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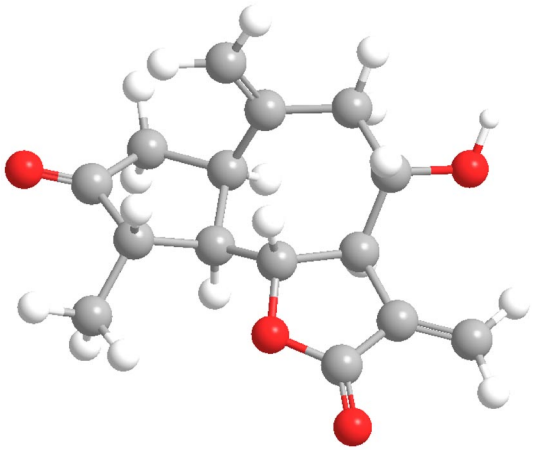

The International Scientific and Practice Conference

"ACHIEVEMENTS AND PROSPECTS FOR THE DEVELOPMENT OF PHYTOCHEMISTRY",

Dedicated to the 20th Anniversary of
International Research and Production Holding
"PHYTOCHEMISTRY"

10-11 of April 2015, Karaganda, Republic of Kazakhstan.

guest editor Professor Gayane A. Atazhanova

THE INTERNATIONAL SCIENTIFIC AND PRACTICE CONFERENCE "ACHIEVEMENTS AND PROSPECTS FOR THE DEVELOPMENT OF PHYTOCHEMISTRY", DEDICATED TO THE 20TH ANNIVERSARY OF "PHYTOCHEMISTRY" INTERNATIONAL SCIENTIFIC AND PRODUCTION HOLDING

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The International Scientific and Practice Conference "Achievements and Prospects for the Development of Phytochemistry" took place on the 10–11 of April 2015, Karaganda, Republic of Kazakhstan. It has been organized on the initiative of the Committee of Science, Ministry of Education and Science of the Republic of Kazakhstan, Ministry of Healthcare and Social Development of Republic of Kazakhstan, National Academy of Sciences, Akimat of Karaganda region and dedicated to the 20th anniversary of "Phytochemistry" international scientific and production holding". The Conference was attended by the Minister of Education and Science of the Republic of Kazakhstan, Akim (Governor) of Karaganda region, Deputy Minister of Healthcare and Social Development of the Republic of Kazakhstan, heads of a number of research institutes and universities, leading scientists and representatives of scientific centers in the field of chemistry of plant substances, pharmacology and drug technology from Germany, USA, China, Pakistan, Czech Republic, Russian Federation, Republic of Belarus, Uzbekistan, Kyrgyz Republic, Azerbaijan and Kazakhstan – in total more than 200 participants.

On the Conference the greeting of President of Republic of Kazakhstan N.A. Nazarbaev was read out

During the Conference, the exhibition within a campaign "Made in Kazakhstan" was organized with the participation of 10 domestic pharmaceutical enterprises which presented more than 400 drug products.

The Test Centre «National Centre of drugs inspection, medical devices and equipment» of the Ministry of Healthcare and Social Development of the Republic of Kazakhstan and "International Research and Production Holding Phytochemistry JSC signed memorandum of understanding and cooperation". The main objective of the memorandum is cooperation in the field of development and research of new medicinal herbal products and standard samples for their testing, development of national pharmacopeial standards for medicinal herbal.

Zhandos Akylserikovich Abishev
Deputy Akim of Karaganda Region

Signing of Memorandum between NCM MHSD and "Phytochemistry" holding JSC

165 plenary, oral and poster reports were presented and discussed at the Conference in the following fields: medicinal plants *ex situ*, *in situ* and *in vitro*, issues of pharmacognosy and pharmaceutical chemistry; low-molecular compounds in plants, chemical modification of molecules and their properties; pharmacological researches of plant substances and clinical trials of new phytopreparations; current issues of phytopreparation technology and introduction of quality control in pharmaceutical production.

The participants of the Conference noted a high level and the actuality of the presented reports and messages, as well as a wide range of the addressed issues. Most of the

presented reports showed a significant novelty, high quality and reflected the achievements in the field of fundamental and applied researches in the chemistry of plant substances. The results obtained by authors, have been already applied in practice or can be recommended for applying.

At the exhibition "Made in Kazakhstan"

Over the last 10 years there have been researched the pharmacological properties of 100 drugs based on the natural compounds and their derivatives which passed clinical trials and were registered at the end of 2013. Among these ones, 38 compounds are considered to be potential drugs against oncologic diseases, 26 compounds are used as anti-infective, 19 are for the treatment of cardiovascular system and metabolic disorders, 11 are against an inflammation as well as 6 compounds for the treatment of neurology. However, in the future, new types of drugs are likely to be discovered as it happened with the discovery of five herbal drugs over the last 15 years with new pharmacophores that have passed clinical trials.

In this regard, the report of Doctor of Sciences John A. Beutler from the National Cancer Institute, USA "Development of englerings for cancer" is of interest. Quantity of kidney cancer has increased for the last ten years and patient survival at the advanced stages remains very low. Guaianoic sesquiterpene englerings isolated from plants *Phyllanthus*, induce necrotic death of kidney tumor cells without covering normal cells. Englerings have strong and selective cytotoxic properties against kidney cancer cells at all stages, which makes them promising as a new anti-cancer drug.

In his report Professor Ludger Wessjohann (Germany) describes a new modern approach of fast and efficient technique for determining biologically active compounds without its preliminary isolation from plant extracts using modern computing correlation and chemoenzymatic techniques.

Directional chemical modification of native plant substance molecules opens prospects for the new derivatives with a high and purposefully altered biological activity at a relatively low toxicity. For example, the report of Doctor of Chemical Sciences, Professor E.E. Shults (Russian Federation), describes some opportunities of the directional synthetic transformations of available plant

Professor Ludger Wessjohann, Germany

diterpenoids – lambertianic and isopimaric acids and peucedanin furocoumarin using Cu-catalyzed reactions of 1,3-dipolar cycloaddition at the key stage.

Professor V.V. Veselovsky, Russian Federation

Widespread distribution among the natural metabolites of compounds with cyclopentane fragment with unique biological properties explains the interest of synthetic chemists of leading scientific centers in directed compound synthesis of this structure. The report of Doctor of Chemical Sciences, Professor V.V. Veselovsky (Russian Federation), describes the results of works performed at the N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, on investigation of synthetic potential of intramolecular dipolar [3+2] cycloaddition reaction of unsaturated silyl nitronic esters which ensures various stereoselective functionalization of cyclopentane ring.

The reports of Professors T. Macek (Czech Republic) and V.A. Khripach (Belarus Republic) present data of biological activity and use of steroid hormones. Discovered properties point at the prospectivity of the mentioned phytosterols as a base of new preparations for medicine. The special attention should be paid to their anti-cholesterol effect as well as adaptogenic, immunomodulatory, antiviral and anticancer activity.

Special aspects of biosynthesis of plant sesquiterpenoids and particularly possible pathways of biosynthesis of sesquiterpene lactone arglabin have been analyzed in the

Academician V.A. Khripach, Belarus Republic

Professor E. Kmonickova, Czech Republic

report of professor S.M. Adekenov (Kazakhstan). In addition to the above, there has been demonstrated an advantage of the enantio- and stereospecifity of biosynthesis of arglabin molecule from kauniolide in comparison with chemical synthesis which occurs forming a racemate.

In the reports of Professor P. Drašar (Czech Republic), Doctor of Chemical Sciences, Professors N. F. Salakhutdinov (Russian Federation), Zh. A. Abilov, K. M. Murzagulova, Yu. A. Shapovalov, G. Ye. Zhusupova, A. Zh. Turmukhambetov, B. I. Tuleuov (Kazakhstan) and others there have been summarized the results of extraction of biologically active compounds from plant raw materials, demonstrated the latest achievementsin contemporary organic synthesis of plant compound molecules and determined its structure and preparation of drugs on its basis.

Great progress has been achieved in the use of tissue cultures (methods *in vitro*) for bioscreening of natural compound samples and its derivatives. The models have been developed using isolated organs and tissue sections. A.V. Rat'kin (Russian Federation) presented in his report results of the research of hypolipidemic action of sesquiterpene γ-lactone achillin *in vitro* on a cell culture of rats' hepatoma.

Professor P. Drašar, Czech Republic

The report by Professor E. Kmonickova (Czech Republic), dedicated to the study of immunomodulatory activity of natural compounds using nitric oxide as a biological marker raised high interest.

The report of the famous scientist, pharmacologist D. A. Sychev (Russian Federation) dictated his work on the Conference to the issues of introduction of pharmacogenetic tests into clinical practice. He has developed a number of pharmacogenetic tests; however, the pace of implementation of pharmacogenetics into real clinical practice is low. In order to develop a pharmacogenetic test that forecasts the efficiency and safety of the anticancer drug "Arglabin", there are required the researches in the field of molecular biology, which are aimed to search for target molecules for this drug (pharmacodynamics) as well as molecular basis of its pharmacokinetics in a patient's body.

The latest achievements in search for leading compounds are related to a virtual screening. Improving of the computer technologies enables to search for successful compounds in the vast databases more efficiently. The Conference participants deem appropriate use of virtual docking of natural compounds and their derivatives. At the present time, Holding "Phytochemistry" is going to carry out a virtual docking of 15 derivatives of arglabin on 20 key enzymatic targets (ATP-ase inhibitors (antitubercular); COX-1 and 2 inhibitor (anti-inflammatory); P53-MDM2 inhibitors (anticancer); EGFR inhibitors (anticancer); CCK2 antagonist; Factor-XA inhibitors, etc.) to determine potential compounds – sources of new drugs. In addition, it is planned modeling the interrelations of 15 derivatives of arglabin with matrix proteins (complex "ligand-protein") and calculation of a constant for binding ligand and protein.

While standardizing raw materials, pharmaceutical substances and complete dosage forms there are used both pharmacopoeial standard samples and universal standard samples of active pharmaceutical substances and their impurities, standard samples of plant origin as well as physical and chemical standard samples to calibrate the measuring equipment. Standard samples are a factor that has a direct effect on quality of laboratory results, which allow to control the quality of all stages of drug production according to GMP standards. A comparative analysis of standard samples of drugs of plant origin according to the Pharmacopoeia of Europe, USA and Russia is presented in the report of G.Kh. Toleuova and colleagues (Kazakhstan). In addition, results of standardization of terpenoids, flavo-

noids, alkaloids and ecdysteroids to develop a temporary analytical regulatory document for standard samples (cynaropicrin, menthol, artemisinin, grossheimin, achillin, glaucine) are provided. Information on analytical standard samples that are used for quality control of drug raw materials, pharmaceutical substances and complete dosage forms has been summarized.

In the field of modern phytochemistry and nutriceuticals a significant attention is paid to creation of complex drugs containing different classes of biologically active substances by using standardized plant extracts. Producing the drugs with nanotechnology methods can improve the efficiency of active ingredients due to their selective delivery to the target organ and reduce possible side effects. Experimental data received at the "Russian Research Center for Molecular Diagnostics and Therapy" JSC (Moscow, Russian Federation) regarding the development of docetaxel-, silybin-, vinblastine-containing polymer nanocompositions demonstrate prospectivity for new approaches for creation high-performance systems for delivery of drugs, which, in turn, reduce their toxicity and increase the treatment efficiency of socially significant diseases.

One of the promising directions of phytochemical production is the development of new technologies for phytopreparation manufacture using solid-phase mechanochemical methods presented in the reports of Yu.S. Chistyachenko and L.P. Suntsova (Russian Federation). Implementation of mechanochemical processing technique of drug raw material will enable to increase solubility of native substances.

Report of Professor Kh.I. Itzhanova (Kazakhstan) presents a production technology of original phytopreparations as tablets using modern auxiliary ingredients and equipment for film tablet coating onto oral forms.

Professor Sh.Sh. Sagdullayev, Uzbekistan

Commercial output of substances of original drugs "Allapinin", "Aklezin", "Axaritmin", "Lycorine hydrochloride", "Galantamine hydrobromide", "Aconitine" with output of 80–85 % of content in the plant raw materials has been organized at the Institute of Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. Within the framework of new technology "Lipid concentrate" is produced from residuals of "Allapinin" and "Acsaritmin" drugs, which is used as a biologically active additive into the household chemistry products (report of Doctor of Technical Sciences, Professor Sh.Sh. Sagdullayev, Uzbekistan).

Professor V.F. Korsun (Russian Federation) points out in his report that today phytotherapy is an integral part of the modern clinical medicine of all countries of the modern world. It is suggested to include implementation of phytotherapy into the standards (protocols) for administering medical aid and to limit the time for processing the applications for new phytopreparations up to 1 year in the Pharmacological Committee of Ministry of Healthcare and Social Development of the Russian Federation. Also it was suggested to prepare and consider the state program of development of the phytopharmacology as one of the primary problem of population health.

Professor V.F. Korsun, Russian Federation

The Conference participants specifically noted the main achievements over 20 years of the International Research and Production Holding "Phytochemistry". Within 1997–2015 more than 500 plant species growing in Kazakhstan have been investigated. It has been established that 362 plant species are promising in terms of search for new biologically active compounds; more than 1000 natural compounds have been isolated and identified, on their basis over 2000 new derivatives have been synthesized which are categorized as terpenoids, flavonoids, steroids and alkaloids, some of which showed significant antimicrobial, antiviral, antifungal, antitumor, analgesic, phagocytosis-stimulating and other kinds of activities; 72 new original phytopreparations have been developed and production organized. Among these phytopreparations there are antitumor drug "Arglabin", hepatoprotector "Salsocollin", antiparasitic agent "Sausalin", hypolipidemic agent "Aterolid", adaptogenic drug "Ecdyphyt", etc. More than 1500 scientific papers have been published in the peer-reviewed and top-rated journals. 25 monographs have been released. More than 100 applications for inventions have been submitted, more than 85 patents for the Republic of Kazakhstan, 10 USA patents, 3 European patents, 3 Eurasian patents, as well as patents for China, Georgia and Ukraine have been obtained.

Within 1995–2015 14 theses of doctoral and 64 Candidate's among holding's co-workers have been defended.

The Karaganda Pharmaceutical Plant was built and put into operation on the base of Holding, where a full technological cycle has been developed and implemented, from the production of drug raw materials, including its processing to the manufacture of complete dosage forms of phytopreparations. The Republican Database of biologically active compounds and drugs has been established.

While the Conference took place, the participants visited laboratories of the International Research and Production Holding "Phytochemistry" and production facilities of the Karaganda Pharmaceutical Plant.

The Conference was on high scientific level, reports of contributors gave rise to active discussion and on the basis of work the appropriate decision was made.

The conference's participants recommended to held next international research and practice conference "Achievements and Prospects for the Development of Phytochemistry" on the basis of Irkutsk A. E. Favorsky Chemistry Institute of Siberian Branch of RAS in the June of 2017.

From Organizing Committee of
International Research and Production Conference
«Achievements and prospects for development of Phytochemistry»
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COMPARATIVE ANALYSIS OF CHEMICAL COMPOSITION OF PLANTS OF THE GENUS *ARTEMISIA* CONTAINING ARGLABIN OF RUSSIAN (BURYATIA) AND KAZAKHSTAN FLORAS

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Keywords: *Artemisia*, arglabin, CO_2 extraction, centrifugal chromatography, essential oil, chamazulene, matricarine.

This review presents data on the quantitative content of arglabin in aerial parts of *Artemisia jacutica* Drob., *A. adamsii* Bess. of flora of Buryatia, with carrying out the comparative analysis of the component composition of the essential oil and CO_2 extracts mentioned species of Artemisia and *A. glabella* Kar. et Kir. growing in Republic of Kazakhstan. Sesquiterpene lactone arglabin isolated for the first time from *A. adamsii*. Bess. with the method of centrifugal chromatography.

Introduction

In the world flora there are more than 500 species of the species *Artemisia* (*Compositae*)[1,2]. Most of species of *Artemisia* are applied to Central and West Asia, including Buryatia and Kazakhstan. The territory of Kazakhstan is a part of the Central Asian and Buryatia-Angarsk center of the origin of *Artemisia* in Eurasia[3]. From a wide range of biologically active substances, containing in *Artemisia*, the greatest attention of researchers is attracted to sesquiterpene lactones and essential oils[4,5]. From sesquiterpene lactones a specific interest is attracted to arglabin with antitumor activity and isolated from the aerial parts of endemic species of Kazakhstan *Artemisia glabella* Kar. et Kir.[6]. Essential oil of *A. jacutica* is a source of chamazulene – a non-toxic compound possessing an anti-inflammatory, bactericidal, regenerative effects[7].

The aim of this study is to reveal new sources of arglabin among the plants of *Artemisia* of Buryatia flora (Russia) and to provide comparative analysis of its chemical composition with *Artemisia glabella* Kar. Et Kir.

Experimental part

Data on the objects of study and characteristics of collecting ground are shown in Table I.

All mentioned *Artemisia* species are endemic. Systematic position of *Artemisia subviscosa* Turch. is currently comprehended by different authors at both levels, species *Artemisia subviscosa* Turch. ex Bess.[2,8] and subspecies of *A. obtusiloba* Ledeb. subsp. *subviscosa* (Turch. ex Bess.) Krasnob. comb. nov. et stat[9]. Previously, *Artemisia glabella* Kar. et Kir. was also considered as A. *obtusiloba* Ledeb.[2], currently it is considered as separate species[10].

Previously there was carried out screening on the presence of sesquiterpene lactones for which out of 5 g of mentioned raw materials aqueous, alcoholic, and chloroform extracts were extracted three times at heating. After cooling of aqueous extraction the extractive compounds were converted into chloroform. Sesquiterpene lactones detection was performed by using TLC method on the Sorbfil plates in the system containing acetonitrile:water (1:1). Identification was performed after treating chromatographic plates with 2 % solution of potassium permanga-

Table I

Objects of study (aerial parts)	District, collection date and phase of plant development
Artemisia adamsii Bess.	Russia, Republic of Buryatia, Ivolginsk district, surroundings of Gurulba village, 31.07.2014, flowering stage
Artemisia jacutica Drob.	Russia, Republic of Buryatia, Yeravninsk district, surroundings of Shiringa village, 21.08.2014, flowering stage
Artemisia glabella Kar. et Kir.	Republic of Kazakhstan, Karaganda, surroundings of Karaganda Botanical garden, flowering stage
Artemisia subviscosa Turch. ex Bess.	Russia, Republic of Buryatia, Barguzinsk district, surroundings of Ulun village, 01.08.2014, beginning of flowering stage

nate with largest R_f (0.39), in comparison with a standard solution of arglabin. Arglabin was detected in chloroform, alcohol and aqueous extracts of *Artemisia jacutica* and *Artemisia adamsii* however in *Artemisia subviscosa* it was not detected.

The presence of sesquiterpene lactones in these extracts was also confirmed by IR spectroscopy (Thermo Scientific Avatar 360 spectrometer, potassium bromide tablets, shooting area from 3800 to 600 cm^{-1}). The IR spectrum of *Artemisia adamsii* (chloroform extract) has absorption bands at 3394 (free OH^-), 2916, 2848 (joint by hydrogen bond OH^-), 1760 (C = O γ-lactone); *Artemisia jacutica* (chloroform extract), a broad band at 3444 (free OH^-), 2917, 2848 (joint by hydrogen bond OH^-), 1772 (C = O γ-lactone), 1505 (Ar), 1741 and 1239 (CH_3C = O), 756 (CH-); *Artemisia subviscosa* 2917, 2849 (joint by hydrogen bond OH^-), 1693 (α, β unsaturated -C=C-CHO), 1623 (-COO-), 1510 (Ar), 756 (CH-). The presence of the absorption bands in the range of 1740–1800 cm^{-1} (typical for C=O γ-lactone) indicates the presence of sesquiterpene lactones in the CO_2 extracts of *Artemisia jacutica* and *Artemisia adamsii*, and their absence in *Artemisia subviscosa*.

Carbon dioxide extracts were obtained on the device USFE-200Z. CO_2 extracts were obtained in two modes, at temperature of 40 °C, pressure 15 MPa and 35 MPa. All CO_2 extracts are pasty mixtures of yellow color with characteristic odour of Artemisia. The absorption bands were detected in IR spectra of carbon dioxide extracts of *Artemisia jacutica*, in *Artemisia adamsii* were also detected absorption bands typical for carbonyl of γ-lactone.

The quantitative content of arglabin in CO_2 extracts was determined by the method of reversed-phase HPLC on HEWLETT PACKARD Agilent 1100 Series in isocratic mode under the following conditions: analytical column filled with sorbent Zorbax SB-C_{18}, 4,6 x 150 mm, particles size 5 μm, composition of mobile phase: acetonitrile – water in correlation of 50:50; detection at wavelength of 204 nm; room column temperature, speed of mobile phase 0.5 ml/min; sample injection volume 20 μl. The content of arglabin in studied samples was determined by the comparison with the external standard. The content of arglabin in CO_2 extract isolated from the aerial part of *Artemisia adamsii* at 15 MPa was 23.2 % and at 35 MPa was 23.3 %. In carbon dioxide extract of *Artemisia jacutica* (p=35 MPa) the content of arglabin was 3.6%. The comparison of the obtained data with the literary ones showed that in *Artemisia adamsii* of Buryatia flora arglabin is produced in amount equivalent to *Artemisia glabella* (from 9 to 30.2 %) (ref.[11]).

Comparative evaluation of the chemical composition of *Artemisia adamsii*, *Artemisia jacutica* and *Artemisia glabella* consisted in the comparison of the component composition of essential oil and carbon dioxide extract. The qualitative composition and quantitative content of the substances in carbon dioxide extracts and essential oil of

Table II
Component composition of easy volatile fraction of CO_2 extract

Components	*Artemisia adamsii*	*Artemisia jacutica*	*Artemisia glabella*
Monocyclic monoterpenes			
1,8-Cineole	3.9	4.9	1.8
Terpineol-4	–	–	1.2
α-Terpineol	–	–	1.6
Bicyclic monoterpenoids			
Filifolone	0.8	–	–
Camphor	9.9	8.6	–
Borneol	1.3	–	–
Acyclic monoterpenoids			
cis-α-Ocimene	–	1.7	–
Linalool	–	–	1.4
Bicyclic sesquiterpenoids			
β-Eudesmol	–		0.6
β-Selinene	–	15.9	–
Long-chain hydrocarbons			
n-Tricosane	–	1.8	–
Pentacosane	–	2.2	–

Artemisia glabella were analyzed by the method of chromatography–mass spectrometry on gas chromatograph with mass selective detector Agilent 7890/5975C. The column HP-5MS 5 % Phenyl Methyl Silox (30 m × 0.25 mm) at a rate of carrier gas of helium of 1 ml/min was used. The temperature of evaporator was 230 °C. Gas chromatography column was tolerated at 40 °C for 10 min; with programming the temperature up to 240 °C at a rate of temperature change 2 °C/min and then tolerated in isothermal mode for 20 min. Sample injection mode – flow division 100:1. The conditions of recording mass spectra are 70 eV, mass range *m/z* 10–350. The percentage of the components was calculated automatically based on peak areas of total ion chromatogram. The quantitative analysis was performed by the method of internal normalization by the areas of peaks without using correction coefficients.

Components were identified by mass spectra and retention time using Wiley GC/MS library. The main components of essential oil of *Artemisia glabella* and volatile fraction of carbon dioxide extract are shown in Tables II and III. In CO_2 extract of *Artemisia adamsii* were identified 4 volatile compounds making 15.9 % of the sum of all components. Carbon dioxide extract *of Artemisia jacutica* represented by 6 components making 35.1 % of the sum of all components. Least of all components identified in CO_2 extract of *Artemisia glabella* (5 components) making only 6.6 % of the sum of all compounds. Such a low value of identified compounds in all three samples may be connected with the fact that CO_2 extract contains a large amount of "heavy" compounds difficult for separation by GC-MS. In the row of identified compounds the only common to all three samples is 1,8-cineole (1.8–4.9 %). In the composition of carbon dioxide extracts the presence of a significant number of bicyclic sesquiterpenoid β-selinene (15.9 %) in *Artemisia adamsii* and bicyclic monoterpenoid of camphor in *Artemisia jacutica* (9.9 %) and *Artemisia adamsii* (8.6 %) is noteworthy (Table II).

From air-dry raw materials of *Artemisia adamsii* and *Artemisia jacutica* by the hydrodistillation method the essential oils were extracted. The component composition of the oil of *Artemisia adamsii* and *Artemisia jacutica* were determined by the method of chromatography–mass spectrometry on gas chromatograph Agilent Packard HP 6890 N with quadruple mass spectrometer (HP MSD 5973) as a detector. There was used 30-meter-long quartz column HP-5 MSD with internal diameter 0.25 mm, film thickness 0.25 μm (copolymer of 5 % diphenyl of 95 % dimethylsiloxane), gas-carrier – helium, flow rate 1.5 ml/min, evaporator temperature 280 °C, column temperature 50 °C (2 min), 50–200 °C (4 °C/min), 200–280 °C (20 °C/min). 280 °C (isotherm 5 min), ion source 170 °C, interface between gas chromatograph and mass selective detector 280 °C. The energy of ionizing electrons equals to 70 eV, sample volume equals to 1 μl of solution with flow separation 20:1. The calculation of the linear retention indexes (*J*) was performed according to literature[12]. The percentage of the components was calculated automatically from peak areas of total ion chromatograms. Quantitative analysis was performed by the method of internal normalization by the areas of peaks without using correction coefficient. Components were identified by mass spectra and retention times using the library of chromatography-mass-spectrometry data NIST 11, A.V. Tkachev's library of volatiles of plant origin[12].

Essential oils of *Artemisia adamsii*, *A. jacutica*, *A. subviscosa* have common components, such as monocyclic monoterpenoids – α-terpineol, terpineol-4, 1,8-cineole, *p*-cymene, α-terpinene. Essential oils of *Artemisia adamsii* contain a large amount of a bicyclic ketone car-3-ene-5--one (12.4 %). Essential oils of *Artemisia glabella*, *A. adamsii* have common bicyclic monoterpenoid: camphor, borneol, bornyl acetate, α-pinene. Linalool (5.7 %), geranyl butyrate (1.5 %) were detected only in the composition of the oil of *Artemisia glabella* and neryl-2-methylbutanoate (4.8 %), neryl pentanoate (7.0 %), neryl-3--methylbutanoate (1.5 %) only in the oil of *Artemisia jacutica*.

The composition of "heavy" (sesquiterpenoids) compounds in the oils of three genera of *Artemisia* differs as well. Primarily the interest is attracted to a compound having anti-inflammatory properties – chamazulene. "Major" sesquiterpenoids in essential oil of *Artemisia adamsii* are alicyclic bicyclo germacrene (2.8 %) and tricyclic α-copaen (less than 0.5 %), β-copaen (3.1 %), spathulenol (2.6 %), in *Artemisia glabella* are monocyclic β-bisabolene (3.6 %), bicyclic β-selinene (1.1 %), the alicyclic aromadendrene (2.1 %) and (*E*)-nerolidol (1.1 %). In the essential oil of *Artemisia glabella* were found the representatives of not only terpene compounds but also of aromatic compounds, cuminol (2.3 %), *p*-isopropylbenzaldehyde (6.3 %) (Table III).

The separation of carbon dioxide extracts of *Artemisia adamsii* and *Artemisia jacutica* were performed using centrifugal partition chromatography on the installation FCPC-A200 (fast centrifugal partition chromatography). Further, a solvent system for separating CO_2 extract of *Artemisia jacutica* on the installation FCPC-A200 was selected; herewith the following solvent mixtures were used: hexane-ethyl acetate-ethanol-water at different ratios. The most optimal for separating CO_2 extract of *Artemisia jacutica* on the installation FCPC-A200 is a mixture of hexane: ethanol: water (6:5:1), as test sample is highly soluble in this solvent system and the compounds included in it are distributed between the upper and lower layer.

Conditions of separating 1,64 g of CO_2 extract of *Artemisia jacutica*: a sample of 1.5 g in 5 ml of upper phase, 5 ml in lower phase. UV detection at 210, 220, 254, 289 nm, rotary speed at 1600 rpm, method of separation is double, elution and extrusion: elution – tail-to-head (bottom-up eluent input), mobile phase – upper layer (nonpolar), eluent feed rate 7 ml/min for 70 min, fractions were collected according to the obtained chromatogram. Extrusion – tail-to-head (bottom-up eluent input), eluent feed rate 20 ml/min for 29 minutes, fractions were collected according to the obtained chromatogram, 101 fractions were collected. From the combined fractions 8–22 white

Table III
Main component of essential oils of *Artemisia adamsii*, *A. jacutica*, *A. glabella*

Components*	*J*	*Artemisia adamsii*	*Artemisia jacutica*	*Artemisia glabella*
Monocyclic monoterpenoids				
α-Terpinene	1017	1.3	+	1.4
p-Cymene	1024	1.5	+	6.8
α-Phellandrene	1028	+	+	1.6
1,8-Cineole	1031	24.4	3.0	9.3
γ-Terpinene	1058	2.3		1.9
Terpineol-4	1058	6.3	+	1.2
α-Terpineol	1191	3.2	+	2.7
Isopiperitenone	1273	1.1		
Bicyclic monoterpenoids				
α-Pinene	932	+	+	5.8
Camphene	947			1.4
Filifolone	1103	5.7		
Chrysanthenone	1126	6.3		
Camphor	1144	2.5		2.6
Borneol	1166	3.3	+	5.2
Bornyl acetate	1287	+		2.3
Car-3-en-5-on	1315	12.4		
Aromatic compounds				
p-Isopropyl-benzaldehyde	1222			6.3
Cumenol	1293			2.3
Acyclic monoterpenoids				
β-Myrcene	991	1.5	1.5	
cis-α-Ocimene	1038		+	2.5
Linalool	1100			5.7
Neryl 2-methyl-butanoate	1579		4.8	
Geranyl butyrate	1562			1.5
Neryl pentanoate	1636		7.0	
Neryl 3-methyl butanoate	1585		1.5	
Tricyclic sesquiterpenoids				
α-Copaene	1378	+	1.8	
α-Cubebene	1351		1.3	
β-Copaene	1432	3.1		
Spathulenol	1580	2.6	+	+
Monocyclic sesquiterpenoids				
Elemol	1553		2.6	
β-Bisabolene	1511			3.6
Bicyclic sesquiterpenoids				
Caryophyllene	1422		1.4	
Selina-4,11--diene	1477		2.1	
β-Selinene	1488			1.1
γ-Eudesmol	1633		29.8	
Chamazulene	1730		17.1	
Acyclic sesquiterpenoids				
Aromadendrene	1440			2.1
Bicyclogermacrene	1500	2.8	+	
(*E*)-Nerolidol	1565	+		1.1
Regrouped monoterpenoids				
Lavandulyl-*n*--hexanoate	1657		1,84	

Note: *in the table are given the components, the content of which is ≥0.5%. Sign "+" indicates components the content of which is less than 0.5%.

crystalline substance was isolated after column chromatography. On the basis of spectral data (IR, UV, NMR ^{1}H, ^{13}C), the substance was identified as a sesquiterpene lactone matricarin.

Also there was separated a CO_2 extract of *Artemisia adamsii* via centrifugal partition chromatography. For the separation were taken 2.00 g of CO_2 extract of *Artemisia adamsii*, the system of solvents heptane: ethyl acetate: acetonitrile (2:1:2), the sample 1.00 g in 3 ml of upper phase, 7 ml of mobile phase, 10 ml of sample was introduced into the chromatograph, UV detection at 210, 220, 254, 289 nm, rotary speed at 1600 rpm, method of separation is double, elution and extrusion, 94 fractions were collected. From fractions 18–44 white crystalline substance was isolated. The IR spectrum data (KBr, ν_{max}, cm^{-1}): 3510; 3036; 2994; 2926; 2850; 2740; 1871; 1767; 1667;

1648; 1442; 1408; 1307; 1254; 1155; 1134; 1112; 1091; 1028; 996; 883; 839; 813; 799; 653; 590; 535; 448; 431; 408. The UV analysis data: 201 nm. Substance melting temperature is 99–101 °C. On the basis of spectral data (IR, UV, NMR ^{1}H, ^{13}C), the substance was identified as a sesquiterpene lactone arglabin. The purity of isolated arglabin by HPLC is 99.8 %.

Thus, the data obtained by us indicates that *Artemisia adamsii* Bess.*, Artemisia jacutica* Drob. contain sesquiterpene lactones, including arglabin in quantities close to its content in *Artemisia glabella*. Lack of arglabin in *Artemisia subviscosa* Turch. ex Bess. can testify in favor of the autonomy of this species. Essential oil of *Artemisia jacutica* Drob. is perspective for further use as it contains a large amount of chamazulene.

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DEVELOPMENT OF ENGLERINS FOR CANCER THERAPY

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Key words: the sesquiterpene englerin A, antitumor agent, *Phyllanthus engleri*

In this review the discovery and development of the sesquiterpene englerin A and its analogs for cancer treatment are summarized.

Introduction

Discovery

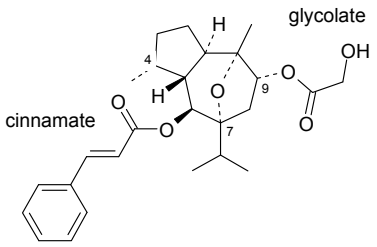
The National Cancer Institute has conducted a screening program against 60 human cancer cell lines for the last 25 years[1]. The cells were selected from eight common organs of tumor origin: non-small cell lung cancer, colon cancer, CNS cancers, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer. Several leukemia cell lines have also been utilized, as a reference to previous NCI anti-leukemia drug discovery efforts. The initial concept was to find agents which affected only a single tumor type; however it was rapidly found that very few compounds met that criterion. Recent advances in cancer biology have shown just how genetically and biologically diverse cancers are[2,3], making the NCI 60 premise that one could find organ-specific antitumor agents entirely untenable.

Nonetheless, we chose to re-examine the specificity hypothesis for renal cancers, due to the relative genetic and metabolic homogeneity of cancers of the kidney[4]. We asked the bioinformatics question – out of all natural product extract samples tested in the NCI 60 screen, which extracts showed selectivity for the nine renal cancer cells in the panel over all other cancer cell lines? Out of 68,000 tests, 34 samples met the criteria and were examined manually. One extract stood out among the rest, an organic solvent extract of the root bark of the Tanzanian tree *Phyllanthus engleri* Pax (Euphorbiaceae). The stem bark extract showed similar activity to the root bark, but none of the extracts of other plant parts of the collection were active[5].

Phyllanthus engleri has a documented history of ethnomedical use in eastern Africa, the most common use being smoking the bark to relieve chest congestion or cough, with the proviso that it must be used very carefully, as inhaling too much drug can be fatal. It has been reported to be used for suicide in the same way[6], and to be poisonous to cattle and sheep who consume the plant. Other medical uses include tooth removal, and treatment of bilharzia, gonorrhea, and epilepsy[7].

Bioassay-guided fractionation using a two day cell growth inhibition assay with kidney cancer cells led to the isolation of englerin A as the active compound, with the related englerin B having a similar structure but having no activity. A sequence of diol batch elution, silica gell flash chromatography and preparative C-18 HPLC was used. Englerin A reproduced the pattern of NCI 60 selectivity seen in the crude extracts of the plant. The planar structure was solved by standard two-dimensional NMR experiments, and the relative configuration established by nOe experiments, however it was not possible to obtain the absolute configuration at this stage[5].

Englerin A is a guaiane sesquiterpene diester, with an uncommon oxygen bridge spanning the seven membered ring. Two alcohols of the core structure are esterified respectively by a cinnamic acid moiety and a glycolic acid moiety[5]. Due to the unique structure and potent biological activity, patents were filed covering the composition of matter and utility in cancer, and US, European, Australian and Japanese patents have recently issued[8–11].



Englerin A

During one instance of bioassay guided fractionation of the extract, we found a series of chlorinated englerins which were determined to be artifacts arising from the use of aged chloroform as a chromatography solvent. Since the artifactual compounds possessed renal specificity, they were purified and elucidated. The compounds were apparently formed by dichlorination across the cinnamate double bond, and subsequent dehydrohalogenation to monochloroenglerin A, the most active of the series[12].

Despite the presence of 176 samples from 33 other *Phyllanthus* species in the NCI extract collection, no other extracts derived from the genus has shown renal cancer selectivity.

Synthesis and Reisolation

The publication of the structure and biological activity of englerin A in early 2009 was followed in short order by the total synthesis of the (+)-isomer by the Christmann group. This isomer was found to be biologically inactive, thereby indicating that the absolute configuration of the natural material was (–)[13]. Subsequent synthesis of the (–)-isomer by numerous groups using several different synthetic methodologies[14–24] confirmed the assignment. The synthesis developed by the Chain group stands as the most step and mass-efficient preparation of the natural product to date, requiring only eight steps in 20 percent overall yield[20].

The plant has thus far been a reliable source of the compound. A recollection made in Tanzania at the same site as the original collection yielded 6.4 g of englerin A from 7 kg of stem and root bark. No survey has yet been made of englerin content of different populations of the plant in Tanzania and other adjacent countries.

Development

Spectrum of antitumor activity

Englerin A is a potent inhibitor of cell growth in most renal cancer cell lines, in a few breast cancer cell lines, two CNS tumor cell lines, and two ovarian cancer cell lines, all in the NCI 60 screen[5]. Activity against bladder cancer cell lines has been reported as well. A high throughput screen for inhibitors of the Ewing sarcoma target EWS-Fli1conducted in our lab[25] identified the extract of *P. engleri* as active, and testing of englerin A confirmed that was potently active as well. Recently, we screened englerin A against a large panel of sarcoma cell lines, and found that 10 of 20 Ewing sarcoma cell lines were sensitive, while most other sarcoma cell lines were resistant.

In vivo testing

Englerin A has demonstrated activity in mouse models of kidney and prostate cancer[26]. While the lack of sensitivity of prostate cancer cell lines would seem a poor prognosis for in vivo activity, inhibition of prostate cancer cell growth in vitro by englerins has been shown to vary with different media conditions. We have linked this to the effects of englerin A on glycolysis (see below). Several other mouse studies are in progress.

Mechanism of action

A detailed study of englerin A effects on signaling pathways in renal cancer cells found that the compound stimulated the activity of protein kinase C, in particular, isoform theta. This agonism was far more selective than that of phorbol esters[27,28], or bryostatin[29], which are relatively non-selective PKC ligands.

In 786-0 kidney cancer cells, englerin A induces an insulin-resistant phenotype, causing phosphorylation of heat shock factor 1 (HSF1), while down-regulating the insulin transporter Glut1 through phosphorylation of insulin receptor substrate 1 (IRS1), thereby blocking the PI-3 kinase/Akt pathway. This dual effect leads to metabolic catastrophe[26]. Note that both pathways appear to be required for cell growth inhibition; if either HSF1 or IRS1 is lacking in tumor cells, the compound has no effect[26].

A recent paper proposed a different target, activation of transient receptor potential channels 4 and 5 (TRPC4/5), which was shown to lead to abrupt influx of calcium in A498 kidney cancer cells[30]. An effect on calcium had also been noted by the Ramos group in A498 cells[31].

The relative importance of the two postulated mechanisms to inhibition of cancer cell growth in vivo remain to be determined. It is possible that they are connected, although PKCθ is not a calcium-sensitive isoform. It should be noted that we have observed hypoglycemia and modulation of the phosphorylation of two targets of PKCθ (IRS1 and GSK3β) in tumors from treated animals, giving further support to the PKC θ hypothesis[26].

Medicinal Chemistry

Development of several different synthetic methods has led to the synthesis of numerous analogues of the natural product[22,32–38]. Pertinent structure activity relationships include the following:

1) Loss of the glycolate ester leads to complete loss of activity[35]. Modification of the glycolate with small isosteres, such as an acetate ester[22], retains the pattern of selectivity, but often with substantial loss of potency. The most interesting and active modification at this site is a reverse ester prepared by the Chen group[32].
2) Modification of the cinnamate ester, on the other hand, is well-tolerated. Replacement of the cinnamate double bond with a cyclopropyl group leads to a potent compound[32], while incorporation into a β-napthoate ester also maintains potency[34]. Aromatic substitution on the cinnamate is generally poorly tolerated[34].
3) The humble 7-isopropyl substituent is critical for activity, since a change to ethyl- or methyl-substitution rapidly reduces potency[34]. Deletion of the fused cyclopentyl ring is also fatal to activity[37], as is deletion of the 4-methyl group[38].

Pharmaceutical Parameters

At the NCI, formulation studies and preliminary pharmakokinetic studies have been conducted, but as yet there have been no publications reporting this work. Toxicology studies have not yet been commenced.

Conclusion

Englerin A and its derivatives present a compelling case for pharmaceutical development as drugs to treat a limited range of cancers, particularly renal cancers and Ewing sarcomas. The fundamental requirements for drug development are largely in place for rapid development with appropriate investment.

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INSECT REPELLENT AND FEEDING DETERRENT ACTIVITY OF NATURAL SESQUITERPENE LACTONES AND THEIR DERIVATIVES

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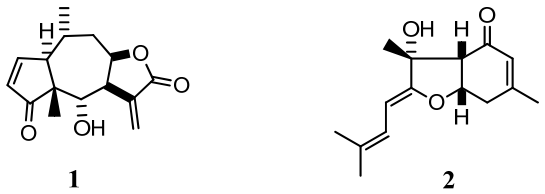

1 **2**

Fig. 1

Keywords: Insect antifeedants, stored products pests, *Sitophilus granarius*, *Trogoderma granarium*, *Tribolium confusum*, *Trialeurodes vaporariorum*, *Tenebrio molitor*, sesquiterpene lactones

Introduction

The study of biological activity in extracts from different plant species, as reported in several reviews[1–3], proved that sesquiterpene lactone-containing species have an expressed effect on insect feeding[3–10]. Significant antifeedant activity was reported to be found in bakkenolide-A, alantolactone and helenalin[6–8]. Sesquiterpenoids: helenaline (1) and bisabolangelone (2) (see Fig. 1) are moreover toxic to larvae and cause morphogenetic changes in pupae of *Tribolium confusum* Duv. and *Trogoderma granarium* Ev. The adults treated by those compounds laid fewer eggs and had shorter survival[6].

Sesquiterpenes of plant origin belong to major determinants of the plant-insect interactions manifesting as a significant factor of chemical ecology[9–11]. Attempts to explain the mode of action, or even advances to elucidate the mechanism of activity, are still only at the level of consideration[10–16]. For a better explanation, it is still more and purposeful investigation required.

In this paper are reported and discussed recent results of our studies on antifeedant and repellent activities of a series of so far unexplored sesquiterpene lactones and their chemically transformed derivatives.

Table I
Sesquiterpene lactones selected for testing, and their origin

Compounds	Plant species	References
Inuchinenolide C (3)	*Inula caspica* Blume	17
Britanine (6)	*Inula caspica* Blume	17
Pulchelline C (29)	*Inula caspica* Blume	17
Alantolactone (23)	*Inula helenium*	18
Isoalantolactone (25)	*Inula helenium*	18
α-Santonine (27)	*Artemisia gracilescens* Krasch. et Iljin	19
Achilline (11)	*Achillea micrantha* Willd;	20
Grossmisine (15)	*Achillea micrantha* Willd;	20
Hanfilline (30)	*Achillea nobilis* L:	21
Estafiatine (12)	*Achillea nobilis* L:	21
Stizolicine (31)	*Stizolophus balsamita* (Lam., Cass. ex. Takht)	22
Arglabin (16)	*Artemisia glabella* Kar. et Kir.	23
Argolide (32)	*Artemisia glabella* Kar. et Kir.	23
Grosheimin (21)	*Chartolepis intermedia* Boiss	24
Gaigranine (9)	*Gaillardia grandiflora* Hort.	25
Spatuline (10)	*Gaillardia grandiflora* Hort.	25

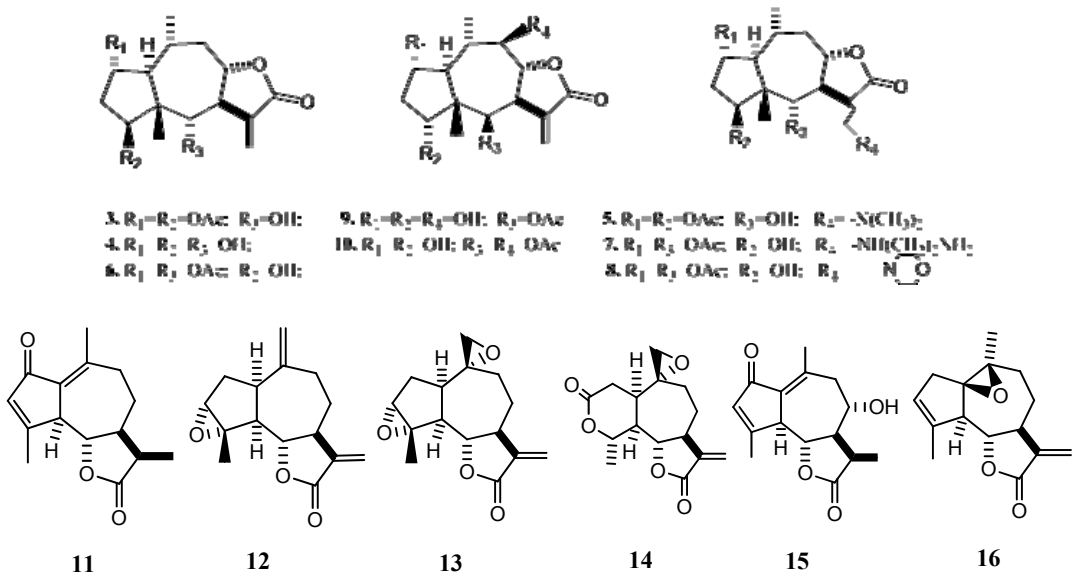

Fig. 2

Materials and methods

Chemicals

The tested compounds were isolated from various plants, as shown in Table I. Majority of these sesquiterpene lactones are already known compounds, as reviewed and characterized e.g., in ref.[16].

For the structure-activity relationship study the following derivatives of the compounds listed in Table I were used in our bioscreening: didesacetylinuchinenolide C (4), dimethylaminoinuchinenolide C (5), ethylendiaminobritanine (7), morpholynobritanine (8), epoxyestafiatine (13), epoxy-δ-lactone of estafiatone (14), epoxyarglabin (17), dimethylaminoarglabin (18), morpholinoarglabin (19), hydrochlorid dimethylaminoarglabin (20), acetate of grosheimin (22), epoxyalantolactone (24), epoxyisoalantolactone (26), semicarbazone α-santonine (28). Their preparation and structural identification was published in our previous communications[26–29].

Test methods

The laboratory tests were carried out in two stages.

The first stage test was performed on two insect test-objects: on a confused flour beetle (*Tribolium confusum*) and on an imago of greenhouse whitefly (*Trialeurodes vaporariorum*) with gnawing and sucking systems, respectively. The experiments were carried at 24 °C for about 18 daylight hours. The two-week old imagoes of 36 hours-unfed flour beetles were involved in tests. The cut of kidney bean leaf with 25 mm diameter was treated by 0.5 % solution of the tested compound. After drying, the cuts were placed to Petri dishes where the beetles were added. The tests were carried out in two replicas, in two cuts and fifteen beetles for each compound.

In the cases of whitefly, the cut pieces of young kidney bean plants were treated by dissolved compounds, dried, and then placed into glasses with water. The imagoes of whitefly were ice-anesthetized and placed near treated plants and covered by exposimeter. The activities of compounds were evaluated by findings of the first two recordings. During the first recording (24 hours) the rate of species' deaths in the hunger control after 24 hours was almost 100%. Second set of recordings was carried out in 10 days after beginning of the tests, where the presence of still alive individuals, and born of first age larvae per leaf were measured.

The repellent activity in trials on *Tenebrio molitor* L. (Tenebrionidae) was evaluated through the observation of the specimen's movements within the first hour of the experiment. The clearly expressed repellency (R) was shown in beetle's preferred grouping near the farther part of the dish. In control group, the beetles ate all food within the first 15–20 minutes.

The second stage test concerns feeding preferences. At this test, it was used a method of capsules in order to check how 9 selected compounds affected the feeding inhibition of *Sitophilus granarius* L. (Curculionidae) adults and *Tribolium confusum* Duv. (Tenebrionidae) adults, and also *Tribolium confusum* larvae and *Trogoderma granarium* Duv. (Dermestidae) larvae. The test compounds were

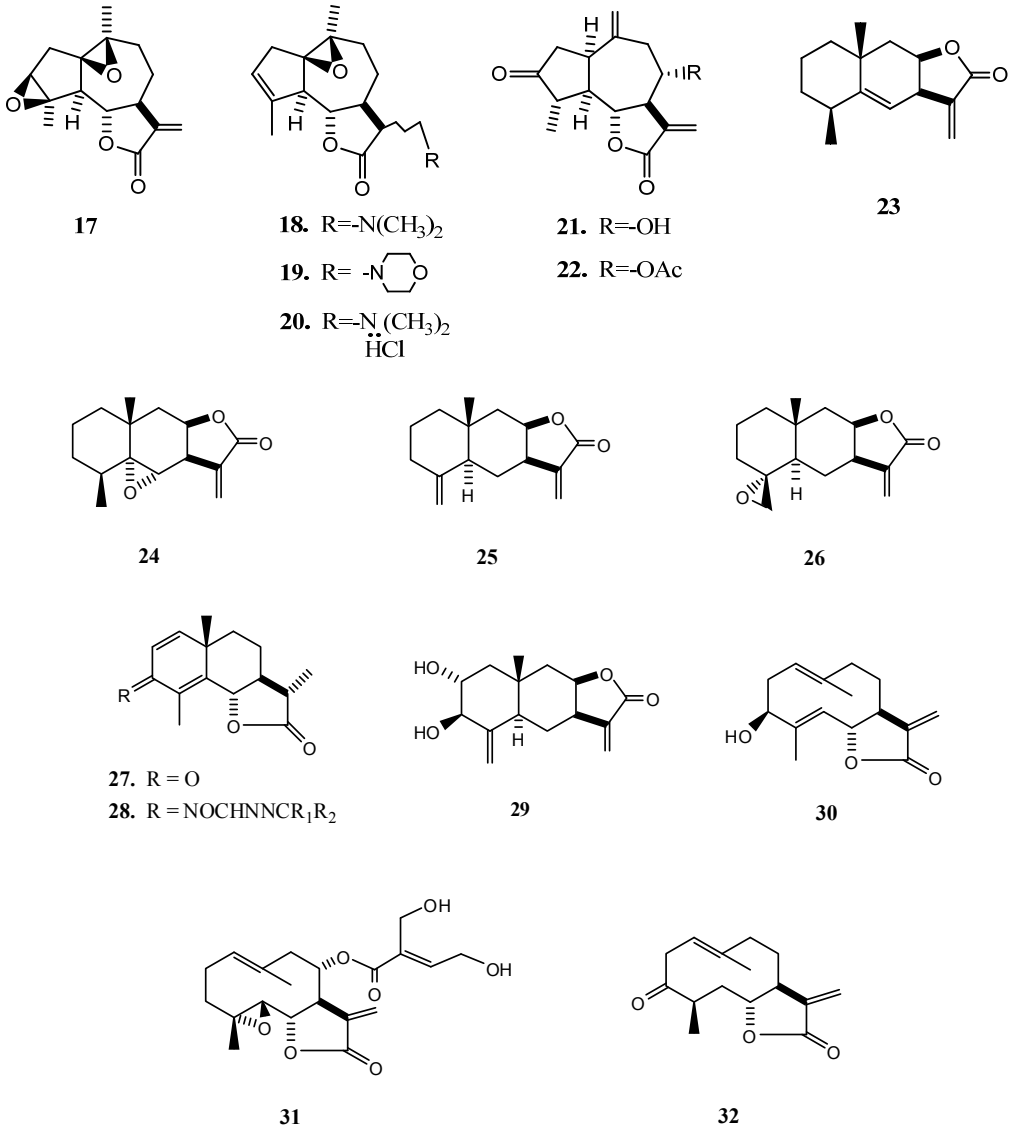

Fig. 3

six sesquiterpene lactones isolated from the Compositae (Asteraceae) family, and three derivatives of those natural compounds. From the structural type point of view, these compounds ranged in: two guaianolides (16) and (20), six pseudoguaianolides: (3), (4), (5), (6), (9), (10), and 1 eudesmanolide (29) (for classification see ref. 16).

Disks with wheat capsules were used for feeding the insects. Disks (1 cm in diameter) were saturated by ethanol solutions of the compounds (concentration 10 mg/ml). All experiments were carried out by the same scheme. The imagoes (representing the adult stage of insects) of *Sitophilus granarius* (3 adults) and *Tribolium confusum* (20 adults) and also the larvae of *Trogoderma granarium* (10 larvae), *Tribolium confusum* (10 larvae) were used as the test models. The insects were fed in three variants:

(1) – clean food (control test: with two untreated disks: KK);
(2) – food with possibility of choice (choice test: with one untreated disk: K and one disk treated with the tested compound: E);
(3) – food with the two disks: EE, treated with tested compounds in question only (test without choice: non-choice test).

The treated and untreated disks were placed into boxes (two in each box; at five replications); upon which there was conducted a test without a choice, and then in combi-

nation with the test containing the untreated disk giving the possibility to choose (choice test).Wafer disks were weighed after 30 min air-drying prior to the experiment and once again in 7 days after their feeding to the beetles and larvae. On the basis of the weight of the food eaten there was obtained the activity index of the tested compound as follows. Three coefficients were calculated from the weight of food which had been eaten during the variant KK, during the test without choice EE and with the possibility of choice KE (for details, see ref. 6):

a) absolute coefficient of deterrence,

$$A = \frac{KK - EE}{KK + EE} \times 100$$

b) relative coefficient of deterrence,

$$R = \frac{K - E}{K + E} \times 100$$

c) general (total) coefficient of deterrence,
T=A+R

The general coefficient was used as an index of activity. Strong (very good) antifeedants had an index: 150–200, good antifeedants: 100–150, average antifeedants: 50–100, while the index of the compound which had been neutral during the experiment was 0–50. Negative value of the coefficient (< 0) mens, that it concerns a feeding attractant.

Statistical analysis: The values of deterrence coefficients were statistically analyzed by means of one-way analysis of variance ANOVA. In the cases where ANOVA

Table II
Test results of repellent activity of sesquiterpene lactones and their derivatives on the flour beetle (*Tenebrio molitor*)

Compound	Repellence	% rate of eaten fodder in 48 hours
Inuchinenolide C (3)	R	67
Didesacetylinuchinenolide (4)	-	84
Britanine (6)	R	5
Ethylendiaminobritanine (7)	-	19
Morpholynobritanine (8)	-	68
Gaigranine (9)	-	57
Spatuline (10)	-	37
Achilline (11)	-	93
Estafiatine (12)	-	87
Epoxyestafiatine (13)	-	95
Epoxy-δ-lactone of estafiatone (14)	-	85
Grossmisine (15)	-	83
Arglabin (16)	R	7
Epoxyarglabin (17)	R	7
Dimethylaminoarglabin (18)	R	3
Morpholinoarglabin (19)	-	93
Dimethylaminoarglabin hydrochloride (20)	R	62
Grosheimin (21)	-	83
Grosheimin acetate (22)	-	87
Alantolactone (23)	-	87
Epoxyalantolactone (24)	R	12
Isoalantolactone (25)	-	85
Epoxyisoalantolactone (26)	R	12
α-Santonine (27)	-	91
Semicarbozon α- santonine (28)	-	75
Pulchelline C (29)	-	82
Hanfylline (30)	-	81
Stizolicine (31)	-	59
Argolide (32)	-	92
Control (water-ethanol)	-	87

results were statistically significant, Tukey's test was performed.

Results and discussion

The effects of sesquiterpene lactones and their derivatives on *Tenebrio molitor* are listed in Table II. The compounds (3), (6), (16) and (20) have clearly expressed repellency (R). Compounds (17) and (18) have also repellent activity, but to the less extent. The antifeedant activity was evaluated per the amount of eaten food. The compounds (3), (6), (16), (17), (18), and (20) displayed antifeedant activity to *Tenebrio molitor*. The presence of intestinal activity was estimated by the death rate of beetles within 48 hours. The studied compounds have no intestinal activities.

Test results (summarized in Table III) obtained by experiments on the greenhouse whitefly (*Trialeurodes vaporariorum*) attested the compound (6) with a rather high activity. Compounds (16), (23) and (25) exhibited also antifeed-ant activities in the same test, as well as the compound (12), but to the less extent. It should be noted, that alantolactone (23) was registered earlier[6] as a compound, which inhibits the feeding of *Sitophilus granarius, Tribolium confusum* beetles (adults), and also larvae of *Tribolium confusum* and *Trogoderma granarium*.

Six compounds: (3), (4), (5), (16), (20) and (29) attracted our attention by their activities, which were probably enhanced by the particularities of their structures.

Table III
Test results of activities of sesquiterpene lactones and their derivatives on imago of greenhouse whitefly (*Trialeurodes vaporariorum*)

Compound	% of death in 24 hours	Presence of imago and larvae of the first stage on the plants in 10 hours
Inuchinenolide C (3)	37	+/+
Didesacetylinuchinenolide (4)	41	+/+
Britanine (6)	35	+/+
Ethylendiaminobritanine (7)	23	+/+
Morpholynobritanine (8)	21	+/+
Gaigranine (9)	14	+/+
Spatuline (10)	10	+/+
Achilline (11)	16	+/+
Estafiatine (12)	32	–/+
Epoxyestafiatine (13)	17	+/+
Epoxy-δ-lactone of estafiatone (14)	25	+/+
Grossmisine (15)	24	+/+
Arglabin (16)	75	–/–
Epoxyarglabin (17)	22	+/+
Dimethylaminoarglabin (18)	34	+/+
Morpholinoarglabin (19)	5	+/+
Dimethylaminoarglabin hydrochloride (20)	14	+/+
Grosheimin (21)	15	+/+
Grosheimin acetate (22)	34	+/+
Alantolactone (23)	36	–/–
Epoxyalantolactone (24)	35	+/+
Isoalantolactone (25)	49	–/–
Epoxyisoalantolactone (26)	19	+/+
α-Santonine (27)	4	+/+
Semicarbozon α- santonine (28)	13	+/+
Pulchelline C (29)	31	+/+
Hanfylline (30)	4	+/+
Stizolicine (31)	30	+/+
Argolide (32)	7	+/+
Control (water-ethanol)	–	+/+

Table IV
Deterrent activity of the tested compounds (General coefficients of deterrence)

	Variant 1 *Tribolium confusum* Duv. (beetles)	Variant 2 *Tribolium confusum* Duv. (larvae)	Variant 3 *Trogoderma granarium* Ev. (larvae)	Variant 4 *Sitophilus granarius* L. (beetles)
Britanine (6)	163	196.7	127	156
Inuchinenolide C (3)	124.6	143.8	87.56	107.9
Pulchelline C (29)	137.2	162.3	71	98.8
Arglabin (16)	171.1	180	69.5	88.2
Gaigranine (9)	124.4	106.5	148.6	97.7
Spatuline (10)	128.2	89.6	104.8	79.4
Dimethylamino-inuchinenolide C (5)	69.6	166.7	126.7	135.2
Didesacetyl-inuchinenolide C (4)	–29.4	99.5	24	42.7
Dimethylamineарglabin hydrochloride (20)	74.7	131.7	66.6	150.5

The stated compounds were studied at the second stage of experiments. The results of those tests (see Table IV) showed that britanine (6) was the best antifeedant against all pest species; inuchinenolide C (3) and pulchelline C (29) were good feeding deterrents (antifeedants) against the tested species. The weakest antifeedant effect against the tested insects showed compounds (4) and (20).

The best antifeedants against the adults of *Tribolium confusum* were britanine (6) and arglabin (16), good deterrents were compounds (3), (9), (16) and (29), average deterrents were compounds (5) and (20).

It was shown, that amine addition to the lactone moiety of the natural compounds de-creased their activities against the adults of *Tribolium* sp. The same decreased activity was registered in case of didesacetylized derivative (4) of the natural compound (3), which turned out to be even an attractant to the adults of *Tribolium confusum*.

The best antifeedants against the larvae of *Tribolium confusum* were compounds (5), (6), (16) and (29). The compounds (4) and (10), on the contrary, showed the weakest deterrent effect.

The highest index of deterrence was registered in case of larvae *Trogoderma granarium*, when the capsules were saturated by compounds (5), (6) and (9). The other natural lactones and their derivatives were only average or weak deterrents.

The best antifeedants against the adults of *Sitophilus granarius* were compounds (5), (6) and (20). The rest of compounds turned out to be only average or weak deterrents. In general, it was shown, that the compounds with similar molecular structure, but with different substituents or additional structure moieties demonstrated the influence of those constituents on their activities. An exception was only the compound (4), a derivative of the native lactone (3), whose activity was significantly lower than the activity of the natural one. The chemically prepared dimethylamino adduct (5), as well as the natural arglabin (16) showed also lower activities compared to that of other natural compounds.

Effect on reproduction.

Based on the above referred tests, the highly effective lactones (3), (6) and (29) were chosen for treating the grains of wheat in a dose of 10 mg / 100 g granule = 100 ppm (see Tables V and VI).

Female and male insects, 3 pairs of *Sitophilus granarius* beetles and 5 pairs of *Tribolium confusum* pupae were used as follows:

– on clean grains (control group),

– on grains treated by the compounds.

After 30 days of the feeding in these experiments (with *T. confusum, S. granarius*), the quantity of posterity and the weight of the wasted dust were checked.

The first parameter in question was the number of posterity. *Sitophilus granarius* females were less fecund on the grains treated by antifeedants. The posterity of *Tribolium confusum* was somewhat less numerous on the grains treated by pulchelline C (29).

The highly effective antifeedants tested on grain, did not shown obvious influence on the posterities of the insects tested.

The second parameter tested, was a weight of wasted dust which could identify the intensity of feeding. Feeding of *Sitophilus granarius* beetles on the grain pollinated by the best antifeedants was as intensive as in the case of the control grain.

Compound (29) obviously decreased the weight of the wasted dust, and only this sesquiterpene lactone inhibited the feeding of *Tribolium confusum* beetles almost to 50 %.

Table V
Feeding inhibition of *Sitophilus granarius* L. (experiments on the wheat-grain granules treated by dosing 10 mg / 100 g granule = 100 ppm)

	VARIANT 1	VARIANT 2
Control	100,0	100,0
Pulchelline C (29)	14,7	0,1
Britanine (6)	64,1	59,2
Inuchinenolide C (3)	21,8	–13,8

Table VI
Experiments with *Sitophilus granarius* L. (experiments on the wheat grain granules treated by dosing 10 mg / 100 g granule = 100 ppm)

	VARIANT 1	VARIANT 2
Control	24	24
Pulchelline C (29)	17	22
Britanine (6)	17	10
Inuchinenolide C (3)	17	15

The observed difference can be caused by a difference in the way of feeding of those pests. *Sitophilus granarius* ate through the coat of grain in several places, and after having biting through the pericarp saturated by the compounds, they ate the clean endo-sperm. Contact of the beetles with antifeedants, which were pol-linated on the grain surface, was very short, and if there was no other food for feeding, perhaps, it has come to overcoming of the protecting barrier. *Tribolium confusum* gnawed out a large hole on the grain surface and had to be in contact with the antifeedant for a long period. That's why the sensitivity of this class was stronger and more distinct in contact with the antifeedants.

The active antifeedants were mixed with flour in proportion of 100 mg/kg, and after adding the dextrin and maltose, there were formed into wheat granules as big as grains. The material prepared for the tests filled the whole volume of granules, and insects of *Sitophilus granarius* had been fed on them during the whole period of experiment, i.e. 30 days. Compounds (3), (6) and (29) were used in the first series of experiments. Feeding intensity, which was evaluated by the number of eaten granules, reduced to 60% for britanine (6) and to 20% for the rest of antifeedants. The number of posterity decreased to 30 %.

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S. M. Adekenov[a], G. M. Mukhametzhanova[a], G. A. Atazhanova[a], and Juraj Harmatha[b] *([a] JSC "International Research and Production Holding "Phytochemistry",4 Gazaliyev Str., Karaganda, Republic of Kazakhstan, [b] Institute of Organic Chemistry and Biochemistry ASCR, Prague, Czech Republic):* **Insect Repellent and Feeding Deterrent Activity of Natural Sesquiterpene Lactones and their Derivatives**

Thirty compounds were investigated in order to detect their repellent and antifeedant activity. The substances were tested on two insect test-objects: 1. flour beetle (*Tenebrio molitor*) and 2. imago of greenhouse whitefly (*Trialerodes vaporariorum*) using gnawing and sucking apparatus, respectively. Furthermore, it was used a method of capsules made from flour, in order to evaluate their influence on feeding inhibition of *Sitophilus granarius* beetles (adults) and *Tribolium confusum* beetles, as well as on larvae of *Tribolium confusum* and *Trogoderma granarium.* Sesquiterpene α-exometylene-γ-lactones: inuchinenolide C, britanine, pulchelline C, arglabin, epoxyarglabin and structurally transformed dimethylaminoarglabin showed high antifeedant and repellent activities. Some other compounds, as e.g., the transformed dimethylaminoarglabin hydrochloride, have only a low antifeedant activity, however, high repellent activity. Some regularity between the structure and activity was also revealed.

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MOLECULAR DOCKING OF ARGLABIN AND ITS DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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Key words: sesquiterpene lactone, arglabin and its derivatives, molecular docking, topoisomerase, biological screening, modelling.

Introduction

Virtual screening is one of the modern approaches to the directed search for new drugs. The key procedure of virtual screening, based on knowledge of spatial structure of biological target, is the molecular docking. Docking allows to predict spatial structure of the complex and to evaluate the quality of the complex binding ligand-protein. Therefore, it is possible to predict the presence or absence of biological activity for natural compounds, including a number of terpenoids, which are widespread in plants.

One of practically available terpenoids is a sesquiterpene lactone arglabin (1), obtained in industrial conditions at Karaganda Pharmaceutical Plant from raw material of *Artemisia glabella* Kar. et Kir.[1–3]. In the capacity of renewable material on the basis of this guaianolide there is carried out a series of chemical modifications of its molecule. As a result, over 100 new compounds were obtained[4].

The article presents the results of molecular docking on the enzyme systems of human DNA topoisomerase I and IIB type for 19 molecules of sesquiterpene lactone arglabin and its chemically modified derivatives. Also there were compared the results of computer prediction with conducted biological researches in test systems *in vitro* and *in vivo*.

Materials and metods

Methods of molecular docking conduction

Objects of research were sesquiterpene lactone of guaian-type – arglabin (1) and its 18 derivatives: dimethylaminoarglabin hydrochloride (2), dichlorocarbene arglabin (3), tetrachlorocarbene arglabin (4), pentachloroarglabin (5), dibromocarbene arglabin (6), dimethylaminoarglabin (7), dimethylaminoarglabin methyl iodide (8), diethylaminoarglabin (9), diethylaminoarglabin methyl iodide (10), dimethyl phosphonate arglabin (11), diethyl phosphonate arglabin (12), dipropyl phosphonate arglabin (13), *(E)*-phenylarglabin (14), citizinilarglabin (15), *(Z)*-phenylarglabin (16), hydroxyarglabin (17), phenylarglabin (18), 3(4)α-epoxyarglabin (19).

Molecular docking was performed on the enzyme systems of human DNA topoisomerase I and IIB type. This class of enzymes was not chosen by chance since it is known that topoisomerases are targets of many drugs, in particular, with antitumor activity.

Virtual spatial structures of human topoisomerases were obtained from a public database of protein molecules PDB (Protein Data Bank)[5].

Three-dimensional spatial structures (3D) of sesquiterpene lactone arglabin (1) and its derivatives were made via LigPrep program from the developer Schrodinger Small-Molecule Drug Discovery package (http://www.schrodinger.com). The protonated molecules positions were attributed to physiological conditions.

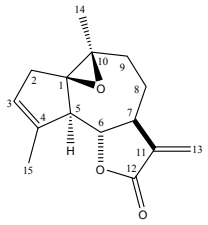
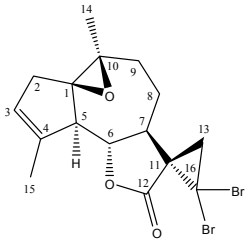
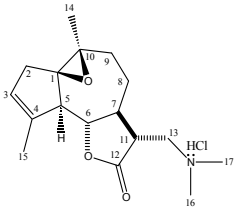
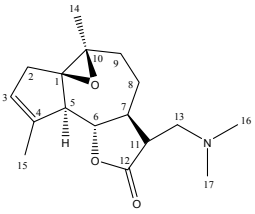
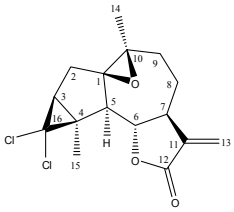
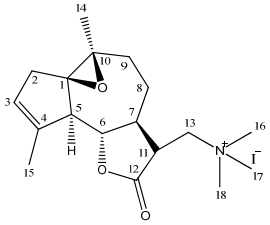
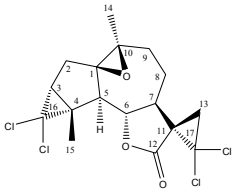
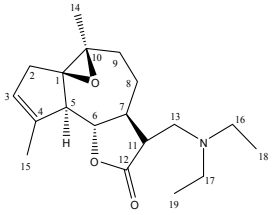
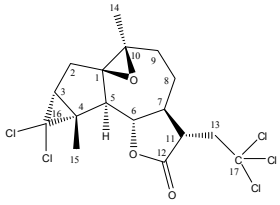
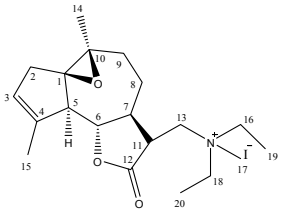
For molecular docking were used three models of topoisomerase: human DNA topoisomerase I with indolocabazole SA315F (PDB-ID: 1SEU)[6], human DNA topoisomerase I with indenoisoquinoline AI-III-52 (PDB-ID: 1TL8)[7], human DNA topoisomerase IIB with etoposide (PDB-ID: 3QX3) (Fig. 1)[8].

The ligands were removed from all active sites of all three enzymes – topoisomerases I and IIB type. Meanwhile, the DNA molecules were retained. Molecular docking was performed via Glide program from the developer Schrodinger Small-Molecule Drug Discovery package (http://www.schrodinger.com). For each compound of sesquiterpene lactone arglabin (1) and its derivatives were predicted ten positions of binding and were calculated and analyzed values of G-score for enzymes – DNA topoisomerases. Herewith, a low (negative) value of G-score indicates a strong intermolecular force.

Methods of biological screening conduction

Antitumor activity of compounds was studied on white outbred rats with transplantable tumors of mice and rats. Antitumor activity of studied compounds was determined by daily intraperitoneal injection of 2 % solution of dimethyl-sulfoxide (DMSO) within 5 days at maximum tolerated dose. For evaluation of antitumor activity of the compounds there was used the percentage inhibition of tumor growth and the value of increasing life expectancy determined directly after treatment. Examination results were processed statistically with calculating t-criterion

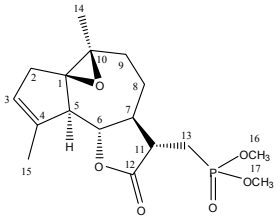
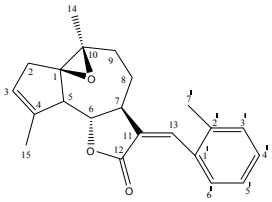
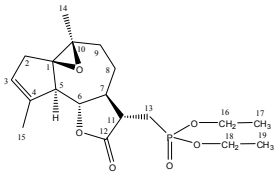
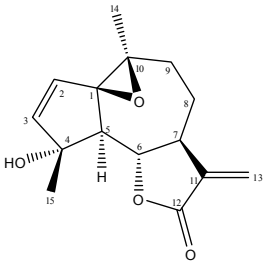
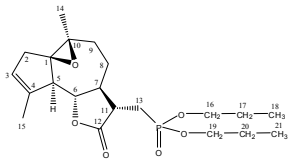
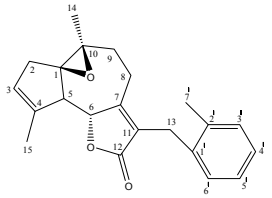
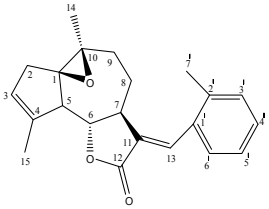
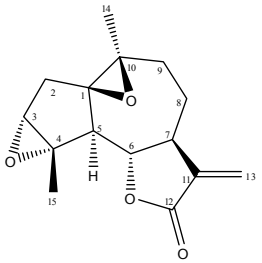
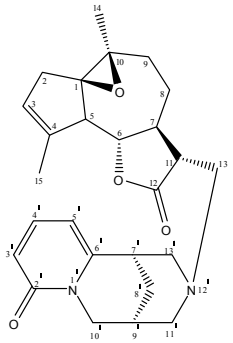
Table I
Structural formulae of sesquiterpene lactone arglabin (1) and its derivatives

№	Name of compound	Two-dimensional molecular structure	№	Name of compound	Two-dimensional molecular structure
1.	Sesquiterpene lactone arglabin $C_{15}H_{18}O_3$		6.	Dibromocarbene arglabin $C_{16}H_{18}O_3Br_2$	
2.	Dimethylamino-arglabin hydro-chloride $C_{17}H_{25}O_3NHCl$		7.	Dimethylaminoarglabin $C_{17}H_{25}O_3N$	
3.	Dichlorocarbene arglabin $C_{16}H_{18}O_3Cl_2$		8.	Dimethylaminoarglabin methyl iodide $C_{18}H_{28}O_3NI^-$	
4	Tetrachloro-carbene arglabin $C_{17}H_{18}O_3Cl_4$		9.	Diethylaminoarglabin $C_{19}H_{29}O_3N$	
5.	Pentachloro-arglabin $C_{17}H_{19}O_3Cl_5$		10.	Diethylaminoarglabin methyl iodide $C_{20}H_{32}O_3NI^-$	

according to Student's test.

The cytotoxic activity of compounds was evaluated in the test on survival of marine crustaceans larvae *Artemia salina* (Leach). The experiments were conducted on 2 day old larvae under conditions of cultivation *in vitro*. The larvae were grown by immersion of eggs of marine crustaceans *Artemia salina* (Leach) in artificial seawater and were incubated for 48 hours at temperature 37 °C. The sample of test specimen was dissolved in 2 ml of methanol, then out of this solution was taken 500 µl (3 parallels),

Continuation of Table I

№	Name of compound	Two-dimensional molecular structure	№	Name of compound	Two-dimensional molecular structure
11.	Dimethyl phosphonate arglabin $C_{17}H_{25}O_6P$		16.	*(Z)*-phenylarglabin $C_{22}H_{24}O_3$	
12.	Diethyl phosphonate arglabin $C_{19}H_{29}O_6P$		17.	Hydroxyarglabin $C_{15}H_{18}O_4$	
13.	Dipropyl phosphonate arglabin $C_{21}H_{33}O_6P$		18.	Phenylarglabin $C_{22}H_{24}O_3$	
14.	*(E)*-phenylarglabin $C_{22}H_{24}O_3$		19.	3(4)α-epoxyarglabin $C_{15}H_{18}O_4$	
15.	Citizinilarglabin $C_{26}H_{32}O_4N_2$				

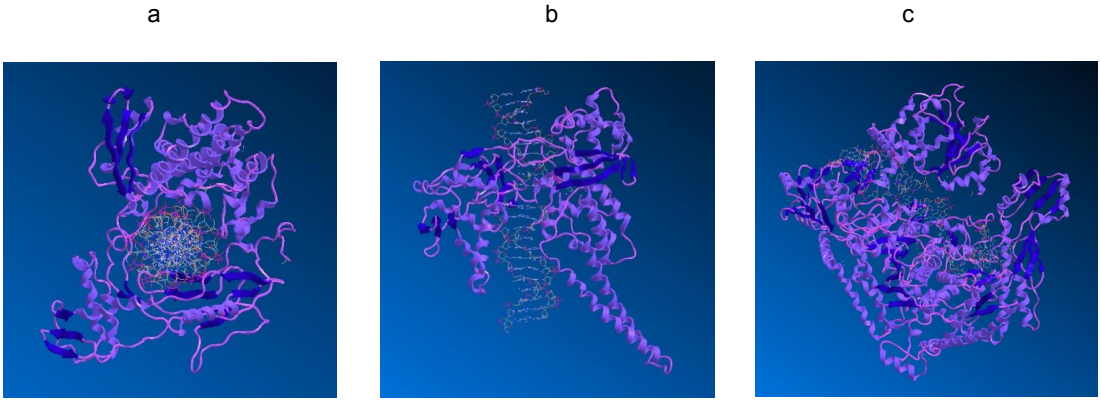


Fig. 1. **a) Human DNA topoisomerase I with indolocabazole SA315F; b) Human DNA topoisomerase I with indenoisoquinoline AI -III-52; c) Human DNA topoisomerase IIB with etoposide (PDB-ID: 3QX3)**

50 µl (3 parallels), 5 µl (3 parallels). After evaporation of methanol into each vial was added 5 ml of artificial sea-water. Thus, if the initial mass of a sample was 2 mg, the final sample concentration was 100 µg/ml, 10 µg/ml and 1 µg/ml, respectively, each concentration in 3 repetitions. Into each bottle with sample via Pasteur pipette were placed 10 marine crustacean larvae *Artemia salina* (Leach) 2 days of age. After 24 hours survived and dead larvae were counted. Then using the obtained data on the upper and lower toxic limit, a half toxic dose of the sample was calculated[9].

The study of derivatives of arglabin (1) on phagocytic activity of blood cells was held in whole blood. 0.1 ml of heparinized venous blood (20 ED per 1 ml of blood) was taken from the ulnar vein of donor, brought into the hole of immunological plate and incubated with 0.05 ml of test substance for 10 minutes in a thermostat at 37 °C. Then diurnal culture of staphylococcus *Staphylococcus aureus* in volume of 0.05 ml were put into the holes with blood and 0.025 ml into the holes with leukocytic suspension, concentration of staphylococcus was 20 microbial bodies per cell. Again samples were incubated in a thermostat at 37 °C for 15 minutes.

Then the contents of holes were transferred to defatted glass slide and were thermostated for 20 minutes at 37 °C under conditions of moist chamber. Then dabs were dried in the air in a vertical position, the hemolysis of erythrocytes with distilled water for 3 minutes was performed, after that the dried dabs were fixed for 30 minutes in a mixture of Nikiforov (ether: 96%, alcohol 1:1) and stained with azure-eosin for 10 minutes. When microscoping (10x40 magnification, oil immersion) there was counted a number of phagocytic neutrophils (phagocytic index) per 200 phagocytes and a number of staphylococci absorbed by one neutrophil (phagocytic number)[10].

Determination of anti-inflammatory properties was carried out on white outbred male rats 180–200 g in weight. The experimental and control animals were kept under standard conditions of vivarium with temperature 18 –20 °C and free access to water and food.

Antiexudative action of lactones was studied on a model of aseptic inflammation. Acute inflammatory edema was simulated by subplantar injection into rat rear paw of phlogogenic agent – 0.1 ml of 1% carrageenan solution. Measurement of paw volume was performed *via* plethysmometer before the experiment, in 4, 24 hours after experiment beginning and thereafter every other day. The test substances were administered intraperitoneally at doses of 1 and 10 mg/kg. The control group of animals was injected saline in equivalent quantities. As the comparison drug was used a solution of Diclofenac sodium at a dose of 25 mg/kg. Results were compared in groups. The pronounced anti-inflammatory effect was judged by the degree of edema inhibition[11].

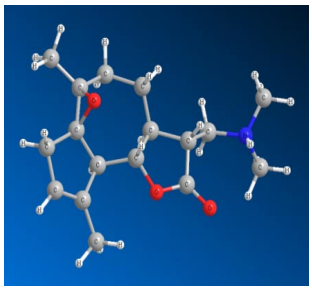
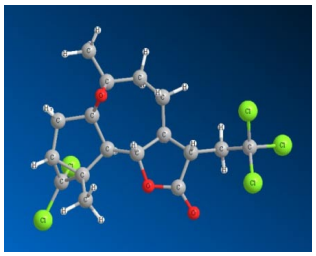
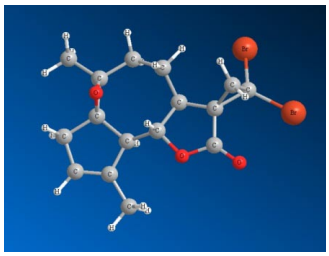
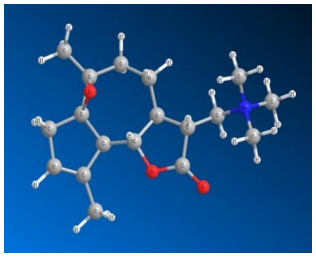
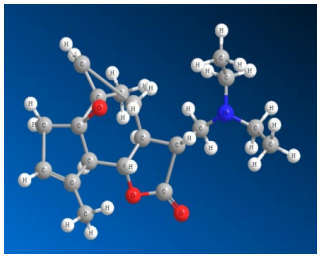
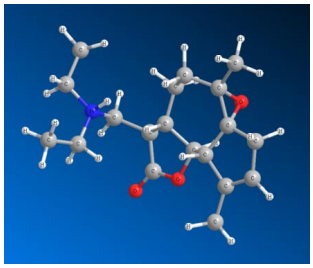
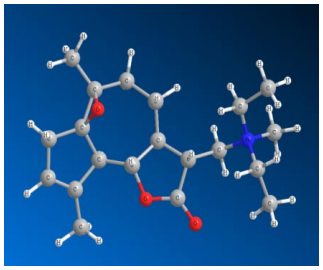
Results and discussions

Docking allows evaluating the quality of the binding of molecule with the target only in the presence of spatial structure of the intermolecular complex ligand-target. Therefore, for conduction of molecular docking on the enzyme systems of human DNA topoisomerase I and IIB type, it was necessary to carry out full three-dimensional (3D) modeling and visualization of studied compounds (Table II).

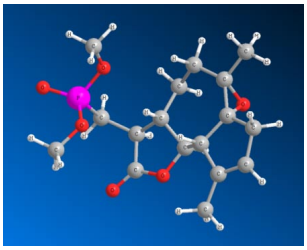
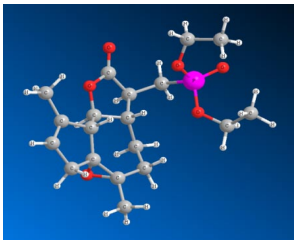
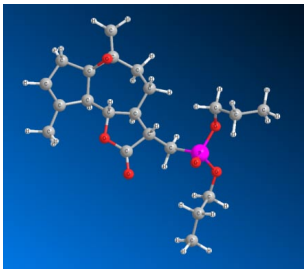
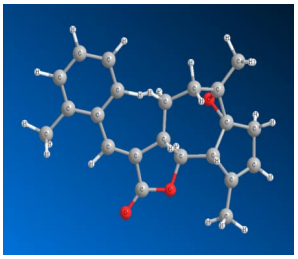
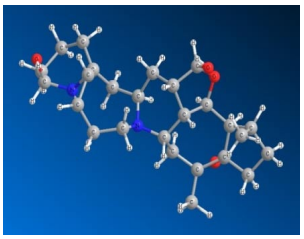
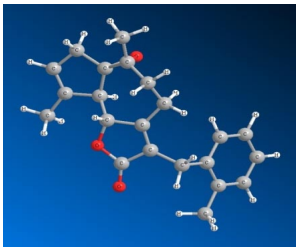
Conversion of two-dimensional (2D) molecular structures of sesquiterpene lactone arglabin (1) and its 18 derivatives to three-dimensional (3D) spatial structures was carried out via the program LigPrep. Herewith, an indispensable condition was the protonated molecules position of the compounds under study.

When performing docking of the molecules of sesquiterpene lactone arglabin (1) and its derivatives on the first model, namely, on human DNA topoisomerase I type with indolocarbazole SA315F (PDB-ID: 1SEU), the fol-

Table II
(3D) spatial structure of studied compounds

Compound No.	Three-dimensional (3D) spatial structure	Compound No.	Three-dimensional (3D) spatial structure
1.		2.	
3.		4.	
5.		6.	
7.		8.	
9.		10.	

Continuation of Table II

Compound No.	Three-dimensional (3D) spatial structure	Compound No.	Three-dimensional (3D) spatial structure
11.		12.	
13.		14.	
15.		16.	
17.		18.	
19.			

lowing values of G-score (Gold score) were obtained. Meanwhile, the values of G-score reflect the cohesion from more stable to less stable in descending order (Table III).

As it is shown in the table, the values of G-score are not great and indicate a weak intermolecular bond. The predicted binding modes were inhomogeneous, i.e. there

Table III
Data on ligand-protein docking for the model of DNA topoisomerase I type (PDB-ID: 1SEU)

Compound No.	G-score value in descending order
Dipropyl phosphonate arglabin (13)	–7.14
Hydroxyarglabin (17)	–6.92
Dimethyl phosphonate arglabin (11)	–6.61
(E)-Phenylarglabin (14)	–6.58
Dimethylaminoarglabin hydrochloride (2)	–6.56
(Z)-Phenylarglabin (16)	–6.56
Dimethylaminoarglabin methyl iodide (8)	–6.51
Phenylarglabin (18)	–6.25
Diethylaminoarglabin methyl iodide (10)	–6.23
Citizinilarglabin (15)	–6.18
Diethyl phosphonate arglabin (12)	–5.79
Diethylaminoarglabin (9)	–4.88
Dimethylaminoarglabin (7)	–4.72
Tetrachlorocarbene arglabin (4)	–4.64
Dichlorocarbene arglabin (3)	–4.59
Dibromocarbene arglabin (6)	–4.57
Sesquiterpene lactone arglabin (1)	–4.41
Pentachloroarglabin (5)	–4.32

Table IV
Data on ligand-protein docking for the model of DNA topoisomerase I type (PDB-ID: 1TL8)

Compound No.	G-score value in descending order
Dimethylaminoarglabin hydrochloride (2)	–6.79
(Z)-Phenylarglabin (16)	–6.62
Phenylarglabin (18)	–6.61
Hydroxyarglabin (17)	–6.44
Dimethyl phosphonate arglabin (11)	–6.39
Citizinilarglabin (15)	–6.34
Diethylaminoarglabin methyl iodide (10)	–6.24
Dipropyl phosphonate arglabin (13)	–6.21
Dimethylaminoarglabin methyl iodide (8)	–5.54
Tetrachlorocarbene arglabin (4)	–5.22
Diethyl phosphonate arglabin (12)	–5.19
Sesquiterpene lactone arglabin (1)	–5.08
Pentachloroarglabin (5)	–4.75
Diethylaminoarglabin (9)	–4.67
Dimethylaminoarglabin (7)	–4.44
Dibromocarbene arglabin (6)	–4.04
Dichlorocarbene arglabin (3)	–3.44
(E)-Phenylarglabin (14)	–2.46

Table V
Data on ligand-protein docking for the model of DNA topoisomerase IIB type (PDB-ID: 3QX3)

Compound No.	G-score value in descending order
Dimethylaminoarglabin methyl iodide (8)	–10.42
Diethylaminoarglabin (9)	–9.82
Dimethylaminoarglabin hydrochloride (2)	–9.45
Diethyl phosphonate arglabin (12)	–8.94
(E)-Phenylarglabin (14)	–8.53
Hydroxyarglabin (17)	–8.45
Diethylaminoarglabin methyl iodide (10)	–8.44
Dimethyl phosphonate arglabin (11)	–8.27
(Z)-Phenylarglabin (16)	–8.21
Phenylarglabin (18)	–7.99
Dipropyl phosphonate arglabin (13)	–7.50
Sesquiterpene lactone arglabin (1)	–7.43
Pentachloroarglabin (5)	–7.12
Citizinilarglabin (15)	–6.87
Dibromocarbene arglabin (6)	–6.20
Tetrachlorocarbene arglabin (4)	–5.65
Dichlorocarbene arglabin (3)	–5.19
Dimethylaminoarglabin (7)	–4.66

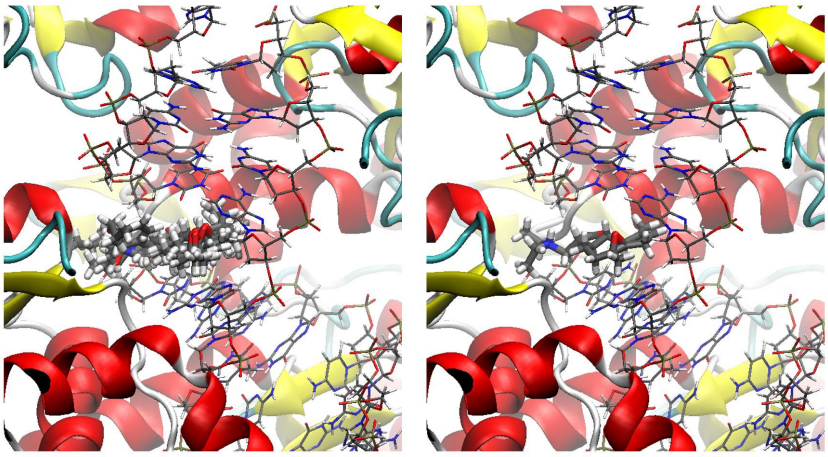


Fig. 2. **3D picture of spatial arrangement of compounds for which the best binding positions are predicted**

was a difference in the position of binding between compounds. Aromatic component in compounds dipropyl phosphonate arglabin (13) and hydroxyarglabin (17) was intercalated based on DNA, and that proved the link being nonspecific and not being an interlink of functional group of arglabin.

When carrying out docking on the model of human DNA topoisomerase I with indenoisoquinoline AI-III-52 (PDB-ID: 1TL8) were obtained the following values of G-score (Table IV).

In the process of docking was detected high degree of inhomogeneity of binding among the tested compounds. Also, a low affinity for structures cocrystallized with different ligands was predicted.

The third model of human DNA topoisomerase IIB type with etoposide (PDB-ID: 3QX3) showed the best values of G-score (Table V).

Compounds sesquiterpene lactone arglabin (1), dimethylaminoarglabin hydrochloride (2), pentachloroarglabin (5), dimethylaminoarglabin methyl iodide (8), diethylami-

Table VI
Antitumor activity of sesquiterpene lactone arglabin (1) and its dimethylaminoarglabin hydrochloride (2)

№	Name of compound	Dose mg/kg	Tumor cells growth inhibition, %											
			Pliss lymphosarcoma	Walker carcinosarcoma	Geren carcinosarcoma	Sarcoma-45	M-1 sarcoma	Alveolar mucous liver cancer PC-1	P-388 Leukemia	Leukemia L-1210	Resistant variants			
											Sarcoma-45		Pliss lymphosarcoma	
											to 5-fluorouracil	to sarcolysine	to rubomycinum	to prospidinumy
1	Comparative drug Colchamine	2	54.4	30.1		20.4		26.5			19.6			
2	Sesquiterpene lactone arglabin	30	57.6	41.1	48.0	23.0	55.6	32.1	43.0	34.0		59.7	44.1	31.0
3	Dimethylaminoarglabin hydrochloride (2)	50	52.0	76.1	86.5	83.1		80.0	109.0		13.2			

noarglabin (9), diethylaminoarglabin methyl iodide (10), diethyl phosphonate arglabin (12), and hydroxyarglabin (17) showed comparable positions of binding, as these compounds have substantially high values of G-score pointing to the specific intermolecular bond (Fig. 2).

Based on the results of molecular docking on enzyme system of human DNA topoisomerase IIB type with etoposide, was selected a number of compounds, namely, sesquiterpene lactone arglabin (1), dimethylaminoarglabin hydrochloride (2), pentachloroarglabin (5), dimethylaminoarglabin methyl iodide (8), diethylaminoarglabin (9), diethylaminoarglabin methyl iodide (10), dipropyl phosphonate arglabin (13), phenylarglabin (18). These compounds showed relatively high values and stable positions of binding energy which are of great interest for further research.

Antitumor activity of sesquiterpene lactone arglabin (1) and its dimethylaminoarglabin hydrochloride (2) was confirmed experimentally in *in vivo* tests on white outbred rats with transplantable tumors of mice and rats.

Herewith, dimethylaminoarglabin hydrochloride (2) showed relatively high antitumor activity against M-1 sarcoma, Walker carcinosarcoma, sarcoma-45, Geren carcinosarcoma, alveolar mucous liver cancer PC-1 and Pliss lymphosarcoma. It also had a significant effect on P-388 leukemia (increase of longevity 109%) (Table VI)[12].

Derivatives of sesquiterpene lactone arglabin (1) dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) showed cytotoxic activity in the test on survival of marine crustaceans larvae *Artemia salina* (Leach) under conditions of cultivation *in vitro*.

Studies were carried out on cytotoxic activity of the derivatives (8) and (10) obtained by alkylation of diethylaminoethylamine (9). Within the experiment there was studied the effect of different concentrations of investigated material on the survival of marine crustaceans larvae *Artemia salina* (Leach)[13].

It was experimentally determined that derivatives of arglabin (1) dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) show cytotoxicity at a concentration of 100 μg/ml. Herewith, LD_{50} of the derivative (8) is 26.9 μg/ml, while LD_{50} of the derivative (10) is 72.7 μg/ml. The results of the study of cytotoxic properties of investigated compounds are presented in Table VII.

As can be seen from the table, the derivatives of arglabin dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) show high cytotoxic activity. However, the cytotoxic activity of the derivative (8) is considerably higher than the derivative (10).

Table VII
Cytotoxic activity of arglabin derivatives dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10)

№	Compound	Percentage of dead marine crustaceans larvae, after 24 hours, %			LD_{50}, μg/ml	95% fiducial interval
		100 μg/ml	10 μg/ml	1 μg/ml		
1.	Dimethylaminoarglabin methyl iodide (8)	100	3.33	0	26.9	18.29–40.17
2.	Diethylaminoarglabin methyl iodide (10)	60	6.66	0	72.7	42.13–16.6

Table VIII
Phagocytic activity of sesquiterpene lactones of dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10)

№	Compound	Concentration	Phagocytic index	Phagocytic number
1.	Saline	–	49.96 ± 12.05	4.22 ± 1.04
2.	Comparative drug Levamisole	10	60.7 ± 1.95	2.4 ± 0.3
		1	80.26 ± 4.9	2.76 ± 0.1
		0.1	72.5 ± 5.9	2.58 ± 0.3
3.	Comparative drug Immunal	1:1	72.36 ± 5.0	4.5 ± 0.23
4.	Dimethylaminoarglabin methyl iodide (8)	1 μg/ml	36.88 ± 9.86	2.53 ± 0.13
		0.1 μg/ml	48.36 ± 12.6	3.33 ± 1.1
		0.01 μg/ml	31.84 ± 9.3	2.34 ± 0.25
5.	Diethylaminoarglabin methyl iodide (10)	1 μg/ml	41.1 ± 10.32	2.18 ± 0.13
		0.1 μg/ml	48.92 ±3.03	2.56 ± 0.25
		0.01 μg/ml	47.08 ± 6.15	2.24 ± 0.2

When evaluating the effect of derivatives of arglabin (1) dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) on phagocytic activity of neutrophils, relatively high phagocytic activity of the studied compounds was determined. Herewith, dimethylaminoarglabin methyl iodide (8) has the most phagocytosis stimulating activity at a dose of 0.1 mg/ml. Stimulating activity of (10) is registered at a dose of 1 mg/ml and almost undetected in two lower concentrations. Thus, we can assume that dose-dependent type of substances impact on phagocytosis reflects some of their immunomodulatory activity (Table VIII).

As it is known that many sesquiterpene lactones show anti-inflammatory properties connected with their ability to modulate oxidative phosphorylation, platelets aggregation and change the secretion of histamine and serotonin, sesquiterpene lactone arglabin (1) and its derivatives dimethylaminoarglabin hydrochloride (2), dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) were studied on this type of biological activity (Table IX).

Test specimen of arglabin (1) and its derivatives showed anti-inflammatory activity in varying degrees of intensity, which indirectly indicates of the required presence of lactone ring for the implementation of the mentioned effect. Hereby, it was determined that for the detection of anti-inflammatory effect it is important to have the presence of free exomethylene group of lactone cycle.

Table IX
Anti-inflammatory activity of sesquiterpene lactone arglabin (1) and its derivatives

№	Compound	Dose, μg/kg	Degree of edema inhibition %	
			4 hours	24 hours
1.	Diclofenac	1	29.0 ± 5.0*	43.0 ± 6.0*
2.	Sesquiterpene lactone arglabin (1)	1	31.0 ± 4.0*	40.0 ± 3.0*
		10	57.0 ± 2.0*	82.0 ± 4.0*
3.	Dimethylaminoarglabin hydrochloride (2)	1	29.0 ± 3.0*	39.0 ± 3.0*
		10	51.0 ± 4.0*	79.0 ± 4.0*
4.	Dimethylaminoarglabin methyl iodide (8)	1	28.0 ± 4.0*	30.0 ± 3.0*
		10	49.0 ± 3.0*	70.0 ± 2.0*
5.	Diethylaminoarglabin methyl iodide (10)	1	19.0 ± 5.0	22.0 ± 5.0
		10	33.0 ± 3.0*	37.0 ± 4.0*

Note: * – $p<0.05$ in comparison with control

Conclusion

Data obtained at the result of molecular docking of sesquiterpene lactone arglabin (1) and its chemically modified derivatives dimethylaminoarglabin hydrochloride (2), dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) on enzyme systems of human DNA topoisomerase I and IIB type were confirmed by conducted biological studies on models *in vitro* and *in vivo*. Perhaps this is due to the fact that in the molecule of arglabin are present such functional groups as α,β-unsaturated γ-lactone cycle, triple-substituted double bond and an epoxy cycle, the presence of which determines its high biological activity. In accordance with the results of the experiments it was determined that arglabin inhibits farnesyl protein transferase, the enzyme responsible for the formation of malignant tumors.

Results of the molecular docking in point of derivatives of arglabin (1) dimethylaminoarglabin methyl iodide (8) and diethylaminoarglabin methyl iodide (10) showed among presented compounds relatively high values of energy forces of binding (G-score) of the complex ligand-target –10.42 and of –8.44 respectively, and which demonstrate high intermolecular bond between the molecule of compound and the target of human DNA topoisomerase IIB type with etoposide, proved by experimental studies on *in vitro* models in test on survival of marine crustaceans larvae *Artemia salina* (Leach). Thus, cytotoxic activity of the derivative (8) is higher than the values of the derivative (10).

On *in vivo* model in test of transplantable tumors in white outbred rats the antitumor activity of sesquiterpene lactone arglabin (1) and its dimethylaminoarglabin hydrochloride (2) was proved. Thus, sesquiterpene lactone arglabin (1) showed relatively high antitumor activity against strains of cancer cells of Pliss lymphosarcoma, sarcoma M-, Leukemia L-1210, sarcoma-45 to sarcolysine.

Its dimethylaminoarglabin hydrochloride (2) showed high antitumor activity against such strains of transplantable cancer cells as M-1 sarcoma, Walker carcinosarcoma, sarcoma-45, Geren carcinosarcoma, alveolar mucous liver cancer PC-1, Pliss lymphosarcoma, also it had significant effect on P-388 leukemia.

The phagocytic and anti-inflammatory activity of sesquiterpene lactone arglabin (1) and its derivatives dimethylaminoarglabin methyl iodide (8), diethylaminoarglabin methyl iodide (10) and dimethylaminoarglabin hydrochloride (2) was evaluated.

The comparative analysis of molecular docking of sesquiterpene lactone arglabin (1) and its derivatives with our results of biological studies conducted by us allows to note the reliability and prospect of molecular docking application, in particular, molecular docking, as a means of selecting from the vast number of compounds – derivatives of arglabin which, most probably, will possess some kinds of promising biological activities, particularly antitumor and immunomodulatory.

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S. M. Adekenov[a], A. K. Dairov[a], R. B. Seidakhmetova[a], and V. Spiwok[b] *([a] JSC "International Research and Production Holding "Phytochemistry",4 Gazaliyev Str., Karaganda, Republic of Kazakhstan, [b] University of Chemistry and Technology, Prague, Czech Republic):* **Molecular Docking of Arglabin and its Derivatives and Their Biological Activity**

The article presents the results of molecular docking of sesquiterpene lactone arglabin (1) and its chemically modified derivatives on enzyme systems of human DNA topoisomerase I and IIB type. The comparative analysis of the data of computer prediction with conducted biological researches in test systems *in vitro* and *in vivo* was carried out.

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SCREENING OF KAZAKHSTAN PLANTS ON THE CONTENT OF POLYPRENOL COMPOUNDS

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Keywords: polyprenols, extraction, HPLC

1. Polyprenols and their sources

Despite the diversity of modern principles of drugs development, the search for perspective compounds among the substances of plant origin remains one of the fundamental principles in the development of highly effective and safe therapeutic and prophylactic preparations. Currently polyprenol compounds of plants considered as a new class of low-molecular bioregulators, which are involved in the biosynthesis of various carbohydrate-containing polymers.

Over the past 25 years the drugs on the basis of polyprenols have been developed intensively due to their low toxicity and a wide spectrum of biological effect. It is conditional on the fact that they are membrane active substances and contribute to the opening of calcium channels in the bilayers of membrane. Thus, plant polyprenols are prospective for the development of drugs with antiulcer, immunotropic, antiviral and many other kinds of activity[1–7].

The polyprenols are known as acyclic terpene alcohols of the general formula H-$(C_5H_8)_n$-OH, where n > 4 is the quantity of isoprenoid units related by the type "head to tail". The presence in the chain of three units of *trans*-configuration is typical for the vast majority of plant polyprenols (1), whereas the rest contain *cis*-trisubstituted double bond[8–10].

In plants polyprenols are found in the form of mixtures of oligomer-homologs, whose composition and ratio is typical for each plant species. Some of them are named after the corresponding plant source. They differ by the quantity and geometric configuration of double bonds[11–16].

(1)

In nature the polyprenols are distributed in herbage, mostly in plant leaves and needles, both in free and esterified form. The content of polyprenols in some species of angiosperms and gymnosperms fluctuates from 10^{-7} to 2 % (ref.[17–24]).

2. Screening of plants on the content of polyprenols

For the first time in the Republic of Kazakhstan a systematic study of plants, including endemic species, on the content of polyprenol compounds was carried out; their composition was described, the pilot batches of raw materials containing polyprenol compounds were collected.

On the content of polyprenols were studied:

needles of *Pinus sylvestris* L., leaves of *Hippophae rhamnoides* L., leaves of *Lonicera tatarica* L., leaves and inflorescences of *Hipericum perforatum* L., leaves of *Betula pendula* Roth., collected at the Botanic garden of IRPH "Phytochemistry" in May 2015; leaves, shoots and blooms of *Spiraeanthus schrenkianus* Maxim. and the leaves of *Fraxinus sogdiana* Bunge. collected in the vicinity of the ridge of Lower Boralday in June 2015; leaves of *Spiraea hypericifolia* L. collected in the vicinity of Kyzylkain village of Bukhar-Zhyrau district of Karaganda region in May 2015; needles of *Juniperus sabina* L. collected in the vicinity of Spassk mounds of Abay district of Karaganda region in May 2015.

Above mentioned samples of plants were processed by the following way. Tree greenery of plant raw materials is ground, 31.5 g of milled greenery is mixed with 390 g of 5 % aqueous solution of sodium hydroxide (ratio of wood foliage : alkaline solution is 1:12) at room temperature (t= 20–25 °C). The mixture is agitated in a magnetic stirrer for 2 h. The obtained heterogeneous mass is separated: solid phase is extracted, the filtrate is transferred into a separating funnel and extracted three times with petroleum ether at a ratio of petroleum ether : filtrate equal to 1:1. As a demulsifier the ethyl alcohol at the amount of 7–10 % with respect to the filtrate is used. From the obtained extract, the solvent is distilled off on a rotary evaporator. The results of the extractions are presented in Table I. All products are oily, of yellow to light orange color, all of similar smell, except for *Juniperus sabina* extract with the smell of the essential oil.

In the extracts of *Hipericum perforatum*, *Hippophae rhamnoides*, *Betula pendula*, *Pinus sylvestris*, *Fraxinus sogdiana* and *Spiraeanthus schrenkianus*, the following absorption bands are revealed: 3393–3360 cm^{-1}, stretching vibrations in the bound -OH group; 2974–2972 cm^{-1}, symmetric stretching vibrations in -CH_2 group; 2926–2924 cm^{-1}, asymmetric stretching vibrations in -CH_3 and -CH_2 groups;

Table I
Data on extracts of studied plants

Plant denomination	Extract weight, g	Extract yield, %
Pinus sylvestris	0.3	0.93
Spiraeanthus schrenkianus	0.05	0.17
Spiraea hypericifolia	0.06	0.2
Hippophae rhamnoides	0.09	0.28
Lonicera tatarica	0.23	0.73
Hipericum perforatum	0.24	0.75
Betula pendula	0.18	0.57
Fraxinus sogdiana	0.11	0.34
Juniperus sabina	0.13	0.42

1089–1084 cm^{-1}, stretching vibrations in -COH group.

IR spectra of extracts of *Spiraea hypericifolia*, *Juniperus sabina* and *Lonicera tatarica* are characterized by the presence of absorption bands at 2927–2920 cm^{-1}, belonging to asymmetric stretching vibrations in -CH_3 group, 2853–2850 cm^{-1}, belonging to symmetric stretching vibrations in -CH_2 group, and 1463–1456 cm^{-1}, related to the asymmetric deformation vibrations in CH_3 group.

HPLC for the determination of the quantitative content of polyprenols in extracts of *Pinus sylvestris* and *Spiraeanthus schrenkianus* was performed. The quantitative analysis of samples was performed by the method of reversed-phase HPLC on the equipment HEWLETT PACKARD Agilent 1100 Series in isocratic mode under the following conditions: analytical column, filled with sorbent Separon SGX CN, 4 x 150 mm, particles size 5 µm; mobile phase composition, methanol – isopropyl alcohol 50:50; detection at wavelength of 210 nm; room column temperature; mobile phase rate of 0.5 ml/min; injected sample volume of 20 µl. Tap: concentrate of polyprenols isolated from pine resin.

Data calculations performed via ChemStation software. The quantitative content of concentrate of polyprenols in studied samples was determined by the method of comparison with an external standard (Fig. 1–3).

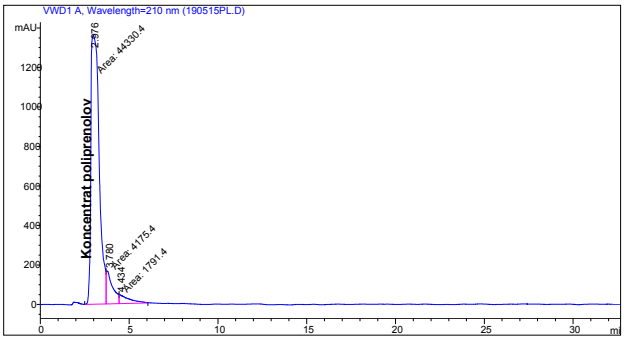

Fig. 1. **Chromatogram of standard of polyprenols concentrate**

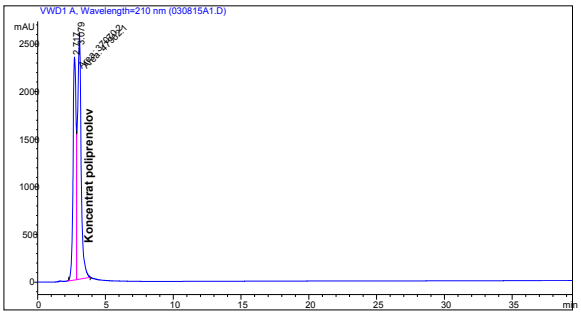

Fig. 2. **Chromatogram of *Pinus sylvestris* extract**

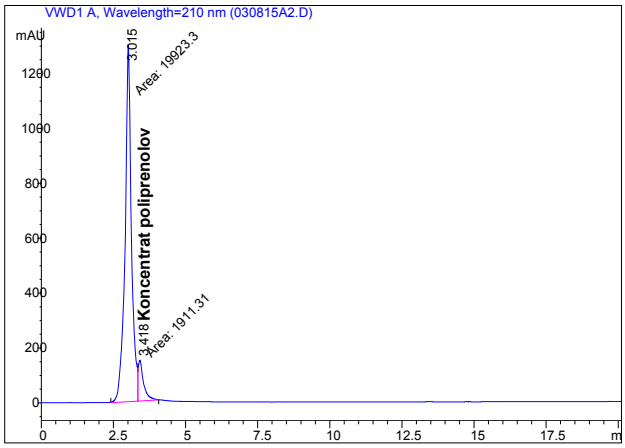

Fig. 3. **Chromatogram of *Spiraeanthus schrenkianus* extract**

Analysis by HPLC showed that studied extracts include polyprenols which are the part of polyprenol concentrate from pine resin, but in a small amount (Table II). In the extract of *Pinus sylvestris* the outcome of polyprenols was 0.09 % of air-dry basis, in the extract of *Spiraeanthus schrenkianus* was 0.002 % of air-dry basis.

By HPLC on the Separon TM column with reversed-phase SGX C18, 7 m, via the elution with a mixture of ethanol – hexane, 9:1, 1 ml/min, at UV detection (l 213 nm), the composition of polyprenol fractions of *Spiraea hypericifolia* and *Juniperus sabina* was studied. As standard samples enriched fraction of *Abies sibirica* (Fig. 4, Table III) and a mixture of homologs (Fig. 5), comprising the polyprenols with 13–19 units were used (Table IV).

Comparison of the retention times of peaks on chromatogram of *Spiraea hypericifolia* extract (Fig. 6) with this standard allows identifying polyprenol with 19 units in quantity of 84.84 % of mixture weight (Table V), 3.9 % of

Table II
Quantitative content of polyprenol concentrate in extracts of *Pinus sylvestris* and *Spiraeanthus schrenkianus*

Sample	Quantitative content of polyprenols, %
Pinus sylvestris extract	9.5
Spiraeanthus schrenkianus extract	1.2

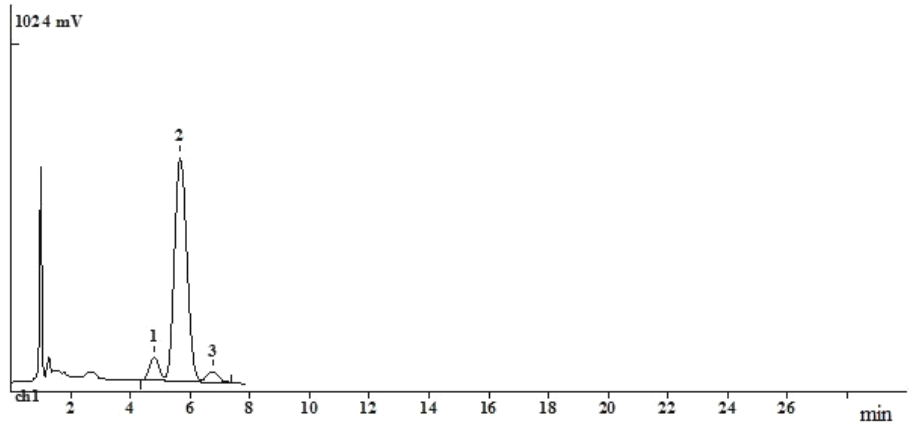

Fig. 4. **Chromatogram of 16-polyprenol (enriched fraction of *Abies sibirica*)**

Table III
Composition of polyprenols of enriched fraction of *Abies sibirica*

Retention time, min	Intensity	Area, %	Identification
4.79	1563.87	7.30	15-polyprenol
5.69	18936.58	88.44	16-polyprenol
6.76	912.08	4.26	17-polyprenol
7.89	21412.53	100.00	

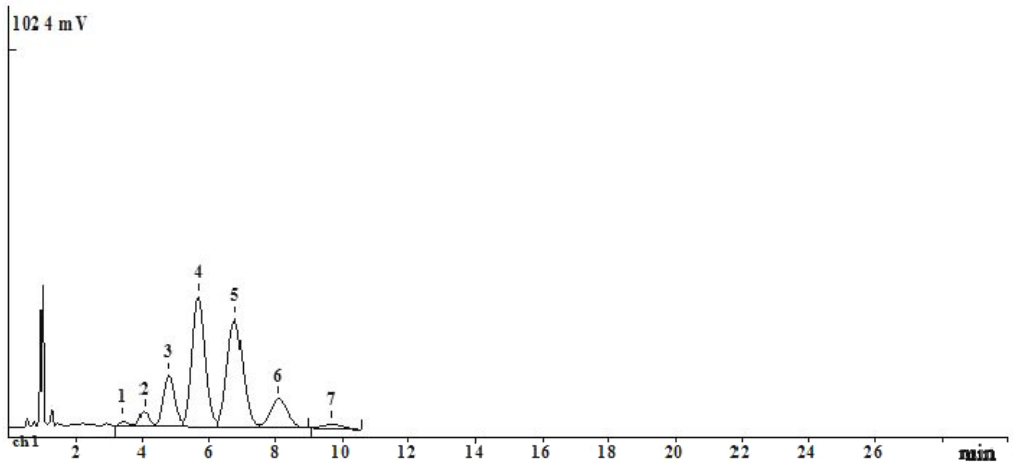

Fig. 5. **Chromatogram of polyprenols from *Abies sibirica***

Table IV
Composition of mixture of polyprenols from *Abies sibirica*

Retention time, min	Intensity	Area, %	Identification
3.44	207.14	0.79	13-polyprenol
4.05	714.40	2.72	14-polyprenol
4.79	3181.49	12.10	15-polyprenol
5.69	9631.52	36.63	16-polyprenol
6.78	9225.71	35.09	17-polyprenol
8.10	2758.54	10.49	18-polyprenol
9.70	506.59	1.93	19-polyprenol
10.6	26225.38	100.00	

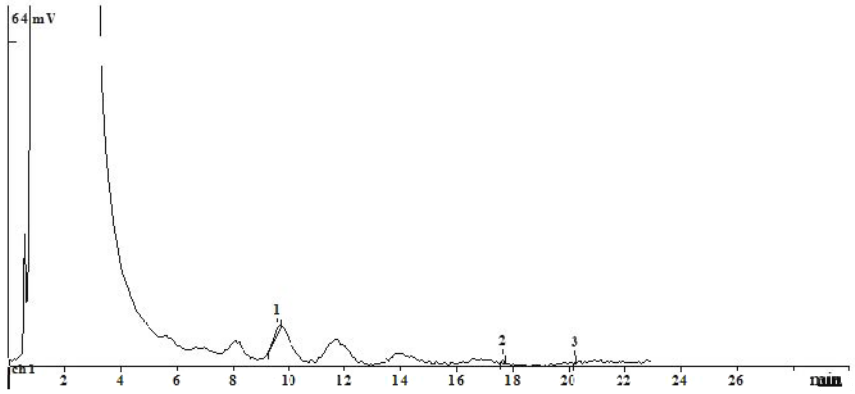

Fig. 6. **Chromatogram of polyprenol fraction of *Spiraea hypericifolia* extract**

Table V
Composition of polyprenol fraction of *Spiraea hypericifolia*

Retention time, min	Intensity	Area, %	Identification
9.68	20.69	84.84	19-polyprenol

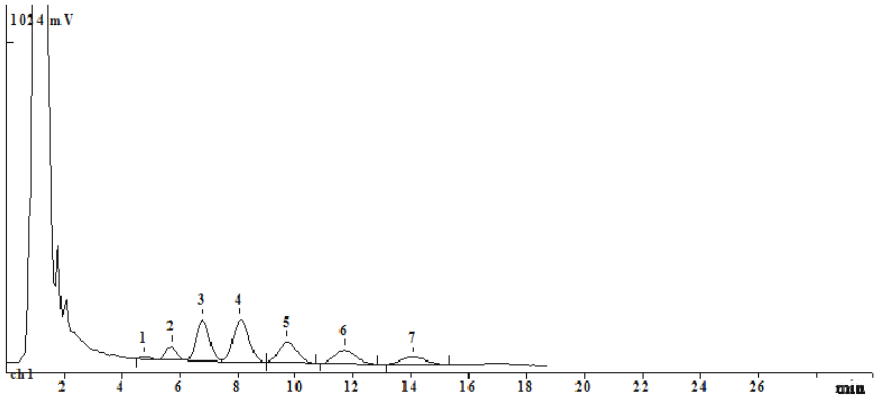

Fig. 7. **Chromatogram of polyprenol fraction of *Juniperus sabina* extract**

Table VI
Composition of polyprenol fraction of *Juniperus sabina*

Retention time, min	Intensity	Area, %	Identification
4.8	144.28	0.90	15-polyprenol
5.69	1094.35	6.80	16-polyprenol
6.78	3954.07	24.57	17-polyprenol
8.10	4807.03	29.87	18-polyprenol
9.71	2753.39	17.11	19-polyprenol
11.69	2050.72	12.74	20-polyprenol
14.09	1289.98	8.02	21-polyprenol
18.77	16093.82	100.00	

extract mass weight and 0.01 % of air-dry basis. Polyprenol components of *Juniperus sabina* with the number of isoprene units from 15 to 21 were similarly identified (Fig. 7), 17,18-polyprenols are prevailing in quantity of 24.6 % and 29.9 % respectively, minor components is 15-polyprenol, 0.9 % of mixture weight (Table VI). Total amount of polyprenols in *Juniperus sabina* is 9 % of extract weight and 0.04 % of air-dry basis.

3. Conclusion

Thus, a chemical study of 9 plant species of the families *Pinaceae*, *Betulaceae*, *Rosaceae*, and others: *Pinus sylvestris* L., *Spiraeanthus schrenkianus* Maxim., *Spiraea hypericifolia* L., *Hippophae rhamnoides* L., *Lonicera tatarica* L., *Hipericum perforatum* L., *Betula pendula* Roth., *Fraxinus sogdiana* Bunge. and *Juniperus sabina* L. was studied. Via HPLC the content of polyprenols in extracts of *Pinus sylvestris*, *Spiraeanthus schrenkianus*, *Spiraea hypericifolia* and Juniperus *sabina* was determined. The maximum content of polyprenols was determined in *Pinus sylvestris* and *Juniperus sabina* (0.09 % and 0.04 % of absolute dry basis respectively).

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SESQUITERPENE LACTONES WITH NON-TYPICAL STRUCTURES AND FEATURES OF THEIR MOLECULES

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Keywords: sesquiterpene lactones, biosynthesis, biogenetic route, farnesyl pyrophosphate, bisabolane, drimanes, lactaranes, lindenanes, marasmanes, eremophilanes, dimer molecules

The review presents data on new sesquiterpene lactones with non-typical structure, isolated from various natural sources – fungi, plants, marine organisms, etc., on their distribution, biological activity and estimated biogenetic route of their formation. The review presents a large chemical diversity of new sesquiterpene lactones with non-typical structure.

1. Introduction

In recent years in search for new drugs the interest of researchers more and more related to sesquiterpene lactones, the vast group of natural compounds possessing large number of biological activity, such as: antitumor, anti-inflammatory, anti-parasitic, antispasmodic, hypoglycemic, cardio tonic, etc.[1-9]. Most of them are isolated from plants of *Asteraceae* family; it is also known of their presence in fungi, mosses, marine organisms, corals and some fauna representatives[10-16].

By 2014 from a variety of sources there were discovered and described over 7,000 sesquiterpene lactones belonging to seven major structural types[17]. However, there is no doubt that much more remains unexplored and will be identified later. This is due to the improvement of the methods of isolation and analysis of natural compounds, which allows obtain representatives of new structural types of sesquiterpene lactones.

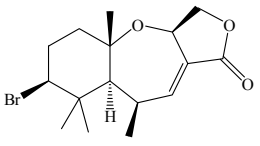
It should be noted that the main structural element of molecules of isolated compounds is a sesquiterpene skeleton formed biogenetically through farnesyl pyrophosphate route. In accordance with the works of many authors there is a plan of terpenoid molecules formation, developed by nature at the early stages of evolution, according to which plants and animals carry out such biochemical transformations, which lead to a surprising diversity of terpenes and their derivatives[18]. The structure's singularity of individual molecules can be explained by the intramolecular rearrangement and other biosynthetic transformations of intermediates generated by enzymatic modifications in plant organisms, marine metabolites[19].

The abundance of terpene carbon skeletons can be attributed to the diversity of enzymes known as terpene synthases. These catalysts are able to convert prenyl diphosphates and squalene into a variety of cyclic and acyclic forms[20].

2. Non-typical sesquiterpene lactones of marine organisms

Representatives of all major groups of sesquiterpenes are found in marine plants and animals. There is a number of peculiarities of biosynthesis and metabolism of biologically active substances in marine organisms in comparison with terrestrial plants and animals. The first of these peculiarities is that the inorganic components of the marine environment often involves in biochemical processes. As it is known, sea water is a habitat for several hundred thousand species of organisms, contains in dissolved form a gigantic amount different kinds of salt. A number of these elements and groups are included in the molecules of natural compounds. Thus, halogenated metabolites are widely represented in macroalgae, especially in red, also in sponges, mollusks, ascidians and other marine invertebrates.

A relatively small number of monocyclic and other simple sesquiterpenes are discovered in the extracts of algae. Mono-cyclofarnezsan skeleton has aplisistatin (**1**), a halogenated derivative of mollusk *Aplysia angasi*, which attracted attention by cytotoxic activity[21]. Aplisistatin (**1**) and its derivatives (**2**) have one carbocycle. Additional seven-membered ring in these compounds is a heterocyclic one. Alike other secondary metabolites of mollusks, aplisistatin (**1**) is of exogenous origin, as it's found in red algae, eaten by Aplysia mollusks. Thus, the study of chloroform-methanolic extract of algae *Laurencia* showed the presence of this substance. The compound (**1**) inhibits efficiently progression of experimental P-388 leukemia in test animals. Unlike most plant lactones, aplisistatin (**1**) does not have exomethylene group.



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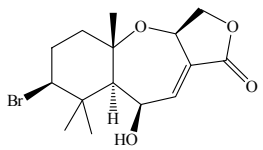
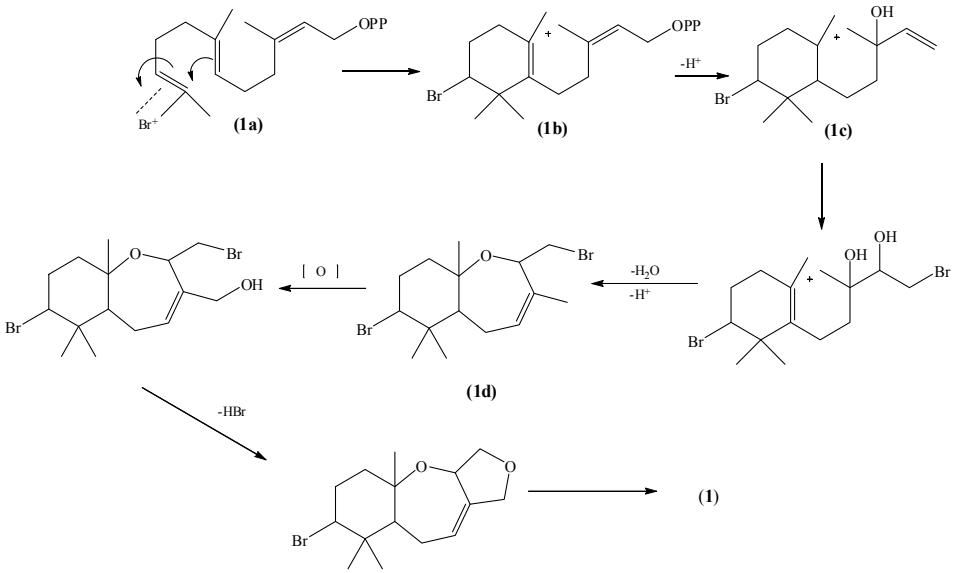


(2)

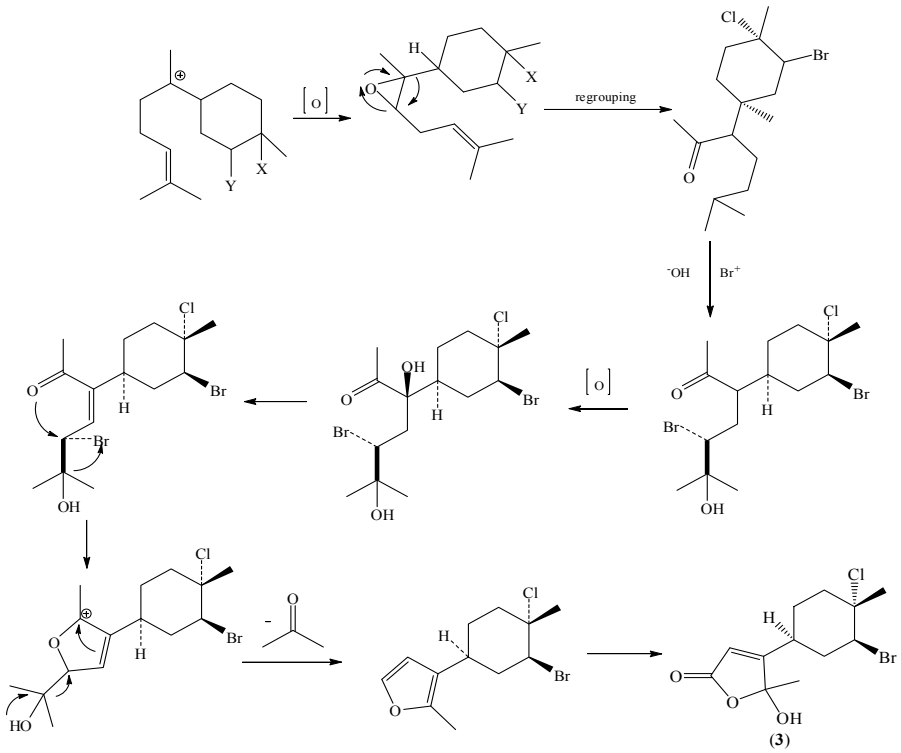
According to biogenetic scheme, the formation of unusual halogenated compound comes from trans, trans-



farnesyl pyrophosphate, conformation (**1a**) after cyclization under the action of bromonium ion, saponification and allyl rearrangement, herewith it may give monocyclofarnesan carbocation (**1b**), a potential key compound in the biogenesis of palisadines, aplisistatin and related compounds.

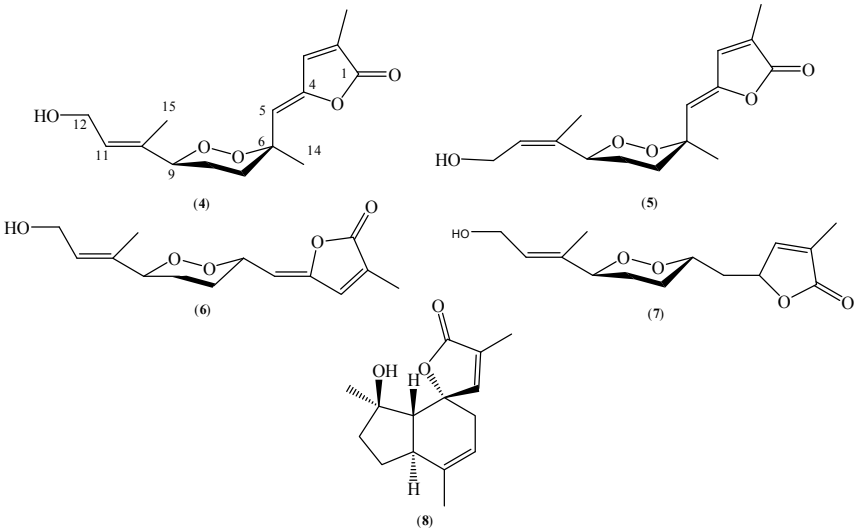
Bisabolane sesquiterpenes are widespread in natural sources. The biosynthesis of cyclohexanone sesquiterpenoids of bisabolane type from pyrophosphate trans-trans-farnesol prevail in a living cell. They are typical for many plants and found in marine organisms, insects and microorganisms. Bisabolane sesquiterpenoid (**3**), with non-typical structure and the atoms of bromine and chlorine in the molecule, is isolated from marine invertebrate mollusks *Aplysia dactylomela*[22].

The formation process of compound (**3**) passes due to transformations of the possible predecessor of bisabolane cation, the true producer of this type of metabolites in *Aplysia dactylomela*. In this process the tricyclic bond of known compounds is oxidized successively, starting from the conjunction of chlorine and bromine to the endocyclic



double bond.

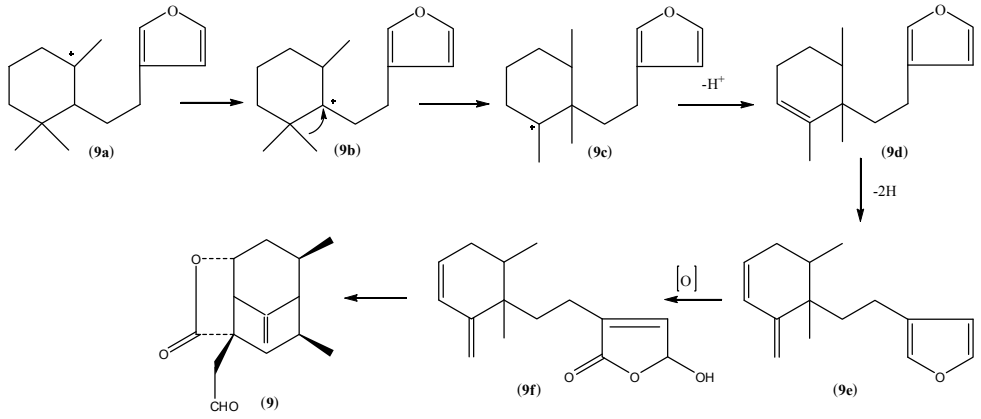
Soft corals *Sinularia sp.* are an abundant source of biologically active secondary marine metabolites, as well attracted much attention recently due to the isolation from them – sesquiterpenes, diterpenes, polyhydroxylated steroids and polyamine compounds. These metabolites possess a range of biological activities, such as: antimicrobial, anti-inflammatory and cytotoxic activity. Non-typical type of sesquiterpene lactones (**4-7**) with peroxy group was isolated for the first time from soft corals *Sinularia sp.*[23]. In continuation of the works of isolation the secondary metabolites from this coral species, the same scientists have isolated a new spirobutenolide (**8**) with valerian skeleton. The information of the isolation similar lactones from the soft corals was not mentioned previously[24].

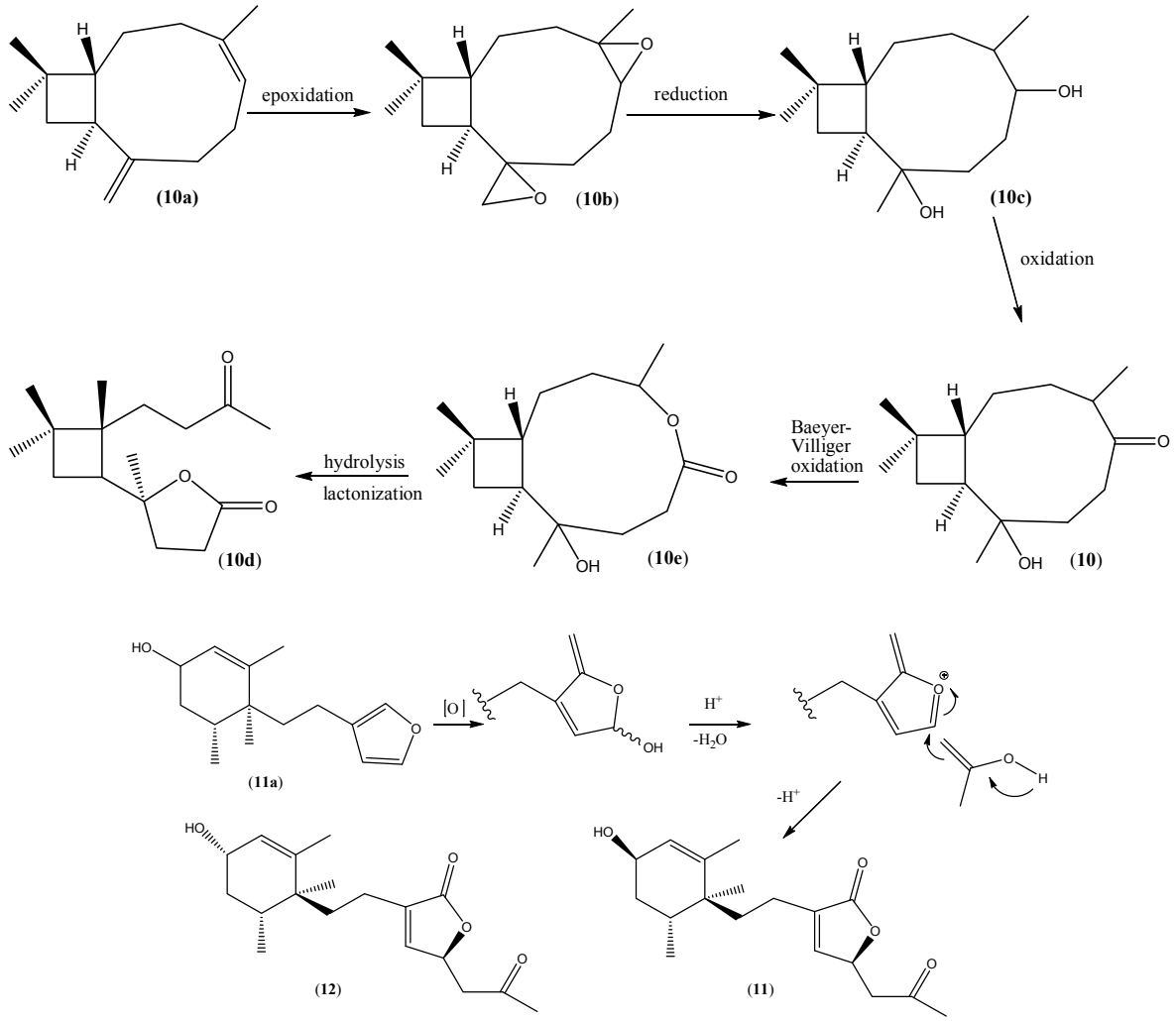


Several sesquiterpenoids of sponges have a regrouped non-isoprenoid skeleton, for instance, upial (**9**) from *Dysidea fragilis*. The structure of upial (**9**) was determined by detail spectral study via lanthanide shift agents, double resonance. The compound is biosynthesized, probably, through the stage of carbocation (**9a**). The displacement of the charge into (**9a**) and 1,2-methyl group gives the ion (**9c**), whose proton cleavage leads to microcyanine-2 (**9d**). Dehydrogenation (**9d**) can give a monocyclic diene (**9e**), which is a potential original substance in the biosynthesis of upial (**9**). Oxidation of the furan ring in (**9e**) and cyclization of the formed one, according to P.J. Scheuer and collaborators, leads to upial (**9**)[25].

New 4,5-seco-caryophyllan sesquiterpene lactone (**10**) was isolated from the coral sea gorgonian *Rumphella antipathies*. The authors suggested biogenetic formation route of unusual compound (**10**), which starts through the epoxidation of caryophyllene (**10a**) with the disclosure of epoxy rings; the next stage is Baeyer-Villiger oxidation, and then esterification proceeds with the formation of a new lactone (**10**). According to their data, previously such lactones of caryophyllene type were not isolated[26].

Two new unusual sesquiterpene lactones (**11,12**) with cyclo-farnesane skeleton which were isolated from the acetone extract of the sponge *Dysidea fragilis*. The structures of new compounds were determined on the basis of a detailed analysis of spectral data and in comparison with NMR data on structurally related compounds. It should be noted that the presence of acetonyl function in these molecules is unusual case among natural compounds. Neverthe-





less, the formation of compounds (**11,12**) may also be an artifact, because in the process of isolation acetone was used. They also proposed the biogenetic route for compounds (**11,12**) which were formed by the oxidative addition of acetonyl function to the furan ring of known compound (**11a**). In order to determine whether (**11,12**) are true sponge metabolites or their artifacts, the authors analyzed chloroform extract of the sponge by two methods: TLC and HPLC, but could not detect the presence of (**11,12**), which means that they were obtained through the extraction process[27].

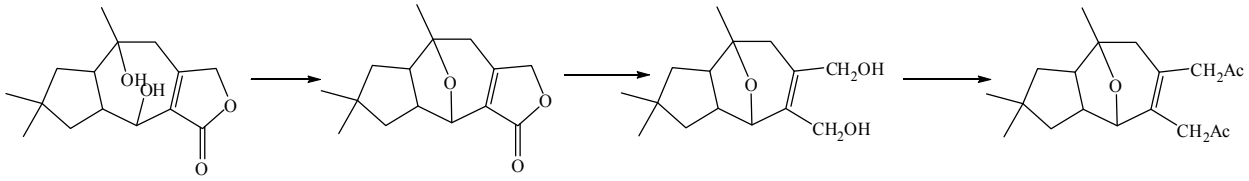
Thus, non-typical sesquiterpene lactones of marine metabolites are formed in accordance with the biogenetic isoprene rule (farnesyl) and contradicting it (non-farnesyl). Interestingly, that most of the marine sesquiterpene lactones are derived from new skeletal systems, unknown to terrestrial natural compounds.

3. Sesquiterpene lactones with non-typical structure of fungi and bacteria

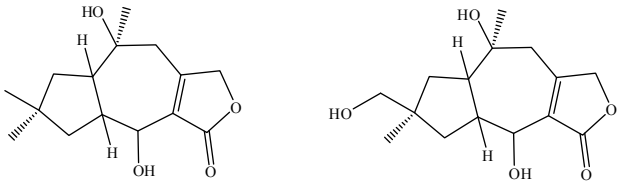
Bacteria and fungi also biosynthesize terpenoids with diverse structural types and with asymmetric centers in their molecules, which determine biological activity and accordingly, have physico-chemical and biological features. First information of the isolation sesquiterpene lactones from fungi is reported in works of Polish scientist W.M. Daniewski, since 1971. He isolated a new sesquiterpene lactone lactarofurin (**13**) and on its basis new derivatives (**14-16**) were synthesized. Chemical modification of the molecule (**13**) was supposed to facilitate the spectrum decoding of the original compound[28].

The structures of lactarofurin A (**17**) and lactarofurin B (**18**) were corrected by the results of X-ray analysis, as their structures previously mentioned were incorrect[29].

Anhydrolactarofurin A (**19**) was obtained from a plant source. Cleaved anhydrolactarofurin A is also obtained as a by-product of the dehydration of monoacetate of lac-



(13) **(14)** **(15)** **(16)**



(17) **(18)**

tarofurin A. Further, the sesquiterpene lactone lactaronecatorin A (**20**) was isolated from *Lactarius necator*[30].

3-O-ethylfurandiol (**21**) is a sesquiterpene lactarane formed during the ethanol extraction of fertile parts of fungus *Lactarius*. Probably, it's formed through enzymatic conjugation of ethanol to molecule of furanol, which is the result of transformation of veletinal; last one is the most common lactone of genus *Lactarius*. This compound was isolated in 1974 and is considered the "artifact", but showed sufficiently strong antifeedant activity, which gave an impetus to subsequent trans-modification of 3-*O*-ethylfurandiol[31].

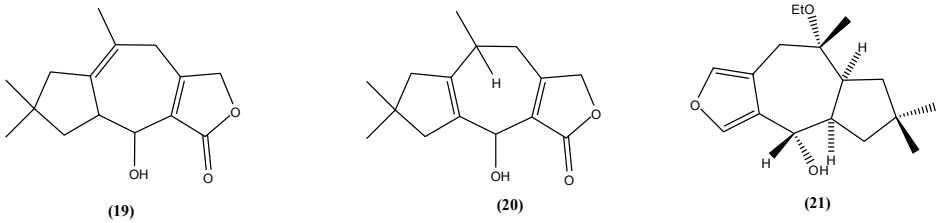
A joint work of Chinese and Australian scientists led to the isolation of sesquiterpene lactones from the fungi *Lactarius piperatus*: 5 known – 7α,8β-dihydroxy-5,13--marasmanolide, 7α,8α-dihydroxy-5,13-marasmanolide, isolactarufin, blennine A и D and 2 new – 7α,8β,13-trihydroxy-5,13-marasmanolide (**22**) and 8-hydroxy-8,9-

from marasmane are formed sesquiterpenoids of isolactarane and lactarane types, and then secolactaranoids[32].

In the review of 2015, devoted to the extraction of biologically active terpenes isolated from marine fungi over the last 5 years there is also information about sesquiterpene lactones. Basically, these are sesquiterpene lactones of drimane type isolated from ethanol extract of fermentation broth of the fungus *Aspergillus ustus*, from the marine sponges *Suberites domuncula* collected in the Adriatic sea, from the fungus *Aspergillus aculeatus* CRI 323-04**,** from the sponge *Xestospongiates tudinaria*, from the fungus *Aspergillus insuetus* (OY-207), isolated from the mediterranean sponge *Psammocinia sp.*, from the endophytic fungus *Aspergillus carneus* KMM 4638, obtained from the brown algae *Laminaria sachalinensis*, etc.

It is considered, that drimane sesquiterpenes are typical only for marine organisms (sponges) and microorganisms[33]. The drimane's skeleton formation passes by the route from acyclic trienone-hydrocarbon of pharnesone series. A typical structural element of drimane terpenoids is a hem-dimethyl fragment in one of the cycles in combination with the methyl group at the junction of bridge and with two vicinal methyl groups in the second cycle. However, the last two methyl groups are frequently oxidized up to alcohol or aldehyde condition.

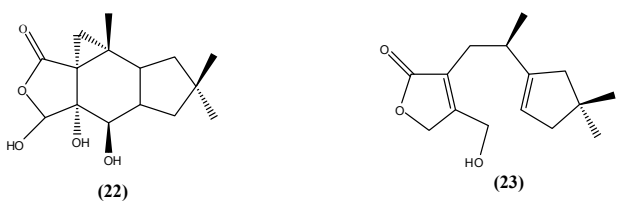
It is known, that a wide range of fungi also produce a great deal of oxidized eremophilanes which perform important physiological functions, mainly the regulation of spore formation and the creation of favorable environment.



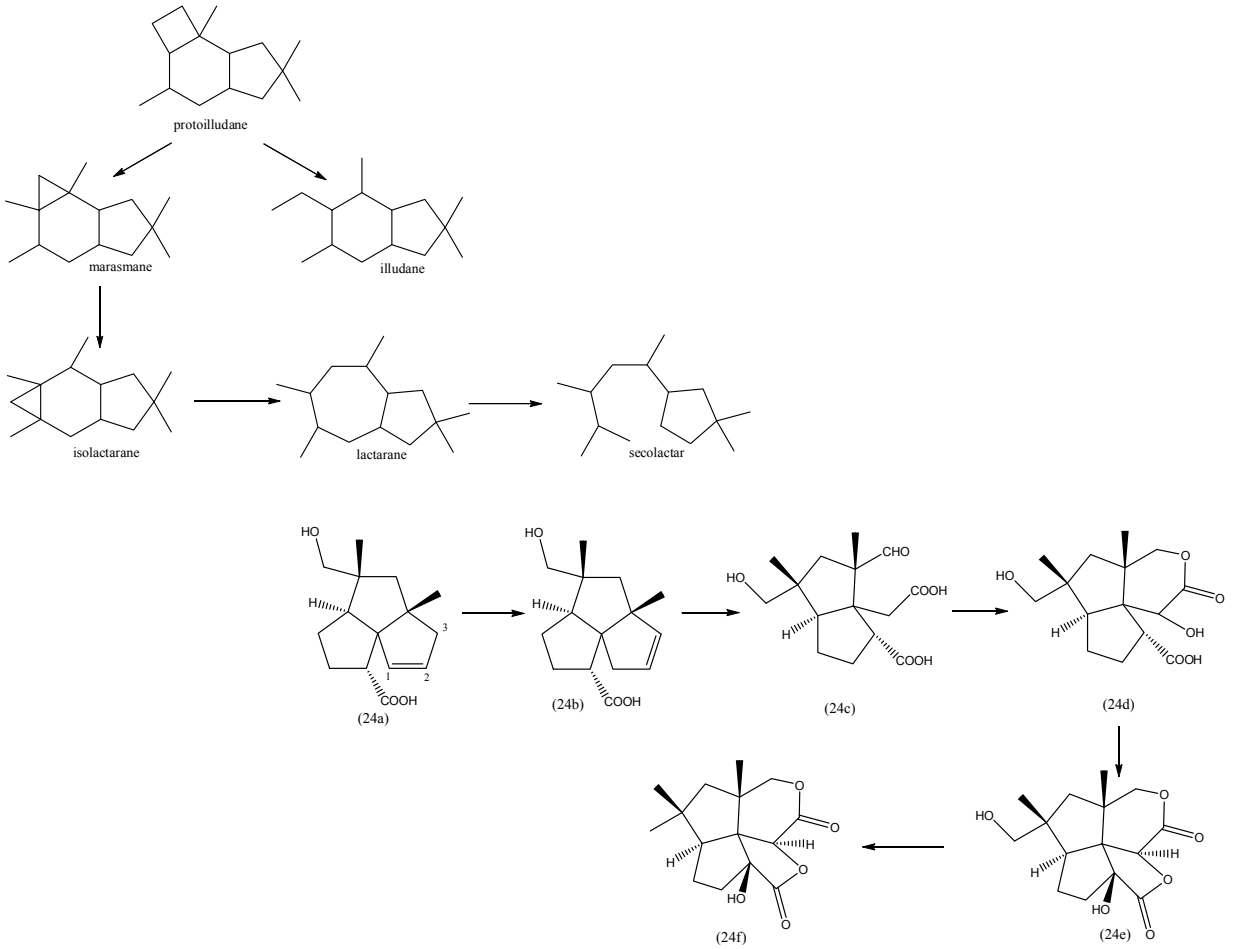
(19) **(20)** **(21)**

-secolactar-1,6-diene-5,13-olide (**23**). The simultaneous presence of these diverse structural types, which include marasmane, lactarane, isolactarane, secolactarane, protoilludane and illudane types are interesting for study biogenetic route of the formation these compounds. According to their data, none of protoilludane and illudane sesquiterpene types were found in the genus *Lactarius*.

The biogenetic route starts from sesquiterpenes of protoilludane type to marasmane, and then to isolactarane,



(22) **(23)**

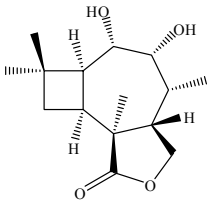


So, for example, there are presented data on the isolation of a new sesquiterpene lactone of eremophilane series from the fungus *Penicillium sp.* PR 19N-1, which is obtained from the abyssal marine mud of the Adriatic Sea[33].

Entophytic microorganisms such as bacteria or fungi living inside of plant tissues are acknowledged as important sources of new biologically active compounds with antitumor, antimicrobial and other types of activities. Entophytic fungi are of great interest in both biological and in applied aspect, but studied very poorly. In the review of Brazilian authors the terpenoids isolated from entophytic fungi and bacteria are summarized. For instance, new sesquiterpene lactones obtained from *Xylaria sp.*, isolated from *Cupressus lusitanica***,** from *Xylaria sp*. BCC21097, from *Licuala spinosa*, etc[34].

From marine fungus *Aspergillus aculeatus* CRI323-04 was isolated lactone (**24**) with unusual structure, asperaculine A with new [5,5,5,6]fenestrane ring. Asperaculine A (**24**) is probably synthesized from silphinenes which are metabolites of plants and fungi. Proposed by the authors biosynthesis of asperaculine A (**24**) begins with the migration of the double bond (C1-C2 to C2-C3) from the intermediate silphinenes (**24a**). Thereafter double bond C2-C3 undergoes oxidative cleavage with obtaining the intermediate (**24c**), which in its turn undergoes successive oxidation and lactonization, followed by the formation of asperaculine A (**24**)[35].

Three new non-typical sesquiterpene lactones (**25-27**) were isolated from basidiomycetes *Marasmiellus troyanus*. These unusual metabolites, probably, came from sesquiter-



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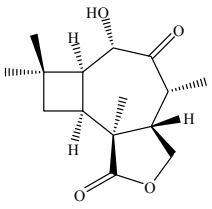

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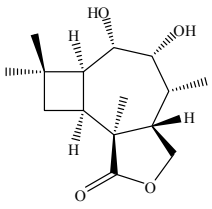


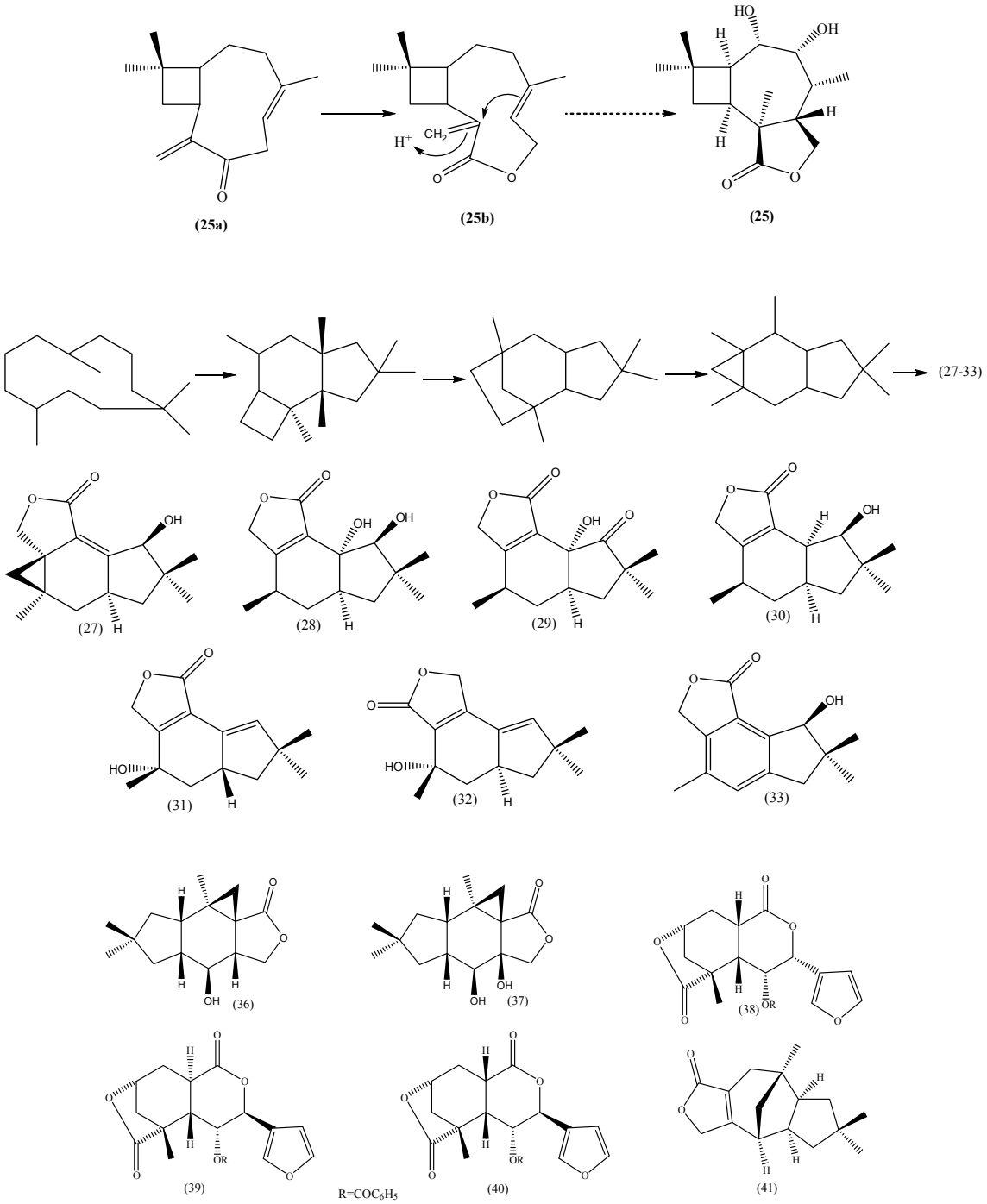
(27)

penes of caryophillane type[36].

The biosynthesis of compounds (**25-27**), probably arises out of caryophyll-4,8(15)-diene precursor (**25a**), which can undergo oxidation via Baeyer-Villiger reaction with obtaining lactone (**25b**). After the attacking exocyclic methylene 4,5 double bond, the ring is closed and may lead to a stable tertiary carbocation in C-4. Oxidation at positions 2 and 3 may lead to the formation of metabolites (**25-26**)[36].

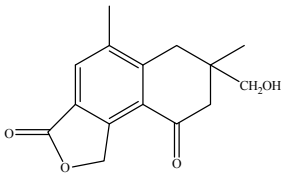
Fungal sesquiterpenoids, formed via humulane-protoilludane biosynthesis, are typical for basidiomycetes. Obtained from protoilludanes, sesquiterpenes with different skeletons such as ceratopicanes, isolactaranes and sterpuranes, can be synthesized in fungi. Compounds (**27-33**) are isolactarane sesquiterpenoids[37].

There were also isolated new marasmane lactones (**34,35**) from fungi *Russula foetens*[38], sollybolides (**36-38**)



from *Collybia maculata* and *Collybia peronata*[39], rufuslactone (**39**) from *Lactarius rufus*[40].

It is reported on the isolation of new terpene alcohol - stenotarsol (**42**) from the beetle *Stenotarsus subtilis* (*Endomychidae*)[41]. Its structure was determined on the basis of spectral data. This compound represents a new type of terpene skeleton and is the first of secondary metabolites isolated from the beetles family. It is known about the isolation of a new antibacterial component (**43**) from the strain *Agrocybe sp.* HKI 0259 (ref.[42]).



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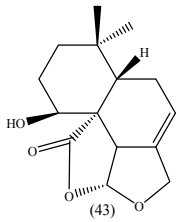


(43)

Thus, fungal terpenoids proved to be a potential source of valuable biologically active natural compounds. Progress in the study of the biosynthesis included deep understanding of terpene synthases, which are the first step in the biogenetic path of the terpenoids, and also in the biosynthesis of genes cluster. However, despite their importance, there are rather few reports on the studies of the biosynthesis of fungal terpenoids[43]. All examined unusual sesquiterpene lactones of fungi, bacteria are formed in accordance with the biogenetic isoprene rule.

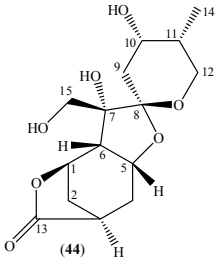
4. Sesquiterpene lactones of unusual type from plants

Recently, there have been published a great number of articles about the isolation from plants of new sesquiterpene lactones, which are non-typical and interesting types such as bisabolane, drimane and others.

Bisabolanes are the monocyclic sesquiterpenes where the farnesane chain is closed into the cyclohexane ring; the additional cyclizations increase the diversity of the given series. Currently, there are known over 100 bisabolane sesquiterpenes. Hereby, two unusual sesquiterpene lactones were isolated. Among them is glochicokcyne A (**44**) from the ethanol extract of the rhizomes *Glochidion coccineum* (Buch.-Ham.) Müll. Arg., its molecule structure and relative configuration was determined by X-ray diffraction analysis. ^{13}C-NMR characteristics of new lactone, including quaternary hemiacetal carbon atom at C-8, are similar to the spectrum data of the compound bisabolane type, isolated from *Phyllanthus emblica* L.[44]. From this plant previously were isolated lignans, triterpenes and norbisabolane sesquiterpenes.

From the ethanol extract of *Abies holophylla* Maxim., new abiesesquine A (**45**) of bisabolane type was isolated, hereby, the structure of the molecule (**45**) was determined using ^{1}H- and ^{13}C-NMR, NOESY, in order to determine the absolute configuration there were made calculations of electronic circular dichroism according to the density functional theory for non-stationary systems (TDDFT) using a software package Gaussian 03 (ref.[45]). The lactone (**45**) showed a significant inhibition of nitric oxide production in RAW264.7, stimulating the LPS.

Abiesesquine A (**45**) is the first example of a sesquiterpene lactone of bisabolane type, consisting of benzene



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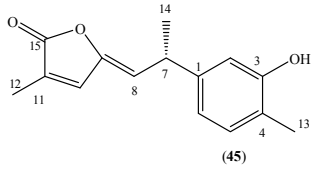

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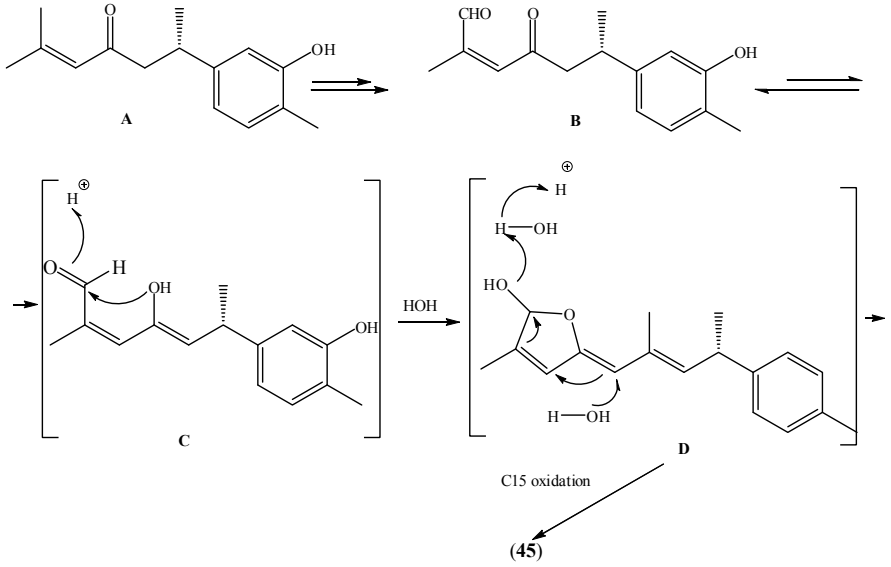
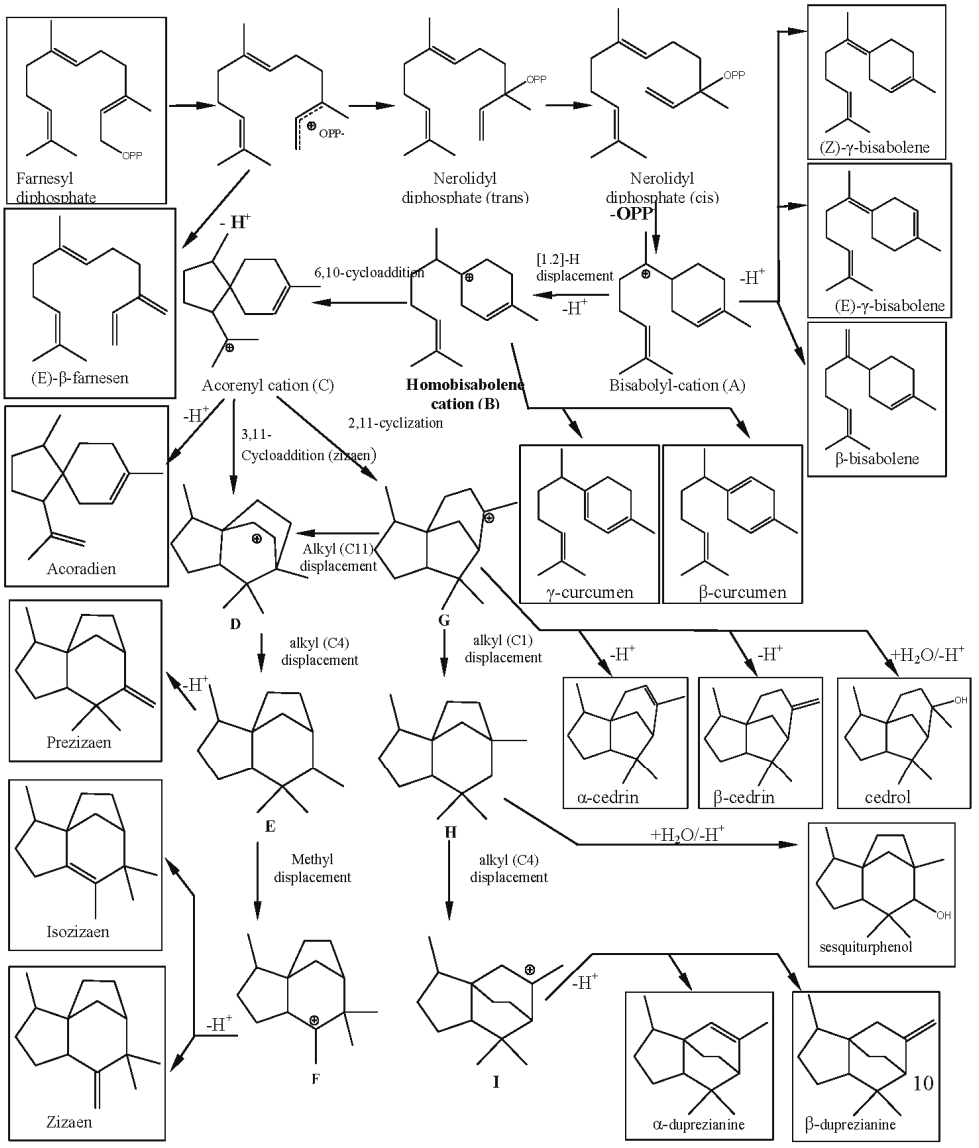


ring and lactone fragment. Hypothetically, the biogenetic route passes through the oxidation of bisabolane (+)-turmerone A (**A**) up to 12-carbaldehyde (**B**). Intermolecular reaction of conjugation their enol isomers (**C**) leads to (7S,8Z)-3,15-dihydroxy-1,3,5,8,10-penten-9,15-epoxybisabolane (**D**). The oxidation of these intermediate products may lead to abiesesquine A (**A**). It's interesting to note, that the absolute configuration at C-7 in abiesesquine A (**45**) is analogous to (**7S**)-(+)-turmeronole A obtained synthetically. The extracted compound (**45**) is a new representative of sesquiterpene lactones of bisabolane type, which molecule structure is containing lactone and benzene rings, that was determined first for the compounds of this group.

In the work of J. Tantillo[46] there are described the quantum-chemical calculations of the cyclization mechanisms for several groups of sesquiterpenes, closely related to each other in a biogenic sense. On the basis of these results, they assumed that the bisabolyl-cation is the basic structural unit, which determines the structure and relative stereochemistry of the formation many sesquiterpenes, such as, (7*S*)-isozizaene, (7*S*,10*R*)-zizaene, (7*R*)-isozizaen, (7*R*,10*R*)-zizaene, (7*S*,10*S*)-zizaene, (7*R*,10*S*)-zizaene, (7*S*,10*S*)-prezizaene, (7*R*,10*S*)-prezizaene, (7*S*,10*R*)--prezizaene, (7*R*,10*R*)-prezizaene, (7*S*,10*S*)-acoradien, (7*R*,10*S*)-acoradien, (7*S*,10*R*)-acoradien, (7*R*,10*R*)--acoradien, (7*S*,10*R*)-R-cedrene, (7*S*,10*R*)-cedrene, (7*R*,10*R*)-R-cedrene, (7*R*)-α-cedrene, (7*S*,10*S*)-R-cedrene,



Scheme 1. **The intended scheme of sesquiterpens formation through bisabolyl-cation**

(7*S*,10*S*)-*a*-cedrene, (7*R*,10*S*)-R-cedrene, etc. (Scheme 1).

In the review devoted to terpene synthases**,** a large amount of monoterpene and sesquiterpene synthases is presented, and as it turned out, bisabolyl-cation contributes to the formation small amounts of sesquiterpenes[20].

A similar validation has in the article of the American scientists[47] who made experiments with the enzyme which takes a part in formation of resins in *Abies grandis* - gamma-humulene synthase. This enzyme consists of 593 aminoacids and synthesizes from farnesyl pyrophosphate a mixture of 52 different sesquiterpenes. The researchers began with the construction of a three-dimensional model of gamma-humulene synthase's molecule. Having a three-dimensional model, it can be determined where the active center is and what aminoacids are included in. Then, from the total number of aminoacids of the active center were excluded those which constitute a so-called "conservative motif" - these aminoacids are identical in all known terpene synthases, whereof it may be concluded that they are necessary for the synthesis of any terpenes, and the change of each of them is likely to result general reduction in enzyme efficiency.

As the result 19 amino acids, included in the active center and not included in the conservative motif, were left for making experiment on. Theoretically predicted combinations were synthesized and tested *in vitro*, at the end it became possible to obtain seven new enzymes suitable for the production individual sesquiterpenes. Four of them do not have natural analogs, including sibirene synthase (produces 78.1 % of sibirene, while the initial gamma-humulene synthase produces only 23.1 % of this sesquiterpene) and alpha-longipinene synthase (produces 61.5 % of alpha-longipinene against the initial 4.7 %).

There were obtained two new gamma-humulene synthases producing perceptible more gamma-humulene than the original enzyme (54.6 % and 85.7 % against the initial 45.1 %). Apparently, in the coming years, the technology of artificial production of new enzymes will develop fast. The authors[47] believe that very soon it will be possible also to design enzymes for the production of substances missing in nature.

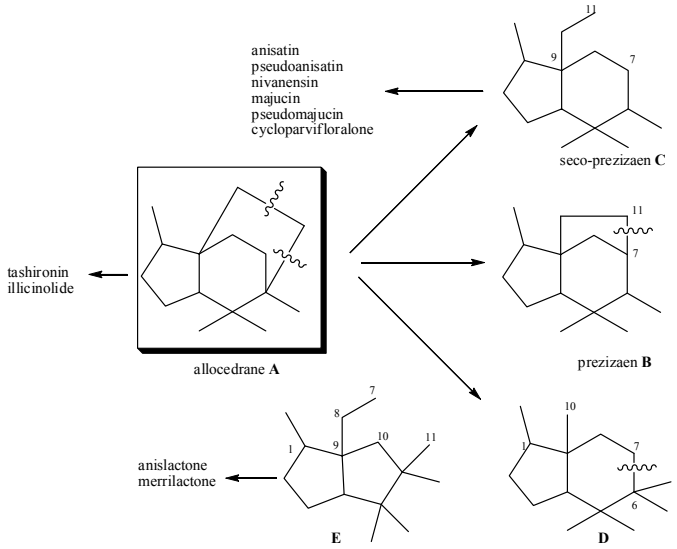
A great variety of terpenes the plants produce due to the enzyme of terpenoid-synthase and cytochrome P450. Scientists focused on the study of two families of genes encoding these proteins. Thus, for example, researchers from Great Britain said that the change in the structure of natural substrates of the terpene synthases' enzymes may lead to the creation of new pesticides having lower risk to the environment, and new important biologically active compounds[48].

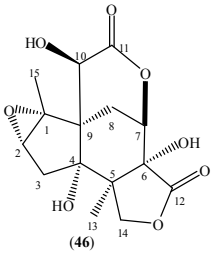
Non-typical prezizaane, seco-prezizaane, merrilactone and anislactone structure is in possession of sesquiterpene lactones isolated from plants of the genus *Illicium* mainly distributed in Eastern Asia and Southeast of North America. In Southern China grow over 25 species of *Illicium*. Some of them were found to be compounds with high neurotrophic and neurotropic activity.

In the monograph of Atta Rahman et al.[49], devoted to the chemistry of compounds isolated from the species of *Illicium*, a possible biogenetic path for the formation of unusual sesquiterpene lactones from tricyclic carbon skeleton of allocedrane is conjectured.

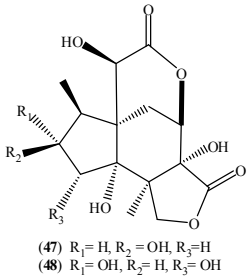
Cleavage of the bond between C-10 and C-11 in **A** serves as a source of bicyclic carbon skeleton of **D**, that duplicates a bond cleavage C6-C7, and then of a pentacyclic between C6 and C10. This leads to the formation of anislactone carbon skeleton E. This biogenic hypothesis, probably, does not have contradictions in the explanation of the inversion of configuration C-9 and the becoming of methyl group C-8. Therefore, allocedrane **A** can be considered as an important intermediate metabolite for the biosynthesis of all *Illicium* sesquiterpenes.

Currently, from the species of the genus *Illicium* were isolated more than 10 sesquiterpene lactones of seco-prezizaane type, for example, from aqueous-ethanol extract of *Illicium icranthum* Dunn were isolated and iden-

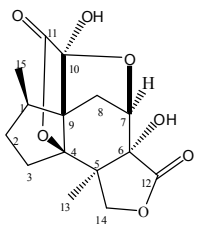




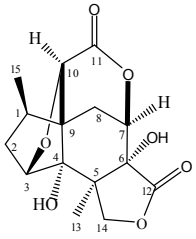
(**46**)



(**47**) R_1= H, R_2 = OH, R_3=H
(**48**) R_1= OH, R_2= H, R_3= OH



(**49**)



(**50**)

tified new compounds (**46-48**) (ref.[50]).

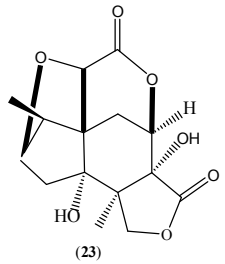
Three new sesquiterpene lactones of seco-prezizaane type (**49-50**) were isolated from methanolic extract of *Illicium jiadifengpi* B. N. Chang with the using by column chromatography over silica gel, Sephadex LH-20 and preparative HPLC. The compounds (**49-50**) have high neurotrophic activity[51], and (**49**) shows this activity at low doses.

From methanolic extract of this plant by Japanese and Chinese scientists in common, two new nor-sesquiterpene lactones (**52-53**) of seco-prezizaane type were isolated. The structure and absolute configuration of (**52**) were determined on the basis of the spectral data analysis. In addition, compound (**52**) has shown a high neurotrophic activity[52].

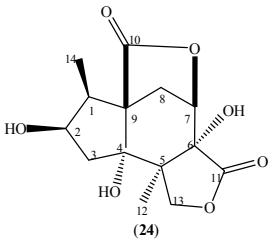
Probably, the biosynthetic route for formation of compounds (**52**) and (**53**) is due to that the hydroxy group at C-10 of compound neomajucin is oxidized to a keto-group of lactone A, which leads to the formation of a keto-carboxylic acid and the formation of acetal group between C-7 and C-10 followed by the creation of (**52**) and (**53**).

Besides, two seco-prezizaane sesquiterpenes (**54-55**), from ethanol extract of *Illicium oligandrum* were isolated 4 new merrillianin sesquiterpene lactones (**56-59**) by Chinese researchers Merr. and Chun, meanwhile the structures (**56**) and (**57**) were determined via X-ray diffraction analysis[53].

Due to high neurotrophic activity of sesquiterpene lactones of seco-prezizaane type, H.M. Shinde[54] suggests a general strategy for obtaining these compounds from chiral



(**23**)



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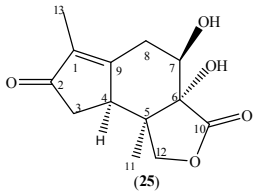
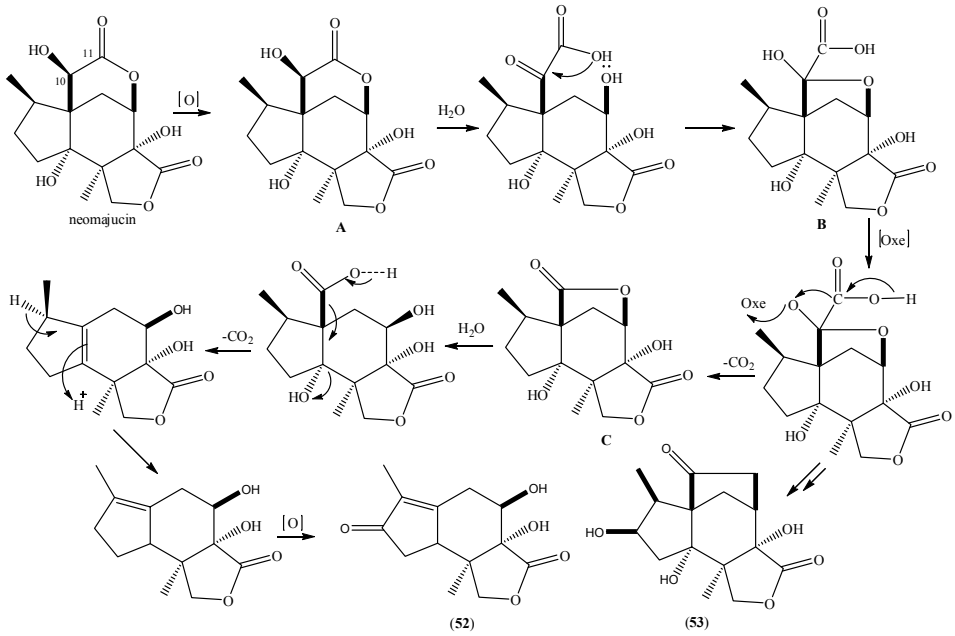
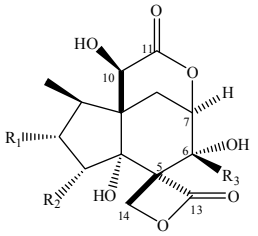


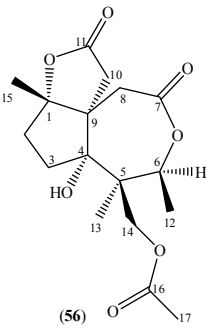
(**25**)



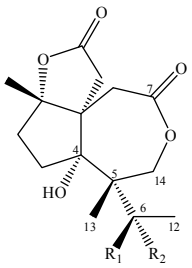
(**52**) (**53**)



(**54**) R_1=H R_2=OH R_3=CH_2OCH_3
(**55**) R_1=OH R_2=H R_3=$COOCH_3$

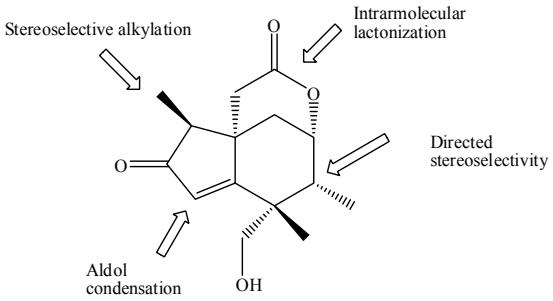


(**56**)



(**57**) R_1=H R_2=$OCOCH_3$
(**58**) R_1=H R_2=OH
(**59**) R_1= R_2=O

tricyclic synthon, which can be easily obtained at the expense of enzymatic separation of the adducts formed by the Diels-Alder reaction of cyclopentadiene and parabenzoquinone. Developed approach allows the using stereogenity of norbornyl system for obtaining the target stereochemical configuration in all key stereogenic centers. The new approach is an interesting strategy of passing from stereo-divergence to stereo-convergence, the possibilities of this approach allowed carrying out a full synthesis of (+)-1*S*-minwagenone.



Thus, isolated and synthesized new sesquiterpene lactones of prezizaen type can be recommended for further in-depth researches on the development of new drugs with neurotrophic action, and also sesquiterpene lactones of this type from plants of the genus *Illicium* can be considered as chemotaxonomic markers for this genus.

A special place among sesquiterpene lactones is occupied by the dimers. For nowadays, information about the isolation, total synthesis, chemical modification of the molecules of dimeric sesquiterpene lactones and their biological activity is too poor. In the works of K.S. Rybalko[55], F. Seaman[56], S.Z. Kasymov[57] were published data of two or more dimeric lactones. By Chinese scientists Z.-K. Zhan, Y.-M. Ying et al. was published a review on natural dimeric sesquiterpenoids[58]. The review includes reference information on this series of natural terpenoids isolated by 2010 inclusively.

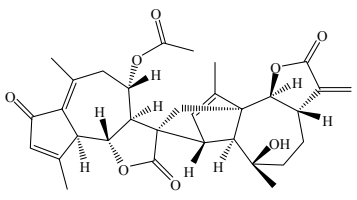
The structure of disesquiterpenoids, whose carbon skeleton consists of 30 carbon atoms, should be considered as a product of dienone synthesis by two molecules of sesquiterpenoids. The formation of dimeric molecules, probably, goes due to the biosynthesis with enzymes catalyzing the Diels-Alder reaction. There is some known information about biomimetic synthesis of these natural complex metabolites, and therefore a complete synthesis of their molecules and finding out the biogenetic transformations of dimeric sesquiterpenoids is an important task in the field of natural isoprenoids' chemistry.

Analysis which was carried out by us of the available information shows that, at the present time from natural sources were isolated over 200 dimeric sesquiterpene lactones. A large number of molecules have traditional structural types of guaian, lindenane, eremophilane[17].

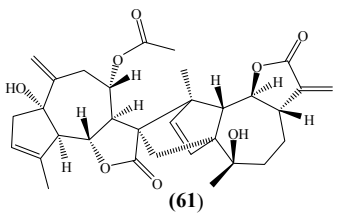
In comparison with monomeric sesquiterpene lactones, dimeric compounds have a relatively limited distribution, for example, guaian dimers are mainly typical for the plants of the genus *Artemisia* and genus *Inula*, which consist of eudesmane and guaian sesquiterpenoids. Eremophilane dimers are a typical chemotaxonomic feature for the plants of the genus *Ligularia* and lindenanes are for *Chloranthus*.

From the foregoing review, the dimeric sesquiterpene lactones of guaian and psuedoguaian types are presented with 59 compounds, of which 27 were isolated from the plants of the genus *Artemisia*. The first natural dimeric sesquiterpene lactone of guaian type, absinthin was isolated in 1956 by Czech scientists V. Gerout with his colleagues, from Artemisia (*Artemisia absinthium* L.).

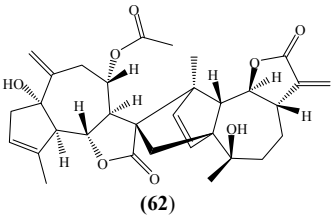
A new dimeric guaianolide (**60**), named artanomadimer A, and its five analogues were isolated from the aerial



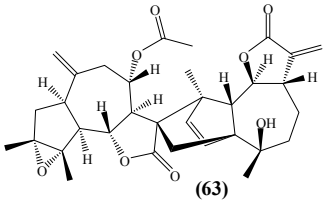
(**60**)



(**61**)



(**62**)



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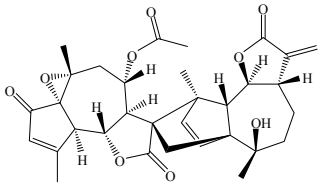

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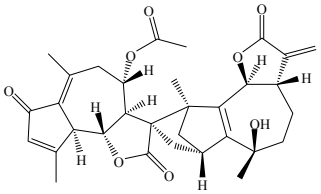

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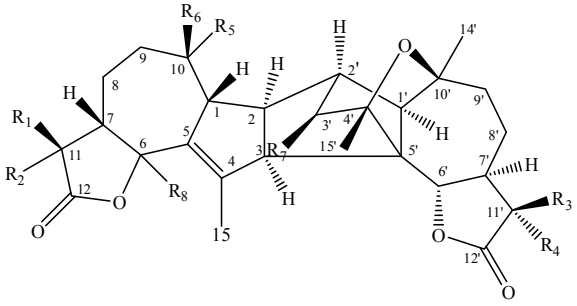
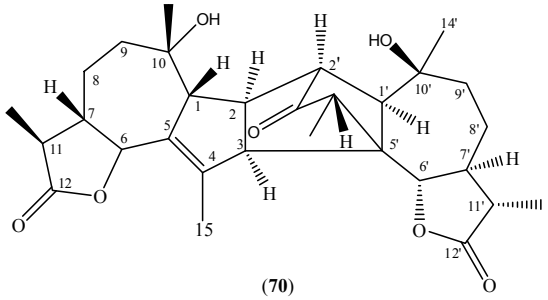




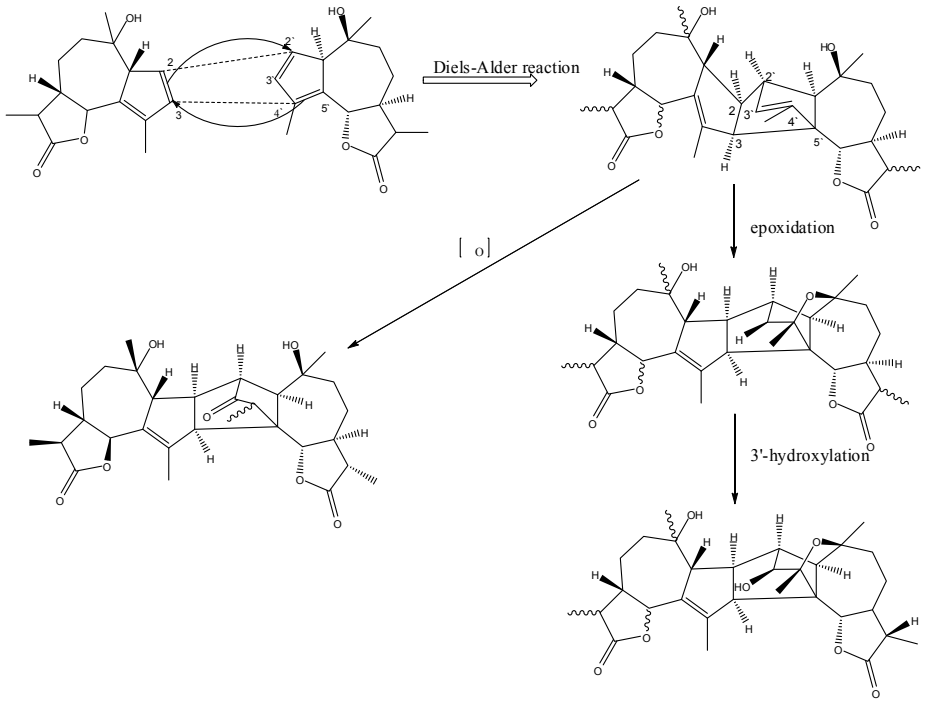
(**70**)

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
(**66**)	H	CH_3	H	CH_3	OH	CH_3	H	α–H
(**67**)	CH_3	H	CH_3	H	OH	CH_3	H	α–H
(**68**)	CH_3	H	H	CH_3	OH	CH_3	OH	β–H
(**69**)	H	CH_3	H	CH_3	CH_3	OH	OH	α–H

flowering part of *Artemisia anomala* S. Moore (**61-65**).

Structures and stereochemistry of these substances were determined spectrally, and absolute stereochemistry of the compound (**63**) was confirmed via X-ray diffraction analysis. The biosynthesis of artanomadimer A (**60**), probably, goes via the Diels-Alder reaction with the formation of new carbon-carbon bond between C-11/C-2' and C-13/C -5'. During the screening on cytotoxicity, it was revealed that the compounds (**60**) and (**65**) show significant inhibitory effects against the growth of cells BGC-823 with IC_{50} with values 2.71 and 6.25 μm, respectively[59].

One of the sources of dimeric guaianolides is *Artemisia absinthium* L., widespread in Europe, Siberia, Asia and Northern Africa and widely used as anti-parasitic prepara-



tion. Previously, from this genus of Artemisia were isolated sesquiterpenes[60], dimeric guaianolides[61], flavones[62], lignanes[63] and essential oils[64]. In the continuation of search for biologically active compounds from this genus of Artemisia, 5 new dimeric guaian lactones (**66-70**) were isolated[65]. Compound (**69**) showed inhibitory activity against LPS-induced NO production in cells BV-2.

The biosynthesis of isolated guaian dimers goes, hypothetically, via the Diels-Alder reaction.

Another source of dimeric guaian sesquiterpene lactones is the plants of the genus *Achillea*. Two new sesquiterpene dimers (**71, 72**), with previously not described skeletal systems, were isolated from *Achillea millefolium* L. The structures of these compounds were determined via spectral methods and on the basis of supposed scheme of biogenetic transformation[66].

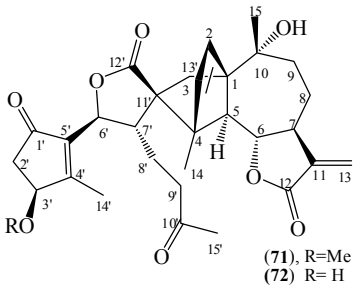
Two new sesquiterpene dimers, ethers A (**73**) and B (**74**) were isolated from the flowering tops of *Achillea millefolium*. The structures (**73**) and (**74**) were determined via comprehensive spectral analysis[67].

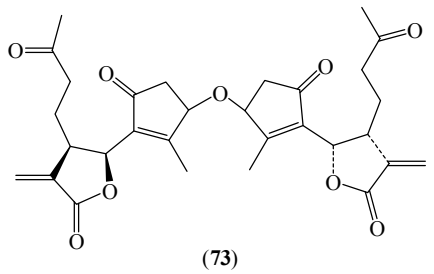
The formation of dimer (**72**), probably, goes due to the biosynthesis with the participation of enzymes, catalyzing the Diels-Alder reaction. The predecessors proposed iso-seco-tanapartolid (**A**) and guaianolide (**B**).

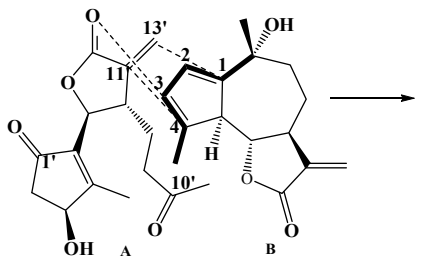
From ethanol extract of perennial herbaceous plant *Chrysanthemum indicum* L. of the family *Astereceae* a new dimeric guaianolide (**75**) was isolated, its structure was determined by spectral methods and X-ray analysis[68].

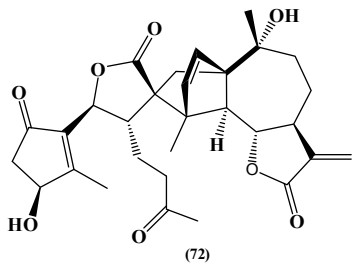
A new sesquiterpene lactone (**76**) is the first furanoheliangolide dimer isolated from the leaves of *Piptocoma rufescens*, collected in the Dominican Republic. Its structure was determined by the interpretation of the spectral data, and the absolute configuration via CD spectrum analysis. This compound showed high cytotoxicity with IC_{50} when tested against HT-29 human colon cancer. The authors proposed a biogenetic route of this compound[69]. Dimeric sesquiterpene lactones are rare natural products, mostly found in the family of Compositae, and have a range of constructional types. It is assumed that a new dimeric sesquiterpene lactone is formed via the Diels-Alder reaction.

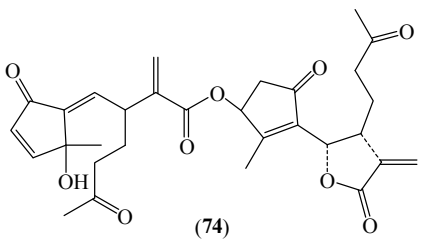
A new dimeric eremophilane sesquiterpene lactone with cyclobutane ring, biliguhodgsonolide (**77**), and 4S,5S,6R,10R)-8,9-seco-12-hydroxyeremophil-7(11)-en--14,6;12,8-diolid-9-al (**78**),were isolated from ethanol extract of the roots and rhizomes of *Ligularia hodgsonii*

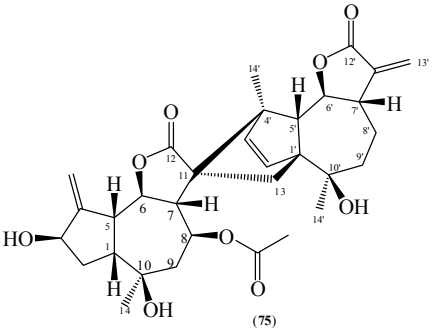


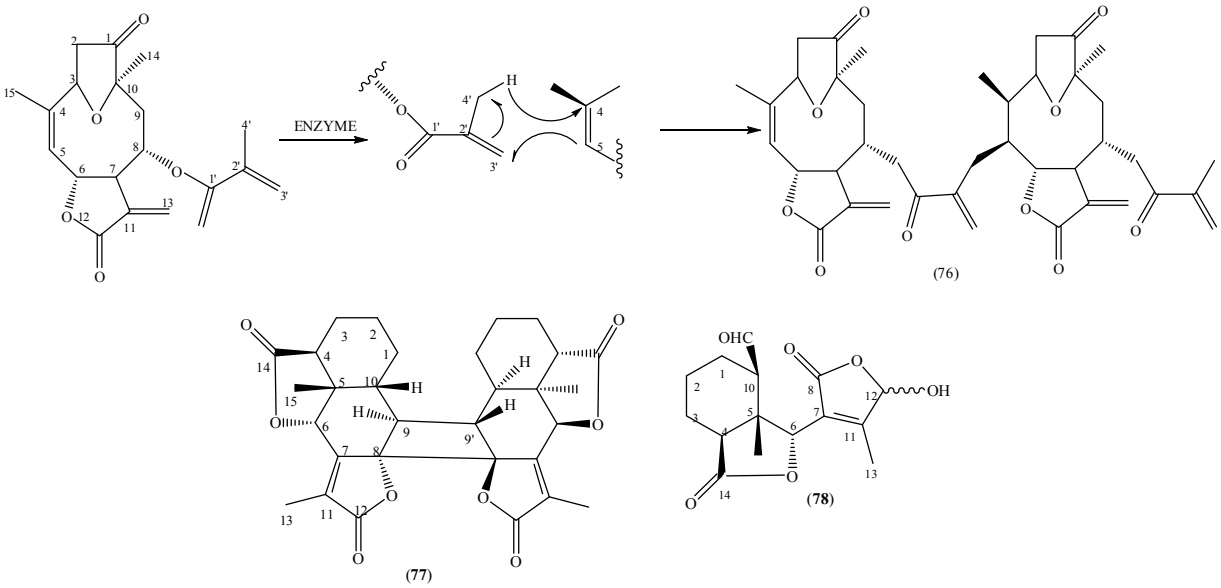












Hook. Their structures, including absolute stereochemistry, were determined with the help of spectral data[70].

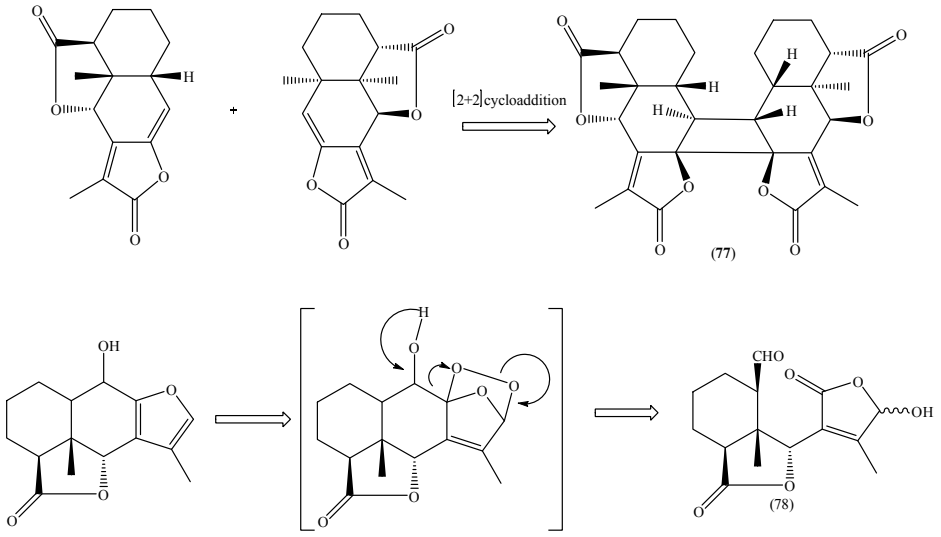
Compound (**77**) is an illustrative example of dimer formation, which is considered as a natural product of dienone synthesis of two molecules.

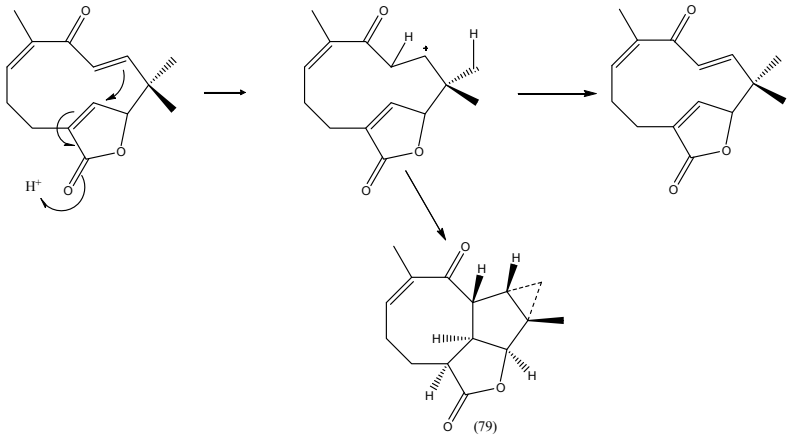
In this work it is first reported about the isolation of unusual lactone seco-eremophilane diolide from the plants of the genus *Ligularia*. Possible mechanism of formation to (**78**) is shown in the scheme below:

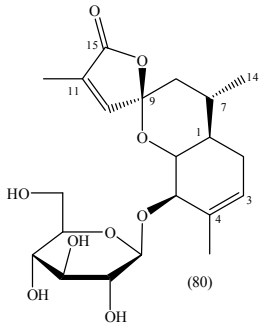
An unusual lactone (**79**), with functional groups that are rare for natural products, was isolated from *Nauplius graveolens subsp. odorus* (Schousb) Wikl and conforms to the new skeleton of 14,15-dimethyl-7,13-dioxotricyclic [6.4.0.09,11]dodeca-12,13-olide and was determined on the basis of spectral methods including 2D-NMR. The authors proposed a biosynthesis of this compound (**79**). The acid induces cyclization of known compound arteriscunolide C, and leads to intermediate carbocation, which can develop in the direction 1 or 2, with the loss of H9 or H14, respectively[71].

2(5*H*)-furanones are involved as intermediates in organic synthesis. Such compounds have various types of bioactivity, such as, anti-inflammatory, antitumor, antibacterial, insecticidal, etc. Molecules with spiro-2(5*H*)-furanone fragment are rarely isolated from natural sources, especially with sesquiterpenoide skeleton. However, from *Abies dalavayi* Franch. var. *Delavayi* a new sesquiterpene lactone (**80**) was isolated[72].

It's known that there are irregular acyclic sesquiterpene lactones, in the biosynthesis of which the regular order of alternation of isoprene units is broken. These acyclic molecules, like farnesyl pyrophosphate, are able to





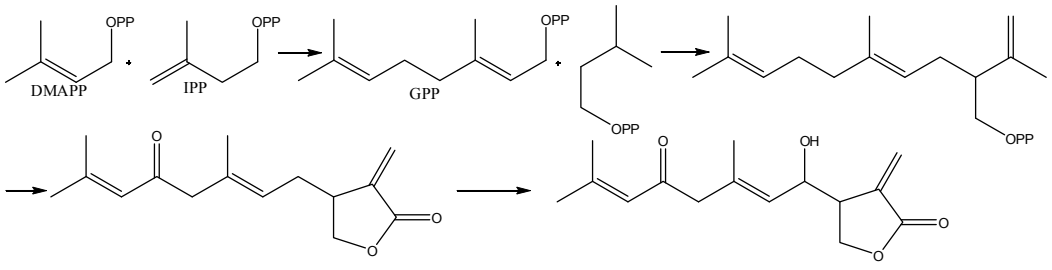


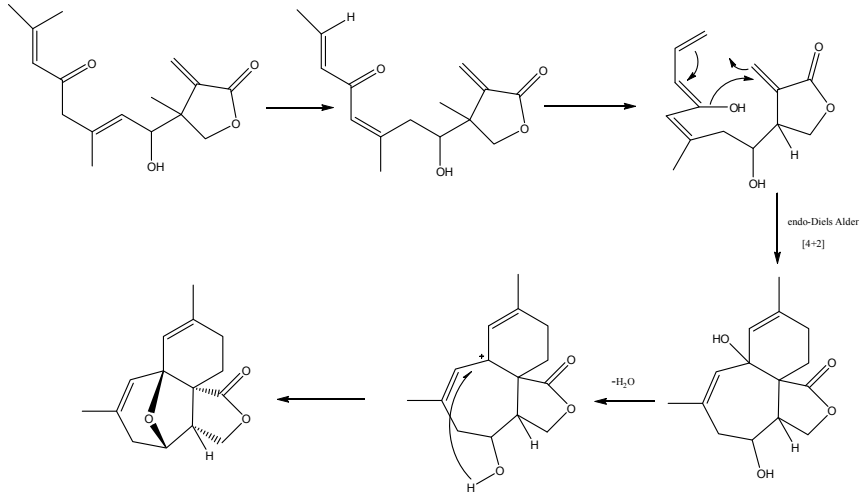
undergo the reaction of cyclization and rearrangement, forming to an irregular cyclic sesquiterpenoids. For example, there is shown an interesting way of the formation new compound (**81**) with an unusual structure. Previously it was proved, that isoprenoid compounds are derived from isopentyphosphate, which, in its turn, from mevalonic acid, and in the early 2000s this route was cancelled due to the discovery of methylerythritol phosphate. Modern publications confirm the existence of two routes and a new route goes in plastids and is responsible for the biosynthesis of monoterpenoids, diterpenoids and carotenoids, and the formation of sesquiterpenoids, triterpenoids and steroids goes in the cytoplasm. As a rule, all sesquiterpenoids come from farnesyl pyrophosphate; however there is some information that certain sesquiterpenes are formed outside farnesyl pyrophosphate route. An example is the isolation of sesquiterpene lactone with an unusual structure, anthecularine (**81**) from *Anthemis auriculata.*

Currently, there are rather few works devoted to the study of biosynthesis such unusual sesquiterpene structures, although these works will not only contribute the solution to actual issues concerned with the peculiarities of their metabolism and biosynthesis, but also will allow to confirm the molecular structure of certain compounds. Given sesquiterpene lactones with a non-typical molecular structure are distinguished primarily by their structural type and, respectively, by physicochemical and biological properties. Isolated from marine organisms, sesquiterpene lactones of unusual structure are optically active compounds, mostly oily and colorless, rarely crystalline. Among them are also found the heteroatomic representatives. Sesquiterpene lactones from plants and higher fungi are colorless crystalline compounds, rarely oily.

Within individual groups of living organisms, the majority of secondary compounds have only a limited distribution. They are typical for certain families or even genera, species, subspecies and chemical races. The more chemical reactions are required for the synthesis of any secondary compound, usually the more limited its distribution is. Therefore, germacranolide costunolide (**82**), the product of the relatively simple biosynthetic route, is produced in many plant species[55,56], while a complicated guaianolide as tapsigargine (**83**) is isolated from *Thapsia garganica* L.[74].

Costunolide (**82**) is considered the most widespread precursor of sesquiterpene lactones, as well as some enzymes involved in the biosynthesis of lactones. H. Bouwmeester et al. argue that costunolide is involved in the biosynthetic route of many lactones, for example, being





a precursor of cynaropicrin in *Cynara cardunculus* L. and of three synthases, germacrene synthase, germacrene oxidase and costunolide synthase[75]; the predecessor of lettucenine A *in Lactuca sativa* L.[76], and of parthenolide in *Tanacetum parthenium* L. Schulz Bip[77], of arglabin in *Artemisia glabella* Kar. et Kir.[78].

Thus, in summary, the following should be noted:

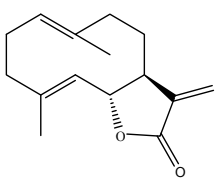
Most of the described compounds are enclosed in plant raw materials; a great number of lactones are isolated from marine organisms, the least number is the secondary metabolites of microorganisms. There is some information on the discovering sesquiterpene lactones in *Disidea pallescens*, representative of the animals' family *Aplysillidae*;

A great number of new structural types of sesquiterpene lactones of unusual type have been found recently and the predecessor for many of them is farnesyl pyrophosphate. Until recently it was considered that the biosynthesis of these simple predecessors is carried out via the stage of mevalonic acid formation. For many lactones of marine metabolites, fungi and plants is also typical the non -mevalonate pathway of formation, i.e. meanwhile non-farnesyl lactones (apliacanes, tremulanes, preziranes, strigolactones, pingvisanes, etc.) are formed;

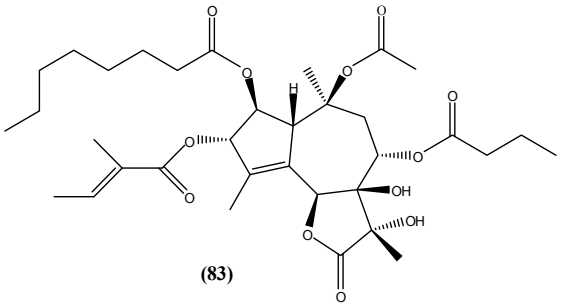
The main part of isolated lactones was examined for biological activity, and it was shown that the majority of them has antimicrobial, antifeedant, insecticidal, antioxidant, anti-inflammatory, anticancer properties, for example, lindenane dimers, mono- and dimeric eremophilanoids have antitumor properties, seco-prezizaanes have neurotrophic properties, lactaranes have antifungal ones, the antimicrobial and antitumor properties are typical for the lactones of marine organisms;

There is revealed a certain regularity of sesquiterpene lactones' groups to one or the other taxons. This provides ample possibilities for using sesquiterpene lactones as chemotaxonomic markers. For instance, furanosesquiterpenoids have found in sponges, the halogenated sesquiterpene lactones have found only in red algae, lactarane compounds are typical for many fungi, dimeric compounds have found in plants of the family *Asteraceae*, lactones of prezizaane type have found in the plants of the genus *Illicium*, etc.;

Many sesquiterpene lactones of unusual type from plants can be obtained by the engineering method in heterologous hosts via enzymatic groundwork, for example, there was developed the producing of anti-malarial drug, artemisinine in *E. coli* and in yeast[79]. Artemisinine from *Artemisia annua* is effective against *Plasmodium* species which is resistant to many drugs, but is very expensive for one-third of patients in the world. The Keasling group[79] obtained an *E. coli* strain, the precursor producing of it, is artemisinic acid, at concentrations over 300 mg/L. It required intensive work, including the construction of mevalonic route for the producing sufficient quantities of iso-



(82)



(83)

prenoid synthesis' precursor, the optimization expression of amorphadiene synthase (a key cyclase of terpenes) and inclusion the modified version of amorphadiene oxidase (P450, transforming amorphadiene to artemisinic acid).

Thus, further studies of sesquiterpene lactones' chemistry with unusual carbon skeletons, including search of new and unusual structural types among them, determining their structure, studying the biogenetic route of formation, their biological activity, is a prerequisite for creating the effective drugs with a broad-spectrum of pharmacological action.

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SYNTHESIS OF GROSHEIMIN FLUORODERIVATIVES

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Keywords: Sesquiterpene lactone, grosheimin, reactive center, fluoroderivatives.

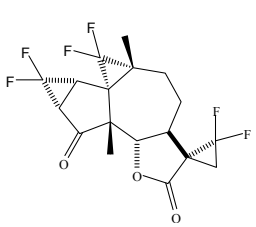
Introduction

Sesquiterpene lactones have a big group of the natural terpenoids, which are often found in flora and possess a wide range of biological activities, including such activities as antitumor, anti-malarial, antimicrobic, antifungal, anti-inflammatory, allelopathic, growth-stimulating and antifeedant. Among them the attention of researchers is drawn by the molecule structures containing heteroatoms such as chlorine, fluorine, nitrogen, sulfur, etc. In our available literature the fluorine atoms in the molecule structures of isolated sesquiterpene lactones have been not found.

It is known that about 10 % of all pharmaceutical drugs in the market contain fluorine in their structures. Introduction of fluorine atoms to a molecule of natural compounds changes their physical and chemical properties, which are closely connected with biological functions. Changes in properties influence on absorption, distribution and interaction with a target cell/tissues/organs. It gives the chance to change a pharmaceutical profile of these compounds in the huge range and explains importance of their application. In 1979, I. Salazar et.al[1] firstly synthesized the new difluorocarbenederivatives of anhydroparhtenin sesquiterpene lactone. Pyrolysis reaction of sodium chorodifluoroacetate in the boiling diglyme forms a reactive difluorocarbene, attacking double bonds of this pseudoguaianolide with formation of derivatives 1–5.

Recently the new fluoroderivatives based on sesquiterpene lactones of parthenolide[2] and artemisinin[3] were synthesized by several reactions.

One of almost available sesquiterpene lactones is a guaianolide grosheimin 6 isolated firstly from *Grossheimia marcocephala* (Muss.-Puschk. ex Willd.), later found in *Chartolepis biebersteinii* Jaub. et Spach., *Ch. glastifolia* (L.) Cass., *Ch. intermedia* Boiss., *Ch. pterocaula* Trautv., and also in some species of *Centaurea*[4–9]. Therefore sesquiterpene lactone grosheimin 6 is of interest to chemical modification of its molecule as renewable material.



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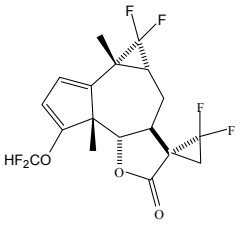

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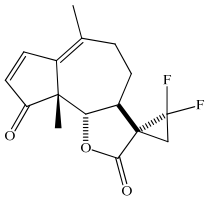

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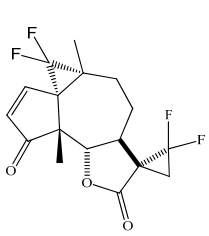

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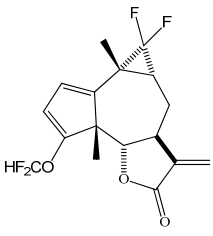

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The distinctive feature of this molecule structure of (6) is its polyfunctionality, first of all, linked with existence of α-methylene-γ-lactone cycle, hydroxyl keto-groups, and exocyclic double bonds that are the main reactive centers.

According to the quantum and chemical calculations, a high electronic density is determinated in atoms of oxygen. Also it is characteristic for end atoms of carbon C-13 and C-14 of double bonds of grosheimin molecule 6. The A-ring of grosheimin 6 has no sp^2 – hybrid atoms. Therefore it is non-planar (dihedral angles of $C^1C^2C^3C^4$ make – 8.2° and 21.4° in the lactone molecule). Adjacent part with a seven-membered cycle also is non-planar (dihedral angle of $C^5C^1C^{10}C^9$ is equal 91.4°, $C^6C^5C^1C^{10}$ = –25.6° in the lactone). While a lactone cycle of this molecule is more planar ($C^6C^7C^{11}C^{12}$ is about 16°) because of presence of two sp^2 – hybrid atoms (Fig. 1).

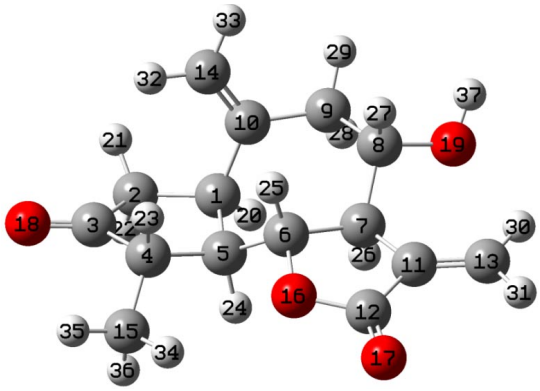
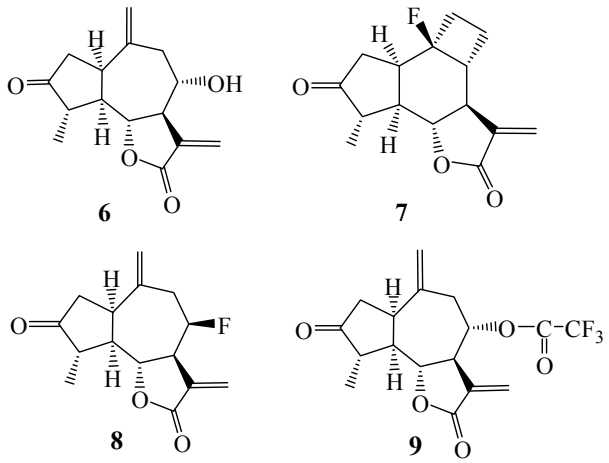


Fig. 1. **Grosheimin conformations by optimized method PM6**

Atoms of oxygen in grosheimin molecule 6, in general, are proton acceptor and the oxygen atom of a hydroxyl group works as a proton-donor.

This paper discusses the ways of synthesis of fluoroderivatives (7–9) of grosheimin molecule 6.



Results and discussion

The fluoration reaction of sesquiterpene lactone grosheimin 6 was performed by diethylaminosulfur trifluoride (DAST). Earlier American researchers of H. Zifer, et.al[10] synthesized some new fluoroderivatives of a sesquiterpene lactone dihydroartemisinin by the fluoration reaction with this reagent.

Mixture of two substances is formed in the reaction of grosheimin 6 with diethylaminosulfur trifluoride (DAST) in a chloride methylene. The received mixture of substances was chromatographed on silica gel and formed derivatives of 7 and 8.

Grosheimin fluoroderivative 7 is a colourless crystal substance, $C_{15}H_{17}O_3F$. ^1H NMR-spectrum of molecule 7 has signals of lactone protons (δ_H =3.99 ppm), protons of

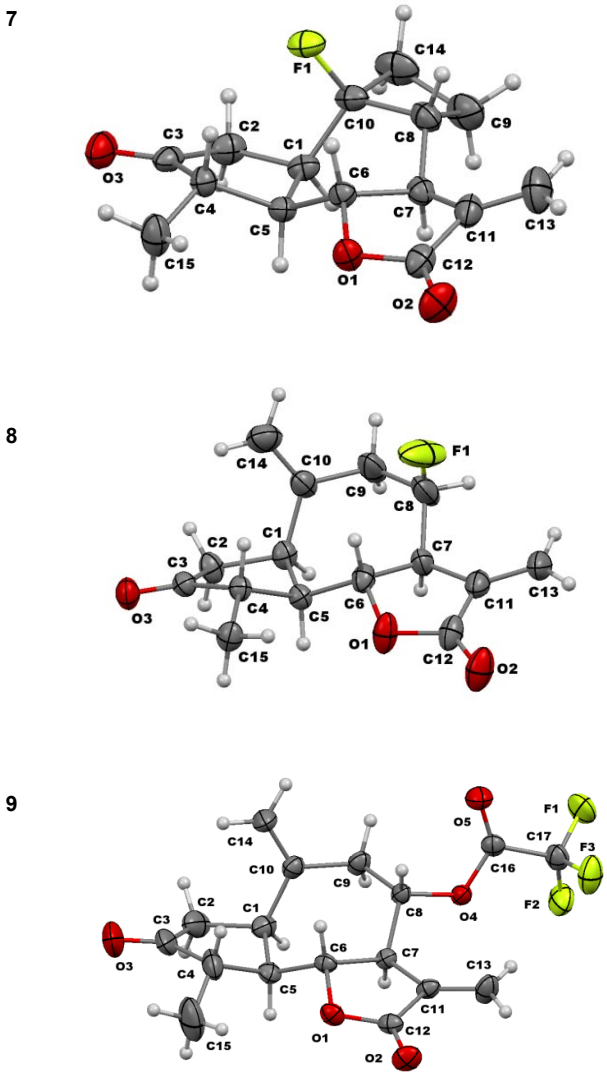


Fig. 2. **Spatial molecule structures of 7–9** (colored, red: O, yellow: F, white: H)

exomethylene group (δ_H=5.50 ppm, δ_H=6.18 ppm) and protons of methyl group (δ_H=1.19 ppm). ^{13}C NMR-spectrum has signals of 15 carbon atoms, from them one signal is for methyl, four – for quarternary, six – for tertiary and four – for secondary carbon atoms. Signals of carbon atoms of keto-groups were observed at δ_C=217.40, δ_C=170.09 and atom of carbon linked with fluorine at δ_C=96.41.

Data of ^{1}H, ^{13}C NMR-spectra of molecule 7 is shown in Table IV.

Grosheimin fluoroderivative 8 is a colourless crystal substance, $C_{15}H_{17}O_3F$. ^{1}H NMR-spectrum of molecule 3 has signals of lactone proton (δ_H =4.45 ppm), protons of olefinic groups (δ_H=5.71 ppm, δ_H=6.43 ppm, δ_H=4.82 ppm, δ_H=5.18 ppm) and protons of methyl group (δ_H=1.26 ppm). ^{13}C NMR-spectrum has 15 carbon atoms, among them one signal is methyl, four are for quarternary, six – for tertiary and four – for secondary carbon atoms. Signals of keto-groups were observed at δ_C=218.61, δ_C=168.89 and atom of carbon linked with fluorine at δ_C=86.63.

Data of ^{1}H, ^{13}C NMR-spectra of molecule of 8 is given in Table V.

Based on the determined molecular structures of 7 and 8, including data of X-ray analysis (Fig. 2), it is possible to note that formation of 8 proceeds as substituting of hydroxyl group in structure of the initial grosheimin 6. And molecule of 7 is formed via carbocationic mechanism of an intramolecular regrouping.

Diethylaminosulfur trifluoride (DAST) is a widely used as stable and effective fluorinating agent[11]. It should be noted that fluorine reagents usually are bad nucleophiles. Fluorine is a weak anion, which can form strong hydrogen bonds. It is known that diethylaminosulfur trifluoride (DAST) induces formation of carbocations of homoallyl alcohols and further expansion of a cycle. Thus the primary way of addition by hydroxyl group of 6 forms a carbocationic complex A, and further intermediate 6a passes into non-classical cyclopropylmethyl carbothion 6b being in balance with cyclobutonic ion 6s. Then reaction of 6s with fluoranion forms 7.

The acylation reaction of sesquiterpene lactone grosheimin 6 with trifluoroacetic anhydride in diethyl ether with formation of a new fluoroderivative 9 was performed.

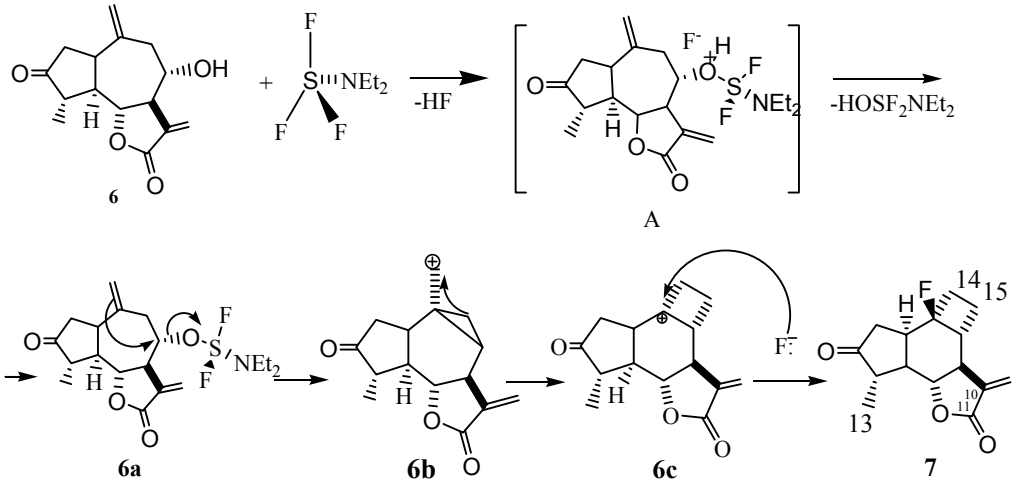
Grosheimin fluoroderivative 9 is a colourless crystal substance, $C_{17}H_{17}O_5F_3$. ^{1}H NMR-spectrum of molecule 9 has signals of lactone proton (δ_H =4.06 ppm), protons of exomethylene groups (δ_H=5.83 ppm, δ_H=6.38 ppm, δ_H=4.93 ppm, δ_H=5.18 ppm) and protons of methyl group (δ_H=1.19 ppm). ^{13}C NMR-spectrum has 17 carbon atoms, from them one signal is for methyl, six – for quarternary, six – for tertiary and four – for secondary atoms. Signals of carbon atoms of carbonyl groups were observed at δ_C=217.79, δ_C=168.32, δ_C=156.11 ppm and atom of carbon linked with fluorine at δ_C=115.18 ppm.

Conclusion

Thus, three new fluoroderivatives, based on sesquiterpene lactone grosheimin, were synthesized, the structure of the received compounds of 7–9 was determined on the basis of the physical and chemical constants and spectral data (^{1}H, ^{13}C NMR, two-dimensional NMR-spectra ^{1}H-^{1}H (COSY, NOESY) and ^{13}C-^{1}H (COSY, COLOC), and the X-ray analysis.

Experimental part

Grosheimin 6 – guaiane sesquiterepene lactone which is isolated from aerial parts of *Chartolepis intermedia* Boiss.[8], colourless crystal substance, m.p. 200–202 °C



Scheme 1. **Estimated mechanism of formation of derivative 7**

Table I
The geometrical characteristics (lengths of bonds (r, Å), bond angles (grad)) of molecules of grosheimin 6 according to calculations by PM6 method

Characteristics of angles	R, Å
$r(C^1\text{-}C^2)$	1.538
$r(C^1\text{-}C^{10})$	1.517
$r(C^2\text{-}C^3)$	1.519
$r(C^3\text{-}C^4)$	1.530
$r(C^4\text{-}C^5)$	1.548
$r(C^{11}\text{-}C^{12})$	1.496
$r(C^{11}\text{-}C^{13})$	1.329
$r(C^{12}\text{-}O^{16})$	1.400
$r(C^{12}\text{-}O^{17})$	1.199
$r(C^8\text{-}O^{19})$	1.444
$C^1C^2C^3$	106.8
$C^2C^3O^{18}$	124.5
$C^2C^3C^4$	109.7
$C^3C^4C^5$	105.4
$C^4C^5C^1$	106.4

(ethanol), $C_{15}H_{18}O_4$, [α] + 119°.

1 G of grosheimin 6 was dissolved in 10 ml of CH_2Cl_2, and then 0.75 ml of diethylaminosulfur trifluoride was added. Reaction was performed at room temperature (20 °C) during 12 hours. Reaction process was controlled by the TLC method. Conversion is partial. It was neutralized with $NaHCO_3$ solution. An organic layer was dried under Na_2SO_4, filtered and solvent was stirred off. The residue (0.633 g) was chromatographed on silica gel column (8 g). Column was eluted with mixture of solvents of petroleum ether and ethyl acetate (9:1), thus compounds 7, 8 were formed. The yield was 3 and 5 % respectively.

262 Mg of grosheimin 6 was added into a flask, dissolved in 5 ml of Et_2O. Then 0.19 ml $(CF_3CO)_2O$ and 0.20 ml of Et_3N were added there. Reaction was performed with continuous stirring and at room temperature. 180 Mg of crystal substance of 9 was received. The yield was 81 %.

Compound 7: m.p. 185–187 °C (petroleum ether:ethyl acetate 2:1). Data of ^{13}C and ^1H NMR are presented in Table IV. The peak of molecular ion $[M]^+$ 264.11 is in the mass spectrum.

Compound 8: m.p. 81–83 °C (petroleum ether:ethyl

Table II
Crystal data and refinement parameters of compounds 7–9

	7	**8**	**9**
Crystal size (mm)	0.14×0.17×0.56	0.32×0.41×0.52	0.24×0.43×0.55
Empirical formula	$C_{15}H_{17}FO_3$	$C_{15}H_{17}FO_3$	$C_{17}H_{17}F_3O_5$
M_w	264.29	264.29	358.31
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	*P*212121	*P*212121	*P*212121
Temperature [K]	200	200	200
a [Å]	6.7564(3)	6.6454(8)	8.1397(2)
b [Å]	7.8295(3)	9.4641(9)	11.9706(3)
c [Å]	24.7956(10)	20.629(2)	16.8447(4)
α [°]	90	90	90
β [°]	90	90	90
γ [°]	90	90	90
V [Å^3]	1311.67(9)	1297.4(2)	1641.30(7)
Z	4	4	4
Dc [Mg·m^{-3}]	1.338	1.353	1.450
μ [mm^{-1}]	0.101	0.102	0.127
F(000)	560	560	744
Reflns collected / unique	15286 / 2978	9187 / 2921	16723 / 3766
*R*int	0.0338	0.0382	0.0360
θ_{max}, reflns $I > 2\sigma(I)$	27.51, 2558	27.51, 2523	27.50, 3473
Data / parameters	2978 / 174	2921 / 174	3766 / 228
Goodness on fit	0.977	1.067	0.911
*R*1, *wR*2 [$I > 2\sigma(I)$]	0.0354, 0.0974	0.0410, 0.1100	0.0309, 0.0813
*R*1, *wR*2 [all data]	0.0461, 0.1131	0.0516, 0.1270	0.0352, 0.0858
Δρ (e·Å^{-3})	0.22 / –0.19	0.32 / –0.28	0.24 / –0.17
Flack parameter	–0.4(9)	–0.3(11)	–0.7(5)
CCDC	1044658	1044659	1044660

Table III
Hydrogen Bonds C-H…X (X=O, F) of compounds 7–9

Compound	D-H...A	distance D-H [Å]	distance H…A [Å]	distance D…A [Å]	angle D-H…A [°]
7	C6-H6…O2[a]	1.00	2.56	3.435(2)	146
	C14-H14A…O1[b]	0.99	2.48	3.389(2)	153
8	C6-H6…F1	1.00	2.36	2.828(3)	108
	C13-H13A…O3[c]	0.95	2.59	3.456(2)	151
9	C6-H6...O3[d]	1.00	2.34	3.063(2)	128
	C13-H13B...O4	0.95	2.39	2.950(2)	118
	C15-H15C...O1	0.98	2.56	3.145(3)	118

Symmetry: [a] 1–x,–1/2+y,3/2–z; [b] x,–1+y,z; [c] 3/2–x,2–y,–1/2+z; [d] –x,1/2+y,1/2–z

Short[13] contacts in compound 9: O2...C16 (–1+x,y,z) 2.796(2) Å, delta=–0.42 Å;
C12...H1 (–1/2+x,1/2–y,1–z) 2.65 Å, delta=–0.25 Å

acetate 2:1). Data of ^{13}C and ^{1}H NMR are shown in Table V. The peak of molecular ion $[M]^+$ 264.11 is in the mass spectrum.

Compound 9: m.p. 161–163 °C (petroleum ether:ethyl acetate 1:1). Data of ^{13}C and ^{1}H NMR are given in Table VI.

Single-Crystal X-ray Analysis: Crystal data and refinement parameters of 7–9 are given in the Supporting Information. CCDC-1044658 (for 7), –1044659 (for 8), and –1044660 (for 9) contain the supplementary crystallo-

Table IV
Data of ^{13}C (150.96 MHz) and ^{1}H (600 MHz) ($CDCl_3$) for molecule 7

№ atom	$\delta_{C,}$ type	$\delta_{H,}$ multiplicity (J in Hz)
1	49.12, CH	2.25-2.20, m, H-1, H-4
2a	35.6, CH_2	2.51-2.36, m, H-2a, H-2b, H-5, H-14b
2b	–	2.51-2.36, m, H-2a, H-2b, H-5, H-14b
3	217.40, C	–
4	35.87, CH	2.25-2.20, m, H-1, H-4
5	46.38, CH	2.51-2.36, m, H-2a, H-2b, H-5, H-14b
6	83.32, CH	3.99, ddd (4.0, 8.0, 11.0), H-6
7	47.21, CH	2.74, dddd (4.0, 9.0, 18.0), H-7
8	43.5, CH	2.89, m, H-8
9	96.41, C	–
10	137.76, C	–
11	170.09, C	–
12 a	119.84, CH_2	5.50, d (4.0), H-12a
12 b	–	6.18, d (4.0), H-12b
13	13.61, CH_3	1.19, d (7.0), H-13
14 a	32.28, CH_2	2.19-2.13, m, H-14a
14 b	–	2.51-2.36, m, H-2a, H-2b, H-5, H-14b
15 a	18.66, CH_2	1.4, m, H-15a
15 b	–	2.3, m, H-15b

Table V
Data of ^{13}C (150.96 MHz) and ^{1}H (600 MHz) ($CDCl_3$) for molecule 8

№ atom	$d_{C,}$ type	$d_{H,}$ multiplicity (J in Hz)
1	39.47, CH	3.02, ddd (3.0, 9.0), H-1
2a	43.67, CH_2	2.57, d (8.0), H-2a
2b	–	2.6, d (8.0), H-2b
3	218.61, C	–
4	46.89, CH	2.35-2.24, m, H-4; H-5
5	50.11, CH	2.35-2.24, m, H-4; H-5
6	80.63, CH	4.45, dd (8.0, 10.0), H-6
7	47.19, CH	3.22-3.13, m, H-7
8	86.63, CH	5.15, dt (46.0, 1.0) H-8
9a	44.78, CH_2	2.54-2.38, m, H-9a
9b	–	2.92, ddt(2.0, 6.0), H-9б
10	142.24, C	–
11	134.17, C	–
12	168.89, C	–
	122.38, CH_2	5.71, d (4.0), H-13a
13 a		
13 b	–	6.43, d (4.0), H-13b
14a	116.62, CH_2	4.82, s, H-14 a
14b	–	5.18, s, H-14 b
15	14.02, CH_3	1.26, d (6.0), H-15

Table VI
Data of ^{13}C (150.96 MHz) and ^{1}H (600 MHz) ($CDCl_3$) for molecule 9

№ atom	$\delta_{C,}$ type	$\delta_{H,}$ multiplicity (J in Hz)
1	39.86, CH	3.18, m, H-1
2a	42.90, CH_2	2.49, ddd (1.0, 3.0, 18.0), H-2a
2b	–	2.57, dd (8.0, 18.0), H-2b
3	217.79, C	–
4	46.85, CH	2.38-2.30, m, H-4, H-5, H-9a
5	50.78, CH	2.38-2.30, m, H-4, H-5, H-9a
6	81.45, CH	4.06, dd (8.0, 10.0), H-6
7	45.76, CH	3.46, m, H-7
8	78.29, CH	5.08, m, H-8
9a	42.40, CH_2	2.38-2.30, m, H-4, H-5, H-9a
9b	–	3.05, dd (4.0, 6.0), H-9b
10	140.79, C	–
11	134.57, C	–
12	168.32, C	–
13 a	125.76, CH_2	5.83, d (3.0), H-13a
13 b	–	6.38, d (3.0), H-13b
14a	117.84, CH_2	4.93, s, H-14 a
14b	–	5.18, s, H-14 b
15	14.69, CH_3	1.25, d (6.0), H-15
16	156.11, C	–
17	115.18, C	–

graphic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

The quantum and chemical calculations of geometry and the physical and chemical characteristics of grosheimin 6 were executed by the semi-empirical PM6 method with the subsequent single-point calculation according to the density functional B3LYP/6-31G method within the GAUSSIAN program. The structure of grosheimin 6 that was determined theoretically and opti-

mized, corresponds to its structure according to X-ray analysis. Calculation data on geometry of molecules are presented in Table I.

Crystal data and refinement parameters of 7–9

The data were collected on a Bruker Kappa Apex II CCD diffractometer using φ,ω-scans of narrow (0.5°) frames with MoK_α radiation (λ = 0.71073 Å, graphite monochromator). The structures were detected by direct methods and refined by full-matrix least-squares method against all F^2 in anisotropic approximation using the *SHELX-97* programs set[12]. The hydrogen atoms positions were detected with the riding model. CCDC 1044658-1044660 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Final *R*-values and selected refinement details are given in Table II.

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Synthesis of Grosheimin Fluoroderivatives

In this article are presented three new fluoroderivatives on the basis of natural sesquiterpene lactone grosheimin. And also there is given a mechanism of formation of molecule 7, comprising replacing the hydroxyl group of molecule 6, which is accompanied by rupture of the double bond with following formation of carbocation in 6 b-c intermediates and attaching the halide anion with the formation of the final product. The structures of the obtained compounds were proved by the method of NMR ^1H, ^{13}C and X-ray analysis.

Czech Chem. Soc. Symp. Ser. *13*, 163–234 (2015) Karaganda

THE STRUCTURE AND THERMODYNAMIC PROPERTIES OF MOLECULES OF TERPENES. QUANTUM AND CHEMICAL CALCULATIONS BY THE HARTREE-FOCK METHOD AND DENSITY FUNCTIONAL

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Keywords: terpenes of essential oils, quantum-chemical non-empirical calculations, density functional theory, thermodynamic properties

By quantum-chemical methods of Hartree-Fock and density functional using split valence bases 6-31G(d) and 6-31G(d,p) the calculations of structure and properties of five isomers – monoterpenes of the general molecular formula $C_{10}H_{16}$: limonene, 3-carene, sabinene and α,β-pinenes were made. The stability was evaluated and the comparative stability of the isomers was determined. According to the calculations, the structure of limonene is the most stable energetically and thermodynamically. The change in molecule geometry, related with the distortion of bond lengths, valence and torsion angles, increases the energy of terpene molecules. The relative stability of the isomers is in the agreement with the known experimental data.

Introduction

The work presents the results of applying the methods of non-empirical molecular orbital theory and the density functional theory (DFT) to a very widespread class of organic compounds, the isoprenoids, in particular, the terpenes. Essential oils of plants, having a wide range of biological effects, are rich in terpenes[1,2]. Terpenoid compounds are characterized by a great variety of structural types. Among them are monoterpenes, sesquiterpenes, acyclic, bicyclic terpenoids, and many others. The main characteristic feature of terpenoid molecules is rarely high reactivity. The main factor, determining the relative reactivity in a series of related compounds, is the structure of the molecule: the presence of multiple bonds, functional groups, nature of substituents, their electronic and spatial influence on the reaction center. In this regard, the determination of electronic and stereochemical structures of terpenoids is extremely important for the study of their reactivity and biological activity. For this purpose, various instrumental methods such as X-ray analysis, the method of nuclear magnetic resonance (NMR) as well as the design-theoretical methods of modern computer quantum chemistry are used.

Quantum-chemical modeling of NMR spectra of a series of terpenoid molecules was performed by the authors[3] on the basis of non-empirical method B3LYP/6-311G (d, p) within the framework of the density functional theory. The adequacy of the approach is shown by comparing theoretical spectra with experimental spectra with known assignment of NMR signals. In the works[4–8] by calculating parameters of NMR spectra by the precise molecule geometry by the methods of molecular mechanics (MM2, MMP2, MMX) and semi-empirical quantum-chemical calculations (PM3, MNDO) it was determined that the stable conformation of 3-carene molecule is not bath-like conformation, as previously considered, but the flat form in which all 6 carbon atoms of six-membered cycle are in the same plane (intracyclic torsion angles – no more than 6.5°). As the result of quantum-chemical calculation of terpenoid molecules (α-terpineol, menthol, limonene) by the method *ab initio* in the basis 6-311G** with full geometry optimization, the geometric and electronic structure of these compounds was determined[9]. Calculations by the methods of Hartree-Fock and density functional for a number of terpenoids in ref.[10–12] were made.

The objects of this study are so-called true terpenes, unsaturated hydrocarbons $C_{10}H_{16}$ of different structural types. Among them are: monocyclic monoterpene, limonene (**1**), bicyclic monoterpenes, Δ^3-carene (**2**), sabinene (**3**), α,β-pinene (**4, 5**) (Fig. 1). The studied compounds are structural isomers belong to certain groups of terpenes and have various physical and chemical properties.

The purpose of the study is the determination of comparative stability of monoterpene isomers, prediction of their structural, energetic and thermodynamic characteristics by the design-theoretical way. Their experimental values for the concerned terpenes are unknown, so from the point of view of organic chemistry, the quantum-chemical calculations of these properties are of interest both in theoretical and in practical terms.

Calculation methods

Quantum-chemical calculations of electronic and spatial structure as well as the thermodynamic properties of molecules of terpene nature, taking into account the contribution to the thermodynamic properties of the vibrations and rotations of molecules, were carried out by the methods of *ab initio* Hartree-Fock and DFT on the split valence bases 6-31G, for improving the calculation accuracy – with the inclusion of polarization functions of *d*-type for carbon atoms and *p*-type for hydrogen atoms. Due to the asymmetry of the molecules, in whole the calculation

of local symmetry of methylene and methyl groups was not made. For the determination of equilibrium geometry of terpene molecules a complete optimization of their structural parameters on the level of Hartree-Fock with the basis set 6-31G(d,p) and B3LYP/6-31G(d, p) method in the framework of GAUSSIAN 03W program was carried out[13]. The latter method was also used for calculating the thermodynamic characteristics of compounds.

Results and discussions

Geometric, energetic and thermodynamic parameters of the optimized structures of monoterpenes calculated using the methods B3LYP/6-31G (d,p) and HF/6-31G(d,p) are shown in Table I. Since the numbering of the atoms in molecules varies, the structural data are given with the specification of the type of atom hybridization. The calculation of vibrational frequencies of the molecules of studied compounds showed the absence of imaginary frequencies in IR spectra, that was the proof of the compliance of optimized geometry of molecules with the local minima on the potential energy surface. The structure of the molecules is reflected by the geometry corresponding to the energy minimum.

Three-dimensional models of the optimal structures with stereochemical centers are shown in Fig. 1. The calculated values of spatial characteristics correspond to the standard lengths of chemical bonds and valence angles. Optimized internuclear distances R are in the value area: 1.518–1.560 Å (C_{sp}^3 - C_{sp}^3), 1.487–1.525 Å (C_{sp}^2 - C_{sp}^3), 1.335–1.341 Å (C=C) according to the B3LYP method. The Hartree-Fock method due to the neglect of electron correlation gives underestimated values of the lengths of valence bonds. Unlike the latter, the values of valence angles are almost similar at both levels of calculation, and this, in our opinion, is a consequence of using polarization functions. The results of calculations of torsion angles also coincide well with each other. The above-mentioned neglect of electron correlation in Hartree-Fock method leads to the overestimation of the total molecule energy (E_n in brackets, Table I). However, both methods give qualitatively similar results: in the order of the magnitude of energy and the relative energy stability of the molecules (Table I, Fig. 2).

The most energetically and thermodynamically stable isomer is limonene (1-methyl-4-isopropenyl-1-cyclohexene) (**1**), whose structure is characterized by the presence of the only cycle (semi-chair type form) and simultaneous presence of *endo*- and *exo*cyclic double bonds. It should be noted that according to DFT and HF methods, the angles between the planes of these ethylene linkages are 109.82° and 107.77°, which corresponds to almost orthogonal arrangement of these bonds in relation to each other. The molecule has one asymmetric R-configuration center. The structure optimized by us corresponds to the molecule of optically active *D*-limonene ((R)-enantiomer).

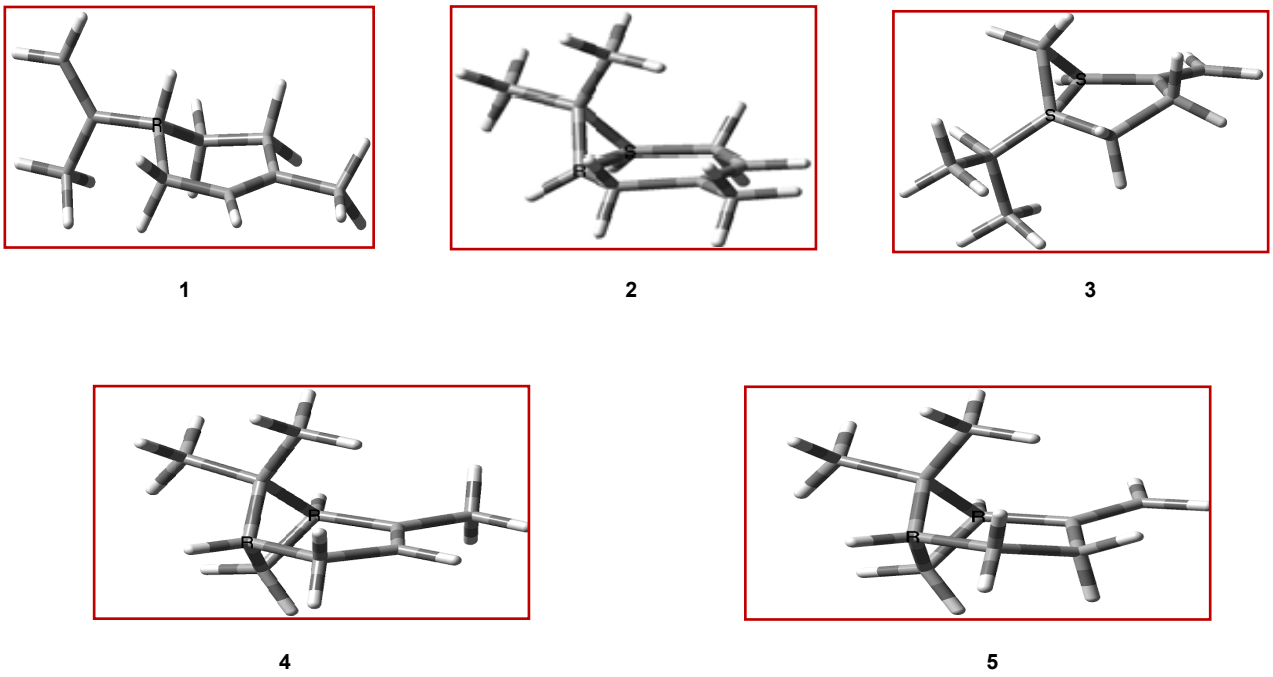

Fig. 1. **Models of the optimized structures of monoterpenes:** limonene (**1**), Δ^3-carene (**2**), sabinene (**3**), α-pinene (**4**), β-pinene (**5**) (B3LYP method)

Table I
Structural, energetic (total energy E_n, zero-point energy E_0 fluctuations) and thermodynamic properties of monoterpenes according to calculations by B3LYP / 6-31G (d, p)

Parameter	Monoterpenoids				
	Limonene	Δ^3-carene	Sabinene	α-Pinene	β-Pinene
Internuclear distances *R*, Å					
C_{sp}^2-C_{sp}^3	1.506 (1.507*)	1.507 (1.508)	-	1.502 (1.502)	-
- " -	1.515 (1.513)	1.513 (1.511)	1.521 (1.517)	1.523 (1.523)	1.525(1.525)
- " –	1.508 (1.509)	1.506 (1.505)	1.487 (1.488)	1.514 (1.514)	1.509(1.509)
- " –	1.520 (1.520)	-	-	-	-
- " -	1.511 (1.511)	-	-	-	-
C_{sp}^3-C_{sp}^3	1.545 (1.537)	1.522 (1.519)	1.528 (1.509)	1.541 (1.536)	1.538 (1.533)
- " –	1.542 (1.534)	1.518 (1.505)	1.535 (1.531)	1.555 (1.546)	1.555 (1.551)
- " -	1.535 (1.529)	1.522 (1.519)	1.550 (1.544)	1.559 (1.550)	1.560 (1.558)
endo-C=C	1.340 (1.323)	1.338 (1.322)	-	1.341 (1.323)	-
exo-C=C	1.337 (1.322)	-	1.335 (1.319)	-	1.336 (1.320)
Valence angles α, degree					
$C_{sp}^2C_{sp}^2C_{sp}^3$	124.64(124.66)	125.83(125.90)	126.25(126.25)	124.4(119.9)	123.1(123.2)
$C_{sp}^2C_{sp}^3C_{sp}^3$	113.08(112.86)	116.19(115.92)	108.15(108.26)	110.3(110.5)	113.3(113.4)
$C_{sp}^3C_{sp}^3C_{sp}^3$	110.91(110.66)	119.77(120.25)	105.70(105.73)	111.2(111.4)	111.2(111.5)
Torsion angles β, degree					
$C_{sp}^2C_{sp}^2C_{sp}^3C_{sp}^3$	-14.69 (-15.15)	2.01 (0.62)	-	2.49 (2.36)	-
$C_{sp}^2C_{sp}^3C_{sp}^3C_{sp}^3$	45.90 (46.06)	2.12 (0.59)	-25.31 (-24.65)	45.61(42.34)	22.92(20.21)
$C_{sp}^3C_{sp}^3C_{sp}^3C_{sp}^3$	-61.35(-61.86)	-0.10 (0.02)	14.91 (14.58)	-82.69(-83.0)	-59.6 (-58.1)
- E_n,** ат.ед	390.699839 (387.997835*)	390.690137 (387.985896)	390.682756 (387.980040)	390.682667 (387.97718)	390.677672 (387.97361)
E_0, kkal/mole	147.744	147.798	147.734	148.196	148.636
$\Delta_f H_{298}^0$, kkal/mole	-1377.22	-1371.25	-1366.76	-1366.57	-1363.14
S_{298}^0, J/(mole·K)	419.04	406.56	408.53	391.25	388.16
ΔG_{298}^0, kkal/mole	-1328.15	-1321.29	-1316.94	-1315.52	-1311.87
C_{p298}^0, J/(mole·K)	18.18	18.14	18.11	17.91	17.79

***** Data of calculations by HF/6-31G(d,p) method are provided in brackets.
****** For crystal graphite – a standard condition of carbon Ep+n298 = –37.893757 ат.ед.[15], for H_2 according to quantum chemical calculation $E_p + H_{298} = -1.165065$ au $E_0 = 6.382$ kcal/mol

Limonene stability is confirmed by the experimental fact[2]: *D*- and *L*-limonenes are relatively stable under thermal influence and do not transform into other hydrocarbons at heating up to 250–400 °C. Contrariwise, all the examined bicyclic terpenes, having more energy in comparison with limonene, are much more reactive and can be transformed into low-lying isomers (Fig. 2), that is also confirmed by the experimental data[14]. Indeed, it is known that at strong heating without the access for air (400–500 °C) terpene rings open, meanwhile from the bicyclic terpenes can be obtained monocyclic terpenes. In particular, pinenes are very reactive. At mild heating or in the presence of platinum sponge β-pinene is transformed easily into α-pinene.

The product of non-catalytic isomerization of α-pinene is the limonene[14]. The diagram in Fig. 2, displaying the relative energy position of isomers – monoterpenes, shows the possibility of such interconversions.

Bicyclic monoterpenes 3-carene, sabinene and α,β-pinenes characterized by the presence of two condensed cycles and one, endo-or exocyclic, double bond. The most stable of them and the second for stability after limonene according to calculations, is Δ^3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-en) (**2**). The difference between the energies of the last is 25,47 kJ/mol (Fig. 2).

Geometry optimization of Δ^3-carene molecule leads to the structure which is coordinated with data[4–8] according to which as it was mentioned above, in the stable conformation of a molecule of 3-carene all atoms of carbon of six-membered cycle are coplanar (intra cyclic torsion corners do not exceed 6,5°). According to our calculations, the corresponding torsion corners do not exceed 2,2° (B3LYP method). The angle between the planes of three – and six-membered cycles of a molecule of 3-carene is equal 113,05°, i.e. they are located almost perpendicularly to each other. Connection of C-C along which there is a cis-joint of cycles is formed by asymmetric atoms of R-carbon and S-configuration.

The next among $C_{10}H_{16}$ isomers on energy ascending – sabinene (1-isopropyl-4-metilenbicyclo[3.1.0]hexane) (**3**). The molecule differs from the previous isomers by the presence in the structure not of six- but a five-membered cycle which, in our opinion, destabilizes a molecule because it leads to distortion of the bond lengths and angles. Two cycles are located on the relation to each other at an angle 109.72°. The five-membered cycle has an envelope form. The bond of C-C stated above is formed by asymmetric atoms of S-configurations carbon. As it can be seen from the diagram, practically flush with sabinene is α-pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) (**4**), which, however, are quite different on structure from the last. Pinenes – compounds of Pinane type – belong to separate subgroup within the bicyclic monoterpenes. They are characterized by the presence of seven-membered cycle. α-Pinene and β-pinene (2-methylene-6,6-dimethylbicyclo [3.1.1]heptane) (**5**) differ in the position of the double bond: the first – endo second – exocyclic ethylene bond. The latest exocyclic C=C bond is much more accessible for the attack, which increases its reactivity. Two asymmetric atoms of carbon which are present at their cycles have R-configuration.

According to Table I, fundamental thermodynamic properties – enthalpy of formation, entropy, free energy of Gibbs, a molar thermal capacity also are in direct dependence on geometry of terpenes. The enthalpy of formation was calculated on a formula:

$$\Delta H = (E_{\text{п}}+H_{298})_{\text{izom}} - \sum(E_{\text{п}}+H_{298})_{\text{C, H2}}$$

where $E_{\text{n}} + H_{298}$ – the sum of the electron energy and the thermal amendment to an enthalpy of isomer and simple substances at 298K, including energy of zero-point fluctuations and contributions of transactional, vibrational and rotational movements (the enthalpy of formation of isomers calculated relatively simple substances $10C+8H_2$).A parallel change of the standard enthalpy of formation of isomers, total electronic energy indicates identical sequence of molecular thermodynamic stability. The enthalpy of formation also increases from limonene to β-pinene. In contrast, entropy decreases in the row, i.e. the lowest value S^0_{298} has β-pinene, which confirms its high reactivity. For the calculated zero-point energy and entropy observed some inversion. In other ranks there is a monotonic change of sizes.

Comparative analysis of isomers-monoterpenes structure allows making some assumptions about the factors

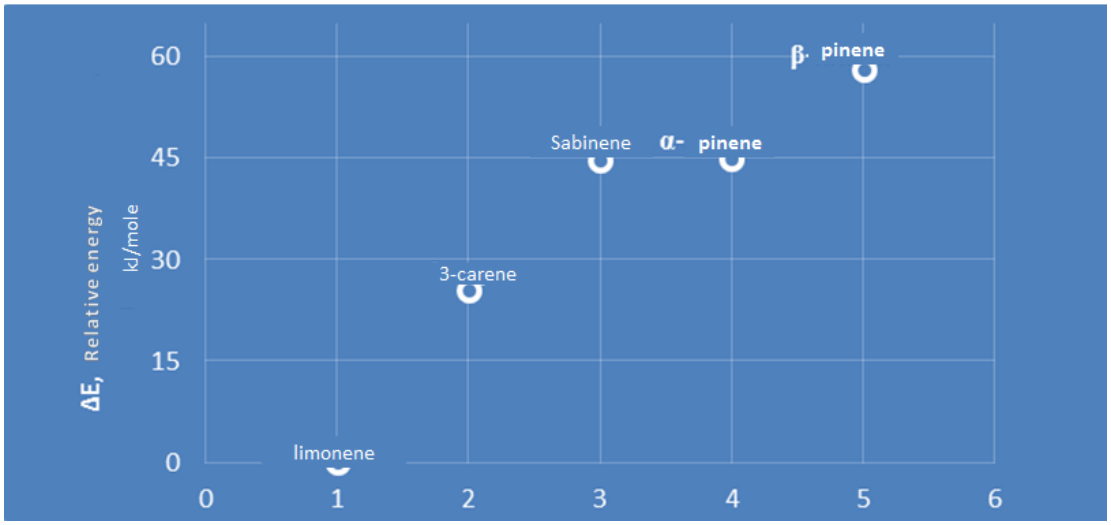

Fig. 2. **Chart of power stability of isomers $C_{10}H_{16}$** (method B3LYP/6-31G(d,p))

Table II
Energy of internuclear and intra-electron repulsion in a molecule monoterpenes according to calculations by the B3LYP/6-31G (d, p)

Energy ($x10^2$at.unit.)	Molecule				
	Limonene	Δ^3-carene	Sabinene	α-Pinene	β-Pinene
$E_{яя}$	5.2727469	5.4838711	5.4644245	5.6567167	5.6795235
$E_{ээ}$	6.5274463	6.7389529	6.7174384	6.9131359	6.9347294

that determine the stability of the molecules. One of the explanations is based on the concept of the voltage cycle. Apparently, the lack of the dual cycle, Pitze's tension and Prelog, determine the stability of limonene. The presence in 3-carene of conjugated three-membered cycle with an angular, torsion and trans annular tension leads to its destabilization. Reduction of number of atoms in a cycle and the presence of all mentioned types of tension increases energy of sabinene molecule. Resection of Bayer and Prelog voltage in a 7-membered cycle α-pinene promotes stabilization, which entails a very slight increase of energy, while the olefinic bond in the core makes it more robust in comparison with β-pinene (**5**).

The alternative explanation for the relative stability of monoterpenes consists in comparison with energies of mutual repulsion of nucleons and electrons (Tab. II). The smallest nuclear repulsion energy ($E_{яя}$) and electrons ($E_{ее}$) is a molecule limonene, most – molecule of β-pinene. In the latter case strong electron-electron interaction destabilizes a molecule, making it energetically unstable. Another electronic factor can be a reason of isomers stability changes – is the influence of character of hybridization of carbon atoms, expressed in a change of length and energy of the connections formed by them. Csp2-Csp3 bond noticeably shorter and stronger than Csp^3-Csp^3 bond (Table I). For the considered compounds there is dependence of stability of the molecules on the number of Csp^2-Csp^3 bonds: their number is maximal at limonene (**5**), minimum (**2**) – at β-pinene. Accordingly, the thermodynamic stability decreases.

Conclusion

On the basis of quantum-chemical calculations by the methods of Hartree-Fock and functional of density estimated stability and comparative stability of $C_{10}H_{16}$ isomers-monoterpenes is established. The most energetically and thermodynamically stable isomer is limonene. Changing the geometry of molecules associated with the distortion of bond lengths, valence and torsion angles increases energy of molecules terpenes. In the analysis of structure and properties of molecules of terpenes expediently to use methods of density's functionality theory and the advanced bases which give the best results; it is especially important in the absence of experimental data.

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CONTENTS

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