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NEW BIOCIDES FOR COOLING WATER TREATMENT

PART. III. QUATERNARY AMMONIUM SALTS DERIVATIVES OF GLYCINE ESTERS***

The paper presents the results of the successive stage of research on new biocides for abatement of microorganisms forming biological fouling in industrial cooling systems [4, 5]. The experiments were carried out on 16 quaternary ammonium salts, derivatives of glycine esters — mainly the long-chain ones, i.e. C₁₀–C₁₆. The above compounds acted as growth inhibitors of both algae (*Oscillatoria* sp.) and bacteria (*Sphaerotilus natans*) which occur in mass in cooling systems. It has been stated that the biological activity of the compounds tested depends on their chemical structure.

1. INTRODUCTION

The problem of abatement of microorganisms forming biological foulings in cooling systems and its importance in industry was discussed in the paper [4].

Nowadays quaternary ammonium salts are more and more widely used as biocides. From the so far performed investigations it follows that ammonium chlorides earlier synthesized and tested by us [4, 5] are an efficient group inhibiting the growth of algae and bacteria inhabiting industrial cooling systems.

The analysis of the results obtained allow us to observe a relationship between chemical structure of the given compound and its biological activity. An interesting effect has been stated in a group of esters, derivatives of glycine [4], while in the group of alkoxymethylenammonium chlorides the biological activity increased with the increasing length of alkyl

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chain [5]. For this reason we have decided to synthesize a new group of quaternary ammonium salts, i.e. glycine esters derivatives having an elongated alkyl chain in ester group C_{10} – C_{16} , i.e. within the range of the maximal biological activity. This group of ammonium salts is the subject of the present study.

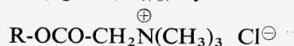
2. MATERIAL AND METHODS

2.1. CHLORIDES OF GLYCINE ESTERS

The tests were performed on 16 ammonium salts, derivatives of glycine esters, the structure of which and results of biological tests are presented in tabs. 1–4.

Table 1

Inhibition of algal and bacterial growth (%) by chlorides of trimethylglycine esters



Hamowanie wzrostu glonów i bakterii (%) przez chlorki estrów trójmetyloglicyny o wzorze ogólnym



Compound number	R	Concentration (mg/dm ³)				
		<i>Oscillatoria</i> sp.			<i>Sphaerotilus natans</i>	
		20.0	2.0	0.4	200	100
1	$C_{10}H_{21}$	100.0	0.0	—	60.5	19.5
2	$C_{12}H_{25}$	100.0	0.0	—	60.8	21.5
3	$C_{14}H_{29}$	100.0	27.7	0.0	72.5	30.1
4	$C_{16}H_{33}$	100.0	24.5	0.0	70.1	22.3

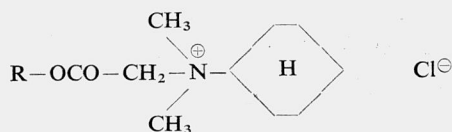
Chlorophyll, control value — 0.65 mg.

Biomass, control value — 10.5 mg.

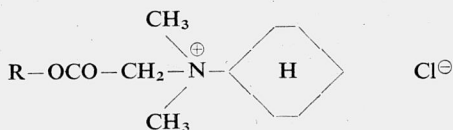
The above compounds have been synthesized in our laboratory by quaternization of tertiary amines (trimethylamine and triethylamine, N-methylmorpholine and N,N-dimethylcyclohexylamine) with the corresponding chloroacetates obtained, according to SMRZ and WINTERNITZ [6], in esterification of the respective alcohol with chloroacetyl chloride. The reaction runs according to the following pattern:

Table 2

Inhibition of algal and bacterial growth (%) by chlorides of N,N-dimethylcyclohexylglycine esters



Hamowanie wzrostu glonów i bakterii (%) przez chlorki estrów N,N-dwumetylocykloheksyloglicyny o wzorze ogólnym



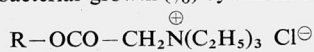
Compound number	R	Concentration (mg/dm ³)				
		<i>Oscillatoria</i> sp.			<i>Sphaerotilus natans</i>	
		20.0	2.0	0.4	200	100
5	C ₁₀ H ₂₁	100.0	17.6	—	10.0	0.0
6	C ₁₂ H ₂₅	100.0	100.0	0.0	45.2	0.0
7	C ₁₄ H ₂₉	100.0	100.0	23.7	49.1	20.4
8	C ₁₆ H ₃₃	100.0	8.0	—	50.5	19.1

Chlorophyll, control value — 0.63 mg.

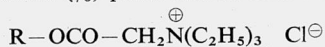
Biomass, control value — 10.5 mg.

Table 3

Inhibition of algal and bacterial growth (%) by chlorides of triethylglycine esters



Hamowanie wzrostu glonów i bakterii (%) przez chlorki estrów trójetyloglicyny o wzorze ogólnym



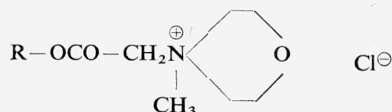
Compound number	R	Concentration (mg/dm ³)				
		<i>Oscillatoria</i> sp.			<i>Sphaerotilus natans</i>	
		20.0	2.0	0.4	200	100
9	C ₁₀ H ₂₁	100.0	0.0	—	50.7	11.8
10	C ₁₂ H ₂₅	100.0	0.0	—	68.9	15.5
11	C ₁₄ H ₂₉	100.0	23.6	—	75.1	20.6
12	C ₁₆ H ₃₃	100.0	100.0	32.4	73.8	30.4

Chlorophyll, control value — 0.6 mg.

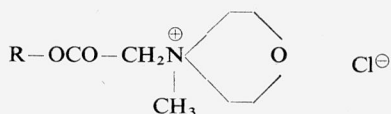
Biomass, control value — 10.8 mg.

Table 4

Inhibition of algal and bacterial growth (%) by chlorides of N-methylmorpholinoglycine esters



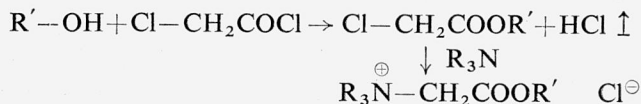
Hamowanie wzrostu glonów i bakterii (%) przez chlorki estrów N-metylomorfolinoglicyny o wzorze ogólnym



Compound number	R	Concentration (mg/dm ³)				
		<i>Oscillatoria</i> sp.			<i>Sphaerotilus natans</i>	
		20.0	2.0	0.4	200	100
13	C ₁₀ H ₂₁	100.0	0.0	—	20.1	0.0
14	C ₁₂ H ₂₅	100.0	0.0	—	18.5	0.0
15	C ₁₄ H ₂₉	100.0	10.0	—	45.5	0.0
16	C ₁₆ H ₃₃	100.0	100.0	6.3	43.8	0.0

Chlorophyll, control value — 0.63 mg.

Biomass, control value — 10.4 mg.



where:

R' — decyl, dodecyl, tetradecyl, and hexadecyl,

R₃N — trimethylamine, triethylamine, N-methylmorpholine, and N,N-dimethylcyclohexylamine.

All the compounds tested were of about 99% purity. The details of the synthesis and the properties of the salts obtained will be published separately.

2.2. BIOLOGICAL TESTS

Biological tests have been performed on microorganisms isolated from industrial cooling systems.

1. Bacteriocidal activity of the compounds investigated was tested on filamentous bacterium *Sphaerotilus natans*. The procedure was described in the former paper [4]. Results presented in tabs. 1-4 are expressed in percent of bacterial growth inhibition by the com-

pound tested.

2. Algicidal activity of the compounds investigated was tested on *Oscillatoria* sp. The detailed description of the procedure used is also given in [4]. The inhibition of algal growth (tabs. 1-4) expressed in percent is referred to control samples (without biocide) in which chlorophyll contents have been assumed as 100%.

3. RESULTS AND DISCUSSION

Biological tests have indicated a high algicidal activity of the group of ammonium salts subject to investigations. At the concentration of 20 mg/dm³ all the salts investigated inhibited completely the growth of *Oscillatoria* sp. By gradually decreasing this concentration to 2.0 mg/dm³ and 0.4 mg/dm³, it is possible to detect the effect of the chain length of alkyl of ester group on algicidal activity of the compound tested. The highest activity has been stated for tetradecyl esters (C₁₄) in cyclohexyldimethylammonium (tab. 2) and trimethylammonium (tab. 1) derivatives or for hexadecyl esters (C₁₆) in N-methylmorpholinium (tab. 4) and triethylammonium (tab. 3) derivatives.

Having in mind the observed effect of the chain length of alkoxymethylammonium salts on their biological activity [5], we have confined our investigations of the group of glycine esters derivatives to those being greater than C₁₀; the esters C₁-C₂ examined earlier and the salts of octadecyl esters (C₁₈) were neglected because of their limited solubility in water. The relationship stated by us suggests that algicidal activity of the compounds examined results from the action of ammonium salts on biological membranes and on the transport across these membranes [2]. In former paper [4] we have shown that esters with short alkyl chains (C₁-C₂) are characterized by a relatively weak activity which can take place only in the highest concentrations (10⁻³ mol/dm³). Within the group of esters examined we have found some compounds (7, 12) whose algicidal activity was observed even at concentrations as low as 0.4 mg/dm³ (ca 10⁻⁶ mol/dm³). A high activity of dichloroethyl esters suggests that these esters act according to another mechanisms and to explain it some new experiments are required.

While considering the effect of the remaining fragments of the molecule on algicidal activity we have stated that the trimethylammonium derivatives are characterized by the lowest activity (tab. 1). Similar algicidal activity has been stated in the remaining three groups of esters.

In the four groups of the compounds investigated the effect of alkyl length on bacteriocidal activity was similar. The highest activity has been observed for esters C₁₄-C₁₆ (tabs. 1-4), this relationship is not so distinct as in the case of algicidal activity. Within the groups of esters investigated, trimethylammonium (tab. 1) and triethylammonium (tab. 3) derivatives show the highest activity, since at the concentration of 200 mg/dm³ the growth inhibition of *Sphaerotilus natans* was higher than 70%. The activity of salts with ring substituent, thus morpholinium salts, was distinctly weaker (tab. 2).

The stated effect of the length of alkyls of ester group on algicidal and bacteriocidal activities of the salts investigated is consistent with the relationship found for alkoxymeth-

ylammonium salts [5]. Similar effect of the length of alkyls of both groups of salts on their fungicidal activity was stated in [4, 5], and that of alkoxyethylammonium salts on the transport of ions across liposome membranes in [2]. This suggests that the mechanism of biological activity in both groups of ammonium salts is similar. This hypothesis will be investigated in the nearest future.

Summing up, the investigated group of ammonium salts — the glycine ester derivatives — has shown some effective biological properties and possibilities of their practical application because of their high algicidal and bacteriocidal activity. Slightly lower bacteriocidal activity of ammonium salts tested in the present work and in [4, 5] seems to result from the choice of the bacterium tested (gram-negative *Sphaerotilus natans*). As is well known [3], bacteria of this kind are more resistant to disinfectants.

REFERENCES

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- [7] Unpublished data.

NOWE BIOCYDY W OCZYSZCZANIU UKŁADÓW CHŁODNICZYCH CZĘŚĆ III. CZWARTORZĘDOWE SOLE AMONIOWE POCHODNE ESTRÓW GLICYNY

Przedstawiono wyniki badań kolejnego etapu prac poszukiwawczych nad nowymi biocydami do zwalczania mikroorganizmów tworzących obrosty biologiczne w przemysłowych układach chłodniczych [4, 5]. Badaniom poddano 16 czwartorzędowych soli amoniowych, pochodnych estrów glicyny — głównie estrów długołańcuchowych (C₁₀–C₁₂). Związki te wykazały działanie hamujące zarówno na wzrost glonów (*Oscillatoria* sp.), jak i bakterii (*Sphaerotilus natans*) występujących masowo w układach chłodniczych. Potwierdzono zależność między działaniem biologicznym a budową chemiczną badanej grupy związków.

NEUE BIOZIDE IM KÜHLWASSERKREISLAUF TEIL. III. QUARTÄRE AMMONIUMSALZE, DERIVATE VON GLYZINESTER

Dargestellt werden weitere Ergebnisse von Forschungsarbeiten mit neuen Bioziden zur Bekämpfung von Mikroorganismen, welche biologische Bewuchse in industriellen Kühlwasserkreisläufen bilden [4, 5]. Getestet wurden 16 quartäre Ammoniumsalsalze Derivate von Glyzinester — vorwiegend mit langkettigen

Estern (C_{10} - C_{12}). Diese Verbindungen wirkten hemmend auf den Aufwuchs von Algen (*Oscillatoria* sp.) und Bakterien (*Sphaerotilus natans*), welche in Kühlkreisläufen massenhaft vorkommen. Es besteht ein Zusammenhang zwischen der biologischen Wirkung und der chemischen Konstitution der untersuchten Verbindungen.

НОВЫЕ БИОЦИДЫ В ОЧИСТКЕ СИСТЕМ ОХЛАЖДЕНИЯ ЧАСТЬ III. ЧЕТВЕРТИЧНЫЕ АММОНИЕВЫЕ СОЛИ, ПРОИЗВОДНЫЕ ЭФИРОВ ГЛИЦИНЫ

Представлены результаты исследований последовательного этапа работы над новыми биоцидами, предназначенными для борьбы с микроорганизмами, обрастающими промышленные системы охлаждения [4, 5]. Было исследовано 16 четвертичных аммониевых солей, производных эфиров глицины — прежде всего — длинноцепочных эфиров (C_{10} - C_{12}). Эти соединения задерживают так рост водорослей (*Oscillatoria* sp.), как и бактерий (*Sphaerotilus natans*) выступающих массами в системах охлаждения. Подтверждена зависимость между биологическим действием исследуемой группы соединений и их химическим строением.