INFLUENCE OF TYPE OF CHEMICAL BOND BETWEEN MACROMOLECULE AND MEDICINAL PREPARATION ON THE PROPERTIES OF PHYSIOLOGICALLY ACTIVE COMPOUNDS

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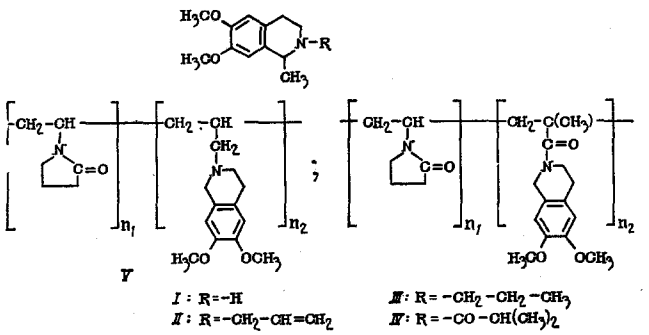
The type of chemical bond between a macromolecule and medicinal preparation is one of the more important molecular parameters, determining the properties of physiologically active polymers (PAP) [1-5]. The present work describes a search for more effective and modifications of the hypotensive preparation salsolidine — 1-methyl-6,7-dimethoxytetrahydroisoquinoline (I) — among its polymer derivatives.

For our investigation we synthesized water soluble PAP, in which I is bound to the main chain of the macromolecule through the N—CH_2 and N—CO groups.



V

I: R=-H
II: R=-CH_2-CH=CH_2
III: R=-CH_2-CH_2-CH_3
IV: R=-CO-$CH(CH_3)_2$

EXPERIMENTAL CHEMICAL

Salsolidine (I) is prepared by adding 40 ml of a 25% ammonia solution to 100 ml of a 20% solution of the hydrochloride of I. The precipitate of I is washed with cold water to a negative reaction for chloride ions, and then dried. Yield 15 g (88.5%), mp 72-73°C. Found, %: C 69.2; H 7.95; N 6.70. Calculated, %: C 69.5; H 8.2; N 6.75.

N-Allylsalsolidine (II) was synthesized by the method described in [6] by adding 10.53 g of I to a solution of 1.08 g of metallic sodium in 15 ml of xylene at 140°C, with vigorous stirring for 6 h. Then 3 g of allyl bromide are added in the course of 1 h, NaBr separating is filtered, and the filtrate is concentrated and distilled *in vacuo*. Compound II has the form of an oily liquid. Yield, 4.3 g (34%), bp 143-145°C at 1 mm Hg. Found, %: C 72.8; H 8.40; N 5.62. Calculated, %: C 73.0; H 8.5; N 5.63.

N-Propylsalsolidine (III) was synthesized similarly to II from I and propyl bromide. Yield 39%, bp 120-121°C at 1-1.5 mm Hg. Found, %: C 72.0; H 8.7; N 5.61. Calculated, %: C 72.7; H 9.3; N 5.76.

Isobutyryl salsolidide (IV) was synthesized by adding a solution of 2.61 g of isobutyryl chloride at 20°C in the course of 2 h to a solution of 5.17 g of I in 75 ml of chloroform, containing 3 g of triethylamine. After chloroform distillation the remaining oily liquid was distilled *in vacuo*. Yield, 4 g (58%), bp 195-196°C at 1-1.5 mm Hg. Found, %: C 69.9; H 8.21; N 5.08. Calculated, %: C 69.4; H 8.30; N 5.05.

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TABLE 1. Acute Toxicity of Derivatives of I

Compound	Content of I, mole %	Molecular weight	LD_{50}, mg/kg
I	—	207	170
II	—	247	16
III	—	249	21
IV	—	277	167
V	10	2800—3000	82
VI	19	3700—4000	3050

Note. Content of I was determined only in PAP.

Copolymer of vinylpyrrolidone (VP) with II (V) is prepared by copolymerization of VP (0.35 mole) and II (0.65 mole) in dioxane in the presence of azo-bis-butyrodinitile (1% of the weight of the monomers) at 60°C in the course of 24 h in evacuated glass ampuls. The product is precipitated by ether. Yield of V, 11.2%. The composition of the copolymer was determined by UV spectroscopy from the "benzene absorption" of the repeating units of II, according to a procedure previously developed [7], and by elemental analysis. Composition: 90 mole % of repeating units of VII and 10 mole % of repeating units of II. Average molecular weight 2800-3000.

Copolymer of VP with methacryloyl salsolidide (VI) is prepared by a procedure similar to that used in the reaction of poly(methacryloyl chloride) with I [8]. Instead of poly-(methacryloyl chloride), a copolymer of VP with methacryloyl chloride (VIII) was used. The synthesis of VIII has already been described in [9]. A 11 g portion of VIII is dissolved in 30 ml of DMFA, and at 20°C, 8.3 g of I in 20 ml of DMFA are added, and the mixture is stirred for 2 h. The product is precipitated with ether. Yield, 14 g (92%). The composition of the copolymer is determined by UV spectroscopy [7] and by elemental analysis. The composition of the copolymer is: 80 mole % of repeating units of VII and 20 mole % of repeating units of methacryloyl salsolidide. The average molecular weight was 3700-4000.

The UV and IR spectra were run on SF-4A and UR-20 (GDR) spectrophotometers, respectively.

EXPERIMENTAL PHARMACOLOGICAL

The toxicity of the preparations was studied in acute experiments on 200 mice of both sexes, weighing 18-24 g each, with intravenous administration. The toxicity factors were determined according to Leachfield and Wilkoxon [10].

The influence of the preparations on the arterial pressure and respiration was studied on 76 cats of both sexes, weighing 2.5-4.5 kg each, under urethane (1.2-1.5 g/kg, intraperitoneally) narcosis. The arterial pressure was recorded from the general carotid artery through systems of polyethylene tubes, by a mercury tonometer of the firm Ludwig. The polyethylene tube was filled with a 5% solution of sodium citrate. The respiration was recorded in parallel with the arterial pressure by means of a Morey capsule, connected to the trachea. All the preparations studied were in the form of 1-10% solution and were administered intravenously at approximately the same rate — 1 ml per 10 sec.

The chronic experiments with a model of an experimental hypertonia were carried out on 20 rabbits of both sexes, weighing 4-4.5 kg each, in which the general carotid artery was preliminarily removed onto the the skin surface. The arterial pressure was measured by the Riva-Rocci mercury manometer. After the operational wound had healed, the pressure in the experimental rabbits was periodically measured, and its mean value was calculated for each animal. Then, hypertonia was induced by prolonged daily intravenous administration of pituitrin into the otic regional vein of rabbit in a dose of 1 unit/kg. After 40-44 injections of pituitrin, the arterial pressure in rabbits increased by 40-45%, and during the following 8-11 days the dynamics of the arterial pressure was observed.

RESULTS AND DISCUSSION

In compound V, the active compound I is bound to the polymer carrier through the $N-CH_2$ group, and in compound VI, through an amide bond. In the IR spectra of V there are vibra-

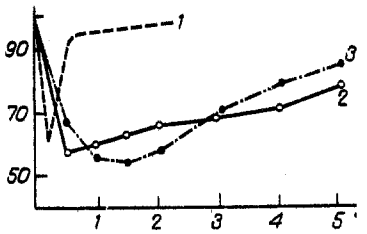

Fig. 1. Decrease in arterial pressure in cats during intravenous administration of derivatives of I. On abscissa — time (in h); on ordinate — decrease in arterial pressure (in %). 1) I (30 mg/kg); 2) V (10 mg/kg); 3) VI (100 mg/kg).

tion bands, characteristic of the repeating units of the two monomers (see structural formulas): of the amide group of VP at 1685 cm^{-1}, and the stretching vibration bands of the isoquinoline ring at 1620, 1525 and 1425 cm^{-1}. In the UV spectrum of the polymers there is an absorption band at 285 nm, characteristic of aromatic compounds of the benzene series. The polymers dissolve well in water, alcohols, DMFA, but are insoluble in benzene and ether.

In copolymers VI, the number of repeating units of I reaches 45 mole %. These polymers are in the form of slightly yellowish powders. In the IR spectrum of VI there are vibration bands of the VP residues (1685 cm^{-1}) and the amide group (1640 cm^{-1}) of methacryloyl salsolidide residues. The latter have an intense absorption at 283 nm. For comparison, we synthesized and studied compounds modeling the active repeating units of PAP, i.e., amine III and amide IV.

The acute toxicity of the synthesized compounds was studied by intravenous administration (Table 1). The investigations showed that during the administration of small doses of PAP, a certain quieting and decrease in the motive activity were observed in the mice, while large, close to lethal doses caused convulsions. Table 1 shows that the toxicity of compound VI, i.e., the polymer derivative of I with an amide bond, decreased by a factor of 18, while the toxicity of compound with the N—CH_2 group (compound V) was twice that of I itself.

To clarify the problem as to how these opposite effects are related to the polymer state, we determined the toxicity of the model compounds.

The change in toxicity can be related to both the polymer state, and to substitution of the hydrogen atom in the NH group of I, or to the simultaneous effect of these two factors. We found that the acylation of I did not affect the toxicity. Hence, the considerable decrease in the toxicity of VI is related to the polymer character of the preparation. We showed, with compound III as an example, that alkylation of I leads to a strong increase in toxicity (Table 1). As expected, the toxicity of II is higher than that of its saturated analog III. The corresponding polymer V has an acute toxicity, one quarter of that of compound III. Hence, increase in the toxicity of the alkylamino polymer compared with compound I is not due to the effect of the polymer state, but to the effect of alkylation.

To clarify the problem as to how the main pharmacological action of the preparation changed when it was introduced into the composition of the polymer, we studied the hypotensive action of PAP, in acute and chronic experiments. The acute experiments on the influence of PAP on the arterial pressure were carried out on cats by intravenous administration at various doses. It was found that the hypotensive effect of V develops, beginning at a dose of 5 mg/kg. Thus, decrease in the arterial pressure was observed for 60–80 min, and the maximal decrease was 25.5%. A further increase in the dose led to a corresponding, but not proportional increase in the hypotensive action. We should note that with increase in the dose, the time of the hypotensive effect of V also lengthens. Similar results were also obtained in the study of the action of compound VI. However, its noticeable hypotensive effect was observed at larger doses (beginning at 25 mg/kg), and in this case a more concentrated (10%) solution of the polymer in water was used. The results obtained, given in Fig. 1 and in Table 2, show the differences in the activity of the polymer compounds depending on the type of bond between I and the polymer chain.

TABLE 2. Hypotensive Effect of Derivatives of I on Cats

Compound	Dose, mg/kg		Decrease in arterial pressure	Duration of hypotensive action of compound, min
	total	calculated per content of I		
I	15	15	28	
I	30	30	43,4	
III	5	4,2	32	6—60
IV	6	4,5	10—15	12—30
V	5	0,8	25,5	60—108
V	10	1,6	37,8	120—210
V	15	2,0	42,3	180—270
V	20	3,3	74,1	240—300
VI	50	15	38,2	120—180
VI	100	30	42,0	180—300
VI	200	60	66,0	300

TABLE 3. Influence of Polymer Derivatives of I on Arterial Pressure in Rabbits with Experimental Hypertonia

Group of animals	Compound	Total dose, mg/kg	Arterial pressure, mm Hg			
			initial	45 days after introduction of pituitrin	11 days after administration of derivatives of I	decrease in arterial pressure, %
1st	I	15	100,5	146,4	113,3	22,6
2nd	VI	150	99,2	137,5	106,6	22,5
3rd	I	30	102,1	137,6	117,5	14,6
4th (control)	Distilled water	—	97,5	136,6	136,6	—

Note. For the method of preparation of the animals in groups, see explanation in text.

Compound VI has a clearly pronounced prolonged hypotensive activity in doses of 50-200 mg/kg, i.e., in doses comprising 1/60-1/15 LD_{50}, respectively. We should note that if it is calculated for the content of the active component, the hypotensive activity of the polymer compounds did not change. This is confirmed by the activity of the model compound. Compound V has a stronger hypotensive action in doses equal to or smaller than doses of I causing these effects. If we consider that the content of the active principle in polymer V is not more than 10 mole %, it is understandable why 1-2 mg of I contained in the polymer in a bound form has a stronger action than 10-20 mg of free I.

For a more thorough investigation of the hypotensive action of the polymer preparations, we studied their action in chronic experiments on an experimental model of hypertonia. The experiments were carried out on rabbits weighing 4-5 kg each. The animals were divided into four groups. The rabbits of three groups received compounds V, VI and I, respectively, daily, in doses shown in Table 3. The rabbits of the fourth group served as control. The treatment of the rabbits was discontinued on the fifteenth day, but the arterial pressure of the animals in the first and second groups was lower for 40-45 days. The increased arterial pressure in the animals of the third group was restored 10-12 days after discontinuation of the treatment. The arterial pressure in the control group of rabbits remained high during the entire experiment. Thus, polymer derivatives of I studied during treatment of the experimental hypertonia had a strong and prolonged hypotensive action, considerably greater than that of the initial I.

The synthesized PAP differ considerably from I in both toxicity and hypotensive action. If for compound VI, we can still assume splitting of the active component from the polymer, this assumption is difficult for compound V. It is possible that in the last case, the physiological action is caused by the macromolecule as a whole, as this would explain the high activity of the polymer with this type of bond.

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