SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF COPOLYMERS BASED ON NEW DIALLYL MONOMERS AND SULFUR DIOXIDE

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A series of copolymers of 2,2-diallyl-1,1,3,3-tetramethylguanidinium chloride and tris(diethylamino)diallylaminophosphonium chloride and tetrafluoroborate with sulfur dioxide have been obtained by free-radical polymerization reactions. The antimicrobial activity of the synthesized compunds with respect to several bacteria, spores, and fungi was determined by the method of double serial dilutions.

Key words: diallyl monomers, copolymers, antimicrobial activity, synthesis.

A guanidine group in the repeat unit of polymers is known to impart to them high biocidal activity [1]. This enables such polymers to be widely used as antibacterial preparations [2, 3]. For example, new alkylene- and hydroxyalkyleneguanidine antiseptics have been developed [4].

Herein we study the antimicrobial activity of copolymers of 2,2-diallyl-1,1,3,3-tetraethylguanidinium chloride and tris(diethylamino)diallylaminophosphonium chloride and tetrafluoroborate with sulfur dioxide.

EXPERIMENTAL CHEMICAL PART

NMR spectra were recorded on a Bruker AM-300 spectrometer at 75.5 MHZ operating frequency (^{13}C). Spectra were recorded with broad-band proton decoupling and in JMOD mode. The solvents were DMSO-d_6 and D_2O; internal standards TMS and 2,2-dimethyl-2-silapentane-5-sulfonic acid, respectively.

2,2-Diallyl-1,1,3,3-tetraethylguanidinium chloride (AGC) was prepared by the published method [5]. The purity of AGC was monitored by elemental analysis and ^{13}C NMR (Table 1). $C_{15}H_{30}ClN_3$.

TABLE 1 lists chemical shifts (δ, ppm) and multiplicities in ^{13}C NMR spectra of AGC.

We used initiators azoisobutyrylnitrile and potassium persulfate. Solvents DMSO, MeOH, THF, and dichloromethane had characteristics corresponding to those in the literature after purification by the usual methods [6].

Sulfur dioxide was dried by passage through conc. H_2SO_4 and freshly calcined $CaCl_2$.

Solvents and other standard reagents had properties corresponding to those in the literature after purification by the usual methods.

Tris(diethylamino)diallylaminophosphonium chloride (EAAP-Cl). Tris(diethylamino)phosphazohydride (392 g, 1.5 mol) was stirred vigorously, treated with freshly distilled allyl chloride (600 g, 7.5 mol), and stirred until the temperature of the mixture stopped rising, at which point the stirring mixture was treated with an aqueous solution of NaOH (255 g, 6.4 mol, 50%) keeping the temperature below 38°C. The mixture was stirred for another 30 – 45 min and then held for 10 – 12 h under gentle reflux. The mixture was cooled. The middle layer was separated and extracted with CH_2Cl_2 to remove EAAP-Cl. The extract was evaporated stepwise in a rotary evaporator at 100°C, first using a water aspirator and then an oil pump (5 – 7 mm Hg), to afford EAAP-Cl (490 g, 86.5% of theoret.). The purity was monitored by elemental analysis and ^{13}C NMR (Table 1). $C_{18}H_{40}ClN_4P$.

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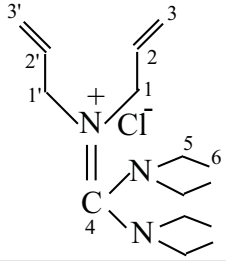
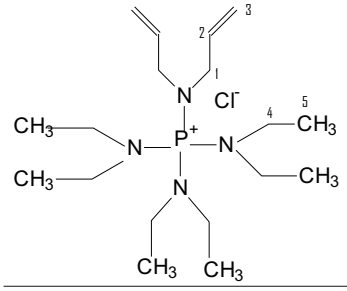
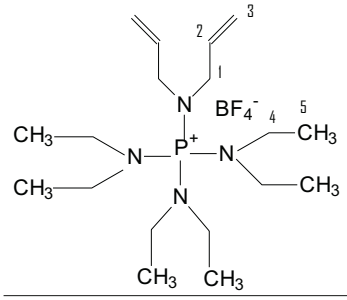
TABLE 1. Chemical Shifts and Multiplicities of ^{13}C NMR Resonances of AGC, EAAP-Cl, and EAAP-BF_4

Structure	C_1, $C_{1'}$	C_2, $C_{2'}$	C_3, $C_{3'}$	C_4	C_5	C_6
	54.56 t	133.76 d	123.41 t	165.71 s	45.83 t	14.48 q
	49.27 t	132.54 d	119.58 t	40.58 t	13.09 q	
	49.65 t	134.08 d	119.65 t	41.02 t	13.73 q	

Tris(diethylamino)diallylaminophosphonium tetrafluoroborate (EAAP-BF_4). EAAP-Cl (490 g) was dissolved in distilled water (2.0 L), stirred vigorously, treated with an aqueous solution of $NaBF_4$ (960 g, 20%), stirred for 15 – 20 min, and left for 1 d at room temperature. The precipitate was filtered off, washed with distilled water, and dried in vacuo at 80°C to constant mass to afford EAAP-BF_4 (390 g, 70.1% of theoret.). The purity was monitored by elemental analysis and ^{13}C NMR (Table 1). $C_{18}H_{40}F_4N_4PB$.

Copolymerization of AGC and the diallylaminophosphonium salts with SO_2 was carried out in a glass reactor as before [7]. The polymers were purified by double reprecipitation from a solution in the appropriate solvent (MeOH) added to a precipiting solvent (THF) and dried in vacuo at 50°C to constant mass. The composition of the copolymers was calculated from the elemental analysis.

The structures of the copolymers were confirmed by ^{13}C NMR spectroscopy. The spectra indicated that AGC, EAAP-BF_4, and EAAP-Cl copolymerized with SO_2 through both double bonds and intramolecular cyclization to form copolymers **I**, **II**, and **III** with pyrrolidine structures (Table 2).

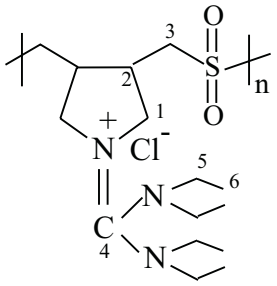
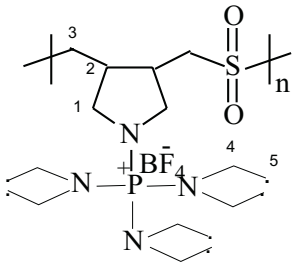
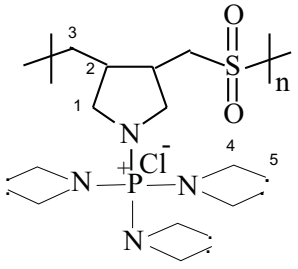
Copolymers of EAAP-BF_4 with SO_2 (copolymer **III**) were soluble in MeOH, DMSO, and DMF; copolymers of AGC with SO_2 and EAAP-Cl with SO_2 (copolymers **I** and **II**), also in water.

EXPERIMENTAL BIOLOGICAL PART

Acute toxicity of the copolymers upon peroral administration was studied in white mongrel mice of both sexes (18 – 22 g) by the literature method [8].

Antimicrobial activity was determined using the double serial dilution method [9] against test cultures of *Escherichia coli* str. 25922; *Staphylococcus aureus* str. 906; *S. saprophyticus* ATCC 15305; *Micrococcus luteus* ATCC 4698; *Bacillus antracoides* 1312; *B. subtilis* ATCC 6633; *B. proteus* str. "Flowers"; *Candida albicans* 264/624; and *Salmonella* spp. (Tarasevich State Research Institute for Standardization and Monitoring of Medical Biological Preparations). We used an 18-h agar culture (2.5×10^5 microbes per mL of medium). Solutions of compounds in water or DMSO were used in the tests. The maximum test concentrations were 1000.0 mg/mL. Tubes were incubated at 37°C with subsequent innoculation after 24 h into slanted tubes with meat peptone agar. Results were calculated from the presence and nature of culture growth in the nutrient medium.

TABLE 2. ^{13}C NMR Spectra of Copolymers **I**, **II**, and **III** (DMSO-d_6, TMS)

No.	Polymer	Chemical shifts and multiplicities of resonances, ppm						
		*	C_1	C_2	C_3	C_4	C_5	C_6
I			55.05 t	36.76 d	50.96 t	162.0 s	46.23 t	14.30 q
II		*cis* *trans*	50.57 51.04 t	34.90 36.94 d	50.42 52.14 t	39.40 39.35 t	12.93 12.43 q	
III		*cis* *trans*	52.60 53.34 t	36.71 38.77 d	51.98 54.82 t	41.27 40.37 t	13.94 13.94 q	

* geometric isomers.

TABLE 3. Antimicrobial Activity of Copolymers of AGC with SO_2 (Copolymer **I**), EAAP-BF_4 with SO_2 (Copolymer **II**), and EAAP-Cl with SO_2 (Copolymer **III**)

No.	Name of microorganism species (strain)	Antimicrobial activity (MIC), mg/mL		
		Copolymer I	Copolymer II	Copolymer III
1	*Escherichia coli*, str. 25922	500.0	62.5	62.5
2	*Staphylococcus aureus*, str. 906	7.8	<31.2>15.6	7.8
3	*Micrococcus luteus*, ATCC 4698	7.8	<15.6>7.8	>7.8<15.6
4	*Staphylococcus saprophyticus*, ATCC 15305	15.6	15.6	<31.2>15.6
5	*Candida albicans*, 264/624	15.6	<62.5>31.2	31.2
6	*Bacillus antracoides*, 131	–	500.0	<500.0>250.0
7	*Bacillus proteus*, str. “Flowers”	–	>250.0<500.0	<1000.0>500.0
8	*Bacillus subtilis*, ATCC 6633	>31.2<62.5	<62.5>31.2	<15.6>7.8
9	*Salmonella spp.*	–	125.0	<125.0

Copolymers **I** – **III** were nontoxic (LD_{50} upon injection into the stomach was > 1000 mg/kg).

The study of the antimicrobial activity of copolymers **I** – **III** showed that they inhibited most effectively growth of

Staphylococcus and *Micrococcus* species and the yeast-like fungus *Candida albicans* (Table 3).

Thus, the copolymers of the new allyl monomers with SO_2 are nontoxic, exhibit pronounced antimicrobial activity, and can be used for drug production and biotechnology.

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