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Reaction of Arabinoogalactan from the Sibirian Larch with Kanamycin

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Abstract—By means of the UV, IR, and NMR spectroscopy, densimetry and viscometry reaction of arabinogalactan from Sibirian larch with kanamycin in water solutions was studied. The composition and the stability constant of the complex formed were evaluated, and optimal conditions of its synthesis were established.

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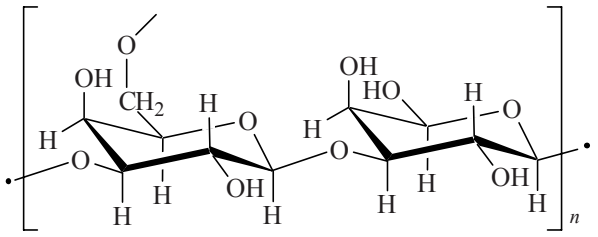
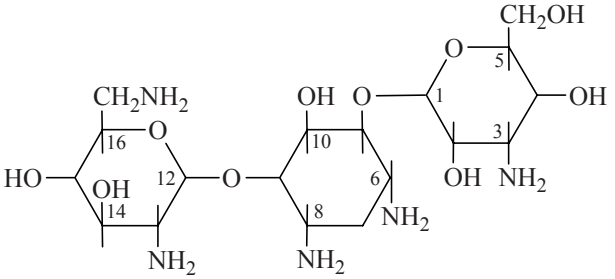
Natural polysaccharide arabinogalactan attracts great attention from the point of view of using it as a polymeric matrix for immobilization of various therapeutic preparations. Interest to this substance and the products of its chemical transformations as the objects of investigation and of practical application is first of all caused by the unique properties of this polysaccharide: the biological activity, nontoxicity, and the ability to biodegradation [1]. Investigations of biological activity of arabinogalactans was begun recently, and the results obtained were discussed in reviews [2, 3]. H. Yamada et al. showed that polysaccharides of this type exhibit high and varied physiological activity directly connected with their structural features [3–8]. Therefore the arabinogalactane can be considered a promising matrix for the synthesis of new chemical structures and creation of drugs of a varied spectrum of action.

Arabinogalactan of the Sibirian larch has a highly branched macromolecule with the backbone chain consisting mainly of the 1→3 linked β-D-galactopyranose residues. The greater part of them carries branches at C-6. Side chains contain 3,6-di-O- and 6-O-substituted residues of β-D-galactopyranose and 3-O-substituted residues of β-L-arabinofuranose. The terminal nonreducing residues are β-D-galactopyranose, β-D-arabinofuranose, and β-L-arabinopyranose [9]. Structure of the elementary unit of arabinogalactan may be described by the following formula.



The aim of this work is the investigation of the reaction of arabinogalactan with kanamycin by means of spectrophotometry, viscometry, and densimetry, and synthesis of new compounds exhibiting physiological activity.

Kanamycin belongs to the aminoglycoside antibiotics. It exhibits the bactericidal action towards the majority of Gram-positive and Gram-negative microorganisms, and also the acid-resistant bacteria (including tuberculosis mycobacterium) [10].



Absorption spectra of kanamycin and its mixture with arabinogalactan in water solutions in the presence of 0.1 M NaCl were taken. The spectrum of kanamycin

in 1×10^{-1} M water solution is characterised by the absorption band with λ_{max} 275 nm. At the addition of arabinogalactan to the solution of this aminoglycoside an increase in the intensity of the absorption band of kanamycin and the batochromic shift of λ_{max} to 286 nm are observed. These facts show that the reaction between the substances under study takes place. Most probably it leads to the formation of a complex compound. Besides the complex formation such shift in the electron absorption spectrum may be caused by the change in pH of the solutions under investigation. But adjustment of pH of the medium in the range from 12 to 1 leads only to the insignificant decrease in the intensity of the kanamycin absorption band, while its location does not change.

The composition of complexes was studied in water solutions at pH 7 at the wavelength 286 nm. For the evaluation of composition spectrophotometrical method of molar ratios was used [11].

To this purpose dependence of the absorption of solutions on the concentration of kanamycin at a constant concentration of polysaccharide was studied. According to the molar ratios method spectral changes are described by the following equation.

$$[AG]_0/(A - A_0) = 1/(\varepsilon - \varepsilon_0) + 1/\{(\varepsilon - \varepsilon_0)K[R]\}.$$

Here A and A_0 are optical densities of solutions in the presence and in the absence of R, $[AG]_0$ is the starting concentration of arabinogalactan, ε and ε_0 are molar extinctions for the corresponding composition, K is the stability constant, [R] is the concentration of the second reagent.

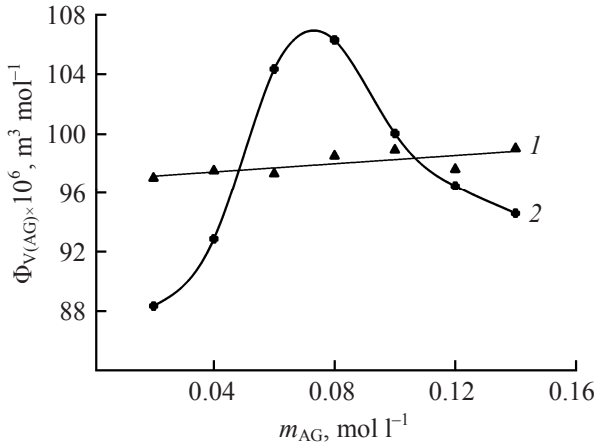


Fig. 1. Dependence of the apparent molar volume of arabinogalactan on its concentration in (*1*) water and (*2*) water solution of kanamycin at 25°C, m_{CN} 0.04 mol l^{-1}.

Stability constant of complexes was evaluated from the the slope of the dependence of $[AG]_o/(A - A_o)$ on 1/[R]. Its value was 1.01×10^3 showing the formation of a fairly stable compound. The plot of the optical density of kanamycin solutions vs the concentration of arabinogalactan shows that the formation of complexes of the 2:1 composition takes place. That means that one molecule of kanamycin reacts with two elementary units of arabinogalactan. Besides, the composition of arabinogalactan complex with kanamycin was evaluated by densimetry and viscometry. Values of the apparent molar volumes of arabinogalactan $[\Phi_{v(AG)}]$ in water and water solution of kanamycin were found from the known relationship.

$$\Phi_{V(AG)} = M/\rho - 1000(\rho - \rho_0)/m_{AG}\rho\rho_0,$$

where M is the molecular mass of arabinogalactan, and ρ_o are the densities of the three-component solution and water solution of kanamycin with the fixed concentration.

Dependences of the apparent molar volume of arabinogalactan $\theta_{v(AG)}$ on its concentration for the binary (arabinogalactan + water) and three-component systems (arabinogalactan + water + kanamycin) at the fixed concentration of kanamycin are presented in the Fig. 1. It is seen that the ability of arabinogalactan to complex formation with kananycin is reflected in the volume properties of solutions. The dependence of the apparent molar volume of arabinogalactan on its concentration at the fixed kanamycin content has maximum point corresponding to the 2:1 arabinogalactan–kanamycin ratio. For water solutions of arabinogalactan no extremum points were found. Changes in the properties of solutions because of the interaction between the components of the system were also observed in the changes of their viscosity.

Dynamic viscosity values were found from the relationship:

$$\eta = K\tau\rho,$$

where K is the viscometer constant, τ is the solution flow time, ρ is the density of this solution. Dependence of the dynamic viscosity of kanamycin solutions on the polysaccharide content for the arabinogalactan–water–kanamycin system has complex character with the expressed discontinuity point at the 2:1 component ratio (Fig. 2). According to the reported data [12] the most informative is the dependence of the first derivative of the solution viscosity on its composition. With this purpose dependence $\eta = f(m_{AG})$ for the arabinogalactan–water–kanamycin system was des-

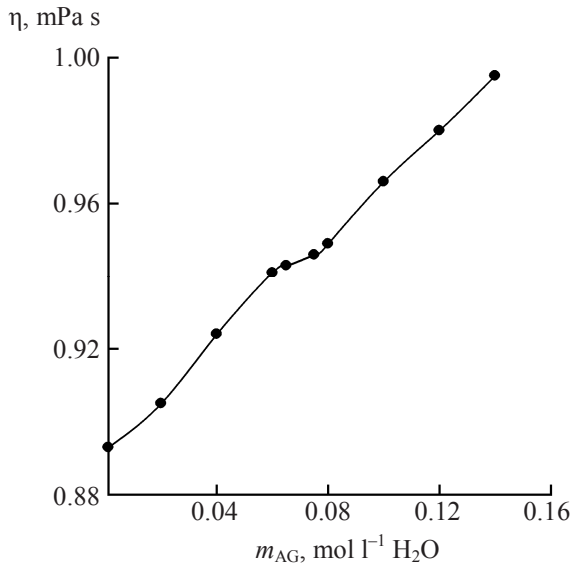


Fig. 2. Dependence of dynamic viscosity of the arabinogalactan solution on its concentration in water solution of kanamycin at 26°C, m_{CN} 0.04 mol l^{-1}.

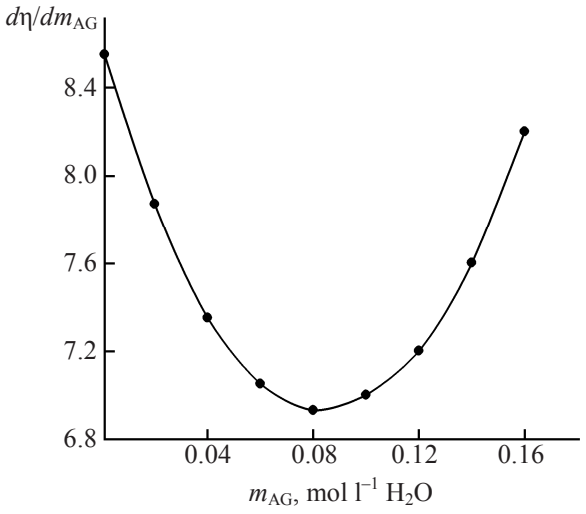


Fig. 3. Dependence of the first derivative of viscosity on the concentration of arabinogalactan in the kanamycin water solution at 25°C, m_{CN} 0.04 mol l^{-1}.

cribed by the third degree polynom. In the Fig. 3 dependence of $d\eta/dm_{AG} = f(m_{AG})$ for this system is presented. From this picture it is seen that the rate of change in the solution viscosity passes through the minimum point at the arabinogalactan concentration corresponding to the 2:1 stiochiometric composition of complex.

Hence, the densimetric, the viscometric, and the spectral studies showed that arabinogalactan forms a stable 2:1 complex with kanamycin in the diluted water solution.

^{13}C NMR spectra show that the reaction of arabinogalactan with kanamycin causes the shift of signals of practically all the carbon atoms of the product as compared to the starting substances. The largest downfield shifts are observed for the C^1 and C^{12} (more than 2 ppm), C^9 (0.672 ppm), and C^{11} (0.435 ppm) carbon atoms taking part in the formation of the glycoside bond. Significant shift (0.702 mmp) takes place for the signal of C^7 cabon atom located between two amino groups on the C^6 and C^8 atoms of kanamycin. Signal of the C^{15} atom carrying terminal hydroxy group also changes. Instead of a doublet it becomes a broadened singlet at 73.23 ppm.

Hence, chemical shifts of signals in the ^{13}C NMR spectrum of the arabinogalactan–kanamycin complex due to the interaction between the polysaccharide and the antibiotic significantly differ from the standard values characteristic of kanamycin. These changes show that complex formation between the starting substances proceeds with participation of the hydroxy and the glycoside groups of the biopolymer and the antibiotic.

Reaction of polysaccharide with kanamycin is characterized by significant changes in the IR spectra. Spectra of the starting substances and the complex obtained were studied, and the attribution of the most important bands was carried out. It was found that in the spectra of complexes isolated an intense absorption band appears at 1525 cm^{-1}. It is characteristic of the bending vibrations of the kanamycin amino groups. Absorption bands of the amino group bond vibrations are revealed at 3500–3400 cm^{-1} and 900–650 cm^{-1} [13]. Besides, the low frequency shift of maximum point of the OH group absorption band (3600–3050 cm^{-1}) by 100 cm^{-1} and of the glycoside C–O bond (1135–1070 cm^{-1}) by 15-20 cm^{-1} is observed. These data indicate the H-bonding between the hydroxy and the glycoside groups of the polymeric matrix molecule and the antibiotic. In other words, the addition of kanamycin to arabinogalactan proceeds mainly due to the interaction of these groups.

Summarizing the above-reported data it may be suggested that the interaction between arabinogalactan and kanamycin is fairly intricate considering the possibility of formation of H-bonds of different types between the components. The formation of the

complex proceeds through the coordination of one kanamycin molecule by two carbohydrate units of arabinogalactan by means of the intermolecular H-bonding with participation of practically all hydrogen-containing groups.

In the course of the optimization of synthetic procedure for preparation of the arabinogalactan–kanamycin complexes effects of the reaction temperature, of the reaction time, and of the reagent ratio on the composition of the products obtained were studied. The results obtained are presented in the table.

It follows from the results that the reaction temperature and the reaction time practically do not effect the antibiotic content in the complex. At the increase of the arabinogalactan–kanamycin molar ratio to 1:2 the amount of the bound antibiotic to some extent increases. Further increase in the amount of antibiotic brought into the reaction does not lead to the increase in its content in the complex formed.

The complex compounds synthesized were isolated by precipitation with ethanol from water solution, purified and studied by spectral methods. All the compounds obtained are well soluble in water and practically insoluble in ethanol, acetone, and ether.

Acute toxicity studies of the preparations obtained were carried out. The largest dose introduced was 1500 mg kg^{-1}. No further studies were performed because of the evident nontoxicity of substance. Hence, the compounds obtained belong to weakly toxic compounds of the 4th group. Preliminary studies showed that these complexes exhibit high tuberculocidal activity.

Effect of the complex formation conditions on the kanamycin content in the reaction products

Arabinogalactan: kanamycin molar ratio	τ, h	*T*, °C	Kanamycin content mol/basic mol of polymer
1:1	0.25	20	1.21
1:1	0.5	20	1.20
1:1	1	20	1.21
1:1	3	20	1.22
1:1	24	20	1.23
1:0.25	2	20	1.18
1:0.5	2	20	1.20
1:1	2	20	1.23
1:2	2	20	1.33
1:1	2	0	1.18
1:1	2	40	1.20
1:1	2	80	1.23

EXPERIMENTAL

^{13}C NMR spectra were taken on a Bruker AM-300 spectrometer (75.47 MHz) with the wide-band proton decoupling and in the JMODXH mode from the 3–5% arabinogalactan solutions in D_2O against internal TMS. Spectra were recorded at 25°C with 15 s delay between the pulses. UV spectra of water solutions were measured on a UV–VIS SPECORD M-40 spectrophotometer in quartz cells in the range 220-900 nm. IR spectra were obtained on a Shimadzu spectrometer in mineral oil at 700–3600 cm^{-1}; pH of the solutions was controlled on an ANION 4100 pH-meter. Content of the antibiotic in the reaction mixtures was evaluated by elemental analysis.

Arabinogalactan with the molecular mass 40000 was isolated by water extraction from the Sibirian larch.

Complexes were obtained as follows. Polysaccharide, 1 g, was dissolved in 20 ml of water. Kanamycin, 3.18 g, was dissolved in 20 ml of water, and pH of the solution was adjusted at 7. The resulting solution was added dropwise under the intense stirring to the solution of polysaccharide at room temperature. The reaction was carried out for 3 h, and then the product was precipitated with ethanol. The preparation obtained was dissolved in water and precipitated with ethanol, the solid phase was filtered off, washed 3 times with ethanol, then with diethyl ether, and dried in a vacuum.

Densities of the solutions under study were evaluated by means of a picnometer at 25°C. NaCl solutions, 0.05 M, were used as solvents. In the three-component arabinogalactan–water–kanamycin solutions the kanamycin concentration was fixed (0.04 mol l^{-1}). Concentration of arabinogalactan (m_{AG}) varied in the range 0–0.16 mol l^{-1}.

Viscosity of the solutions was measured on an Ubbelohde viscometer with a suspended level at 25°C. In the three-component arabinogalactan–water–kana-

mycin solutions the kanamycin concentration was fixed (0.04 mol l^{-1}), and the concentration of arabinogalactan (m_{AG}) varied in the range 0–0.16 mol l^{-1}.

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