EXPERIMENTAL BIOLOGY

The antimicrobial activity of the compounds synthesized was studied in experiments *in vivo* [2], by the method of double serial dilutions in liquid culture medium, using as examples 16 strains of various types of bacterial (see Table 3).

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SYNTHESIS AND BACTERICIDAL ACTIVITY OF CATIONIC SURFACE-ACTIVE AGENTS CONTAINING AN ASYMMETRIC NITROGEN ATOM

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At present, great attention is being paid to the interrelationship between the chemical structure of surface-active agents (SAA) and their bactericidal activity. The change in the antimicrobial activity of SAA has been studied in relation to the length of the hydrocarbyl radical [2, 6], the nature of the anion [4, 5, 7], the presence of unsaturated bonds [1, 3] and certain other factors.

The aim of the present work was to synthesize cationic SAA containing an asymmetric nitrogen atom in the molecule, and to study their bactericidal activity.

For the investigation, we chose hydrochlorides of tertiary amines with asymmetric nitrogen atoms with the following structures

$RR'R^2N$ (I — IX; R = everywhere dodecyl; I: $R^1 = CH_3$, $R^2 = C_2H_5$; II: $R^1 = CH_3$, $R^2 = C_3H_7$; III: $R^1 = CH_3$, $R^2 = C_4H_9$; IV: $R^1 = CH_3$, $R^2 = \beta\text{-}(CH_2)_2OH$; V: $R^1 = CH_3$ $R^2 = \beta\text{-}(CH_2)_2CN$; VI: $R^1 = CH_3$, $R^2 = \beta\text{-}(CH_2)_2CONH_2$; VII: $R^1 = CH_3$, $R^2 = \beta\text{-}(CH_2)_2OCOCH_3$; VIII: $R^1 = \beta\text{-}(CH_2)_2CN$, $R^2 = \beta\text{-}(CH_2)_2OH$; IX: $R^1 = CH_3$, $R^2 = CH_2C_6H_5$).

For comparison we studied the bactericidal activity of hydrochlorides of amines with the same lower radicals of type

RR^1_2N (X — XVII; R = everywhere dodecyl; X: $R^1 = CH_3$; XI: $R^1 = C_2H_5$; XII: $R^1 = C_3H_7$; XIII: $R^1 = C_4H_9$; XIV: $R^1 = \beta\text{-}(CH_2)_2OH$; XV: $R^1 = \beta\text{-}(CH_2)_2OCOCH_3$; XVI: $R^1 = \beta\text{-}(CH_2)_2CN$; XVII: $R^1 = \beta\text{-}(CH_2)_2CONH_2$).

The compounds were synthesized by the reaction of secondary derivatives of dodecylamine with an alkyl halide or derivatives of acrylic acid.

Since the boiling points of the tertiary amines obtained and those of the initial secondary alkylmethylamines are similar, the usual method for the separation of these compounds at the end of the reaction by distillation was found to be unsuitable. To separate the tertiary amines, the reaction mixture was treated with a mixture of acetic acid and acetic anhydride, excess of unreacted acetylating agent was removed, and the tertiary amines were isolated in the form of hydrochlorides. Dry hydrogen chloride was passed through the solution of the reaction mixture in ether or petroleum ether, or the reaction mixture was dissolved in a minimal amount of hydrochloric acid, and the impurities were removed by extraction by ether or petroleum ether from the aqueous solution. The free amines were isolated from the hydrochlorides by treatment with aqueous alkali, followed by the distillation of the organic layer. The characteristics of the newly obtained tertiary amines are given in Table 1, and of the

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TABLE 1. Tertiary Amines

Compound	Yield, %	bp, °C (mm Hg)	n_D^{24}	Found, %		Empirical formula	Calculated, %	
				C	H		C	H
I	83	128—129/3	1,4372	79,32	14,75	$C_{15}H_{33}N$	79,22	14,63
II	51	156—158/10	1,4408	79,74	14,53	$C_{16}H_{35}N$	79,59	14,61
III	45	163—165/5	1,4412	80,11	14,73	$C_{17}H_{37}N$	79,92	14,60
IV	64	155—158/2	1,4419	74,18	13,74	$C_{15}H_{33}NO$	74,01	13,67
V	98	182—183/5	1,4498	76,27	12,89	$C_{16}H_{32}N_2$	76,13	12,78
VI*	52,2	—	—	71,31	12,74	$C_{16}H_{34}N_2O$	71,06	12,67
VII	45	179—182/9	1,4508	71,68	12,43	$C_{17}H_{35}NO_2$	71,53	12,36
VIII	57	125—126/6	1,4360	72,14	12,01	$C_{17}H_{34}N_2O$	72,29	12,13
IX	87	198—201/10	1,4852	83,15	12,25	$C_{20}H_{35}N$	82,98	12,19
XII	65	151—153/5	1,4421	80,29	14,67	$C_{18}H_{39}N$	80,22	14,59
XIII	83	190—192/12	1,4468	81,05	14,73	$C_{20}H_{43}N$	80,73	14,57
XIV	86,6	193—195/3	1,4614	70,02	13,06	$C_{16}H_{35}NO_2$	70,28	12,90
XV	39	192—196/6	1,4318	67,38	11,13	$C_{20}H_{39}NO_4$	67,19	11,00
XVI†	85,7	215—220/3	—	74,36	11,53	$C_{18}H_{33}N$	74,18	11,41
XVII‡	95	—	—	66,13	11,44	$C_{18}H_{37}N_3O_2$	66,01	11,39

*The product is a paste.
†The product solidifies.
‡mp 105-107°C.

TABLE 2. Hydrochlorides of Tertiary Amines

Initial amine	Yield, %	mp, °C	Analysis for chlorine		
			found, %	Empirical formula	Calculated, %
I	95	138—139	13,32	$C_{15}H_{34}ClN$	13,45
II	93	Paste	12,96	$C_{16}H_{36}ClN$	12,77
III	98	102—103	12,28	$C_{17}H_{38}ClN$	12,16
IV	74	157—159	12,55	$C_{15}H_{34}ClNO$	12,68
V	72	75—76	12,11	$C_{16}H_{35}ClN_2$	12,29
VI	89	174—176	11,69	$C_{16}H_{35}ClN_2O$	11,57
VII	67	120—122	10,89	$C_{17}H_{36}ClNO_2$	11,03
VIII	58	145—147	10,96	$C_{17}H_{35}ClN_2O$	11,13
IX	77	Paste	11,02	$C_{20}H_{36}ClN$	10,89
X*	94	181—183	14,37	$C_{14}H_{32}ClN$	14,21
XI†	97	117—119	—	$C_{16}H_{36}ClN$	—
XII‡	92	41—45	11,83	$C_{18}H_{40}ClN$	11,60
XIII	81	Paste	10,89	$C_{20}H_{44}ClN$	10,63
XIV	87	74—76	11,47	$C_{16}H_{36}ClNO_2$	11,61
XV	79	84—86	8,93	$C_{20}H_{40}ClNO_4$	9,01
XVI	84	64—66	10,81	$C_{18}H_{34}ClN_3$	10,83

Note. + means that in the given concentration the compound is ineffective. Remainder of legend the same as in Table 1.

hydrochlorides in Table 2. It should be noted that the hydrochlorides of most of the tertiary amines that we obtained were found to be soluble in nonpolar solvents, such as benzene or ether. This is particularly characteristic of compounds with lower radicals containing four or more carbon atoms.

As salts of tertiary amines readily racemize in aqueous solutions, we did not attempt to isolate optically active compounds.

The antimicrobial activity of the compounds obtained was studied by generally accepted procedure, by disinfecting batiste test objects according to the requirements of the "Instructions for the determination of bactericidal properties of new disinfecting agents" approved by the Ministry of Public Health of the USSR in 1968. As model test organisms we used *St. aureus* strain No. 906 and *E. coli* strain No. 1257. The results of the investigation are given in Table 3. It was found that even when compounds with a similar structure are compared, the bactericidal activity of compounds containing an asymmetric nitrogen atom is higher than that of salts of dodecylalkylamines with the same lower radicals. Hydrochlorides of X and XI are effective for vegetative forms of microorganisms in a concentration of 0.05% in the course of 15 min and are inactive in a concentration of 0.025% towards gram-negative bacteria, while the hydrochloride of I causes the destruction of the test organisms in a concentration of 0.05% in the course of 5 min, and in a concentration of 0.025% in the course of 15-25 min (compare compounds X and XI with compound I, see Table 3). Compounds with a

TABLE 3. Bactericidal Activity of Hydrochlorides of Tertiary Amines

Initial amine	Concentration, %	Time of destruction, min	
		St. aureus	E. coti
I	0,05	5	5
	0,025	15	25
II	0,05	5	5
	0,025	15	25
III	0,05	5	5
	0,025	5	5
	0,01	20	+
IV	0,05	5	5
	0,025	10	15
V	0,05	5	5
	0,025	20	20
VI	0,05	15	25
	0,025	20	30
VII	0,05	5	5
	0,025	10	10
VIII	0,05	10	5
	0,025	20	15
IX	0,05	15	30
	0,025	25	+
X	0,05	15	25
	0,025	25	+
XI	0,05	10	15
	0,025	30	+
XII	0,05	30	25
XIII	0,05	25	20
XIV	0,1	20	25
XV	0,1	20	20
XVI	0,1	20	20
XVII	0,05	20	15

Note. + means that in the given concentration the compound is ineffective.

large difference in the length of the lower radicals, for example, on transition from hydrochloride of I to hydochloride of IV (see Table 3), or those containing functional groups in the composition of the molecule, for example, an ester group (compound IV in Table 3) are still more effective than dodecyl(alkyl)amines with the same lower radicals (compare compounds X and XV with compound VII in Table 3).

We were thus the first to find that the introduction of an asymmetric nitrogen atom into the composition of the molecule of a cationic SAA leads to a marked increase in the bactericidal action of the compounds, compared with compounds with a similar chemical structure without an asymmetric nitrogen atom. The reason for this phenomenon can be conclusively clarified only after the mechanism of the action of the compounds on the microbial cell has been studied.

EXPERIMENTAL

Dodecyldibutylamine Hydrochloride (XIII). A mixture of 10 g (0.049 mole) of dodecyl chloride, 15 g (0.116 mole) of dibutylamine and 25 ml of isopropanol is heated for 16 h in a sealed ampule at 130°C. The mixture is cooled and the contents of the ampule are made alkali to a strong alkaline reaction. The mixture is extracted with ether, and after removal of the solvent, the amine obtained is distilled *in vacuo*. bp 192-195°C/12 mm, n_D^{24} 1.4468. Yield 6.9 g (83%). Found, %: C 81.05; H 14.73. $C_{20}H_{43}N$. Calculated, %: C 80.72; H 14.57. A 2-g portion of the amine is dissolved in hexane and a current of dry hydrogen chloride is passed through the solution. The oil which precipitates is separated, and the remaining solvent is removed *in vacuo*. Yield 2.2 g (97%). Paste, which solidifies after prolonged standing at room temperature. Because of strong hygroscopicity, the melting point of the product could not be determined. The compound is soluble in most organic solvents, including ether and benzene. Found, %: Cl 10.89. $C_{20}H_{44}ClN$. Calculated, %: Cl 10.63.

Dodecylmethylbutylamine Hydrochloride (III). A mixture of 20 g (0.1 mole) of dodecylmethylamine, 7 g (0.05 mole) of butyl bromide, and 30 ml of isopropanol is boiled for 12 h. The mixture is cooled, and 15 ml of 10% aqueous alkali are added to the reaction mixture, which is then extracted 5 times with ether. The solvent is removed, and to the residue a

mixture of 10 ml of acetic acid and 8 ml of acetic anhydride is added. The reaction mixture is left to stand for two days at 20-25°C. Excess of acetylating agent is removed *in vacuo*. To the residue, 10 ml of concentrated hydrochloric acid are added, the impurities are removed from the aqueous solution by extraction with ether, and the aqueous layer is separated and distilled *in vacuo*. The boiling point of dodecylmethylbutylamine is 163-165°C/5 mm, n_D^{24} 1.4412. Yield 5.6 g (45%). Found, %: C 80.11; H 14.73. $C_{17}H_{37}N$. Calculated, %: C 79.92; H 14.60. An aliquot portion of the amine obtained is dissolved in a minimal amount of concentrated hydrochloric acid, the impurities are extracted from the aqueous concentrate by ether, and water is distilled *in vacuo*. The residue is reprecipitated from ether by petroleum ether. The product is recrystallized from acetone. mp 102-103°C. Yield 89%. Found, %: Cl 12.28. $C_{17}H_{38}ClN$. Calculated, %: Cl 12.16.

Dodecylmethyl(β-cyanoethyl)amine Hydrochloride (V). A mixture of 1.14 g (0.057 mole) of dodecylmethylamine and 10 g (0.14 mole) of acrylonitrile is distilled, and the tertiary amine is distilled *in vacuo*. bp 182°C/5 mm, n_D^{24} 1.4498. Yield, 14.1 g (98%). Found, %: C 76.27; H 12.89. $C_{16}H_{32}N_2$. Calculated, %: C 76.13; H 12.78. The hydrochloride is obtained from the tertiary amine by passing a current of dry hydrogen chloride through a solution of the amine in ether. The precipitate is filtered and purified by reprecipitation from alcohol by ether, mp 75-76°C. Found, %: Cl 12.11. $C_{16}H_{33}ClN_2$. Calculated, %: Cl 12.29.

Dodecyl(β-cyanoethyl)(β-hydroxyethyl)amine hydrochloride (VIII). A mixture of 6 g (0.026 mole) of dodecyl(β-hydroxyethyl)amine and 15 g (0.3 mole) of acrylonitrile is boiled for 3 h. When cool, the mixture is acetylated as described in the preceding experiment. Dodecyl(β-cyanoethyl)(β-hydroxyethyl)amine is isolated by distillation *in vacuo*. bp 125-126°/6 mm, n_D^{24} 1.4360. Yield 3.8 g (57%). Found, %: C 72.14; H 12.25. $C_{17}H_{34}N_2O$. Calculated, %: C 72.29; H 12.13. The hydrochloride of the tertiary amine is obtained by the above procedure. mp 145-147°C. Yield 58%. Found, %: Cl 10.96. $C_{17}H_{35}ClN_2O$. Calculated, %: Cl 11.13.

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