Plants polyols and polyoxysteroids: Optimization of extraction, chemical modification and biological activity

The dissertation is devoted to the optimization of methods for the isolation and complex extraction of polyoxysteroids and polyols from plant materials, theoretical and experimental research on the directed synthesis of new supramolecular encapsulated and hydrophilic derivatives based on them with high biological activity.

Methods of IR-, UV-, Mass-, NMR-spectroscopy, X-ray diffraction and HPLC analysis, quantum chemical calculations of reactivity and PASS-prediction of biological activity and bioscreening were used in this work.

The relevance of the topic of dissertation research. Steroid compounds play an extremely important role in the life of plants, animals and humans. In this regard, they have attracted the attention of researchers working in the field of bioorganic chemistry, pharmaceutical chemistry, and the chemistry of supramolecular compounds in recent decades. The unique properties of molecules characterized by the presence of a wide range of biological activity, structural features and the availability of sources for their preparation made it possible for them to become practically important source of renewable material for the development of new highly effective phytopreparations based on them.

Such multifunctionality is especially pronounced in a relatively new group of natural compounds, polyoxysteroids, which are a fairly large group (about 500 compounds).

However, the development of modern research is hampered by the limited availability of polyoxysteroids, the content of which in the organisms of plants and animals is low, and is also hampered by their water insolubility. Therefore, the main way to develop drugs based on them is directed organic synthesis or chemical modification. In addition, studies show that the modification of the molecules of steroid compounds sometimes leads to an increase in biological activity compared with natural analogues. This is the main reason for the need for chemical and biological studies of the vast series of polyoxysteroids.

In recent years, interest has also grown in other structurally similar cyclic polyhydroxylated biologically active substances, namely resveratrol (3,5,4-trihydroxy-trans-stilbene) and D-pinitol (1S, 2S, 4S, 5R)-6-methoxycyclohexane-1,2,3,4,5-pentol). These compounds showed pronounced hypoglycemic and antidiabetic properties.

Recent studies have clearly shown that the main trends in the synthesis of drugs are aimed at the chemical modification of compounds in order to manifest one or
another type of biological activity. It is known that a combination of several different chemical structures in one molecule allows one to achieve a significant synergistic effect, which, ultimately, will allow one to obtain substances with completely new practically useful properties.

One of the promising directions in terms of modification and creation of new water-soluble substances of medicines is supramolecular complexation-nanocapsulation.

Currently, the application of cyclodextrins (CDs) to obtain inclusion complexes of biologically active compounds (BAC) and medicines is one of the main ways of developing and creating new forms of pharmaceuticals. CDs differ in a number of advantageous properties due to their low cost and special structure among other widely known encapsulating receptors for BAC such as cucurbiturils, crown ethers, calixarenes, etc.

From the point of view of comparing similar structures, the main distinguishing feature of CDs is their ability to hydrophobically bind a guest molecule in their cavity in an aqueous medium with the formation of inclusion complexes with substrates that are less polar than water and if their geometry and structure are complementary to cavity of cyclodextrin receptor.

Available literature data on the study of ecdysteroid-containing plants, polyoxysteroids and polyols, methods for their extraction, modification and biological activity show the promise of studying these secondary plant metabolites. In this regard, the study of polyoxysteroids and polyols from available plant materials of the Republic of Kazakhstan, the modification of their molecules with subsequent bioscreening of the compounds obtained, as well as the development of high-tech and less expensive technologies for creating new supramolecular encapsulated and hydrophilic forms based on them remains relevant and in demand.

The subject of these studies is the optimization of isolation methods, directed chemical modification, the study of the fine structure of the extracted and synthesized compounds and the study of their biological activity.

The purpose and objectives of the study. The purpose of this dissertation is to optimize the isolation of polyoxysteroids and polyols from plant materials, directed synthesis of new supramolecular encapsulated and hydrophilic derivatives with high biological activity based on them.

Achieving this goal involves solving the following tasks:

- Carrying out research on the extraction of polyoxysteroids and polyols from plants growing and introduced in the territory of the Republic of Kazakhstan;
- Optimization of extraction of ecdysterone, which is a major polyoxysteroid from a selected promising type of plant material;
- Prediction of biological activity and the investigation of the reactivity of the selected substances in order to carry out chemical modifications based on them;
- Directional modification of selected synthon molecules and the establishment of the fine structure of the new substances obtained;
- Study of the biological activity of the resulting new supramolecular encapsulated and hydrophilic derivatives.
Scientific novelty of the work:

There was carried out for the first time optimization of the extraction of ecdysterone, an almost important polyoxysteroid from the plant *Serratula coronata* L. of *Asteracea* Dumort. family, by varying the methods of extraction of raw materials and the phases of growth of the species;

- A cyclic polyol was extracted for the first time from the plant *Silene brahuica* Boiss of the *Caryophyllaceae* Juss family. The fine structure of D-pinitol (1S, 2S, 4S, 5R)-6-methoxycyclohexane-1,2,3,4,5-pentol), which has hypoglycemic and antidiabetic properties, is fully characterized using $^1$H, $^{13}$C NMR (HSQC, COSY, NOESY)-spectroscopy;

- A strategy for the targeted modification of polyols and polyoxysteroids has been developed for the first time as a result of the integrated application of modern methods of mathematical modeling of biological activity using the PASS online program and quantum chemistry;

- New water-soluble compounds on the basis of ecdysterone (2β, 3β, 5β, 22R) - 2, 3, 14, 20, 25 - hexahydroxy - cholest - 7 - ene-6 - one) and α-, β- and γ-CDs, were obtained for the first time and their structures were established by NMR spectroscopy. When studying changes in the chemical shifts of protons of substrates and receptors, it was found that ecdysterone interacted with CD to form supramolecular inclusion complexes of stoichiometric composition 1: 1 and 1: 2. It was found that the inclusion complex with β-CD exhibited high solubility in water more than 100 times than the initial polyoxysteroid;

- New supramolecular inclusion complexes were synthesized for the first time on the basis of polyoxysteroids, namely 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, 3-epi-2-deoxyecdysone and modified synthon 2, 3, 22 - acetoxy - 14, 20, 25 - hydroxy - 5, 9 (H) - cholest - 7 - ene - 6 - one and their fine structures were fully confirmed by the data of two-dimensional correlation of $^1$H - $^1$H TOCSY, $^1$H - $^1$H ROESY, $^1$H - $^{13}$C HMQC and $^1$H-$^{13}$C HMBC;

- Water-soluble mixed complexes of 1: 1 composition were formed for the first time by the supramolecular interaction of the polyol D - pinitol with CD. The structure of supracomplexes was studied by NMR spectroscopy;

- It was revealed as a result of the bioscreening that the supramolecular complex of 3-epi-2-deoxyecdysone with β-CD at a dose of 25 mg/kg had a pronounced anti-inflammatory activity in the model of acute exudative reactions and exceeded the activity of the comparison drug “Sodium Diclofenac” in 1.6 times, and inclusion complexes of 2-deoxyecdysone with α-, β- and γ-CD in this dose had an anti-inflammatory activity comparable to the activity of the reference drug.

- The investigation of the antiradical properties by inhibiting the reaction of the DPPH radical in the presence of a complex of ecdysterone with γ-CD showed a pronounced antiradical activity (ARA) in vitro comparable to the effect of butylhydroxyanisole (BHA). The study of antioxidant activity (AOA) in the series of α-, β-, and γ-CD of ecdysterone complexes showed that the antioxidant property was most pronounced for the complex with γ-CD.
The scientific and practical significance of the study lies in the fact that its results can become the basis for the creation of new highly effective phytopreparations of a new generation with desired properties.

Water-soluble encapsulated forms based on a number of practically accessible cyclodextrins and phytoecdysteroids can be used in the pharmaceutical industry as dosage forms with desired properties for the first time.

The first synthesized water-soluble supramolecular inclusion complex of 3-epi-2-deoxyecdysone with β-CD according to the results of bioscreening can be considered as a potential anti-inflammatory agent.

The scheme proposed for the complex processing of raw materials of *Serratula coronata* L. with the aim of obtaining practically valuable ecdysteroids and flavonoids has been implemented in Karaganda Pharmaceutical Plant LLP.

**Conclusions from the results of the dissertation research:**

1. There was carried out a comprehensive study of the aerial part of *Serratula coronata* L. cultivated in the collection site of medicinal plants of ISPH “Phytochemistry” (Karaganda) in various phases of growth and using the most optimal methods of extraction and the content of the main active component of ecdysterone (20-hydroxyecdysone). It has been established that the ecdysterone content decreases from the beginning of the growing season to the final phase, which is confirmed by the data of high-performance liquid chromatography. The data on the quantitative content of the target component are given.

2. The results of a comprehensive study of the aerial part of *Serratula coronata* L., the plant basis of the first Kazakhstan anabolic and adaptogenic preparation Ecdifit, cultivated on the site of medicinal plants of ISPH “Phytochemistry” have been presented. The data on the optimal yields of ecdysterone, the main substance of many actoprotective drugs from various types of extracts and the complex extraction of flavonoids and ecdysteroids, have been presented. It was shown that separately isolated flavonoids and ecdysteroids could be used as new substances and working reference samples.

3. It was established that during water-ethanol, and then isobutanol extraction of the aerial part of *Silene brahuica*, the main components were phytoecdysteroids and a cyclic polyol, D-pinitol, isolated with quantitative yield from this plant for the first time. The fine structure of D-pinitol (1S, 2S, 4S, 5R)-6-methoxycyclohexane-1,2,3,4,5-pentol) was fully characterized using $^1$H, $^{13}$C NMR (HSQC, COSY, NOESY) spectroscopy. It is shown that it is promising to use plant raw materials from *Silene brahuica* as an industrially significant source for obtaining the target product due to the high content of D-pinitol (1.0 g by weight of air-dried raw materials), which has antidiabetic and hypoglycemic properties.

4. The geometric, energy, and electronic parameters of 2-deoxyecdysterone were calculated by the quantum-chemical method of the density functional DFT / B3LYP / 6-31G. A comparative analysis of the physico-chemical properties of the molecules of 2-deoxyecdysone, 2-deoxyecdysterone and ecdysterone that are synthons for regioselective modifications was performed. Virtual bioscreening of these phytoecdysteroids was carried out.
5. 20-hydroxyecdysterone - (2β, 3β, 5β, 22R) -2,3,14,20,22,25-hexahydroxy-5β- (H)-cholesta-7-ene-6-one was isolated in quantitative yield by ethanol extraction from the aerial part of *Silene wolgensis* (Hornem.) Otth; sometimes *Silene wolgensis* (Willd.) Bess. ex Spreng. The complexation of phytoecdysteroid with β-cyclodextrin was studied by NMR spectroscopy. When studying changes in the chemical shifts of protons of substrates and receptors, it was found that ecdysterone interacted with cyclodextrins with the formation of supramolecular inclusion complexes of stoichiometric composition 1: 1 or 1: 2. Ecdysterone-β-cyclodextrin complexes exhibit 100 times higher solubility in water than the starting compound.

6. 2-deoxy-20-hydroxyecdysone (2-deoxyecdysterone) was isolated from the aerial part of *Silene fruticulosa* (Pall.) Schischk of Caryophyllaceae Juss family for the first time. Using NMR spectroscopy, the complexation of phytoecdysteroid with γ-cyclodextrin was studied. By changing the chemical shifts of the protons of the substrate and the receptor, it was found that 2-deoxy-20-hydroxyecdysone interacted with γ-cyclodextrin to form a supramolecular inclusion complex of stoichiometric composition 1: 1 with the entry of fragment A of the substrate molecule into the internal cavity of the receptor.

7. 2-deoxyecdysone-3β, 14α, 22R, 25-tetrahydroxy-5β- (H)-cholesta-7-ene-6-one was isolated from *Silene wolgensis* (Hornem.) Bess. Ex. Spreng. of Caryophyllaceae Juss. for the first time. Using NMR spectroscopy, the complexation of phytoecdysteroid with α-, β- and γ-cyclodextrins was studied. By changing the chemical shifts of the protons of the substrate and receptors, it was found that 2-deoxyecdysone interacted with α-, β- and γ-cyclodextrins with the formation of supramolecular inclusion complexes of stoichiometric composition 1: 1 with the entry of ring A of the steroid nucleus of the substrate molecule into the internal cavity of the receptor.

8. An ecdysteroid 3-epi-2-deoxyecdysone was extracted from the aerial part of *Acanthophyllum gypsophiloides* Regel. It was shown by NMR spectroscopy that the supramolecular complexation of an ecdysteroid with α-, β- and γ-cyclodextrins was accompanied by the formation of mixed complexes of the composition 1: 1 (β-cyclodextrin), inclusion complexes of the composition 1: 2 (γ-cyclodextrin) and external complexes of the composition 1: 2 (2-hydroxypropyl-β-cyclodextrin).

9. A water-soluble mixed inclusion complex 1: 1 was formed for the first time through intermolecular interactions of ecdysterone 2,3,22-triacetate molecule with β-cyclodextrin. The fine structure of the synthon molecule was established using modern spectral methods and X-ray diffraction analysis.

10. For the first time, water-soluble mixed complexes of 1: 1 composition were formed by supramolecular interaction of the practically valuable polyol D-pinitol with cyclodextrins. The structure of supracomplexes was studied by NMR spectroscopy.

11. A method has been developed for the determination of related impurities in ecdysterone RS by HPLC and its validity has been proved. According to the results of the study, a quality specification was developed for ecdysterone RS as a unique standard sample of domestic production, which is included in the State
Pharmacopoeia of the Republic of Kazakhstan, which is necessary to ensure proper quality control of the substance for pharmaceutical use and drugs.

12. It was revealed as a result of the bioscreening that the supramolecular complex of 3-epi-2-deoxyecdysone with β-CD at a dose of 25 mg/kg had a pronounced anti-inflammatory activity in the model of acute exudative reactions and was 1.6 times higher in activity than the reference drug Sodium Diclofenac and inclusion complexes of 2-deoxyecdysone with α-, β- and γ-cyclodextrins at this dose had anti-inflammatory activity comparable to the activity of the reference drug.

13. The results of studies of the antiradical properties by inhibiting the reaction of the DPPH radical in the presence of a complex of ecdysterone with γ-cyclodextrin showed a pronounced antiradical activity in vitro comparable with the effect of butylhydroxyanisole (BHA).

14. It was also revealed as a result of the bioscreening that the inclusion complex of ecdysterone triacetate with β-cyclodextrin in the model of acute tetrachloromethane hepatitis had an insignificant hepatoprotective effect, and the initial triacetate derivative of ecdysterone did not show significant changes in the inhibition of animal liver mass growth and in the restoration of blood serum rat parameters.

The main provisions of the dissertation submitted to the defense:
- The results of optimization of the extraction of polyoxysteroids from plant materials and their identification;
- Isolation and identification of an individual polyol from a polyoxysteroid-containing plant material;
- Directional chemical modifications of polyoxysteroids and polyol, taking into account the peculiarities of their structure and the structure of the molecules derived from them based on spectral data;
- Results of bioscreening on the biological activity of new supramolecular complexes of polyoxysteroids with cyclodextrins;
- Laboratory technological regulations for the production of the amount of separately purified ecdysteroids and flavonoids from the substance of the drug “Ecdifit” (thick extract of Serratula coronata L.).

Connection of work with the plan of state scientific programs.
The work was carried out as part of grant projects: “Search for new natural sources and development of a method for obtaining the substance of steroid preparations” for 2015-2017. (State registration number 0115 RK 00185) and “Synthesis, structure and biological activity of new water-soluble derivatives of polyoxysteroids” for 2018-2020. (No. of state registration 0118 RK 00011).

Approbation of work. The main provisions, conclusions and scientific results of the dissertation were reported and discussed at the All-Russian and international conferences: IX All-Russian scientific conference “Chemistry and technology of plant substances” (Moscow, RF, 2015), 23rd Conference on Isoprenoids (Minsk, Belarus, 2016), All-Russian scientific conferences with international participation “Modern problems of organic chemistry” (Novosibirsk, Russia, 2017), 12th, 13th International Symposium on the Chemistry of Natural Compounds (Tashkent, Uzbekistan 2017, Shanghai, China 2019), International scientific conference
“Medicines based on natural compounds” (Tashkent, Republic of Uzbekistan, 2018).

The main results of the dissertation research. The main provisions of the dissertation are reflected in 15 published works, of which 3 articles are in the list of publications approved by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 2 articles in foreign journals with impact factor (Q3 and Q4), 1 article in a specialized journal of neighboring countries, 9 works in materials of national and international conferences.

Volume and structure of the dissertation. The dissertation is presented on 174 typescript pages, includes 26 figures, 29 tables, 7 diagrams; consists of an introduction, analysis of domestic and foreign literature on this topic, 6 sections of our own experimental and theoretical research, experimental part, conclusion, list of sources used, including 327 works, 8 applications.