Biol. Stud. 2020: 14(3); 65–78 • DOI: https://doi.org/10.30970/sbi.1403.630
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 541.49:546.791.6 +546.73

FUNGICIDAL AND BACTERICIDAL ACTIVITY OF ALKYL-SUBSTITUTING POLYETHERGUANIDINES

***M. Ya. Vortman*[1]*, *Yu. B. Pysmenna*[2], *A. V. Rudenko*[3], *V. V. Tretyak*[3], *V. N. Lemeshko*[1], *V. V. Shevchenko*[1]**

[1] *Institute of Macromolecular Chemistry, NAS of Ukraine*
48, Kharkivs'ke Shose St., Kyiv 02160, Ukraine

[2] *D. K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine*
154, Zabolotny St., Kyiv 03143, Ukraine

[3] *Institute of Urology of the National Academy of Medical Sciences of Ukraine*
9A, Vynnychenko St., Kyiv 04053, Ukraine

**Corresponding author: e-mail: vmar1962@i.ua*

Vortman M. Ya., Pysmenna Yu. B., Rudenko A. V., Tretyak V. V., Lemeshko V. N., Shevchenko V. V. Fungicidal and bactericidal activity of alkyl-substituting polyetherguanidines. **Studia Biologica**, 2020: 14(3); 65–78 • DOI: https://doi.org/10.30970/sbi.1403.630

Background. Polyhexamethylene guanidines are widely used as biocides and disinfectants due to the wide range of their antimicrobial activity against Gram-positive and Gram-negative bacteria, viruses, fungi, and molds. The mechanism of biocidal action of polyguanidines is similar to that of quaternary ammonium compounds and has a membrane toxic nature. The advantages of polyhexamethylguanidines salts include their moderate toxicity and a lack of cumulative action against living organisms. The convenience of such polymeric biocides lies in their high water solubility and in the absence of volatility, which allows disinfectant work to be carried out in the presence of people. It is known that the introduction of alkyl radicals into the polymer chain leads to an increase in the bactericidal and fungicidal action of the obtained compounds. In order to enhance these properties, it seems promising to obtain polyetherguanidines with alkyl radicals in their structure. The aim of this work was to study the fungicidal and bactericidal activity of synthesized alkyl-substituted polyetherguanidinium iodides against a number of bacteria and microscopic fungi.

Methods. Bacteria were grown on the meat-peptone agar for 48 hours at 28±2 °C. Test cultures of micromycetes were cultured on agar beer wort (6 ° B), incubated for 14 days at 28±2 °C. Antimicrobial and fungicidal activities of the newly synthesized alkyl-substituting polyetherguanidines were determined by the standard disco-diffusion method.

Results. The synthesis of polyetherguanidinium iodide is carried out in four stages. The first stage is the synthesis of a guanidine-containing oligoether with terminal guanidine moieties by the reaction between aromatic oligoepoxide and guanidine. The second stage is the synthesis of polyetherguanidinium chloride by the reaction between guanidinium-containing oligoether with terminal guanidine fragments and oligooxyethylenediamine of different molecular weight. In the third stage, the obtained polyetherguanidinium chloride is converted from the salt form to the base form by the reaction with an equivalent amount of alkali in ethanol. In the fourth stage, the basic polyetherguanidine is reacted with methyl iodide at a molar ratio of polyetherguanidine: methyl iodide components of 1:2. The bactericidal and fungicidal activities of alkyl-containing polyetherguanidinium iodides against various heterotrophic bacteria and microscopic fungi has been shown. It was found that polyetherguanidinium iodides at a concentration of 1–3% inhibited the growth of Gram-negative (*Escherichia coli* 475, *Klebsiella pneumonia* 479) and Gram-positive (*Staphylococcus aureus* 451) bacteria. The obtained alkyl-containing polyetherguanidinium iodides at a concentration of 1% for 14 days showed fungicidal activity in almost all studied isolates. With an increasing length of the oligoethylene oxide component, the obtained polymers with n = 10 and 50 did not show a fungicidal effect against *Aspergillus niger*, and the polymer with n = 50 – against the micromycete *Aspergillus versicolor.* If we determine the fungicidal effect as a whole, the polymer with n = 6 had the highest activity against all studied isolates after 14 days, while the fungicidal effect of polymers with n = 10 and n = 50 tends to decrease. All synthesized alkyl-containing polyetherguanidinium iodides showed the highest activity against such microscopic fungi as *Cladosporium cladosporioides*, *Acremonium strictum*, *Alternaria alternate*, *Cladosporium sphaerospermum*, *Paecilomyces variotii*, *Stachybotrys chartarum.* Also noteworthy is the selective effect of the obtained polymers on individual isolates.

Conclusions. The obtained polyetherguanidinium iodides of different molecular weight at a concentration of 1–3% showed bactericidal activity against *Staphylococcus aureus* 451*, Escherichia coli* 475, *Klebsiella pneumoniae* 479 and fungicidal effect against all fungi studied by us and can be used as disinfectants for indoor treatment.

***Keywords**:* polyetherguanidinium iodides, antibactericidal activity, antifungal activity

INTRODUCTION

Polyhexamethylene guanidines (PGMG) are widely used as biocides and disinfectants due to the wide range of their antimicrobial activity against Gram-positive and Gram-negative bacteria, viruses, fungi, and molds [5–7, 19, 20]. The mechanism of biocidal action of polyguanidines is similar to that of quaternary ammonium compounds and has a membrane toxic nature [14, 17].

The advantages of PGMG salts include their moderate toxicity and the absence of cumulative action against living organisms (hazard class 4 according to GOST 12.1.007-76) [6, 10, 12]. The convenience of using such polymeric biocides lies in their high solubility in water and in the absence of volatility, which allows for disinfection in the presence of humans [12, 14, 16, 17].

Previously, we investigated the fungicidal activity of guanidine-containing oligoether (GO) against Gram-positive and Gram-negative bacteria and isolates of microscopic fungi that caused damage to rubber materials. GO with terminal guanidinium fragments was obtained on the basis of aromatic oligoepoxide and guanidinium chlo-

ride. It was found that guanidine-containing oligoether at a concentration of 3% inhibited growth of bacteria and most of the studied micromycetes [4, 8, 18]. Fungicidal effect of the functionalized polymer based on the specified oligoetherguanidinium chloride and aliphatic oligooxyethylenediamine, which inhibited growth of most of studied micromycetes isolated from the premises in Kyiv, was also investigated. It is known that the introduction of alkyl radicals into the polymer chain leads to an increased bactericidal and fungicidal effect of the obtained compounds. In order to enhance these properties, it seems promising to obtain polyetherguanidines with alkyl radicals in their composition. The simplest method of introducing an alkyl radical into a polymer chain involves using the most common alkylating agent, methyl iodide. It can be assumed that the introduction of methyl iodide into polyguanidine chain will produce a polymer with a good bactericidal and fungicidal properties.

The aim of this work was to study the fungicidal and bactericidal activities of synthesized alkyl-substituted polyetherguanidinium iodides against a number of bacteria and microscopic fungi.

MATERIALS AND METHODS

The objects of the study were:

- strains of Gram-positive *Staphylococcus aureus* 451, *Enterococcus faecalis* 422 and Gram-negative bacteria *Escherichia coli* 475, *Klebsiella pneumonia* 479, *Pseudomonas aeruginosa* 465 isolated from biomaterial of patients and stored in the laboratory of bacteriological researches of Institute of Urology, National Academy of Medical Sciences of Ukraine;
- microscopic fungi *Acremonium strictum* W. Gams F-16703, *Alternaria alternata* (Fr.) Keissl. F-41225*, Aspergillus niger* Tiegh. F-73001*, Aspergillus versicolor* (Vuill.) Tirab. F-41250*, Cladosporium cladosporioides* (Fresen.) G.A. de Vries F-41252, *Cladosporium sphaerospermum* Penz. F-41255, *Paecilomyces variotii* Bainier F-16724, *Penicillium chrysogenum* Thom F-16719ZAS, *Penicillium funiculosum* Thom F-16728 and *Stachybotrys chartarum* (Ehrenb.) S. Hughes F-41212 stored at the Department of Physiology and Systematics of Micromycetes of D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine [4].

Research materials. Diane epoxy oligomer DER-331 (DOW Chemical Company, Germany) with MW 365 g/mole and mass fraction of epoxy groups 23.5%, hydroxyl groups 0.6% was dehydrated by heating in vacuum for 2–6 h at 80–90 °C and 2 mm residual pressure of mercury column. Guanidine hydrochloride (GD) (Aldrich, 99.9% purity) was used without further purification. Oligooxyethylenediamine D-230 MW 230, D-400 MW 400 and D-2000 MW 2000 (Aldrich, 99.9% purity) were used without further purification. Methyl iodide (Aldrich, 99.9% purity) was used without further purification. Medical ethanol-rectified 96% was used without further purification. Dimethylformamide (DMF) was purified by distillation.

For the synthesis of guanidinium-containing oligoether – GO based on aromatic epoxy oligomer DER-331 MW 365.5 and guanidinium chloride, 10 g of epoxy oligomer was dissolved in 30 ml of dimethylformamide and uploaded to the reactor and a solution of guanidine (3.3 g) was added, which was pre-converted from salt to basic form by alkali. The reaction was carried out for 2–3 h at 50–60 °C. The completeness of the reaction was monitored by IR spectroscopy for the disappearance of epoxy groups

absorption at 920 cm^{-1}. To the solution of the resultant product was added 5 mL hydrochloric acid (37%) and the reaction mixture was mixed to convert oligoether into an acid form, the solvents were separated from the reaction mixture under reduced pressure. The obtained GO was precipitated from dimethylformamide to diethyl ether. To remove solvent residues, the product was kept in vacuum at a pressure of 1 mbar at 80 °C for 12 h. The product yield constituted 95%.

To obtain polyetherguanidinium chloride (PEG-GC), a mixture of 10 g of GO and 8.2 g of oligooxyethylenediamine with terminal amino groups MW 400 was heated to 80 °C and stirred for 4 h, then the reaction was continued for 4 h at 130–140 °C and 4 h at 180 °C. After cooling the reaction mixture, an amorphous vitreous polymer of PEG-GC was obtained. To purify the product from residual starting reagents, it was dissolved in 40 mL of water and precipitated by adding 20 mL of saturated sodium chloride solution. The purified polymer was separated by decantation, washed with water (20 mL) and dried in vacuum at a pressure of 1 mbar at 80 °C for 24 h. The product represents an amorphous polymer of dark yellow color. The intrinsic viscosity of PEG-GC, determined in 0.1 N aqueous NaCl solution at 25 °C, is 0.065 Dl/g. The obtained polyetherguanidinium chloride was converted from the salt form to basic form by reaction with an equivalent amount of alkali in ethanol. The obtained sodium chloride was removed from the reaction mixture by filtration. The product was dried and reacted with methyl iodide at a molar ratio of PEG: methyl iodide 1 : 2 in methanol at 50 °C. The final product was dried at room temperature and represented an amorphous polymer of dark yellow color. Alkyl-substituted polyetherguanidinium iodides based on oligooxyethylene glieols MW 230 and 2000 were obtained by a similar method.

Cultivation of microorganisms. Bacteria were grown on meat-peptone agar for 48 hours at 28±2 °C. Test cultures of micromycetes were cultured on agar beer wort (6 ° B), incubated for 14 days at 28±2 °C.

The antimicrobial activity of newly synthesized polyetherguanidinium iodides was determined by the standard disco-diffusion method [6]. In our study we invistigated 1 and 3% solutions of polymers in distilled water. The solutions were applied in 0.2 mL on standard paper disks with a diameter of 6 mm and placed on the surface of meat-peptone agar inoculated with the appropriate test culture of bacteria. Incubation was performed for 18 hours at 28±2 °C. Antimicrobial activity was expressed by the diameters (mm) of the zones of growth retardation of microorganisms.

Fungicidal activity of the newly synthesized polyetherguanidinium iodides was determined by the method of diffusion of the investigated polymer solution into agar of nutrient Chapek-Dox medium. Test cultures were taken from the collection of fungal cultures of the Department of Physiology and Systematics of Micromycetes of the Institute of Microbiology and Virology of NAS of Ukraine, isolated from technical, polyethylene and construction materials and stored in the Testing Laboratory of Fungal Resistance and Microbiological Research of Technical and Medical Products and Materials.

A suspension with a concentration of 1×10^6 spores of each species was prepared from a working batch of fungal cultures aged 14 to 28 days. The suspension was prepared as follows: spores from a pure culture tube were transferred to a flask containing 25±5 cm^3 of sterile water. The suspension of spores of each species was mixed, the concentration of spores was calculated using a Goryaev counting chamber. Next, 1 ml of the spore suspension was added to sterile Petri dishes and filled with Chapek-Dox warm medium for preparation of lawns of cultures by a deep method.

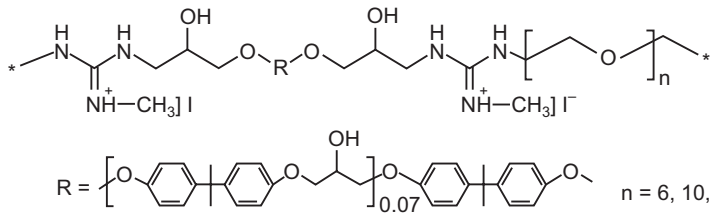
After solidification of the medium, holes were made with a sterile drill with a diameter of 5 mm, which filled 0.1 mL of the test drug. Petri dishes were kept in a thermostat at a temperature of (29±2) °C for 14 days.

Evaluation of the sensitivity of cultures isolated from technical and building materials to the studied compounds was carried out by measuring the diameter of the zone of growth retardation of micromycetes: > 25 mm – high; 25–15 mm – average; <15 mm – low; 0 mm – absent.

IR Fourier spectroscopy. The IR spectra of oligomers with Fourier transform were recorded on a TENSOR 37 spectrophotometer in the spectral region of 6000–400 cm^{-1} in KBr tablets. ^1H NMR spectra were recorded on a Varian VXR-400 MHz instrument (USA) in a $CDCI_3$ system.

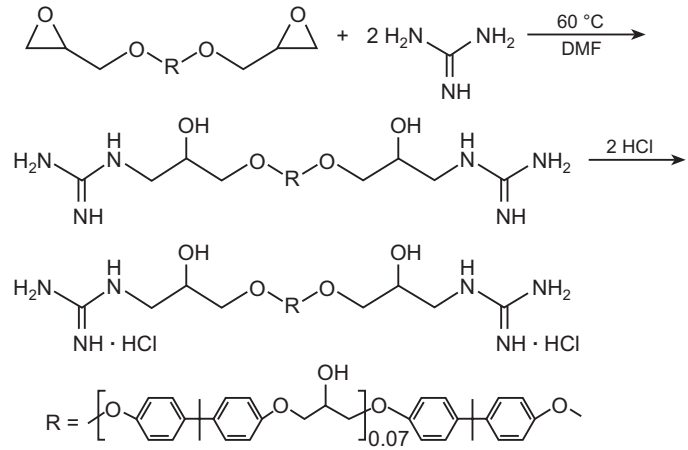
Statistical processing of the results. The experiments were performed in triplicate, the results were expressed using the standard deviation M±n. Data processing was performed using the software package Excel 2016 (MS Office) and Origin 8.5 (MS Office).

Research results. The synthesized alkyl-containing polyetherguanidinium iodides can be represented by the following structural formula:



The synthesis of alkyl-containing polyetherguanidinium iodides was carried out in four stages.

The first stage was the synthesis of guanidine-containing oligoether with terminal guanidine moieties by the reaction between aromatic oligoepoxide and guanidine



The structure of the obtained oligomer was confirmed by IR (**Table 1**) and ^1H NMR spectra (**Fig. 1**).

Table 1. **IR spectroscopy functional groups of guanidine-containing oligoether**

Таблиця 1. **Функціональні групи ІЧ-спектроскопії гуанідинзаміщеного олігоетеру**

Major functiomal group	Absorbtion frequency region, cm^{-1}
O–H	3550
N–H	3200
C–H	2949
CH_2	2896
CH_3	2868
C = C aromatic	1450, 1560,
C = N	1650
C–O–C	1100, 1250,1300
C–H	770

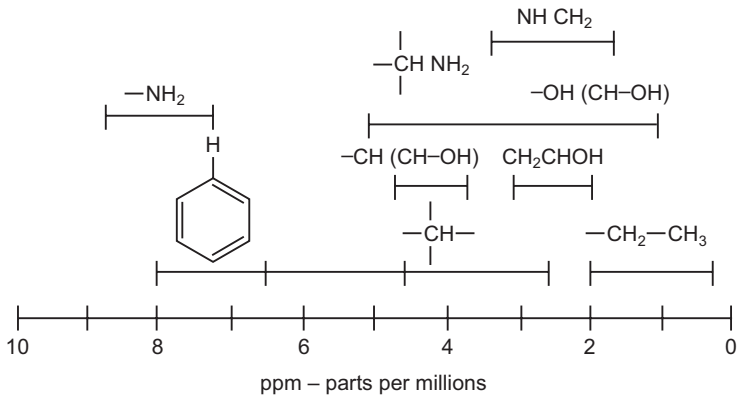


Fig. 1. Functional groups and chemical shifts in ^1H NMR spectroscopy of guanidine-containing oligoether

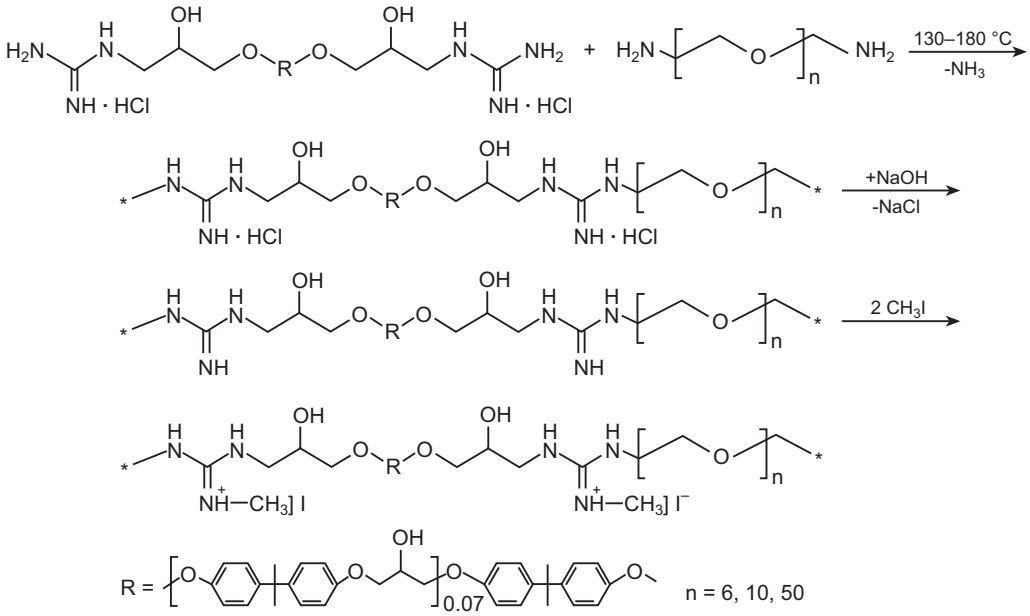
Рис. 1. Функціональні групи ЯМР-спектроскопії гуанідинзаміщеного олігоетеру

GO is a light yellow viscous liquid that is soluble in water, ethanol, methanol, methyl ethyl ketone, dimethylformamide, dimethylsulfoxide, dimethylacetamide and insoluble in diethyl ether, hexane, acetone.

The second stage is the synthesis of polyetherguanidinium chloride by the reaction between guanidinium-containing oligoether with terminal guanidine fragments and oligooxyethylenediamine of different molecular weight.

In the third stage, the obtained polyetherguanidinium chloride is converted from the salt form to the base form by an equivalent amount of alkali in ethanol.

In the fourth stage, the basic polyetherguanidine is reacted with methyl iodide at a molar ratio of PEG : methyl iodide components of 1:2.



The structure of the obtained polyetherguanidinium iodides was confirmed by IR (**Table 2**) and ^{1}H NMR spectra (**Fig. 2**).

Table 2. **IR spectroscopy functional groups of alkyl-containing polyetherguanidinium iodides**

Таблиця 2. **ІЧ-спектроскопія функціональних груп алкіловмісних поліетергуанідину йодидів**

Major functiomal group	Absorbtion frequency region, cm^{-1}
O–H	3400
N–H	3156
C–H	2900
CH_2	2880
CH_3	2860
C=C aromatic	1420, 1520,
C = N	1610
C–O–C	1120, 1250,1320
C–H	760

The obtained polymers are resinous products of dark yellow color that are soluble in water, ethanol, methanol, methyl ethyl ketone, dimethylformamide, dimethyl sulfoxide, dimethylacetamide and insoluble in diethyl ether, hexane, acetone. Data on the study of bactericidal activity of the obtained polyetherguanidinium iodides in relation to a number of Gram-positive and Gram-negative bacteria are given in **Table 3** and **Fig. 3**.

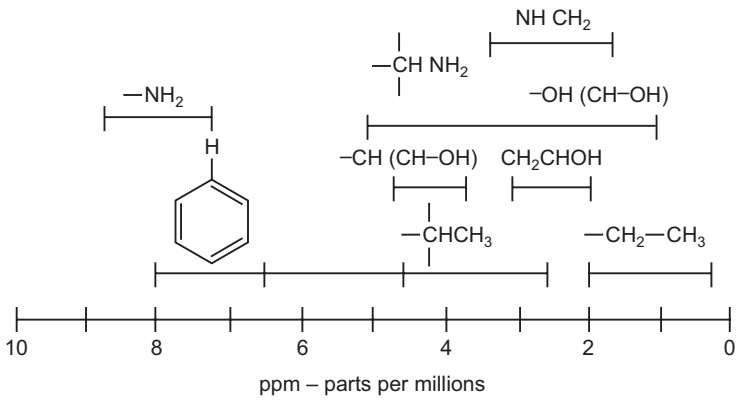


Fig. 2. Functional groups and chemical shifts in ^{1}H NMR spectroscopy of alkyl-containing polyetherguanidinium iodides

Рис. 2. Функціональні групи ^{1}H ЯМР-спектроскопії алкілозаміщених поліетергуанідиній йодидів

Table 3. **Antimicrobial activity of alkyl-substituted polyetherguanidinium iodides**

Таблиця 3. **Антимікробна активність алкілозаміщених поліетергуанідиній йодидів**

Chemical group / Bacterial strains swarm	Diameter of the zone of growth inhibition, mm					
	PEG-1, n = 6		PEG-2, n = 10		PEG-3, n = 50	
	Polymer concentration , %					
	1	3	1	3	1	3
Gram-negative bacteria						
E. coli 475	15.0±0.2	18.0±0.2	12.0±0.1	14.0±0.2	11.0±0.2	12.0±0.1
K. pneumoniae 479	14.0±0.1	16.0±0.2	10.0±0.2	12.0±0.1	9.0±0.1	11.0±0.1
P. aeruginosa 465	15.0±0.1	20.0±0.2	12.0±0.3	14.0±0.2	9.0±0.1	10.0±0.1
Gram-positive bacteria						
S. aureus 451	16.0±0.1	20.0±0.3	14.0±0.2	18.0±0.3	12.0±0.2	15.0±0.2
E. faecalis 422	18.0±0.2	20.0±0.3	15.0±0.2	15.0±0.2	12.0±0.1	12.0±0.1

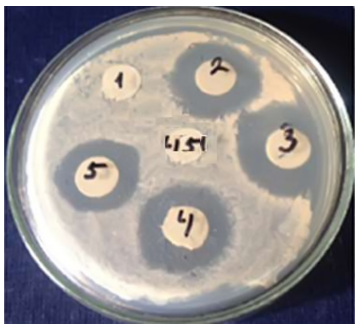


Fig. 3. Zones of growth retardation of the bacterium *S. aureus* 451 (mm) under the influence of alkyl-containing polyetherguanidinium iodide: 1 – control (distilled water); 2, 3 – PEG-1 (1 and 3%); 4, 5 – PEG-2 (1 and 3%)

Рис. 3. Зони затримки росту бактерії *S. aureus* 451 (мм) під впливом алкілозаміщеного поліетергуанідиній йодиду: 1 – контроль (дистильована вода); 2, 3 – ПЕГ-1 (1 і 3%), 4, 5 – ПЕГ-2 (1 і 3%)

According to the results of the microbiological studies, alkyl-containing polyetherguanidinium iodides show antimicrobial activity against the tested crops in concentrations

of 1–3%, which are recommended for commercial disinfectants based on salts of polyhexamethylene guanidine hydrochloride. As can be seen from table 1, with increasing polymer concentration from 1% to 3% in aqueous solution, the bactericidal activity increases. If we compare the three newly synthesized PEG-iodides, which differ in the length of the oligooxyethylene component, we can conclude that the zones of bacterial growth retardation decrease with increasing molecular weight of the oligooxyethylene component and, accordingly, the molecular weight of the polymer. For the polymer PEG-1 at a concentration of 3%, the zone of growth retardation of bacteria *E. coli* 475, *K. pneumoniae* 479, *P. aeruginosa* 465, *S. aureus* 451, and *E. faecalis* 422 is 16–20 mm.

The fungicidal activity of alkyl-containing polyetherguanidinium iodides in relation to different types of microscopic fungi is shown in **Table 4** and in **Fig. 4–7**.

Table 4. **Fungicidal activity of alkyl-containing polyetherguanidinium iodides**

Таблиця 4. **Фунгіцидна активність алкілозаміщених поліетергуанідиній йодидів**

N	Type of micromycete	Composition and concentration of the solution		
		PEG-1, n = 6	PEG-2, n = 10	PEG-3, n = 50
	Zone of growth retardation of fungi, mm			
1	*C. cladosporioides* F-41252	20.0±2.3	12.0±0.3	10.0±0.3
2	*A. strictum* F-16703	12.0±1.3	15.0±0.7	20.0±1.3
3	*A. alternate* F-41225	15.0±1.7	10.0±1.3	21.0±1.7
4	*A. niger* F-73001	10.0±0.1	0.0	0.0
5	*A. versicolor* F-41250	15.0±1.3	10.0±0.3	0.0
6	*C. sphaerospermum* F-41255	15.0±1.3	15.0±1.3	15.0±1.7
7	*P. variotii* F-16724	20.0 ±2.3	15.0±1.7	18.0±1.3
8	*P. chrysogenum* F-16719	10.0±0.1	13.0±1.3	11.0±0.1
9	*P. funiculosum* F-16728	10.0±1.3	20.0±1.7	10.0±0.3
10	*S. chartarum* F-41212	20.0±2.3	18.0±1.7	20.0±1.7

PEG-1 Control (distilled water)

Fig. 4. Zones of growth retardation of *Paecilomyces variotii* (mm) under the influence of alkyl-containing polyetherguanidinium iodide PEG-1 (triplicate of the same fungus strain – three repetitions)

Рис. 4. Зони затримки росту гриба *Paecilomyces variotii* (мм) за впливу алкілозаміщеного поліетергуанідиній йодиду ПЕГ-1 (три экземпляри того самого штаму гриба – три повтори)

PEG-3 Control (distilled water)

Fig. 5. Zones of growth retardation of *Cladosporium shaerospermum* (mm) under the influence of alkyl-containing polyetherguanidinium iodide PEG-3 (triplicate of the same fungus strain – three repetitions)

Рис. 5. Зони затримки росту гриба C*ladosporium shaerospermum* (мм) за впливу алкілозаміщеного поліетергуанідиній йодиду ПЕГ-3 (три экземпляри того самого штаму гриба – три повтори)

PEG-1 Control (distilled water)

Fig. 6. Zones of growth retardation of *Aspergillus versicolor* (mm) under the influence of alkyl-containing polyetherguanidinium iodide PEG-1 (triplicate of the same fungus strain – three repetitions)

Рис. 6. Зони затримки росту гриба *Aspergillus versicolor* (мм) за впливу алкілозаміщеного поліетергуанідиній йодиду ПЕГ-1 (три экземпляри того самого штаму гриба – три повтори)

PEG-2 Control (distilled water)

Fig. 7. Zones of growth retardation of *Acremonium strictum* (mm) under the influence of alkyl-containing polyetherguanidinium iodide PEG-2 (triplicate of the same fungus strain – three repetitions)

Рис. 7. Зони затримки росту гриба *Acremonium strictum* (мм) за впливу алкілозаміщеного поліетергуанідиній йодиду ПЕГ-2 (три екземпляри того самого штаму гриба – три повтори)

The fungicidal activity of alkyl-containing polyetherguanidinium iodides at a concentration of 1% was determined with respect to micromicety isolates, which dominated or often occurred in Kyiv and could pose a significant threat to human health.

According to the obtained data, alkyl-containing polyetherguanidinium iodides at a concentration of 1% showed fungicidal activity against almost all studied isolates after 14 days. With increasing length of the oligoethylene oxide component, the obtained polymers PEG-2 and PEG-3 did not show a fungicidal effect against *A. niger*, and PEG-3 against the micromycete *A. versicolor*. If we determine the fungicidal effect as a whole, the polymer PEG-1 showed the highest activity against all studied isolates after 14 days, while the fungicidal effect of PEG-2 and PEG-3 decreased. All synthesized alkyl-containing polyetherguanidinium iodides showed the greatest activity against *C. cladosporioides*, *A. strictum*, *A. alternata*, *C. sphaerospermum, P. variotii, S. chartarum.* Noteworthy is the selective effect of the obtained polymer PEG-2 on the isolate *P. funiculosum* (diameter of growth retardation more than 20 mm), as well as of PEG-3 on isolates *A. strictum*, *A. alternata* (diameter of growth retardation more than 20 and 21 mm, respectively).

DISCUSSION

The synthesized alkyl-containing polyetherguanidinium iodides showed rather high zones of growth retardation of Gram-negative bacteria *E. coli* 475 and *K. pneumoniae* 479 and lower for the Gram-positive bacterium *S. aureus* 451. It can be assumed that the mechanism of biocidal action of alkyl-containing polyetherguanidinium iodides is similar to that of polyguanidines and has a membrane toxic nature [1, 21]. It is known that the biocidal activity of polyguanidines is due to the cooperative interaction of neighboring guanidine groups of the polycation with the microbial cell and is also influenced by the structure of the guanidine group [7, 10] in contrast to the cation of quaternary ammonium compounds [13, 15] where the positive charge is distributed between three nitrogen atoms. Delocalization of the positive charge softens the action of the biocide and reduces its toxicity.

The fungicidal effect of alkyl-containing polyetherguanidinium iodides is probably due to the presence of guanidine moieties and a diphenylolpropane group. It is known that in the molecules of organic substances under the influence of different atoms or atomic groups present in them there is a redistribution of the electronic density of chemical bonds (positive or negative induction effect) [2, 3, 9].

The presence of a diphenylolpropane group in the polymer molecule causes a negative induction effect – the substituent reduces the electron density on the carbon atom to which it is attached. In this case, the substituent acquires a partial negative charge (δ-), and the carbon atom acquires a partial positive charge (δ+) [10]. According to the literature, the value (δ+) may be one of the factors that enhances the interaction of fungicidal substances with the cell wall of fungi [19].

The data above show that with increasing length of the oligoethylene oxide component in the molecules of alkyl-containing polyetherguanidinium iodides, the fungicidal activity generally decreases. This is probably due to a decrease in the concentration of guanidine and diphenylolpropane groups in the polymer chain that promote the fungicidal action.

The obtained data indicate the selectivity of the fungicidal action of solutions on different species of microscopic fungi, which may be due to differences in their metabolic processes and mechanisms of adaptation. Since we observed a change in the color of micromycete colonies under the influence of the studied solutions, we can assume that the main mechanism of resistance is the formation of pigments.

CONCLUSIONS

The obtained alkyl-containing polyetherguanidinium iodides at a concentration of 1 and 3% showed bactericidal activity against *S. aureus, E. coli. K. pneumoniae* and fungicidal effect at a concentration of 1% against all fungi under study and can be used as disinfectants for indoor treatment and protection against fungal and bacterial infections.

COMPLIANCE WITH ETHICAL STANDARDS

Human Rights: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

1. *Asyamova A.V., Gerunov V.I.* Guanidine derivatives in medicine and agriculture. **Vestnik Omskogo gosudarstvennogo agrarnogo universiteta**, 2017; 4(28): 130–135. (In Russian) Google Scholar
2. *Baibakova E.V., Nefedjeva E.E., Suska-Malawska M., Wilk M., Sevriukova G.A., Zheltobriukhov V.F.* Modern Fungicides: Mechanisms of Action, Fungal Resistance and Phytotoxic Effects. **Annual Research & Review in Biology**, 2019, 32(3): 1–16. Crossref • Google Scholar
3. *Brauer V.S., Rezende C.P., Pessoni A.M., De Paula R.G., Rangappa K.S., Nayaka S.C., Gupta V.K., Almeida F.* Antifungal Agents in Agriculture: Friends and Foes of Public Health. **Biomolecules**, 2019, 9(10): 521. Crossref • PubMed • Google Scholar
4. *Chuienko A.I., Vortman M.Y., Shevchenko V.V.* Fungicidal activity of the elderly guanidines, promising until stagnant in the humane industry. **Microbiology and biotechnology**, 2013; 22(2): 97–106. Crossref
5. *Gembitskiy P.A., Vointseva I.I.* Polymer biocidal preparation polyhexamethylene guanidine. Zaporozhye: Polygraph, 1998. 44 p. (In Russian)
6. *Gembitsky P.A.* Guanidine. Chemical encyclopedia: in 5 vol. Moscow: Soviet encyclopedia, Vol. 1, 1988; 617 p. (In Russian)
7. *Gembitsky P.A., Koryavov Ya.I., Erusalimsky P.M.* About the synthesis of poly (alkylene guanidines) and poly (alkylene biguanidines). **Applied Chemistry Journal**, 1975; 48: 1933–1938. (In Russian)
8. *Gorobets A.V., Vakulyuk P.V., Furtat I.M., Vortman M.Ya.* Bactericidal fluorine-containing membranes formed in the presence of guanidine-containing low molecular weight polymers. Scientific Notes. **Chemical Sciences and Technologies**, 2009, 92: 48–52
9. *Jochen Z., Bundschuh P., Arts M., Brühl G., Carsten A. Knäbel G., Payraudeau A., Rasmussen S., Rohr J., Scharmüller J., Smalling A., Stehle K., Schulz S., Schäfer R., Ralf B.* Fungicides: An Overlooked Pesticide Class? **Environ. Sci. Technol**, 2019, 53(7): 3347–3365. Crossref • PubMed • Google Scholar
10. *Jung H.N., Zerin T., Podder B., Song H.Y., Kim Y.S.* Cytotoxicity and gene expression profiling of polyhexamethylene guanidine hydrochloride in human alveolar A549 cells. **Toxicology in Vitro**. 2014; 28(4): 684–692. Crossref • PubMed • Google Scholar
11. *Kim J-Y., Kim H.H., Cho K.H.* Acute cardiovascular toxicity of sterilizers, PHMG, and PGH: severe inflammation in human cells and heart failure in zebrafish. **Cardiovasc Toxicol**, 2013; 13: 148–160. Crossref • PubMed • Google Scholar
12. *Kitamaki R., Shirai K., Sugino K.* Preparation and Properties of Polyhexamethyleneguanidine. **Bull. Chem. Soc. Japan.**, 1968; 41(6): 1461–1463. Crossref • Google Scholar

13. *Mulder I., Siemens J., Sentek V., Amelung W., Smalla K., Jechalke S.* Quaternary ammonium compounds in soil: implications for antibiotic resistance development. **Rev Environ Sci Biotechnol**, 2018; 17: 159–185.
Crossref • Google Scholar
14. *Oulè M.K., Azinwi R., Bernier A.M., Kablan T., Maupertuis A.M., Mauler S.* Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant *Staphylococcus aureus* and nosocomial infections. **J. Med. Microbiol**., 2008; 57: 1523–1528.
Crossref • PubMed • Google Scholar
15. *Rajkowska K., Koziróg A., Otlewska A., Piotrowska M., Nowicka-Krawczyk P., Brycki B., Kunicka-Styczyńska A., Gutarowska B.* Quaternary ammonium biocides as antimicrobial agents protecting historical wood and brick. **Acta Biochim Pol**, 2016; 63(1): 153–159.
Crossref • PubMed • Google Scholar
16. *Stelmakh S.A., Bazaron L.U., Grigorieva M.N.* Hydrogels based on polyhexamethylene guanidine hydrochloride. **Bulletin of the Buryat State University. Chemistry, physics**, 2010; 3: 80–82. (In Russian)
Google Scholar
17. *Svetlov Ye.S. Erofeev V.T, Morozov E.A.* Biocidal preparations based on polyhexamethylene guanidine derivatives. Biodamage and biocorrosion in construction: materials of the Second Intern. scientific and technical conf., 2006: 270–273
18. *Vakulyuk P.V, Vortman M.Ya. , Furtat I.M, Burban A.F.* Track polyethylene terephthalate membranes with antibacterial properties. **Polymer Journal**, 2008, 30(1): 46–51.
19. *Vointseva I.I.* Polyhexamethylene guanidine hydrochloride for water purification and disinfection as an alternative to oxidizing reagents. Part 1. **Water: Chemistry and Ecology**, 2011; 7: 39–45. (In Russian)
20. *Vointseva I.I., Gembitsky P.A.* **Polyguanidine disinfectants and multifunctional additives in composite materials**. Moscow: Paint and varnish industry, 2009, 303 p. (In Russian)
21. *Wang J., Yu Y., Dong Y.* Disinfection of Ready-to-Eat Lettuce Using Polyhexamethylene Guanidine Hydrochloride. **Microorganisms**, 2020; 8(2): 272.
Crossref • PubMed • Google Scholar

ФУНГІЦИДНА І БАКТЕРИЦИДНА АКТИВНІСТЬ АЛКІЛОЗАМІЩЕНИХ ПОЛІЕТЕРГУАНІДИНІВ

***М. Я. Вортман*[1], *Ю. Б. Письменна*[2], *А. В. Руденко*[3], *В. В. Третяк*[3], *В. М. Лемешко*[1], *В. В. Шевченко*[1]**

[1] *Інститут хімії високомолекулярних сполук НАН України Харківське шосе, 48, Київ 02160, Україна*

[2] *Інститут мікробіології і вірусології ім. Д. М. Заболотного НАН України вул. Заболотного, 154, Київ МСП03680, Україна*

[3] *Інститут урології НАМН України, вул. Володимира Винниченка, 9А, Київ 04053, Україна*

**Кореспондуючий автор: e-mail: vmar1962@i.ua*

Вступ. Полігексаметиленгуанідини широко використовують як біоциди та дезинфектанти завдяки широкому спектру їхньої антимікробної активності щодо грампозитивних і грамнегативних бактерій, вірусів, грибів, цвілі. Механізм біоцидної дії полігуанідинів подібний до механізму четвертинних амонієвих сполук і має мембранотоксичний ефект. До переваг солей полігексаметилгуанідинів належать їхня помірна токсичність і відсутність кумулятивної дії щодо живих організмів (4 клас небезпеки за ГОСТом 12.1.007-76). Зручність застосування таких полімерних біоцидів полягає у їхній високій розчинності у воді й у відсутності леткості, що дає

змогу проводити дезинфікувальні роботи за присутності людей. Відомо, що введення у ланцюг алкільних радикалів підвищує бактерицидну та фунгіцидну дії отриманих сполук. Для посилення цих властивостей перспективним є отримання поліетергуанідинів з алкільними радикалами у їхньому складі. Мета роботи – дослідити фунгіцидну та бактерицидну активності синтезованих алкілозамісних поліетергуаніdиній йодидів щодо деяких бактерій і мікроскопічних грибів.

Методи. Бактерії вирощували на м'ясо-пептонному агарі протягом 48 год за температури 28±2 °С. Тест-культури мікроміцетів культивували на агаризованому пивному суслі (6°Б), інкубували 14 діб за температури 28±2 °С. Антимікробну та фунгіцидну активність новосинтезованих алкілозамісних гуанідиновмісних олігомерів визначали стандартним диско-дифузійним методом.

Результати. Синтез поліетергуанідиній йодидів проводять у чотири стадії. Перша стадія полягає у синтезі гуанідиновмісного олігоетеру з кінцевими гуанідиновими фрагментами в реакції між ароматичним олігоепоксидом і гуанідином. Друга стадія – синтез поліетергуанідиній хлориду в реакції між гуанідиновмісним олігоетером із кінцевими гуанідиновими фрагментами й олігооксіетилендіаміном різної молекулярної маси. На третій стадії отриманий поліетергуанідиній хлорид переводять зі сольової форми в основну завдяки обробці еквівалентною кількістю лугу, розчиненого в етанолі. На четвертій стадії проводять реакцію основного поліетергуанідину з йодистим метилом за мольного співвідношення компонентів ПЕГ : йодистий метил 1 : 2. Виявлено бактерицидну та фунгіцидну активності алкіловмісних поліетергуанідиній йодидів щодо різних гетеротрофних бактерій і мікроскопічних грибів. Встановлено, що поліетергуанідиній йодиди в концентраціях 1–3% інгібували ріст грамнегативних (*Escherichia coli* 475, *Klebsiella pneumonia* 479) і грампозитивних (*Staphylococcus aureus* 451) бактерій. Отримані алкіловмісні поліетергуанідиній йодиди у концентрації 1% за 14 діб проявляли фунгіцидну активність майже щодо всіх досліджених ізолятів. Зі зростанням довжини олігоетиленоксидної складової отримані полімери з n = 10 та 50 не проявляли фунгіцидного ефекту щодо *Aspergillus niger*, а полімер з n = 50 і щодо мікроміцету *Aspergillus versicolor*. Якщо визначати фунгіциднмй ефект загалом, то полімер ПЕГ-1 має найвищу активність за 14 діб щодо всіх досліджених ізолятів, для ПЕГ-2 та ПЕГ-3 фунгіцидний ефект зменшується. Найбільшу активність усі синтезовані алкіловмісні поліетергуанідиній йодиди проявляють до таких мікроскопічних грибів: *Cladosporium cladosporioides*, *Acremonium strictum*, *Alternaria alternate*, *Cladosporium sphaerospermum*, *Paecilomyces variotii*, *Stachybotrys chartarum*. Проте варто відмітити вибіркову дію отриманих полімерів щодо окремих ізолятів.

Висновки. Отримані поліетергуанідиній йодиди різної молекулярної маси у концентрації 1–3% проявляли бактерицидну активність проти *Staphylococcus aureus* 451, *Escherichia coli* 475, *Klebsiella pneumonia* 479 і фунгіцидний ефект у концентрації 1% до всіх досліджених нами грибів і можуть бути використані як дезинфіканти для обробки приміщень.

Ключові слова: поліетергуанідиній йодиди, бактерицидна активність, фунгіцидна активність

Received / Одержано 06 August 2020 | Accepted / Прийнято 31 August 2020 | Published / Опубліковано 30 September 2020