A PREPARATION FROM HIPPOPHAE RHAMNOIDES WHICH INHIBITS GROWTH OF TRANSPLANTED TUMORS IN ANIMALS

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As has previously been reported, alcoholic extracts of the bark of Hippophae rhamnoides, prepared by M. F. Petrova in the Laboratory of Chemistry of Natural Substances in our Institute, inhibits the growth of transplanted tumors in animals [1, 2].

The object of the present investigation was to isolate the active principle of the alcoholic extract of the bark of Hippophae rhamnoides, and to study its properties, with particular regard to its biological action.

EXPERIMENTAL METHOD

Production and properties of the preparation. The finely ground bark of young shoots of H. rhamnoides was extracted with alcohol. The alcohol extract was evaporated in vacuo (20 mm) in a current of nitrogen on a water bath in which the water temperature did not exceed 40°. The dark colored tarry residue was treated with water, the insoluble portion filtered off and the solution neutralized with 10% sodium bicarbonate solution to a pH = 7.

The precipitate thus formed was filtered off. The neutral solution was acidified with hydrochloric acid to pH = 6.0 and passed through a column filled with cationic resin (mark KB-4 n – 20) previously treated with a 5% solution of hydrochloric acid solution. After passage of the whole of the solution, the cationate was carefully washed with distilled water, and the material adsorbed was washed out with a 5% solution of hydrochloric acid, a silicotungstic acid test being used as a control of complete desorption. The acid eluate was neutralized with sodium bicarbonate solution to pH = 7.0, the resulting precipitate was filtered off, and the filtrate was shaken up with a small quantity of powdered skin in order to make quite sure that it was free from tannins. After filtration, the solution was evaporated to dryness in a current of nitrogen in vacuo (10 – 15 mm) on a water bath (water temperature not above 35-40°). The firm, dry residue was extracted with absolute alcohol. The alcohol extract was filtered to remove the inorganic residue, and the alcohol was then driven off in vacuo (10 – 15 mm) on a water bath (water temperature not above 40°) in a current of nitrogen. The residue – a pale yellow, firm, amorphous mass, readily soluble in water and alcohol – is the preparation whose antitumor activity was investigated as described below. Judging by the paper chromatogram, obtained on slow filter paper by the system: n-butyl alcohol – acetic acid – water in proportions 4:1:5, the preparation is a mixture of two compounds with R_f values of 0.25 and 0.4, moreover the stains may be detected in ultraviolet light (green luminescence) and they may also be revealed by treatment with the following: 1) an ammoniacal solution of silver nitrate, 2) a 1% solution of potassium permanganate with sodium carbonate, 3) a solution of a diazonium salt derived from aniline.

TABLE 1

The Action of the Preparation Hr and of Sarcolysin on Tumors*

Expt. No.	Tumor	Mode of inoculation	Day after inoculation on which administration of the preparation began	Number of animals		Preparation	Dose (in mg/kg)	Mode of administration	Duration of treatment (in days)	Inhibition of growth of tumors in % of their weight in control animals
				expt.	control					
1	Ehrlich's tumor	Subcutaneously	5-й	10	10	Hr	250	Subcutaneously, every day	12	72
2	The same		5-й	10	10	Sarcolysin	3	Every 48 hours, intraperitoneally	12	42
3	Ehrlich's tumor	Intramuscularly	5-й	12	12	Hr	300	Subcutaneously, every day	14	40
4	The same	The same	5-й	12	12	Hr (pasteurized)	300	The same	14	39
5	" "	» »	5-й	12	12	Sarcolysin	3	Every 48 hours, intraperitoneally	14	28
6	Ehrlich's tumor	Intramuscularly	5-й	10	10	Hr	100	Intraperitoneally, every day	12	20
7	The same	The same	5-й	10	10	»	150	Subcutaneously, every day The same	12	26
8	» »	" "	5-й	10	10	»	200		12	36
9	Mouse hepatoma	Subcutaneously	7-й	14	14	Hr	300	Subcutaneously, every day	18	62
10	The same	"	7-й	14	14	Sarcolysin	3	Every 48 hours, intraperitoneally	18	26
11	Sarcoma 45	Subcutaneously	7-й	14	14	Hr	150	Subcutaneously, every day	14	86
12	The same	"	7-й	14	14	Sarcolysin	5	Every 48 hours, intraperitoneally	14	99
13	Sarcoma 45	Subcutaneously	7-й	10	10	Hr	75	Intraperitoneally, every day	14	48

* Groups of experiments delineated by horizontal lines were carried out at the same time and with a common control.

TABLE 2

The Action of the Preparation Hr and of the Crude Extract of Hippophae rhamnoides on Ehrlich's Tumor, Inoculated Intramuscularly

Expt. No.	Preparation	Dose (in mg/kg) subcutaneously	No. of mice		Average weight of tumor		Inhibition of growth of tumors as % of controls
			expt.	control	expt.	control	
1	Crude extract	100	10	10	5,8	6,8	15
2	Crude extract	250	10	10	4,3	6,8	37
3	Hippophae rhamnoides	100	10	10	4,3	6,8	30
5	Hippophae rhamnoides	250	10	10	3,9	6,8	42

EXPERIMENTAL RESULTS

In consequence of these results and of the method of preparation, the hypothesis is well grounded that the preparation is a mixture of the hydrochlorides of two bases. We are to continue the further study of these bases.

In the following description of the biological action of the preparation it will be designated conventionally as Hr (the initial letters of the generic and species names of the plant from which it is isolated).

For injection into an animal (subcutaneously or intraperitoneally) the preparation was dissolved in distilled water. The toxic action in animals, previously described in the case of the crude extract [2], is of the same general character in the case of Hr, for injection of this substance into rats, mice and rabbits was followed by an action of varying severity on respiration (increased rate and subsequent weakening of the respiratory movements); at the same time a general sluggishness and restriction of the movements of the animal was observed. A noteworthy feature was the considerable difference in the magnitude of the single lethal dose when injected into mice in different ways – intraperitoneally and subcutaneously. By intraperitoneal injection, the LD 100% (the dose lethal for 100% of animals) varied in the region of 500 mg/kg body weight, and by subcutaneous injection of a dose of 1 g/kg body weight only individual mice perished, and the LD 100% was impossible to determine, in general, for a larger dose could not be administered to the mouse subcutaneously. Rats were more susceptible to the toxic action of Hr and they died after intraperitoneal injection of a dose of 140 mg/kg. Death took place 30-50 minutes after administration of the lethal dose.

We tested the inhibiting action of Hr on three different strains to transplanted tumors in mice and rats: on two carcinomas (an Ehrlich's tumor and a hepatoma in mice of the C_3HA strain) and one sarcoma (sarcoma 45 in mongrel rats). It was thus shown that doses, well tolerated on repeated intraperitoneal injection (100 mg/kg for mice and 75 mg/kg for rats) give only a feeble antitumor effect (Table 1, experiments 6 and 13). Larger doses only, tolerated when administered subcutaneously, give good antitumor effects: for mice 250-300 mg/kg and for rats 150 mg/kg daily (Table 1, experiments 1, 3, 4, 9 and 11). Whether the antitumor effect was strong or weak was determined as a rule by the degree of susceptibility of the strain of tumor transplanted to the inhibitory action of the preparations previously studied, mainly chloroethylamine and ethylenimine derivatives. Sarcoma 45 of rats is known to be much more susceptible to the latter than Ehrlich's tumor. In sarcolysin treatment, for instance, complete absorption of sarcoma 45 may be obtained, i.e. 100% inhibition of growth [3], whereas Ehrlich's tumor shows considerably greater susceptibility to the action of this preparation and also to the action of others, well-known preparations (Dopan, TEM, etc.). In view of this, we refer to a 48% inhibition of the growth of sarcoma 45 (see Table 1, experiment 13) as a feeble antitumor effect, and a 40% inhibition of the growth of an Ehrlich's tumor (see Table 1, experiment 3) as a considerable antitumor effect. It must particularly be borne in mind that in the latter case the tumor was inoculated intramuscularly, and from our own observation this greatly increases the resistance of the tumor to the damaging action of the preparation (see below). On experiment on the action of Hr on Ehrlich's tumor was carried out with solution from ampules which had been heated to 70° on a water bath for 3 hours. The result of this experiment showed that heat, in this form, does not affect the antitumor activity of the preparation (see Table 1, experiment 4).

Simultaneously with certain of the experiments described above, and with the same controls, experiments were carried out with sarcolysin, used here as a preparation whose action on tumors has been well studied [3], in order to compare it with our new preparation. Comparison of the action of Hr and sarcolysin on tumors of the same strain showed that Hr significantly falls behind sarcolysin in its action on sarcoma 45 (see Table 1, experiments 11 and 12), but slightly exceeds in its action on carcinomas – both Ehrlich's tumor and mouse hepatoma (see Table 1, experiments 1-5, 9 and 10). The relationship of the strength of action of both of these preparations which were being compared could be convincingly shown to depend on the mode of inoculation of the Ehrlich's tumor: when inoculated intramuscularly (experiments 3-5) the inhibiting action of both sarcolysin and Hr fell by comparison with their action on tumors inoculated subcutaneously (experiments 1 and 2).

The experimental results given in the table showed that the active principle contained in the crude alcoholic extract of the bark of Hippophae rhamnoides was also present in the preparation tested and gave an antitumor effect of approximately the same order as that previously obtained with the crude extract [2].

The results of the experiments to compare the antitumor action of Hr and the crude extract of the bark of Hippophae rhamnoides (Table 2) showed that the active principle is more highly concentrated in Hr than in the crude extract, for it required a dose of 250 mg/kg of the latter to produce almost the same inhibition of the tumor as did Hr in a dose $2\frac{1}{2}$ times smaller.

We do not believe that Hr is the final form of the preparation, since the active principle which it contains requires further purification. Furthermore, the prolonged subcutaneous injection of this preparation is tolerated satisfactorily only by mice, and in rats it often leads to the formation of areas of infiltration and of dermatitis.

SUMMARY

The active principle of the alcohol extract of Hippophae rhamnoides bark was studied. The chemical method of obtaining the Hr preparation representing the mixture of chlorhydrates of 2 bases is described. The results of investigation of the inhibiting effect of Hr on tumors is compared with the action of the well known preparation sarcolysin.

LITERATURE CITED

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* Original Russian pagination. See C.B. Translation.