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Synthesis and Study of Antimicrobial Activity of Sulphamic Pectin Derivatives

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Abstract

Preparation of new derivatives of polysaccharides showing biological activity, precisely, antimicrobial properties remains quite a priority task not only in chemistry of high molecular mass compounds but also in pharmaceutics and medicine, since some antimicrobial compounds obtained on the basis of natural polymers are superior to synthetic low molecular mass antimicrobial compounds on some criteria. New water soluble pectin derivatives containing sulphamic groups in their structure were obtained in this paper by the interaction of sulphamic acid with polysaccharide aldehyde groups. The structure and composition of the resulting compounds were studied by IR spectroscopy, elemental (nitrogen, sulphur) and X-ray crystal analyses. Sulphamic pectin derivatives with various contents of sulphamic groups were obtained by changing the concentration of sulphamic acid in relation to dialdehyde pectin derivatives. The optimum ratio of reactive components that is CHO/NH_2SO_3H = 1.0 : 2.5 and the reaction time of 45 min were found. Study results of antimicrobial action of the synthesized sulphamic pectin derivatives were presented. Biological activity of the resulting compounds was studied by the disk diffusion method in relation to Gram positive and Gram negative bacteria. Direct dependence of antimicrobial activity of the studied drugs on the quantitative content of sulphamic groups in pectin was found. It was determined that sulphamic pectin derivatives with a degree of substitution of 35.0 showed antimicrobial activity in relation to *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris, Streptococcus faecalis, Streptococcus pyogenes, Streptococcus faecalis, Pseudomonas aeruginosa* with a concentration of 50 мg/mL. Antimicrobial action begins to decrease with the decrease in the number of sulphamic groups. Study results of acute toxicity of sulphamic pectin derivatives were presented. According to the results, they can be referred to class V almost nontoxic substances.

Key words: polysaccharides, pectin, sulphamic acid, periodate oxidation, antimicrobial activity

INTRODUCTION

Research devoted to the synthesis of derivatives of polysaccharides showing the antimicrobial activity has been widely developed. It is known that we manage to obtain compounds showing the antimicrobial activity by the introduction of various functional groups (SO_3^-, COO^-), metal ions (Ag^+, Hg^+, Cu^+) or bactericidal substances [1–4].

Oxidised polysaccharides containing aldehyde groups can be used for preparation of new antimicrobial compounds, since it is known that they enter into condensation reactions with substances that have the NH_2–R structure. In this way, based on dialdehyde polysaccharides, a

series of compounds with antibacterial activity has been obtained after the introduction of various antimicrobial specimens or low molecular mass substances into the polymer structure [5–8].

Citrus pectin dialdehyde obtained by selective oxidation using sodium periodate was used by us in the present paper, sulphamic acid (NH_2SO_3H) containing a primary amino group and entering into the composition of disinfectants was used as a nucleophilic reagent.

Pectin derivatives containing sulphamic groups were obtained according to the scheme in Fig. 1.

Infrared spectra of the resulting compounds were recorded using a Bruker Vector-22 FT-IR spectrometer in the wavelength region of 400–4000 cm^{-1} in KBr pellets (3 mg sample/300 mg KBr). The physical structure was studied using a XRD-6100 diffractometer (Shimadzu, Japan).

The composition of the resulting sulphamic pectin derivatives was studied according to sulphur and nitrogen contents. Sulphur content was determined using a Carlo Erba EA 1108 elemental analyser (Italy), the amount of nitrogen – by Kjeldahl method [9]. The degree of substitution of sulphamic pectin derivatives was determined according to nitrogen content [10]. Antimicrobial activity of sulphamic derivatives was studied *in vitro* by the disk diffusion method [11], toxicity – by the Prozorsky method.

EXPERIMENTAL

Periodate oxidation of pectin

A sample weight of 3 g of citrus pectin was dissolved in 50 mL of water, then 100 mL of acetate buffer solution with pH of 4.3 was added and stirred for 2 h followed by the addition of 127 mL of a 0.2 M $NaIO_4$ solution therefrom at 25 °C with a molar ratio of the components of pectin/$NaIO_4$ = 1.0 : 1.5 [12, 13]. The reaction was carried out for 1–3 h. The products were precipitated with acetone. The residues were washed with 75 % until the negative reaction for IO_4^- and IO_3^- ions (potassium iodide test paper control). The content of aldehyde groups was determined by the iodometric method. Reaction products were dried in the dark under vacuum over P_2O_5. The obtained samples had a degree of oxidation of 20–42 %.

Condensation of sulphamic acid with pectin dialdehyde

To a solution of 0.97–3.88 g (0.01–0.04 mol) of sulphamic acid, 1.74 g (0.1 mol) of pectin dialdehyde (PD) with an oxidation degree of 20–42 % calculating from 1 mol of dialdehyde units per 1.0–4.0 mol of NH_2SO_3H was added at a temperature of 25 °C and the resulting mixture was stirred for 2 h.

Reduction reaction of the azomethine bond

A 10 % solution of Na_2CO_3 was added to a reaction mixture containing product **III**, and pH was brought to 8.0–8.5. Afterwards, 0.16–0.35 g (0.004–0.01 mol) of $NaBH_4$ was dissolved in the required volume of a 0.05 M solution of Na_2CO_3 and slowly added to the reaction mixture (sodium borohydride was taken in the twofold excess in relation to the number of aldehyde groups of pectin). A reduction reaction

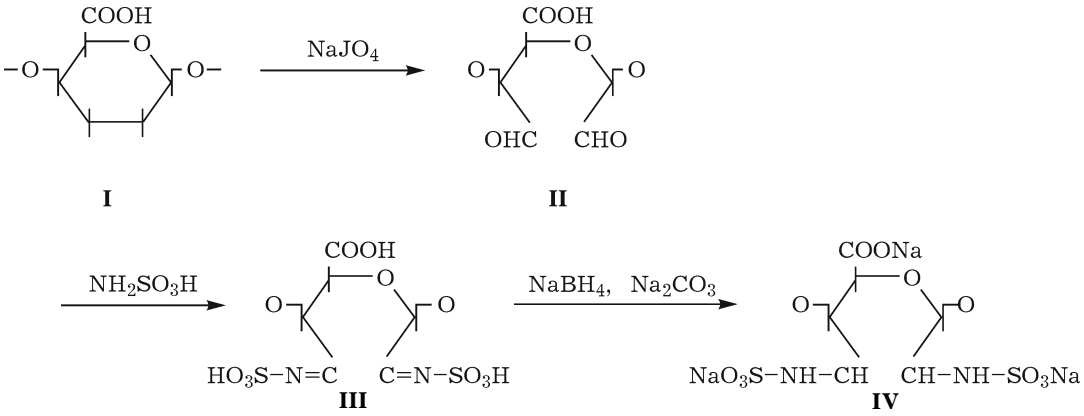

Fig. 1. Scheme for the synthesis of sulphamic pectin derivatives.

TABLE 1

Composition of the interaction products of pectin dialdehyde (PD) with sulphamic acid

Exp. No.	Degree of oxidation of pectin, %	PD/NH_2SO_3H ratio	Content, %		Degree of substitution (DS)
			Nitrogen	Sulphur	
1	20	1 : 1.0	1.1	1.2	7.0
2	23	1 : 2.0	2.4	2.7	16.0
3	23	1 : 2.5	3.2	3.6	20.0
4	30	1 : 1.5	2.8	3.2	19.0
5	33	1 : 2.0	3.6	4.1	25.0
6	34	1 : 2.5	4.3	4,8	30.0
7	34	1 : 3.0	4.8	5.5	31.0
8	38	1 : 4.0	4.8	5.5	34.0
9	42	1 : 4.0	5.1	5.8	35.0

of the C=N bond took 3 h. Reaction products were precipitated and rinsed with acetone. The resulting residues were dissolved in water, purified from impurities by the dialysis method for 18 h and dried by lyophilisation.

RESULTS AND DISCUSSION

It can be seen from Fig. 1 data that the C=N bond is formed after the condensation reaction of NH_2SO_3H with aldehyde groups of pectin. The IR spectrum of compound III contains adsorption bands (cm^{-1}): 3475 (−OH), 1747 (−CHO), 1630 (−C=N−) 1242 (SO_2), 803 (SO).

After reduction of the azomethine bond, an adsorption band in the area of 1570 cm^{-1} (−NH) appears in the IR spectrum of compound **IV**, the adsorption bands at 1747 and 1630 cm^{-1} typical for the −CHO group and −C=N− bond disappear.

From the data of Table 1, it can be seen that with an increase in the oxidation degree of pectin and the ratio of PD/NH_2SO_3H, the degree of substitution (DS) of the final products is increased. An increase in the concentration of sulphamic acid in the reaction medium to 2.5 mol leads to an increase in the content of sulphamic groups in the polysaccharide. A further increase in the concentration of NH_2SO_3H does not affect changes in the amount of sulphamic groups. According to the results of the IR spectrum and the data of Table 1, one can say that the −CHO group (compound **III**) is present in the polysaccharide structure after the interaction of oxidized pectin with sulphamic acid even with the ratio of PD/NH_2SO_3H = 1.0 : 4.0. Apparently, this is due to the formation of electrostatic effects in repulsion of sulphamic acid, which does not allow fully reacting sulphamic acid with PD. It was found that the interaction of pectin dialdehyde with sulphamic acid proceeded intensively for the first hour and ended almost completely for 45 min; further, the content of sulphamic acid was not changed.

It is known that the X-ray method allows studying the physical structure (amorphous or crystalline condition) of polysaccharides and their derivatives. For this purpose, the physical structure of the synthesized compounds was studied. The studies carried out on the study of the physical structure of sulphamic pectins showed that these compounds were amorphous substances (Fig. 2). Amorphization of pectin happens resulting from a decrease in the number of −OH groups and the destruction of ordered polysaccharide-based packing in the periodate oxidation process and the condensation reaction with NH_2SO_3H [14, 15]. Thus, from the

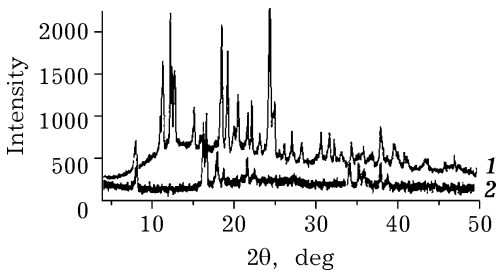

Fig. 2. Roentgenograms of pectin **I** (*1*) and final product **IV** (*2*).

TABLE 2

Microbial sensitivity towards pectin derivatives containing sulphamic groups (DS = 20.0–35.0) under *in vitro* conditions

Microorganisms	Sensitivity of the microbial growth inhibition zone, mm		
	DS = 20.0	DS = 30.0	DS = 35.0
Staphylococcus aureus	10.0±0.1	10.0±0.2	12.0±0.2
Staphylococcus epidermidis	9.0±0.1	10.0±0.1	12.0±0.1
Escherichia coli (lactose-positive)	8.0±0.1	10.0±0.1	10.0±0.2
Escherichia coli (lactose-negative)	10.0±0.2	11.0±0.1	
Proteus vulgaris	13.0±0.1	16.0±0.1	20.0±0.2
Streptococcus pyogenes	11.0±0.1	15.0±0.2	16.0±0.1
Streptococcus faecalis	10.0±0.1	11.0±0.1	12.0±0.2
Pseudomonas aeruginosa	9.0±0.1	10.0±0.1	12.0±0.2

Note. The drug's concentration of 50 μg/mL.

data of Fig. 2, it can be seen that the maximum diffraction peak for pectin is observed in the regions of 11–12.5, 18–19.5 and 24.8°, which is probably due to the formation of intramolecular hydrogen bonds of hydroxyl and carboxyl groups of pectin. The above-mentioned peaks in the roentgenogram of product **IV** disappear.

Antimicrobial activity of sulphamic pectin derivatives was studied by the paper disc method under *in vitro* conditions against gram-positive and gram-negative strains. Samples with SD = 20.0–35.0 were selected for the study.

The diameter of the zones of microbial growth inhibition was used for the study of antimicrobial activity of the studied compounds. A zone diameter of less than 10 mm was assessed as the absence of antimicrobial activity, 10–15 mm is a week activity, 15–20 mm is moderate expressed, above 20 mm is pronounced activity.

According to the data of Table 2, antimicrobial activity of sulphamic pectin derivatives depends on their substitution degree. Thus, a compound with DS = 35.0 at a concentration of 50 μg/mL shows a more pronounced antimicrobial activity against *Proteus vulgaris* and a moderate expressed activity against *Streptococcus pyogenes*. Antimicrobial activity is week against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Streptococcus faecalis* and *Pseudomonas aeruginosa.*

Sulphamic derivatives with DS = 20.0 and 30.0 show week and moderate expressed antimicrobial activities against *Proteus vulgaris*. They show a week antimicrobial activity against other microbes.

Acute toxicity study in white mice of both sexes weighing 18–21 g was performed by the Prozorsky method. The tested grugs were introduced intragastrically once by a special probe in the doses from 1000 to 8000 mg/mL. The results demonstrated that LD_{50} of sulphamic pectin derivatives was 7000 mg/kg, which gives grounds to attribute them to class V almost nontoxic substances.

CONCLUSION

Sulphamic pectin derivatives containing groups were obtained by the interaction of sulphamic acid with pectin dialdehyde. The composition, structure, and bactericidal efficiency of the obtained samples were determined using IR spectroscopy, elemental and X-ray analysis, as well as antimicrobial studies. It was found that sulphamic pectin derivatives showed antimicrobial activity and were attributed to class V almost nontoxic substances.

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