SYNTHESIS AND BACTERICIDAL ACTIVITY OF AMINO ACID HIGHER ESTER HYDROCHLORIDES

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Cationic surface-active agents (SAA) have long been known to be effective bactericides [7, 13]. Several salts of higher alkylamines with high antimicrobial properties have also been described [9, 10]. The main disadvantage of the above cationic SAA is their considerable skin-irritating action. This prevents their wide application as antiseptics in the medicinal practice.

The aim of the present work was to search for effective and slightly toxic bactericides among cationic SAA. For the investigation we selected hydrochlorides of higher esters of amino acids. We assumed that these compounds are less toxic for warm-blooded animals than other cationic SAA, since the starting materials for their synthesis, the amino acids, are considerably less toxic than amines used for the preparation of alkylamine salts and corresponding quaternary ammonium compounds. We have already shown that these compounds have an antimicrobial activity, especially with respect to gamma-positive microorganisms [2, 4]. According to the data of [3, 5], as bactericides the most interesting are the hydrochlorides of lauric esters of valine, β-alanine, β-aminobutyric acid, and lysine. The methods for their preparation are very imperfect. A synthesis has been described of amino acid ester hydrochlorides by the reaction of amino acid hydrochlorides with higher alcohols. It was shown that by direct esterification only glycine higher ester hydrochlorides can be obtained, and it was proposed that the derivatives of other amino acids be prepared by transesterification of amino acid lower ester hydrochlorides with higher alcohols [12]. A method of synthesis of these compounds has been described by the reaction of cesium salts of amino acids with higher alkyl halides [14]. Instead of hydrogen chloride, it was proposed to use strong cation exchangers [11], and also chlorosulfonic acid [8] as catalysts. It was found that amino acid higher ester hydrochlorides can also be obtained by treating a suspension of an amino acid in alcohol with thionyl chloride, phosphorus trichloride, or phosphorus oxychloride [15]. Because of the complexity of the methods of synthesis and low yields of the desired products, one of the first tasks of the investigation was to search for a simple and convenient method for their preparation. When studying the esterification of amino acids by higher alcohols, we found that the limiting stage of the process is the formation of the amino acid hydrochloride. It was therefore proposed to obtain the hydrochlorides of higher esters of amino acids by the reaction of amino acid hydrochlorides with higher alcohols. A simple method has been proposed for synthesizing amino acid hydrochlorides by short-term bubbling of hydrogen chloride through a suspension of the amino acid in a small volume of concentrated hydrochloric acid. By carrying out the reaction with hydrochlorides of the amino acids instead of the free acids, the time of the synthesis could be considerably shortened, the temperature of carrying out the process and the consumption of the higher alcohol could be decreased, and, what is most important, the yield of the desired compounds could be increased to 90-95% [1]. It was also shown that with this method, hydrogen chloride can be bubbled periodically and not continuously, not more than 3-4 times for 10-15 min during the course of the reaction. Hydrogen chloride must be added to the reaction mixture because the initial hydrochlorides of amino acids are built according to a sandwich principle — two molecules of the amino acid are bound to one molecule of hydrogen chloride [6] — while in the final compounds, the higher ester hydrochlorides, the molecule of hydrogen chloride, as in the case of usual amine salts, is bound to each molecule of the amino acid ester. Addition of hydrogen chloride is necessary to make up the deficiency of the salt-forming component in the esterification process.

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TABLE 1. Characteristics of Hydrochlorides of Higher Esters of Amino Acids

Compound	Hydrochloride of	Yield, %	mp, °C	Found, %		Empirical formula	Calculated, %	
				C	H		C	H
I	Octyl ester of glycine	92	68—70	53,85	9,98	$C_{10}H_{22}ClNO_2$	53,67	9,82
II	Decyl " " "	89	87—9	56,96	10,31	$C_{12}H_{26}ClNO_2$	57,19	10,41
III	Dodecyl " " "	90	94—5	60,27	10,68	$C_{14}H_{30}ClNO_2$	60,08	10,80
IV	Tetradecyl ester of glycine	91	97—9	62,29	10,95	$C_{16}H_{34}ClNO_2$	62,41	11,12
V	Decyl ester of DL-α-alanine	93	70—1	58,42	10,60	$C_{13}H_{28}ClNO_2$	58,73	10,61
VI	" " " L-α-alanine	88	66—7	58,37	10,72	$C_{13}H_{28}ClNO_2$	58,73	10,61
VII	" " " D-α-alanine	90,5	95—7	58,62	10,36	$C_{13}H_{28}ClNO_2$	58,73	10,61
VIII	" " " β-alanine	92	79—81	58,52	10,57	$C_{13}H_{28}ClNO_2$	58,73	10,61
IX	" " " α-aminobutyric acid	92	98—100	60,24	10,73	$C_{14}H_{30}ClNO_2$	60,07	10,80
X	" " " γ-aminobutyric acid	88	87—9	60,12	10,93	$C_{14}H_{30}ClNO_2$	60,07	10,80
XI	" " " ω-aminocaproic acid	94	95—7	62,12	10,96	$C_{16}H_{34}ClNO_2$	62,40	11,13
XII	Octyl " " ω-aminocaproic acid	82	48—50	59,87	10,63	$C_{14}H_{30}ClNO_2$	60,08	10,80
XIII	Decyl " " DL-valine	91	58—9	60,99	10,89	$C_{15}H_{32}ClNO_2$	61,30	11,05
XIV	" " " DL-norvaline	89	72—3	60,88	11,67	$C_{15}H_{34}ClNO_2$	60,88	11,58
XV	" " " L-β-phenyl-α-alanine	88	85—8	66,98	9,73	$C_{19}H_{32}ClNO_2$	66,73	9,43
XVI	" " " L-leucine	79	56—7	62,17	11,59	$C_{16}H_{36}ClNO_2$	62,00	11,71
XVII	" " " D-leucine	83	64—5	62,09	11,63	$C_{16}H_{36}ClNO_2$	62,00	11,71
XVIII	" " " DL-leucine	92	83—4	61,91	11,54	$C_{16}H_{36}ClNO_2$	62,00	11,71
XIX	" " " DL-norleucine	87	79—81	62,53	11,27	$C_{16}H_{34}ClNO_2$	62,40	11,13
XX	" " " glutamic acid	71	56—7	64,36	10,87	$C_{25}H_{50}ClNO_4$	64,69	10,86

A detailed study of the reaction showed that to obtain higher yields of the desired products, the ratio of amino acid hydrochloride to higher alcohol must be 1:3. The time of carrying out the process, depending on the nature of the amino acid, should be from 12 to 20 h, and the temperature from 120 to 130°C. To decrease resinification, esterification must be carried out in a nitrogen or other inert gas atmosphere. The physicochemical characteristics and yield of products obtained are listed in Table 1.

The bactericidal activity of the compounds obtained was determined according to the requirements of "instructions on the determination of bactericidal properties of new disinfecting agents" approved by the Ministry of Public Health of the USSR in 1968. As model test microorganisms, we used *St. aureus* strain 906 and *E. coli* strain 1257. The results of the investigation are shown in Table 2. With the glycine derivatives as an example, we studied the influence of the length of the higher residue of the ester group. The maximum bactericidal activity is observed in the case of glycine decyl ester hydrochloride, and therefore mainly compounds containing the decyl radical were further studied. We found that the bactericidal activity of the α-alanine and β-alanine and α- and γ-aminobutyric acid decyl ester hydrochlorides is commensurable with the effectiveness of glycine decyl ester hydrochloride. With further increase in the number of methylene units between the amino and carboxylic groups of the amino acids, the bactericidal activity of the amino acid ester hydrochlorides decreases (compounds II and VIII, compared with compound XI in Table 2). It was interesting to study the influence of the presence of optical isomerism in the amino acid fragment on bactericidal activity. We therefore synthesized hydrochlorides of decyl esters of L-, D-, and LD-α-alanines (VI, VII, and V) and the corresponding leucines (XVI-XVIII). It was shown that compounds obtained from the L-isomers of amino acids are considerably more active than the corresponding salts of higher esters of amino acids synthesized from D-amino acids. L-α-Alanine decyl ester hydrochloride (VI) destroys vegetative forms of microbes at a concentration of 0.01% in the course of 15-20 min, while the compound obtained from the D-isomer (VII) at the same exposure is effective only at a concentration of 0.05%. We should note that we obtained similar results in the case of alkylguanidine- and amidinecarboxylic acids, which indicates a certain regularity of this phenomenon.

For tests over a wide medicinal practice, we selected two preparations, glycine decyl ester hydrochloride (II) and glycine (decyl—tridecyl) ester hydrochloride (XXI). Their toxicity was studied, and it was found that on intrastomachic administration, to white mice, the LD_{50} is equal to 1500 mg/kg. The skin-irritating properties were studied on rabbits and guinea pigs. Multiple deposition of 1% aqueous solutions (working concentrations for these compounds) did not cause any changes in the integument of the rabbits. Deposition of 5% aqueous solutions on rabbits caused a pronounced peeling with weak hyperemia, and in guinea pigs only a slightly pronounced peeling. Allergic properties are not pronounced in the preparations. It was thus shown that amino acid higher ester hydrochlorides have bactericidal properties and

TABLE 2. Bactericidal Activity of Amino Acid Higher Ester Hydrochlorides

Compound	Concentration, %	Time of destruction, min	
		St. aureus	E. coli
I	0,1	n	10
	0,05	n	15
II	0,05	10	5
	0,025	20	10
III	0,05	10	5
	0,025	20	10
IV	0,05	15	10
	0,025	n	20
V	0,1	25	15
	0,05	n	n
VI	0,05	5	5
	0,025	10	15
VII	0,05	15	15
	0,025	n	n
VIII	0,05	5	10
	0,025	15	20
IX	0,025	5	5
	0,01	25	20
X	0,05	5	5
	0,025	10	10
	0,01	20	25
XI	0,05	15	15
	0,025	n	n
XII	0,1	20	25
	0,05	n	n
XIII	0,05	5	10
	0,025	15	20
XIV	0,05	5	15
	0,025	10	20
XV	0,1	15	20
	0,05	25	30
XVI	0,05	5	5
	0,025	10	10
XVII	0,025	5	5
	0,01	20	25
XVIII	0,05	15	20
	0,025	30	n
XIX	0,05	30	n
	0,025	n	n
XX	0,05	20	25
XXI	0,05	5	5
	0,025	10	15
	0,01	15	20
XXII	0,05	20	30

Note. XXI) Glycine (decyl—tridecyl) ester hydrochlorides; XXII) hydrochlorides of esters of a mixture of amino acids; n) compound at this concentration is ineffective.

are slightly toxic towards warm blooded animals. A convenient method for their preparation with high yields has been developed.

EXPERIMENTAL

Glycine Decyl Ester Hydrochloride (III). Hydrogen chloride is bubbled for 15-20 min through a suspension of 100 g of glycine in 30 ml of concentrated hydrochloric acid heated to 100-120°C to complete homogenization of the reaction mixture. The mixture is poured into a crystallizer, cooled, and filtered. The precipitate is washed on the filter with acetone, and dried in air. The yield of glycine hydrochloride is 147 g (99%), mp 184-185°C, which corresponds to the data in [14].

A mixture of 11.5 g (0.1 mole) of freshly prepared glycine hydrochloride and 55.8 g (0.3 mole) of decyl alcohol is heated to 110-120°C, with constant stirring, while a current of dry nitrogen is bubbled through the reaction mixture. The mixture is held at this temperature for 8 h. After 30 min from the beginning of the holding time, dry hydrogen chloride is passed through the reaction mixture for 10-15 min at a rate of 20-40 bubbles per min. This operation is repeated after 2-3 h, and then 1 h before the end of the process. The solution is completely homogenized after 5 h from the beginning of the holding time. The end of the reaction is

determined from cessation of separation of water in the Dean–Stark adapter, and complete dissolution of the probe of the reaction mixture in hot acetone. At the end of the reaction, 100 ml of acetone are added to the reaction mixture, which is heated to 60-70°C for 10-15 min with constant stirring. It is then cooled to 0°C and the precipitate is filtered, washed with acetone, and dried in air. Mp 94-95°C. Yield, 27.1 g (95%).

The remaining compounds were obtained in a similar way.

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COMPARATIVE BACTERICIDAL ACTIVITY OF QUATERNARY AMMONIUM SALTS

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The bactericidal activity of alkyldimethylbenzylammonium chloride (alkyl represents a mixture of C_{10}-C_{16}), discovered about 50 years ago [6], stimulated a wide search for effective bactericides among quaternary ammonium salts (QAS). A large number of publications pertaining to this class of organic compounds have been devoted to the generalization of information on their synthesis and bactericidal activity [2-5]. However, there are no data in the literature permitting a quantitative comparison of substances within the same homologous series and much less of compounds from different series. This is due to the peculiarities of the usual procedure used for evaluating microbiocidal activity of compounds, when the time of their action is determined with a variable concentration of the active substances. Moreover, both characteristics cannot be kept constant for different compounds, which makes it impossible to obtain comparable data.

Under such conditions a calculation method can be used for evaluating the biocidal activity of disinfectants [1].

The concentration of the solutions producing 100% death of microorganisms at set exposure (C_{100}), the relative activity (A_{rel}), and the coefficient of selective action (K_{sa}) should be used as the parameters for evaluating the antimicrobial action at the stage of preliminary selection of promising compounds.

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