Chemistry of Natural Compounds, Vol. 33, No. 5, 1997

PLANT POLYSACCHARIDES. IX. ISOLATION AND ANTICOAGULANT ACTIVITY OF THE POLYSACCHARIDES OF *Lagochilus usunachmaticus*

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UDC 547.917

A polysaccharide possessing pronounced direct-action anticoagulant activity has been isolated from the epigeal part of Lagochilus usunachmaticus.

We have previously reported a study of the pectin from the epigeal part of *Lagochilus usunachmaticus* Korr. (fam. Labiatae) [1]. *Lagochilus* grows in the mountain and foothill regions of Central Asia, and there are 13 species of this genus in Uzbekistan [2]. In the present paper we describe a method of obtaining a polysaccharide with an anticoagulant activity.

The isolation of the polysaccharide included twofold extraction of the raw material with water and precipitation by alcohol in a ratio of 1:3, with intermediate purification by centrifugation. The yield of polysaccharide was 4.0% of the weight of the air-dry plant. It consisted of an odorless light brown powder, had a neutral pH in 1.0-5.0% aqueous solutions, was insoluble in alcohol and acetone, melted with decomposition at 265-270°C, and had a molecular mass of 30,000. By PC and GLC we identified rhamnose, arabinose, xylose, mannose, glucose, and galactose as components of the polysaccharide in a ratio of 3:9:1:3.3:13:17, respectively.

The anticoagulant activity of the polysaccharide was determined in experiments on dogs (*in vitro* and *in vivo*) from its influence on the thromboelastographic (TEG) indices after intravenous injection in doses of 10-25-50-100 mg/kg in the form of a 5% solution. For this purpose we used a TROMB-2 apparatus produced by the L'vov factory (Ukraine). After preliminary novocaine [procaine hydrochloride] anesthesia, 0.36 ml of blood was taken from the exposed femoral vein before, and 30-60-120-180 min and 24 h after, the injection of the preparation, and it was then transferred to a TEG cell and the recording mechanism was switched on. After the end of an experiment, four TEG indices — R, K, C_i, and I — were analyzed. It was found that the index R — the reaction time — had already increased only 30 min after the injection of the preparation, and the effect lasted for 24 h and more. The index K — the blood clotting time — increased to a maximum 180 min after the injection of 10 mg/kg of the preparation (Table 1) and 120 min after the injection of 100 mg/kg. The index C_i — the coagulation index — decreased appreciably from the first observation after the injection of 10 and 100 mg/kg of the preparation (see Table 1). The most important index, I — the thromboelastographic index — rose sharply from the first period of observation and was a maximum 180 min after the injection of 10 mg/kg of the preparation (see Table 1) and 120 min after the injection of 100 mg/kg, like the indices R and K. It must be mentioned that in doses of 10-100 mg/kg the polysaccharide acted for more than 24 h; i.e., none of the indices had returned to their initial levels 24 h after the injection of the preparation (see Table 1).

In various doses — 0.01-0.05-0.1-0.5-1-5-10 mg in 0.26 ml of blood *in vitro* (in direct contact with the blood) — the polysaccharide also lowered the clottability of the blood; i.e., the thromboelastographic indices changed downwards. In its structure and action, this polysaccharide has no analogs among plant substances. However, among polysaccharides of animal origin heparin is the closest in chemical structure and biological activity. For comparison, we studied the influence of heparin (in an effective dose of 130 activity units/kg, intravenously) on the TEG indices. With respect to all the TEG indices, the polysaccharide was superior to heparin in the duration of its action: it acted for more than 24 h, while on the injection of heparin a "ricochet" (reverse) effect was observed after only 180 min. This was not observed on the injection of effective doses of the polysaccharide during observation for 24 h.

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 0009-3130/97/3305-0534$18.00 ©1998 Plenum Publishing Corporation

TABLE 1. Influence of the Polysaccharide (10 mg/kg, intravenously) on Some TEG Indices of Canine Blood

TEG index	Initial values	Times after injection of the preparation, min				
		30	60	120	180	1440
R, min	1.92±0.30	5.55±2.81	4.25±0.62	5.55±1.24	8.20±0.34	3.77±0.17
%	100	288.3	221.0	288.3	426.0	195.8
K, min	1.13±0.36	3.10±1.96	2.38±0.50	2.88±0.62	4.25±1.99	3.13±0.38
%	100	274	211	255	376	277
Ci, %	2.30±0.60	1.00±0.43	0.93±0.25	0.63±0.16	0.70±0.11	0.68±0.07
	100	43.5	40.4	27.4	30.4	29.6
I, %	0.37±0.19	5.50±5.40	1.86±0.62	3.80±1.85	6.80±3.67	2.50±0.38
	100	1486.5	503	1027	1838	675.7

The influence of the polysaccharide on the total behavior of animals and its toxicity were determined on white mice weighing 18-22 g by the intravenous injection of a 5% solution. When it was injected in toxic doses, the mice died from acute suppression of respiration. The mean lethal dose of the substance, LD_{50}, was 800 mg/kg.

Thus, the polysaccharide possesses a marked direct-action anticoagulant activity, exceeding heparin in the duration of its effect, is characterized by the absence of a "ricochet" effect, and has a low toxicity (provisional Republic of Uzbekistan patent No. 2722).

EXPERIMENTAL

General Observations. For the PC and GLC conditions, see [3].

Isolation of Polysaccharides. The air-dry comminuted (to 2 mm) epigeal part of the plant was extracted by stirring with water at room temperature for 2 h (twice). The water was taken in sixfold amount in relation to the weight of the raw material. The aqueous extracts were filtered, combined, concentrated to 1 liter, and treated with ethyl alcohol at a ratio of 1:3 by volume, followed by removal of the resulting precipitate (by centrifugation at 5000 rpm for 10 min).

For purification, the precipitate was repeatedly dissolved in water and centrifuged. The polysaccharide was precipitated from the solution by ethyl alcohol (1:3 by volume). Complete precipitation was achieved by the addition of 3.5-4 volumes of alcohol, while three volumes in relation to the volume of the extract precipitated the bulk of the polysaccharide. Then the polysaccharide was filtered off, dewatered with alcohol (85-96%), dried, and ground to a powder.

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