The Complexes of Drugs with Carbohydrate-Containing Plant Metabolites as Pharmacologically Promising Agents

T.G. Tolstikova*, M.V. Khvostov and A.O. Bryzgalov

Novosibirsk Institute of Organic Chemistry, Lavrentjev ave., 9., 630090, Novosibirsk, Russia

Abstract: Complexation of known drugs with carbohydrate-containing plant metabolites is a promising way to synthesize new drugs that does not only save pharmacological properties of initial agent but also acquire a number of advantageous features such as increased water solubility, bioavailability and decreased toxicity. This review reports on the development and pharmacological evaluation of novel complexes of various well-known drugs with vegetable coplexation agents: glycyrrhizic acid, *Stevia* glycosides, gypsogenin tetraoside, pectin, xyloglucan, arabinogalactan. The aim of this review is to describe advantages of the new approach, suggested by authors, in the development of low toxic and high-performance drugs.

Key Words: Complexation, plant metabolites, glycyrrhizic acid, arabinogalactan, stevioside.

1. INTRODUCTION

Complexation of pharmacons with carbohydrate-containing compounds is a promising way to synthesize drugs that do not only save pharmacological properties of an initial agent but also acquire a number of advantageous features such as increased water solubility, bioavailability and protection from quick metabolism in the body, etc.

The exemplary carbohydrate-containing complex formers are cyclodextrins (CD), semisynthetic derivatives used in the synthesis of more than 70 known pharmacons [1-3]. More than 20 complexes of cyclodextrins are produced commercially as novel dosage forms, including seven forms for parenteral administration [3].

Availability of excellent reviews and non-vegetable origin of cyclodextrins spare us the necessity to discuss these compounds.

2. 18βH-GLYCYIRRHIZIC ACID AS A COMPLEX FORMER OF PHARMACONS

2.1. Physicochemistry of Glycyrrhizic Acid Solutions

18βH-glycyrrhizic acid (GA), a triterpen glycoside produced by widespread fabaceous plants, *Fabaceae Glycyrrhiza glabra L.*, *G. uralensis Fisch*, *G. korshinskyi Grig* (licorice) belongs to few plant metabolites combining availability with a unique variety of pharmacological activity [4-6]. Glycyrrhizic acid (Fig. (**1**)), whose molecule consists of hydrophobic (aglycon) and hydrophilic (carbohydrate chain) parts, manifests the properties typical for micelle-forming substances [7-12]. According to [9], the micellar critical concentration (MCC) of GA water solution is 10^{-3} M. The study of rheological properties of GA solutions by the viscometric method resulted in a conclusion that their properties

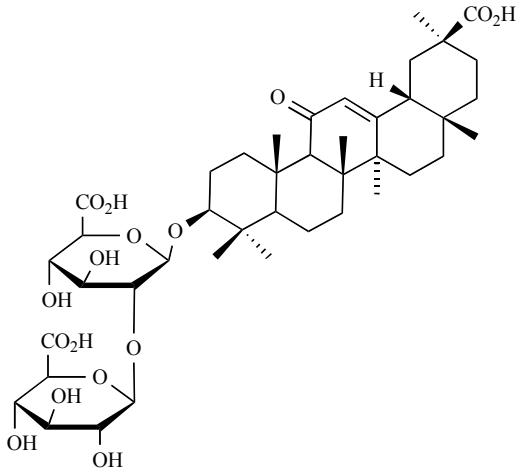

Fig. (1). Structure of glycyrrhizic acid.

were similar to those of Newton liquids [12]. The solution viscosity at the concentration lower than $3\cdot10^{-5}$M does don't change. With increase of the concentration above that for MCC (> $3\cdot10^{-5}$ M) the solution viscosity and turbidity increase. Thus, GA associates existing in water solution start to group into micelles. When ethanol is added to water solution, at 20 volume percent of ethanol MMC increases to 10^{-4}M.

Ethanol is characterized as a structure-creating solvent. Thus, dissolution of GA in ethanol lead to complex formation where GA plays the role of a "host" and ethanol - a "guest".

Apparently, the presence of ethanol prevents from micelle formation at GA concentration ranging from $3\cdot10^{-5}$M to $1\cdot10^{-4}$M.

Different behavior of GA molecules depending on a solvent was demonstrated in the study of glycoside rheology in ethanol and in dimethylformamide (DMF) solutions. It is

*Address correspondence to this author at the Novosibirsk Institute of Organic Chemistry, Lavrentjev ave., 9., Zip code: 630090, Novosibirsk, Russia; Tel: +7 - 383 - 330-07-31; Fax: +7 - 383 - 330-07-31;
E-mail: mihail.hvostov@gmail.com

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Table 1. Anti-Inflammatory Dose Range of Glycyrrhizic Acid Complexes and NSAID

Compounds	Dose Range of the Complex*	Dose Range of Initial NSAID
GA : ASA (1:1)	4500/82=54.8	1900/98=19.4
GA: OF (1:1)	1750/12.5=140	310/8=33.7
GA : BD (1:1)	3150/62=50.8	880/56=15.7
GA : AN (1:1)	8000/68=117.6	570/55=10.3
GA	-	5000/100 =50

*LD_{50} /ED_{50}; ED_{50} – effective dose.

established that for solutions in DMF complexation occurs in concentration $1{,}4 \cdot 10^{-2}$M [13].

GA association in solutions was studied by NMR methods [14, 15]. The experimental data indicate that small micelles with aggregation number M≤ 10 may form. This applies to water-methanol (20% MeOH), neutral or acid solutions with GA concentrations ≤ 0.5 мМ. In 50% water solution, micellation does not occur. The study of water solutions GA by small-angle X-ray scattering method showed that at the concentration of about 1.0 mM glycoside forms rod-like associates with the radius ~ 1.4 nm and length up to 60 nm. The associates are stabilized by intermolecular hydrogen bonds.

2.2. Complexes of Pharmacons with Glycyrrhizic Acid. Pharmacological Properties

The pioneering works on complexation are [16, 17], where solubilizing effect of glycyrrhizic acid and its monoammonium salt, glycyrrham, was investigated for a series of water insoluble pharmacons. In the above-stated works as well as in [18], it was demonstrated that it is possible to obtain water solutions of practically water insoluble antibiotics such as oxytetracycline, nystatin, actinomycin C, corticosteroids such as hydrocortisone and prednisolone, and sulfazine, a sulfanilamide drug. GA demonstrates solubilizing properties at concentrations higher than MMC [17]. It should be noted that none of the above works discusses the ability of glycyrrhizic acid to form complexes with pharmacons. For the first time the issue was discussed in [19], where GA complexes were called new carriers for drugs [19]. In earlier studies, the solution method of complex synthesis prevailed. Mechanochemical activation was introduced later to synthesize complexes directly from solid substances, without any vehicles [20], so all described complexes synthesized in solid phase using an AGO-2 planetary mill (grinding acceleration of 60 g for 10 minutes). The drum volume is 40 cm^3, steel balls 6 mm in diameter with the weight of 75 g are used as grinding bodies.

2.2.1. Complexes of Non-Steroid Anti-Inflammatory Drugs (NSAID) with Glycyrrhizic Acid

Complexes of GA with acetylsalicylic acid (ASA), ortophenum (OF), butadionum (BD) and indometacin (IM) with the molecular composition (GA:NSAID) 1:1 and 2:1, were synthesized [20].

All the above-stated complexes demonstrate anti-inflammatory (AI) activity at the doses lower than the source pharmacon. The dose range of the complexes (LD_{50} /ED_{50}) is 3-11 times higher than that of the initial NSAID [21].

Complexes with acetylsalicylic acid and ortophenum (GA:ASA, GA:OF) produce an expressed AI effect in six models of acute inflammation induced by carragenin, formalin, histamine, serotonin, agar (Difko), tripsin, as well as at chronic inflammation ('cotton pellet' and 'pocket' granulomas) of intact and adrenalectomized animals [21]. A complex of GA with indometacin 1:1 has a more pronounced AI effect as compared with initial drug if administered in equal doses (10 mg/kg).

At complexation of NSAID with GA the potentiation of other kinds of biological activity (analgesic, antipyretic) has been observed [21-24]. At electrical and thermal irritation the analgesic effect of GA complex with ortophenum was more pronounced than that of ortophenum and at thermal pain irritation it exceeded the effect of amidopyrine. The analgesic effect of GA complex with analgin (AN) was 11.4 stronger than that of analgin [24]. The analgesic effect of complex GA:ASA exceeds the effect of aspirin at thermal pain irritation. At acetylcholine convulsions the analgesic dose range of GA complexes with ASA and OF is 3 and 2.3 times broader than that of NSAID, respectively. In the acetum convulsion model the therapeutic ratio of complex GA:ASA was 4 times higher than that of aspirin. Complexes GA:ASA and GA:OF have pronounced antipyretic action. Their antipyretic dose range was 2 times broader than that of initial pharmacons [21, 23, 24].

Thus, water-soluble complexes GA:ASA and GA:OF have pronounced AI and analgesic effects with the pharmacological spectrum and therapeutic dose range exceeding those of initial non-steroid AI drugs. The complexes have pronounced membrane stabilizing action, decrease accumulation of primary and secondary lipid peroxidation products in animals with chronic inflammation.

Complexes with GA have less irritant effect on mucous coat of stomach as compared with original NSAID. Thus, complex GA:ASA stimulates ulcerous lesions reparatio and complex GA:OF shows low ulcerogenic activity. Both complexes decrease the level of prostaglandins E_1 and E_2 in the blood of animals with chronic inflammation. The complexes can be recommended as anti-inflammatory drugs for clinical

Table 2. Acute Toxicity of Complexes GA with NSAID in Mice (per os)

Drugs	LD_{50}, mg/kg	Pharmacon LD_{50}, mg/kg
GA:ASA (1:1)	4500	1900
GAOF (1:1)	1750	310
GA:BD (1:1)	3150	880
GA:AN (1:1)	8000	570
GA	5000	-

trials, including the trial in patients with stomach and duodenal ulcers.

Acute toxicity of GA complexes with NSAID is 2-14 times lower as compared with original drugs (see Table **2**) [21, 23-25]. At complexation of NSAID with GA synergetic effect can be observed, manifested in an increase of water-solubility and biological activity and simultaneous decrease of toxicity and ulcerogenic action on gastrointestinal tract.

2.2.2. Complexes of Prostaglandins with Glycyrrhizic Acid as a New Group of Uterine Tonics

Prostaglandins have been widely used in medicine and veterinary practice due to their ability in small doses to stimulate uterine muscles.

Use of prostaglandin in veterinary practice is one of the most important advantages of prostaglandin studies. Chloprostenol is one of the main drugs used in veterinary practice. Together with other prostaglandins it is obtained by multistage synthesis, which results in high prices of the drug. Reduction of therapeutic active dose is an actual task. Besides, it is necessary to increase stability of labile prostaglandins in the finished drugs.

Both problems were successfully resolved by complexation of prostaglandins with GA. GA complexes with prostaglandins of the E and F series (PGE_1, PGE_2, $PGF_{2\alpha}$, sulprostone (SP) and chloprostenol) have been obtained and investigated.

Unlike the initial prostanoids, the complexes synthesized by the solution method in the form of water-soluble amorphous substances are highly stable both in solid state and in solutions [26]. Complexation has been proved by spectral analysis methods and chromatographic homogeneity.

In experiments on uterus of rats and guinea-pigs, complexes GA:PGE_1 (1:1) and GA:$PGF_{2\alpha}$ (1:1) double uterine contraction amplitude as compared with sodium PGE_1 at the same concentrations (10^{-8} g/ml). Sulprostone and PGE_2 in the form of complexes with GA (1:1) strengthen uterine contraction amplitude by 3 times while increasing uterus tonus [27, 28].

On the base of GA complexes with chloprostenol, a known synthetic luteolytic prostaglandin, highly effective veterinary drug "Chlatraprostin" was developed, the active agent dosage of which is 5 times lower than that accepted in the world practice. In comparison with imported analogues the drug is cheaper and its action is more physiological.

"Chlatiram" containing amino acid tyrosine along with GA and chloprostenol is even more efficacious [29]. Its effect is stronger than that of estrofan the well-known veterinarian drug, at a 100 times reduced dose of prostaglandin.

2.2.3. Complexes of Glycyrrhizic Acid with Cardio Active Drugs

The structure and pharmacological properties of GA complexes with antiarrhythmic drugs allapinin (hydrobromide of lappaconitine, diterpenoid alkaloid) (LA) and antihypertensive drug nifedipine have been investigated.

The process of complexation of lappaconitine with glycyrrhizic acid in solutions was investigated by photo-CIDNP method [30-32]. Photolytic destruction of lappaconitine, which sharply slows down in the presence of GA, was chosen as a reaction simulating the complexation effect. The reaction leads to protection of the complex-bonded alkaloid. The experiment demonstrating that complexation results in the photodestruction slowdown and is a sufficiently correct simulates metabolism of pharmacon *in vivo*. The stoichiometry 1:1 and stability constant $K_s = 2 \cdot 10^5 \pm 0.13 \cdot 10^5 M^{-1}$ for the complex of glycyrrhizic acid with lappaconitine base in water-methanol solution (20% MeOH) was established. In pure methanol the constant is lower by an order, $K_s = 1.3 \cdot 10^4 M^{-1}$. Complex of lappaconitine hydrobromide with GA has the stability constant $K_s = 2.6 \cdot 10^3 M^{-1}$.

It should be noted that average values of the stability constants of pharmacon complexes with cyclodextrins are about $10^3 M^{-1}$.

Allapinin has been included in the list of antiarrhythmic drugs. It is recommended at different forms of cardiac rhythm disturbance, especially in ventricular arrhythmias, paroxysmal atrial fibrillation, monofocus atrial tachycardia [33, 34]. The disadvantage of allapinin is its high toxicity.

In a special series of experiments, it was shown that complexes GA:LA produce an effect on antiarrhythmic activity and that complex GA:LA of molecular composition 4:1, patented as "alaglyzin", has the most potent antiarrhythmic effect [35]. In the calcium chloride and aconite arrhythmia models, "alaglyzin" demonstrated high antiarrhythmic activity and the antiarrhythmic index (LD_{50}/ED_{50}) higher than that of all known antiarrhythmic drugs. "alaglyzin" is 10 times less toxic than allapinin. In an extended study of "alaglyzin" antiarrhythmic activity using the calcium chloride and aconite arrhythmia models was found that ED_{50} for "alaglyzin" and allapinine is 0.125 and 0.290

mg/kg, respectively, as follows from the effective dose of "alaglyzin" it contains 15 times less lappaconitine than the effective dose of allapinine [36].

Nifedipine (NF) (2.6-dimethyl-3.5-dicarbomethoxy-4-(2'-nitrophenyl)-1.4-dihydropyridine) belongs to calcium antagonists. The mere fact of continuing development of novel dosage forms of nifedipine indicates that as the simplest derivative of 1.4-dihydropyridine with proven production technology will be used in the future as a reliable alternative of more expensive drugs of this type [37].

The study of complexation of GA with nifedipine using NMR method showed the existence of complex with the molecular composition GA:NF = 2:1 in water-alcohol solutions at the concentrations of GA ≤ 0,5 мМ. At higher GA concentrations associates are formed, which include equal number of molecules of both substances. The complex is highly stable as indicated by its stability constant (K_s= $1{,}2 \cdot 10^5 M^{-1}$) [38].

Nevertheless, the preliminary study of hypertensive activity of nifedipine convincingly demonstrated that GA:NF = 4:1 is an optimal molecular composition. The complex synthesized by the mechanochemical activation method is an amorphous substance consisting of 20-50 μm vitreous particles. Complexation was also confirmed by radiographic and thermogravimetric data, according to which traces of crystalline phase of both reagents disappear. Water-solubility of nifedipine in the complex increases 8.5 times.

The study of antihypertensive action showed that at intravenous administration of the complex in water solution to rats the desired effect is manifested at the dose of nifedipine 10 times lower than usual dose [39].

As stated above, an important consequence of complexation could be enhancement of pleiotropic properties of a pharmacon, for nifedipine this is antiarrhythmic action. The study of antiarrhythmic action of GA:NF complex showed that protective antiarrhythmic effect can be reached at a dose 29 times lower than that providing antihypertensive effect.

Thus, GA:NF 4:1 complex is promising for the development of the first universal drug capable of arresting hypertension and arrhythmia.

2.2.4. Complexes of Glycyrrhizic Acid with Psychotropic Drugs

Complexation effect has been found in the study of pharmacological properties of GA complexes with fluoxetin, an antidepressant, and phenibut, an anxiolytic. Fluoxetin (FL) (N-methyl-3-(4′-trifluormethylphenoxy)-3-phenylpropylamin hydrochloride) is known to cause an antidepressant action connected with inhibition of capture of serotonin by neurons in CNS. Antidepressants are known to have such disadvantages as rather high doses, a narrow range of therapeutic index, high toxicity and long elimination period, which negatively effect kidney functions and damage hepatocytes. The study of GA complexes with fluoxetin was conducted with the main aim to determine whether the side effects of this pharmacon could be decreased. Preliminary tests showed that the complex with the molecular composition GA:FL 4:1 was the most active, this complex is patented under the name "fluoglyzin" (FG) [40]. The complexes with the molecular compositions 4:1 and 2:1 are characterized by LD_{50} higher than 5000 mg/kg as compared with 248 mg/kg for fluoxetin.

"Fluoglyzin" in the Porsolt test at a single and prolonged administration was found to have a more pronounced antidepressant action as compared with fluoxetine. In comparison with fluoxetine, "fluoglyzin" produces a stronger inhibiting effect on serotonin structures. For example, in the test with 5-oxytriptophan, "fluoglyzin" inhibits chloral hydrate action to a greater extent than fluoxetine. Both drugs produce no anxiolytic effect.

In the social depression model on mice, "fluoglyzin" was shown to have an antidepressant effect similar to that of fluoxetine, which was manifested in a twofold increase of communicability of individuals (the number and duration of contacts) in response to a known and strange partner. The dose of fluoxetine in complex GA:FL 4:1 is 1.08 mg/kg as compared with 15 mg/kg for the sample drug. "fluoglyzin", similarly to fluoxetine, prevents a decrease of glucose content in blood and lowers the intensity of peroxidation, thereby normalizing antioxidant status of depressed individuals [41-43].

To elucidate the action mechanism of complexes GA:FL 4:1 и 1:1 as compared with fluoxetine, their effect on the content of catecholamines and their precursors in different parts of cerebrum was studied at a single and therapeutic administration at the dose of 25 mg/kg. A decrease of fluoxetine dose by 4 times (complex 1:1) and 17 times (complex 4:1) in complexes with GA was found to lower the effect on serotonin metabolism and to activate dopamine metabolism in cerebrum [44, 45].

In [45], the nootropic activity of fluoxetine was established for the first time. Both complexes GA:FL 1:1 and 4:1 demonstrate the same effect but at a slightly lowers level than reference drugs.

Phenibut (PhB) (γ-amino-β-phenylbutyric acid hydrochloride) is a nootropic sedative medication relieving tension and anxiety and improving sleep. In clinical practice it is used at asthenic syndrome, anxious neurotic conditions, sleep disorders and as an anti-naupathia preparation in presurgery procedures. As a nootropic drug phenibut has serious flaws, e.g. it provokes sleepiness and allergic reactions.

Complexes GA:PhB 2:1 and 4:1, the toxicity of which is twice as lower than that of phenibut, produce a cognitive effect similar to that of the pharmacon and GABA. Unlike phenibut and GABA, the complexes increase mnestic capabilities in animals by 20% and decrease sedative effect [45].

2.2.5. Complexes of Glycyrrhizic Acid with Antitumor Drugs

Synthesis by the solution method of complexes of glycyrrhizic acid with 5-fluorouracil, fluorofur, and rubomicine hydrochloride with molecular composition 1:1 is described in [46]. Composition of the complexes, which chromatographically are homogeneous amorphous substances, was proved by spectral methods. Complexation resulted in water solubility and lower toxicity. The complex of fluorofur has

an antitumor action on Pliss lymphosarcoma, melanoma B-16 and Heren's carcinoma. The efficiency indices for these diseases are equal to 3.05, 2.11 and 1.7, respectively. Inhibition of growth for these tumors is 67.2%; 53.4 %; 87.1 %, respectively.

2.2.6. Complexes of Glycyrrhizic Acid with Antimicrobial Drugs

The 1:1 complexes of GA with antibiotics such as levomycetin, sulfapyridazin, sulfadimethoxine, sulfamonomethoxine, sulfadimesine, sulgin, sulfanilamides; and drugs isoniaside and furacillin were synthesized by the solution method. All the complexes are chromatographically homogeneous amorphous substances; complexation is proved by IR spectral data indicating that the spectral lines of hydroxylic and carbonylic groups are shifted to the short-wave band.

Comparative data are available on antimicrobial action of the complexes. In staphylococcosis, on the 10^{th} day after contagion the survival rate was the highest (90%) in the group of animals receiving the complex of GA with levomycetin at the dose of 50 mg/kg. In the group receiving only levomycetin the survival rate was 30%. The survival rate among the animals infected with *Pseudomonas aeruginosa, Proteus vulgaris* and *E.coli* was about 80% in the group receiving the complex. The group receiving levomycetin the survival rate ranged between 20 and 50%. The complex was shown to be able to stimulate humoral and cellular immunity [47].

2.2.7. Complexation with Hypocholesterolemic Agent Symvastatin

The inhibitors of 3-hydroxi-3-methylglutaril-CoA reductase (3HMG-CoA reductase), so called statins, are known as efficacious drugs lowering low-density lipoprotein secretion, which explains their wide use in anti-atherosclerosis therapy. At the same time, most statins are known to have side effects. That is why the development of safer drugs with a prolonged action is a present-day necessity. An NMR study of symvastatin (SMS) behavior in solutions in the presence of GA led to a conclusion on the formation of stable complexes. Complex GA:SMS = 4:1 was synthesized, which demonstrated stability in water solutions at GA concentration more than 0.2 mM [48]. The complex patented under the name "symvaglyzin" (SMG) [49] showed 3HMG-CoA reductase non-competitive inhibiting activity. Effective at the doses containing one third of statin's normal dose, "symvaglyzin" is a more efficacious and safer agent as compared with symvastatin.

Thus, "symvaglyzin" is a noncompetitive inhibitior/proinhibitor of 3HMG-CoA reductase, arresting the synthesis of cholesterol in the microsomal fraction of rat liver *in vitro* on a par with symvastatin. At the inhibition constant ranging between 100 and 300 nM, "symvaglyzin" inhibits the formation of mevalonat by 37.7 – 42.0 %. After a 14-day course of treatment of hypercholesterolemic rats with "symvaglyzin" at the doses containing one third of symvastatin normal dose, "symvaglyzin" decreases total cholesterol level by 31 – 33 %, which is comparable to the effect of symvastatin in the therapeutic dose.

Higher safety of "symvaglyzin" follows from the fact that after 14 days of intake at the doses containing "symvaglysin" the content of which was 2-5 times lower the increase of creatine phosphokinase level in blood of hypercholesterolemic rats was 2-5 times lower [50].

2.2.8. Antidote and Antiradical Activity of Complexes of Glycyrrhizic Acid with Uracil Derivatives

GA forms stable complexes with uracil derivatives with the molecular composition 1:1. The complex with 2-tiouracil is a dehydrate, complexes with salts are crystallized with one molecule of water. Complexes with 5-oxi-6-methyl-uracil and aminouracil are waterless [51]. All complexes are 1.8-1.9 times less toxic than uracil. By the range of antidote action determined using the model of male mice poisoning with sodium nitrite, the complexes exceeded initial uracils on average twice and cystamin - 2.6-17.4 times. Complexes with uracils have a lower value of effective dose (ED_{50}) than cystamin, i.e. they are more efficacious than the reference drug. Complexes with 2-tiouracil and 5-oxi-6-methyluracil, although having a higher value of ED_{50} as compared with cystamin, exceed it in the range of pharmacological action (see Table **3**).

Antiradical activity of GA complexes *in vitro* was studied by chemiluminescence method by determining the reaction rate constant (K_7) for the interaction of ethylbenzol per-

Table 3. Antidote Activity of Pyrimidines and their Complexes with GA After Poisoning Mice by Sodium Nitrite

Pyrimidines	LD_{50}, mg/kg		LD_{50}/ED_{50}	
	Complexes with GA	Pyrimidine	Complexes with GA	Pyrimidine
2-thiouracil	6000	2850	58,2	27,6
6-amino-2-thiouracil	4100	2150	290,7	153,5
6-aminouracil	3700	1250	189,7	86,2
6-methyl-2-thioutacil	5200	2125	208	85
5-oxi-6-methyluracil	20 000	10000	400	200
5-aminouracil	2500	1000	200	80
Cistamin	-	770	-	22,6

oxide radicals with the compounds studied. It was found out that antiradical activity of GA complexes with aminopyrimidines and 2-tiouracil was on average 10 times higher than that of ionol.

High antidote activity of GA complexes with uracil derivatives in combination with their antiradical action makes these compounds promising for the development of drugs protecting living beings from toxic exposures.

3. PHARMACOLOGICAL PROPERTIES OF COMPLEXES OF PHARMACON WITH DIFFERENT TERPENE GLYCOSIDES

3.1. Complexes of *Stevia* Glycosides

South American plant *Stevia rebaudiana* has been cultivated to obtain non-toxic sweeteners. The sweet taste of its leaves is due to eight glycosides of the diterpen series, primarily stevioside (Fig. (**2**)) and rebaudioside A (Fig. (**3**)), a tetrasaccharide [52]. As the objects of pharmacological research, both glycosides have been thoroughly investigated. In particular, they were shown to be absolutely safe (LD_{50} > 10000 mg/kg for stevioside and 8000 mg/kg for rebaudioside), a hypotensive activity has been shown for stevioside and the lack of such for rebaudioside [53], metabolism in human gut organisms has been investigated [54].

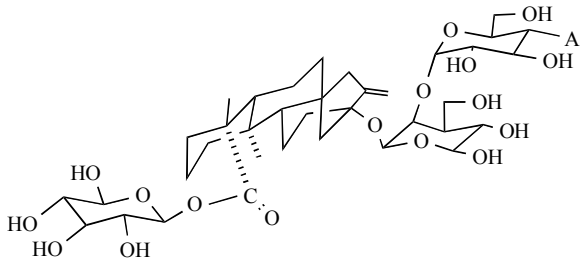

Fig. (2). Structure of stevioside.

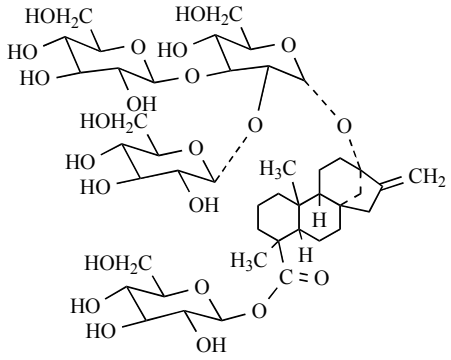

Fig. (3). Structure of rebaudioside A.

Recently, an antiarrhythmic effect has been demonstrated for both glycosides [55, 56]. For example, preventive intravenous administration of these glycosides at the dose of 0.120 mg/kg secures survival of up to 50% of the animals given a lethal dose of calcium chloride. In the adrenaline arrhythmia model with similar administration regimen only stevioside was shown to be effective. The survival rate was 50%. In the same arrhythmia models, both glycosides administrated at the dose of 0.120 mg/kg after the administration of the arrhythmogen failed to block arrhythmia that had already developed [56].

Complexes of lappaconitine with stevioside were made with the molecular ratios ST:LA = 1:1, 4:1, 8:1 and 16:1. Their antiarrhythmic activity was studied using the above-stated arrhythmia models.

As can be seen from Table **4**, complexes with the molecular composition 1:1 and 4:1 when administered preventively ensure full protection from the development of calcium chloride and adrenaline arrhythmias in animals. Both complexes showed low activity in the developed calcium arrhythmia model and no activity at all in the adrenaline arrhythmia model.

Noteworthy results were obtained for the complex with molecular composition 8:1, which ensured full protection of animals when administered preventively and after the development of calcium chloride arrhythmia. In the calcium chloride arrhythmia model the complex with molecular composition 16:1 retains high activity (80%) only when administered preventively.

Data of Table **5** on quantitative characteristics of the complexes indicate that complexation of lappaconitine with stevioside, like in the case of glycyrrhizic acid, allows decreasing the therapeutic dose of the pharmacon significantly.

It is to be noted that the molecule of stevioside, unlike GA, has no free carboxyl groups. Association of glycoside is likely to occur only due to the bonding of carbohydrate fragments. However, it may not be excluded that *in vivo* ester bonds hydrolysis takes place with the formation of acid diglucoside capable of forming associates acting as pharmacon complexing agents.

Complexation of stevioside with fluoxetine allows decreasing the pharmacon toxicity by 32 times. For example, at intragastrical administration LD_{50} of fluoxetine is 248 mg/kg, while LD_{50} of complex 4:1 is higher than 8000 mg/kg.

The study of pharmacological properties of complexes of fluoxetine with stevioside ST:FL with molecular composition 1:1 and 4:1 demonstrated a reduction of depressive effect of L-DOPA on emotional and motor activity in mice, similar to that of fluoxetine. The effect of complexes was shown to depend on the dose. Both complexes act on the dopaminergic system in the same way as fluoxetine.

The effect of fluoxetine complexation with stevioside is also manifested in a reduced sedative action of the pharmacon on the background of chloral hydrate, a somnific drug.

Similarly to GABA, complex ST:FL = 4:1 containing a 10 times reduced dose of the pharmacon, produces a positive effect on the mnestic abilities in animals [56].

Complexes of rebaudioside (RB) with nifedipine with the RB:NF ratio 2:1 and 4:1, containing 9 and 18 weight percent of the pharmacon, respectively, were synthesized using the solution method. Doses ensuring high antiarrhythmic and standard antihypertensive activity were found for the com-

Table 4. Antiarrhythmic Activity of Stevioside and its Complexes with Lappaconitine. Arrhythmogens: $CaCl_2$ (250 mg/kg), Adrenaline (0.3 mg/kg) Administered Intravenously

Agent	Dose, mg/kg	The Rat Survival Rate (%)			
		Regimen of Administration:			
		Complex + Arrhythmogen		Arrhythmogen + Complex	
		$CaCl_2$	Adrenaline	$CaCl_2$	Adrenaline
Stevioside	0.120	40	50	0	0
Complex 1:1	0.150	100	100	20	0
Complex 4:1	0.150	100	100	20	0
Complex 8:1	0.135	80	100	100	0
Complex 16:1	0.128	80	40	20	20
Lappaconitine	0.290	50	0	0	0

plexes. The overall (100%) antiarrhythmic effect was demonstrated by both complexes containing a 10- and 5-times reduced dose of nifedipine, respectively. Antiarrhythmic activity similar to that of nifedipine was shown for complex RB:NF 2:1 at a 5-times reduced dose of the pharmacon.

3.2. Complexation of Prostaglandin with Gypsogenin Tetraoside

Acanthophyllum gypsophylloides is a plant endemic for the Central Asia, the root of which is used as an emulsifying agent in food industry and contains triterpen glycosides [57]. One of them, gypsogenin tetraoside (Fig. (**4**)) was used for synthesizing a complex with a furan-type prostaglandin with molecular composition 4:1. In the experiments in mice the complex at the doses of 50 and 100 μg/kg increases the amplitude of uterine contractions by 100 and 120%, respectively, as compared with well-known drug prostenon. Notably, this complex is more active than that with glycyrrhizic acid with molecular composition 4:1 containing the same dose of prostaglandin [58-60].

4. COMPLEXATION OF PHARMACONS WITH POLYSACCHARIDES

4.1. Complexation with Pectin and Xyloglucan

Pectin, which can be extracted from the cell wall of various plants, is an anion-type polysaccharide with the main chain from poly-α-(1-4)-galacturonic acid. In the market, pectin is represented by two groups - metoxypectin with carboxyl group etherification < 50% and > 50% [61].

Gel-forming properties of pectins are used to synthesize gel-like peroral forms of drugs. Such forms are complexes with the acid-base interaction of the polysaccharide carrier and pharmacon and hydrogen bonds between the polymer hydroxyl groups and electron-acceptors assembles of pharmacon molecules. Peroral forms based on pectin complexes have been proposed for non-steroid anti-inflammatory drugs [62], nonnarcotic analgetic paracetamol [62, 63] and its modification acetamidofenol [64], antiulcer blocker of histamine H_2-receptors cimetidine [65], myotropic spasmolytic theophyllinum [65], and pulmonary surfactant ambroxol [66]. The development of pectin-based matrix pills containing anesthetic ropivacaine for delivery of drugs to large intestine is described in [67]. The authors emphasize that the use of amidated pectin produces the best results. Recent works describe the development of pectin nano-conjugates of anticancer drugs. Nano-conjugates of cisplatin with pectin in the form of nanoparticles sized 100 nM has been synthesized. This is actually a novel drug characterized by slow continuous release of cisplatin during the whole period of circulation. The drug introduced *per os* at a dose of 10 mg/kg body weight can be traced 72 hours after the administration. The preparation shows a lower accumulation in kidneys as compared with cisplatin and, therefore, has reduced nephro-

Table 5. Quantitative Characteristics of Stevioside – Lappaconitine Complexes

Molecular Composition	Dose, mg/kg	Lappacontine Content in the Dose (mg)	Decrease in Therapeutic Dose (Times)
Complex 1:1	0.150	0.063	4.6
Complex 4:1	0.150	0.023	12.0
Complex 8:1	0.135	0.011	16.0
Complex 16:1	0.128	0.006	48.0

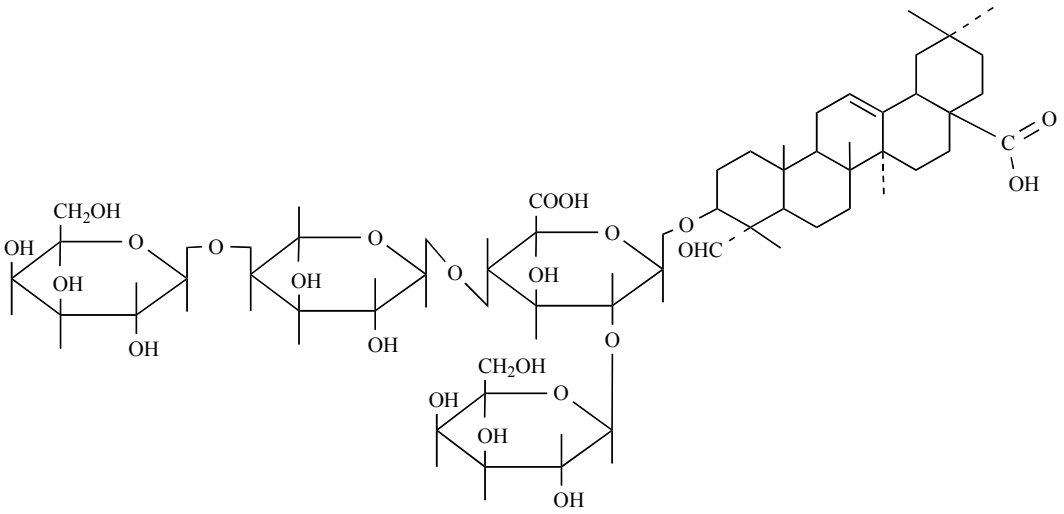

Fig. (4). Structure of gypsogenin tetraoside.

toxicity. The complex tends to accumulate in the lungs, which makes it worth studying as a drug against pulmonary carcinomas [68].

Complexes of 5-fluoruracil with pectin were synthesized in the form of microspheres by dispersion method at high stirring rates. Release of the pharmacon after administration in large intestine was shown to be pH-dependent. The novel form is suggested for study as a rectal agent for 5-fluoruracil administration [69].

Xyloglucan, a polysaccharide derived from the seeds of tamcrinda, is (1-4)-β-D-glucan with elements of (1-6)-α-D-xylose and (1-2)-β-D-galactoxylose. Thus, its molecular structure is formed of hepta-, octa- and nonasaccharide segments [70].

Due to its branching, xyloglucan macromolecule can be used as a pharmacon complexing agent. According to the authors of [71], xyloglucan gels do have a potential as drug carriers. Complexes of xyloglucan with paracetamol [72] and theophillin [73] have been developed for peroral administration. A gel-like complex of xyloglucan with pilocarpine hydrochloride has been developed for ophthalmologic practice [74]. A new transport form of thymol β-adrenaline blocker is its complex with xyloglucan [75]. The mixtures of xyloglucan and pectin form stable gels able to hold pharmacons more tightly, which was demonstrated in experiments at oral administration of complex-bonded paracetamol [76].

4.2. Complexes of Arabinogalactan

Arabinogalactans belong to polysaccharides, which are widespread in plants [77-79]. Larch arabinogalactans, the content of which in the wood reaches 15%, stand apart from these compounds. Arabinogalactan macromolecule is widely branched, Fig. (**5**). The backbone chain consists of D-galactose segments connected by β-(1-3)-glycoside bonds. Side branches connected with the backbone chain by β-(1-6) linkages are D-galactose blocks connected by β-(1-6)-bonds and L-arabinose blocks connected by β-(1-3)-bonds. In arabinogalactan commercially derived from Western larch *Larix occindentalis*, the galactose to arabinose fragments ratio increases to 7:1 from 2.33:1 with growth of molecular mass [80].

Arabinogalactan (AG) of Russian larches *Larix sibirica* and *Larix gmelinii* is characterized by a monomodal curve of molecular mass distribution with a maximum within 13-18 kDa. Practicable methods have been proposed to extract high purity arabinogalactan [81-84]. Oxidative destruction of arabinogalactan derived from Siberian larch *Larix sibirica* in water-peroxide solution under the action of molecular oxygen made it possible to synthesize the polymeric and oligomeric products enriched with carbonyl and carboxyl groups. Destruction proceeds with the breaking of glycoside bonds, cycle opening and oxidation of the anomer carbon atom to carboxyl group and detachment of formic acid molecules in a stepwise manner [85-88].

Arabinogalactan and its oxidates were used for complexation of 5-aminosalicylic acid, an antituberculous compound. The complexes were synthesized in the form of water-soluble amorphous substances. The authors believe bonding into complexes proceeds due to the acid-base interaction between the pharmacon aminogroup and carboxyl groups of oxidates. As for arabinogalactan itself, complexation occurs through pharmacon retention in the side chains by intermolecular hydrogen bonds and interaction of aminopharmacon with a small number of carboxyl groups, which are present in the natural polysaccharide. As a result, complexes have been synthesized with the content of 5-aminosalicylic acid as follows: arabinogalactan – 1.4%, polymer oxidates – 8.5 %, oligomer oxidades – 16.0%. Pharmacon complexes with arabinogalactan and oligomer oxidades show high *in vitro* activity against mycobacteria [89, 90].

In [91, 92] it was shown for the first time that the complexation effect discovered during investigations of complexes of terpenoid glycosides with pharmacons is also manifested by complexes of arabinogalactan as a reduction of the therapeutic dose of pharmacon and appearance of additional positive pharmacological properties.

Complexation of AG with indometacin, a non-steroid anti-inflammatory drug, tranquilizers sibazon (7-chlor-2,3-

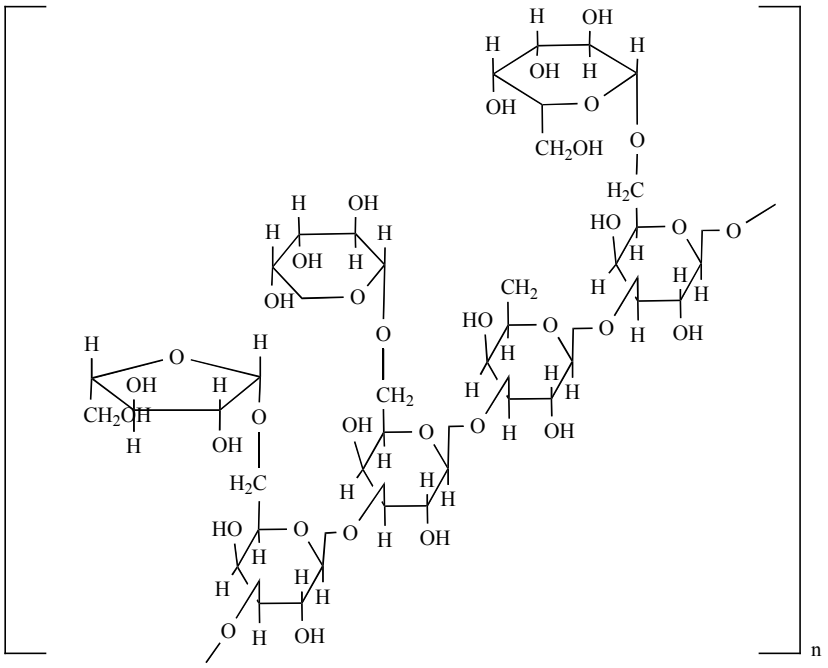

Fig. (5). Structure of arabinogalactan.

dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-2-on) and mezapam (7-chlor-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin), azaleptin (8-chlor-11(4-methyl-1-piperazinyl)5H-dibenzo-[b,e]-1,4-diazepin), a neuroleptic, was studied in the three modes of complex synthesis.

The first mode is intensive stirring of components in water solution. The second is the mechanochemical activation of a mixture of solid components by the method described in [93]. The third mode is the mechanochemical pretreatment of arabinogalactan followed by mechanochemical activation of the mixture of pharmacon with treated AG. The samples of initial AG have unimodal molecular mass distribution with average molecular mass of 13.5 kDa. AG treated mechanochemically is characterized by an intricate chromatogram with several maximums in the interval of 1.2-30 kDa. The average MM value is 5 kDa. Although the chromatographic characteristics of AG treated separately or together with pharmacon do not differ significantly, the water solubility of the pharmacon is the highest in the composition of complexes synthesized by in second mode.

It was shown that water solubility for the complexes with the AG:pharmacon having the weight ratio of 10:1 increases for indometacin, sibazon, mezapam, azaleptin by 9.9, 2.4, 19.1, 20.5 times, respectively. For complexes of AG:pharmacon 20:1 these values are as follows: indometacin – 16.8, sibazon – 3.0, mezapam – 46.8, azaleptin – 38.8. All modes of complex synthesis have to be compared in terms of solubility ratio of one of the pharmacons. For example, in complex AG:mezapam 20:1 the solubility ratios are 10.8, 46.8, and 17.5 for the first, second and third modes respectively. Therefore, mechanochemical activation of the solid component of the mixture produces optimal conditions for the synthesis. The complexes are powders consisting of vitreous particles sized 2-50 μm.

No traces typical for the crystalline phase of pharmacons can be found in the X-ray photographs and thermograms of complexes, indicating that pharmacon molecules have underwent molecular dispersion into the polysaccharide matrix, i.e. complexation has taken place. The stability of intermolecular complexes of AG with pharmacons increases in the series sibazon < indometacin < mezapam < azaleptin. Complexation proceeds mainly due to the intermolecular hydrogen bonds and Coulomb interaction at the ionization of pharmacon molecules. The investigation by the NMR-relaxation method showed that mobility of the pharmacon molecules dramatically decreases in solutions, which is evidence of complex existence [91, 94].

The study of pharmacological properties of the complexes produced the main results as follows. The basic activity of indomethacin within the complexes remains high at the doses of pharmacon reduced 10 (complex 10:1) and 20 (complex 20:1) times. Destructive mucosal involvement of the stomach halves. The basic activity of sibazone within the complexes holds at the dose reduced 10 times. Besides, the anxiolythic effect of pharmacon increases. For complexes of AG with mezapam the standard antianxious activity was observed at the pharmacon doses reduced 20 times. The complexation effect for the complex of AG with azaleptin is manifested in the halved dose and amplification of the sedative component.

Thus, the study of pharmacological properties shows that the results for AG complexes agree well with those for complexes with terpenoids (glycyrrhizic acid, stevioside, rebaudioside). In both cases complexation results in enhanced basic activity, reduced dose, appearance of new properties of pharmacons. It is suggested that this phenomenon, which is of the general nature, be called the effect of complexation (clathration) of pharmacon with carbohydrate-containing plant metabolites.

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REFERENCES

[1] Loftsson, T.; Másson, M.; Brewster, M.E. Self-association of cyclodextrins and cyclodextrin complexes. *Am. J. Drug Deliv.*, **2004**, *2*, 266-275.

[2] Challa, R.; Ahuja, A.; Ali, J.; Khar, R.K. Cyclodextrins in drug delivery: an updated review. *AAPS Pharm. SciTech*., **2005**, *6*, article 43.

[3] Loftsson, T.; Duchene, D. Cyclodextrins and their pharmaceutical applications. *Int. J. Pharm.*, **2007**, *329*, 1-11.

[4] Tolstikov, G.A.; Baltina, L.A.; Grankina, V.P.; Kondratenko, R.M.; Tolstikova, T.G. *Licorice: biodiversity, chemistry and application in medicine*, Academic Publishing Hous "GEO": Novosibirsk, **2007**.

[5] Tolstikov, G.A.; Baltina, L.A.; Schults, E.E.; Pokrovsky, A.G. Glycyrrhizin acid. *Bioorg. Chem.*, **1997**, *23*, 691-709.

[6] Baltina, L.A. Chemical modification of glycyrrhizic acid as a route to new bioactive compounds for medicine. *J. Curr. Med. Chem.,* **2003**, *10*, 155-171.

[7] James, K.C.; Stanford, J.B. The solubilising properties of liqurica. *J. Pharm. Pharmacol*., **1962**, *5*, 445-450.

[8] Gilbert, R.J.; James, K.C. Preparation and surface-active properties of glycyrrhizic acid and its salts. *J. Pharm. Pharmacol*., **1964**, *16*, 394-399.

[9] Azaz, E.; Segal, R. Glycyrrizin as gelling agent. *Pharm. Acta Helv.*, **1980**, *55*, 183-186.

[10] Kondo, M.; Minamino, H.; Okiyama, G. Physicochemical properties and applications of α- and β-glycyrrhizins, natural surface active agents in licorice root extract. *J. Soc. Cosmet. Chem.*, **1986**, *37*, 177-189.

[11] Maskan, M. Rheological behavior of liqorice (Glycyrrhiza glabra) extract. *J. Food Process Eng.*, **1999**, *39*, 389-393.

[12] Romanenko, T.V.; Murinov, Yu.I. Some features of diluted glycyrrhizic acid solutions flux. *J. Phys. Chem.*, **2001**, *75*, 1601-1604.

[13] Kolyadina, O.A.; Murinov, Iu.I. Partial molal volumes of glycyrrhizic acid in ethanol and dimethyl formamide. *J. Phys. Chem.*, **1997**, *71*, 460-463.

[14] Kornievskaya, V.S.; Kruppa, A.I.; Polyakov, N.E.; Lyoshina, T.V. Effect of glycyrrhizic acid on lappaconitine phototransformation. *J. Phys. Chem.*, **2007**, *111*, 11447-11452.

[15] Kornievskaya, V.S.; Kruppa, A.I.; Polyakov, N.E.; Lyoshina, T.V. Research of influence near areas on the reaction possibilities of radicals of model organic compound of methyl ether N-acil-antranil acid by methods H NMR and CPN. *Vestn. NGU. Ser.: Phys.,* **2007**, *2*, 21-29.

[16] Soltesz, G.; Uri, G. Solubilisierende Wirkung des Monoammonium glycyrrhizinats auf Antibiotika und einige andere Stoffe. *Naturwissenschaften*, **1963**, *50*, 691.

[17] Krasova, T.G.; Bashura, G.S.; Murav'ev, I.A. Hydrocortisone and prednisolone solubilization in aqueous solutions of glyciram. *Pharmacy*, **1978**, *27*, 32-35.

[18] Sasaki, Y.; Mizutani, K.; Kasai, R.; Tanaka, O. Solubilizing properties of glycyrrhizin and its derivatives solubilization of saikosaponin-a the saponin of bupleuri radix. *Chem. Pharm. Bull*, **1988**, *36*, 3491.

[19] Tolstikov, G.A.; Murinov, Y.I.; Baltina, L.A. Complexes of β-glycyrrhizic acid with drugs as a novel transport forms. *Pharm. Chem. J.,* **1990**, *24*, 26-27.

[20] Dushkin, A.V.; Karnatovskaya, L.M.; Chabueva, E.N.; Boldyrev, V.V.; Sorokina, I.V.; Tolstikova, T.G.; Dolgikh, M.P.; Shults, E.E. Production and analysis of ulcerogenic action of quick-dissolving solid dispersed systems based on acetylsalicylic acid and biologically active licorice derivatives. *Pharm. Chem. J.*, **2001**, *11*, 18-21.

[21] Bondarev, A.I.; Bashkatov, S.A.; Davydova, V.A.; Zarudiy, F.S.; Lazareva, D.N.; Tolstikova, T.G.; Baltina, L.A.; Tolstikov, G.A.; Murinov, Y.I.; Rusakov, I.A. Antiinflammatory and analgesic action of complexes of antiflogistics with glycyrrhizic acid. *Pharmacol. Toxicol.*, **1991**, *5*, 47-50.

[22] Baltina, L.A.; Davydova, V.A.; Tolstikova, T.G.; Sharipova, F.V.; Murinov, Yu.I.; Zarudiy, F.S.; Lazareva, D.N.; Tolstikov, G.A.; Bondarev, A.I. AC 1566700 USSR, **1991**.

[23] Baltina, L.A.; Sharipova, F.V.; Davydova, V.A.; Tolstikova, T.G.; Zarudiy, F.S.; Lazareva, D.N.; Tolstikov, G.A.; Bondarev, A.I. AC 1566696 USSR, **1991**.

[24] Baltina, L.A.; Davydova, V.A.; Tolstikova, T.G.; Murinov, Yu.I.; Lazareva, D.N.; Tolstikov, G.A.; Bondarev, A.I. AC 1566699 USSR, **1992**.

[25] Tolstikov, G.A.; Baltina, L.A.; Murinov, Yu.I.; Davydova, V.A.; Tolstikova, T.G.; Bondarev, A.I.; Zarudiy, F.S.; Lasareva, D.N. Complexes of with non-steroid anti-inflammatory drugs as new transport forms. *Pharm. Chem. J.*, **1991**, *2*, 29-32.

[26] Tolstikov GA, Tolstikov, G.A.; Murinov, Yu.I.; Baltina, L.A.; Saitova, M.Yu.; Zarudiy, F.S.; Davydova, V.A.; Lasareva, D.N. Complexes of β-glycyrrhizic acid with prostaglandins – new class of uterotonic agents. *Pharm. Chem. J.*, **1991**, *3*, 42-44.

[27] Tolstikov, G.A.; Murinov, Y.I.; Baltina, L.A.; Saitova, M.Yu.; Zarudiy, F.S.; Miftakhov, M.S.; Vostrikov, N.S.; Lazareva, D.N.; Cheremisinov, G.A. *Collected works "The study and use of licorice in the USSR"*, Galym: Alma-Ata, **1991**.

[28] Baltina, L.A.; Saitova, M.Yu.; Murinov, Yu.I.; Adler, M.E.; Miftakhov, M.S.; Lazareva, D.N.; Tolstikov, G.A. Inventors certificate of USSR No 1566697, **1988**.

[29] Tolstikov, A.G.; Tolstikov, G.A.; Murinov, Yu.I.; Karpyshev, N.N.; Saitova, M.Yu.; Karamyshev, V.A. RF Patent No 2123336, 1997.

[30] Kornievskaya, V.S.; Kruppa, A.I.; Leshina, T.V. Effect of glycyrrhizic acid on lappaconitine phototransformation. *J. Phys. Chem. B.*, **2007**, *111*, 11447-52.

[31] Kornievskaya VS, Kruppa AI, Polyakov NE, Leshina TV NMR and photo-CIDNP investigations of the glycyrrhizinic acid micelles influence on solubilized molecules. *J. Inclusion Phenom. Macrocycl. Chem.*, **2007**, *60*, 123-130.

[32] Polyakov, N.E.; Khan, V.K.; Taraban, M.B.; Leshina, T.V.; Salakhutdinov, N.F.; Tolstikov, G.A. Complexation of lappaconitine with glycyrrhizic acid: stability and reactivity studies. *J. Phys. Chem. B.,* **2005**, *109*, 24526-24530.

[33] Sadritdinov, F.S.; Kurmukov, A.G. *Pharmacology of Plant Alkaloids and their Application in Medicine*, Medicine of UzSSR: Tashkent, **1980**.

[34] Mashkovsky, M.D. *Medicinal Agents*, Novaya Volna: Moscow, **2005**.

[35] Bryzgalov, A.O.; Tolstikova, T.G; Sorokina, I.V.; Zhukova, N.A.; Volkova, E.B.; Dolgikh, M.P. Antiarrhythmic activity of alaglizin. *J. Expert Clin. Pharm.*, **2005**, *68*, 4, 24-28.

[36] Yunusov, M.S.; Tolstikov, G.A.; Murinov, Yu.I.; Tsyrlina, E.M.; Tolstikova, T.G.; Sorokina, I.V.; Voevoda, T.V.; Yunusova, S.G.; Dokichev, V.A.; Kaverina, N.V.; Turilova, A.I. RF Patent No 2180583, 2002.

[37] Pogosova, G.V. Nifedipine in treatment of cardiovascular system deseases: new about well known. *Clin. Pharm. Ther.*, **2006**, *3*, 2-6.

[38] Polyakov, N.E.; Leshina, T.V.; Salakhutdinov, N.F.; Kispert, L.D. Host-guest complexes of carotenoids with beta-glycyrrhizic acid. *J. Phys. Chem.,* **2006**, *110*, 6991-6998.

[39] Tolstikova, T.G.; Sorokina, I.V.; Bryzgalov, A.O.; Dolgikh, M.P.; Lifshits, G.I.; Khvostov, M.V. The usage of new approach of known drugs complexation with plant glycosides in prophylaxis and relief of acute arterial hypertension (experimental study). *J. Rational Pharmacother. Cardiol.*, **2006**, *1*, 55-58.

[40] Tolstikov, A.G.; Tolstikova, T.G.; Sorokina, I.V.; Dolgikh, M.P.; Tolstikov, G.A.; Fedoseeva, L.A. RF Patent No 2232574BI, **2004**.

[41] Tolstikova, T.G.; Sorokina, I.V.; Kovalenko, I.L.; Tolstikov, A.G. Influence of clathrate formation to the pharmacon activity in com-

plexes with glycyrrhizic acid. *Doklady Akad. Nauk*, **2004**, *394*, 707-709.

[42] Avgustinovich, D.F.; Kovalenko, I.L.; Sorokina, I.V.; Tolstikova, T.G.; Tolstikov, A.G. Ethological study of antidepressant effect of fluaglizin in conditions of chronic social stress in mice. *Bul. Exp. Biol. Med.*, **2004**, *137*, 99-103.

[43] Kovalenko, I.L.; Avgustinovich, D.F.; Sorokina, I.V.; Tolstikova, T.G.; Tolstikov, A.G. Influence of chronic introduction of fluoglizin on a biochemical blood values in mice in chronic social stress conditions. *Issues Med. Biol. Pharm. Chem.*, **2004**, *11*, 44-47.

[44] Shishkina, G.T.; Dygalo, N.N.; Yudina, A.M.; Kalinina, T.S.; Tolstikova, T.G.; Sorokina, I.V.; Kovalenko, I.L.; Anikina, L.V. Effects of Fluoxetine and Its Complexes with Glycyrrizhinic Acid on Behavior and Brain Monoamine Levels in Rats*. I. P. Pavlov J. High. Ner. Act.*, **2005**, *55*, 207-212.

[45] Shishkina, G.T.; Dygalo, N.N.; Yudina, A.M.; Kalinina, T.S.; Tolstikova, T.G.; Sorokina, I.V.; Kovalenko, I.L.; Anikina, L.V. The effects of fluoxetine and its complexes with glycerrhizic acid on behavior in rats and brain monoamine levels. *Neurosci. Behav. Physiol.*, **2006**, *36*, 329-333.

[46] Baltina, L.A.; Murinov, Yu.I.; Ismagilova, A.F.; Davydova, V.A.; Zarudiy, F.S.; Tolstikov, G.A. Production and antineoplastic action of complex compounds of β-glycerrhizic acid with some antineoplastic drugs. *Pharm. Chem. J.*, **2003**, *37*, 3-4.

[47] Kondratenko, R.M.; Baltina, L.A.; Mustafina, S.R.; Ismagilova, A.F.; Zarudiy, F.S.; Davydova, V.A.; Bazekin, G.V.; Suleimanova, G.F.; Tolstikov, G.A. Complex compounds of glycerrhizic acid with antimicrobial drugs. *Pharm. Chem. J.*, **2003**, *37*, 32-35.

[48] Vavilin, V.A.; Salakhutdinov, N.F.; Ragino, Yu.I.; Polyakov, N.E.; Taraban, M.B.; Leshina, T.V.; Stakhneeva, E.M.; Lyakhovich, V.V.; Nikitin, Yu.P.; Tolstikov, G.A. Hypocholesteremic properties of complex compound of simvastatin with glycerrhizic acid (simvaglizin) in experimental models. *Biomed. Chem.*, **2008**, *54*, 301-313.

[49] Tolstikov, G.A.; Nikitin, Yu.P.; Lyakhovich, V.V.; Salakhutdinov, N.F.; Ragino, Yu.I.; Vavilin, V.A. Drug with hypocholesterolemic effect *Symvaglyzin*. RF Patent RU2308947 C1, 2007.

[50] Ragino, Yu.I.; Vavilin, V.A.; Salakhutdinov, N.F.; Makarova, S.I.; Stakhneva, E.M.; Safronova, O.G.; Nikitin, Yu.P.; Tolstikov, G.A. Examination of the hypocholesteremic effect and safety of simvaglizin on the model of hypercholesterolemia in rabbits. *Bull. Exp. Biol. Med.*, **2008**, *145*, 285-287.

[51] Tolstikov, G.A.; Myshkin, V.A.; Baltina, L.A.; Murinov, Yu.I.; Srubilin, D.V.; Vakaritsa, A.F.; Alyokhin, E.K. Antidotal and antiradical activity of complexes of β-glycyrrhizic acid with pyrimidine derivatives. *Pharm. Chem. J.*, **1996**, *30*, 320-322.

[52] Handro, W.; Ferreira, C.M. *Stevia rebaudiana Bertoni: Production of Natural Sweeteners: Biotechnology in Agriculture and Forestry*,*V7, Medicinal and Aromatic Plants,* Springer-Verlag: Berlin-Heidelbery, **1989**.

[53] Dyrskog, S.E.U.; Jeppensen, P.B.; Chen, J.; Christens, L.P.; Hermansen, K. The diterpene glycoside, rebaudioside A, does not improve glycemic control or affect blood pressure after eight weeks treatment in the Goto-Kakizaki rat. *Rev. Diabetes Stud.*, **2005**, 2, 84-91.

[54] Koyama, E.; Kitazawa, K.; Izawa, O.; Kakegawa, K.; Fujino, A.; Jui, M. *In vitro* metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. *Food Chem. Technol.*, **2003**, *41*, 359-374.

[55] Tolstikova, T.G.; Bryzgalov, A.O.; Sorokina, I.V.; Morozova, E.A.; Tolstikov, S.E.; Al'fonsov, A.V.; Tolstikov, A.G. Stevioside is a novel stimulator of pharmacon clathration with glycosides. *Doklady Akademii Nauk*, **2005**, *403*, 274-276.

[56] Tolstikova, T.G.; Tolstikov, A.G.; Tolstikov, G.A. On the way to low-dose drugs. *Bull. RAS*, **2007**, *77*, 867-874.

[57] Putieva, Zh.M.; Mzhelskaya, L.G.; Gorovits, T.T.; Kondratenko, E.S.; Abubakirov, N.K. Structure of *Acanthophyllum gypsophyloides* glycosides. *Chem. Nat. Comp.*, **1975**, *6*, 728.

[58] Tolstikov, G.A.; Murinov, Yu.I. AC USSR No 1679772 , **1991**.

[59] Tolstikov, G.A.; Murinov, Yu.I. AC USSR No 1685094, **1991**.

[60] Tolstikov, G.A.; Murinov, Yu.I. AC USSR No 17902642, 23101989, **1993**.

[61] Thakur, B. R.; Singh, R.K.; Handa, A.K. Chemistry and uses of pectin – a review. *Crit. Rev. Food Sci. Nutr.,* **1997**, *37*, 47-73.

[62] Kubo, W.; Konno, Y.; Miyazaki, S.; Attwood, D. *In situ* gelling pectin formulations for oral sustained delivery of paracetamol. *Drug Dev. Ind. Pharm.,* **2004**, *30*, 593-599.

[63] Miyazaki, S.; Kubo, W.; Itoh, K.; Konno, Y.; Fujiwara, M.; Dairaku, M.; Togashi, M.; Mikami, R.; Attwood, D. The effect of taste masking agents on *in situ* gelling pectin formulations for oral sustained delivery of paracetamol and ambroxol. *Int. J. Pharm.*, **2005**, *297*, 38-49.

[64] Itoh, K.; Kubo, W.; Fujiwara, M.; Hirayama, T.; Miyazaki, S.; Dairaku, M.; Togashi, M.; Mikami, R.; Attwood, M.; Watanabe, H.; Miyazaki, S.; Attwood, D. The influence of variation of gastric pH on the gelation and release characteristics of *in situ* gelling pectin formulations. *Biol. Pharm. Bull.,* **2006**, *29*, 343-347.

[65] Kubo, W.; Itoh, K.; Miyazaki, S.; Attwood, D. Oral Sustained Delivery of Theophylline and Cimetidine from *In Situ* Gelling Pectin Formulations in Rabbits. *Drug Dev. Ind. Pharm.*, **2005**, *31*, 819-825.

[66] Kubo, W.; Miyazaki, S.; Dairaku, M.; Togashi, M.; Mikami, R.; Attwood, D. Oral sustained delivery of ambroxol from *in situ*-gelling pectin formulations. *Int. J. Pharm.*, **2004**, *271*, 233-240.

[67] Ahrabi, S.F.; Madsen, G.; Dyrstad, K.; Sande, S.A., Graffner, Ch. Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. *Eur. J. Pharmac. Sci.*, **2000**, *10*, 43-52.

[68] Verma A.K.; Sachin, K. Novel Hydrophilic Drug Polymer Nano-Conjugates of Cisplatin Showing Long Blood Retention Profile - Its Release Kinetics, Cellular Uptake and Bio-Distribution. *Curr. Drug Deliv.*, **2008**, 5, 120-126.

[69] Paharia, A.; Yadav, A.K.; Rai, G.; Yain, S.K.; Pancholi, S.S.; Agrawal, G.P. Eudragit-coated Pectin Microspheres of 5-Fluorouracil for Colon Targeting. *AAPS Pharm. Sci. Tech.*, **2007**, *8*, 12.

[70] York, W.S.; van Halbeek, H.; Darwill, A.G.; Albersheim, P. Structural analysis of xyloglucan oligosaccharides by 1H-n.m.r. spectroscopy and fastatom-bombardment mass spectrometry. *Carbohydr. Res.,* **1990**, *200*, 9-31.

[71] Kawasaki, N.; Ohkura, R.; Miyazaki, S.; Uno, Y.; Sugimoto, S.; Attwood, D. Thermally reversible xyloglucan gels as vehicles for oral drug delivery. *Int. J. Pharm.*, **1999**, *181*, 227-234.

[72] Miyazaki, S.; Endo, K.; Kawasaki, N.; Kubo, W.; Watanabe, H.; Attwood, D. Oral sustained delivery of paracetamol from *in situ* gelling xyloglucan formulations. *Drug Dev. Ind. Pharm.*, **2003**, *29*, 113-119.

[73] Miyazaki, S.; Kawasaki, N.; Endo, K.; Attwood, D. Oral sustained delivery of theophylline from thermally reversible xyloglucan gels in rabbits. *J. Pharm. Pharmacol.*, **2001**, *53*, 1185-1191.

[74] Miyazaki, S.; Suzuki, S.; Kawasaki, N.; Endo, K.; Takahashi, A.; Attwood, D. *In situ* gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. *Int. J. Pharmacol.*, **2001**, *229*, 29-36.

[75] Burgalassi, S.; Chetoni, P.; Panichi, L.; Boldrini, E.; Saettone, M.F. Xyloglucan as a novel vehicle for timolol: pharmacokinetics and pressure lowering activity in rabbits. *J. Ocul. Pharmacol. Ther.*, **2000**, *16*, 497-509.

[76] Itoh, K.; Yahaba, M.; Takahashi, A.; Tsuruga, R.; Miyazaki, S.; Dairaku, M.; Togashi, M.; Mikami, R.; Attwood, D. *In situ* gelling xyloglucan/pectin formulations for oral sustained drug delivery. *Int. J. Pharmacol.*, **2008**, *356*, 95-101.

[77] Ovodov, Yu.S. Polysacharides of flower plants: structure and physiological. *Bioorg. Chem.*, **1998**, *24*, 483-501.

[78] Clarke, A.E.; Anderson, R.I.; Stone, B.A. Form and function of arabinogalactans and rabinogalactan-proteins. *Phytochemistry*, **1979**, *18*, 521-540.

[79] Arifkhodzhaev, A.O. Galactans and galactan containing polysaccharides of higher plants. *Chem. Nat. Comp.*, **2000**, *3*, 89-96.

[80] Prescott, J.H.; Graman, E.V. New molecular weight forms of arabinogalactan from Larix occidentalis. *Carbohydr. Res.*, **1997**, *301*, 89-93.

[81] Tsvetaeva, I.P.; Paskerova, E.D. Change in structure of arabinogalactan from Larix sibirica subject to its extraction regime from wood. *J. Appl. Chem.*, **1962**, *35*, 1128-1131.

[82] Medvedeva, E.N.; Babkin, V.A.; Ostroukhova, L.A. Arabinogalactan from larch – properties and usage perspectives (review). *Chem. Plant Stock*, **2003**, *1*, 27-37.

[83] Antonova, G.F.; Tyukavkina, N.A. Synthesis of high-clean arabinogalactan from larch wood. *Wood Chem.*, **1976**, *4*, 60.

[84] Medvedeva, E.N.; Babkin, V.A.; Makarenko, O.A.; Nikolaev, S.M.; Khobrakova, V.B.; Shulunova, A.M.; Fyodorova, T.E.; Eskova, L.A. The production of high-clean arabinogalactan from larch and examination of its immunomodulatory property. *Chem. Plant Stock*, **2004**, *4*, 17-24.

[85] Borisov, I.M.; Shirokova, E.N.; Babkin, V.A.; Tolstikov, G.A.; Monakov, Yu.B. About mechanism of arabinogalactan peroxidation in aquatic environment . *Izvestiya RAS*, **2002**, *383*, 774-776.

[86] Borisov, N.M.; Shirokova, E.N.; Mudarisova, R.H.; Muslukhov, R.R.; Zimin, Yu.S.; Medvedeva, S.A.; Tolstikov, G.A.; Monakov, Yu.B. Peroxide oxidation of arabinogalactan: Kinetics and Products. *Izvestiya RAS*, **2004**, *2*, 305-311.

[87] Monakov, Yu.B.; Borisov, I.M.; Zimin, Yu.S.; Mudarisova, R.Kh.; Tolstikova, T.G.; Shirokova, E.N.; Medvedeva, S.A.; Zaikov, G.E. *In: Peroxides at the Beginning of the Third Millenium*, Nova Science Publishers: New York, **2004**.

[88] Mudarisova, R.H.; Badykova, L.A.; Tolstikova, T.G.; Alexandrova, G.P.; Borisov, N.M.; Monakov, Yu.B. Modification of arabinogalactan and its oxidized forms by 5-aminosalicilic acid. *J. Appl. Chem.*, **2005**, *78*, 1721-1724.

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[89] Badykova, L.A.; Mudarisova, R.H.; Khamidullina, G.S.; Aminev, H.K.; Borisov, N.M.; Monakov, Yu.B. Modification of arabinogalactan and its oxidized forms by n-aminosalicilic acid. *J. Appl. Chem*., **2006**, *79*, 1647-1649.

[90] Badykova, L.A.; Mudarisova, R.H.; Tolstikova, T.G.; Borisov, I.M.; Monakov, Yu.B. Modification of poly – and oligosaccharides based on arabinogalactan by 5-aminosalicilic acid. *Chem. Nat. Comp.*, **2005**, *3*, 219-222.

[91] Dushkin, A.V.; Metelyova, E.S.; Tolstikova, T.G.; Tolstikov, G.A.; Polyakov, N.E.; Neverov, N.A.; Medvedeva, E.N.; Babkin, V.A. Mechanochemical production and pharmacological action of water soluble complexes of arabinogalactan and drugs. *Izvestiya RAS*, **2008**, *6*, 1274-1282.

[92] Dushkin, A.V.; Tolstikova, T.G.; Tolstikov, G.A.; Metelyova, E.S. Water-soluble compositions and method of its production. RF Patent RU 2006143081A, 2008.

[93] Shakhtshneider, T.P.; Boldyrev, V.V. *Reactivity of Molecular Solids*, John Wiley and Sons: England, **1999**.

[94] Fielding, L. Determination of association constants (Ka) from solution NMR data. *Tetrahedron*, **2000**, *56*, 6151-6166.