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Supramolecular Complexes of Glycyrrhizic Acid, its Monoammonium Salt with Diterpenoid Lagochilin and their Hemostatic Activity

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Abstract

Data on the synthesis of supramolecular complexes of lagochilin with glycyrrhizic acid and its monoammonium salt, and their haemostatic activity are presented. It is shown that the hemostatic activity of complexes depends not only on the nature of the ligand and guest molecule, but also on their relationship.

Keywords: Lagochilus inebrians; Lagochilin; Supramolecular complexes; Glycyrrhizic acid; Hemostatic activity

Introduction

It is known that extracts of the medicinal plant *Lagochilus Inebrians* (Lagochilus intoxicating) have a fairly effective haemostatic activity. The active substance of this plant is four atomic alcohol-diterpenoid of the lab Dane type-lagochilin (L). Lagochilin itself is poorly soluble in water and therefore the sodium salt of lagochirzic acid-Lagoden (5% solutions in 5 ml and 10 ml ampoules) was introduced into medical practice. Currently, Lagoden's production has been suspended due to difficulties in obtaining the drug substance and low yield (10%) of the target drug [1].

In this regard, there was a need to search for new, high-tech ways of synthesizing water-soluble derivatives of lagochilin. To achieve this goal, we have chosen the method of molecular encapsulation of biologically active substances with low molecular weight natural substances having effective solubilizing properties. Glycyrrhizic acid (GA) is one of such natural compounds of plant origin, contained in large quantities in the root of licorice. GA and its mono ammonium salt (MASGA) forms supramolecular complexes with many drug substances that are readily soluble in water [2]. In this regard, it was important for us to synthesize a number of supramolecular complexes of GA and MASGA with lagochilin, suggesting, at the same time, the possibility of enhancing their water solubility.

Experimental

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GA and MASGA, we obtained by known methods from a thick extract of licorice root, bringing the final products to 92% purity [3]. Lagochilin was isolated from the 2014 collection of the plant *Lagochilus inebrians*. The main content of lagochilin-1.2% is localized in the leaves and flowers of the plant. The isolation was carried out by extraction with anhydrous dichloroethane, while lagochilin was purified by recrystallization from acetone. The structure and degree of purity were determined by X-ray diffraction analysis of the monohydrate forms of lagochilin [4]. Determination of the hemostatic activity of supramolecular complexes with bleeding from the tail of rats was carried out according to the Akopov method [5]. The obtained results are shown in TABLE 2. All the substances taken for testing (supramolecular complexes and 5% oily solution of lagochilin) were administered intraperitoneally to white outbred rats of both sexes weighing 160 g ± 16 g in a dose of 5 mg/kg, and preparation of Lagoden in a dose of 25 mg/kg.

As a control, the animals were injected with physiological solution in a bioequivalent volume; as drugs of comparison were given the data on Lagoden and 5% oily solution of lagochilin (TABLE 2).

Results and Discussion

Synthesis of supramolecular complexes (FIG.1) of GA, MASGA with lagochilin was carried out in water: alcohol (1:2) medium for 12 h, after removing the solvent, the aqueous part was lyophilized. Some physicochemical parameters, the solubility in water of the obtained complexes in different ratios are given in TABLE 1.

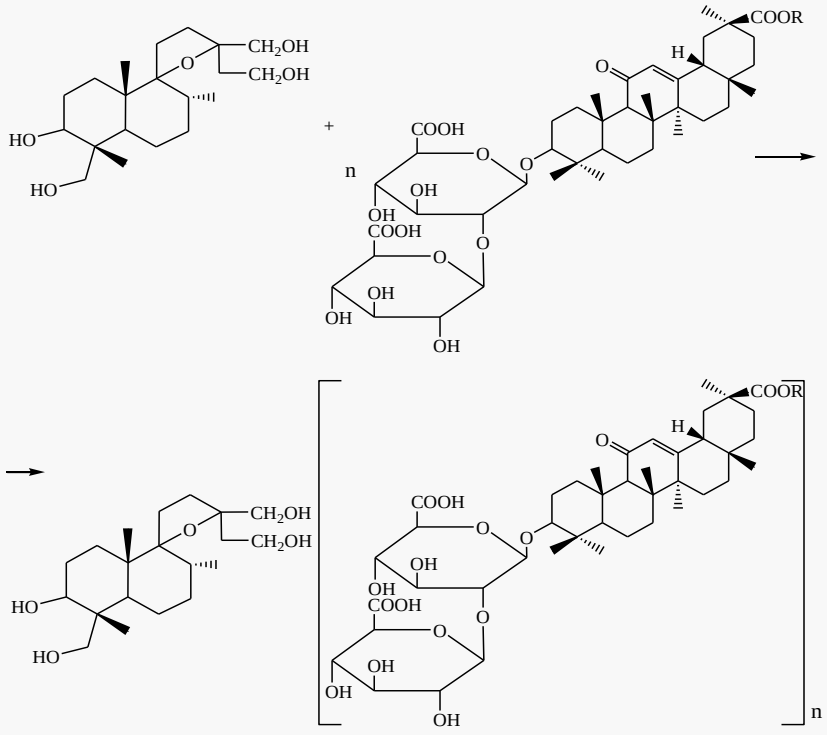

FIG.1. **Scheme for the synthesis of supramolecular complexes of GA, MASGA with lagochilin in different ratios.**

TABLE 1. **Some physicochemical parameters of supramolecular complexes of GA, MASGA with lagochilin.**

N	Supramolecular complex	M.P.	*Rf	(+)	Solubility in water**	UV
		°C				(nm)
1	GA:L (2:1)	173-175	0.71	12	s	251-254
2	GA:L (4:1)	175-178	0.73	16	s	251-254
3	GA:L (9:1)	180-183	0.75	14	s	251-254
4	MASGA:L (2:1)	197-200	0.72	24	f.s	251-254
5	MASGA:L (4:1)	202-204	0.71	20	f.s	251-254
6	MASGA:L (9:1)	205-207	0.72	22	f.s	251-254
*System: Methanol-acetone (2:1); Developer: Iodine vapor; ** s: Soluble; f.s: Fairly soluble.						

The formation of supramolecular complexes of GA, MASGA with lagochilin is clearly visible in a comparative analysis of the IR spectra of GA, lagochilin and GA:L (2:1). Thus, the IR spectrum of GA is characterized by the presence of a band of the valence vibrations of OH groups at 3380 cm^{-1} to 3400 cm^{-1} (broad, intense). The IR spectrum of lagochilin has the following characteristic bands of vibrations of functional groups: hydroxyl groups at 3494 cm^{-1} to 3233 cm^{-1}; -CH, $-CH_2$, $-CH_3$ at 2960 cm^{-1} to 2850 cm^{-1}, and C-O-C at 1053 cm^{-1}. In the IR spectrum of MASGA, the most characteristic vibration bands are: 3400 cm^{-1} to 3250 cm^{-1} -OH groups, 2970 cm^{-1} to 2950 cm^{-1} -CH,$-CH_2$,$-CH_3$; 1715 cm^{-1}, 1656 cm^{-1} -C=C-C=O.

In IR spectra of synthesized supramolecular complexes of GA, MASGA with lagochilin, there are absorption bands of valence vibrations at 1670 cm^{-1} to 1650 cm^{-1}, characteristic to carbonyl groups of GA, MASGA with a shift to the low-frequency region of 10 cm^{-1} to 15 cm^{-1} relative to the original components. Also, the change in the signal of valence vibrations of the hydroxyl groups of lagochilin, GA, MASGA at 3580 cm^{-1} to 3010 cm^{-1} indicates to the formation of intermolecular hydrogen bonds between the guest molecule and ligand, respectively of the supramolecular complexes.

Characteristic for the formation of hydrogen bonds in the GA molecule is the carbonyl group, conjugated with the double bond and the carboxyl groups of the carbohydrate and aglycone parts of the latter, for which the signal broadening is also observed in this region.

In the NMR spectrum of the GA:L (2:1) complex taken in DMSO, there are the following resonant frequencies: the signal in the region of 0.5 m.d. belongs to the protons of $-C-CH_3$ at C_{17}; doublet in the range of 0.7 m.d. to 1.8 m.d. belongs to the protons of $-HC-CH_3$ at C_4 and C_{10} in the molecule of lagochilin. A characteristic for the GA molecule is the signal in the region of 1.0 m.d. belonging to the protons of$-C-CH_3$ at C_4, the singlet in the region of 1.3 m.d. belongs to the protons of $-C-CH_3$ at C_{20}. No fissionable signal in the region of 1 m.d. to 2 m.d. belongs to all the protons of the lagochilin and GA molecules skeletons. The signal of protons in the region of 2.8 m.d. to 3.0 m.d. belongs to protons of the solvent (acetone+deuterated water). Multiple signals in the region of 3.0 to 4.5 m.d. belongs to all protons of CH_2O, CHO groups,

available in the molecules of GA and lagochilin. The singlet in the range of 5.0 m.d. to 5.45 m.d. belongs to olefinic protons at C_{12}. Comparison of the NMR spectrum of the GA:L (2:1) complex with those of the original did not in fact reveal any difference.

UV spectra of the obtained compounds taken in water: alcohol (50:50) medium, are characterized by an absorption band with a maximum at 251nm to 254 nm. The melting point usually proceeds with decomposition. Supramolecular complexes of GA:L are dissolved in water much worse than the complexes of MASGA:L.

Further, the hemostatic activity of obtained GA, MASGA with lagochilin was determined, and the most active ratios of the interacting components needed to be revealed.

Introduction of water-soluble complexes of GA:L as a whole almost twice reduces the duration of bleeding. In this case, the greatest effect can be traced for the supramolecular complexes of MASGA:L. They have more than three times less bleeding time than control. An increase in the amount of ligand from 2 to 9 leads to a decrease in hemostatic activity. The higher efficiency of MASGA:L complexes is possibly associated with faster solubility in water, which indicates its high bioavailability and the ability to diffuse rapidly into the bloodstream.

TABLE 2. **The amount of parenchymal hemorrhage in rats after intraperitoneal injection of supramolecular complexes of GA, MASGA with Lagochilin.**

N	Supramolecular complex (ratio)	Dose, mg/kg	Bleeding time		P
			Second	%	
1	GA:L (2:1)	5	160 ± 13	44	≤ 0.01
2	GA:L (4:1)	5	165 ± 14	46	≤ 0.01
3	GA:L (9:1)	5	185 ± 16	51	≤ 0.01
4	MASGA:L (2:1)	5	85 ± 6	23	≤ 0.01
5	MASGA:L (4:1)	5	100 ± 10	28	≤ 0.01
6	MASGA:L (9:1)	5	120 ± 12	33	≤ 0.01
7	Control physical solution		360 ± 20	100	--
8	Lagoden	25	98 ± 10	27	≤ 0.01
9	Lagochilin (5% solution in oil)	5	130 ± 12	36	≤ 0.01

Thus, we have shown the possibility of synthesis of supramolecular complexes of GA, MASGA with Lagochilin in various ratios of interacting components, and their high water solubility. It was found that the haemostatic activity of the studied molecular complexes depends not only on the nature of the ligand and guest molecule, but also on their ratio.

Conclusion

Supramolecular complexes of GA, MASGA with lagochilin in various ratios were obtained. The structures of the obtained complexes were studied by UV, IR and NMR spectroscopy methods in comparison with the initial materials. It is shown that in the IR spectra of complexes of GA, MASGA with lagochilin there are absorption bands of valence vibrations characteristic to carbonyl groups of GA and MASGA at 1670 cm^{-1} to 1650 cm^{-1}, with a shift to the low-frequency region by 10 cm^{-1} to 15 cm^{-1} relative to the original components.

The study of the hemostatic activity of supramolecular complexes showed that the complexes of MASGA are more active than the complexes of GA with lagochilin. Apparently, this is due to their solubility in water (complexes with MASGA are readily soluble in water in comparison with the complexes of GA).

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