3
XX	>1,9	>1,9	1,0	>1,9
XXI	0,2	1,0	0,2	2,0
XVIIIc	1,3	2,6	1,6	2,6
XXII	0,18	0,45	0,45	>2,0
XXIII	2,6	2,6	2,3	2,6
XXIV	3,1	>3,1	1,6	>3,1
XXV	0,43	0,65	0,43	2,2
XXVI	>2,8	>2,8	>2,8	>2,8

TABLE 4. Bacteriostatic Activity (in mmole/liter)

Compound	E. coli strain 60)	Staph. aureus strain 40499)
II	>3,3	>3,3
III	>3,9	>3,9
IV	>3,5	3,5
V	1,4	2,7
VI	2,7	2,7
VIIa	1,4	2,8
VIIb	0,7	0,7
XII	>2,8	>2,8
XVI	>4,1	>4,1
XVIIIa	2,8	>2,8
XVIIIb	2,0	>2,0
XIXe	>2,6	>2,6
XIXf	>1,9	>1,9
XIXg	>2,6	>2,6
XIXh	>2,3	>2,3
XIXi	>1,8	>1,8
XIXj	>2,3	>2,3
XVIII	1,0	1,0
XVIIIc	>2,6	>2,6
XXIII	>2,6	>2,6
XXV	1,1	1,1

An analysis of the data in Tables 1-3 shows that 1,3,5-dioxaphosphorinanes and their derivatives have the lowest activity. Replacement of oxygen atoms by nitrogen in 6-membered rings does not lead to appreciable change in activity. However, the introduction of a boron-containing fragment into 1,3,5-dioxaphosphorinane ring leads to fungicidal activity. For compounds with no boron-containing fragment, an increase in activity is observed on transition from compounds with 3-coordinated phosphorus atoms to phosphonium salts (compare VIII and XI, XIIIa, and XVa, XXIV and XXV). At the same time, on transition from a compound with a 3-coordinated phosphorus to compounds with the P=O, P=S, or P=Se bond, there is no increase in the inhibiting action of the compounds with respect to fungi. No appreciable changes are observed in the fungicidal activity when the ring is changed from a 6- to 8-membered one.

Table 4 shows the results of the study of the bacteriostatic properties of some of the compounds. The data show that in the action of the compounds studied in bacteria, the same regularities are observed as in the action on fungi, i.e., the phosphonium salts and the boron-containing heterocyclic compounds have the highest bacteriostatic activity.

The compounds studied are moderately toxic to white mice, the LD_{50} is 240-800 mg/kg.

The data obtained in the study of the fungicidal and bacteriostatic activity and toxicity show that there are good prospects for compounds based on hydroxy and amino derivatives of phosphines to be studied in the search for new antimicrobial agents.

EXPERIMENTAL CHEMISTRY

The methods of synthesis of compounds I-VIIb, XIIIa-XIVb, XVI-XX, XVIIIc-XXIV are described in [4-6, 11-19].

Complex of 2,5-Diphenyl-2-bora-5-thio-1,3,5-dioxaphosphorinane with Pyridine (XII). Compounds IV are crystallized from pyridine. Yield of XII, 80%, mp 134°C, ^{31}P NMR spectrum; 29 ppm (pyridine, acetonitrile, DMFA). Found, %: C 61.40; H 5.30; P 8.33; N 3.59; B 2.89; S 8.92. $C_{19}H_{19}PNBO_2$. Calculated, %: C 62.13; H 61.8; P 8.45; N 3.81; B 3.05; S 8.72.

1,3,5,7-Tetraphenyl-3,7-dithio-1,5-diaza-3,7-diphosphacyclooctane (XIVc). An excess of sulfur is added to 0.9 g of XIIIa in 10 ml of CH_3CN. The mixture is heated to boiling and left to stand at room temperature overnight. The next day, excess of sulfur is removed. On standing, a precipitate separates from the filtrate, and is crystallized from CH_3CN. Yield, 0.7 g (70%), mp 222°C, ^{31}P NMR spectrum; 36 ppm (DMFA). Found, %: C 64.75; H 5.40; P 12.09. $C_{28}H_{28}P_2N_2S_2$. Calculated, %: C 64.86; H 5.41; P 11.97.

1,3,5,7-Tetraphenyl-3,7-dimethyl-1,5-diaza-3,7-diphosphoniacyclooctane Diiodide (XVa). A 2-ml portion of MeI is added to 2 g of IIIa in 8 ml of CH_3CN, and the mixture is heated to boiling. When cool, crystals precipitate, and are filtered and washed with CH_3CN. Yield, 2.9 g (89%), mp 195°C, ^{31}P NMR spectrum: 13 ppm (DMFA). Found, %: C 48.77; H 4.72; P 7.75; N 4.17. $C_{30}H_{34}P_2N_2S_2$. Calculated, %: C 48.78; H 4.61; P 8.40; N 3.79.

1,3-Di-p-bromophenyl-3,7-diphenyl-3,7-dimethyl-1,5-diaza-3,7-cyclooctane Diiodide (XVb). A 1-ml portion of MeI is added to 1.2 g of 1,5-di-p-bromophenyl-3,7-diphenyl-1,5-diaza-3,7-diphosphacyclooctane [16] in 5 ml of CH_3CN. After 2 h, the solution is evaporated, and the residue is crystallized from CH_3CN. Yield, 0.6 g (71%), mp 189-190°C, ^{31}P NMR spectrum: 16 ppm (DMSO). Found, %: C 40.27; H 3.68; P 6.62; N 3.66. $C_{30}H_{32}P_2N_2I_2Br_2$. Calculated, %: C 40.17; H 3.57; P 6.92; N 3.13.

1,3-Dibenzyl-5-phenyl-5-n-butyl-1,3,5-diazaphosphorinanium bromide (XXI). A 2-ml portion of p-BuBr is added to 0.8 g of XVIIIa. After XVIIIa had dissolved, the solution is evaporated, and the residue is crystallized from CH_3CN. Yield, %: C 65.04; H 6.72; P 6.26; N 5.60. $C_{27}H_{34}P_2N_2Br$. Calculated, %: C 65.19; H 6.84; N 5.63.

Di(anilinomethyl)phenylmethylphosphonium Iodide (XXV). A 1-ml portion of MeI is added to 1.2 g of XXIV in 6 ml of CH_3CN. After removal of CH_3CN, the material crystallizes. The crystals are filtered and washed with CH_3CN. Yield, 1.09 g (63%), mp 154°C, ^{31}P NMR spectrum: 22 ppm (DMFA). IR spectrum, ν: 3200 cm^{-1} (NH, oil). Found, %: C 55.08; H 5.52; P 7.01; N 5.80. $C_{21}H_{24}PN_2I$. Calculated, %: C 54.55; H 5.19; P 6.71; N 6.06.

Di(p-Toluidinomethyl)phenylphosphine Oxide (XXVI). A 5-ml portion of acetone and 3 ml of H_2O_2 are added to 4.6 g of a reaction mixture, obtained in the reaction of 2 g (12 mmoles) of XI with 2.6 g (24 mmoles) of p-toluidine. After 2 h, the crystals are filtered. Yield,

2.3 g (53%), mp 167-168°C, ^{31}P NMR spectrum: 33 ppm (DMFA), IR spectrum, ν: 3250 cm^{-1} (NH, oil). Found, %: C 72.44; H 7.16; P 8.62; N 7.41. $C_{22}H_{23}PN_2O$. Calculated, %: C 72.53; H 6.84; N 7.69.

EXPERIMENTAL BIOLOGY

The fungicidal activity of the compounds was studied by a method described in [7]. In the determination of the antifungal properties, two-week cultures of dermatophytes and a three-day culture of *C. albicans* were used. The test tubes with the medium containing compounds in increasing concentrations and control test tubes without the compound were inoculated by a culture of fungi, and then were placed in a thermostat at 26°C. After 14 days, the inoculated material was reinoculated from tubes in which no growth was observed into a pure Saburo culture. The fungicidal activity (the lowest concentration stopping the growth of fungi) was indicated from the absence of the growth of fungus after reinoculation in the course of 30 days. Repetitivity, 3-4-fold.

The bacteriostatic properties were studied by the method of serial dilutions by the method described in [8]. The bacterial charge in the experiment was 300,000 microbial bodies per ml. The experimental results were recorded every 24 h for 5 days, registering the presence of growth (turbidity) of retardation of growth of the culture in the medium.

The toxicity of the compounds was determined on white mice at peroral administration of acetone-oil solutions with calculation of LD_{50} by the method of Leachfield and Wilcoxin [9].

The experimental results were statistically processed [10].

LITERATURE CITED

1. K. A. Petrov and V. A. Pershina, Usp. Khim., 37, No. 7, 1218-1243 (1968).
2. K. A. Petrov, V. A. Chauzov, and T. S. Erokhina, Usp. Khim., 43, No. 11, 2045-2088 (1974).
3. A. Sh. Sharifullin, R. K. Valetdinov, A. S. Salivanova, et al., in: Synthetic Polymers of Medicinal Designation [in Russian], Tashkent (1973), p. 74.
4. B. A. Arbuzov, O. A. Erastov, and G. N. Nikonov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 12, 2771-2773 (1979).
5. B. A. Arbuzov, O. A. Erastov, and G. N. Nikonov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 9, 2129-2131 (1980).
6. B. A. Arbuzov, O. A. Erastov, and G. N. Nikonov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 4, 952-953 (1980).
7. S. N. Milovanova and Z. G. Stepanishcheva, in: Methods of Experimental Chemotherapy [in Russian], G. N. Pershin, ed., Moscow (1971), pp. 318-320.
8. S. N. Milovanova, in: Methods of Experimental Chemotherapy [in Russian], G. N. Pershin, ed., Moscow (1971), pp. 104-106.
9. M. L. Belen'kii, Elements of Quantitative Evaluation of Pharmacological Effect [in Russian], 2nd edn., Leningrad (1963).
10. I. A. Oivin, Pat. Fiziol., No. 4, 76-85 (1960).
11. B. A. Arbuzov, O. A. Erastov, I. P. Romanova, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 12, 2803-2806 (1981).
12. B. A. Arbuzov, O. A. Erastov, S. Sh. Khetagurova, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 9, 2136-2139 (1979).
13. B. A. Arbuzov, O. A. Erastov, S. Sh. Khetagurova, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 7, 1626-1630 (1980).
14. B. A. Arbuzov, O. A. Erastov, S. Sh. Khetagurova, et al., Dokl. Akad. Nauk SSSR, 244, No. 3, 610-614 (1979).
15. B. A. Arbuzov, O. A. Erastov, G. N. Nikonov, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 7, 1545-1549 (1981).
16. B. A. Arbuzov, O. A. Erastov, and G. N. Nikonov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 735 (1980).
17. B. A. Arbuzov, O. A. Erastov, and G. N. Nikonov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 4, 954-957 (1980).
18. B. A. Arbuzov, O. A. Erastov, G. N. Nikonov, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 721-723 (1980).
19. B. A. Arbuzov, O. A. Erastov, T. A. Zyablikova, et al., Izv. Akad. Nauk SSSR, Ser. Khim., No. 1, 127-131 (1982).