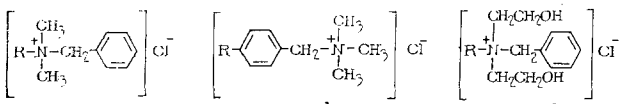
ANTIMICROBIAL AND SURFACE-ACTIVE PROPERTIES OF CATIONIC SURFACTANTS BASED ON CHLOROALKANES AND ALKYLBENZENES

M. A. Chernyavskaya, V. V. Stefanovich,
I. A. Sergeeva, and A. S. Belova

UDC 615.281:547.532'021].074

Cationic surfactants have recently been finding increasingly wide use not only as aseptic and disinfecting agents but also as additives to drugs and cosmetics in connection with the fact that they possess a broad spectrum of antimicrobial activity and comparatively low toxicity with respect to the animal and human organism [1, 4-6, 8-10, 11]. However, the assortment and volume of production of cationic surfactants is extremely limited. In the search for new effective antimicrobial agents, the criterion for selection should be not only high activity of the compounds but also availability and cost of the raw materials for their production [2]. Of substantial interest as an initial raw material for the production of cationic surfactants are chloroalkanes and alkylbenzenes, which are intermediates of the production of catamine AB and alkylbenzenesulfonates [3]. To search for new effective antimicrobial agents, the production of which might be based on available raw materials and to determine the nature of the high antimicrobial activity of cationic surfactants, we conducted investigations studying the relationship between the structure of the cationic surfactant molecule and their biological action. For this purpose we synthesized compounds belonging to three homologous series of quaternary ammonium bases (QAB), which differ in the nature of the substituents at the quaternary nitrogen atom:



I a-e II a-d III a-d

a: R=C_8H_{17}; b: R=$C_{10}H_{21}$; c. R=$C_{12}H_{25}$; d: R=$_{16}H_{33}$; e: R=$C_{18}H_{37}$.

The data presented in Table 1 and Fig. 1 give information on the influence both of substituents at the quaternary nitrogen atom and of the length of the hydrocarbon radical on the antimicrobial action of cationic surfactants. The influence of the nature of the substituents at the nitrogen atom on the antimicrobial activity of synthesized QAB is most graphically revealed on the example of compounds Ia, IIa, and IIIa. The presence of a benzene ring in compound IIa seems to lengthen the alkyl chain, which leads to an intensification of the antimicrobial and surface activity (Tables 2 and 3). We observed the same effect when the length of

TABLE 1. Antimicrobial Activity of Cationic Surfactant

Compound	Minimum bacteriostatic concentrations, μg/ml			Minimum bactericidal concentrations, μg/ml		
	exposure, min					
	5	15	30	5	15	30
Ia	100	250	125	5000	4000	4000
Ib	62	62	31	2000	1000	1000
Ic	31	16	8	250	250	250
Id	1	1	0,5	62	62	62
Ie	2	2	2	500	500	500
IIa	62	31	16	1000	500	500
IIb	16	16	8	500	250	250
IIc	8	4	2	250	250	125
IId	1	1	0,5	125	125	62
IIIa	125	62	31	3000	3000	2000
IIIb	31	16	8	1000	1000	1000
IIIc	16	8	4	125	125	125
IIId	1	0,5	0,5	62	62	62

All-Union Scientific-Research Institute of Disinfection and Sterilization, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 18, No. 11, pp. 1344-1348, November, 1984. Original article submitted May 4, 1984.

 0091-150X/84/1811-0784$08.50

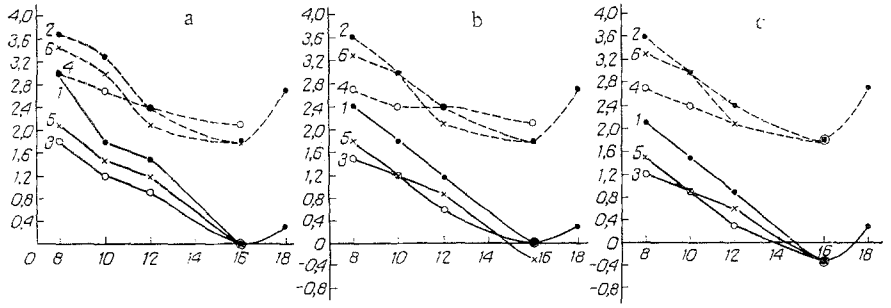


Fig. 1. Dependence of the antimicrobial activity of cationic surfactants on the length of the hydrocarbon radical. a) Exposure 5 min; b) exposure 15 min; c) exposure 30 min. Along x axis: n) the number of carbon atoms in the radical; along y axis: logarithm of the concentration, μg/ml. Ia-Ib) Bacteriostatic concentrations (1); Ia-Ie) bactericidal concentrations (2); IIa-IId) bacteriostatic concentrations (3); IIa-IId) bactericidal concentrations (4); IIIa-IIId) bacteriostatic concentrations (5); IIIa-IIId) bactericidal concentrations (6).

TABLE 2. Surface Tension of Aqueous Solutions of Alkyldimethylbenzylammonium Chlorides and Alkylbenzyltrimethylammonium Chlorides

Concentration, g/liter	Surface tension, σ, mN/m								
	Alkyldimethylbenzylammonium chlorides					Alkylbenzyltrimethylammonium chlorides			
	Ia	Ib	Ic	Id	Ie	IIa	IIb	IIc	IId
5,0	49,22	39,89	33,89	34,27	—	27,79	30,31	36,34	60,78
2,5	53,78	43,24	33,26	35,29	—	28,11	31,83	39,14	56,44
1.25	61.46	52,05	46,56	36,15	51,6	30,57	31,72	39,70	56,72
0,625	68.04	60,18	51,92	37,42	51,92	38,70	37,13	40,60	58,99
0,321	69.25	65,08	59,43	38,36	53,47	48,21	50,55	43,69	59,75
0,156	70,48	69,44	65,32	39,55	55,02	57,62	58,46	49,79	63,14
0,078	71.26	69,79	66,92	41,52	55,48	66,17	67,66	57,08	65,62
0,039				42,20	68,80	69,06	68,96	62,02	66,24
0,020				52,00	69,35	70,23	70,90	70,43	69,55
0,010				60,70	69,72	71,94	71,66	71,33	71,07

TABLE 3. Critical Micelle Concentrations of Alkyldimethylbenzylammonium Chlorides and Alkylbenzyltrimethylammonium Chlorides

Compound	CMC, g/liter
Ia	—
Ib	3,82
Ic	2,50
Id	0,62
IIa	1,20
IIb	0,91
IIc	0,35
IId	0,20

the hydrocarbon radical was increased by two methyl groups. However, in this case there are no sharp differences in the values of the bacteriostatic and bactericidal concentrations in a comparison of compounds Ib, c, IIb, c, and IIIb, c. The difference in activity between compounds Ib and IIb is the most distinct in the case of the bacteriostatic action of the preparations and affects primarily the rate of influence of the substances on the bacterial cell. The latter fact explains the higher bactericidal activity of compound IIIb in comparison with compound Ib in the case of a 5 min exposure.

The maximum bactericidal and bacteriostatic activity in all the homologous series was possessed by compounds with a radical containing 16 carbon atoms. In this case increasing the alkyl radical to 16 carbon atoms has a greater effect on the increase in the bactericidal activity of compounds Id and IIId than of compound IId. Compounds Id and IIId had a bactericidal effect after 5, 15, and 30 min contact with cells in a concentration of 62 μg/ml, whereas IId in this concentration produced 100% death of *E. coli* only after 30 min exposure. All the preparations with a C_{16} radical are characterized by inhibition of the growth of *E. coli* culture in extremely negligible concentrations (0.5-1.0 μg/ml), i.e., 8-16 times lower in the case of the compound with a C_{12} radical. Further increasing the length of the alkyl radical by two methyl groups leads to compound Ie, the antimicrobial activity of which is one-fourth the activity of Id. Evidently in the series studied, the compounds with a C_{16} radical are characterized by an optimum combination of hydrophobic, hydrophilic, and steric factors, which determine the minimal activity of these compounds. From the data obtained, it follows that cationic surfactants exert antimicrobial effects on bacterial cells in concentrations significantly below the critical micelle concentrations (CMC) of these compounds (see Tables 1, 2, and 3). This is an indication that cationic surfactants interact with the cells in monomer form, and the surface activity of the compounds may be of definite significance for the biological action of the surfactants. This conclusion agrees with the literature data on the fact that surface-active substances possess surface activity in the form of monomers, i.e., at concentrations below their CMC, when the formation of micelles is practically not observed [7, 9]. From this it follows that an unsubstantiated increase in the concentration of the surfactants during the creation of bactericidal compositions is inadvisable.

Thus, we studied the bacteriostatic and bactericidal activities in three homologous series of cationic surfactants, differing in the nature of the substituents at the quaternary nitrogen atom. Enlargement of the hydrocarbon radical, determining the hydrophobicity of the surfactant, depending on the structure of the compounds, has different effects on the change in their activity. In the series of alkyldimethylbenzylammonium chlorides, the antimicrobial activity of the preparation is determined by the size of the alkyl radical. Enlargement of the hydrophobic radical has less of an effect on the activity of alkylbenzyltrimethylammonium and alkyldiethoxybenzylammonium chlorides. This is an indication that the antibacterial activity of cationic surfactants is determined by the structure and properties of the molecule as a whole, which depend both on the hydrophobic and on the hydrophilic portions of the surfactant molecule and their mutual influence. In the series studied, the lowest activity was exhibited by the compound containing a radical with eight carbon atoms and the greatest by the compound containing a radical with 16 carbon atoms. Further increasing the size of the alkyl radical led to a decrease in the antimicrobial activity of the preparations.

EXPERIMENTAL CHEMISTRY

Dodecyldimethylbenzylammonium Chloride (Ic). A mixture of 18.6 g dodecyl alcohol and 0.75 g zinc chloride was heated in a flask to 140°C, and a stream of hydrogen chloride was passed through with mixing for 13 h. The dodecyl chloride formed was separated from the aqueous layer, washed with water, dried, and redistilled under vacuum. A 20.4-g portion of dodecyl chloride (with chloride content 17.3%) was loaded into a steel ampul, 4 moles of 40% aqueous dimethylamine solution was added, and the mixture exposed at 140-150°C for 16 h. Then dodecyldimethylamine was separated from the aqueous layer, washed with a 10% solution of NaOH, with water, and redistilled under vacuum. A mixture of 21.3 g dodecyldimethylamine, 12.6 g benzyl chloride, and 13.0 ml of water was heated in a flask to 60-65°C and exposed with mixing for 4 h. Yield was 67.5 g of an aqueous solution of dodecyldimethylammonium chloride with a 49.8% content of the active substance.

Octyl-, Decyl-, Hexadecyl-, and Octadecyldimethylbenzylammonium Chlorides Ia, b, d, e. These were produced analogously.

Decyldihydroxyethylbenzylammonium Chloride (IIIb). Decyl chloride was produced by the reaction of decyl alcohol with hydrogen chloride in the presence of zinc chloride. A mixture of 17.65 g (0.1 mole) decyl chloride, 31.5 g (0.3 mole) diethanolamine, and 36 g isopropyl alcohol was exposed in a steel ampul at 140°C for 5 h. The isopropyl alcohol was distilled, water was added to the residue, the tertiary amine was removed, redistilled under vacuum, and after redistillation subjected to interaction with benzyl chloride analogously to the production of Ic. Decyldihydroxyethylbenzylammonium chloride was produced in a form of an aqueous solution with a 48.95% content of the main substance.

Octyl-, Dodecyl-, and Hexadecyldihydroxyethylbenzylammonium Chlorides IIIa, c, d. These compounds were produced analogously.

Octylbenzyltriammonium Chloride (IIa). A mixture of 19.0 g octylbenzene, 3.9 g paraformaldehyde, 14.0 g zinc chloride, and 30 g acetic acid was heated in a flask with a reflux condenser to 70°C and a stream of hydrogen chloride was passed through for 6 h. Octylbenzyl chloride was washed with water to a neutral pH and redistilled under vacuum. A mixture of 23.85 g octylbenzyl chloride and 300 g of a 24.6% aqueous solution of trimethylamine was heated slowly with mixing to 60°C and exposed for 3 h. Octylbenzyltrimethylammonium chloride was obtained in the form of an aqueous solution with a 44.3% content of the main substance.

Decyl-, Dodecyl-, and Hexadecylbenzylammonium Chlorides (IIb-d). These were produced analogously.

EXPERIMENTAL BIOLOGY

For a determination of the bacteriostatic and bactericidal activities we used a suspension method, using a microbial load of 10^8 microbial cells in 1 ml and exposures of 5, 15, and 30 min at 37°C. To prepare the microbial suspension and dilutions of the preparations we used sterile 0.005 M Tris-SO_4 buffer, pH 7.5. After the corresponding exposure, 0.1 ml of the mixture was transferred under sterile conditions to test tubes each containing 5 ml of casein broth and placed in an incubator at 37°C. The experiments were conducted in eight repetitions. Growth of the cultures was monitored according to the change in the optical density of the broth samples on a photoelectrocolorimeter in comparison with growth of the control culture for 5-6 h on the day of the experiment, 24, 48 h, and 7 days after the experiment was set up.

In the course of the investigations we used a test culture of *E. coli* 1257, used in the All-Union Scientific-Research Institute of Disinfection and Sterilization for estimating antimicrobial activity of disinfectants. The minimum bacteriostatic concentration was considered to be the concentration that produced a lag in the growth of the culture in comparison with the control. The minimum bactericidal concentration was considered to be the concentration at which there was no growth in all the repetitions (24-32) for seven days.

The surface tension of the surfactant solutions was determined by the method of measuring the greatest pressure of a bubble, based on the fact that the surface tension of the investigated liquid is proportional to the greatest pressure when an air bubble is forced out of the capillary, immersed in the investigated liquid to a definite depth. The initial concentration was 5 g/liter (0.5% by weight). Subsequent concentrations were prepared by diluting the original solution.

LITERATURE CITED

1. A. A. Abramzov, Surface-Active Substances [in Russian], Leningrad (1981).
2. A. I. Gershenovich, V. A. Filimonov, L. N. Sergeeva, et al., in: All-Union Symposium on the Physicochemical Bases of the Use and Directed Synthesis of Surface-Active Substances Summaries of Results [in Russian], Tashkent (1974), pp. 140-141.
3. A. I. Gershenovich and V. V. Stefanovich, Khim. Prom-st', No. 6, 16-18 (1964).
4. O. V. Gudz' and G. T. Pis'ko, Antibiotiki, No. 9, 672-674 (1982).
5. V. Zablotskii, Novosti Farm. Med., No. 4, 175-180 (1976).
6. M. D. Mashkovskii, Drugs [in Russian], Eighth edn., Vol. 2, Moscow (1978), pp. 352, 365-367.
7. K. L. Mittel and P. Mukerdzhi, in: Micelle Formation, Solubilization, and Microemulsions [in Russian], Moscow (1980), p. 11-31.
8. A. G. Nekhorosheva, Antimicrobial and Disinfectant Properties of Certain Cationic Surfactants and Compositions Based on Them. Author's Abstract of Candidate's Dissertation [in Russian], Moscow (1971).
9. A. G. Florens, in: Micelle Formation, Solubilization, and Microemulsions [in Russian], Moscow (1980), p. 42-62.
10. M. A. Chernyavskaya, A. S. Belova, and V. V. Stefanovich, in: Theory and Practice of Disinfection and Sterilization [in Russian], Moscow (1983), pp. 30-34.
11. A. E. Épshtein, Synthesis of Bactericidal Quaternary Ammonium Salts Containing Hydrophobic Radicals. Candidate's Dissertation [in Russian], Moscow (1978).