ABSTRACT

of the dissertation for the degree of the doctor of philosophy (PhD) on the specialty 6D060600 – Chemistry

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The new derivatives of available monoterpenoids of Kazakhstan plants and their biological activity

General characteristics of the dissertation research. The thesis is devoted to the search and preparative isolation of natural monoterpenoids, which are abundantly found in the essential oils of the plants of Kazakhstan, as well as their chemical transformation, followed by a study of the biological and catalytic activity of the obtained derivatives.

Two monoterpenoids sabinol and terpinen-4-ol were selected for chemical transformations, which were preparatively isolated in pure form from the essential oil (EO) of the *Juniperus sabina* L. plant. A number of derivatives of sabinol and terpinen-4-ol were stereoselectively synthesized and tested for cytotoxic and anti-radical activity. In addition, synthesized 3-amino-1,2-diols, diols and triols based on sabinol were investigated as chiral catalysts in the asymmetric reaction of Et_2Zn addition to benzaldehyde.

Relevance of the research topic. Modern science focuses mainly on innovative therapeutic agents derived from natural compounds, which can be widely used in medicine, pharmaceuticals, as well as in the cosmetic and perfume industries. Owing to the change in the chemical structure of natural biologically active compounds, it is possible to increase, decrease or modify their biological activity. Based on this basic idea, a wide range of different structures can be obtained, leading to the development of more effective therapeutic agents. In this regard, it is promising to use monoterpenoids as starting molecules for the synthesis of medicinal substances due to their availability, renewability and a wide range of biological activity. In addition to understanding that this resource is practically inexhaustible, it should also be mentioned that the use of monoterpenoids for drug development often reduces the toxicity of the resulting compound, which may indicate the presence of complementarity between natural molecules and living organisms that have existed for thousands of years.

Studies have shown that both natural monoterpenes and their synthetic derivatives have various pharmacological properties, including antifungal, antibacterial, antioxidant, antitumor, antiarrhythmic, anti-aggregative, local anesthetic, anti-inflammatory, antihistamine and antispasmodic activities. Monoterpenes also act as regulators of growth, heat, transpiration, tumor inhibitors, oxidative phosphorylation inhibitors, insect repellents, and antidiabetic agents. These interesting properties can be potentially used not only in the pharmaceutical industry, but also in the food and cosmetic industries.

The presence of monoterpenes in natural sources in pure enantiomeric forms makes these compounds convenient precursors for the synthesis of optically active ligands for use in asymmetric catalysis. Monoterpenes are optically active compounds that have multiple stereocenters. In addition to their commercial availability, the advantage of these molecules is that the existing chiral centers are preserved in the newly formed molecules, and the transfer of chirality usually occurs with high stereoselectivity. All these advantages make this class of compounds very attractive in the development of chiral ligands (catalysts) for the purposes of organic synthesis.

The degree of the problem elaboration. Interest in natural monoterpenes does not cease to fall and numerous research papers published annually in international scientific journals serve as evidence of this fact. An analysis of the scientific work on the pharmacological properties of monoterpenes has shown that the majority of chemically modified compounds of this class in most cases show anti-tuberculosis, anti-inflammatory, anti-microbial, antiviral and anti-tumor effects. Large-scale studies on chemical transformation and the study of the biological activity of natural monopenoids are carried out by research teams led by Salakhutdinov N.F. (Russia), Zsolt S (Hungary), Dimitrov V (Bulgaria), Hu Y., Wang Sh (China), Rivas F (USA) and etc.

If we talk about the use of monoterpenes in asymmetric catalysis, in recent years several synthetic strategies have been developed to obtain enantiopure 3-amino-1,2-diols and 1,3-amino alcohols derived from naturally occurring monoterpenes, such as (+)-limonene, (+)-and (-)-α-pinene, (-)-β-pinene, (1R)-(-)-myrtenol, (+)-pulegon, (+)-camphor, (-)-fenchone, (-)-menton, (+)-3-caren and (-)-perilaldehyde. These useful synthons were effectively used as chiral ligands, and their catalytic activity was thoroughly tested and interpreted.

We carried out a detailed analysis of the literature data on the monoterpenoids sabinol and terpinen-4-ol. According to these data, sabinol is well known for its anthelmintic activity and as a means of stimulating menstruation, and terpinen-4-ol has an antitumor effect on human lung cancer cells, and also induces apoptosis in the cell lines of colorectal cancer HCT116 and RKO. However, despite the fact that these compounds have been intensively studied from a biological point of view, they have poorly studied from the point of chemical modifications. There is also no information on the synthesis of chiral catalysts based on the sabinane bicyclic system.

Interrelation of work with the plan of state scientific programs. The thesis was carried out in the framework of joint research projects carried out at the Institute of Applied Chemistry at the Department of Chemistry of the L.N. Gumilyov Eurasian National University, at the Institute of Pharmaceutical Chemistry of the University of Szeged (Szeged, Hungary) and at the Department of Chemistry of the Catholic University of Leuven (Leuven, Belgium).

The purpose and objectives of the research. The main goal of the research work is to obtain synthetically new derivatives of natural monoterpenoids, which are abundantly found in the essential oils of the plants of Kazakhstan, and their practical use as biologically and catalytically active substances.

To achieve this goal, the following tasks were set and solved:

- to investigate the composition of the essential oil (EM) of the *Juniperus sabina* L. plant by gas chromatography to establish the quantitative content of sabinyl acetate and terpinen-4-ol;
- preparatively isolate monoterpenoids terpinen-4-ol and sabinol in pure form, which is contained in EO in the form of sabinyl acetate;
- to synthesize stereoselectively and study the chemical transformations of 3-amino-1,2-diols based on the sabinane system;
- to synthesize stereoselectively and study the chemical transformations of diols and triols based on the sabinane system;
- to synthesize triazoles and urea based on sabinol;
- to synthesize 2-amino-1,4-diols based on the *p*-menthane system;
- to give a full spectral characteristic of the synthesized derivatives, including ^{1}H, ^{13}C, 2D NMR, Mass, X-ray, elemental analysis;
- to determine the cytotoxic, anti-radical and other types of biological activity of essential oils and sabinol derivatives;
- to study the catalytic activity of 3-amino-1,2-diols, diols and triols based on the sabinane system in the model reaction of diethyl zinc addition to benzaldehyde.

The objects of this research are monoterpenoids (+) - sabinol and R-terpinen-4-ol, isolated from *Juniperus sabina* L. essential oil.

The choice of research objects was based on the presence of a high content of these molecules in plant raw materials and the absence of chemically modified derivatives for them (in the literature).

Sabinol and terpinen-4-ol were practically not subjected to synthetic transformations earlier. In addition, these monoterpenoids are found in high concentrations in *Juniperus sabina* L. essential oil, which determined the choice of this plant as a source of sabinol and terpinen-4-ol.

The subject of research in this thesis is the chemical conversion of monoterpenoid (+)-sabinol via Overman rearrangement reaction, reactions of dihydroxylation, hydroboration-oxidation, epoxydation, protection-deprotection, creating heterocyclic systems (oxazine, triazole) and a three-stage chemical transformation of R-terpinen-4-ol to obtain a series of 2-amino-1,4-diol derivatives through chemical reactions of the oxirane system creation, followed by aminolysis of the resulting epoxide.

The scientific novelty of the research results is stated by the fact that for the first time:

- the optimal technology of isolation and purification of (+)-sabinol and R-terpinen-4-ol from the essential oil of *Juniperus sabina* L. was developed;
- 19 new enantiomerically pure compounds based on the sabinane bicyclic framework (diols, triols, aminodiols, triazoles, urea, etc.) were stereoselectively synthesized with the establishment of their fine chemical structure;
- 6 new compounds based on terpinin-4-ol werestereoselectively synthesized in high yields with a detailed analysis of the spectral characteristics of their molecular structure;

- bioscreening of new synthesized derivatives was carried out for cytotoxic and anti-radical activity;

- synthesized 3-amino-1,2-diols, 1,3-diol and triols based on (+) - sabinol were used as catalysts in the ethylation of benzaldehyde with diethylzinc.

The theoretical significance of the study lies in the fact that methods have been developed for the preparative purification of monoterpenoids from natural sources. In addition, the results obtained on the synthetic transformations of (+)-sabinol and R-terpinene-4-ol have expanded the general knowledge of the chemical properties of mono- and bicyclic monoterpenes with hydroxyl and alkenyl functional groups. The structures of new compounds and their characteristics are included in the global data bank as new compounds, in particular, the structure of the diol based on (+)-sabinol, characterized by X-ray structural analysis, was registered by the Cambridge structural data bank.

The practical significance of the study lies in the possibility of using the synthesized compounds as potential therapeutic substances. Also promising is the use of synthesized aminodiols based on the sabinane bicyclic system as chiral catalysts for the synthesis of enantiopure compounds, which is very important for obtaining synthetic drugs with high purity and has a direct impact on their biological action.

The personal contribution of the author of the work consists in collecting, processing and analyzing literary data on the topic of the thesis, direct planning and carrying out the experimental part. The author participated in the analysis, interpretation and presentation of the obtained research results and their discussion, as well as in the preparation of scientific articles for publication in national and international journals.

The main provisions for the defense:

- study of the composition of essential oil isolated from the aerial parts of plants *Juniperus sabina* L.;

- methods for the isolation and purification of monoterpenoids (+)–sabinol and R-terpinen-4-ol;

- chemical transformation of (+)-sabinol;

- chemical transformation of R-terpinen-4-ol;

- biological activity (antiradical and cytotoxic) of synthesized derivatives;

- catalytic activity of aminodiols, diols and triols, stereoselectively synthesized from (+)-sabinol.

Conclusions on the results of the dissertation research:

1. The chemical composition of the essential oils of plants *Juniperus sabina* L. was investigated by chromato-mass spectrometry. As a result of the experiment, it was found that the volatile mixture of *Juniperus sabina* L. contains 42 compounds, giving a total of 100% of identified components. Sabinen, trans-sabinyl acetate, and terpinen-4-ol in *Juniperus sabina* L. EO are of interest for preparative isolation with quantitative contents of 37.92%, 31.47% and 10.87%, respectively.

2. An efficient procedure has been developed for the preparative separation of oxygen-containing monopenes, which made it possible to isolate (+)-sabinol and

R-terpinen-4-ol from *Juniperus sabina* L. EO with a purity of at least 95% in quantities (more than 1 g), sufficient for use as starting compounds for chemical transformation.

3. A series of 2-amino-1,3-diols based on a sabinane bicyclic system was synthesized stereoselectively. The synthetic pathway to the primary aminodiol (*136*) lay through the main reaction of Overman's rearrangement, the replacement of the trichloroacetamide protective group with the tert-butylcarbonate group, the dihydroxylation reaction and the removal of the protection group. The whole process of synthesis of the target molecule (*136*) consisted of 7 stages with indicators of the yields of reaction products more than 77%. Next, aminodiol (*136*) was transformed into secondary aminodiol (*137*) - N-benzyl derivative, and then the final ring-closure product spirooxazolidine (*138*) was obtained with a yield of 90%.

4. (+)-Sabinol was used as a starting compound for the stereoselective preparation of diols and triols. The diol was synthesized by a hydroboration-oxidation reaction using borohydride complexes BH_3*THF or BH_3*Me_2S, leading to the product in 47% and 70% yields, respectively. Synthesis of 2 diastreomeric triols was carried out in different synthetic pathways: first - by stereoselective dihydroxylation of sabinol with the OsO_4/N-methylmorpholine N-oxide system and, second - by alkaline hydrolysis of regioisomeric esters formed during the epoxidation reaction of sabinol with m-CPBA.

5. Two 1,5-disubstituted 1,2,3-triazoles were selectively obtained by a three-component reaction consisting of a primary amine based on a bicyclic monoterpene system, enolizable ketones and 4-nitrophenylazide using an organocascade process with product yields more than 50%. Also, as a result of the interaction of the primary amine and hexyl isocyanate, the ureid was obtained as a yellow oily substance with 95% yield.

6. Six new 2-amino-1,4-diols based on the p-menthane system were synthesized stereoselectively and in high yields using successive epoxidation reactions of R-terpinen-4-ol, followed by the opening of the oxirane ring with primary benzyl- and phenylamines, substituted in aromatic rings by different functional groups.

7. The chemical structures of the new synthesized compounds based on (+)-sabinol and R-terpinen-4-ol have been proven and analyzed using a combination of ^{1}H, ^{13}C NMR and Mass Spectroscopy, elemental analysis, GC-MS, HPLC. The absolute configurations of the molecules and the positions of the atoms were established using two-dimensional NMR spectra (^{1}H–^{1}H COSY, HSQC, HMBC, NOSY). In addition, the structure of the diol was investigated by X-ray diffraction analysis.

8. The new derivatives of sabinol (*131*), (*132*) and (*146*) were tested for antiradical and cytotoxic activity and it was established that all of them have cytotoxicity and do not possess the property of free radical scavenging.

9. Synthesized new chiral aminodiols, diols and triols based on (+)-sabinol were used as asymmetric catalysts in the model reaction of diethylzinc addition to benzaldehyde. As a result of the study, it was revealed that aminodiols turned out

to be more effective catalysts as compared to diol or triol derivatives with indicators *ee*>44%.

Approbation of the results. The main results of the thesis were tested in the form of an oral and poster presentation in the following conferences and symposia: Republican scientific-practical conference "Post-industrial world: green growth and green economy" (Ust-Kamenogorsk, November 24-25, 2016), V Scientific-practical conference "Modern aspects of the use of plant materials and raw materials of natural origin in medicine" (Moscow, Russian Federation, March 15, 2017), VI International scientific conference "Theoretical and experimental chemistry" (Karaganda, June 15-17, 2017) and the XVI International scientific symposium "16th Belgian Organic Synthesis Symposium" (Brussels, July 8-13, 2018), there were also discussed at a seminar at the University Szeged during research internship (Szeged, Hungary, 2018).

Publications. The main results of research on the topic of the thesis are presented in 9 published works, including 2 articles in international journals that have a non-zero impact factor according to the database of scientific journals Scopus (Q3) and Web of Science (Q2), 3 articles in journals included in the list recommended by the Monitoring Committee in the field of education and science of the MES RK, and 4 papers in collections of scientific papers of international and republican conferences.

The structure and scope of the thesis. The thesis work consists of an introduction, a literature review, an experimental part, the results of a study and their discussion, a conclusion a list of sited sources and annexes. The total amount of work is 117 pages of typewritten text and includes 45 figures, 21 tables and 168 names of sources used.