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NEW BIOCIDES FOR COOLING WATER TREATMENT

I. SELECTED QUARTERNARY AMMONIUM SALTS

The paper presents the results of the research on new biocides to control microbial growth in cooling systems. The investigations included 62 quarternary ammonium chlorides belonging to 5 structural groups. Biological tests were performed under laboratory conditions, using microorganisms isolated from industrial cooling system, i.e. *Oscillatoria* sp. and *Anabaena variabilis* (algae and *Sphaerotilus natans* (bacteria). It has been stated that some of the compounds investigated inhibit microbial growth even at the concentration lower than 10^{-4} mol/dm^3, thus their practical application seems to be possible.

1. INTRODUCTION

Surface waters are utilized by the industry in various technological processes, of which cooling belongs to the most important ones. Specific conditions existing in cooling systems promote the development of mass microbial growths which, by impeding heat exchange, increasing hydraulic resistances in pipes and intensifing the corrosion, contribute to substantial economic losses [7, 16]..

Biological slimes are mainly composed of bacteria, fungi and algae, of which the organisms attached to the substrate are the most troublesome as for instance, filamentous blue-green algae and bacteria [5, 8, 11].

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To counteract the mass development of microorganisms various kinds of measures are applied, chiefly chemicals acting as bacteriocides and algicides. The troublesome and corrosive chlorination process is replaced by organic biocides, of which quarternary ammonium salts are more and more widely applied [1,6, 17].

Quarternary ammonium salts acting as bacteriocides [4], fungicides [18] as well as plant growth regulators [14] show a large spectrum of biological activity. In our earlier investigations we have shown that a number of ammonium salts without a long alkyl radicals act as plant growth regulators, and that some of them reveal also antifungal activity with respect to pathogenic fungi [3, 12, 15]. It may be expected with a high probability that these compounds will also appear active against algae and bacteria forming biological slimes in cooling systems. The group of the above salts is the subject of the present paper.

2. MATERIAL AND METHODS

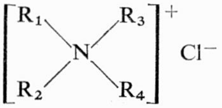
2.1. AMMONIUM SALTS

The investigations included 62 ammonium salts synthetized in our laboratory [15] and belonging to 6 structural groups: analogues of chlorocholine chlorides (tab. 1), morpholinium derivatives (tab. 2), benzylammonium chlorides (tab. 3), derivatives of glycine esters (tab. 4), derivatives of glycine amides (tabs. 5 and 6).

All the salts examined, of purity higher than 98%, were characterized by constant melting points and their elementary analyses gave satisfactory results [15].

Table 1

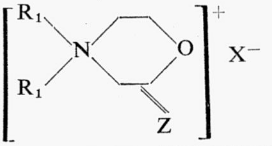
Analogs of chlorocholine chloride
Analogi chlorku chlorocholiny



Compound No.	R_1	R_2	R_3	R_4	M.P. (°C)
1	C_2H_5-	C_2H_5-	$-C_2H_4Cl$	$H-$	210-211
2	C_2H_5-	C_2H_5-	$-C_2H_4OH$	$-C_2H_4OH$	221-222
3	CH_3-	CH_3-	$-C_2H_4OH$	$-C_2H_4OH$	244-245
4	CH_3-	CH_3-	$-C_2H_4Cl$	$-C_2H_4Cl$	217-218
5	C_2H_5-	C_2H_5-	$-C_2H_4OH$	$H-$	129-130
6	CH_3-	CH_3-	$-C_2H_4Cl$	$H-$	207-208

Table 2

Derivatives of 1.4-oxazine
Pochodne 1,4-oksazyny

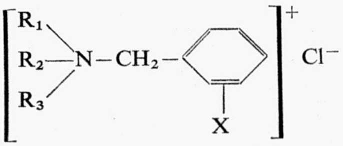


Compound No.	R_1	R_2	Z	X^-	M.P. (°C)
7	CH_3-	$H-$	H_2	Cl	206-208.5
8	CH_3-	$H-$	H_2	Br	175-176
9	CH_3-	CH_3-	H_2	Cl	327-328
10	CH_3-	CH_3-	H_2	Br	340-342
11	CH_3-	CH_3-	H_2	CH_3SO_4	134-135
12	CH_3-	$-C_2H_4OH$	H_2	Cl	—*
13	C_2H_5-	$H-$	H_2	Cl	167-168
14	CH_3-	CH_3-	$-O-$	Cl	220-222
15	C_2H_5-	C_2H_5-	$-O-$	Cl	213-214.5
16	CH_3-	$CH_2=CH-CH_2-$	H_2	Cl	199-201
17	$-C_2H_4OH$	$H-$	H_2	Cl	104-107
18	C_2H_5-	C_2H_5-	H_2	Cl	295-296
19	$-H_2C-CH_2-O-CH_2-CH_2-$		H_2	Cl	179-180.5

* Strongly hygroscopic compound.

Table 3

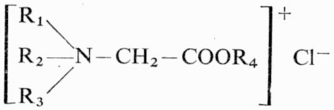
Benzylammonium chlorides
Chlorki benzyloamoniowe



Compound No.	R_1	R_2	R_3	X	M.P. (°C)
20	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		2.4-Cl_2	199.5-200
21	C_2H_5-	C_2H_5-	C_2H_5-	2.4-Cl_2	178.5-179.5
22	CH_3-	CH_3-	CH_3-	2.4-Cl_2	225-226
23	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		H	237-237.5
24	CH_3-	CH_3-	CH_3-	H	240-241.5
25	C_2H_5-	C_2H_5-	C_2H_5-	H	185-186
26	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		4-Cl	192-193
27	C_2H_5-	C_2H_5-	C_2H_5-	4-Cl	172-173
28	CH_3-	CH_3-	CH_3-	2-Cl	208-208.5
29	CH_3-	CH_3-	CH_3-	3-Cl	194-194.5
30	CH_3-	CH_3-	$-C_2H_4OH$	H	80-81
31	CH_3-	CH_3-	$-C_2H_4OH$	2.4-Cl_2	78.5-79.5
32	CH_3-	CH_3-	$-C_2H_4OH$	4-Cl	121.5-122
33	CH_3-	CH_3-	CH_3-	4 Cl	205-206
34	C_2H_5-	C_2H_5-	C_2H_5-	2 Cl	148-149
35	C_2H_5-	C_2H_5-	C_2H_5-	3 Cl	202-202.5
36	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		3 Cl	196-197

Table 4

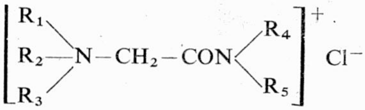
Derivatives of glycine esters
Pochodne estrów glicyny



Compound No.	R_1	R_2	R_3	R_4	M.P. (°C)
37	CH_3-	CH_3-	CH_3-	$-C_2H_4Cl$	175-176
38	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		$-C_2H_4Cl$	189.5-190
39	CH_3-	CH_3-	CH_3-	CH_3-	171-172
40	C_2H_5-	C_2H_5-	C_2H_5-	$-C_2H_4Cl$	141-142
41	CH_3-	CH_3-	CH_3-	C_2H_5-	168.5-169.5
42	C_2H_5-	C_2H_5-	C_2H_5-	C_2H_5-	131-132
43	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		CH_3-	151-152
44	CH_3-	CH_3-	CH_3-	α-fenchyl	238.5-239
45	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		α-fenchyl	190.5-191
46	CH_3-	CH_3-	CH_3-	menthyl	208-209
47	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		menthyl	188.5-190
48	C_2H_5-	C_2H_5-	C_2H_5-	menthyl	186.5-187.5
49	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		isobornyl	206.5-207.5
50	CH_3-	CH_3-	CH_3-	isobornyl	226-227
51	C_2H_5-	C_2H_5-	C_2H_5-	isobornyl	112-113

Table 5

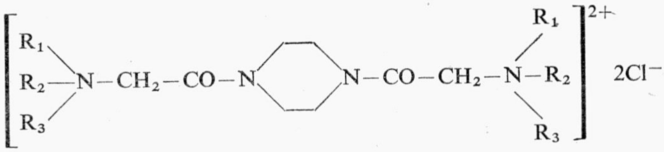
Derivatives of glycine amides
Pochodne amidów glicyny



Compound No.	R_1	R_2	R_3	R_4	R_5	M.P. (°C)
52	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		C_2H_5-	C_2H_5-	213-214
53	CH_3-	CH_3-	CH_3-	C_2H_5-	C_2H_5-	212-213
54	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		$-H_2C-CH_2-O-CH_2CH_2-$		206-208
55	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		$-H_2C-CH_2-CH_2-CH_2-CH_2-$		209-210
56	C_2H_5-	C_2H_5-	C_2H_5-	$-H_2C-CH_2-O-CH_2CH_2-$		171-172.5
57	CH_3-	CH_3-	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		103-104
58	C_2H_5-	C_2H_5-	C_2H_5-	$-H_2C-CH_2-CH_2-CH_2CH_2-$		190-190.5
59	C_2H_5-	C_2H_5-	C_2H_5-	C_2H_5-	C_2H_5-	173-174

Table 6

Derivatives of glycine amides
Pochodne amidów glicyny



Compound No.	R_1	R_2	R_3	M.P. (°C)
60	CH_3-	CH_3-	CH_3-	232-233
61	C_2H_5-	C_2H_5-	C_2H_5-	197-198
62	CH_3-	$-H_2C-CH_2-O-CH_2CH_2-$		219-220

2.2. BIOLOGICAL TESTS

Algicidal and bacteriocidal activities of the salts investigated were tested on dominating filamentous microorganisms isolated from biological slimes of industrial cooling systems. Filamentous *Sphaerotilus natans* was isolated from bacterial slime of cooling system in Wrocław Distillery, while the blue-green algae (*Oscillatoria* sp. and *Anabaena variabilis*) from the cooling system of Blachownia power plant at Kędzierzyn.

I. *Sphaerotilus natans* was isolated on nutrient agar. Preliminary test allowing to select the bacteriocidal substances has been carried out on agar-agar supplemented with a biocide [13]. Eventually either bacterial growth or its lack was observed. The compounds selected in this way were then tested in a nutrient solution of the following composition (in g/dm^3): NaCl – 0.5, NH_4NO_3 – 3.0, KH_2PO_4 – 0.5; K_2HPO_4 – 0.5; $MgSO_4$ – 0.5; glucose – 10.0, $FeSO_4$ – 2–3 crystals.

Culture flasks containing 250 cm^3 of nutrient solution and biocide at an appropriate concentration, inocculated with a homogenized bacterial suspension (0.1 cm^3), were incubated at 20°C for 72 hrs. The increment in bacterial growth in the presence of biocide was determined by filtering the content of flasks through a dried and weighed membrane filter. Thereupon the filter with biomass was dried at 105°C until the weight was stabilized.

The control samples (without biocide) were incubated under the same conditions. While determining the percent of the bacterial growth inhibition by the salt examined, it has been assumed that the biomass increment in control tests was 100%. The experimental and control tests were performed in 5 and 20 replications, respectively.

II. Tests for algicidal activity have been performed using the following nutrient solution (in g/dm^3): KNO_3–0.5; KH_2PO_4–0.2; $MgSO_4 \cdot 7H_2O$–0.16; $FeC_6H_5O_7$–0.003; (OH) $C_3H_4(COOH)_3$–0.03 and microelements. Flasks containing 48 cm^3 of nutrient solution, the tested biocide at the appropriate concentration and 2 cm^3 of homogenized suspension

Table 7

Inhibition of algae growth by quartenary ammonium chlorides (in %)
Hamowanie wzrostu glonów przez czwartorzędowe chlorki amoniowe (w %)

Compound No.	Concentrations [M]					
	Oscillatoria sp.			*Anabaena variabilis*		
	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-5}$
1	2	3	4	5	6	7
1	86.4	—	—	54.8	0.0	—
2	52.5	—	—	25.8	—	—
3	49.2	—	—	25.8	—	—
4	57.6	—	—	35.5	—	—
5	37.3	—	—	22.6	—	—
6	94.9	0.0	—	69.4	0.0	—
7	42.4	—	—	0.0	—	—
8	44.1	—	—	0.0	—	—
9	52.5	—	—	22.6	—	—
10	44.1	—	—	0.0	—	—
11	50.8	—	—	0.0	—	—
12	40.7	—	—	0.0	—	—
13	66.1	—	—	41.9	—	—
14	100.0	100.0	0.0	87.1	48.4	0.0
15	100.0	100.0	44.1	83.9	35.5	0.0
16	49.2	—	—	22.6	—	—
17	32.2	—	—	0.0	—	—
18	0.0	—	—	0.0	—	—
19	0.0	—	—	0.0	—	—
20	0.0	—	—	0.0	—	—
21	0.0	—	—	0.0	—	—
22	0.0	—	—	0.0	—	—
23	0.0	—	—	0.0	—	—
24	0.0	—	—	0.0	—	—
25	0.0	—	—	0.0	—	—
26	0.0	—	—	0.0	—	—
27	0.0	—	—	0.0	—	—
28	0.0	—	—	0.0	—	—
29	0.0	—	—	0.0	—	—
30	0.0	—	—	0.0	—	—
31	0.0	—	—	0.0	—	—
32	0.0	—	—	0.0	—	—
33	0.0	—	—	0.0	—	—
34	0.0	—	—	0.0	—	—
35	0.0	—	—	0.0	—	—
36	0.0	—	—	0.0	—	—
37	100.0	100.0	88.1	87.1	69.4	0.0

1	2	3	4	5	6	7
38	100.0	100.0	86.4	58.1	25.8	0.0
39	54.2	—	—	58.1	0.0	—
40	100.0	100.0	72.9	53.2	0.0	—
41	57.6	—	—	0.0	—	—
42	59.3	—	—	0.0	—	—
43	86.4	—	—	46.8	—	—
44	91.5	—	—	54.8	0.0	—
45	84.7	—	—	50.0	—	—
46	100.0	—	—	83.9	0.0	—
47	89.8	—	—	75.8	0.0	—
48	100.0	100.0	0.0	91.9	72.6	0.0
49	93.2	—	—	67.7	0.0	—
50	93.2	—	—	50.0	—	—
51	100.0	79.7	0.0	85.5	46.8	0.0
52	0.0	—	0.0	0.0	—	—
53	30.5	—	—	0.0	—	—
54	28.8	—	—	0.0	—	—
55	45.8	—	—	0.0	—	—
56	16.9	—	—	0.0	—	—
57	0.0	—	—	0.0	—	—
58	57.6	—	—	21.0	—	—
59	50.8	—	—	0.0	—	—
60	33.9	—	—	0.0	—	—
61	0.0	—	—	0.0	—	—
62	62.7	—	—	27.4	—	—

Control values of chlorophyll: *Oscillatoria* sp. — 0.59 mg, *Anabaena variabilis* — 0.62 mg.

of algae, the optical density of which was determined, were incubated in a thermoluminostate at 20°C for 14 days. Thereupon the content of chlorophyll in biomass was determined by the methods described in [2]. The chlorophyll content in control tests being assumed as 100%, the percent of inhibition given in tab. 7 is referred to the control. The data given in this table are the mean values calculated from experimental (5) and control (20) replications.

3. RESULTS AND DISCUSSION

Tests for algicidal activity included all the compounds investigated. Results are summarized in tab. 7. In preliminary tests carried out at the concentration of 10^{-3} mol/dm^3 the both species of algae were used. Thereupon the procedures applied were different. The compounds which had shown a total inhibitory effect with respect to *Oscillatoria* sp. were first tested at the concentration of 10^{-4} mol/dm^3 and then at 10^{-5} mol/dm^3. In case of *Anabaena* *variabilis* the tests at concentration of 10^{-4} mol/dm^3 were performed with salts which caused the growth inhibition higher than 50%, and those still active at this concentration were subsequently tested at 10^{-5} mol/dm^3.

Bactoriocidal activity of the compounds examined were tested with respect to *Sphaerotilus natans*. In preliminary test performed on nutrient agar all but one compound (No. 39) appeared inactive. This latter inhibited totally bacterial growth in this medium, and in nutrient solution the growth inhibition at 200 mg/dm^3 and 100 mg/dm^3 amounted to 74% and 20%, respectively.

The group of chlorocholine analogs 1–6 (tab. 1) proved to be inactive as algicides. A relatively high activity has been stated only in compounds 1,6 (tab. 1) being given at the highest concentration employed. A total lack of activity has been also stated in benzylammonium salts with short alkyl radicals 20–36 (tab. 3).

A relatively low activities with respect to *Oscillatoria* sp. and practically null activity with respect to *Anabaena variabilis* were found for the group of the derivatives of glycine amids (52–62, tabs. 5 and 6).

The most interesting appeared to be morpholinium salts (7–19, tab. 2) and the derivatives of glycine esters (37–51, tab. 4). In the first group a high activity with respect to the both algae was stated in the compounds 14 and 15. It is characteristic that they show a strong plant growth activity [12], although its mechanism has not been so far elucidated. It is worth noticing that in water solutions these compounds, due to the opening of 2-oxomorpholinum ring, are trasformed to corresponding betaines [19] which are the proper growth substances. The algicidal activity of N,N-dimethylmorpholinium chloride 9 proved to be relatively low, whereas plant growth activity was very high [9, 10, 12]. The above examples as well as the comparison of algicidal and plant growth activities of the remaining compounds have shown the total lack of correlation. The compounds 14 and 15 are the only exception in this respect, their algicidal activity drops, however, substantially at the concentration of 10^{-5} mol/dm^3.

The most valuable results have been obtained for the group of the derivatives of glycine esters (37–51, tab. 4). The above compounds have shown a high activity with both algal species when given at the highest of the concentrations used. A particularly high activity has been stated in the derivatives of 2-chloroethyl esters of glycine (37,38, 40) which were strongly active against the two algae, even at lower concentrations. This activity is considerably reduced by substitution of a low alkyl radical for the chloroethyl one. Some derivatives of terpene esters (48-menthyl and 51-isobornyl ones) containing a triethylammonium group appeared, however, to be active. This fact indicates that the alkyl (cycloalkyl) structure of the ester group is the essential fragment of the molecule that influences its algicidal activity. It suggests the possibility of finding further active compounds within this group of substances by modifications of ester group as well as of substituents in ammonium group.

The described investigations provided interesting data on the influence of the structure of ammonium salts on their activity with respect to filamentous algae and bacteria. The obtained results indicate the direction in which further search for suitable biocides to combat biological slimes in cooling system are to be conducted. Some of the compounds investigated, as for instance 37, 38 and 40, are the potential biocides whose efficiency should be verified in conditions closely resembling those existing in the industry. At present the investigations in the both directions are continued.

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NOWE BIOCYDY W OCZYSZCZANIU UKŁADÓW CHŁODNICZYCH

WYBRANE CZWARTORZĘDOWE SOLE AMONIOWE

Przedstawiono wyniki prac poszukiwawczych nad nowymi biocydami do zwalczania mikroorganizmów tworzących obrosty biologiczne w układach chłodniczych. Badaniami objęto 62 czwartorzędowe chlorki amoniowe należące do 5-ciu grup strukturalnych. Test biologiczny wykonano w warunkach laboratoryjnych na mikroorganizmach (w szczególności na glonach *Oscillatoria* sp. i na bakteriach *Sphaerotilus natans*) izolowanych z przemysłowych układów chłodniczych. Niektóre z badanych związków hamują procesy wzrostowe mikroorganizmów nawet przy stężeniach niższych od 10^{-4} mola/dm^3, co rokuje możliwości ich wykorzystania w praktyce.

NEUE BIOZIDE ZUR REINIGUNG VON KÜHLWASSERSYSTEMEN

SELECTIVE AUSWAHL VON QUARTÖREN AMMONIUMSALZE

Dargestellt werden Ergebnisse der Suche nach neuen Bioziden zur Bekämpfung von Mikroorganismen die den biologischen Bewuchs in Kühlwassersystemen bilden. Untersucht wurden 62 quartäre Ammoniumchloride die zu 5 Strukturgruppen angehörten. Als Testorganismen für die biologischen Versuche im Labor dienten Algen (*Oscillatoria* sp.) und Bakterien (*Sphaerotilus natans*) die aus technischen Anlagen isoliert wurden. Manche Verbindungen hemmen das Wachstum dieser Mikroorganismen sogar bei einer Molarkonzentration die kleiner als 10^{-4} Mol/dm^3 war. Das gibt gute Aussichten für ihre Anwendung in der Praxis.

НОВЫЕ БИОЦИДЫ В ОЧИСТКЕ СИСТЕМ ОХЛАЖДЕНИЯ

ИЗБРАННЫЕ ЧЕМВЕРИНЫЕ АММОНИЙНЫЕ СОЛИ

Представлены результаты изыскательных работ над новыми биоцидами для борьбы с микроорганизмами, образующими биологические обросты в системах охлаждения. Исследованиями охвачены 62 четверичных аммонийных хлорида, принадлежащих к 5 структурным группам. Биологический тест был выполнен в лабораторных условиях на микроорганизмах (в частности на водорослях *Oscillatoria* sp. и на бактериях *Sphaerotilus nantas*), изолированных из промышленных систем охлаждения. Некоторые из исследуемых соединений тормозят ростовые процессы микроорганизмов даже при более низких концентрациях от 10^{-4} моль/дм^3, что позволяет надеяться на создание возможности использования их в практике.