SYNTHESIS AND ANTIBACTERIAL ACTIVITY

OF SEVERAL BISAMMONIUM COMPOUNDS

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In the midst of compounds possessing high antibacterial properties and therefore having broad application, compounds from the group of monoquaternary ammonium salts of the following type should be noted:



where R^1 , R^2 , R^3 are relatively short alkyls (CH₃, C₂H₅, C₆H₅CH₂); R is a long-chain alkyl containing from eight to 18 atoms; X⁻ is most frequently a halide ion [1-4]. The preference for these compounds amongst existing disinfecting compounds (chloroactive compounds and phenol derivatives) is because they are very soluble in water, do not have an odor, and, showing high antibacterial effect, do not corrode metallic objects.

At present, the antibacterial activity of monoquaternary ammonium compounds has been studied quite completely, but only isolated works have been devoted to the study of the antibacterial properties of the bisammonium preparations, from which it is known that the latter compounds show a strong antibacterial effect [5, 6].

With this goal, it seem promising to synthesize the bisammonium salts (I-XXI), which are analagous in structure to the enumerated monoquaternary compounds

$$\begin{bmatrix} R (CH_3)_2 \dot{N} (CH_2)_n \dot{N} (CH_3)_2 R \end{bmatrix} 2 \overline{X}$$

where R is the long-chain alkyl containing from eight to 16 carbon atoms; X^- is the halide ion; n is a whole number, and also to study the antibacterial activity as a dependence on chemical structure.

It was shown [2] that the maximum antibacterial activity in a series of monoquaternary compounds is shown by compounds containing a cetyl radical. Therefore, it was interesting to clarify if this rule is also observed for bisquaternary compounds. It was important to establish what optimum distance between the nitrogen atoms guarantees the highest antibacterial effect.

Data on the synthesized compounds are presented in Table 1.

Synthesis of the compounds was accomplished by two methods: from diamines which were transformed to the corresponding tetramethylditertiarydiamines by the Eschweiler-Clarke method with subsequent condensation with alkyl halides (method A), and from diols by their conversion to the α, ω -dihaloalkyls, which were then condensed with the corresponding tertiary amines (method B).

The antibacterial activity of the synthesized bisquaternary compounds was studied in relation to two forms of microorganisms, <u>Escherichia coli</u> and <u>Staphylococcus</u> <u>aureus</u>, by the generally accepted method of disinfecting infested batiste test objects.

It is known that antibacterial activity appears in monoquaternary ammonium compounds with the introduction of an octyl radical. In this connection, a series of bisquaternary salts having long-chain radicals

All-Union Research Institute of Chemical Reagents and Particularly Pure Chemical Compounds and Central Research Disinfection Institute, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, No. 5, pp. 15-18, May, 1968. Original article submitted September 26, 1967.

UDC 615.777.12-012.5

Compound No.	R	n	x	Method of preparation	Melting point (degre e s)	Yield (%)	Found (%)			Calcu- lated (%)		Antibacterial effect of the compound	
							N	Br or Cl	Empirical formula	N	Br of Cl	Concentration (%)	Staphyloco- [] 'dx a cus aureus Escherichia [] (u'dx coli
I	C8H11	6	Br	A	202-2,5	50,3	4,86	28,40	C28H58Br2N2	5,01	28,61	0,1	15 25
11	C9H19	6	Br	A	2077,5	72,7	4,65	26,90	C ₂₈ H ₄₂ Br ₂ N ₂	4,77	27,24	0.05	15 30
III	C10H21	6	Br	Α	2166,5	71,8	4,37	25,82	C _{so} H _{ee} Br ₂ N ₂	4,55	26,00	0,025	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
IV	C11H23	6	Br	Α	2201	68,3	4,23	24,71	C32H70Br2N2	4,35	24,86	0,025	10 15
v	C12H25	6	Br	А	225	70,4	3,97	28,68	C34H74Br2N2	4,17	23,82	0,025	
VI	C18H33	6	Br	Α	220	78	3,42	20,49	$C_{42}H_{90}Br_2N_2$	3,57	20,41	0,1	20 30
VII	$C_{11}H_{23}$	2	Br	A	102-3	62	4,56	27,45	C ₂₈ H ₆₂ Br ₂ N ₂	4,77	27,24	0,01	15 10
V111	C12H25	2	Br	А	1934	76,1	4,32	26,31	$C_{30}H_{66}Br_2N_2$	4,55	26,01	0,005	15 20
١X	C16H33	2	Br	А	191-2	79,3	3,58	21,82	C ₃₈ H ₈₂ Br ₂ N ₂	3,85	21,98	0,025	
х	$C_{12}H_{25}$	3	Br	А	2089	74	4,51	25,12	C ₃₁ H ₆₈ Br ₂ N ₂	4,45	25,42	0,025	10 15
ХI	$C_{12}H_{25}$	4	Br	A	216-7	70,6	4,15	24,53	C ₃₂ H ₇₀ Br ₂ N ₂	4,35	24,86	0,025	15 20
ХП	$C_{22}H_{2\delta}$	5	Br	В	221-2	63,8	4,43	24,12	$C_{33}H_{72}Br_2N_2$	4,26	24,33	0,025	10 25
хш	CH3	10	Br	В	248-8,5	76,2	6,55	38,57	$\mathrm{C_{10}H_{38}Br_{2}N_{2}}$	6,71	38,49	0,2	20 +
X1V	C_6H_{13}	10	Br	В	119	41	4,85	28,32	C ₂₆ H ₅₈ Br ₂ N ₂	5,01	28,61	0,2	
xv	$C_{12}H_{35}$	10	Br	В	1256	69	4,07	21,73	C _{ss} H _{s2} Br ₂ N ₂	3,86	21,98	0,025	10 15
XVI	CH ₈	20	Br	В	235-7	81,3	4,85	28,88	C ₂₆ H ₅₈ Br ₂ N ₂	5,01	28,61	0,025	5 15
XVII	C_8H_{17}	20	Br	В	116-7	78	3,53	20,75	C40H86Br2N2	3,71	21,17	0,05	15 30
xviii	$\mathrm{C_{12}H_{2\delta}}$	20	Br	В	169-70	77,6	2,94	18,63	C48H102Br2N2	3,23	18,43	0,01	10 30
XIX*	$C_{9}H_{19}$	6	Cl	А	205-7	57,4	5,82	13,99	$C_{28}H_{62}Cl_2N_2$	5,63	14,24	0,01	15 15
XX*	$C_{10}H_{21}$	6	сı	A	211-2	62,3	5,04	13,26	$\mathrm{C_{30}H_{B6}Cl_2N_2}$	5,32	13,48	0,025	5 10
XXI*	$C_{12}H_{2b}$	6	CI	A	214 - 5	59.7	4,69	12,32	C ₃₄ H ₇₄ Cl ₂ N ₂	4,81	12,18	0,01	10 15
ххн	Trimethyl- cetylammo- nium bromide				23941	85	Lite	ra- data				0,025	\$ 15

TABLE 1. Characteristics of Compounds (I-XXII)

Note. + compounds in the given concentration are ineffective: the asterisk signifies that the compound is hygroscopic.

from octyl to cetyl, inclusively, was synthesized. As the starting ditertiarydiamine we used N, N, N^1 , N^1 -tetramethylhexamethylenediamine as the most accessible hexamethylenedramine. Here it was found that the antibacterial activity of bisquaternary compounds having an octyl residue (I) is not large, but it increases sharply with an increase in the length of the radical, reaching a maximum in compounds having a dodecyl residue (V).

A sharp fall in antibacterial effect is observed with further growth of the radical to a cetyl (VI). In order to verify this fact on other examples, several analagous chlorides (XIX-XXI), and also bisquaternary compounds based on ethylenediamine (VII-IX) were investigated.

However, here also the maximum antimicrobic activity corresponds to the compound having two dodecyl residues. Consequently, in contrast to the corresponding monoquaternary compounds, the maximum antibacterial effect in bisquaternary compounds corresponds to the compound having a dodecyl and not a cetyl radical. The second characteristic of bisquaternary compounds is that, in contrast to monoquaternary compounds, the effect of the anion in them shows up significantly less on the antibacterial activity.

However, here also (cf. compounds (II), (III), (V) with (XIX-XXI)), the compounds containing a chloride anion react more strongly than the corresponding bromides of bisquaternary salts.

It was not any less interesting to clarify the effect of the distance between the cationic centers on the antibacterial activity of the bisquaternary salts. Here, the corresponding compounds with n = 2-6, 10, 20

with the dodecyl radical were investigated. It was found that the antibacterial activity of the compounds gradually decreases with increase of n.

Consequently, compounds having a possible smaller distance between the nitrogen atoms are of interest in increasing the antibacterial effect of bisammonium compounds. It should be noted that upon going from compounds having a greater distance between nitrogen atoms, for example, at n = 20, the methylene bridge between nitrogen atoms becomes like a long-chain alkyl. In this case, the antibacterial effect of the compound improves upon decreasing the size of R (XVI-XVIII). At n = 10, evidently, the length of the methylene bridge is still too small to produce an antibacterial effect of the compounds; therefore, for compounds of this type a normal regularity for bisammonium compounds is observed: the maximum effect corresponds to compounds having a dodecyl radical (XIII-XV).

It is also necessary to note that upon comparing compound (VIII), which shows the best results in its antimicrobic activity among bisquaterary compounds, with the monoquaternary compound (XXII), it is seen that the latter is significantly weaker than the bisquaternary. It is interesting to note that the most active monoquaternary compound containing a cetyl radical (XXII) is similar in its biological activity to the bisquaternary compound (VIII).

The studied group of bisquaternary compounds almost does not show a selective effect. The greater portion of compounds are equally active, both in relation to Gram-negative and Gram-positive micro-organisms, which undoubtedly is their advantage over monoquaternary compounds.

EXPERIMENTAL

Synthesis of bisquaternary salts was accomplished by two methods: reaction of ditertiary amines with alkyl halides (method A) and reaction of α , ω -dihalo compounds with tertiary amines (method B). The ditertiary amines based on 1,2-dimethylene-, 1,3-trimethylene-, 1,4-tetramethylene-, and 1,6-hexamethylenediamines were obtained by the Eschweiler-Clarke reaction [7, 8] from the reaction of the mentioned diamines with formaldehyde and formic acid. Synthesis of N,N,N¹, N¹ tetramethyltrimethylenediamine by the Eschweiler-Clarke method was carried out by us for the first time.

The tertiary amines dimethylhexyl- and dimethyloctylamines [9] and dimethyldodecylamine [10] were obtained by the reaction of secondary amines with the corresponding alkyl halides. The alkyl halides nonyl-, decyl-, and dodecyl chlorides were obtained by reaction of thionyl chloride with the corresponding alcohol in the presence of dimethylformamide [11]. Octyl-, nonyl-, decyl-, undecyl-, and dodecyl bromides [12] and the α, ω -dihalo compounds such as 1,10-dibromodecane [13] and 1,20-dibromoeicosane [14] were obtained by the reaction of the corresponding alcohols and glycols with a 43% solution of hydrobromic acid, with the exception of 1,4-dibromobutane, which was obtained by the reaction of tetrahydrofuran with sodium bromide in concentrated sulfuric acid [15].

A. A solution of 9.96 g (0.04 mole) of dodecyl bromide in 15 ml of absolute ethanol was added with stirring to a solution of 3.44 g (0.02 mole) of N,N,N¹, N¹ tetramethylhexanediamine in 10 ml of absolute ethanol heated to 50°C. The reaction mixture was heated for 6 h with a reflux condenser at 90-100°C. After cooling, the precipitated white solid was washed with dry ether and recrystallized two times from acetone with the addition of several drops of absolute ethanol.

B. We heated 2.29 g (0.01 mole) of 1,5-dibromopentane with 6.39 g (0.03 mole) of dimethyldodecylamine in 10 ml of absolute ethanol for 8 h at 90°C. The obtained brown-colored precipitate was dissolved in absolute ethanol and the material was precipitated with dry ether, then recrystallized from acetone.

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