In case compounds showing low activity in vitro should prove to be active in vivo, two compounds inactive in vitro (IIIa and IIIb) were examined in vivo in all three models. Negative results were obtained in single oral doses of up to 400 mg/kg 30 min before infection. In tests on healthy mice, both compounds, like (VI), were well tolerated in single doses of up to 500 mg/kg.

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SYNTHESIS AND BACTERICIDAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS CONTAINING AN ASYMMETRIC NITROGEN ATOM

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Quaternary ammonium compounds (QAC) containing one or two higher radicals in the molecule have long been used as antimicrobial agents in medical practice. Several important patterns have been established with regard to the relationship between the chemical structure of the QAC and their bactericidal activity [2, 3, 8, 11, 12, 17] making it possible to effect the industrial production of several similar products.

It was found that hydrohalides of higher tertiary amines with an asymmetric nitrogen atom in the molecule have much higher bactericidal activity than similar compounds not containing such an atom [4]. It was of interest to find out whether such a pattern also holds for the corresponding QAC.

As starting compounds for the synthesis of these QAC, we used dodecylmethylamine. Several methods of the preparation of these amines have been described having the disadvantage of low yield (not more than 50%) [6, 7, 13, 15] and the necessity of using special apparatus or difficultly obtainable reagents [1, 5, 10, 14, 16]. We have developed a simple and convenient laboratory method for the preparative synthesis of higher alkylmethylamines. It was proposed to hold a mixture of a 5-fold excess of an alcoholic solution of methylamine with a higher alkyl halide at room temperature. When alkyl bromides are used, the mixture is held for 2-3 days, while in the case of alkyl chlorides - for 6-8 days. The yield of the desired end products is thereby 90-94%. The corresponding tertiary amines were synthesized by alkylation of alkylmethylamine with an alkyl halide having a short carbon chain, or by the reaction of the secondary amine with acrylic acid derivatives. In the first case, the reaction was carried out in the presence of powdered alkali acting as a hydrogen halide acceptor. The tertiary amines could thus be obtained in 65-90% yield, using equimolecular amounts of the reagents. It should be noted that the thus obtained tertiary amines, which are distinguished from the starting dodecylmethylamines only by the presence of a radical with a short carbon chain, had boiling points in practically the same temperature range. Therefore, the tertiary amines were separated from their mixture with secondary amines using chemical methods. For this purpose the reaction mixture was acetylated with a mixture of AcOH and Ac_2O, the excess of unreacted acetylating agent was removed, and the tertiary amine was extracted by a dilute solution of an acid, the aqueous extract was made alkaline to pH 9-10, the tertiary amine was separated from the aqueous solution by extraction with an organic solvent, and the desired end product was isolated by a conventional method. The quaternization was carried out by the reaction of the tertiary amine with an alkyl halide in the presence of a solvent, or without it. The temperature of carrying out the reaction varied from room temperature to the boiling point of the solution. The QAC were separated from the reaction mixture by precipitation

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TABLE 1. Physicochemical Properties of Compounds I-XXXV

Compound	Yield, %	mp, °C	Empirical formula
I	87	—	$C_{18}H_{40}NBr$
II	85	—	$C_{19}H_{42}NBr$
III	86	—	$C_{19}H_{42}NBr$
IV	54	—	$C_{21}H_{46}NBr$
V	57	—	$C_{23}H_{50}NBr$
VI	65	—	$C_{25}H_{54}NBr$
VII	73	—	$C_{31}H_{66}NBr$
VIII	88	103—105	$C_{22}H_{40}NBr$
IX	76	77—78	$C_{24}H_{44}NBr$
X	54	—	$C_{18}H_{38}NBr$
XI	42	—	$C_{18}H_{36}NBr$
XII	45	—	$C_{19}H_{36}NBr$
XIII	84	—	$C_{17}H_{38}NBr$
XIV	55	—	$C_{18}H_{37}N_2Br$
XV	85	102—103	$C_{17}H_{36}NBrO_2$
XVI	59	54—56	$C_{20}H_{40}NBrO_2$
XVII	73	50—52	$C_{24}H_{42}NBrO_2$
XVIII	81	175—176	$C_{18}H_{40}NBrO$
XIX	39	—	$C_{17}H_{37}NBr_2$
XX	47	—	$C_{18}H_{41}N_2Br$
XXI	59	—	$C_{20}H_{43}N_2BrO$
XXII	67	173—174	$C_{28}H_{52}NBrO$
XXIII	63	59—60	$C_{22}H_{39}N_2BrO_3$
XXIV	44	—	$C_{25}H_{44}NBrO_2$
XXV	76	142—143	$C_{22}H_{39}NBrO_2$
XXVI	52	—	$C_{23}H_{42}NBr$
XXVII	89	—	$C_{17}H_{38}NBr$
XXVIII	91	122—123	$C_{18}H_{40}NBr$
XXIX	63	—	$C_{22}H_{40}NBr$
XXX	92	228—230	$C_{15}H_{34}NBr$
XXXI	84	—	$C_{21}H_{46}NBr$
XXXII	89	97—98	$C_{19}H_{42}NBr$
XXXIII	66	108—109	$C_{24}H_{42}NBrO_2$
XXXIV	63	—	$C_{27}H_{42}NBr$
XXXV	74	—	$C_{21}H_{37}N_2BrO_2$

Note. Compounds I-VII, X-XIV, XIX-XXI, XXIV, XXVI, XXVII, XXXI, XXXIV, XXXV are pastes or amorphous substances. The results of their elemental analysis data correspond to the calculated values.

with an excess of dry ether from a minimal amount of an alcoholic solution, or by purification by the Stepanenko method [9].

A method has been developed for the preparation and purification of tertiary amines containing phenol radicals in the molecule. The QAC obtained from these amines are odorless, have good detergent properties and are readily soluble in water. In their physicochemical properties, the QAC containing an asymmetric nitrogen atom in the molecule, differ considerably from the corresponding analogs with a symmetric structure. Most of these compounds do not have a crystalline structure but are thick pastes of amorphous products. These QAC are in general soluble not only in polar, but also in several nonpolar solvents, such as, for example, benzene and ether. Some of the compounds are hygroscopic. All this hinders their isolation in a pure state and results in a decrease in yield.

The synthesis was carried out of the following QAC of the general formula $RR^1R^2R^3N^+X^-$, where R is always $C_{12}H_{25}$, R^1 = Me (except for compounds XXVIII, XXXI, and XXXII); R^2 = Et (I-VIII, X, XI, XIII-XV, XVIII-XXVIII, XXXII), n-Bu (IX), $CH_2CH{=}CH_2$ (XII, XVI), CH_2Ph (XVII, XXXIV), Me (XXIX, XXX, XXXIII, XXXV), Pr (XXXI); R^3 = Pr (I, XXXI, XXXII), Bu (II), tert-Bu (III), C_6H_{13} (IV), C_8H_{17} (V), $C_{10}H_{21}$ (VI), $C_{16}H_{33}$ (VII), CH_2Ph (VIII, IX), $CH_2CH{=}CH_2$ (X), $CH_2C{\equiv}CH$ (XI, XII), $(CH_2)_2OH$ (XIII), $(CH_2)_2CN$ (XIV), CH_2COOH (XV), $(CH_2)_2COOMe$ (XVI, XVII), CH_2OEt (XVIII), $(CH_2)_2Br$ (XIX), γ-$NH_2C_3H_6$ (XX), γ-$AcNHC_3H_6$ (XXI), 3,5-$(i\text{-}Pr)_2$-4-OHC_6H_2 (XXII), 2-NO_2-4-OHC_6H_3 (XXIII), 2-Me-5-$MeCOOC_6H_3CH_2$ (XXIV), 4-$NO_2C_6H_4$ (XXV, XXXV), MePhCH (XXVI, XXIX), Et (XXVII, XXVIII), Me (XXX), 2-Me-5-$AcOC_6H_3CH_2$ (XXXIII); X = Br.

TABLE 2. Bactericidal Activity of Quaternary Ammonium Compounds

Compound	Concentration, %	Time of destruction, min	
		S. aureus	E. coli
I	0,025	5	5
	0,01	10	20
II	0,025	15	15
	0,01	30	+
III	0,025	20	25
IV	0,025	10	20
V	0,025	20	30
VI	0,1	5	30
	0,05	10	+
VII	0,1	30	+
VIII	0,025	15	15
IX	0,025	10	20
	0,01	20	30
X	0,025	10	20
	0,01	20	25
XI	0,025	15	20
XII	0,025	5	15
	0,01	15	25
XIII	0,025	5	5
	0,01	5	15
	0,005	20	+
XIV	0,05	30	+
XV	0,1	20	15
XVI	0,1	10	25
	0,05	15	+
XVII	0,1	15	+
XVIII	0,025	15	15
	0,01	25	30
XIX	0,025	15	30
XX	0,025	10	15
XXI	0,025	15	25
XXII	0,025	5	10
	0,01	10	20
XXIII	0,025	10	25
XXIV	0,025	5	15
	0,01	15	20
XXV	0,025	5	15
XXVI	0,05	5	20
	0,025	20	+
XXVII	0,025	20	30
XXVIII	0,025	15	30
XXIX	0,05	15	20
	0,025	+	+
XXX	0,025	10	15
	0,01	30	+
XXXI	0,1	10	+
	0,05	20	+
XXXII	0,025	10	20
XXXIII	0,025	15	30
XXXIV	0,025	10	+
XXXV	0,025	10	25

Note. Sign + means that a compound is inactive in the given concentration during an exposure for 30 min.

The characteristics of the newly obtained compounds are given in Table 1.

The antimicrobial activity of the QAC was studied by a generally accepted procedure, by disinfecting batiste test-samples according to the requirements of the "Instructions on the determination of bactericidal properties of new disinfecting agents," approved by the Ministry of Public Health of the USSR in 1968. The results of the investigation are given in Table 2. In carrying out this investigation, we were interested in the influence on the antimicrobial activity of the introduction of an asymmetric nitrogen atom into the composition of the QAC molecule, the role of the size of the introduced radicals, the presence of unsaturated bonds in their composition, and also these or other functional groups including electron-donor or electron-acceptor substituents.

As known, dioctyl-, didecyl- and didodecyldimethylammonium chlorides have an unusually high bactericidal activity [12], which is comparable with that of the most effective QAC containing one higher radical in the molecule,

for example, cetylpyridinium-, cetyltrimethylammonium- and dodecylbenzylmethylammonium halides. We showed that in QAC with an asymmetric nitrogen atom, this regularity if absent. Of these, compounds I, VIII, IX which contain propyl and benzyl radicals have the highest bactericidal activity. Introduction of radicals with a higher number of carbon atoms into a QAC molecule leads to a sharp decrease of the bactericidal activity (compounds I-VII, see Table 2). Replacement of a normal carbon chain by a branched chain leads to a similar effect. It has previously been found that the introduction of radicals with unsaturated bonds into a molecule of QAC intensifies the bactericidal activity of the preparation [11]. For QAC containing an asymmetric nitrogen atom, this relationship could not be found. Table 2 (compounds I and X-XII) shows that even QAC XII with allyl and propargyl radicals, has a weaker antimicrobial action than compound I, which does not contain radicals with unsaturated bonds. Introduction into the composition of the QAC of radicals containing carboxyl, β-cyanoethyl and certain other functional groups leads to a considerable weakening of the bactericidal activity of the compounds. In several cases it was found that the introduction of electron-donor groups into the composition of the radicals of the QAC leads to a sharp increase in the antimicrobial activity, while introduction of electron-acceptor groups results in weakening of this activity (compounds XIII, VIII, IX, XX-XXV). Among the newly synthesized compounds, compound XIII exhibits the maximal activity; the bactericidal effect with respect to gram negative microorganisms in a concentration of 0.01% occurs after 15 min, and in experiments with gram-positive bacteria in a concentration of 0.005% after 20 min.

It was thus unequivocally established that the QAC containing an asymmetric nitrogen atom in the molecule have a considerably higher bactericidal activity than similar compounds with a symmetric structure. The data obtained, and also the previously established similar regularity in the case of tertiary amine salts permit us to state that the presence of a symmetrical nitrogen atom in the structure of cationic surface-active agents (SAA) results in the intensification of the antimicrobial properties of the compounds.

EXPERIMENTAL (CHEMICAL)

Dodecylmethylamine. A mixture of 4 moles of 30% alcoholic solution of methylamine and 0.8 mole of dodecyl bromide was held for 3 days at room temperature in an Erlenmeyer flask closed with a ground glass stopper. The end of the reaction was observed from the complete dissolution of a sample of the reaction mixture in a 10% HCl. After the end of the reaction, the solution was made alkaline, the solvent and the excess of unreacted methylamine were evaporated, and the residue was suspended in a small quantity of water. The product was extracted with ether, the ether extract was washed with water to neutral reaction, and dodecylmethylamine hydrochloride was separated from it by extraction with dilute HCl. The acid aqueous extract was made alkaline to pH 9-10, the product was extracted with ether, and distilled under vacuum, bp 135-137°C/10 mm, n_D^{25} 1.4380, yield 94%.

Dodecylmethylethylamine. A mixture of 0.1 mole of dodecylmethylamine, 0.1 mole of EtBr, 5 g of NaOH and 60 ml of iso-PrOH was boiled for 5 h. After the end of the reaction, the solvent was evaporated, the residue was suspended in a small amount of water, the product was extracted with ether, the solvent was evaporated from the extract, and the residue was acetylated with a mixture of an excess of AcOH and Ac_2O (boiling for 1 h). The unreacted acetylating agent was evaporated under vacuum. The product was extracted from the residue with dilute HCl. The acid solution obtained was treated with ether to remove impurities, and then was made alkaline to pH 9-10, and the compound was extracted with ether, and distilled under vacuum, bp 141-142°C/5 mm, n_D^{24} 1.4371, yield 66%.

Dodecylmethylethylpropylammonium bromide (I). A mixture of 0.01 mole of dodecylmethylethylamine, 0.021 mole of PrBr and 15 ml of iso-PrOH was boiled for 10 h. The solvent and the unreacted alkyl halide were evaporated under vacuum, the residue was dissolved in a minimal amount of water, and the impurities were removed by extraction with ether. Water was then distilled from the solution under vacuum. The residue (compound I) was a thick white paste. The product is hygroscopic, soluble in polar and nonpolar solvents. Yield, 87%.

Dodecylmethyl(β-carbomethoxyethyl)amine. A 0.25 mole portion of methyl acrylate was added dropwise to 0.1 mole of dodecylmethylamine. The mixture warmed up spontaneously to 60-70°C. After being cooled to room temperature, the reaction mixture was boiled for 3 h, the excess of unreacted methyl acrylate was distilled off under a water pump vacuum, and the residue was acetylated as described above. Fractional distillation gave the desired end compound, bp 185-186°C/10 mm, yield 95.2%. The product is thick viscous oil. $C_{17}H_{35}NO_2$.

Dodecylmethyl(β-carbomethoxyethyl)benzylammonium bromide (XVII). A mixture of 0.02 mole of dodecylmethyl(β-carbomethoxyethyl)amine, 0.025 mole of benzyl bromide and 30 ml of iso-PrOH was boiled for 10 h. The solvent was evaporated under vacuum, the residue was dissolved in a minimal amount of alcohol, and the

product was precipitated by an excess of dry ether. The thick viscous oil obtained was separated by decantation. After grinding with petroleum ether, product XVII crystallized, mp 50-52°C. Yield 73%.

Dodecylmethyl(4-hydroxy-3,5-diisopropylbenzyl)amine. A 0.09 mole portion of formaldehyde (in the form of 37% formalin) was added with stirring to a solution of 0.05 mole of 2,6-diisopropylphenol and 0.138 mole of dodecylmethylamine in 50 ml of alcohol, cooled to 7°C, at such a rate that the temperature of the reaction mixture did not rise above 10°C. The mixture was then stirred for 30 min at room temperature and for 10 h at the boiling point. the mixture was poured into 1 liter of water acidified to pH 2. The organic layer was separated, and the residue of the unreacted phenol was removed from the aqueous solution by extraction with ether. The aqueous solution was made alkaline to pH 9-10, and the excess of dodecylmethylamine was removed by extraction with organic solvent. The aqueous solution was again acidified strongly and the upper organic layer of the hydrohalide of the desired end product was separated. The material was suspended in a small amount of water, the suspension was made alkaline to pH 9-10, and the organic compound was separated by extraction with ether. After evaporation of the solvent under vacuum, the residue was in the form of a light-yellow oil, which solidified on standing. Yield, 76%. $C_{26}H_{47}NO$.

Dodecylmethylethyl(4-hydroxy-3,5-diisopropylbenzyl)ammonium bromide (XXII). A mixture of 0.01 mole of dodecylmethyl(4-hydroxy-3,5-diisopropylbenzyl)amine, 0.015 mole of EtBr and 20 ml of acetonitrile was boiled for 15 h. The solvent and the unreacted alkyl halide were removed under vacuum, the residue was dissolved in a minimal amount of water, the aqueous solution was purified from impurities by extraction with hexane, and then water was evaporated from it under vacuum to dryness. The residue was a thick viscous oil. After standing for 2 days, the material crystallized, mp 173-175°C. The yield of compound XXII was 67%.

EXPERIMENTAL (BIOLOGICAL)

The bactericidal activity of the synthesized QAC was determined by the method of batiste test-samples using the cultures of *E. coli* strain 1267 and *St. aureus* strain 906. The batiste test samples 0.5 × 1 cm in size were infected with a 2 billion units suspension of the above microorganisms, dried in a thermostat at 37°C, and then submerged in a solution of the compound studied. Every 5 min, test samples were withdrawn from the solution, washed successively in a 0.1% solution of sulfanol and tap water, and then were inoculated in a liquid culture medium. The inoculates were placed in a thermostat at 37°C for 7 days. Turbidization of the medium indicated the growth of the microorganisms.

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