ANTIBACTERIAL AND FUNGICIDAL ACTIVITY OF GUANIDINIUM OLIGOMERS

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Guanidinium oligomers are a poorly studied class of organic compounds and attract attention due to their antimicrobial properties. Strengthening the antimicrobial properties and simplifying and reducing the cost of the synthesis of these compounds is promising for obtaining functional guanidine-containing oligomers with alkyl radicals of different lengths in their composition. **The aim of this work** is to study the bactericidal and fungicidal activities of newly synthesized oligomeric guanidinium bromides with alkyl radicals of various lengths. **Methods.** The synthesis of tetraalkyl-substituted guanidine-containing oligomers with an aromatic and aliphatic oligoether component was carried out by the reaction of guanidine-containing oligomers with terminal guanidine fragments and alkyl bromides ($\text{Alk} = -\text{C}_3\text{H}_7$, $-\text{C}_7\text{H}_{15}$, $-\text{C}_{10}\text{H}_{21}$) at a molar ratio (1:4) of components. Different types of microorganisms (clinical isolates, gram-positive and gram-negative bacteria, microscopic fungi) were used as test cultures to determine the biocidal activity of the obtained compounds. The bacteria were grown on meat-peptone agar for 48 hours, micromycetes – on beer wort agar (6 °B) for 14 days. The hydrocarbon-oxidizing bacteria and micromycetes were incubated at a temperature of 28 ± 2 °C, and clinical bacterial isolates – at a temperature of 37 ± 2 °C. Antimicrobial activity of oligomers was determined by the standard disco-diffusion method, and fungicidal – by the method of wells in agar. **Results.** Tetraalkyl-substituted guanidinium bromide oligomers with various radicals ($-\text{C}_3\text{H}_7$, $-\text{C}_7\text{H}_{15}$, $-\text{C}_{10}\text{H}_{21}$) were obtained and their bactericidal and fungicidal activity against various groups of microorganisms was shown. It was found that the obtained oligomers at a concentration of 1–3 % in aqueous solution inhibited the growth of gram-negative and gram-positive bacteria. Antimicrobial and fungicidal properties depended on the length of the alkyl radical, and as its length increased, the diameter of growth inhibition zones of bacteria and micromycetes were increased. For 3 % solutions of tetraalkyl-substituted guanidine oligomer with aromatic oligoepoxide ($\text{Alk} = \text{C}_{10}\text{H}_{21}$), the growth inhibition zones of bacteria were 18–21 mm. The bactericidal effect of oligomer based on aromatic oligoepoxide with alkyl radicals $\text{Alk} = \text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$ was 20–25 % higher than that for variants with aliphatic oligoepoxide. All the tetraalkyl-substituted ($\text{Alk} = \text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$) guanidine-containing oligomers at a concentration of 1 % solution showed fungicidal activity to almost all micromycetes, the growth inhibition zones for microscopic fungi on the 7th day were 7–20 mm. The largest growth inhibition zones of micromycetes (in the range 15–20 mm) were observed for oligomers with aromatic oligoepoxide and radicals $\text{Alk} = \text{C}_{10}\text{H}_{21}$ and C_7H_{15} and aliphatic oligoepoxide with radical $\text{Alk} = \text{C}_{10}\text{H}_{21}$ (in the range 15–20 mm). **Conclusions.** The length of the alkyl radical and the nature of the oligoether component affected the bactericidal and fungicidal properties of newly synthesized oligomers. With an increase of the length of the alkyl radical of guanidine-containing oligomers, their bactericidal and fungicidal properties increase, tetraalkyl-containing oligomers are promising for use as disinfectants for indoor treatment and as additives in polymer compositions to protect them from bio-damage.

Keywords: tetraalkyl-substituted guanidine-containing oligomers, heterotrophic bacteria, clinical isolates, micromycetes, bactericidal and fungicidal activity.

Organic nitrogen-containing compounds are widely used as antimicrobial agents. Among them, quaternary ammonium compounds are the most common and effective. The biological effect of quaternary ammonium salts is that they disrupt the structure of cell membranes and cause denaturation of cellular proteins. These same substances can reduce the activity of key enzymes of cellular metabolism [1–4]. Along with them, guanidine derivatives are known as nitrogen-containing compounds that are part of disinfectants. Derivatives such as arginine and creatinine are of biological value to the animal and human bodies. They are also widely used as antiseptics, insecticides, medicines, and preservatives [1–4]. In particular, some of the known medicinal substances based on guanidine are biguanidins, sulfaguanidine and chlorhexidine.

Among polymer derivatives of guanidine, polyhexamethylene guanidinium chloride (PGMG) has received the greatest practical use due to its biocidal properties [5–8]. It is synthesized by polycondensation of hexamethylenediamine and guanidinium chloride. The mechanism of biocidal action of polyguanidins is similar to quaternary ammonium compounds and has a membrane-toxic character: guanidine polycations are adsorbed on the negatively charged surface of bacterial cells, diffuse through the cell wall, and bind to acid phospholipids, proteins of the cytoplasmic membrane, which leads to its rupture and death of the microbial cell [9–13]. The increase in the biocidal activity of PGMG compared to low-molecular-weight biocides is due to the cooperative interaction of neighboring chains of guanidine groups of the polycation with the microbial cell. In comparison with quaternary ammonium salts, the increase in the biocidal activity of polyguanidins is also due to the peculiarities of the structure of the guanidine grouping: in the guanidine cation, the positive charge is delocalized and distributed among three nitrogen atoms, as well as additionally delocalized according to the σ -bond system. Delocalization of the positive charge softens the effect of the biocide and reduces its toxicity. The macromolecular nature of the reagent causes intramolecular interaction of macromolecule fragments removed along the chain, conformational transformations of polymer chains, and changes in the complementarity of the polycation and protein macromolecules of the bacterial cell, which contributes to further delocalization of the positive charge and reduces the toxicity of the compound. It is known that guanidinium polymers have lower

toxicity compared to guanidine and belong to the fourth hazard class [14–19].

Of undoubted interest from the point of view of bactericidal properties is the previously practically unexplored class of guanidinium oligomers. It can be assumed that by analogy with ammonium derivatives of organic compounds and polyguanidins, guanidinium oligomers will exhibit bactericidal activity against both gram-positive and gram-negative bacteria and fungicidal properties against microscopic fungi.

Previously, we investigated the fungicidal and bactericidal activity of dialkyl-substituted guanidine-containing oligomers based on aromatic oligoepoxide against a number of gram-positive and gram-negative bacteria and microscopic fungi. It was found that synthesized dialkyl-substituted guanidine-containing oligomers at a concentration of 1–3 % inhibited the growth of most of the studied bacteria and micromycetes [20]. It is known, that the introduction of alkyl radicals of different lengths into the carbon chain leads to increasing of the bactericidal and fungicidal effects of the obtained compounds [6–8]. To enhance these properties, it seems promising to obtain reactive functional tetraalkyl-substituted guanidine-containing oligomers with alkyl radicals of different lengths in their composition.

The aim of this work is to study the bactericidal and fungicidal activities of our newly synthesized oligomeric tetraalkyl-substituted guanidinium bromides with radicals of different carbon chain lengths.

Materials and methods

The objects of study were bacterial gram-positive strains of *Staphylococcus aureus* 451, *Enterococcus faecalis* 422, gram-negative strains of *Escherichia coli* 475, *Pseudomonas aeruginosa* 465, and *Klebsiella pneumonia* 479, isolated from pathogenic material (urogenital system) of sick people and stored in the Laboratory of Bacteriological Research of the Institute of Urology of the National Academy of Medical Sciences of Ukraine. Gram-positive bacterial strain *Pseudomonas pseudoalcaligenes* 109 and gram-negative bacteria *Rhodococcus erythropolis* 102 and *Bacillus subtilis* 138 were isolated and identified by us earlier from damaged protective coatings of gas pipelines and deposited in the collection of the Department of General and Soil Microbiology of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine [21].

Microscopic fungi *Cladosporium cladosporioides* 41450, *Cladosporium sphaerospermum* 41451, *Penicillium funiculosum* 41452, *Paecilomyces varioti* 41453, *Penicillium chrysogenum* 41454, *Aspergillus niger* 41455, *Aspergillus versicolor* 41456, *Alternaria alternate* 41457, *Stachybotrys chartarum* 41458 and *Acremonium strictum* 41459 were isolated from the premises of Kyiv, which were affected by Mycological damage and stored in the collection of the Department of Micromycete Physiology and Systematization of Micromycetes of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine [23].

Cultivation of microorganisms. Strains of hydrocarbon-oxidizing bacteria were grown on meat peptone agar medium during 48 hours at a temperature of 28 ± 2 °C and clinical bacterial isolates at a temperature of 37 ± 2 °C. Test cultures of micromycetes were cultured on beer wort agar medium (6 °B) for 14 days at a temperature of 28 ± 2 °C.

Synthesis of oligomers. Diane epoxy oligomer DER-331 (DOW Chemical Company, Germany), MM 365 g/mol, mass fraction of epoxy groups 23.5 %, hydroxyl groups 0.6 %, aliphatic epoxy oligomers DEG-1 (LLC "Special contract") mass fraction of epoxy groups 28.6 %, hydroxyl groups 1.3 % were dehydrated by heating in vacuum for 2–6 hours at 80–90 °C and the final

pressure 2 mmHg. Guanidine hydrochloride (GH) ("Aldrich", degree of purity 99.9 %) was used without additional purification. Follow alkyl bromides as propyl- ($-C_3H_7$), heptyl- ($-C_7H_{15}$), and decyl- ($-C_{10}H_{21}$) bromides ("Aldrich", degree of purity 99.9 %) were used without additional purification. Methanol and dimethylformamide was purified by distillation. The synthesis of tetraalkyl-substituted guanidine-containing oligomers was carried out in two stages. At the first stage, guanidine-containing oligomers with terminal guanidine fragments were obtained by the reaction of guanidine, previously converted by alkali from the salt form to the base one, and aromatic DER-331 or aliphatic DEG-1 oligoepoxide in dimethylformamide at a temperature of 70 °C for 4 hours and a molar ratio of components of 2:1. At the second stage, a reaction was performed between guanidine-containing oligomers and alkyl bromides ($Alk = -C_3H_7$, $-C_7H_{15}$, $-C_{10}H_{21}$) in methanol at a temperature of 50 °C for 2–3 hours and a molar ratio of components of 1:4. The resulting products were redefined from methanol to diethyl ether. To remove solvent residues, the product was kept in vacuum at a temperature of 60 °C for 5 hours. The yield of products was 93–95 %. Consequently the following tetraalkyl-substituted guanidine-containing oligomers were obtained:

Tetraalkyl-substituted guanidine-containing
oligomers

based on aromatic oligoepoxide

based on aliphatic oligoepoxide

Determination of antimicrobial activity. Bactericidal activity was determined by the standard disco-diffusion method. Disks with deposited aqueous solutions of newly synthesized oligomers at concentrations of 1 and 3 % were used. An oligomer solution (0.2 mL – 2 mg for 1 % solution and 6 mg for 3 % solution) was applied to standard paper disks with a diameter 6 mm, which were placed on the surface of meat-peptone agar inoculated with a bacterial test-culture. Incubation was performed for 48 hours at 37 ± 2 °C for clinical

isolates and at 28 ± 2 °C for carbohydrate-oxidizing bacteria, antimicrobial activity was expressed as the diameter (mm) of the microbial growth inhibition zone. Fungicidal activity was determined by the well's method in agar. Test-cultures of microscopic fungi were grown on beer wort agar (6 °B) for 14 days at 28 ± 2 °C. A suspension of each type of fungi strain was prepared in sterile distilled water with a concentration of $1 \cdot 10^6$ CFU. The prepared suspension in volume 1 mL were placed into a Petri dish with 20 mL of Chapek-Dox molten medium and thoroughly mixed. 0.2 mL of the test newly synthesized oligomers was added to sterile wells with a diameter of 5 mm. Fungicidal activity was expressed in mm of the diameter of the microbial growth inhibition zone. The diameter of the growth inhibition zone of micromycetes was measured from the end of the wells edge.

The results were obtained on the 7th day of experiment.

Fourier transform IR spectroscopy. Infrared spectra with Fourier transformation of oligomers were recorded on “TENSOR 37” spectrophotometer (“Brukeroptik” GmbH, Germany) in the spectral region 6000–400 cm⁻¹ in KBr tablets.

¹H NMR spectra were recorded on “Varian VXR-400 MHz” (USA) device in CDCl₃ solution.

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

Results. The scheme for alkyl-substituted guanidinium oligomers obtaining can be presented as follows:

The completeness of the reaction was monitored by IR spectroscopy (Fig. 1). IR spectra showed such groups as: 3200–3550 cm⁻¹ – valence bands of -OH (hydroxyl) and -NH (amino) groups; 2869 cm⁻¹, 2926 cm⁻¹, 2964 cm⁻¹ – absorption bands of -CH, -CH₂ and -CH₃ groups, respectively; 1460 cm⁻¹ – deformation vibrations of the -C–H bonds; 1640 cm⁻¹ – valence vibrations of -C=N guanidine fragments and deformation vibrations of -NH groups. 1450–1650 cm⁻¹ – absorption bands of -C=C- bonds of the benzene ring for an oligomer with aromatic oligoepoxide; 1640 cm⁻¹ – deformation vibrations of -NH groups, 1100–

1300 cm⁻¹ – vibrations of -C–O–C- bonds of ether groups.

The structure of the obtained oligomers was confirmed by ¹H NMR spectrometry (Fig. 2). In the ¹H NMR (CDCl₃) spectrum of tetraalkyl-substituted guanidine-containing oligomers, proton signals are present at 1.72 ppm (t, 3H, -CH₃), 2.73 ppm-NH (-NH-CH₂), 2.58 ppm -CH₂ (CH₂CHOH), 3.58 ppm -OH (CH-OH), and 3.96 ppm -CH (CH-OH), 6.8 ppm and 7.2 ppm – benzene ring for an oligomer based on aromatic oligoepoxide, 7.8 ppm and 8.2 ppm -NH (NH₂) group.

Fig. 1. IR spectrum of tetraalkyl-substituted oligomers: 1 – with aromatic oligoepoxide; 2 – with aliphatic oligoepoxide (Alk= C₁₀H₂₁)

The results of the bactericidal activity studies of the obtained synthesized tetraalkyl-substituted guanidine-containing oligomers were showed antimicrobial activity against range of gram-positive and gram-negative bacteria (see Table 1 and Fig. 3).

On the Fig. 3 growth inhibition zones of *R. erythropolis* 102 (I) and *B. subtilis* 138 (II) under the action of tetraalkyl-substituted guanidine-containing oligomers with aliphatic and aromatic oligoepoxides and the radical $\text{Alk}=\text{C}_{10}\text{H}_{21}$ is shown.

As we see from data in Table 1, tetraalkyl-substituted guanidine-containing oligomers based on aromatic oligoepoxide showed antimicrobial activity against the studied test-cultures in the concentration range of 1–3 %. Activity depends on the length of the alkyl radical, and the diameter of the bacterial growth inhibition zones increases

with length increasing. Large values of the growth inhibition zones (18–20 mm) of *E. coli* 475, *K. pneumonia* 479, *P. aeruginosa* 465 and *S. aureus* 451 was observed for 3 % solutions of tetraalkyl-substituted guanidine oligomer based on aromatic oligoepoxide with $\text{Alk}=\text{C}_{10}\text{H}_{21}$. Inhibition of the growth of soil bacteria *R. erythropolis* 102 (17 mm) and *B. subtilis* 138 (21 mm) was observed under the action of oligoether guanidinium tetradecyl bromide ($\text{Alk}=\text{C}_{10}\text{H}_{21}\text{Br}$) at a concentration of 3 %.

Tetraalkyl-substituted guanidine-containing oligomers based on aliphatic oligoepoxide exhibit antimicrobial activity against the studied test cultures in the range of concentrations of 1–3 %, which also depend on the length of the alkyl radical, and the diameter of the bacterial growth inhibition zones increases with length increasing. Growth inhibition zones for *E. coli*, *K. pneumoniae*,

Fig. 2. ^1H NMR spectrum of tetraalkyl-substituted oligomers: 1 – with aromatic oligoepoxide; 2 – with aliphatic oligoepoxide ($\text{Alk}=\text{C}_{10}\text{H}_{21}$)

Fig. 3. Growth inhibition zones of *R. erythropolis* 102 (I) and *B. subtilis* 138 (II) under the action of 3 % solutions of tetraalkyl-substituted guanidine-containing oligomers with a radical ($\text{Alk}=\text{C}_{10}\text{H}_{21}$): K – control (distilled water); I – 1, 2 – solution of oligomer with aliphatic oligoepoxide; 3, 4 – solution of oligomer with aromatic oligoepoxide; II – 1, 2 – solution of oligomer with aromatic oligoepoxide; 3, 4 – solution of oligomer with aliphatic oligoepoxide.

Table 1

Antimicrobial activity of tetraalkyl-substituted guanidine-containing oligomers, diameter of the growth inhibition zone (mm) (disco-diffusion method)

Oligomer	Oligomer concentration, %											
	Aromatic						Aliphatic					
	Alk=C ₃ H ₇			Alk=C ₇ H ₁₅			Alk=C ₃ H ₇			Alk=C ₇ H ₁₅		
Bacterial strains	1	3	1	3	1	3	1	3	1	3	1	3
	Gram-negative bacteria											
<i>E. coli</i> 475	10±0.09	11±0.12	10±0.12	15±0.14	13±0.14	18±0.13	8±0.08	9±0.08	12±0.14	13±0.16	11±0.11	14±0.14
<i>P. aeruginosa</i> 465	8±0.07	10±0.11	9±0.11	14±0.16	12±0.10	19±0.18	9±0.12	9±0.12	9±0.06	9±0.11	9±0.08	12±0.10
<i>K. pneumoniae</i> 479	9±0.10	9±0.08	11±0.13	17±0.15	14±0.17	20±0.27	10±0.10	10±0.10	11±0.08	11±0.13	11±0.14	13±0.12
<i>P. pseudocaligenes</i> 109	–	–	–	–	–	–	–	–	22±0.18	24±0.23	20±0.22	22±0.24
	Gram-positive bacteria											
<i>S. aureus</i> 451	11±0.11	13±0.15	16±0.17	19±0.18	17±0.20	22±0.22	14±0.16	14±0.12	16±0.14	18±0.17	16±0.17	18±0.17
<i>E. faecalis</i> 422	10±0.07	12±0.16	10±0.09	15±0.15	13±0.14	16±0.15	12±0.13	12±0.09	14±0.17	14±0.10	13±0.13	15±0.15
<i>R. erythropolis</i> 102	11±0.10	12±0.12	12±0.10	14±0.20	15±0.12	17±0.20	9±0.11	10±0.13	10±0.10	12±0.13	13±0.11	18±0.19
<i>B. subtilis</i> 138	14±0.13	15±0.14	15±0.20	17±0.16	19±0.19	21±0.22	18±0.17	20±0.21	20±0.18	25±0.19	25±0.26	26±0.22

Legend: growth inhibition diameter 0–10 mm (highlighted in pink) – no sensitivity was detected, 11–25 mm (highlighted in light green) – sensitivity to the oligomer, above 25 mm – high sensitivity (highlighted in green), “–” – was not determined, n=3.

P. aeruginosa, *S. aureus* under 3 % solutions of tetraalkyl-substituted guanidine oligomer with aliphatic oligoepoxide with $\text{Alk}=\text{C}_{10}\text{H}_{21}$ were 12–14 mm. These values are lower than for solutions of tetraalkyl-substituted guanidine oligomer with aromatic oligoepoxide with $\text{Alk}=\text{C}_{10}\text{H}_{21}$. *P. pseudoalcaligenes* 109, *B. subtilis* 138 growth inhibition zones were 22–26 mm, large values for almost all the alkyl radicals.

If we compare the bactericidal effect of newly synthesized tetraalkyl-substituted oligomers based on aromatic and aliphatic oligoepoxides, then for the former with alkyl radicals $\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$, it is slightly larger, which can be explained by the different flexibility of the oligoeter chain, which affects the permeability to bacterial cells. An exception was observed for tetraalkyl-substituted oligomers with aliphatic oligoepoxide with $\text{Alk}=\text{C}_7\text{H}_{15}$ and $\text{Alk}=\text{C}_{10}\text{H}_{21}$ in relation to its effect on *P. pseudoalcaligenes* 109 and *B. subtilis* 138 strains, the growth retardation zones of this bacterium were 20–26 mm.

In comparison with the previously synthesized dialkyl-substituted guanidine-containing oligo-

mers [20], tetraalkyl-substituted oligomers exhibit slightly lower activity, which can be explained by the fact that tetra-substitution creates spatial obstacles to the penetration into the pathogenic bacterial cells.

Data from studies of the fungicidal activity of tetraalkyl-substituted guanidine-containing oligomers with aromatic and aliphatic oligoepoxides in relation to a number of microscopic fungi are shown in Table 3 and Fig. 4.

On the Fig. 4 the of growth inhibition zones of fungi *P. chrysogenum* F-41454 and *P. variotii* F-41453 under the action of tetraalkyl-substituted guanidine-containing oligomers with aromatic and aliphatic oligoepoxides ($\text{Alk}=\text{C}_{10}\text{H}_{21}$) are shown.

Tetraalkyl guanidine-containing oligomers based on aromatic oligoepoxide ($\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$) at a concentration of 1 % exhibit fungicidal activity to almost all the studied isolates. Diameter of the growth inhibition zones for *C. cladosporioides* 41450, *C. sphaerospermum* 41451, *P. funiculosum* 41452, *P. varioti* 41453, *P. chrysogenum* 41454, *A. versicolor* 41456, *A. alternate* 41457, *S. chartarum* 41458, and *A. strictum* 41459 under the

Fig. 4. Growth inhibition zones of the fungi *P. chrysogenum* F-41454 (a, b) and *P. variotii* F-41453 (c, d) under the action of an alkyl-substituted guanidine-containing oligomer with aromatic oligoepoxide $\text{Alk}=\text{C}_{10}\text{H}_{21}$: a, c – control without adding of oligomers; b, d – 1 % solution of an oligomer with aromatic oligoepoxide.

Table 2

Fungicidal activity of tetraalkyl-substituted guanidine-containing oligomers, diameter of the growth inhibition zones (mm) (well's method in agar)

Oligomer	Oligomer concentration 1%					
	Aromatic			Aliphatic		
Type of fungi	Alk=C ₃ H ₇	Alk=C ₇ H ₁₅	Alk=C ₁₀ H ₂₁	Alk=C ₃ H ₇	Alk=C ₇ H ₁₅	Alk=C ₁₀ H ₂₁
<i>C. cladosporioides</i> F-41450	17±0.33	20±1.33	20±2.33	13±1.13	15±1.13	20±1.67
<i>C. sphaerospermum</i> F-41451	13±1.33	15±1.67	20±1.33	12±1.33	16±1.67	25±3.1
<i>P. funiculosus</i> F-41452	10±1.33	19±0.67	19±1.33	10±1.33	15±1.67	21±1.33
<i>P. variotii</i> F-41453	10±1.33	15±1.67	20±1.33	7±0.33	10±0.33	13±0.34
<i>P. chrysogenum</i> F-41454	12±1.22	17±1.67	19±1.33	12±0.17	15±1.33	18±1.67
<i>A. niger</i> F-41455	8±0.32	9±0.33	8±0.33	7±0.24	8±0.25	9±0.33
<i>A. versicolor</i> F-41456	8±0.10	15±1.34	16±1.33	9±0.33	10±0.34	15±0.67
<i>A. alternata</i> F-41457	10±0.10	19±0.67	20±1.67	12±0.1	10±0.33	22±2.33
<i>S. chartarum</i> F-41458	20±2.67	15±1.33	18±1.67	3±1.33	3±0.1	15±1.67
<i>A. strictum</i> F-41459	15±1.33	18±1.33	18±1.33	13±0.34	15±1.67	15±1.33

Legend: growth inhibition zone diameter 0–10 mm (highlighted in pink) – no sensitivity detected, 12–25 mm (highlighted in light green) – sensitivity to the oligomer.

influence of the obtained oligomers after 7 days was from 7 to 25 mm, depend on the fungi isolate. The significant inhibition effect (average diameter of the growth inhibition zone – 15–20 mm) for almost all the micromycetes under the action 1 % solution of guanidine-containing oligomers with $\text{Alk}=\text{C}_{10}\text{H}_{21}$. For the tetraalkyl-substituted guanidine-containing oligomer with $\text{Alk}=\text{C}_3\text{H}_7$, the growth inhibition zones were lower (average diameter of the growth inhibition zone was 10–13 mm). The diameter of the growth inhibition zones for micromycetes depended on the length of the alkyl radical of the oligomer, and the diameter of the bacterial growth inhibition zone increased with increasing length.

The largest diameter of the growth inhibition zone for a 1 % solution of oligomers after 7 days of the experiment was observed for guanidine-containing oligomers with aromatic oligoepoxide with $\text{Alk}=\text{C}_{10}\text{H}_{21}$ (15–20 mm) except for fungus *A. niger* (8–9 mm) (Table 2). For an aliphatic oligoepoxide-based oligomer with $\text{Alk}=\text{C}_7\text{H}_{15}$, the growth inhibition zones for micromycetes non-significantly reduced. The diameter of the growth inhibition zone of micromycetes also depended on the length of the alkyl radical of the oligomer, with increasing length, the diameter of the bacterial growth inhibition zone increased, but the value was less than for oligomers with aromatic oligoepoxide.

Discussion. Tetraalkyl-substituted guanidine-containing oligomers are viscous liquids of light yellow color that are soluble in water, ethanol, methanol, methyl ethyl ketone, dimethylformamide, dimethyl sulfoxide, dimethylacetamide and insoluble in diethyl ether, hexane, and acetone. From the above-mentioned structural formula, the synthesized tetraalkyl-substituted guanidinium-containing oligomers are multifunctional compounds with a hydrophobic aromatic component and alkyl substituents containing hydroxyl groups and guanidinium fragments. They are polyfunctional substances and similar in composition to polyguanidines, with a variance of oligoether aromatic or aliphatic component and hydroxyl groups along the chain. Therefore, it can be assumed that the mechanism of biocidal action of such oligomers will be similar to polyguanidines and will be membrane-toxic.

It is known that the base of biocides is substances that can inhibit the life activity of biological objects. Strains of opportunistic microorganisms that are most often found as pathogens of chronic infections, as well as strains

of carbohydrate-oxidizing bacteria were selected for the study. The latter formed extracellular poly- and lipopolysaccharides, thanks to which bacteria adhere to the surface of protective materials, which ensures their rapid reproduction and contributes to the survival of the population in extreme conditions. As a result of the metabolism of carbohydrate-containing compounds, these bacteria accumulate organic acids, which are potential factors for the biodegradation of protective materials. Analysis of the fatty acid composition of the bacterial exopolymeric complex revealed the presence of saturated, unsaturated fatty acids and oxy acids with a carbon chain length of 11 to 18 carbon atoms [22].

If we compare the bactericidal effect of newly synthesized tetraalkyl-substituted oligomers based on aromatic and aliphatic oligoepoxides, then for the former with alkyl radicals $\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$, it is larger, which can be explained by the different flexibility of the oligoether chain, which affects the permeability of pathogenic bacteria inside the cell. Compared to the previously synthesized dialkyl-substituted guanidine-containing oligomers, tetraalkyl-substituted oligomers exhibit slightly lower activity, which can be explained by the fact that tetra-substitution creates spatial obstacles for penetration into bacterial cells [20]. The fungicidal effect of the studied oligomers with aromatic and aliphatic oligoepoxides is probably due to the presence of a diphenylpropane group, as well as guanidine and alkyl bromide fragments in their composition. It is known that in the molecules of organic substances, under the influence of different atoms or atomic groups present in them, the electron density of chemical bonds is redistributed (positive or negative induction effect). The presence of a diphenylpropane group in the oligomer molecule causes a negative induction effect – the substitute reduces the electron density on the Carbon atom with which it is bound. In this case, the substituent acquires a partial negative charge (δ^-), and the carbon atom acquires a partial positive charge (δ^+) [6]. According to the literature, the value (δ^+) may be one of the factors that enhance the interaction of fungicidal substances with the fungal cell wall [23].

It is known that macromolecules of guanidine polymers are adsorbed on the negatively charged cell surface, thereby blocking the processes of respiration and nutrition (transport of metabolites through the cell wall and cytoplasmic membrane). They diffuse through the cell wall, causing irreversible damage inside the cell and inactivating a number of enzymes [19]. It is worth noting the

low toxicity of such compounds to humans and warm-blooded animals [19]. It can be assumed that the oligomers studied by us also exhibit fungicidal activity by a similar mechanism.

Conclusions. Bactericidal and fungicidal activity of tetraalkyl-substituted guanidine-containing oligomers against various heterotrophic bacteria and microscopic fungi was detected. Alkyl-substituted guanidine-containing oligomers at concentrations of 1–3 % inhibited the growth of gram-negative and gram-positive bacteria. Tetraalkyl-substituted ($\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$) guanidine-containing oligomers at a concentration of 1 % showed fungicidal activity to almost all the studied fungal isolates. It was found, that antimicrobial properties depend on the length of the alkyl radical. The radical length increasing promotes the inhibition effect, i.e. growth of bacterial and fungal growth inhibition zones. The newly synthesized oligomers are promising for use as disinfectants for indoor treatment and as additives in polymer compositions to protect them from bio-damage.

АНТИБАКТЕРІАЛЬНА ТА ФУНГІЦИДНА АКТИВНІСТЬ ТЕТРААЛКІЛЗАМІСНИХ ГУАНІ- ДИНІЙВМІСНИХ ОЛІГОМЕРІВ

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Резюме

Гуанідинієві олігомери, малодосліджений клас органічних сполук, привертають увагу завдяки їх бактерицидним властивостям. Посилення антимікробних властивостей та спрощення і здешевлення процесу синтезу даних сполук є перспективним для отримання функціональних гуанідинієвмісних олігомерів з алкільними радикалами різної

довжини в своєму складі. **Метою** даної роботи є дослідження бактерицидної і фунгіцидної активностей новосинтезованих олігомерних гуанідинієвмісних олігомерів з алкільними радикалами різної довжини. **Методи.** Синтез тетраалкілзамісних гуанідинієвмісних олігомерів з ароматичною та аліфатичною олігоетерною складовою проводили по реакції гуанідинієвмісних олігомерів з кінцевими гуанідиновими фрагментами та алкілбромідами ($\text{Alk}=\text{C}_3\text{H}_7$, C_7H_{15} , $\text{C}_{10}\text{H}_{21}$) за мольного співвідношення компонентів 1:4. Як тест-культури для визначення біоцидності отриманих сполук було використано штами клінічних ізолятів і вуглеводеньокиснювальних бактерій грам-позитивних та грам-негативних бактерій. Мікроскопічні гриби виділені з приміщень м. Києва, які зазнали мікологічного ураження. Бактерії вирощували на м'ясопептонному агарі протягом 48 годин, мікроміцети – на агаризованому пивному суслі (6 °Б) протягом 14 діб. Штами вуглеводеньокиснювальних бактерій та мікроміцети інкубували за температури 28 ± 2 °С, а клінічні ізоляти – за температури 37 ± 2 °С. Антимікробну активність олігомерів визначали стандартним диско-дифузійним методом, а фунгіцидну – методом лунок в агар. **Результати.** Отримано тетраалкілзамісні гуанідинієвмісні олігомери з різними радикалами (C_3H_7 , C_7H_{15} , $\text{C}_{10}\text{H}_{21}$) та показано їх бактерицидну та фунгіцидну активності відносно різних груп мікроорганізмів. Встановлено, що отримані олігомери в концентрації 1–3 % водного розчину інгібували ріст грам-негативних і грам-позитивних бактерій. Антимікробні та фунгіцидні властивості залежали від довжини алкільного радикалу, зі збільшенням його довжини зростав діаметр зони затримки росту бактерій і мікроміцетів. Для 3 % розчинів тетраалкілзамісного гуанідинієвмісного олігомеру на основі ароматичного олігоетерного оксиду ($\text{Alk}=\text{C}_{10}\text{H}_{21}$) зони пригнічення росту бактерій складали 18–21 мм. Бактерицидна дія олігомеру на основі ароматичного олігоетерного оксиду з алкільними радикалами $\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$ більша на 20–25 % за такі з варіантами з аліфатичним олігоетерним оксидом. Всі тетраалкілзамісні ($\text{Alk}=\text{C}_7\text{H}_{15}$, $\text{C}_{10}\text{H}_{21}$) гуанідинієвмісні олігомери у концентрації 1 % у водному розчині проявляли фунгіцидну активність практично до всіх тест-культур мікроміцетів, зони затримки росту культур яких на 7 добу становили 7–20 мм. Найбільші зони затримки росту мікроміцетів спостерігали за дії олігомерів на основі ароматичного олігоетерного оксиду з радикалами $\text{Alk}=\text{C}_{10}\text{H}_{21}$ та C_7H_{15}

(в середньому 15–20 мм) та аліфатичного оліго-епоксиду з радикалом $\text{Alk}=\text{C}_{10}\text{H}_{21}$ (в середньому 15–20 мм). **Висновки.** Довжина алкільного радикалу та природа олігоетерної складової впливає на бактерицидні та фунгіцидні властивості новосинтезованих олігомерів. Зі збільшенням довжини алкільного радикалу гуанідинійвмісних олігомерів підвищуються їх бактерицидні та фунгіцидні влас-

тивості, Тетралкілзамісні олігомери є перспективними до використання як дезінфектанти для обробки приміщень та як добавки в полімерні композиції для захисту їх від біопшкоджень.

Ключові слова: тетраалкілзамісні гуанідинійвмісні олігомери, гетеротрофні бактерії, клінічні ізоляти, мікроміцети, бактерицидна та фунгіцидна активності.

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