Supramolecular Complexes Of Angydrolagochiline With Glycyrrisic Acid And Its Salts And Study Of Their Specific Hemostatic Activity

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Abstract: For the first time, water-soluble supramolecular complexes based on diterpenoid lactone anhydrolagochiline with triterpene saponin glycyrrhizic acid (GA) and its monoammonium and monopotassium salts in various molar ratios have been obtained. The physicochemical and spectral characteristics of the obtained complex compounds were studied. Screening studies were carried out to study the specific hemostatic activity of the obtained supramolecular complexes.

Keywords: Lagochilus Inebrians Bge, anhydrolagochiline, supramolecular compound, glycyrrhizic acid.

INTRODUCTION

It is known that hemostatic agents are highly demanded drugs that have found wide application in many areas of surgery, gynecology, therapy of internal organs and other areas of practical medicine. In the folk medicine of Central Asia, the plant *Lagochilus Inebrians* Bunge, an intoxicating one, is used as a hemostatic agent. The hemostatic (hemostatic) effect of drugs of the genus *Lagochilus* (Lamiaceae) is due to the content in its composition of the four atomic alcohol diterpenoid lagochilin, stachydrin alkaloid, vitamin K and tannins. The flowers and leaves of the *Lagochilus* plant (Lamiaceae), (Latin Flores et folia *Lagochili*), collected during the flowering period, are used as medicinal raw materials [1, 5, 2001].

The agents regulating hemostasis and fibrinolysis can be divided into two main groups: the first group - substances that inhibit hemostasis and enhance fibrinolysis (antithrombotic drugs) and the second group - agents that increase blood clotting and suppress fibrinolysis (hemostatic agents) [6, 2000; 10, 2002].

THE MAIN FINDINGS AND RESULTS

A number of acetyl and isopropylidene derivatives have been obtained on the basis of lagochiline. It was shown that the hemostatic activity in a certain way depends on the number of free hydroxyl groups of lagochilin and its derivatives. However, lagochilin derivatives are poorly soluble in water, which limits their bioavailability. Chemical modification of lagochilin is carried out mainly at hydroxyl groups to obtain esters or succinates with dibasic acid anhydrides [11, 2005; 17, 2002].

According to literature data, 18 β -H glycyrrhizic acid (GA) and some of its salts form clathrate compounds with a number of active principles of drugs. At the same time, the obtained supramolecular complexes have a higher bioavailability, due to an increase in their water solubility, a relatively lower effective dose and prolonged pharmacological action [18, 2001; 22, 2005].

Based on the above, the aim of this work was to synthesize anhydrolagochilin and obtain water-soluble supramolecular complexes of anhydrolagochilin, to study their specific hemostatic activity.

Methodology. For identification, the method of thin layer chromatography on "SILUFOL" plates was used. TLC solvent systems: water: ethanol 1: 1. Iodine vapor was used as a developer. For column chromatography, we used silica gel with a particle size of 100/160. IR spectra were recorded on a Perkin-Elmer System-2000 IR-Fourier spectrometer on KBr tablets.

Results and its discussion

Anhydrolagochiline was synthesized according to a previously known method from lagochilin [23]. Some physicochemical properties of the synthesized anhydrolagochiline are compared with the literature data, and the results are shown in Table 1.

Table 1

Nº	Substance	Mp. °C	R _f * (system)	/α /D water: ethanol 1: 1	Solubility	IR spectrum cm-1	Yield %
1	Anhydrolagochi i-lin	97-98	0,58 (I)	+20	acetone, dioxane	1652(COC), 2946(CH ₂) 3450- 3350(OH)	90%

Some physical and chemical indicators of anhydrolagochilin

* System water: acetonitrile: acetone (1: 1: 1)

As can be seen from the data given in table 1, anhydrolagochiline is not soluble in water. This, in a sense, limits its bioavailability. However, despite this, it was dissolved in oil and the specific hemostatic activity was studied [17].

Water-soluble supramolecular complexes of anhydrolagochiline with GA and its salts were obtained. The general production scheme is shown in Scheme 1.

Scheme 1

General scheme for the preparation of supramolecular complexes of anhydrolagochiline with GA, monoammonium and monopotassium salts

Scheme 1 shows a general scheme for the preparation of complex compounds of anhydrolagochiline with GA and its salts in various molar ratios. The physicochemical and spectral characteristics of the obtained complex compounds are shown in Table 2.

Table 2

Some physicochemical characteristics of supramolecular complexes of GA and its monoammonium and monopotassium salts with anhydrolagochilin

Nº	Complexes	Mp. °C decomp osition	α_D / 0,5% water : etanol 1:1	IC cm^{-1}	UV Hm	Yield %
1	AHL- GA 1:2	180-183	+28	1043 (COC), 1725 (CO) 2946 (CH ₃), 3421 (OH)	251	94,6
2	AHL- GA 1:4	186-189	+40	1043, (COC), 1728 (CO) 2877 (CH ₃), 2932 (OH)	251	90,6
3	AHL- GA 1:9	192-195	+38	1043 (COC), 1730 (CO) 2880 (CH ₃), 2935 (OH)	250	93,9
4	AHL- MASGA 1:2	200-203	+40	1042 (COC), 1659 (CO) 2944 (CH ₃), 3417 (OH)	250	80,8

5	AHL- MASGA 1:4	206-209	+42	1042,(COC),1658(CO) 2945 (CH ₃),3414 (OH)	250	87,3
6	AHL- MASGA 1:9	210-212	+40	1042,(COC),1657(CO) 2946(CH ₃),3412(OH)	250	83,6
7	AHL- MASGA 1:2	220-223	+22	1044(COC),1657 (CO) 2945 (CH ₃),3417 (OH)	251	91,2
8	AHL- MPSGA 1:4	225-228	+24	1044(COC),1658(CO) 2946(CH),3418(OH)	251	85,7
9	AHL- MPSGA 1:9	229-232	+22	1044,(COC)1660 (CO) 2948 (CH ₃)3420 (OH)	251	88,2

*AHL-anhydrolagochilin,GA- glycyrrhizic acid, MASGA- monoammonium salt of glycyrrhizic acid, MPSGA – monopotassium salt of glycyrrhizic acid.

As can be seen from the data in Table 2, in the UV spectra of the obtained compounds absorption bands are observed with a maximum at 250-251 nm in a water: alcohol medium (50:50), corresponding to the " π - π *" transition of the double conjugated bond at C (carbon) rings of the skeleton of the aglycone part of the HA molecule and its salts.

In the IR spectra of supramolecular complexes of anhydrolagochiline with HA and its salts, the following vibration frequencies are observed: stretching vibrations of carboxyl groups not participating in the formation of HA hydrogen bonds in the range of vibrations of functional groups at 1735-1725 cm^{-1} . Stretching vibrations of carbonyl groups participating in the formation of hydrogen bonds are observed in the range of 1660-1657 cm^{-1} . In this case, a shift of vibrations of carbonyl groups relative to the initial ones in a low-frequency field by 70-65 cm^{-1} is observed, which indicates the formation of hydrogen bonds. Stretching vibrations of hydroxyl groups of the lagochiline skeleton at positions 3 and 18, participating in hydrogen bonds, appear as broad bands at 3420-3370 cm^{-1} .

The melting points of the obtained complex compounds are from 180 to 230 ° C at which their decomposition occurs. Complex compounds were identified by thin layer chromatography. The α / D of the obtained compounds was also studied at 0.5% concentration in water: ethanol solution (1: 1 by volume). The data obtained show that all analyzed complexes are optically active and dextrorotatory.

Previously, the specific hemostatic activities of a number of lagochilin derivatives were studied. It was shown that all studied compounds of this group possess, to one degree or another, hemostatic activity. Moreover, it should be noted that lagochilin is insoluble in water and therefore it has not been studied as a hemostatic. However, a 0.5% solution of lagochilin in peach oil shows 67% activity compared to the reference drug.

The hemostatic activity of anhydrolagochiline and an isopropylidene derivative of lagochiline, exhibit weak hemostatic activity (17%). Protection of hydroxyl groups at C3 and C18 leads to a complete loss of hemostatic activity. The protection of hydroxyl groups at C3 and C18 in the lagochirzine molecule to acetyl groups does not lead to a particular change

in their hemostatic activity (67%). The introduction of the isopropylidene group into the lagochirzine molecule relatively reduces the hemostatic activity (51%), but the opening of the lactone ring with the formation of the corresponding sodium salt -3,16,18-trihydroxy-9,13-epoxylabdan-15-oic acid leads to a sharp increase in hemostatic activity (100%), which was used as a reference drug. Comparison of the hemostatic activity of lagochilin, anhydrolagochilin, lagochirzine using aqueous-alcoholic solutions indicates that lagochirzine has the greatest activity [21, 22].

Thus, it was shown that the protection of 3 out of 4 hydroxyl groups of the lagochilin molecule on the acetyl groups does not affect the hemostatic activity, only the complete protection of the hydroxyl groups leads to a loss of hemostatic activity. The introduction of isopropylidene groups negatively affects the manifestation of hemostatic activity, which is possibly associated with an increase in the hydrophobicity of the lagochilin molecule. Apparently, in the manifestation of hemostatic activity, a special role is played by the solubility of lagochilin derivatives, which ensures the high bioavailability of the studied substances [21].

Further, the effect of the supramolecular complexes of Lagochilin, Lagochirzin and anhydrolagochilin with HA and its salts on the blood coagulation process was studied, to determine the pattern of changes in activity from the chemical structure and complexing agent, a screening test was performed for the duration of bleeding and the amount of blood loss was determined.

The experiments were carried out in a thermostat with an open door, with a temperature of $+30^{\circ}$. At this temperature, the rats were kept in a thermostat for 1 hour. Prior to this, the study drug was administered. Then the rats were taken out from the thermostat one by one, and the tip of the tail was cut off with a length of about 10-12 mm with sharp scissors. A sheet of filtered paper was brought to the tail stump. Before this, the paper was dried in a thermostat and weighed on a balance. The blood flowing out of the tail was distributed on filter paper, which was then again dried and weighed. The duration of bleeding was recorded using a stopwatch from the moment the first drop of blood appeared until the complete cessation of bleeding, and the amount of blood loss was assessed by the weight of the dry residue in mg [1].

The experiments were carried out on 205 white outbred rats weighing 200 ± 20 g of both sexes, 5 animals per group. The drugs were administered orally at a dose of 10 mg / kg. The results obtained were processed statistically [1].

The effect of drugs on the blood coagulation process was judged by the test, the bleeding time and the amount of blood loss. This test reflects the vascular-platelet mechanism of hemostasis, and is determined by the number and condition of platelets (their ability to adhere and aggregate). The results are shown in Table 3.

Table 3**Bleeding time and amount of blood loss in rats 60 minutes after drug**

administration ($M \pm m$; $n = 5$)

	Drug name	Bleeding time		The amount of blood loss	
		sec	%	mg	%
1	Control(water)	260 $\pm$ 25,0	100	251 $\pm$ 23,0	100
2	2:1(GA-LG)	160 $\pm$ 12,0*	62	150 $\pm$ 12,0	60
3	4:1 (GA-LG)	200 $\pm$ 16,0	77	400 $\pm$ 34,5	159
4	2:1 (GA-LGh)	178 $\pm$ 15,2*	68	157 $\pm$ 12,5	63
5	4:1 (GA-LGh)	250 $\pm$ 23,0	96	250 $\pm$ 33,0	100
6	2:1 (GA-AHL)	0 $\pm$ 0*	0	0 $\pm$ 0	0
7	4:1 (GA-AHL)	22 $\pm$ 2,3*	8,6	6 $\pm$ 0,4	2,4
8	2:1 (MASGA-LG)	13,3 $\pm$ 7,9*	5,1	13 $\pm$ 1,0	5,2
9	4:1(MASGA-LG) Glilagin	65 $\pm$ 3,0*	30	60 $\pm$ 4,5	27
10	2:1 (MASGA-LGh)	154 $\pm$ 12,0*	59	400 $\pm$ 39,0	159
11	4:1 (MASGA-LGh)	28 $\pm$ 2,3*	11	35 $\pm$ 3,3	14
12	2:1 (MASGA-AHL)	48 $\pm$ 4,1*	18	23 $\pm$ 2,0	9,2
13	4:1 (MASGA-AHL)	190 $\pm$ 17,0*	73	483 $\pm$ 46,5	192
14	2:1 (MPSGA -LG)	195 $\pm$ 15,5*	75	300 $\pm$ 25,5	120
15	4:1 (MPSGA -LG)	300 $\pm$ 26,5	115	416,6 $\pm$ 35,6	166
16	9:1 (MPSGA-LG)	70 $\pm$ 5,6*	27	33,3 $\pm$ 2,3	13,3
17	2:1 (MPSGA-LGh)	73 $\pm$ 6,5*	28	20 $\pm$ 1,5	8
18	9:1 (MPSGA-LGh)	272 $\pm$ 15,3	105	300 $\pm$ 25,5	120
19	4:1 (MPSGA-AHL)	300 $\pm$ 2,5	115	416 $\pm$ 35,0	166

* $P < 0.05$ in relation to control

**AHL- anhydrolagochilin, LGh-lagochirzin, LG-lagochilin, GA- glycyrrhizic acid, -MASGA- monoammonium salt of glycyrrhizic acid, MPSGA- monopotassium salt of glycyrrhizic acid.

Complexing agents promote, firstly, an increase in the solubility of active substances and reduce toxicity; also, due to synergism, they should reduce the therapeutic dose by 10 - 50 times.

As can be seen from the data in Table 3, among the supramolecular complexes **GA-LGh** and **GA-LG** in ratios of 2: 1, relatively weak (60-68%) hemostatic activity, and the ratio of 4: 1 practically did not differ from the indicators in the control group of animals.

We observed even greater activity in the supramolecular complexes of GA-AHL in a 2: 1 ratio, where we could not determine the bleeding time and the amount of blood loss. With the introduction of the drug GA-AHL 4: 1, the bleeding time was reduced, to 22 ± 2.3 sec, or 12 times, and the amount of blood loss decreased to 6 ± 0.4 mg, or 42 times. The highest activity was found in the sample with the highest anhydrolagochiline content.

Among the supromolecular complexes of MSGA-LGh, the most active ratio was 4: 1; with the lowest content of Lagohirzin (28 ± 2.3 sec and 35 ± 3.3 mg or 6.3 and 7.1 times, respectively). The most active of the supramolecular complexes MSGA-LG was the 2: 1 ratio, where the bleeding time was reduced to 13.3 ± 7.9 seconds, or 20 times, and the amount of blood loss decreased to 13 ± 1.0 , or 19 times.

Of the studied supramolecular complexes MSGA-AHL in a ratio of 2: 1, where the bleeding time was reduced to 48 ± 4.1 sec, or 5.4 times, and the amount of blood loss decreased to 23 ± 2.0 mg, or 11 times.

This is clearly seen from Figures 1 and 2, which are presented in the form of a diagram.

Figure 1

Figure 2

Among the supramolecular complexes **MKSGK-LGh**, the most active ratio was 2: 1, the bleeding time in which decreased, to 73 ± 6.5 sec, and the amount of blood loss to 20 ± 1.5 mg, or 3.6 and 12.6 times, respectively in relation to the benchmark.

The most active of the supramolecular complexes **MKSGC-LG** was the 9: 1 ratio, where the bleeding time was reduced, to 70 ± 5.6 sec, or 3.7 times, and the amount of blood loss decreased to 33.3 ± 2.3 or 7.6 times. Thus, the conducted research has led to the following conclusions:

1. Supramolecular water-soluble complexes of anhydrolagochiline with GA and its salts in various molar ratios have been obtained for the first time.
2. The physicochemical and spectral characteristics of the obtained supramolecular complexes and specific hemostatic activity were studied.
3. Among Lagochilin Lagochirzin and their derivatives at a dose of 10 mg / kg, Lagochilin had the most active effect on bleeding time, followed by Anhydrolagohilin - Lagochirzin.

4. The greatest effect on bleeding time at a dose of 10 mg / kg among supramolecular complexes with GA was shown by GA-AHL, which in a 2: 1 ratio it was impossible to determine bleeding time, and in a 4: 1 ratio reduced bleeding time by 12 times, and the amount of blood loss 42 times.

5 The greatest effect on bleeding time at a dose of 10 mg / kg among supramolecular complexes was shown by **MSGA-LH** (2: 1), which reduced bleeding time by 11-26 times, and the amount of blood loss by 19-18 times.

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