Water-Soluble Substances of Arglabin

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Abstract

The article discusses the results of a study of the water solubility of natural sesquiterpene lactone arglabin, in particular, its ability to complex formation with complexing agents polyvinylpyrrolidone, the disodium salt of glycyrrhizic acid, magnesium carbonate. Mechanocomposites with polyvinylpyrrolidone and disodium salt of glycyrrhizic acid, which have increased water solubility, were obtained by the method of mechanochemical treatment of arglabin native. At the same time, the best result of dissolution in water is achieved by a two-hour treatment with polyvinylpyrrolidone and with disodium salt of glycyrrhizic acid. The water solubility of complex compounds of arglabin with polyvinylpyrrolidone increases by 4.61 times, and with disodium salt of glycyrrhizic acid by 4.42 times.

Keywords: Arglabin; Water solubility; Polyvinylpyrrolidone; Mechanocomposite.

1. Introduction

In recent years, when searching for new medicinal substances, the attention of researchers has been increasingly attracted to natural sesquiterpene lactones – an extensive group of terpenoid compounds with a wide range of biological activity: antitumor, anti-inflammatory, antiparasitic, antispastic, hypoglycemic, cardiotonic, among which important representatives are alantolactone, thapsigargin, artemisinin, arglabin, parthenolide [1]. The main difficulty in the introduction of drugs based on sesquiterpene lactones in practical medicine, clinical pharmacology is their practical insolubility in water, as hydrophobic compounds.

One of the practically valuable sesquiterpene lactones of plant origin is arglabin 1(10)β-epoxy-5,7α,6β(H)-guaia-3(4),11(13)-diene-12,6-olide] (1), which in its structure, in addition to the γ-lactone ring, has an epoxy group and an olefinic double bond in the cycle, which is a colorless crystalline substance of the composition $\text{C}_{15}\text{H}_{18}\text{O}_3$ with a melting point of 101–104° (hexane), $\alpha/\beta +45^0$ (c 0,3; chloroform). Natural arglabin (ARG) is insoluble in water [2].

The presence in the structure of arglabin (1) of an exomethylene group (C11-C13), conjugated to the carbonyl of the γ-lactone, an epoxy cycle (C1-C10), as well as an olefinic double bond C3=C4, makes it promising for regio- and stereoselective chemical modification.

According to the results of quantum-chemical non-empirical calculations by the Hartree-Fock method in a 6-31G split-valence basis, the electron density in the arglabin molecule 1 is distributed in accordance with the partial atomic charges given below. A significant dipole moment of the molecule (6.51D) indicates its high reactivity (Fig. 1).

Figure 1 shows three-dimensional models of the arglabin molecule according to the data of quantum-chemical and X-ray structural research methods. According to these calculations, the C-13 atom (0.75 ‒ the B3LYP/6-31G and B3LYP/6-31G (d) method) has the highest frontier density of the lowest free orbital of arglabin (1), when C13 atoms have the highest frontier density of the highest occupied orbital (0.45 ‒ B3LYP/6-31G)/(0.44 ‒ B3LYP (6-31G (d)) and C-4 (0.38 ‒ B3LYP/6-31G)/(0.37 ‒ B3LYP/6-31G (d)) atoms have the highest frontier density of the highest occupied orbital. Hence, nucleophilic reactions predominantly occur along the C11-C13 exomethylene bond, and electrophilic reactions predominantly occur along the C3-C4 olefinic double bond [3].

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The main disadvantage of arglabin, as well as other natural sesquiterpenic lactones, is its poor solubility in water, which negatively affects its bioavailability and reduces the specific pharmacological activity in the organism. Therefore, modification of the natural arglabin molecule by transition it into water-soluble forms is considered practically important. With the chemical modification of the substance molecules, an increase in toxicity is possible, which reduces the practical value of the original medicinal substances. In terms of solving the issue of water solubility, a method for producing dimethylaminoarglabin hydrochloride is proposed, on the basis of which the drug “Arglabin” is developed, which is used in the treatment of tumors of the breast, lungs, liver, etc. [4]. The water-soluble form of the substance of dimethylaminoarglabin hydrochloride is obtained by amination and bubbling with gaseous hydrogen chloride, thereat changing the structure of the initial arglabin and affecting the optically active centers of the molecule, which can reduce the pharmacological activity.

To preserve the pharmacological action and the initial structure of natural arglabin, in our opinion, a mechanochemical method for producing complexes with water-soluble complex-forming agents is considered one of the rational ways to produce a water-soluble form. In this case, due to the Van der Waals forces, complexes with a pharmacologically active substrate are formed, which allows the subsequent and purposeful transporting of the arglabin molecule to the organism. The molecule of the pharmacologically active sesquiterpene lactone, while maintaining the original structure of the molecule, actively interacts with enzymes.

The mechanochemical treatment [5–14] is a solid-phase process and, in comparison with liquid-phase methods, it has several advantages, namely: the possibility of carrying out the process in one technological stage without the use of solvents and melts, without the use of high temperatures, without drying the materials, and also the absence of restrictions on the joint solubility of the components. To ensure water solubility and increase the effectiveness of drugs, modification by polymers is proposed. At the same time, natural polymers have significant advantages over synthetic ones. Polyvinylpyrrolidone is widely used as a carrier of polymers for the production of solid dispersions [15].

In continuation of the earlier [16] experiments on the production of a complex of arglabin with arabinogalactan, the structure and properties of complexes of sesquiterpene lactone arglabin with the disodium salt of glycyrrhizic acid, polyvinylpyrrolidone and magnesium carbonate have been synthesized and studied.

2. Materials and methods

As complex forming the following agents were used:

- Disodium salt of glycyrrhizic acid (2) (Na₂GA) – derivative of plant saponin, (CFS, 98%) production of Shaanxi Sciphar Biotechnology Co., Ltd (Xi’an, China). Gross formula C₄₂H₆₀O₁₆Na₂. Represents a gray powder with a mustard tint. Does not melt. Sublimates at temperature ~400 °C. Easily soluble in water.

- Polyvinylpyrrolidone (3) (PVP) – synthetic polymer, production of Huangshan Bonsun Pharmaceuticals Co., Ltd. (Huangshan, China). General formula (C₆H₉NO)ₙ. Represents as a white, yellowish-white odorless powder. Has a sweet taste.
Melting points = 150 °C. Freely soluble in water, ethanol and methanol.

- The substance of basic magnesium carbonate (MgCO$_3$ basic) (4) of pharmacopeial purity (Manufacturer’s pharmacopoeial monograph 42-3989-08).

Optimal mass ratios of components: ARG/Na$_2$GA – 1:10, ARG/MgCO$_3$ basic – 1:5 and ARG/PVP – 1:10.

The solubility of the obtained composites was determined according to the State Pharmacopoeia of the Republic of Kazakhstan [17], the concentration of the native arglabin mechanocomposite with complex forming agents was 1 g/l, the dissolution medium – purified water, temperature 37 °C. The test was carried out on a solubility tester DT 820 (Germany), the results are presented in Table.

| Substance          | Mixing time, min | The time of mechanochemical treatment, h | Sample injection volume, μl | Peak time, min | Peak area    |
|--------------------|------------------|----------------------------------------|-----------------------------|----------------|--------------|
| ARG                | 30               | 15.146                                 | 16221.10                    | 15.09          | 82149.39     |
|                    | 60               | 15.129                                 | 18829.70                    | 15.07          | 81333.39     |
|                    | 90               | 15.071                                 | 19820.04                    | 15.06          | 84961.53     |
|                    | 120              | 15.056                                 | 20560.10                    | 15.05          | 87649.11     |
|                    | 150              | 15.027                                 | 21147.10                    | 15.04          | 88067.45     |
|                    | 180              | 15.048                                 | 20900.60                    | 15.04          | 87039.39     |
| ARG:MgCO$_3$ = 1:5| 150              | 15.349                                 | 39718.80                    | 15.32          | 17464.56     |
|                    | 2                | 15.32                                  | 34776.86                    | 15.30          | 16120.04     |
|                    | 3                | 15.32                                  | 30761.18                    | 15.31          | 16221.10     |
|                    | 4                | 15.31                                  | 22087.96                    | 15.30          | 16221.10     |
|                    | 6                | 15.30                                  | 17464.56                    | 15.30          | 16221.10     |
| ARG: PVP = 1:10    | 150              | 15.17                                  | 64876.94                    | 15.06          | 82149.39     |
|                    | 1                | 15.04                                  | 92320.41                    | 15.05          | 84811.53     |
|                    | 2                | 15.03                                  | 87469.11                    | 15.05          | 84811.53     |
|                    | 3                | 15.03                                  | 81333.39                    | 15.05          | 84811.53     |
|                    | 4                | 15.05                                  | 80674.85                    | 15.05          | 84811.53     |
|                    | 5                | 15.01                                  | 8105.51                     | 15.01          | 8105.51      |
|                    | 6                | 15.06                                  | 87039.39                    | 15.06          | 87039.39     |
| ARG:Na$_2$GA = 1:10| 150             | 15.086                                 | 92013.13                    | 15.09          | 97495.52     |
|                    | 1                | 15.086                                 | 92013.13                    | 15.09          | 97495.52     |
|                    | 2                | 15.064                                 | 83118.4                     | 15.06          | 83118.4      |
|                    | 3                | 15.064                                 | 84811.53                    | 15.06          | 84811.53     |
|                    | 4                | 15.064                                 | 84811.53                    | 15.06          | 84811.53     |
|                    | 5                | 15.062                                 | 87039.39                    | 15.06          | 87039.39     |
|                    | 6                | 15.062                                 | 87039.39                    | 15.06          | 87039.39     |
High-performance liquid chromatography (HPLC) analysis was performed on a chromatograph Agilent 1200 with a Zorbax SB-C18 column, 150*4.6 mm. Column temperature +30 °C. Detector – diode array. The acetonitrile: water system was used as an eluent: a ratio of 50:50, a flow rate of 0.5 ml/min, and detection at a wavelength of 204 nm. The difference in solubility is determined by the difference in the peak area.

Electron micrographs of the samples were obtained using an Olympus CX41 electron microscope (Tokyo, Japan).

The cytotoxicity of mechanocomposites samples was assessed in the test of the survival of the larvae of the crustaceans Artemia salina (Leach). The experiments were carried out on 2-day-old larvae under in vitro cultivation conditions. The larvae were bred by immersing the eggs of the crustaceans Artemia salina (Leach) in artificial seawater and incubated for 48 h at 37 °C.

The test was carried out using ready-made samples at a concentration of 100 μg/ml, 10 μg/ml and 1 μg/ml, as well as negative controls, where dimethyl sulfoxide and water in 3 parallel experiments were used. Using a Pasteur pipette, 10 larvae of 2-day-old crustaceans of Artemia salina (Leach) were planted in each bottle with samples. After that, all the bottles were left at room temperature in the light for 24 h.

After 24 h, the survived and dead larvae were counted. Then, using the obtained data on the upper and lower toxic limits, the half toxic dose of each sample was calculated.

3. Results and discussions

3.1. Obtaining of arglabin mechanocomposites

Obtaining of solid dispersions of arglabin was carried out in a ball mill LBM-1 with a drum, which has a fluoroplastic lining coating. Treatment mode: acceleration of grinding bodies – 1 g, total loading of components of the treated mixture from 18 to 22 g, drum volume – 300 ml, grinding bodies – steel balls (steel grade 100Cr6, diameter 22 mm, loading 675 g). Treatment time ranged from 1 to 6 h. Regardless of the volume of the drum, the degree of filling with grinding bodies should be approximately 40%, and the degree of filling with the treated material is 10–40%.

As can be seen from the diagram (Fig. 2), the best result of the dissolution of 0.33±0.13 min is a five-hour mechanochemical treatment of arglabin with disodium salt of glycyrrhizic acid in a ratio of 1:10. Arglabin native and its mechanocomposite with magnesium carbonate are practically insoluble in water.

Microphotographs presented in Fig. 3 characterize the surface morphology of the obtained samples. Pure arglabin consists of crystalline particles and their agglomerates. Disodium salt of glycyrrhizic acid consists of spherical hollow particles with a smooth surface. After mechanochemical treatment, the initial particle shape of the starting components has changed and it is impossible to isolate individual components except for the formed agglomerates. As can be seen in Fig. 3, the obtained substances represent as polydisperse powders with particles 5–20 μm in size and their aggregates.

To determine the solubility of arglabin, a study was made of the dissolution kinetics of the initial molecule over time.

A sample of a weight 1 g was mixed in 500 ml of distilled water in a rotating basket with blades at a temperature of 37 °C, for 150 min, at a rotation speed of 200 rpm. After that, samples were taken and filtered through a paper filter. Arglabin concentration in solution was determined by HPLC. The results are presented in Table.

The results presented in Table show that, after a 2-hour mechanochemical treatment, the water solubility of arglabin in complex with disodium salt of glycyrrhizic acid has increased by 4.61 times, in complex with polyvinylpyrrolidone by 4.42 times, and with magnesium carbonate by 1.66 times.
Earlier, we [13] have carried out experiments on the mechanochemical treatment of arglabin with a water-soluble polysaccharide of arabinogalactan from Larix. According to the diffraction pattern of arglabin mechanocomposites with arabinogalactan, where destruction of the crystal lattice of arglabin is observed, molecular dispersion does not occur in the polysaccharide matrix and, accordingly, its solubility does not increase.

When magnesium carbonate (MgCO\(_3\)) is used as a complex forming agent, a slightly alkaline medium is created that increases the water solubility of the arglabin mechanocomposite with magnesium carbonate (5).

The electronegative center in the arglabin molecule, in this case the oxygen atom of the epoxy group, pulls the electron density from the protons of the C-14 methyl group, which become donors of the lone electron pair of the oxygen atom of magnesium carbonate. Another electronegative center is the olefinic double bond at C3-C4, where the protons of the methyl group at C4 also become a donor. A similar mechanism of the formation of coordination bonds is also observed through the protons of the exomethylene group of the γ-lactone.

Structural features of the complex forming agents of the disodium salt of glycyrrhizic acid and polyvinylpyrrolidone make it possible to form complexes with arglabin (6) and (7), whose molecules form hydrogen bonds in the intermolecular space formed by macromolecules of complex forming substances. As a result of the mechanical treatment of powder mixtures after the initial grinding, a process of aggregation of microparticles takes place. Microcomposites are formed, consisting of submicron particles and having a very developed contact between the phases.

The obtained mechanocomposites samples of arglabin with arabinogalactan, basic magnesium carbonate, polyvinylpyrrolidone and disodium...
salt of glycyrrhizic acid were studied for cytotoxicity against larvae of crustaceans *Artemia salina* (Leach).

Thus, the results obtained indicate that an increase in the water solubility of the substance based on arglabin was achieved by the formation of complexes of arglabin by the mechanochemical treatment of natural sesquiterpene lactone with the disodium salt of glycyrrhizic acid, polyvinylpyrrolidone, magnesium carbonate.

4. Conclusions

The mechanochemical interaction of natural sesquiterpene lactone arglabin with polyvinylpyrrolidone, disodium salt of glycyrrhizic acid and basic magnesium carbonate was carried out; complex forming agents, optimal compositions of water-soluble arglabin complexes were selected. The regime of obtaining complexes by the mechanochemical method is determined.

Mechanical treatment of natural arglabin with basic magnesium carbonate, polyvinylpyrrolidone and disodium salt of glycyrrhizic acid transforms the crystalline substance into an amorphous state. The process is accompanied by the formation of coordination bonds between the molecules of arglabin and complex forming agent according to the donor-acceptor mechanism. The process of obtaining complexes occurs in the solid phase, which eliminates the use of organic solvents.

The obtained mechanocomplexes of arglabin with polyvinylpyrrolidone and disodium salt of glycyrrhizic acid have increased water solubility. At the same time the molecular structure of arglabin does not change, which is an important factor for maintaining the pharmacological activity of the sesquiterpene lactone molecule. Based on the experimental results, the cytotoxicity of mechano-composites of arglabin with arabinogalactan, basic magnesium carbonate, polyvinylpyrrolidone and disodium salt of glycyrrhizic acid was determined.

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