ANNOTATION

Dissertations for the degree of Doctor of Philosophy (PhD)
in the specialty 6D060600 – Chemistry

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Phytoecdysteroids. Isolation, identification, synthesis of new biologically active compounds

General characteristics of the work. This dissertation is devoted to a comprehensive study of phytoecdysteroids - natural polyhydroxysteroid compounds of plant origin with a wide range of biological activity. The work considers the issues of isolation and identification of phytoecdysteroids from plant materials, optimization of methods for obtaining target compounds, theoretical and experimental substantiation of targeted chemical modification, as well as the synthesis and study of new supramolecular water-soluble and biologically active derivatives. The study was carried out using an interdisciplinary approach combining methods of phytochemistry , organic and supramolecular synthesis, quantum chemistry, virtual bioscreening and experimental biology. Particular attention is paid to the development of scientifically based approaches to increasing solubility, stability and bioavailability. phytoecdysteroids due to supramolecular complexation with cyclodextrins and other encapsulating agents.

Relevance of the study. Steroid compounds play a crucial role in the lives of plants, animals, and humans. Therefore, in recent decades, they have attracted the attention of researchers working in the fields of bioorganic chemistry, pharmaceutical chemistry, and supramolecular chemistry . The unique properties of these molecules, characterized by a broad spectrum of biological activity, their structural features, and the availability of their sources, have enabled them to become a valuable source of renewable materials for the development of new, highly effective herbal medicines and drugs based on them.

At the same time, despite the high biological activity of phytoecdysteroids , their practical application is limited by low water solubility , instability and insufficient bioavailability , which necessitates the development of new approaches to their chemical modification and supramolecular encapsulation.

Research Objectives and Tasks. The aim of this dissertation is to optimize the method for isolating phytoecdysteroids from plant materials, conduct targeted synthesis based on them, and study new supramolecular encapsulated and hydrophilic derivatives with high biological activity.

Achieving this goal involves solving the following tasks:

1. Conduct phytochemical studies to isolate and identify phytoecdysteroids from wild plants of the flora of Kazakhstan and those introduced to the collection site of JSC Scientific and Production Center " Phytochemistry ".

2. To optimize the process of extracting 20-hydroxyecdysone from a promising plant species – a superproducer of the target major phytoecdysone ;

3. Prediction of biological activity, spatial structure and reactivity of isolated phytoecdysones – the main synthons for chemical modification using virtual bioscreening and modern methods of quantum chemical calculations.

4. Directed chemical modification of phytoecdysteroids and determination of their fine structure.

5. Conduct *in vivo* Bioscreening of the obtained new supramolecular encapsulated

and hydrophilic derivatives of phytoecdysones .

Methodological basis and metrological support for the study. Field and seasonal collection of wild and introduced plant species, solid-liquid extraction of dried and crushed plant materials, column chromatography, thin-layer chromatography, synthesis of supramolecular hydrophilic complexes, calculation and prediction of water solubility (Advanced software) Chemistry Development , Toronto , ACD/ Percepta 14.2.0 Build 2977), prediction of biological activity (PASS Online), quantum chemical calculations (DFT/B3LYP method using GAUSSIAN 09 software), statistical processing of experimental data (Student's method).

The plant species were identified jointly with A.N. Kupriyanov, Director of the Kuzbass Botanical Garden of the Institute of Human Ecology, Siberian Branch of the Russian Academy of Sciences (Kemerovo), in creative collaboration with the Botany and Biotechnology Laboratory of the Scientific and Practical Center for Phytochemistry . Herbarium specimens are stored in the Herbarium Collection of the Scientific and Practical Center for Phytochemistry .

The structure of the substances isolated from plants and their modified forms were identified using modern methods: NMR ^{1}H and ^{13}C at 400 MHz on a JNM-ECA Jeol 400 instrument (Japan), two-dimensional spectra 2D COSY, HSQC, HMBC and ROESY were recorded on an ECZ 500R instrument (JEOL, Japan) with a frequency of 500 MHz at a temperature of 27 ° C, optical rotation of molecules was determined on an Atago polarimeter Polax 2L (Japan), IR spectroscopy (Avatar 360 ESP, USA), melting point (OptiMelt MPA 100, USA), the quantitative content and purity of the target components were determined by HPLC (Hewlett Packard Agilent 1100, USA), the purity of regularly used solvents was determined on a gas chromatograph (Agilent 7890 B , USA), X-ray structural analysis was performed on a diffractometer (Bruker Kappa APEX2 CCD, USA) . The main analytical and metrological work was carried out in the laboratory of control and analytical work and physical testing methods of the Scientific and Production Center " Phytochemistry ", as well as at the University of Chemistry and Technology (Prague). Individual stages of the work were carried out at the Institute of Solid State Chemistry and Mechanochemistry of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk), the NMR Spectroscopy Laboratory of the Kokshetau University named after Sh. Ualikhanov and the engineering laboratory of the Physicochemical Research Methods of the Karaganda University named after Academician E.A. Buketova

The main provisions submitted for defense:

1. Four phytoecdysteroids and one cyclic polyol, D-pinitol, were isolated and identified from plants native to Kazakhstan and introduced to the collection site of JSC SPC Phytochemistry (*Serratula coronata* L.*):* 20-hydroxyecdysone (ecdysterone) from *Serratula coronata* L., 2-deoxyecdysone from *Silene cretaceae* Fisch., 2-deoxy-20-hydroxyecdysone from *Silene fruticulosa* (Pall) Schischk., 3-epi-2-deoxyecdysone from *Acanthophyllum gypsophiloides* Regel., and D-pinitol from *Silene brahuica* Boiss. Despite some technological and preparative difficulties (low yields of ecdysteroids in plants), in order to ensure the reliability and reproducibility of the results, all experiments on their extraction, chromatographic isolation and syntheses based on them were carried out in triplicate and statistically processed.

2. The optimal conditions for the extraction of ecdysterone, a major phytoecdysteroid *of Serratula coronata* L., at a temperature of 80 ° C and an extraction time of 3 hours, during the plant’s vegetation phase, is 2.9%, which proves the high

efficiency of using plant materials as the main industrially significant source of the above-mentioned substance .

3. In order to preliminary predict the biological activity, spatial structure and reactivity of the isolated main phytoecdysteroids 20-hydroxyecdysone (20E), 2-deoxyecdysone (2-DE) and 2-deoxy-20-hydroxyecdysone (2-DE-20E) for their further use as technologically accessible synthons, virtual bioscreening methods (PASS) were used . Online) and quantum chemical calculations (DFT/B3LYP density functional theory in the 6-31 G split-valence basis within the GAUSSIAN 09 program) as the most approximate method.

4. The developed strategy for targeted modification of phytoecdysteroids 20E, 2-DE, 2DE-20E, pre-modified derivative 20E – 2,3,22-triacetoxy-14,20,25-trihydroxy-5,9 (H) – cholest – 7 – ene – 6 – one and rare epimer 3-epi-2-deoxyecdysone (3E-2DE) and polyhydroxy – structurally similar cyclic polyol D - pinitol with α-, β-, γ-, 2-hydroxypropyl-β- cyclodextrins (2-GP-β-CD) and disodium glycyrrhizate (Na 2 GA) resulted $_{in}$ the synthesis of 20 new supramolecular complexes. Fine structures of all obtained inclusion complexes (ICs) in different stoichiometric ratios: 3 ICs 20E with α-, γ- and 2-GP-β-CD, as well as 2 ICs with β-CD in ratios of 1:1 (20E-β-CD) and 1:2 (20E-β-CD), respectively; 3 intraluminal ICs 2-DE with α-, β- and γ-CD in ratios of 1:1; 2 KV 2DE-20E with β-CD in ratios of 1:1 and 1:2; 1 intracavity complex of composition 1:1 with coverage of the side aliphatic tail of 3E-2-DE with β-CD and 1 complex with external interaction, with γ-CD 1 intracavity complex of composition 1:2 covering the side aliphatic tail and fragments of the sterane carbon skeleton of the 3-E-2-DE molecule and with 2-GP-β-CD 1 complex with external structural features of composition 1:2 was obtained; 1 complex of 3-acetoxy derivative 20E with β-CD of composition 1:1, 4 KV D - pinitol with α-, β-, γ- and 2-GP-β-CD in ratios 1:1; 2 mechanocomposites 20E with 2-GP-β-CD and Na_2 GA in a 1:10 ratio were confirmed by two-dimensional correlations of $^{1}H - ^{13}C$ TOCSY NMR spectra , $^{1}H - ^{1}H$ ROESY , $^{1}H - ^{13}C$ HMQC and $^{1}H - ^{13}C$ HMBC .

5. Conducting virtual bioscreening according to the PASS program and experimental *in vivo* Bioscreening results allow us to reveal that the supramolecular complex of 3-epi-2-deoxyecdysone with β- cyclodextrin at a dose of 25 mg/kg has a pronounced anti-inflammatory activity and exceeds the comparison drug " Diclofenac sodium" by 1.6 times, and complexes of 2-deoxyecdysone with α-, β-, γ- cyclodextrins at this dose have this type of activity, comparable to the action of the comparison drug.

Scientific novelty of the work:

– for the first time based on the results of a comprehensive study *of Silene brahuica* Boiss of the family *Caryophyllaceae* Juss , in addition to the major ecdysterone , a structurally similar one was isolated and identified polyhydroxylated compound – D - pinitol , which has hypoglycemic and antidiabetic effects ;

– for the first time, a combination of modern methods of mathematical modeling of biological activity using the PASS program A new strategy for targeted chemical modification of phytoecdysteroids has been developed using online and quantum chemistry;

– For the first time, new water-soluble composites were synthesized and their structure was determined by the interaction of ecdysterone with α-, β-, γ-, and 2-HP-β-cyclodextrins . Using NMR spectroscopy, it was established that ecdysterone interacts with cyclodextrins to form supramolecular inclusion complexes of stoichiometric composition at a ratio of 1:1 and 1:2. It was determined that the inclusion complex of

ecdysterone with β- cyclodextrin exhibits a comparatively high solubility in water, more than 100 times that of the original polyoxysteroid ;

– for the first time, 20 new supramolecular inclusion complexes were synthesized based on phytoecdysteroids 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, and a rare epimer 3-epi-2-deoxyecdysone and a modified synthon 2, 3, 22 – acetoxy –14,20,25–trihydroxy-5,9(H) –cholest – 7 – en –6–one and their fine structure was established using the methods of two-dimensional correlation of NMR spectra ^{1}H - ^{1}H TOCSY , ^{1}H - ^{1}H ROESY , ^{1}H - ^{13}C H M QC and ^{1}H - ^{13}C HMBC ;

, composites with 2-hydroxypropyl-β- cyclodextrin and disodium glycyrrhizic acid salt were obtained based on ecdysterone , and the structure of the synthesized complexes was determined by NMR spectroscopy in deuterated water. It was found that the supramolecular complex of ecdysterone with 2-hydroxypropyl-β- cyclodextrin exhibits a 3-fold improved solubility in water than the original compound;

– For the first time, water-soluble mixed complexes of 1:1 composition were synthesized through supramolecular interaction of D- pinitol with cyclodextrins , the structure of which was studied using NMR spectroscopy;

– According to the results of bioscreening of the synthesized samples, it was revealed that the supramolecular complex of 3-epi-2-deoxyecdysone with β-cyclodextrin at a dose of 25 mg/kg has a pronounced anti-inflammatory activity in the model of acute exudative reactions and exceeds the effect of the comparison drug " Diclofenac sodium" by 1.6 times, and the inclusion complexes of 2-deoxyecdysone with α-, β- and γ- cyclodextrins at this dose have anti-inflammatory activity comparable to the effect of the comparison drug;

– the antiradical property of a complex of ecdysterone with γ- cyclodextrin was determined , comparable to the effect of butylhydroxyanisole (BHA).

Description of the Main Results:

1. A systematic phytochemical investigation of plant species from the flora of Kazakhstan was carried out using modern analytical techniques. Four phytoecdysteroids (20-hydroxyecdysone, 2-deoxyecdysone, 2-deoxy-20-hydroxyecdysone, 3-epi-2-deoxyecdysone) and D-pinitol were isolated using solid–liquid extraction, column chromatography, and thin-layer chromatography. Their structures were confirmed by ^{1}H and ^{13}C NMR spectroscopy (400–500 MHz), 2D COSY, HSQC, HMBC, ROESY experiments, IR spectroscopy, and X-ray diffraction analysis. All experiments were performed in triplicate with statistical evaluation using Student’s t-test.

2. The process for obtaining 20-hydroxyecdysone from *Serratula coronata* L. was optimized. The rational extraction parameters (80°C, 3 hours, vegetative growth phase) were established, providing a reproducible yield of 2.9%. Quantitative determination and purity control were performed by HPLC (Agilent 1100), confirming the technological efficiency and reproducibility of the developed methodology.

3. A scientifically substantiated strategy for the directed chemical modification of phytoecdysteroids was developed based on theoretical prediction. Virtual biological activity screening using PASS Online and quantum-chemical calculations employing the DFT/B3LYP method (6-31G basis set, Gaussian 09 software) were applied to determine the electronic structure features and reactive centers of the molecules. This approach enabled the rational selection of synthetic transformation pathways.

4. Twenty new supramolecular inclusion complexes were synthesized and structurally characterized. Complexes with α-, β-, γ-, and 2-hydroxypropyl-β-cyclodextrins, as well as mechanocomposites with 2-HP-β-CD and disodium glycyrrhizinate, were obtained. The stoichiometry of the complexes (1:1 and 1:2) was established using 2D NMR techniques (TOCSY, ROESY, HMQC, HMBC). A significant enhancement in water solubility was demonstrated: more than a 100-fold increase for the 20E–β-CD complex and a threefold increase for the 2-HP-β-CD complex.

5. High biological activity of the synthesized compounds was experimentally confirmed. In vivo studies demonstrated that the 3-epi-2-deoxyecdysone–β-cyclodextrin complex at a dose of 25 mg/kg exhibited anti-inflammatory activity 1.6 times higher than the reference drug diclofenac sodium. Inclusion complexes of 2-deoxyecdysone with α-, β-, and γ-cyclodextrins showed comparable anti-inflammatory activity. Additionally, the γ-cyclodextrin complex demonstrated antiradical activity comparable to butylated hydroxyanisole.

Importance of the obtained research results. The scientific significance of the results lies in expanding fundamental understanding of the chemical composition and reactivity of plant-derived phytoecdysteroids . The phytochemical studies conducted allowed us to deepen our understanding of the prevalence and diversity of phytoecdysteroids in plants native to the Republic of Kazakhstan and confirm the potential of the studied plant species as sources of biologically active compounds. The obtained data complement existing knowledge about natural steroids and can serve as a basis for further fundamental research in the chemistry of natural compounds. An important scientific result is the establishment of optimal conditions for the extraction of 20-hydroxyecdysone from *Serratula. coronata* L. , which is important not only for the practical production of the target compound but also for the development of methodology for phytochemical studies of ecdysteroids . The proposed approaches improve the efficiency of plant material use and ensure the reproducibility of results.

Results related to the application of quantum-chemical calculations and virtual bioscreening methods to predict the reactivity and biological activity of phytoecdysteroids are of significant scientific significance. The obtained correlations between the electronic structure of molecules, their spatial organization, and the biological activity they exhibit contribute to the development of theoretical foundations for the rational design of biologically active derivatives of natural compounds. Of particular importance is the development and experimental implementation of a strategy for the targeted chemical modification of phytoecdysteroids using supramolecular complex formation.

The synthesis and comprehensive investigation of new inclusion complexes with cyclodextrins and disodium glycyrrhizinate made it possible to obtain fundamentally new data on the nature of intermolecular interactions in “phytoecdysteroid–host” systems, which is of significant importance for the development of supramolecular chemistry of natural compounds. The practical significance of the obtained results is determined by the fact that supramolecular complexation made it possible to substantially improve the physicochemical properties of phytoecdysteroids, primarily their water solubility and bioavailability, which are key factors for expanding their pharmacological applications. The identified supramolecular complexes exhibiting pronounced anti-inflammatory activity and surpassing reference drugs are of interest as

a promising basis for the development of new medicinal products and herbal preparations.

The results obtained in the dissertation contribute to the formation of scientific approaches to the rational use of natural plant raw materials and the creation of effective and safe biologically active compounds based on them. These results may also be used in further fundamental and applied research, in the development of technological regulations, as well as in the educational process for training specialists in chemistry and pharmacy. The implementation of the study within the framework of grant-funded projects confirms the relevance and practical orientation of the conducted research, as well as its compliance with priority areas of development in chemical and pharmaceutical sciences. The work was carried out within the framework of grant projects No. 1716-GF4 “Search for New Natural Sources and Development of a Method for Obtaining Steroid Drug Substances” (2015–2017, state registration No. 0115 RK 00185) and AP05133718 “Synthesis, Structure, and Biological Activity of New Water-Soluble Polyoxysteroid Derivatives” (2018–2020, state registration No. 0118 RK 00011).

The author's personal contribution to the dissertation is reflected in comprehensive research activities: analysis of literary and patent sources, conducting experimental and computational studies, as well as interpreting and summarizing the obtained data. Based on these materials, the author prepared scientific articles and conference abstracts. In the preparation of each publication, the doctoral candidate actively participated: conducting literature and patent searches, selecting appropriate scientific journals, preparing articles, interpreting results, and interacting with journal editors and reviewers.

The main provisions, conclusions, and scientific results of the dissertation were tested and discussed at international and Russian conferences, including the IX All-Russian Scientific Conference “Chemistry and Technology of Plant Substances” (Moscow, 2015), 23rd Conference on Isoprenoids (Minsk, 2016), All-Russian Conference with international participation “Modern Problems of Organic Chemistry” (Novosibirsk, 2017), 12th and 13th International Symposiums on the Chemistry of Natural Compounds (Tashkent, 2017; Shanghai, 2019), as well as the International Conference “Medicinal Preparations Based on Natural Compounds” (Tashkent, 2018), and are reflected in 11 publications: 5 articles in journals approved by the Committee for Quality Assurance in the Field of Science and Education of the Ministry of Education and Science of the Republic of Kazakhstan; 4 articles in foreign journals with impact factors (Q2, Q3, and Q4); 1 article in a specialized journal included in the RSCI database:

1. «Bioavailability and structural study of 20-hydroxyecdysone complexes with cyclodextrins» https://doi.org/10.1016/j.steroids.2018.11.007 - Isolation of 20-hydroxyecdysone from the plant *Silene wolgensis* Hornem Bess. ex. Spreng ., identification, synthesis of supramolecular inclusion complex with β- cyclodextrin and 2-hydroxypropyl-β- cyclodextrin in different stoichiometric ratios, establishment of fine structure of the obtained system using ^{1}H and ^{13}C NMR spectroscopy.

2. «Supramolecular complexes of 3- epi -2- deoxyecdysone with cyclodextrins and their anti - inflammatory activity » https :// doi . org /10.1134/ S 1070363219030095 - Chromatographic study of the amount of ecdysteroids obtained from *Acanthophyllum gypsophiloides* Regel ., extraction of 3-epi-2-deoxyecdysone, supramolecular interaction of the molecule with β- , 2-hydroxypropyl- β - cyclodextrins , interpretation of the

obtained NMR spectroscopy data, establishment of the interaction of the guest-host molecule receptors using 2D NMR.

3. «Supramolecular Complex of 20- Hydroxyecdysone -3- acetate with β-Cyclodextrin and Its Biological Activity» https :// doi . org /10.1134/ S 1070363220120075 - Synthesis of 20-hydroxyecdysone triacetate, chromatographic separation of the reaction mixture, recrystallization of the obtained sample, preparation of crystals for X-ray structural analysis, synthesis of a supramolecular inclusion complex with β- cyclodextrin , study of the complexation process and assignment of proton and carbon signals by NMR spectroscopy.

4. «Synthesis , NMR Spectroscopic Study of α-, β- and γ- Cyclodextrin Inclusion Complexes of 2- Deoxyecdysone and Their Anti - inflammatory Activity» DOI :10.6060/ mhc 200602 t – Column chromatography of the alcoholic extract *of Silene wolgensis* Hornem Bess . ex Spreng ., extraction of 2-deoxyecdysone, synthesis of inclusion complexes with α-, β- and γ- cyclodextrins , interpretation of NMR spectroscopy data, collection of materials for submission to a journal article.

5. «Synhesis , anti - inflammatory and analgesic activity of the supramolecular complex of 3- EPI -2- deoxyecdysone and prospects for its medical application» https :// doi . org /10.1016/ j . fitote .2025.106952 - synthesis of the 3-epi-2-deoxyecdysone inclusion complex, preparative production of supramolecular system samples for transfer to the pharmacology laboratory for studying anti-inflammatory and analgesic activity. Collection of materials for the article, correspondence with the editors, responses to reviewers' suggestions and comments on the manuscript.

6. «Optimization of the technology for obtaining ecdysterone from *Serratula coronata* L . by varying the extraction methods and growth phases » https :// doi . org /10.31489/2018 CH 2/45-50 - Cultivation of Serratula coronata on the territory of the Karaganda Botanical Garden, collection of plant biomass in different phases of plant flowering, extraction of Serratula using various technological methods, chromatographic analysis of the obtained extracts for the presence of 20-hydroxyecdysone, statistical processing of the obtained results, article design.

7. Preparation and Physicochemistry Properties of Supramolecular Complexes of Ecdysterone » DOI:10.14258/ jcprm .20230412999 – Synthesis of an inclusion complex, a mechanocomplex system of 20-hydroxyecdysone with 2-hydroxypropyl - β - cyclodextrin and disodium glycyrrhizic acid . Study of the complex formation process by two-dimensional NMR spectroscopy, study of signals and spin-spin interactions between substrate and clathrate molecules . Study of the water solubility of the obtained samples using interphase dissolution using HPLC.

The main provisions, conclusions and scientific results of the dissertation were presented and discussed at international conferences:

1. Complexes of 20- Hydroecdysone with α -, β - and γ - Cyclodextrins - 23rd Conference on Isoprenoids (Minsk, Belarus) – For the first time, a synthesis of a 20-hydroxyecdysone inclusion complex with cyclodextrins was carried out , and the structure of the complex was identified using NMR spectroscopy.

2. The study of antioxidant activity of ecdysterone inclusion complexes with α -, β - and γ - cyclodextrins – Tashkent, 2017. – A study of the antioxidant and antiradical activity of the obtained complexes was conducted.

3. Study of the spatial structure of 2,3,22-acetoxy-14,20,25-hydroxy-5,9(H)-cholest-7-en-6-one. - Tashkent, - 2018. – The spatial structure of 20-hydroxyecdysone triacetate was studied by X-ray diffraction.

4. Quantum-chemical DFT approach to the study of phytoecdysteroid synthons for regioselective modifications. - Tashkent, 2018. – Torsion and bond angles of the steroid backbone and reaction centers were investigated using modern quantum-chemical calculations.

5. Synthesis, structure, and bioactivity of a new water-soluble 20-hydroxyecdysone derivative. Shanghai, 2019. – Study of the water solubility and bioavailability of the resulting complexes as a finished encapsulated dosage form.

The author independently developed experimental schemes for the isolation, modification and supramolecular complex formation phytoecdysteroids , the spectral data were interpreted and the biological activity of the obtained compounds was assessed.