SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF MONOMERIC AND POLYMERIC QUATERNARY AMMONIUM SALTS BASED ON AMINOALKYL ESTERS OF METHACRYLIC ACID

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We have previously shown that some copolymers of N-vinylpyrrolidone (VP) with (2-methacryloxyethyl) triethylammonium halides that contain quaternary nitrogen atoms in the side chain of the polymer display antimicrobial properties [1]. The ability of these copolymers to inhibit the growth of some Gram-positive bacteria increased as the number of ammonium groups in the polymer chain increased and depended only slightly on the molecular weight of the polymers. On the other hand, with respect to low-molecularweight cation-active detergents, it is known [2-4] that the effectiveness of their antimicrobial action depends to a considerable degree on the character of the substituent attached to the quaternary nitrogen atom. In this connection, it seemed of interest to ascertain the effect of the nature of the substituent attached to the nitrogen atom on the antimicrobial activity of monomeric quaternary ammonium salts of aminoalkyl esters of methacrylic acid and of their homopolymers and copolymers with VP.

With this end in mind, we synthesized some new quaternary ammonium salts of diethylaminoethyl methacrylate (DEAEM) and 1,3-bis(dimethylamino)isopropyl methacrylate (DMAIM) by alkylation of DEAEM and DMAIM with alkyl halides of different structure (and also with dimethyl sulfate) and copolymerized the resulting salts with VP.

The amino esters were alkylated in a solvent or without one, varying the reagent ratio, reaction time, and reaction temperature as a function of the nature of the alkylating agent. To avoid polymerization of the monomers, hydroquinone was added to the reaction medium as an inhibitor. The properties of the monomeric ammonium salts obtained are presented in Table 1.

The IR spectra of I-VI contain absorption bands characteristic for methyl and methylene groups (2880-2900 cm⁻¹) and for a terminal methylene group (1630-1640 cm⁻¹), the carbonyl band of an ester group (1730 cm⁻¹), and the band of the valence vibrations of a C-O bond (1165 cm⁻¹). The monomers obtained are soluble in methanol, ethanol, dimethylformamide, and chloroform. Monomers II, III, and V are only slightly soluble in water, while I, IV, and VI are soluble in water.

The copolymerization of the ammonium salts of DEAEM and DMAIM with VP was carried out in ethanol in the presence of azobisisobutyronitrile (AIBN) via the method previously described in [1]. The composition of the copolymers was calculated from the percentage of ionic halogen, determined by argentometric titration with a TVL-1M titrator. The copolymerization conditions and the characteristics of some of the copolymers are presented in Table 2.

As seen from Table 2, the copolymerization of all of the investigated quaternary ammonium salts with VP proceeds rapidly and gives high yields of polymers; an increase in the fraction of quaternary ammonium salt in the starting mixture of monomers is accompanied by a decrease in the intrinsic viscosity of the copolymers. This phenomenon, which was previously detected in a study of the copolymerization of DEAEM ethohalides with VP [1], is due to the thermal decomposition of the quaternary ammonium groups of the monomers and copolymers in the course of the polymerization. The resulting alkyl halides, the presence of

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TABLE 1. Ammonium Salts of DEAEM and DMAIM

CH ₃	CH3	CH2-N(CH3)2RX
$CH_2 = C - C - OCH_2OH_2 - N(C_2H_5)_2 RX$	CH2=C-C-0-0	с́н +
Ö.	-	$CH_2 - N(CH_3)_2 RX$

R 1	Com-	8	mn °C	Halogen	Empirical	12°	Bromine No.	
	x	Yield,			Iormuta	Haloge calc.,	found	calc.
C ₆ H₅CH₂	Cl	32	159159,5	10,78;	C ₁₇ H ₂₆ CINO ₂	11,39	51,5	51,4
C ₁₄ H ₂₉	I	66	76—7	25,25;	$C_{22}H_{48}INO_2$	25,06	30,7	31,4
C ₁₆ H ₃₃	I	56	845	23,28;	$\mathrm{C_{26}H_{52}INO_2}$	23,71	29,7	29,4
C_2H_5	I	93	2023	48,11;	$C_{15}H_{32}I_2N_2O_2$	48,30	30,6	30,4
C ₆ H ₅ CH ₂	I	98	168,5—170	39,55;	$C_{25}H_{36}I_2N_2O_2$	39,20	24,6	24,6
СН₃ │	CH₂SO₄	98	172—3		$C_{15}H_{36}N_2O_{10}S_2^*$		34,1	34,4
	₆ H ₅ CH ₂ ₁₄ H ₂₉ ₁₆ H ₃₃ ₂ H ₅ ₆ H ₅ CH ₂ H ₃	$\begin{array}{c c} & & & \\ {}_{8}H_{5}CH_{2} & Cl \\ \\ {}_{14}H_{29} & I \\ \\ {}_{16}H_{33} & I \\ \\ {}_{2}H_{5} & I \\ \\ {}_{8}H_{5}CH_{2} & I \\ \\ {}_{8}H_{5}CH_{2} & I \\ \\ {}_{2}H_{3} & CH_{2}SO_{4} \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

*Found, %: S 13.76; 13.34. Calculated, %: S 13.72.

TABLE 2. Copolymerization of VP (M_1) with Quaternary Ammonium Salts of DEAEM and DMAIM (M_2) at $70^{\circ}C$

M ₂	Comp. startin ture of mers, 1 M_1	of the g mix- mono- mole¶ M ₂	Amount of AIBN , wt. %	Reaction time, h	Yield, wt. %	[ŋ] at 25°, d1/g	Percentage of Ma in the co- polymer, mole %
I	80	20	0,3	7	93	0,55	16,6
	70	30	0,3	12	91	0,50	18,4
	65	35	0,3	30	82	0,27	24,2
	60	40	0,3	35	95	0,12	32,1
II	95	5	0,3	16	75	0,55	5,0
	80	20	0,3	10	71	0,36*	18,4
IV	80	20	0,3	10	68	0,32	17,3
	80	20	0,5	12	74	0,21	16,9
	60	40	0,3	10	80	0,27	30,1
v	80	20	0,5	40	56	0,25*	14,2
	60	40	0,3	17	67	0,30*	34,2

*In dimethylformamide at 25°C.

which in the reaction mixture is confirmed by qualitative reactions, participate in chain-transfer reactions: this also leads to a decrease in the molecular weight of the copolymer. It is apparently for this reason that we were unable to obtain polymers with sufficiently high molecular weights in the homopolymerization of DEAEM and DMAIM salts. Such homopolymers were obtained by known alkylation reactions in the chains of poly-DEAEM and poly-DMAIM [5, 6]. The structures of the synthesized copolymers were confirmed by argentometric titration and by their IR spectra, in which absorption bands at 1710-1730 and 1170 cm⁻¹, corresponding to the vibrations of the ester group, 2880-2900 cm⁻¹, characteristic for methyl and methylene groups, and 1650- 1660 cm^{-1} , corresponding to the valence vibrations of the carbonyl group of the pyrrolidone ring, are observed. The copolymers of VP with monomers II, III, and V proved to be water-soluble when they contained no more than 10 mole % of the salts, while the remaining copolymers were water-soluble when any percentage of ammonium salts was present. Aqueous solutions of the polymeric quaternary ammonium salts have neu-

tral pH values. Strongly basic polymeric hydrates of ammonium oxide can be obtained when the halide ions are exchanged by hydroxyl ions on ÉDÉ-10P anion-exchange resin.

The antimicrobial activity of the compounds obtained was determined by serial cultures with a <u>Staph-ylococcus aureus</u> 209P microbe culture. The results of the microbiological tests are presented in Table 3. It is seen from Table 3 that the monomers differ markedly from one another with respect to activity as regards <u>Staphylococcus aureus</u> 209P. While monomer VII has low activity, monomers II and III, on the other hand, display clearly expressed antimicrobial properties. The antimicrobial activity of the monomer increases with the introduction of a second ammonium group and also with the introduction of benzyl and long aliphatic groups and reaches a maximum in compounds with tetradecyl and cetyl groups. This is in good agreement with the general regularities known for low-molecular-weight quaternary ammonium salts [3, 7]. The data in Table 3 attest to the fact that the polymers have appreciable antimicrobial action. As in the case of DEAEM ethohalides [1], more active copolymers and homopolymers were obtained from low-activity monomers IV, V, and VI. The same dependence of the effectiveness of the antimicrobial action on the character of the group attached to the nitrogen atom as was observed in the monomers is retained in the polymers: copolymers containing benzyl (Nos. 4 and 5 as compared with Nos. 2 and 3) and, particu-

TABLE 3. Antimicrobial Activity of Ammonium Salts of DEAEM and DMAIM and of Their Homopolymers and Copolymers with VP (with respect to Staphylococcus aureus 209P)

·····		Copolymers with VP				
Compound	MBC, ¹ μg/m1	No.	ammonium group con- tent, mole	Inj at 25°. 1 M KCI. dl/g	MBC ,1 μg/m1	
VII ² [1]	10 000	1 2	9,8 15,6	0,64 0,64	1 000	
I	37	3 4 5 6	20,3 16,6 32,1	0,58 0,55 0,12	160 37 8	
н	0.03	7	18,4	0,36	9	
III	0,3	8	8,7	0,55	18	
IV V VI	300 300 1 000	9 10 11 12	22,4 100 100 21,5	0,42 0,46 0,85 0,6	37 15 31 100	

¹MBC is the minimum bacteriostatic concentration.

 $^2 The structural formula of this compound is <math display="inline">_{\mbox{CH}_3}$

 $CH_{2} = \overset{|}{\overset{C}{C}} - \overset{-}{\overset{C}{C}} - OCH_{2}CH_{2} - \overset{+}{\overset{N}{N}} (C_{2}H_{3})_{3} Y^{-}.$

larly, tetradecyl (Nos. 6 and 7 as compared with Nos. 1 and 3) groups are the most active. With respect to the strength of their effect, the latter are not inferior to the homopolymers when the ammonium group content in them is 5 mole % or 19 wt. % (MBC of No. 6 \simeq MBC of No. 10). However, this dependence for the polymers is nevertheless expressed less noticeably than in the monomers. The peculiarity of the polymeric quaternary ammonium salts, in which, as it were, the effects of the structure of the link are equalized and the antimicrobial action is caused by the entire macromolecule as a whole, is apparently a result of this.

The results have shown that by using active (with respect to Gram-positive microbes) monomers I-IV, one can obtain copolymers of them with VP (with any composition whatsoever) that have considerable bacteriostatic action. Of these, the most interesting are copolymers with a small percentage of quaternary ammonium groups (up to 20 mole %), which have proved to be compounds of low toxicity [1]. It might be expected that such water-soluble copolymers may be used as polymer-carriers of medicinal substances of the anionic type.

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EXPERIMENTAL

(2-Methacryloxyethyl)diethylbenzylammonium Chloride (I). A mixture of 25 g of DEAEM, 0.3 g of hydroquinone, and 26 g of benzyl chloride was heated at 140-150° for 8 h, after which it was cooled and poured with stirring into 250 ml of diethyl ether. The precipitated powdery product was separated on a filter, washed with ether, and dried in vacuo. Repeated reprecipitation from methanol into ether gave 13.8 g (32%) of I.

Similarly, 10 g of DEAEM and 21.2 g of tetradecyl iodide [8] gave 18.2 g (66%) of (2-methacryloxyethyl)diethyltetradecylammonium iodide (II), while 10 g of DEAEM and 22.8 g of cetyl iodide [8] gave 16.3 g (56%) of (2-methacryloxyethyl)diethylhexadecyl ammonium iodide (III).

<u>1,3-Bis (dimethylethylammonium) isopropyl Methacrylate Iodide (IV).</u> A mixture of 10 g of DMAIM, 72 g of ethyl iodide, and 0.1 g of hydroquinone was heated at 60° for 40 min. The mixture was then cooled and poured into 100 ml of dry acetone to give 25.5 g (86.5%) of IV.

<u>1,3-Bis (dimethylbenzylammonium)isopropyl Methacrylate Iodide (V)</u>. A 5 g sample of DMAIM and 0.1 g of hydroquinone were mixed with 10 ml of methanol and 12.2 g of benzyl iodide. After 20 min, the cooled mixture was poured into 200 ml of diethyl ether to give 13.5 g (77%) of V.

1,3-Bis(trimethylammonium)isopropyl Methacrylate Methosulfate (VI). A mixture of 12 g of DMAIM, 42 g of dimethyl sulfate, 0.15 g of hydroquinone, and 40 ml of ethanol was heated at 80° for 6 h, after which the cooled mixture was poured into 200 ml of diethyl ether to give 19.2 g (73%) of VI.

The copolymerization of monomers I-VI with VP was accomplished by the method previously described in [1]. The homopolymers of monomers IV and V were obtained via the method in [5, 6].

For proof of the formation of alkyl halides during the polymerization, VP was copolymerized with IV, the volatile components were removed from the polymerizate by distillation, and halogen was detected in the distillate by a qualitative Beilstein test.

LITERATURE CITED

- 1. E. F. Panarin, M. V. Solovskii, and O. N. Ékzemplyarov, Khim.-Farm. Zh., No. 7, 24 (1971).
- 2. G. M. Dyson, May's Chemistry of Synthetic Drugs, 5th ed., Longmans, London (1959).
- 3. N. N. Borisova, V. E. Limanov, and A. V. Starkov, Med. Prom. SSSR, No. 7, 16 (1966).
- 4. N. D. Sukhareva and E. K. Chadova, Proceedings of the Central Scientific-Research Disinfection Institute [in Russian], Vol. 8 (1954), p. 127.
- 5. G. S. Kolesnikov, A. S. Tevlina, and N. I. Skripchenko, Vysokomol. Soedin., Ser. A, <u>13</u>, No. 8, 2139 (1971).
- 6. A. S. Tevlina, V. V. Korshak, N. I. Skripchenko, and T. A. Ogneva, Plasticheskie Massy, No. 8, 7 (1972).
- 7. Z. S. Sidenko, Khim.-Farmats. Zh., No. 5, 15 (1969).
- 8. W. Wood, J. Chem. Soc., 3327 (1953).