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Synthesis and Study of Antimicrobial Action of Guanidine Derivatives of Pectin and Carboxymethyl Cellulose

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Abstract

Polymeric guanidine derivatives occupy a special place among antimicrobial compounds. Guanidines are well soluble in water, have a wide spectrum of antimicrobial action and durable effects, nontoxic, and also meet modern medical requirements. Due to the high reactivity of guanidine to interact with various functional groups, we manage to carry out a number of new chemical modifications that allow obtaining new biologically active low and high molecular mass compounds. The present paper presents preparation of water-soluble guanidine derivatives of pectin and carboxymethyl cellulose bound between each other with the amine bond. A process for the preparation of guanidine derivatives of pectin and carboxymethyl cellulose consists of the following stages: periodate oxidation of polysaccharides, the condensation reaction of guanidine with oxidized pectin and carboxymethyl cellulose, −C=N bond reduction. The structure and composition of the resulting compounds were studied by IR spectroscopy, X-ray structural and elemental analysis (by nitrogen content). It was determined that the maximum number of guanidine groups in the resulting products was contained during the interaction of dialdehyde derivatives of pectin and carboxymethyl cellulose with guanidine in a 1.0 : 2.3 molar ratio. Antimicrobial activity of resulting guanidine derivatives of pectin and carboxymethyl cellulose against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella*, *Escherichia coli* and *Streptococcus faecalis* was explored. It was found that bacteriostatic effect began to increase with an increase in the concentration of the obtained compounds. The paper gives acute toxicity studies results on guanidine derivatives of pectin and carboxymethyl cellulose, upon the results of which they can be referred to class IV slightly toxic substances.

Key words: pectin, carboxymethyl cellulose, guanidine, periodate oxidation, acute toxicity, antimicrobial action

INTRODUCTION

The development of studies aimed at the creation of new antimicrobial compounds is related to modern medicine demands [1]. Above all, new antimicrobial drugs obtained should have a wide spectrum of antimicrobial action, be nontoxic, not become accumulated in the organism, show a prolonged effect and selectively act on bacterial cells.

Among antimicrobial compounds meeting the above requirements, one should note polyguanidines that are products of polycondensation reaction of guanidine salts with hexamethylenediamine. Polyguanidines are very soluble in water, solutions are odorless, have a broad spectrum of antimicrobial activity, demonstrate prolongation, do not accumulate in the body and belong to class IV slightly toxic substances [2]. Antimicrobial action of polyguanidines is explained by the presence in a macromolecule of repeating guanidine fragment giving the macromolecule a positive charge. A positively charged macromolecule, in its turn, acquires the capacity to be adsorbed in the surface of the cell membrane of bacteria,

penetrate to the inside of the cell and hinder replication of nucleic acids [3]. Due to the high reactivity of amino groups in the guanidine molecule, opportunities of its wide modification and obtaining therefrom new cationic antimicrobial polymers arise.

Papers [4, 5] wherein polyguanidines are obtained by introducing guanidine groups to biopolymer structure look promising. Compounds obtained in such a way show antimicrobial properties. Synthesis of guanidine-containing derivatives of polysaccharide is carried out in two steps: polysaccharide oxidation and condensation reaction of oxidized biopolymers and guanidine. The coupling of guanidine to polysaccharides is carried out *via* the azomethine −C=N bond. This chemical bond allows not only preserving guanidine antimicrobial activity but also obtaining biologically active compounds with prolonged action [6].

Given the above, obtaining water-soluble guanidine-containing derivatives of pectin and carboxymethyl cellulose, in which the guanidine fragment is bound with a macromolecule through an amine bond and studying their antimicrobial activity are of interest.

EXPERIMENTAL

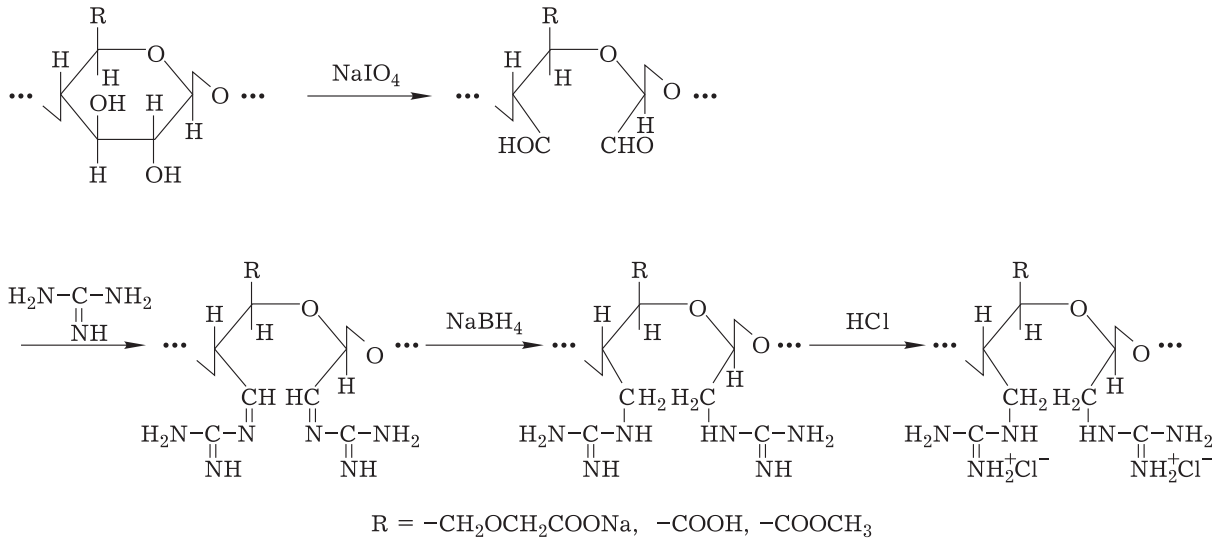
Purified citrus pectin (M = 162 000, $-OCH_3$ group content is 7.6 %), Na-CMC (the degree of polymerisation (DP) = 400, γ = 85±5), and chemically pure reagent grade guanidine hydrogen carbonate ($(H_2N)_2C{=}NH \cdot 1/2H_2CO_3$) were used as initial polysaccharides.

Periodate oxidation of pectin and Na-CMC

In a glass beaker, 1 g of Na-CMC or pectin was poured with 100 mL of distilled water, then 100 mL of acetate buffer solution with pH 4.3 and a 0.2 M solution of $NaJO_4$ was added with a molar solution of Na-CMC, pectin/$NaJO_4$ = 1.0 : 1.5, oxidation process continued for 2.5−3 h at a temperature of 25 °C. Upon the completion of the periodate oxidation reaction, the modified polysaccharides were precipitated with acetone, the precipitates formed were rinsed with 70 % ethyl alcohol until a negative reaction to JO_4^- and JO_3^- ions (monitoring by the reaction with silver nitrate solution) and dried in darkness under vacuum over P_2O_5. The content of aldehyde groups was determined by the iodometric method.

Preparation of guanidine-containing derivatives of pectin and carboxymethylcellulose

To a glass beaker equipped with a stirrer, 50 mL of solutions containing 0.015−0.025 mol of guanidine carbonate was poured, then 0.01 mol of dialdehyde pectin (DAP) or carboxymethyl cellulose (DACMC) was added, the reactions continued for 30 min at a temperature of 25 °C, the resulting azomethine −C=N− bond was reduced using sodium borohydride. For this purpose, 0.02 mol of $NaBH_4$ was dissolved in 5 mL of a 0.1 M solution of NaOH and were added in portions to reaction mixtures. Reduction of −C=N− bond continued for 1.5−2.5 h at room temperature (Scheme 1). The end of reduction was determined by a change in colour intensity of the reaction mixture (gradual discoloration of the



Scheme 1. Synthesis scheme of guanidine-containing derivatives of pectin and carboxymethyl cellulose as hydrochloride.

TABLE 1

Composition of interaction products of DAP with $(H_2N)_2C=N$ (time is 30 min, T = 25 °C)

Degree of oxidation of pectin, mol. %	DAP/$(H_2N)_2C=N$ ratio	Nitrogen content, %	Degree of substitution (DS) by aldehyde groups, mol. %	Amount of guanidine, %
30.0	1 : 1.5	6.2	42.0	26.0
33.0	1 : 2.0	8.5	58.0	35.0
33.0	1 : 2.2	8.8	63.0	37.0
35.0	1 : 2.3	9.7	70.0	40.8
35.0	1 : 2.5	9.9	70.0	41.0

TABLE 2

Composition of interaction products of DACMC with $(H_2N)_2C=N$ (time is 30 min, T = 25 °C)

Degree of oxidation of Na CMC, mol. %	DACMC/$(H_2N)_2C=N$ ratio	Nitrogen content, %	Degree of substitution (DS) by aldehyde groups, mol. %	Amount of guanidine, %
29.0	1 : 2.0	5.8	52.0	24.5
30.0	1 : 2.2	6.4	58.0	27.0
31.0	1 : 2.3	6.9	62.0	30.0
31.0	1 : 2.5	7.0	62.5	30.3

bright yellow colour). Then, the reaction mixture was added dropwise to a 5 % solution of HCl and the pH was brought to 6.0–6.2. Reaction products were precipitated and washed with acetone. The resulting precipitates were dissolved in water, purified from impurities by dialysis for 20 h and dried by lyophilization.

Infrared spectra of the resulting compounds were recorded on Vector-22 FTIR spectrometer in 400–4000 cm^{-1} wave range using KBr pellets (3 mg of sample per 300 mg of KBr). Physical structure was studied using XRD-6100 powder diffractometer (Shimadzu, Japan).

The amount of nitrogen was defined by the Kjeldahl method [7]. The degree of substitution and the amount of nitrogen in the resulting products were calculated by the methods described in [8, 9]. Antimicrobial activities of guanidine derivatives of pectin and carboxymethyl cellulose were studied by the agar diffusion method [10] under *in vitro* conditions, acute toxicity was defined by the Litchfield and Wilcoxon method [11].

RESULTS AND DISCUSSION

The infrared spectrum of guanidine-containing derivatives of pectin and carboxymethyl cellulose contained adsorption bands in the regions of 3180–3423 cm^{-1} (–OH); 2921–2923 cm^{-1} ($-CH_2-$); 1605–1615 cm^{-1} (–NH–); 1660–1680 cm^{-1} typical for –C=N– bond in the guanidine fragment.

The composition of interaction products of DAP and DACMC with guanidine are given in Tables 1 and 2. As can be seen, there is no complete reaction between aldehyde groups of pectin and carboxymethyl cellulose with guanidine with a molar ratio of DAP, DACMC/$(H_2N)_2C=N$ = 1.0 : 1.5–2.2). An increase in guanidine molar ratio to 2.3 leads to a complete reaction of aldehyde groups of polysaccharides and guanidine. Quantitative content of nitrogen suggests that two guanidine fragments fall on each elementary unit of oxidized pectin.

The results presented in Fig. 1 demonstrate that the reaction of guanidine with DAP and DACMC proceeds intensely for the first 5 min. A complete reaction of guanidine with DAP is observed with 15 min reaction time, and with DACMC – 10 min. A further increase in reaction

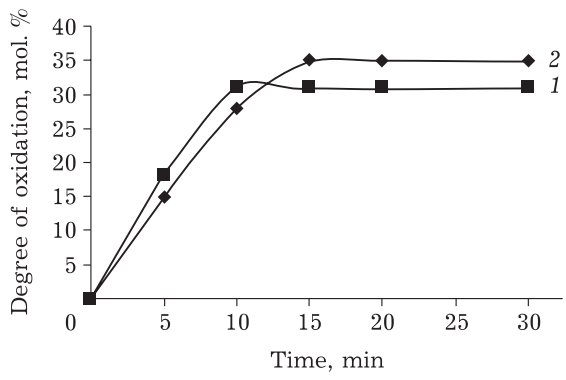


Fig. 1. Reaction rate of guanidine with DACMC (*1*) and DAP (*2*) with a molar ratio DACMC, DAP/$(H_2N)_2C=N$ = 1.0 : 2.3 *vs* reaction time. Degree of oxidation (% mol.): 31.0 (*1*), 35.0 (*2*)

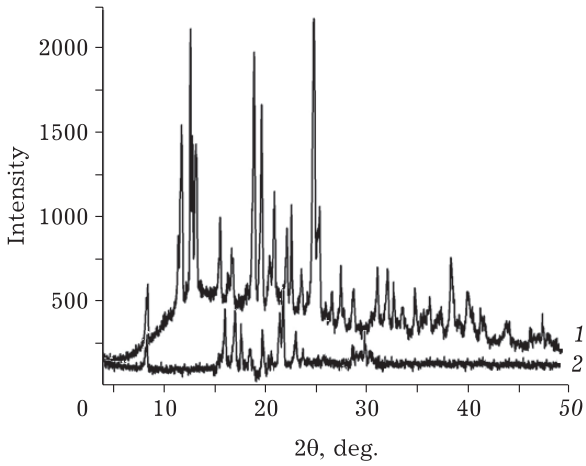


Fig. 2. X-ray patterns of pectin (*1*) and guanidine substituted pectin (*2*).

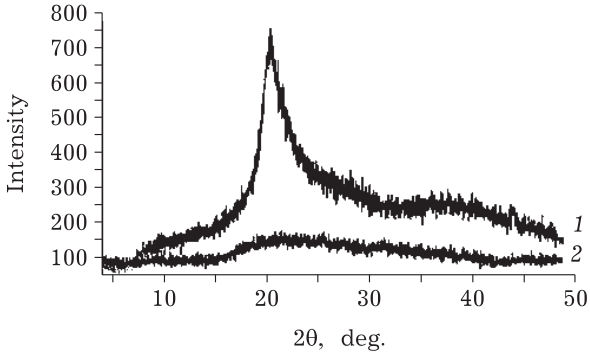


Fig. 3. X-ray patterns of Na-CMC (*1*) and guanidine carboxymethyl cellulose (*2*).

time does not lead to a rise in the content of guanidine groups in final products.

Carried out studies on an examination of the physical structure of guanidine-containing derivatives of pectin and carboxymethylcellulose (amorphous or crystalline state) demonstrated that these compounds were amorphous substances (Figs. 2 and 3). Amorphisation of pectin and carboxymethyl cellulose occurs resulting from a decrease in the number of –OH groups and destruction of ordered packings of polysaccharides during periodate oxidation and the introduction of guanidine groups in macromolecules.

With the aim of studying biological activity of the resulting derivatives of pectin and carboxymethyl cellulose studies were performed against gram-positive and gram-negative bacteria at a concentration of drugs of 50 μg/mL (Table 3). It can be seen that at a concentration of 50 μg/mL, guanidine substituted pectin has weak activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella*, *Escherichia coli* and *Streptococcus faecalis*. Guanidine substituted carboxymethyl cellulose shows moderately pronounced activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*.

It is noteworthy that the antimicrobial activity of the agents under study depends on their concentration (Fig. 4). Thus, there is no antimicrobial activity with agent concentration of 10–25 μg/mL. A further increase in the concentration of the compounds under study to 50 μg/L leads to a drastic increase in the growth inhibition zone of *Staphylococcus aureus*. A further rise in agent concentration to 75–100 g/L results in an increase in bacteriostatic effects against *Staphylococcus aureus*.

It has been determined that LD_{50} for guanidine derivatives of pectin was 3000 mg/kg, for carboxymethyl cellulose – 1800 mg/kg, which gives grounds to attribute them to class IV slightly toxic substances.

Thus, the carried out microbiological studies have demonstrated that the obtained compounds

TABLE 3

Sensitivity of microorganisms to guanidine substituted pectin (DS = 70 mol. %, the amount of guanidine is 40.8 %) and carboxymethyl cellulose (DS = 62 mol. %, the amount of guanidine is 30 %) at a concentration of 50 μg/mL

Drugs	Growth inhibition zone, mm				
	Microorganisms				
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Klebsiella*	*Escherichia coli*	*Streptococcus faecalis*
Guanidine pectin	14.0±0.1	13.0±0.2	12.0±0.2	12.0±0.1	11.0±0.1
Guanidine CMC	16.0±0.1	15.0±0.1	13.0±0.1	16.0±0.1	12.0±0.1

Note. Diameters of growth inhibition zones of less than 10 mm – the absence of antibacterial activity; 10–15 mm – weak activity; 15–20 mm – moderate activity; higher than 20 mm – distinct activity.

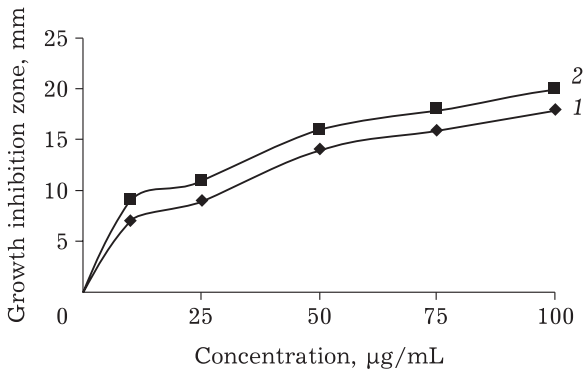


Fig. 4. Bacteriostatic effect of guanidine substituted pectin (*1*) and carboxymethyl cellulose (*2*) against *Staphylococcus aureus vs*. concentration.

wherein the guanidine fragment is bound with a macromolecule of pectin and carboxymethyl cellulose through an amine bond show antimicrobial properties and represent low-toxic substances.

CONCLUSION

Water-soluble derivatives of pectin and carboxymethyl cellulose with various contents of guanidine and a degree of substitution were obtained. The structure and composition of the resulting compounds were studied by IR spectroscopy, X-ray, and elemental analysis (according to nitrogen content). It was found that a complete reaction of aldehyde groups of pectin and carboxymethyl cellulose with guanidine occurred with the molar ratio of dialdehyde carboxymethyl cellulose (DACMC), dialdehyde pectin (DAP)/$(H_2N)_2C{=}N$ = 1.0 : 2.3.

It was established that guanidine-containing derivatives of pectin and carboxymethyl cellulose showed antimicrobial action against *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Escherichia coli* and *Streptococcus faecalis* in a concentration of 50 μg/mL.

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