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BACTERICIDAL ACTIVITY OF PHOSPHONIUM COMPOUNDS

Shamima Khalid, Husan Afroz Rizvi and Yasmeen Badar*

PCSIR Laboratories Complex, Karachi-39

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Bactericidal activity of the seven phosphonium compounds was tested in vitro against 12 different strains of pathogenic bacteria. Of the series of compounds tested only 1-substituted 2-ethoxy-2-alkoxyvinyl triphenyl phosphonium tetrafluroborate (I), carbomethoxy methyl triphenyl phosphonium bromide (II), carbomethoxy methylene triphenyl phosphoran (III) and methyl triphenyl phosphonium iodide (VI) exhibited complete inhibition of growth at 100 µg/ml. Partial inhibition was also noted by compound II, III and VI at lower concentrations. About 50-80% growth was inhibited at 80 µg/ml whereas about 40% inhibition was observed by compound II and VI at a concentration as low as 20 µg/ml. Minimum inhibitory concentrations varied from 40-100 µg/ml. Structure activity relationship has been discussed.

Key words: Phosphonium compounds, Bactericidal activity, Antibacterial activity.

INTRODUCTION

A number of phosphonium salts, phosphorans, substituted phosphoramides, and related organophosphorous compounds have been evaluated for various biological activities. *Tris* (aryl) alkyl phosphonium halides [1, 2] and other related compounds [3] have been reported as useful pesticides and insecticides. Phosphonium salts of phosphonomethylglycine are used as liquid herbicides [4]. Many quaternary phosphonium salts [5-7] and phosphonium hydrazones [8] were found to have antibacterial activity. Thiosemicarbazones and arylhydrazones of triphenylphosphonium salts are antiviral agents [9] and some substituted phenylphosphates and quaternary phosphonium compounds have shown neurotoxicity and anticholinesterase activity [10, 11]. These interesting biological activities of various types of phosphonium compounds have persuaded us to evaluate our phosphonium compounds, which were synthesized for some other purpose [12, 13], for possible biological activity. These compounds have not been evaluated so far for any type of biological activity except our previous report [14] on the toxicity and morphogenetic effect of some of these compounds on yellow fever mosquito. Present communication deals with the antibacterial activity of seven phosphonium compounds against 12 different strains of pathogenic bacteria.

MATERIAL AND METHODS

The phosphonium compounds which have been tested for antibacterial activity are listed in Table 1. These compounds were prepared according to the methods given in the corresponding references. All the test organisms used in the present study were clinical isolates and were obtained partly from the Department of Microbiology, University of Karachi, Karachi, and partly from the Applied Biology and Marine Resources Centre of PCSIR Laboratories Complex, Karachi. These organisms are listed in Table 2. The test organisms were maintained on nutrient agar slants and were subcultured before use. 24 hours broth cultures were prepared in 5 ml sterile nutrient broth.

The activity of the compounds was determined by the agar dilution streak method [15]. The compounds were first tested at the concentration of 1,000 µg/ml, and those which completely suppress the growth were retested at 500 µg/ml

Table 1. List of phosphonium compounds tested for antibacterial activity.

Compound No.	Chemical Name	Chemical formula	Reference
I.	1-substituted 2-ethoxy-2-alkoxyvinyl triphenyl phosphonium tetra fluroborate	$[(C_6H_5)_3\overset{+}{P}CH{=}C(OC_2H_5)_2]\ B\overline{F}_4$	18
II.	Carbomethoxy methyl triphenyl phosphonium bromide	$(C_6H_5)_3\overset{+}{P}{-}CH_2COOCH_3\ \overline{Br}$	19
III.	Carbomethoxy methylene triphenyl phosphoran	$(C_6H_5)_3P{=}CHCOOCH_3$	19
IV.	3-Carbomethoxy-1-methyl prop-2-enyltriphenyl phosphonium iodide	$(C_6H_5)_3\overset{+}{P}CH(CH_3){-}CH{=}CHCOOCH_3\ \overline{I}$	20
V.	3-Carbomethoxy-1-methyl prop-2-enyltriphenyl phosphoran	$(C_6H_5)_3P{=}CH(CH_3){-}CH{=}CHCOOCH_3$	20
VI.	Methyl triphenyl	$(C_6H_5)_3\overset{+}{P}CH_3\ \overline{I}$	21
VII.	Ethyl triphenyl phosphonium iodide	$(C_6H_5)_3\overset{+}{P}C_2H_5\ \overline{I}$	21

* To whom all correspondence may be addressed:

and 100 µg/ml. Two control plates containing 0.2 ml of 50% alcohol and two standard plates containing 10 µg/ml streptomycin sulphate were also treated similarly with each set of experiment. Minimum inhibitory concentration (MIC) of those compounds which completely inhibit the growth of at least one or more organisms at 100 µg/ml was determined against the specified organism by tube dilution technique [16]. All the experiments were repeated thrice at least after a week's interval and the activity of the compound was found to be reproducible.

RESULTS AND DISCUSSION

The phosphonium compounds under study were screened for antibacterial activity against 12 different organisms at a concentration of 1,000 µg/ml, 500 µg/ml and 100 µg/ml by agar dilution streak method [15]. The level of activity of each compound has been represented in Table 3. The criteria of activity was the complete inhibition of growth of one or more organisms, and the reproducibility of the activity in at least two successive series of tests separated by a week in time. The reproducible activity at 1,000 µg/ml and 500 µg/ml was considered to be weak or moderate and was not of much interest as more potent antibacterial agents are available. However, the compounds showing reproducible activity at 100 µg/ml were considered to be potentially active and suitable for further scrutiny of useful bactericidal activity.

The agar dilution streak method is the most convenient method among the various testing procedures in which the sterility of the test compound is not very necessary. This is, however, a qualitative method for determining the activity and is useful only for screening the compounds on the basis of presence or absence of activity at a particular concentration. For determining the activity quantitatively in terms of % inhibition of growth, tube dilution technique was used [16].

As indicated from Table 3, the compound No. I, II, III and VI were found to be active at a dose level of 100 µg/ml. The order of activity with respect to the number of organisms inhibited was II>VI>I>III. Table 4 represents the minimum inhibitory concentration (MIC), which is defined as that concentration which inhibit growth to 50% of the control after 24 hours incubation. The actual percentage of growth at various concentrations has been represented in Fig. 1.

The results have indicated that this class of compounds is generally weakly or moderately active and only 4 compounds (No. I, II, III and VI) out of seven tested, have shown some significant bactericidal activity at lower concentrations. About 50-80% growth was inhibited at 80 µg/ml by compound No. II, III and VI, while compound II and VI inhibit the growth of *Citrobacter freundii* and *S. aureus* respectively upto 40% at a concentration as low as 20 µg/ml. With the exception of VI, which was active against *S. aureus*, all the others were active against gram negative strains, particularly *Shegella* species and *Citrobacter freundii*. The MIC of all the four active compounds vary from 40-100 µg/ml.

Table 2. Organisms used for testing the antibacterial activity of phosphonium compounds.

S. No.	Organisms	Classification
1.	*Staphylococcus aureus*	Gram positive
2.	*Salmonella typhypara* B	Gram negative
3.	*Salmonella typhimurium*	Gram negative
4.	*Shigella boydii*	Gram negative
5.	*Shigella flexneri*	Gram negative
6.	*Shigella sonni*	Gram negative
7.	*Shigella dysenteriae*	Gram negative
8.	*Citrobacter freundii*	Gram negative
9.	*Klebsella pneumoniae*	Gram negative
10.	*Escherichia coli*	Gram negative
11.	*Streptococcus faecalis*	Gram positive
12.	*Pseudomonas aeroginosa*	Gram negative

Table 3. Inhibitory concentrations of phosphonium compounds agianst various strains of bacteria (agar dilution streak method (17)).

Compound No.	Inhibitory Concentrations of the test compounds vs. organism No. (Table 2). 1000 µg/ml	500 µg/ml	100 µg/ml
I	4, 5, 6, 10, 11	5, 6	5
II	1, 3, 4, 5, 7, 8, 10, 11	1, 4, 5, 7, 8, 11	4, 8
III	1, 4, 8, 10, 11	4	4
IV	1, 4, 5, 7, 8, 11	1, 7	–
V	1, 4, 5, 7, 8, 11	1, 7, 8	–
VI	1, 3, 4, 5, 6, 7, 12	1, 6, 7, 12	1
VII	5, 7, 8, 10, 11	8	–

The compound numbers are the same as mentioned in Table 1.
The numbers in the concentrations columns refer to the number of organisms (Table 2) which were completely inhibited at that concentration.
Standard Streptomycine sulphate inhibited all the test organisms at 10 µg/ml. concentration and control plates showed 100% growth.

Table 4. Minimum inhibitory concentration of the active phosphonium compounds (Tube dilution method (22)).

Sample No.	Organism No.	Minimum inhibitory concentration (µg/ml) 20	40	60	80	100
I	5	+	+	+	+	–
II	4	+	+	+	±	–
II	8	+	+	±	±	–
III	4	+	+	±	±	–
VI	1	+	±	±	±	–

Sample No. and organism No. are the same as listed in Table 1 and 2 respectively.
Turbidity of non-drug-control was taken as 100%.
– indicates no growth i.e. 100% inhibition.
± indicates 50% or more inhibition as compared to control.
+ indicates less than 50% inhibition as compared to control.

The results have suggested that the test compounds are not very highly active and comparable to the potent drugs already available. Streptomycine sulphate which we used as standard in our study has inhibited the growth of all the test organisms at a level of 10 µg/ml. The activity of these compounds is, however, suitable enough to be utilized effectively in the preparation of antiseptics and disinfactant soaps. Such type of phosphonium compounds have shown low toxicity for animals and have been incorporated earlier [6, 17] in toilet soaps for controlling sanitation in feeding units.

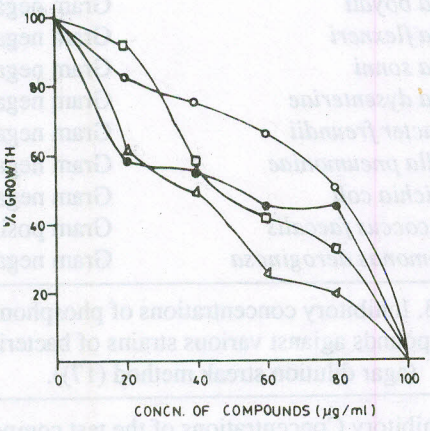

Fig. 1. Inhibitory effect of posphonium compounds at different concentrations. Compd. No. II vs *Shegella boydii* (4); Compd. No. II vs *Citrobacter freundili* (8); Compd. No. III vs *Shegella boydii* (4); Copmd. No. VI vs *S. aureus* (1); Turbidity without test compound (control) was taken as 100%

Regarding the structure activity relationship, it has been observed that the phosphonium salts containing halogen atoms, are more active than non-halogenated phosphorans. The highest activity was obtained with compound II which is an ionic salt containing bromine. Its phosphoran (compound III) which has the same structure but no bromine is less active. It has also been observed that the activity of the compound is highly effected by slight modifications in the chemical structure. Compounds IV and V which are the modified structures of the active compounds II and III, do not possess any activity even at a concentration of 100 µg/ml. Similarly compound VI was highly active, while its analogue (VII), which has an ethyl group instead of methyl group in the molecule, is less active. This suggests that the toxiphoric group in this type of structure (VI) is a methyl group.

These studies have shown that the activity depends on the chemical structure of the compounds tested. The most active phosphonium compound is the one which is in the form of an ionic salt and contains halogen atom in the molecule. Among the halogen atoms, bromine is found to be more reactive than iodine. A fluorinated compound (I) which is structurally different, has shown less activity than iodinated compound, but more activity than non-halogenated compound (III). The mechanism by which these compounds inhibit the growth is not known.

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