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SYNTHESIS OF A HEMOSTATIC DRUG FROM CELLULOSE ACETATE

G. R. Rakhmonberdiev, A. S. Sidikov, R. D. Yusupov, U. N. Zainutdinov, and D. S. Kazantseva

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Hemostatic resorbent polymeric materials have been synthesized from water-soluble acetylcellulose, lagochilin, and lagohirsin. The substances obtained possess an effective hemostatic action and have a water-soluble form.

It is known that by adding (low-molecular-mass) physiologically active substances to the macromolecule of a high-molecular-mass compound it is possible to obtain a prolongation of their action, to decrease their toxicity, to improve their solubility, and to achieve directed transport into determined organs [1].

We have previously [2] reported the results of investigations on the production of a hemostatic material from water-soluble acetylcellulose by the "inclusion" in its structure of a drug — lagoden (homogeneous mixture). Low-molecular-mass natural compounds such as lagochilin and lagohirsin, isolated from plants of the genus *Lagochilus*, possess only a weak hemostatic activity because of their insolubility in water. In order to impart solubility to them and to prolong their action, and also to create polymeric medicinal forms, we have investigated the addition of molecules of lagochilin or lagohirsin to the aldehyde groups of oxidized water-soluble acetylcellulose (OWSAC).

As is known, the molecule of water-soluble acetylcellulose (WSAC), obtained by the procedure of [3], contains two functional groups, $-OH$ and $-OCOCH_3$, which greatly restrict the possibility of polymer-analogous transformations. With the aim of forming in it a new reactive $-HC=O$ group we have carried out the directed oxidation of WSAC with periodic acid, which is capable of oxidizing the two hydroxy groups present at the second and third carbon atoms of the anhydroglucose unit of WSAC [4].

To confirm the formation of an aldehyde group in the WSAC macromolecule we recorded IR spectra of control and oxidized specimens. In view of the fact that the absorption band of the aldehyde group coincides with the absorption band of the acetyl group, to demonstrate the formation of aldehyde groups we recorded IR spectra of saponified samples of WSAC and OWSAC. Saponification was performed with a 0.5 N solution of sodium methanolate in anhydrous methanol [5]. The IR spectra of the OWSAC specimens revealed an absorption band relating to an aldehyde group, the intensity of which depended on the time of oxidation.

The number of aldehyde groups in the product was determined by titrating an aqueous solution of the OWSAC with sodium thiosulfate. The OWSAC contained 100-110 aldehyde groups per 1000 anhydroglucoside units (AGUs) and had retained its solubility in water. A further increase in the number of aldehyde groups led to the formation of intermolecular acetal bonds, as a result of which the OWSAC became insoluble.

To add lagochilin or lagohirsin to the aldehyde groups of OWSAC, a definite amount of the substance was dissolved in dimethylformamide to give a concentration of 5-6%, and lagochilin or lagohirsin was added in a ratio to the polymer of 1:4. As a water-abstracting agent we used anhydrous copper sulfate or phosphoric anhydride. The addition of lagochilin or lagohirsin to OWSAC can be represented in the following way:

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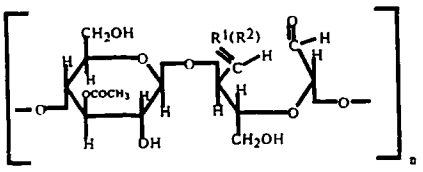

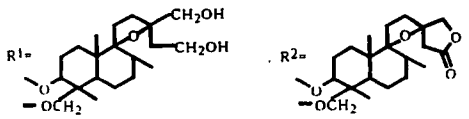

To prove the addition of lagochilin or lagohirsin to the polymer, we determined the numbers of aldehyde groups in the products obtained. The results of analysis are given in Table 1.

In view of the high reactivity of the aldehyde groups in the OWSAC macromolecule, on the addition of lagochilin or lagohirsin about 40-45% of the aldehyde groups reacted with these substances. The large number of unbound aldehyde groups after the reaction is explained by the formation of a hemiacetal bond and by the steric factor (coverage of the aldehyde groups by molecules of the bound preparation). The relatively larger number of aldehyde groups remaining free on passing from lagochilin to lagohirsin is explained by the smaller number of free reactive (hydroxy) groups in lagohirsin.

In the IR spectra of the products obtained, the intensity of the absorption band in the 1740 cm^{-1} region corresponding to the aldehyde group had decreased in comparison with the initial OWSAC. A comparative hemostatic evaluation of the polymeric materials obtained, in the form of films, showed (Table 2) that the OWSAC itself shortened the time of parenchymatous hemorrhage by a factor of 2 in relation to the control.

When deposited on a liver wound surface, the preparations of OWSAC with lagochilin or lagohirsin led to instantaneous blood clotting and shortened the time of parenchymatous hemorrhage by a factor of 5-6 in comparison with a control.

Thus, preparations of OWSAC with lagochilin and lagohirsin have a water-soluble polymeric form and exhibit an effective hemostatic action.

EXPERIMENTAL

Preparation of OWSAC. With vigorous stirring, 2.5 g of WSAC was dissolved in 47.5 ml of distilled water, and, with constant stirring, 1.75 g of periodic acid was added. Oxidation was continued at room temperature for 75 min. The oxidation product was precipitated in acetone, washed several times with the precipitant to neutrality, and dried at room temperature.

Determination of the Aldehyde Groups in the OWSAC. A conical flask was charged with 0.2 g of OWSAC, 15 ml of distilled water, 10 ml of a 0.05 N buffer solution, and 10 ml of a 0.63 N solution of iodine and was left at 20°C for 2 h. Then 15 ml of a 0.1 N solution of hydrochloric acid was added, and the residual iodine was titrated with a 0.003 N solution of sodium thiosulfate. A control experiment was run. From the difference (ml) between the volumes of sodium thiosulfate consumed in the titration of the control and the experimental samples we found the amount of iodine used in the oxidation of the anhydroglucoside groups [sic] of the OWSAC. The numbers of aldehyde groups (i) per 1000 anhydroglucoside units in the OWSAC samples were calculated from the formula:

$$i = \frac{(V_1 - V_2)0.03m}{366} 1000,$$

where V_1 and V_2 are the respective volumes of sodium thiosulfate solution consumed in titration in the control experiment and remaining after the oxidation of the OWSAC, ml; m is the weight of the OWSAC; 366 is the molecular mass of the elementary unit of the OWSAC; and 0.03 is the concentration of sodium thiosulfate.

Addition of Lagochilin or Lagohirsin to OWSAC. In a three-necked flask fitted with a stirrer, reflux condenser, and thermometer, 2.0 g of OWSAC was dissolved in 98 g of anhydrous dimethylformamide. To this polymer solution were added 0.5 g of lagochilin or lagohirsin and 6 g of water-abstracting catalyst (anhydrous copper sulfate or phosphorus pentoxide). The reaction was performed with vigorous stirring in a water bath at 60°C ($CuSO_4$) or 80°C (P_2O_5) for 8 h. The reaction mixture

TABLE 1. Numbers of Aldehyde Groups in the Products Obtained (the OWSAC contained 110 aldehyde groups per 1000 AGUs)

Water-abstracting agent	Number of aldehyde groups per 1000 AGUs	Substance added
$CuSO_4$	60	Lagochilin
$CuSO_4$	74	Lagohirsin
P_2O_5	50	Lagochilin
P_2O_5	65	Lagohirsin

TABLE 2. Influence of the Preparations Obtained on the Time of Parenchymatous Hemorrhage in Rats (mean of six experiments in each case)

Substance investigated	Blood clotting time		Error, p <
	(M±m), s	% of control	
Control (distilled water)	258±5.7	100	–
OWSAC	118±8.7	41	0.01
OWSAC with lagochilin	43±4.4	16	0.001
OWSAC with lagohirsin	52±6.5	20	0.001

was filtered in a special filtering apparatus through three layers of calico under pressure. The solution was precipitated with acetone and the resulting mass was washed repeatedly with the precipitant. The polymeric material obtained was dried at room temperature.

The hemostatic evaluation of the preparations was made according to [6].

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