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SYNTHESIS OF A COPOLYMER POSSESSING AN ANTICOAGULATIVE ACTION

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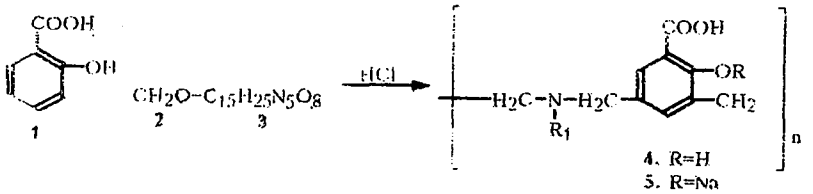
After chemical modification, sericin — a protein product of the silk industry [1] — may find a new use in medicine.

With this aim, we have carried out in one stage the condensation of salicylic acid (**1**), formaldehyde (**2**), and sericin (**3**) in an aqueous medium at pH 1 (35% HCl), followed by washing with water to neutrality and further treatment with an aqueous solution of caustic soda. The highest yield (98%) of water-soluble surface-active compound (**4**) was observed when the reaction was conducted in the temperature interval of 90-98°C at a ratio of the initial substances of 1.0:2.1:1.0 (mole-% of **1**, **2**, and **3**, respectively), for 6-8 h [2].



R_1 - seicin residue

n = 50-70

The copolymer synthesized (**5**) [3] — a light yellow powder with a molecular mass of 6000-8000 Da — was a surface-active agent (SAA) with the following characteristics: surface tension, 34 dyne/cm^2; emulsifying capacity, forms a good water–oil emulsion: foaming capacity, 100 ml of initial foam; critical micelle concentration (CMC), 0.55; readily soluble in water and organic solvents (alcohol, chloroform, dimethylformamide); characteristic viscosity, 0.04-0.06.

The IR spectrum of compound (**5**) had absorption bands at 1600-1690 cm^{-1}, corresponding to the stretching vibrations of an amide carbonyl, and at 3000-3600 cm^{-1}, corresponding to OH and C–N groups.

In the PMR spectrum of (**5**) in D_2O solution, broad signals, difficult to identify and overlapped by the signals of salicylic acid, were observed from the main substance in the weak field at 6.4-7.8 ppm. At 6.1-3.5 ppm there were the signals characteristic of polysaccharides, and at 0.8-4.0 ppm signals of the protein part of sericin. Because of the pronounced overlapping and great breadth of the signals their individual assignment was impossible.

The copolymer synthesized (**4**) and its sodium salt (**5**) were pharmacologically active compounds [4].

A medicobiological investigation carried out in the Moscow State Institute of Blood Substitutes and Medicinal Preparations showed that the copolymer possessed anticoagulative properties and qualified as a compound of low toxicity.

In connection with the presence in the copolymer of salicylic acid, which causes a decrease in the amount of prothrombin in the blood, and shows an inhibiting influence on the aggregation of thrombocytes, we investigated its influence on the hemostasis system. The anticlotting effect of the preparation was studied *in vitro* and *in vivo*.

We took blood from a human subject and determined the number of thrombocytes on stimulation by collagen and ADP (photometric method), the activated partial thromboplastin time, and a number of parameters of the thrombodynamotachogram (after the addition of physiological solution or the preparation to normal thrombocitic plasma). Concentrations of the preparation in plasma harmless for man, ranging from 1/3 to 1/10 LD_{50}, were investigated.

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The results showed that the preparation had no influence *in vitro* on the number of thrombocytes in thrombocytic plasma but caused a dose-dependent decrease in the induced aggregation of the thrombocytes. ADP- and collagen-induced aggregation of thrombocytes was observed in the greatest degree at the highest of the preparation concentrations studied — 3.6 mg/ml. A concentration of 0.9-1.8 mg/ml exerted a pronounced hypocoagulationary action, while 3.6 mg/ml led to noncoagulability of the plasma.

According to the literature, anticoagulant potential is usually characterized by the dose of the substance that doubles the activated partial thrombopalastin time. For compound (**5**) this was 0.7 mg/ml, which is absolutely harmless for the organism according to the *in vitro* results. It is approximately 1/15 of the mean lethal dose, i.e., 5 times less than the maximum tolerated dose.

At a concentration in the plasma of 0.01-1.0 mg/ml *in vitro* the preparation possessed a clear anticoagulant and fibrinolytic activity, and at 0.5 mg/ml an antiaggregation activity in relation to thrombocytes.

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