

Natural Sesquiterpene Lactones as Renewable Chemical Materials for New Medicinal Products

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Abstract

Literature data and own research results on the technology for isolating natural sesquiterpene lactones such as arglabin, alantolactone, artemisinin, grosheimin, isoalantolactone, parthenolide, santonin and potential possibilities of their use as renewable material for obtaining new compounds as well as biologically active derivatives are generalized in this review. Sesquiterpene lactones from plants are promising sources for the development and practical application of new original medical products possessing antitumor, anti-inflammatory, antimalarial, antiulcer, antiviral and immune-stimulating action. The technology for isolating sesquiterpene lactones is based on the extraction of raw plant material by different organic solvents with the subsequent chromatographic purification. The effective and environmentally safe technology for isolation and purification of sesquiterpene lactone arglabin from *Artemisia glabella* Kar. et Kir. by the CO₂-extraction method is developed. Thereat, it was experimentally determined that the method for isolating arglabin from CO₂ extract of *Artemisia glabella* Kar. et Kir. using centrifugal partition chromatography is effective for preparative isolation of the active substance and its manufacturing application. It is practically important to obtain water-soluble derivatives of biologically active sesquiterpene lactones and also to use the nanotechnology achievements for directed transportation of a molecule of the medicine in the human body thereby reducing toxicity of an active component. Promising direction is chemical modification of molecules in sesquiterpene lactones which are renewable material for obtaining new derivatives, thanks to which it becomes possible to solve two problems at the same time. Firstly, these researches help to obtain derivatives with higher biological activity or improved physical and chemical properties. Secondly, these researches enable us to disclose the mechanism of action of different medicines within the framework of "structure-activity" correlation. The article presents the literature data and own results on chemical modification of sesquiterpene lactones of alantolactone, arglabin, artemisinin, grosheimin, isoalantolactone, parthenolide and santonin. Various reactions on functional groups of these molecules were used to obtain a number of new derivatives of sesquiterpene lactones containing haloid-, pyrazole-, triazole-, amino-, dialkylamino-, hydroxy-, dialkyl phosphonate- and cyclopropane groups, which have shown high physiological activity.

Introduction

Sesquiterpene lactones constitute a numerous group of natural terpenoids, mainly from plant origin, and showing antitumor, antiviral, immune-stimulating, antifungal, antimicrobial, anti-inflammatory, antimutagen, growth-stimulating and antifeedant activity [1-12]. Therefore, the search for new compounds with wide spectrum of pharmacological activity in this series opens possibilities for developing effective and novel drugs.

On the other hand natural sesquiterpene lactones represent renewable material for chemical modification. Researches in the field of chemo- and stereoselective modifications of sesquiterpene lactone molecules are of current importance as they allow deeper understanding of chemical properties in terpenoid compounds and studying the "structure-activity" correlation. Modified derivatives of sesquiterpene lactones possessing high biological activity can be used in medicine and agriculture.

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Manufacturing technique of patented drugs ("Alanton", "Santonin", "Qinghaosu", "Parthenolid") based on sesquiterpene lactones are multiphase, labour-consuming, requiring large amounts of toxic organic solvents the application of which is barred by international GMP standards. An effective and environmentally friendly technology for isolation and purification of sesquiterpene lactone arglabin from *Artemisia glabella* Kar. et Kir. according to GMP requirements was developed in International Research and Production Holding "Phytochemistry". In the course of experiments we determined that the method for isolating arglabin from CO₂ extract of *Artemisia glabella* Kar. et Kir. with centrifugal partition chromatography is best for preparative operating time and manufacturing application of its substance.

Method of Isolating Santonin and Chemical Modification

Sesquiterpene γ -lactone α -santonin (1) is a typical representative of eudesmane bicyclic sesquiterpenoids and was the first to be used in medicine [13]. α -Santonin (1) was first isolated from *Artemisia cina* Berg. by the Italian scientist Stanislao Cannizzaro in 1830 and three years later it was already used actively in therapies. In the mid-19th century, there was a number of factories in Germany and England which processed santonin-containing populations of *Artemisia maritima*, growing in the North Africa, India and South America.

In 1892, the santonin factory was built in Shymkent. This factory had been the only pharmaceutical manufacturer in Kazakhstan for a long time. Establishing the pharmaceutical factory in Southern Kazakhstan was based, first of all, on the availability of plant material for santonin manufacturing, namely, availability of endemic species of *Artemisia cina* Berg. at industrial scales.

Technological process of santonin manufacture (1) includes 5 stages [14]:

- the first stage - raw materials (flower heads of *Artemisia cina* Berg.) steeped in water and mixed with lime containing minimum of 60% calcium oxide, with α -santonin (1) being dissolved in alkalies where the lactone ring opens forming a salt of santoninic acid;
- the second stage - sevenfold desalination of calcium santonate by water, the extracted content of α -santonin (1) varies from 0.7 to 1.4%;
- the third stage – upon completion of the extraction and discharging of the extract, the extractor if filled with open steam and essential oil is distilled with aqueous vapor. The oil obtained is left to settle and then dried over sodium sulfate; it is

used as a topical antirheumatic drug. The yield of α -santonin (1) at the extraction stage is 95%;

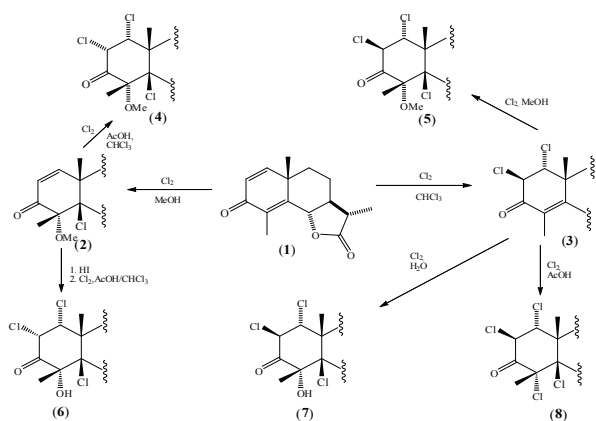
- the fourth stage - concentrated extract containing calcium santonate, resins and other extractive substances is acidified with nitric acid. Thereat, calcium nitrate and santoninic acid are formed, the latter slowly transforms to α -santonin. Crude α -Santonin (1) is washed with water ten times until neutral reaction, then pressed on a centrifuge, transferred to a drying chamber and dried at temperature 66-68°C. The yield at this stage is about 80%;
- the fifth stage – purification of crude α -santonin (1) by repeated crystallization from ethanol and subsequent purification on a specially constructed filter equipment. α -Santonin (1) is transferred from crystallizers to centrifuge, pressed and washed with purified water. The yield of pure α -santonin (1) at this stage is 80-84%.

Nowadays, α -santonin (1) is found in 20 species of *Artemisia* genus [14] and considered by researchers as a renewable chemical material for the synthesis of new compounds [15]. Ketogroup at C-3 and methylene bonds at C1-C2 and C4-C5 are basic reaction centers in a santonin molecule (1).

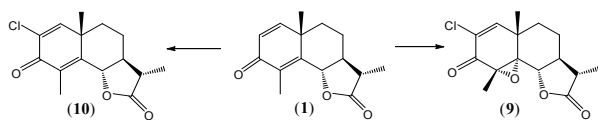
An interesting reaction for the santonin molecule (1) is its chlorination with chlorine gas in the process of which the chlorine atom is coupled at the diene double bond and C4-C5 double bond. Japanese chemists Takayanagi H., et al. [16] found that chemo- and stereoselectivity of chlorine coupling at the double bond directly depended on the nature of a solvent. For example, chlorination of α -santonin (1) in methanol results in the formation of 5 β -chloro-4-methoxysantonin (2) – a C4-C5 double bond coupling product. When CHCl₃ is used as a solvent there is formed 1 α , 2 β -dichlorinesantonin i.e. trans-coupling product at C1-C2 double bond.

Further, derivatives of (5), (7) and (8) were obtained from (3). Thus, the use of various solvents enables chemoselective chlorination of diene system. The same researchers determined that the use of CHCl₃ - AcOH mix leads to stereoselective formation of *cis*-dichlorides.

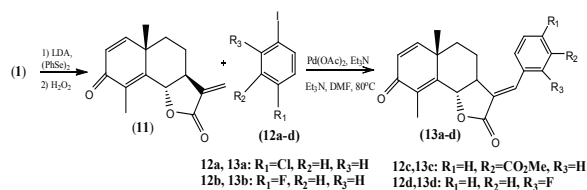
By the further conversions they obtained the products of *cis*-dichlorination (4) and (6) from the derivative (2). Products of *cis*- and *trans*-dichlorination can be easily distinguished by comparative analysis of proton magnetic resonance spectra by the value of Spin-spin coupling constant between H-1 and H-2 protons. In compounds of (4) and (6), spin-spin coupling constant (³J_{H1-H2}) is 6 Hz which is characteristic for *cis*-focused protons. For compounds of (3), (5), (7-8), Spin-spin coupling constant between H-1 and H-2 is 12 Hz which indicates at their *trans*-orientation [16].



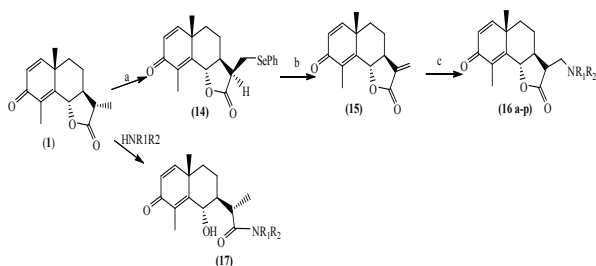
At interaction of α -santonin (1) with chlorine in aqueous acetonitrile, 2-chloro-4,5 α -epoxy- α -santonin (9) and 2-chloro- α -santonin (10) were obtained. Their spatial structure was confirmed by X-ray analysis. It was determined that 2-chloro- α -santonin (10) possesses high antitrichomonas activity [17].



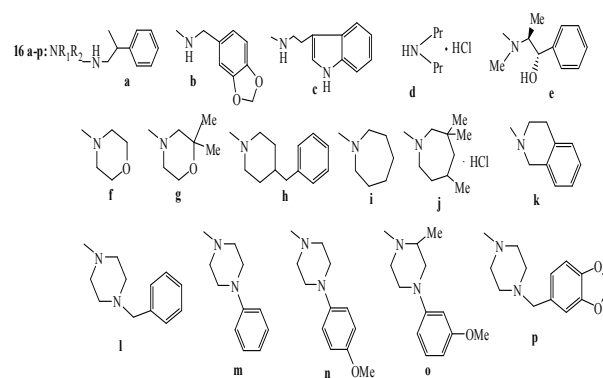
To carry out Heck reaction α -santonin derivative (11) was obtained. At interaction of 11, 13-dehydro-santonin (11) with aryl halides (12a-d) in catalytic system Pd(OAc)₂-Et₃N in dimethylformamide solution we obtained compounds (13a-d) with high yield [18].



Previously unknown amine derivatives (16-17) of α -santonin (1) were synthesized and studied for their activity against tumor human cell lines [19].



17: NR₁R₂=NHMe, NHCH₂Ph, NH(CH₂)₂OH, NMe₂, N(CH₂)₄, N(CH₂)₅, N(CH₂CH₂)₂O



a. BuⁿLi, HNⁱPr₂, THF, -78 °C, Ar, Ph₂Se; b. H₂O₂, AcOH, THF; c. NHR₁R₂, MeON

Artemisinin Isolating Technology and Ways to Obtain its Derivatives

Artemisia annua L. was already used medicinally more than 1000 years ago. However, an active substance from *Artemisia annua* L. was isolated and characterized in 1972. The sesquiterpene lactone artemisinin (18) isolated from *Artemisia annua* L. possesses high antimalarial action. A highly effective antimalarial drug "Qinghaosu" in the form of tablets was developed on its basis in China [20].

Artemisia annua L. is widespread annual weed growing in China, occurring in Siberia, Europe and the USA and is easy to cultivate. Depending on agronomic conditions and other factors, its yield from aerial parts varies from 0.5 to 0.01%.

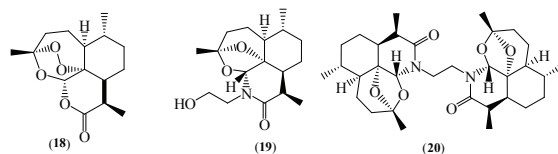
Artemisinin (18) is obtained by extraction of *Artemisia annua* L. followed by column chromatography. Non-polar solvents are used as extractant. There exists a method of isolating artemisinin (18) without column chromatography where the aerial part of *Artemisia annua* L. is extracted with ethanol, then the extract is distilled and the residue is re-extracted with hexane. The extract is evaporated and its residue is dissolved in ethyl acetate, activated carbon is added to the mixture for chlorophyll binding, followed by its filtration and the filtrate is left to crystallize the crystals of artemisinin [21].

There is another known method of manufacturing of artemisinin (18) which is implemented by the following scheme: the air-dried and ground plant material of *Artemisia annua* L. grass is extracted six times within 8 h in Soxhlet with hexane at temperature 60-80°C. Liquid extract is evaporated to 5% of the initial volume. The residue of hexane extract is mixed with water acetonitrile at ratio 1:5 for 3 h. The mixture is left to settle and phases are divided after full demixing. Acetonitrile solution is dried over sodium sulphate and evaporated under vacuum. The residue obtained is chromatographed on silica gel column with ethyl acetate and hexane. At elution

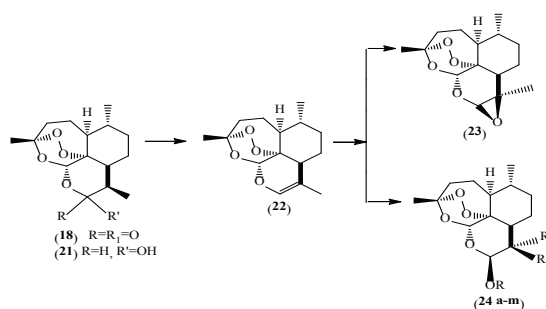
of the column with 8% mixture of ethyl acetate and hexane, artemisinin (18) is obtained which is then recrystallized from the mixture of ethyl acetate and hexane (1:4), the crystals are filtered and dried. The yield is 0.045% [22].

To date, over 200 new derivatives of artemisinin (18) have been synthesized [23]. Thereat, C-10- substituted derivatives of artemisinin (18) were found to be effective for treatment of infections caused by parasites of *Plasmodium*, *Neospora* or *Eimeria*, especially *Plasmodium falciparum*, *Neospora caninum* and *Eimeria tenella* which trigger malaria, neosporosis and *Coccidioides immitis*, respectively. Although derivatives of artemisinin (18) presented in the review [23] possess high antimalarial and antitumor activity, there are some problems connected with the stability, bioavailability and potential neurotoxicity.

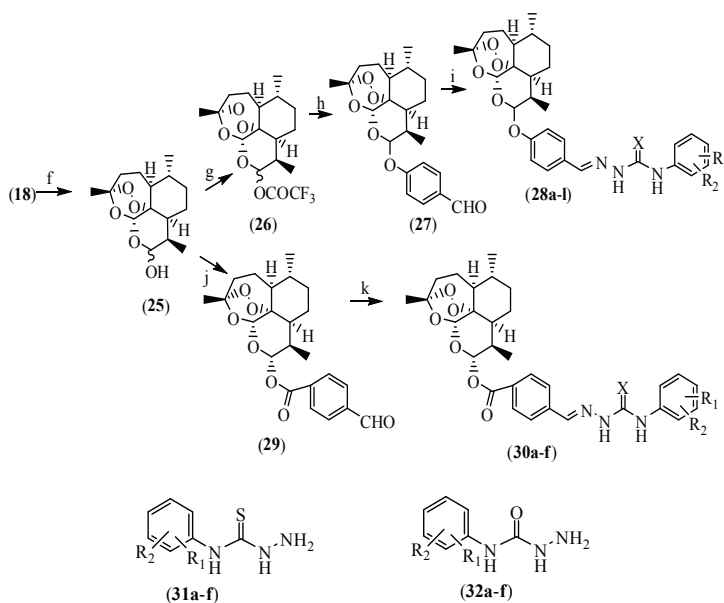
Mai M. Al-Oqail, etc. [24] conducted the reactions of artemisinin (18) with ethanolamine. Authors isolated and identified corresponding lactam (19) from the reaction mixture. Reaction of artemisinin (18) with ethylene diamine led to a dimer (20). It is interesting to note that despite the lack of a pharmacophore group - endoperoxide cycle, only the dimer (20) showed activity for two clones of malaria agents of *Plasmodium falciparum*, comparable to that of the initial substance.



For studying correlation “structure-activity”, modifications of artemisinin lactone cycle (18) and artemisinin derivative (21) were carried out. Water-soluble acids (24 a-m) were among the obtained compounds (22-24) [25].



Chinese scientists Yang Liu, et al. synthesized new derivatives of dihydroartemisinin (28a-1, 30a-f). The antimalarial activity of the synthesized compounds was studied [26].



28a: X=S, R₁=H, R₂=2-F

28b: X=S, R₁=H, R₂=4-F

28c: X=S, R₁=H, R₂=4-OCH₂CH₃

28d: X=S, R₁=2-CH₃, R₂=5-CH₃

28e: X=S, R₁=3-CH₃, R₂=5-CH₃

28f: X=S, R₁=3-Cl, R₂=2-CH₃

28g: X=O, R₁=H, R₂=2-F

28h: X=O, R₁=H, R₂=4-F

28i: X=O, R₁=H, R₂=4-OCH₂CH₃

28j: X=O, R₁=2-CH₃, R₂=5-CH₃

28k: X=O, R₁=3-CH₃, R₂=5-CH₃

28l: X=O, R₁=3-Cl, R₂=2-CH₃

30a: X=O, R₁=H, R₂=2-F

30b: X=O, R₁=H, R₂=4-F

30c: X=O, R₁=H, R₂=4-OCH₂CH₃

30d: X=O, R₁=2-CH₃, R₂=5-CH₃

30e: X=O, R₁=3-CH₃, R₂=5-CH₃

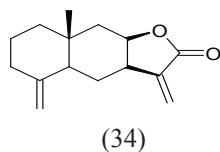
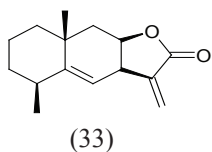
30f: X=O, R₁=3-Cl, R₂=2-CH₃

Reagents and conditions: (f) NaBH_4 , CH_3OH , $0-5^\circ\text{C}$, 3 h; (g) TFFA, Et_3N , CH_2Cl_2 , $-5-0^\circ\text{C}$, 1 h; (h) 4-hydroxybenzaldehyde, $-5-0^\circ\text{C}$, 12 h; (i) (31a-f) or (32a-f) CH_3COOH , EtOH , room temp., 2 h; (j) 4-formylbenzoic acid, DCC, DMAP, ice water bath, room temp., 1 h; (k) (32a-f), CH_3COOH , EtOH , room temp., 2 h.

Thus, the isolation of the highly active compound artemisinin from *Artemisia annua* L., having a peroxide group in its structure, opened a new stage in searches of antimalarial drugs. It was determined that peroxide function has a key value for therapeutic action because its reduction leads to the disappearance of the therapeutic activity of artemisinin (18).

Isolation and Chemical Modification of Alantolactone and Isoalantolactone

“Alanton” is a medicinal product with antiulcer action, containing purified complex of sesquiterpene lactones of *Inula helenium* L. [27]. The following lactones are main components of the product: alantolactone (33) and isoalantolactone (34) whose content should not be below 95 %. At present, “Alanton” is produced in the form of tablets as an antiulcer medicine at Borschagov factory.



“Alanton” substance from underground part of *Inula helenium* L. is manufactured in several stages:

- ground plant material is extracted by 85% ethanol (ratio 1:3) in a percolator battery for 12 h;
- the extract is evaporated under vacuum to 2/5 of the initial volume, water is added and evaporation is continued until 1/5 of the initial volume of the extract;
- terpenoid fraction is isolated by methylene chloride from the water residue. Chloride methylene extracts are poured out, mixed, dehydrated by heat-treated sodium sulphate for 2 h, filtered and evaporated to 1/10 of the initial volume;
- the solution obtained is purified by column chromatography method on aluminium oxide. Elution is carried out with methylene chloride. The eluate is evaporated until the solvent is fully evaporated. Residue is a dense mass with dark yellow colour;
- final stage of obtaining alanton involves addition of 30 parts of rectified alcohol to the residue

and then the same amount of purified water is added gradually at stirring. The mixture becomes turbid, yellow flocks of residue are formed and with further addition of water lumps are formed. Suspension is poured out to the crystallizer, where it is cooled to $0-5^\circ\text{C}$. Alanton crystals are formed within 2 days. Alanton is filtered, washed by 4 parts of gasoline which is cooled to $0-5^\circ\text{C}$. The residue is dried for 10-12 h. Dried alanton is crushed in a ball mill, sifted and packaged.

The yield of alanton is 1% on air-dry basis.

Authors [28] offer an alternative way of manufacturing “Alanton” substance. Technology for obtaining the sesquiterpene lactone complex of alantolactone (33) and isoalantolactone (34) from plant material is carried out by double extraction of roots and rhizomes of *Inula helenium* L. with ethanol and hexane (1:4) at boiling, mass ratio of roots and rhizomes with extragent (1:5) for 3 h. The extracts are mixed, filtered from mechanical impurities and evaporated to 1/4 of the initial volume. Residue falls out at this stage. To purify it from the impurities, the residue is dissolved in the minimum volume (50 ml) of ethanol and hexane (1:4) and sesquiterpene lactones are settled out by adding pure hexane until steady turbidity. Max falling out of sesquiterpene lactone crystals is reached within 24 h at room temperature. Yellowish crystals of purified sesquiterpene lactone complex of alantolactone (33) and isoalantolactone (34), at quantitative ratio of 2:1, melting point $86-88^\circ\text{C}$, are obtained by column chromatography. Silica gel and eluent (a mixture of petroleum-ether and ethyl acetate (9:1) are used as a solvent. This method reduces the time for isolating lactones from 192 h to 58 h and the yield increases from 1.3-1.5 to 1.7-1.9%.

There was developed technology to obtain alantolactone (33) as a reference sample, which includes the following stages: extraction of essential oil from rhizomes and roots of *Inula helenium* L. by steam distillation with the simultaneous extraction in chloroform; isolation of sesquiterpene lactone complex by silica gel column chromatography (eluent-mixture: petroleum ether - ethyl acetate, 9:1); isolation and purifying alantolactone by preparative layer chromatography (stationary phase - Silufol plate, eluent-mixture: benzene - ethyl acetate - metanol (94:3:0.5)). With the use of spectrophotometry, UV - IR-spectra, TLC, HPLC, chromo-mass spectrometry, melting point, polarimetry and qualitative reactions the isolated compound was proved to be alantolactone (33) which contained 98% of the basic substance [29].

Previously, in 1990 I.A. Milman described chemical conversions of lactones (33) and (34) which can be subdivided into reactions on α -methylene group

of a lactone ring and decalin part of the molecule (isomerization, hydroxylation, epoxidation) [30].

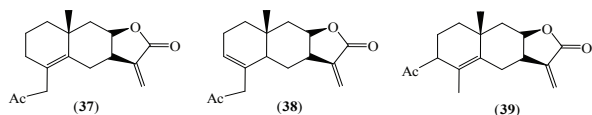
Various positioning of C=C double bond (exo, endo) in decalin part of molecules in (33) and (34) stipulates a number of specific properties characteristic only to one of the two isomer compounds. An example of this can be an acid-catalyzed shift of the double bond C4-C15 to *endo*-position with the formation of isomers (35) and (36).



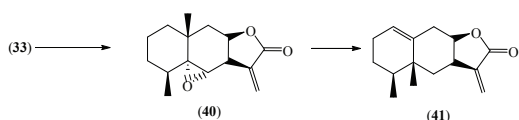
It was found that formation of (50) and (51) is possible at short-time processing of (34) with the solution of $\text{CH}_3\text{SO}_3\text{H}$ diluted in trifluoroethanol. Lactone (33) does not react in the described conditions.

Similar inertness of 5,6-olefinic bond was observed at hydrogenating the mixture of lactones (33) and (34). *Exo* double bond shifts also when lactone (34) is treated with formic acid. If 11,13-dihydroisoalantolactone as the initial compound, the yield of C4-C5 is 85%.

Three isomers (37-39) are formed by acylation of lactone (34) with acetic oxide in the presence of catalyst ZnCl_2 . The synthesis of the derivative (39) is explained by isomerization of the double bond followed by acylation at C-3.

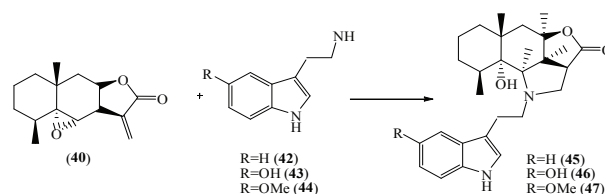


Alantolactone (33) can be used as an initial lactone for the construction of eremophilanolides. Conversion is reached by processing of epoxide (40) with formic acid in acetone.

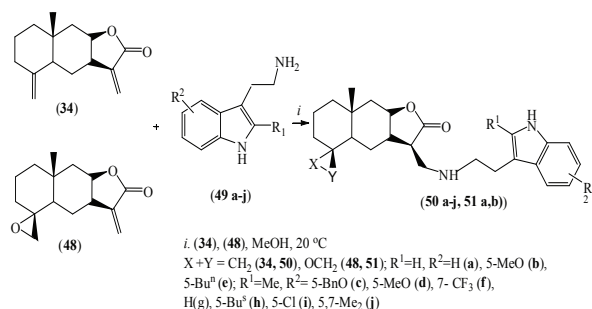


Synthesis of new biologically active compounds based on sesquiterpene lactones of alantolactone, isoalantolactone, santonin and britanin is carried out at the Institute of Physiologically Active Substances of the Russian Academy of Sciences (Russia). Notable are the reactions with alkaloids, in particular, their coupling on the exomethylene group of alantolactone epoxide (40) with epoxy ring opening at

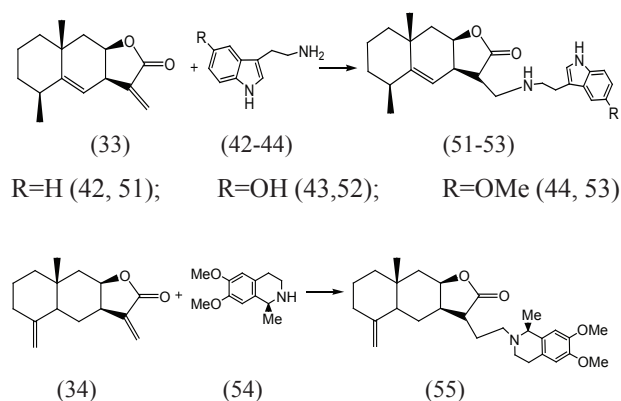
C5-C6 and formation of a new heterocyclic system - hydrogenated benzo[g]furo[4,3,2-cd]indolone [31].



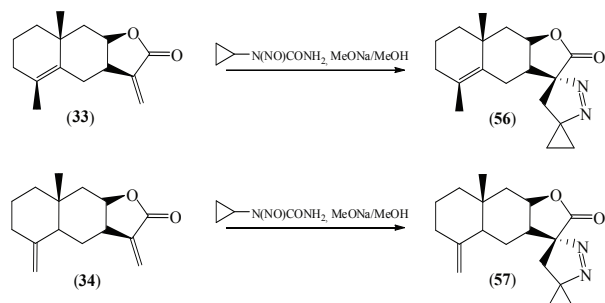
The lactone ring in isoalantolactone (34) and its epoxide (48) contain exocyclic double bond which is activated by carbonyl group. One of ways to modify such derivatives is the use of Michael reaction – coupling of nucleophilic reagents with electron-deficient alkenes. The authors used substituted tryptamines (3-(2-aminoethyl)indoles) to introduce an additional pharmacophore fragment as N-nucleophils into the molecule of lactone [32]. Obtained triptamine derivatives of isoalantolactones are of interest as effective antioxidants. The use of such compounds is promising for the development of new neuroprotective drugs and cytoprotectors with wide spectrum of action [32].



Authors [33] were the first to carry out amination reactions of sesquiterpene lactones alantolactone (33) and isoalantolactone (34). Alkaloids (42-44) were used as aminating agents. As a result a number of compounds (51-53) was obtained.



Interaction of (33, 34) with in situ generated diazocyclopropane from N-nitroso-N-cyclopropylurea under sodium methylate proceeds with high regio- and stereoselectivity with the production of spiro-linked pyrazolines on exomethylene bond of the lactone cycle [34].



Thus, the regio- and stereoselective synthesis of new derivatives with high biological activity on exocyclic bond of methylene- γ -lactone ring was offered for chemical modification of alantolactone and isoalantolactone.

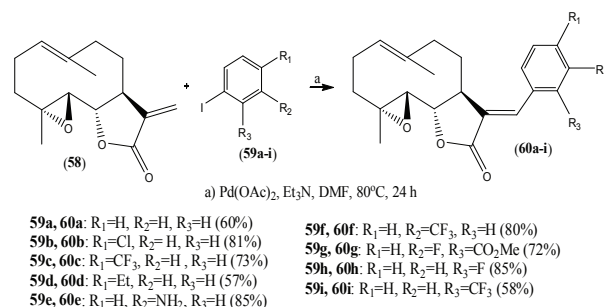
Isolation of Parthenolide and Chemical Conversion of its Molecule

Sesquiterpene lactone parthenolide (58) is a component of the lactone fraction of *Tanacetum parthenium* L. aerial part and constitutes a base of medicinal products for migraine prophylaxis [35]. High pharmacological activity is stipulated by the presence of α -methylene- γ -lactone ring and epoxide fragment in its structure. Over 500 publications [36] issued in the USA, Canada, Great Britain, France, Germany and other European countries about biological activity of parthenolide (58) were identified. Parthenolide (58) can be considered as biologically active substance. Parthenolide (58) induces cell apoptosis in human liver tumor, possesses antiviral activity for virus hepatitis B and C; induces apoptosis of leukaemia cells, possesses antimyeloma effect; inhibits pancreatic carcinoma cells and possesses anti-inflammatory effect. It is used for treatment of fever, migraine and rheumatoid arthritis [36].

Parthenolide (58) is produced by the following scheme: air-dried ground plant material of *Tanacetum parthenium* L. aerial part is extracted at room temperature by 90% water ethanol. The extract is filtered and evaporated under vacuum; the residue is chromatographed on silicagel column with the use of dichloromethane as an eluent. The produced fractions are collected, evaporated and re-chromatographed on silicagel column with hexane and dichloromethane.

Changho Han and other scientists were the first to carry out Palladium catalyzed reaction of arylation

of α -methylene- γ -lactone-containing sesquiterpene lactones of parthenolide (58) and α -santonin (1). As a result, E-olefinic products were received with sufficiently high yield. The obtained sesquiterpene lactone derivatives were studied for cytotoxic activity on HeLa cells [37].



In the USA, researchers synthesized amino derivatives of parthenolide and studied their antileukemic activity (University of Kentucky, the USA) [38]. Palladium catalyzed reaction of arylation of α -methylene- γ -lactone fragment in sesquiterpene lactones (Department of Medicinal Chemistry and Molecular pharmacology, Purdue University, the USA) was also studied [39]. Thus, it was determined that the presence of exomethylene groups at γ -lactone cycle of parthenolide (58) and its derivatives defines their corresponding high antitumor and anti-inflammatory activity.

Technology of Arglabin Isolation and Chemical Modification of its Molecule

An original medicinal product "Arglabin" based on the sesquiterpene lactone arglabin (61) was developed at the International Research and Production Holding "Phytochemistry". "Arglabin" passed final phases of clinical trials and is currently manufactured by Karaganda Pharmaceutical Factory. The method for obtaining "Arglabin" is patented in 11 countries worldwide namely, Japan, China, USA, Great Britain, Germany, Switzerland, France, Austria, Italy, Netherlands and Sweden [40-41].

Escalation of safety requirements to technology processes in pharmaceutical industry necessitated exclusion of highly toxic solvents from technology for production of medicinal products and search for new effective and non-toxic solvents. One of environmentally friendly technology is the use of liquefied carbon dioxide in supercritical mode, which enables the isolation of a complex of biologically active substances from plant material, provides high yield and not any traces of solvent in extract and guarantees sterility of the substance.

Optimum parameters of the extraction mode for *Artemisia glabella* Kar. et Kir plant material with

the use of CO₂-gas in the supercritical condition providing a quantitative yield of arglabin were established as follows: grinding size of the plant material - 5 mm, 22 MPa pressure, 65°C temperature, extraction duration -180 min. Yield of CO₂-extract – 45.6 g (4.56%) from 1 kg *Artemisia glabella* Kar. et Kir. plant materials; arglabin content in the

extract – 13.8 g (30.2%), residual arglabin concentration in the extraction cake - 0.08 g (0.09%, on air-dry basis).

Using the supercritical CO₂-extraction of *Artemisia glabella* Kar. et Kir. to extract arglabin (Table 1) has considerable advantages in comparison with chloroform extraction.

Table 1

Comparative characteristics of arglabin extraction methods from *Artemisia glabella* Kar. et Kir

Extraction method	Yield of extract and quantitative content of arglabin						
	Volume of extracting arglabin, % dry weight	Yield of extract dry weight		Content of arglabin in extract units		Residual arglabin concentration in the extraction cake units	
		g	%	g	%	g	%
CO ₂	92.4	45.6	4.6	13.8	30.2	0.08	0.09
Chloroform	78.0	150.0	15.0	11.6	7.8	2.28	0.26

To improve the productivity and automation, to reduce the duration of the technological process and to exclude the use of toxic solvents, the technology for obtaining arglabin native substance (61) was developed by using centrifugal partition chromatography.

Technological process of isolating arglabin (61) from CO₂-extract of *Artemisia glabella* Kar. et Kir. includes two stages: purification on the industrial unit FCPC-5000 (fast centrifugal partition chromatography) and recrystallization of target product.

To implement the first stage we studied how a number of technological factors would effect the yield of the target product, namely, the mass and the solvent of loaded sample, velocity of the mobile phase and rotor speed.

It was experimentally determined that for isolating arglabin (61) from CO₂-extract of *Artemisia glabella* Kar. et Kir., the following conditions are best: 70.0 g of CO₂- extract of *Artemisia glabella* Kar. et Kir. is dissolved in 100 ml of mobile phase and 700 ml of stationary phase at thorough stirring. Separation was conducted at the eluent flow rate of 100 ml/min and rotor speed of 1000 rpm, UV-detecting at 220 nm. The loaded sample was fully separated within 40 min. Fractions were collected using fractional collector according to the produced chromatogram. Eluent was distilled on the rotary evaporator and returned to the process with minimum losses.

As a result of partition 4 fractions in volume from 300 to 800 ml were received. The solvent was evaporated producing technical arglabin with the concentration of the target product over 95%.

The second stage of purification included recryst-

allization of crude arglabin (61) first from ethanol of 96% and then from hexane.

We determined experimentally that method of isolating arglabin with centrifugal partition chromatography from CO₂-extract of *Artemisia glabella* Kar. et Kir. in comparison with column chromatography, is characterized by the best productivity, all-around automation, reduction of process duration. This method does not call for sorbents and high-purity solvents and guarantees production of up to 200 g of arglabin a day with the purity not less than 99.0%.

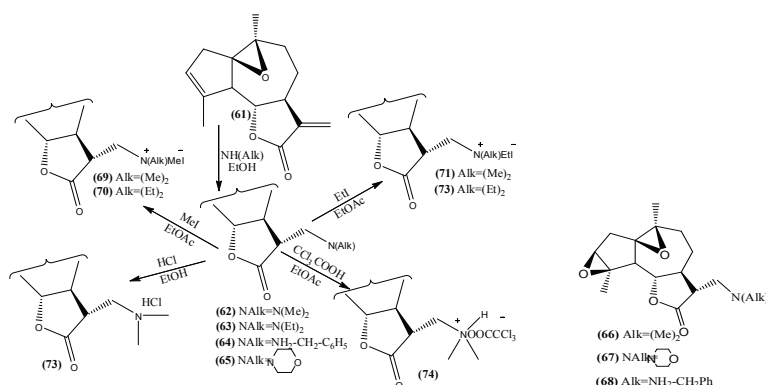
The fact that the structure of arglabin (61) has an exomethylene group conjugated with carbonyl γ -lacton, epoxide cycle, as well as olefinic double bond C3=C4 makes it promising for regio- and selective chemical modification for obtaining new derivatives.

Treating arglabin (61) with aminating reagents we synthesized new amino derivatives (62-65); amino derivatives (66-68) were produced from epoxyarglabin with high yields from 80 to 96%.

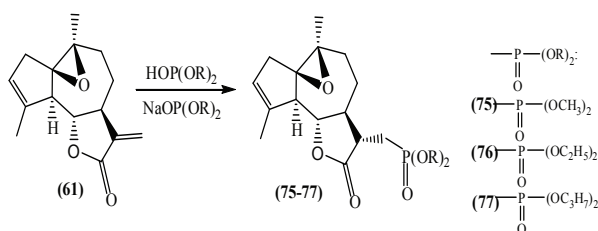
Reaction of dimethylamino- and diethylaminoderivatives of arglabin (62-63) with alkyl iodides was carried out to obtain quaternary salts, whereat corresponding salts of (69-72) were obtained with high yields.

Salt (62) soluble in water and polar organic solvents was obtained by barbotage of dried hydrogen chloride through ethanol solution of dimethylamino arglabin (73).

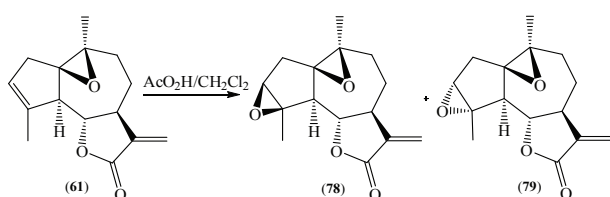
Trichloroacetate of arglabin (74) was synthesized at interaction of arglabin dimethylaminoderivative (62) with trichloroacetic acid in ethyl acetate.



Phosphorylation reaction was studied in various conditions, but the positive result was received only with sodium. As a result dialkyl phosphonate derivatives (75-77) with high yield were received. Their structures were determined by spectroscopy NMR ^1H , ^{13}C , ^{31}P , two-dimensional spectroscopy NMR ^1H - ^1H (COSY) and X-ray analysis.



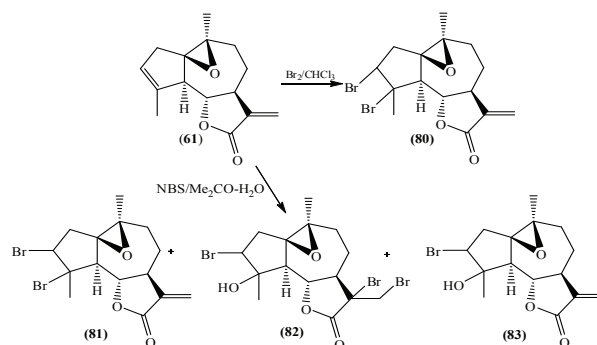
Oxidation of double bond resulted in the formation of epoxy derivatives of arglabin at C3-C4. It is known that double bonds are oxidized to epoxide compounds when treated with peracids. For example, derivatives (78) and (79) are formed at interaction of arglabin (61) with peroxyacetic acid.



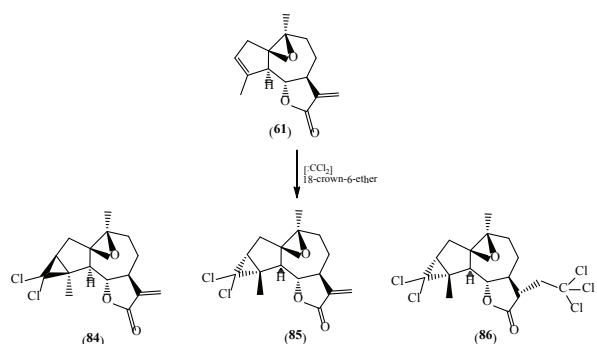
There are two functional groups readily reacting with bromine in the molecule of arglabin (61): an exomethylene group conjugated with carbonyl γ -lactone and olefinic double bond.

Reaction with molecular bromine in chloroform was carried out to obtain bromine derivatives of arglabin (61). This produced one product – arglabin dibromide (80) – which proves regio- and stereoselectivity of that reaction. Other method of obtaining bromine derivatives of sesquiterpene lactone argla-

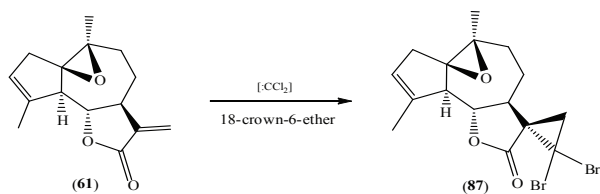
bin (61) is its interaction with N-bromosuccinimide in water acetone. Thereat, all reaction centers of arglabin molecule (61) participated in this reaction. As a result, three bromine derivatives of arglabin (81-83) were obtained.



We studied interaction of arglabin (61) with dichloropolycarbon generated from chloroform in the conditions of interphase catalysis with dicyclohexane-18-crown-6-ether to synthesize cyclopropane derivative. Thereat, compounds (84-86) were synthesized.



To obtain new biologically active derivatives based on guaianolide arglabin (61), in the conditions of interphase catalysis we synthesized new dibrominepolycarbon derivative of arglabin (87) with the yield of 28%.



Thus, derivatives containing pyrazol-, triazol-, amino-, dialkylamine-, hydroxy-, dialkyl phosphate- and cyclopropane groups with antitumor action, immune-modulating, anti-inflammatory, anti-fungal and antibacterial properties were obtained by various reactions on functional groups of arglabin molecule (61).

Isolation of Grosheimin from Available Plant Material and Chemical Modification of Guaianolide Molecule

Water-soluble form of grosheimin (88) isolated from aerial part of *Chartolepis intermedia* Boiss. is promising as new antivirus drug for influenza infection and was recommended for expanded pre-clinical and clinical trials.

We studied effect of some technology factors like mass and volume of loaded sample, velocity of mobile phase and rotor speed to develop the optimum conditions of isolating grosheimin (86) from ethyl acetate extract with the centrifugal partition chromatography.

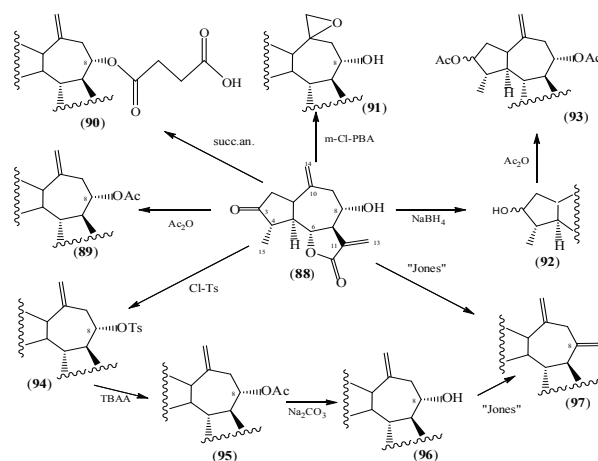
Quantitative yield of grosheimin (88) from ethyl acetate extract of *Chartolepis intermedia* Boiss. is provided with following parameters: 50.0 g of extract was dissolved in 200 ml stationary and 150 ml mobile phases at thorough stirring. Partition was performed at the eluent flow rate of 30 ml/min and rotor speed of 1000 rpm. The loaded sample is fully parted within 1.5 h. UV- detection was at 220 nm. Fractions were collected with fractional collector. Eluent was distilled on rotor evaporator and returned to the process with minimum losses.

As a result of partition, 6 fractions in volume from 100 to 300 ml were obtained and then the solvent was evaporated. Fraction 3 contained over 95 % of grosheimin (88). The second stage of purification included recrystallization of grosheimin from 96 % ethanol. Yield of grosheimin (88) with purity not less than 97.0 % made 2.3 % in terms of mass of *Chartolepis intermedia* Boiss. ethyl acetate extract. [42].

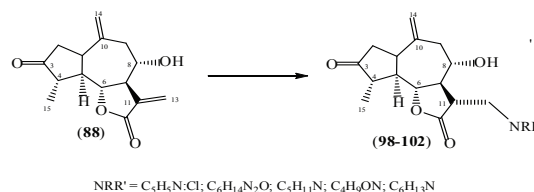
In 1964, K.S. Rybalko was the first to isolate grosheimin (88) from *Grossheimia marcocephala* and study it. Later Czechoslovak, Italian and Polish researchers jointly elaborated molecular structure of grosheimin. In particular, they conducted detailed studies of proton magnetic resonance spec-

tra, its deuterate derivative, acetate, adducts with trichloroacetylisocyanate and 3,5-dinitrobenzoate and also performed circular dichroism spectra and as a result suggested the keto-group position at C-3 and established α -configuration of methyl group at C-4, hydroxylic functions at C-8, protons of H-1 and H-5. Besides, they established trans-coupling of the lactone ring toward carbocycle of the molecule. Exomethylene group conjugated with carbonyl γ -lactone, methylene at C10-C14, hydroxylic function at C-8 and ketogroup at C-3 are the basic reaction centers in the molecule of (88).

According to reference [43] it is known that on the basis of grosheimin (88) reactions of hydrogenation, amination, acylation and oxidation were performed. Thereat, new derivatives (89-97) were synthesized.

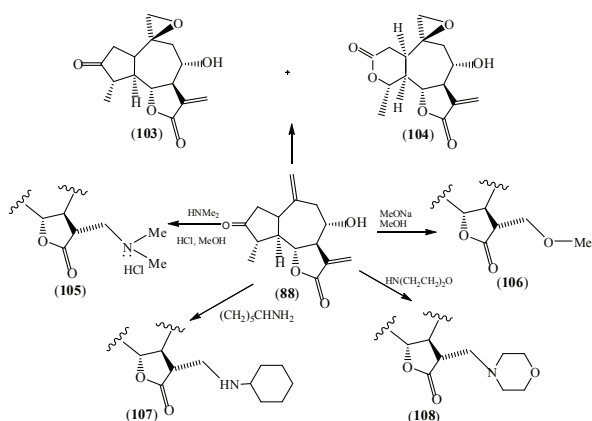


Grosheimin (88) interacts with pyridin, morpholine, cyclohexylamine, piperidine, methyl benzylamine, 2-hydroxyethylpiperazine by Michael reaction with the formation of derivatives (98-102) possessing high antitumor activity [44].

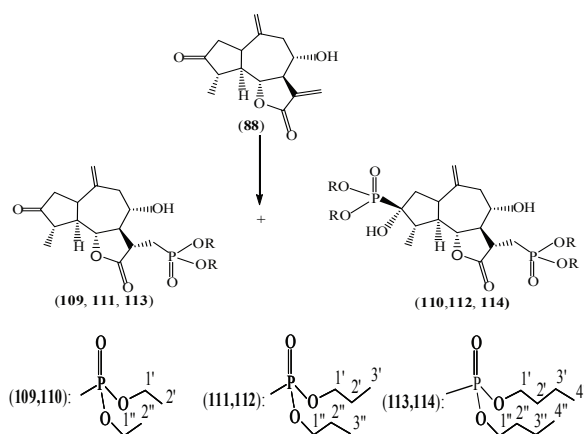


Two compounds were synthesized by epoxidation of grosheimin (88) with *m*-chlorineperbenzoic acid: 10(14)-epoxygrosheimin (103) and 3-keto-8-hydroxyguai-10(14),11(13)-dien-3(4),6(12)-diolide (104). A number of amino derivatives (105-108) were previously obtained by Michael reaction on exomethylene group of γ -lactone cycle

of grosheimin, in particular, with hydroxyethylpiperazidine, piperidine, pyrrolidine, morpholine, N-hexamethyleneimine, cyclohexylamine. 13-Metoxygrosheimin (106) was synthesized by interaction of grosheimin with sodium methylate in methanol.



As to the synthesis of phosphorus-containing derivatives based on guaianolide of grosheimin (88), phosphorylation reaction of its molecule was carried out for the first time. As a result of this reaction, compounds (109-114) were obtained [45-46].



Conclusions

Thus, summarizing the above-stated, the following should be noted:

- sesquiterpene lactones are a promising source for the development of new original medicinal products with wide spectrum of action and their introduction into clinical practice;
- extraction of plant material with various organic solvents followed by chromatographic purification constitute the basis of the technology for isolating sesquiterpene lactones. Therefore, de-

velopment of effective and environmentally friendly methods of isolating sesquiterpene lactones from plant material to GMP requirements - international rules for manufacturing medicinal products is a critical task at present;

- it is important to obtain water-soluble derivatives of biologically active sesquiterpene lactones and to use achievements of nanotechnology for the directed transport of the molecule of a medicinal product in human body, and thereby reducing the toxicity of the active component;
- promising direction is chemical modification of sesquiterpene lactone molecules as they constitute renewable material for obtaining new derivatives and help to solve two problems simultaneously. First, to obtain derivatives with higher biological activity or improved physical and chemical properties. Secondly, these investigations help to understand the mechanism of action of any examined medicinal substance in the context of the "structure-activity" correlation.

References

1. Siddhardha B., Ramakrishna G., Basaveswara M.V. International journal of pharmaceutical, chemical and biological sciences, 3:206 (2012).
2. Jadranin M., Đorđević I., Tešević V., Vajs V., Menković N., Soković M., Glamočlija J., Milosavljević Sl. Rec. Nat. Prod, 7: 234 (2013).
3. Moghadama M., Naghibi F., Atoofi A., Rezaiee M., Irani M., Mosaddegh B.Z. Naturforsch, 1:108 (2013).
4. Rosselli S., Bruno V., Raimondo F.-V., Spadaro V., Varol M., Koparal A., Maggio B. Molecules, 17: 8186 (2012).
5. Ibrahim M., Farooq T., Hussain N., Hussain A., Gulzar T., Hussain I., Akash M. Rehmani F. Chemistry Central Journal, 7: 1161 (2012).
6. Farha A., Geetha B., Mangalam S., Dhanya S., Latha P., Remani P. Asian journal of pharmaceutical and clinical research, 6:2 (2013).
7. Lee D.-S, Choi H., Woo K., Kang D., Lee H. European Journal of Pharmacology, 715:13 (2013).
8. Ghantous A.; Gali-Muhtasib H.; Vuorela H.; Saliba N.A.; Darwiche N. Drug Discov. Tod., 15:668 (2010).
9. Zhang S., Won Y.-K., Ong C.-N., Shen H.-M. Curr. Med. Chem. Anticancer Agents, 5:239 (2005).
10. Merfort I. Curr. Drug Targ., 12:1560 (2011).
11. Wedge D.E., Galindo J.C.G., Macías F.A. Phytochemistry, 53:747 (2000).
12. Canales M., Hernández T., Caballero J., de Viver A.R., Avila G., Duran A., Lira R., J. Ethnopharmacol, 97:429 (2005).
13. Tolstikova T.G., Tolstikov A.G., Tolstikov G.A.

- Medicines from plant substances. Novosibirsk, Geo, 2010, 215 p.
14. Simonsen J.L., Barton D.H.R. The Terpenes. Vol.III. – The University Press, Cambridge. -1951. – P. 249-295.
 15. Arantes F.F.P., Barbosa L.C.A., Alvarenga E.S., Demuner A.J., Bezerra D.P., Ferreira J. R.O., Costa-Lotufo L.V., Pessoa C., Moraes M.O., Eur. Journal of Med. Chemistry. 44:3879 (2009).
 16. Takayanagi H., Irimajiri R., McMurry T.B.H. Chem. Pharm. Bull. 39:780 (1991).
 17. Ivasenko S.A., Edilbaeva T.T., Kuliyasov A.T., Atazhanova G. A., Drab A.I., Turdybekov K.M., Raldugin V.A., Adekenov S.M., Chemistry of natural compounds. 1:30 (2006).
 18. Han C., Barrios F., Riofski M., Colby D., J. Org. Chem. 74:7176 (2009).
 19. Klochkov S.G., Afanas'eva S.V., Pushin A.N., Gerasimova G.K., Vlasenkova N.K., Bulychev Yu.N., Chemistry of natural compounds. 6:684 (2009).
 20. Luo X.-D., Shen C.-C., Medicinal Research Reviews, 7:29 (1987).
 21. Patent 2003/0185914 US. Process for isolating artemisinin from *Artemisia annua* / Kumar Sushil, Gupta Shiv Kumar, Singh Digvijay, Gupta Madan Mohan, Jain Dharam Chand, Kahol Atul Prakash, Khanuja Suman Preet, Singh Ram Govind; published in 02.10.2003.
 22. Patent 1999/005955084A US. Process for the simultaneous production of artemisinin and essential oil from plant *Artemisia annua* / Dharam Chand Jain, Sudeep Tendon, Rajendra Singh, Mohammed Shafiq; published in 06.11.1999.
 23. Chaturvedi D., Goswami A., Saikia P., Barua N., Rao P. Chem. Soc. Rev., DOI: 10.1039/b816679j (2010).
 24. Al-Oqail M.M., Galal A.M., Ahmad M.S., Molecules, 8:901 (2003).
 25. M.Martinez-Vazquez, S.Sepulveda, M.A. Belmont, M.Rubio, P.Joseph-Nathan. J. Nat. Prod., Vol.55, No.7, P. 884-898 (1992).
 26. Liu Yang, Cui K., Lu W., Luo W., Wang J., Huang J., Guo C., Molecules, 16:4527 (2011).
 27. Patent 577034 USSR. Method of obtaining sesquiterpene lactones. Khvorost P.P., Komissarenko N.F., Obolentsova G.V, Vidukova A.I., Hajai Ya.I., Luchkova M.M, Georgievsky V.P., Degtyarev L.D., Zinchenko V.V; 25.10.1977.
 28. Patent 1710062 SU. Method of obtaining sesquiterpene lactones. Babaev N.F., Serkerov S.V.; published in 07.02.1992.
 29. Belyakov K.V., Popov D.M., Pharmacy, 1:37 (2004).
 30. Milman I.A., Chemistry of natural compounds 3:307 (1990).
 31. Klochkov S.G., Afanas'eva S.V., Pukhov S.A., Chemistry of heterocyclic compounds, 2:406 (2012).
 32. Klochkov S.G., Afanas'eva S.V., Bulychev Yu.N., Neganova M.E, Shevtsova E.F. Russian Chemical Bulletin, chemical series, 2:407 (2012).
 33. Klochkov S.G., Afanas'eva S.V., Ermatova A.B., Chudinov A.V, Chemistry of natural compounds. 47:716(2011).
 34. Tomilov Yu.V., Revunov E.V., Shulishov E.V., Semenov V.V., Russian Chemical Bulletin, 61:280 (2012).
 35. Patent 006479080 US. Tanacetum parthenium extract and method of obtaining same / Ezio Bomnardelli, Paolo Morazzoni; published in 12.11.2002.
 36. Konovalova D.S., Konovalov D.A, Ecology of person, 3:3 (2008).
 37. Han C., Barrios F., Riofski M., Colby D., J. Org. Chem 74:7176(2009).
 38. Nasim Sh., Crooks P.A., Bioorganic and Medicinal Chemistry Letters, 18:3870 (2008).
 39. Han Ch., Barrios F.J., Riofski M.V., Colby D.A., 74:7176(2009).
 40. Patent USA 6,242,617,B1, Jun.5.2001; Deutshchen Patent № 697 2504.9-08, 23.10.03; European Patent № 0 946 565, 15.10.03; Patent of China ZL 2006 8 0055852.4, 26.12.2012 Adekenov S.M. "Method and device for production of lyophilized hydrochloride-1 β ,10 β -epoxy-13-dimethylaminoguaia-3(4)-en-6,12-olide".
 41. The Eurasian patent № 015557, 30.08.2011 Adekenov S.M. Method of obtaining 1(10) β -epoxy-13-dimethylamino-5,7 α ,6,11 β (H)-guai-3(4)-en-6,12-olide hydrochloride lyophilized, antitumor drug "Arglabin".
 42. Innovative patent RK № 23472 "Methylodide 13-dimethylamino-3-oxo-8-hydroxy-1,5,7 α ,4,8, 11 β (H)-guai-10(14)-en-6,12-olide, possessing anti-virus and immune-modulting action. Adekenov S.M., Ivasenko S.A., Seidakhmetova R.I, Akhmetova S.B., Atazhanova G.A.
 43. Barbetti G., Fardella G., Chiappini I., Scarcia V., Furlani Candiani A., Farmaco Ed. Sci. 40:23(1985).
 44. Naidenova E., Dryanovska-Noninska L., Drozhzh B., Problems of pharmacy, 5:85 (1977).
 45. Jalmakhanbetova R.I, Ivasenko S.A., Kuliyasov A.T., Khasenov B.B., Kurmankulov N.B., Adekenov S.M., Chemistry of natural compounds 4:311 (2004).
 46. Jalmakhanbetova R.I, Ivasenko S.A., Kuliyasov A.T., Kurmankulov N.B., Adekenov S.M., Chemistry of natural compounds 4:316-318 (2004).

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