SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF COMPOUNDS OBTAINED FROM HIGHER ALKYLAMINES

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Recently great attention has been paid to the products of the reaction of higher polyalkylamines (I) with halocarboxylic acids (II), known in other countries under the name of TEGO-disinfectants. They have a high bactericidal activity towards vegetative forms of bacteria [1, 2], destroy tuberculous mycobacteria [3, 4], fungi [5], and certain forms of viruses [6, 7]. The preparations have detergent properties not inferior to those of anionic surface active agents (SAA), and, which is very important, they are only slightly toxic to warm-blooded animals [8].

Similar properties have been discovered in the reaction products of higher monoalkylamines (III) and (II) [9-11].

According to the data of several authors [10-13], the reaction between (I) and (II) proceeds unequivocally, and as a result ampholytic SAA are formed with the structure of $RNH(CH_2CH_2NH)_n(CH_2)_mCOOH$, where R is a C_8H_{17}–$C_{14}H_{29}$ aliphatic radical with a normal structure, n = 0-2, m = 1-8. However, according to [14], even during the reaction of II with monochloroacetic acid (IV), alkyliminodiacetic acid is obtained together with alkylglycine.

It was therefore interesting to study this reaction in greatest detail, as well as the antimicrobial activity and toxicity of the products obtained. For the investigation, we chose the reaction of (III) with (IV), or its alkali salts. The reaction was carried out by heating the components in a polar solvent. It was found that the reaction mass is a mixture of alkylglycines (V), alkyliminodiacetic acid (VI), and alkylamine hydrochlorides (VII).

$$\underset{\text{III}}{RNH_2}+\underset{\text{IV}}{ClCH_2COOH}\longrightarrow\underset{\text{V}}{RNHCH_2COOH}+\underset{\text{VI}}{RN(CH_2COOH)_2}+\underset{\text{VII}}{RNH_2\cdot HCl}$$

a: R — C_8H_{17}; a: R = $C_{10}H_{21}$; b: R = $C_{12}H_{25}$; c: R = $C_{11}H_{23}$–$C_{13}H_{27}$; d: R = $C_{14}H_{29}$; e: R = $C_{16}H_{33}$; f: R = naphthenyl.

The reaction of the different amines (III) with (IV) proceeds similarly, and therefore the study of this reaction and the alternative synthesis of the products were carried out only with (IIIc).

Since chloroacetates of alkylamines (VIII) are formed when the components are mixed even in the cold, we could assume that compounds (VIII) are intermediate products in the reaction. However, when (VIIIc) was heated under various conditions, only 13-26% of a mixture of (Vc) and (VIc) could be isolated. Hence, the synthesis of (V) and (VI) proceeds only partially in this way. The general scheme of the reaction of (III) with (IV) can be represented as follows:

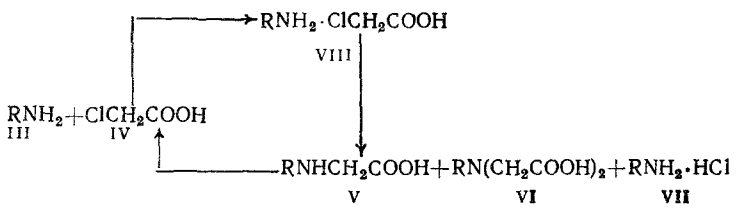

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TABLE 1. Results of Alkylation of Alkylamines with Monochloroacetic Acid (IV) under Various Conditions

Molar ratio III:IV:KOH	Reaction conditions	Amount of compound in reaction mixture, %			
		III	V	VI	VII
1:1:0	Saturated solution of sodium acetate, pH 4.0, 95-98°C	—	—	—	60
1:1:0	Saturated solution of sodium tetraborate, pH 12.0, 95-98°C	20	—	—	—
1:1:0	Water, 95-98°C	—	—	—	50
1:1:1	The same	30	—	—	—
1:1:2	"	20	—	—	—
1:1,1:1,1	"	25	—	—	—
1:1,2:1,2	"	20	—	—	—
1:1,3:1,3	"	17	—	—	—
1:1,5:1,5		10	45	45	—
1:1,3:1,3	Isopropanol, 80°C	35	—	—	—
1:1,3:1,3	Ethanol, 75°C	25	—	—	—
1:1,3:1,3	Methanol, 65°C	18	—	—	—

The results of the investigation of the reaction under different conditions are listed in Table 1. The analytical data show that the reaction is concluded after 1 h in an aqueous medium, and 3-5 h in alcoholic medium. We found that to increase the yield of (Vc) and (VIc), the solution of (IV) or its alkali salt must be added gradually to hot (IIIc), and in this case the rate of formation of (Vc) and (VIc) exceeds the rate of decomposition of (IV). Substitution of the solvent by a less polar one decreases the yield of (Vc) and (VIc).

Thus, despite the change in the ratio of the initial components, temperature, pH of the medium and the nature of solvent, a mixture of (V, VI) and (VII) is always obtained. We should note that in the case of reaction of (IIIc) with alkali salts of (IV), (VIIc) is not formed in the reaction mixture, since even at the end of the reaction the alkaline medium is retained. But the amount of (VIc) increases.

To identify the individual mixture components, we prepared compounds (Vc-VIIIc) by alternative methods. Compound (Vc) was synthesized by two methods. We isolated this compound from the reaction mixture obtained by heating (IIIc) with an alkali salt of (IV), as in the procedure described in [11], with subsequent purification of the compound in the form of zwitterions by the method of Anderson [15].

Compound (Vc) was also prepared by a two-stage synthesis. Dodecylglycinonitrile (IX) was prepared by the reaction of (IIIc) with formaldehyde sodium bisulfite and sodium cyanide [16]. Compound (IX) was saponified in both alkaline and acidic medium. The alkaline hydrolysis was carried out in an aqueous, aqueous-alcoholic medium and in glycerol. We found that hydrolysis in the aqueous medium leads to the formation of amide (V) only, while heating with alkali in glycerol at 230°C causes the decomposition of the product. In a series of experiments it was found that saponification of (IX) in an alcoholic solution of an alkali or by a dilute sulfuric acid was satisfactory. The remaining mixture components were prepared by known methods: (VI) according to [14], (VII) according to [17], and (VIII) according to [18].

The bactericidal activity of the compounds obtained was studied by a generally accepted method, consisting in disinfecting batiste test objects. As model microorganisms we used *St. aureus* strain and *E. coli* strain 1257. To establish the acute toxicity level of the preparation, 1, 2, 4, 6, 15 and 20% solutions of the preparations were introduced by a single intragastric administration to white mice. The LD_{50} was calculated by the Kerber method. The results of the investigation of the antimicrobial activity and acute toxicity are listed in Table 2. It is seen that compounds (VII) and (VIII) have a high bactericidal activity, (V) is only slightly active, especially with respect to gram-positive microorganisms, and (VI) is entirely inactive. As expected, of the compositions obtained (A-G, see Table 2), compositions B, C and D were found to be most effective, although interior in this respect to the corresponding (VII) and (VIII).

TABLE 2. Antimicrobial Activity and Toxicity of Compounds Studied

Compound	Concentration	Exposure, min		LD_{50}, mg/kg for white mice
		St. aureus	E. coli	
V c	0,05	30	15	1500
VI c	1,0	Ineffective		
VII c	0,05	10	5	—
	0,025	20	10	755
	0,01	30	20	
VII d	0,05	10	10	744
	0,025	20	15	
	0,01	30	20	
VII g	0,025	20	10	—
VIII c	0,025	20	10	600
	0,01	30	20	
VIII d	0,025	20	15	667
VIII g	0,025	20	10	—
Composition				
A	0,1	30	15	—
B	0,1	10	5	—
	0,05	20	15	—
B	0,1	10	5	—
D	0,05	20	15	—
	0,1	10	5	—
	0,05	20	15	—
E	0,1	15	10	—
	0,05	25	20	—
F	0,1	30	15	—
G	0,025	20	10	1500

Note. Compositions A-G containing 50% of (VII), 45% of (V) and 5% of (VI) with corresponding radicals (a-g).

An important disadvantage of compounds (VII) and (VIII) should be noted, namely, high toxicity to warm-blooded animals, in particular, a sharply expressed skin irritating action. At the same time, (V) and (VI) were found to be slightly toxic compounds. Compositions A-F have a lower antimicrobial activity than the corresponding (VII) and (VIII). An exception is composition G, whose bactericidal activity is equal to that of (VIIg) and (VIIIg). This fact is probably explained by a synergistic effect of (Vg) and (VIg). Unfortunately, the product was only slightly soluble in water. This drawback was eliminated by the addition of a solubilizer, i.e., oxides of higher tertiary amines. The antibacterial activity of the preparations thus increased. At present, composition G, which was called "amphosept," has been successfully tested under room conditions.

Our study confirms the already known fact that during the reaction of higher alkylamines with monochloroacetic acid, alkylamine hydrochlorides are formed together with alkylglycines and alkyliminodiacetic acids. It was shown that the antimicrobial activity of the preparations obtained, to which the structure of ampholytic SAA has been ascribed, is mainly due to the presence in their composition of salts of higher alkylamines, belonging to the class of cationic SAA. The presence of ampholytic compounds in these mixtures leads to a considerable decrease in the toxicity of the preparations, compared to the toxicity of the alkylamine salts.

EXPERIMENTAL

The reaction products were analyzed by the following method. At the end of the reaction, the reaction mixture is made alkaline and evaporated *in vacuo* to a small volume. Compound (III) is extracted by benzene, the solvent is removed from the extract, and (III) is weighed. The amount of (VIIc) in the reaction mixture is determined by TLC on Silufol UV-254 plates (CSR) in a n-butanol—acetic acid—water (5:2:2) system (system 1) by comparing the detected spots of the standard with those of the sample studied. The optimal results were obtained in the investigation of samples containing 2-12 μg of (VIIc). Good results were also obtained by using a methanol—25% aqueous ammonia (7:1) system (system 2) and the ethanol—methyl ethyl ketone—25% aqueous ammonia (5:5:1) system (system 3).

The amount of (VIc) in the reaction mixture was found by nonaqueous titration with perchloric acid of a sample of the reaction mixture, whose components were first acylated

by a mixture of acetic anhydride and acetic acid. After determination of (VIc) and (VIIc) by the above methods, the amount of (Vc) is determined from the difference.

Dodecylglycine (Vc) is obtained by the method described in [14]; the compound is purified by the method of [15]; yield 30%. R_f = 0.49 (system 3), mp 180-182°C (methanol—acetone). Found, %: C 68.69; H 11.92; N 5.53; $C_{14}H_{29}NO_2$. Calculated, %: C 69.08; H 12.01; N 5.75.

Nitrile of Vc (IX) is obtained according to [16], yield 82.5%.

Alkaline Hydrolysis of (IXc). A 2.27-g (0.01 mole) portion of (IXc) is boiled with 2.0 g of sodium hydroxide in 40 ml of water, the mixture is filtered, and the filtrate is acidified with hydrochloric acid to pH 3.0. The precipitate is filtered, dissolved in alcohol, treated with brand A activated charcoal, and filtered, and the solvent removed. The residue is recrystallized from alcohol to yield 1.05 g (43.2%) of (Vc).

Acid Hydrolysis of (IXc). A 2.27-g (0.01 mole) portion of (IXc) is heated for 3 h at 120-130°C with 12.5 g of 40% sulfuric acid. The reaction mixture is diluted with 150 ml of water, made alkaline while heating, and filtered; the filtrate is treated with ether to remove unreacted (IXc), and acidified with hydrochloric acid to pH 3.0. The precipitate is filtered, dissolved in alcohol, treated with brand A activated charcoal, and filtered, and the solvent is removed. The product is recrystallized from alcohol to yield 1.3 g (53.5%) of (Vc). The properties of the hydrolysis products of (IXc) are similar to those of (Vc) prepared according to [14]. The compounds do not depress the melting point of a mixed probe with an identical sample synthesized and purified by the methods described in [14, 15].

Dodecyliminodiacetic Acid (VIc). The compound is prepared according to [14] at a ratio of (IIIc) to (IVc) equal to 1:5. Yield 95%, R_f = 0.15 (system 3), mp 135-136°C (alcohol), which corresponds to the literature data [14].

Dodecylamine Hydrochloride (VIIc). A 9.2-g (0.05 mole) portion of (IIIc) is dissolved in 100 ml of ether. A current of dry hydrogen chloride is passed for 10 min. The precipitate is filtered, washed with ether, and dried to yield 10.25 g (93%) of (VIIc). R_f 0.40 (system 2), mp 184-186°C, which corresponds to the data of [7].

Compounds (VIId) and (VIIg) are prepared by reacting equimolecular amounts of the corresponding amines (III, g) with hydrogen chloride in ether, and then removing the solvent *in vacuo*, and also by mixing these amines with a calculated amount of hydrochloric acid in an aqueous solution.

Dodecylamine Chloroacetate (VIIIc). A solution of 2.08 g (0.022 mole) of (IV) in 20 ml of ether is added to a solution of 3.7 g (0.02 mole) of (IIIc) in 20 ml of ether. The mixture is stirred for 15 min, the precipitate is filtered, washed with ether, and dried to yield 4.36 g (76.3%) of (VIIIc), R_f 0.64 (system 2), mp 63-66°C, which corresponds to the data of [18]. Compounds (VIIId) and (VIIIg) are prepared similarly to (VIIIc), from (III, g) and (IV).

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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF GUANIDINE AND 2-AMINO 2-IMIDAZOLINE DERIVATIVES

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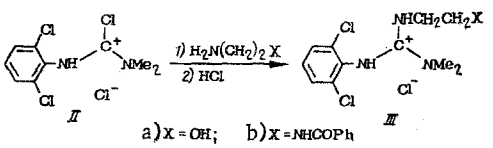
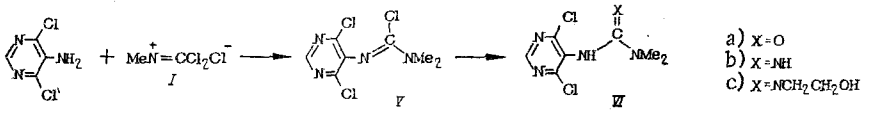
N,N-Dimethyl-N-dichloromethylene-immonium chloride (I) [1, 2] and N,N-dimethyl-N'-(2,6-dichlorophenyl)-C-chloroformamidine hydrochloride (II) [3], which we used in [3] to synthesize the medicinal preparation clofeline (hemiton, catapresan) are readily available starting compounds for the preparation of different derivatives of guanidine and 2-amino-2-imidazoline, which are interesting from the point of view of a search for new hypotensive agents.

In the present work, we synthesized and pharmacologically studied the previously unknown hydrochlorides of N-substituted N'-(2,6-dichlorophenyl)-N",N"-dimethylguanidines (III), obtained from (II) by treatment with 2-aminoethanol and N-benzoylethylenediamine in acetonitrile, according to a method developed by us in [4].



To obtain guanidine derivatives containing a pyrimidine ring as the substituent, N,N-dimethyl-N'-(4,6-dichloro-5-pyrimidyl)-C-chloroformamidine (V) was synthesized by the reaction of 4,6-dichloro-5-aminopyrimidine (IV) [5] with (I); reaction of (V) with dilute sodium hydroxide, aqueous ammonia and 2-aminoethanol gave N,N-dimethyl-N'-(4,6-dichloro-5-pyrimidyl)-urea (VIa) and substituted guanidines (VIb, c), respectively.



Besides the "open-ring" analogs of clofeline, it was interesting to obtain for biological studies derivatives of 2-aminoimidazoline containing 2,6-dichlorophenyl and 4,6-dichloropyrimidyl fragments attached to the nitrogen atom of the imidazoline ring. We therefore studied the reaction of substituted guanidines (IIIa) and (VIc) with thionyl chloride. We found that heating (IIIa) and (VIc) with thionyl chloride is accompanied by cyclization and formation of 1-(2,6-dichlorophenyl)- (VII) and 1-(4,6-dichloro-5-pyrimidyl)-2-dimethylaminoimidazolines (VIII). The action of methyl iodide, benzoyl and benzyl chlorides on (VII) yielded the corresponding quaternary salts (IXa-c). By alkaline hydrolysis of (IXa-c), the N-substituted N'-(2,6-dichlorophenyl)-ethylene-ureas (Xa,b) were synthesized. Transition from (Xa) to 1-(2,6-dichlorophenyl)-2-imino-3-methylimidazoline (XI) was carried out by heating (Xa) with phosphorus oxychloride, followed by treatment of the intermediate amidochloride with ammonia.

The structure of compounds obtained was confirmed by the data of IR and UV spectrometry (Table 1).

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