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SESQUITERPENE LACTONES OF PLANTS OF THE GENUS ACHILLEA L.

Abstract: Plants of the genus *Achillea* L. are considered promising sources of medicinal substances based on essential oils, sesquiterpene lactones, and flavonoids. In the present work, the results of a search for sesquiterpene γ-lactones in 13 Achillea species growing in Kazakhstan are summarized for the first time. The distribution of sesquiterpene lactones among the sections of the genus *Achillea* L. and their classification according to structural types are discussed. Based on chemical and biological screening, the presence of sesquiterpene γ-lactones was established in nine *Achillea* species. Three species –*Achillea inundata* Kondr., *Achillea salicifolia* Besser, and *Achillea tianschanica* Kupr. & Kulemin – were identified as promising sources for the isolation and identification of sesquiterpene lactones. From *Achillea nobilis* L. and *Achillea micrantha* Willd. 11 sesquiterpene lactones have been isolated, including 3 new compounds. The physicochemical characteristics of the isolated sesquiterpene lactones were determined, and their structures were elucidated using IR, UV, ^{1}H NMR, and ^{13}C NMR spectroscopy. An analysis was carried out on the influence of extraction methods, fractionation of crude plant extracts, and purification procedures on the yield of sesquiterpene lactones from *Achillea* species. Data on the biological activities of the isolated sesquiterpene lactones were compiled and summarized, including antitumor, antitrypanosomal, antileishmanial, anthelmintic activities, and cytotoxicity. The biosynthetic pathways of sesquiterpene lactones in *Achillea* species are discussed, along with the dynamics of their localization in different plant organs during various stages of vegetation. It was established that sesquiterpene lactones from *Achillea* species may serve as renewable starting materials for chemical modification and the synthesis of new compounds, representing potential sources of novel antitumor and antiparasitic agents.

Keywords: *Achillea* L., sesquiterpene lactones, qualitative reaction, molecular structure, infrared spectroscopy, nuclear magnetic resonance, X-ray crystallographic analysis, biological activity.

Introduction

Plants, as renewable biological resources, represent a potential source of new natural compounds with a wide range of pharmacological activities. The genus *Achillea* L. comprises approximately 150 species distributed throughout the countries of the Northern Hemisphere, with the greatest diversity occurring in Europe, Central Asia, and North America (Anderberg et al., 2007; Ehrendorfer et al., 2005). On the territory of Kazakhstan, 19 species of the genus *Achillea* L. occur: Sect. Filipendulinae (DC.) Afan.: *A. filipendulina* Lam.; Sect. Micranthae Klok. & Krytzka: *A. micrantha* Willd., *A. arabica* Kotschy; Sect. Nobilia Klok. & Krytzka: *A. nobilis* L.; Sect. Millefolium (Mill.) Koch: *A. asiatica* Serg., *A. kuprijanovii* Stepanov, *A. sergievskiana* Schaulo & Shmakov, *A. inundata* Kondr., *A. kamelinii* Kupr., *A. millefolium* L., *A. schmakovii* Kupr., *A. setacea* Waldst. & Kit., *A. tianschanica* Kupr. & Kulemin, *A. karatavica* Kamelin, *A. stepposa* Klokov & Krytzka, *A.* × *kasakhstanica* Kupr. & Alibekov; Sect. Ptarmica (Mill.) Koch: *A. impatiens* L., *A. ledebourii* Heimerl, *A. salicifolia* Besser.

The results of chemical investigations indicate that the aerial parts of *Achillea* L. species are a rich source of sesquiterpene γ-lactones. According to published data, a total of 103 sesquiterpene lactones have been identified in 27 species of this genus, including 79 guaianolides, 20 germacranolides, and 4 eudesmane-type lactones (Agatha et al., 2024; Alves et al., 2018; Frey et al., 2024). These compounds exhibit a broad range of biological activities, including antiparasitic (Sülsen & Martino, 2018), antiviral (Amen et al., 2025), antimicrobial (Ivanescu et al.,

2015), anti-inflammatory (Ivanescu et al., 2015), and antitumor effects (Laurella et al., 2022).

It is noteworthy that the overwhelming majority of sesquiterpene lactones from *Achillea* L. species are non-linear lactones. Their structures usually contain epoxy-, keto-, acetoxy-, and hydroxyl groups. Ten guaianolides bear a chlorine atom in their structure (Fraga, 2013). 23 of these compounds possess an exocyclic methylene group conjugated with the carbonyl group of the γ-lactone moiety, indicating the potential of searching for new sesquiterpene γ-lactones as promising pharmacologically active agents (Fraga, 2013).

Plants of section Millefolium (Mill.) Koch are widely distributed on the territory of Kazakhstan (Kupriyanov & Kulemin, 2023). The species of section Millefolium (Mill.) Koch are the most chemically studied with respect to the content of sesquiterpene lactones. Thus, from *Achillea millefolium* L., series Millefoliatae (DC.) Klok. & Krytzka, new sesquiterpene lactones with unusual structures have been isolated and described – 5 sesquiterpene dilactones and 12 guaianolides, including 9 halogen-containing guaianolides exhibiting comparatively high anti-inflammatory activity (Li et al., 2021; Li et al., 2023a; Li et al., 2023b; Li et al., 2023c). From *Achillea millefolium* L., a guaianolide with a peroxide bridge, α-peroxyachifolide, has been isolated, which is a skin sensitizer (Strzępek-Gomółka et al., 2021). By methanol extraction of the aerial part of *Achillea millefolium* L., three sesquiterpene lactones of the psilostachyin type, containing, in addition to the γ-lactone ring, a δ-lactone fragment, as well as a guaianolide and a germacranolide, have been isolated for the first time. Two guaianolides containing both γ- and δ-lactone rings exhibited antiproliferative activity towards HeLa, MCF-7, and A-431 cells, while the guaianolide achillinin A was active against the A549, RERF-LC-kj, and QG-90 cell lines (Csupor-Löffler et al., 2009; Yong et al., 2011). On the basis of terpenoids of the essential oil of *Achillea millefolium* L., a wound-healing and anti-inflammatory preparation, Akhizan, has been developed (Kalinkina et al., 1993). Australian researchers, by extraction with dichloromethane of the aerial part of *Achillea aspleniifolia* Vent., series Asplenifoliae Klok. & Krytzka, isolated a new pseudoguaianolide (Kasner et al., 1990). By HPLC analysis of an ultrasound-assisted dichloromethane extract of *Achillea collina* (Wirtg.) Becker ex Heimerl, the presence of five known guaianolides was determined (Rothwangl-Wiltschnigg et al., 2004). From *Achillea asiatica* Serg., three previously undescribed guaianolides were isolated (Glasl et al., 2001). In *Achillea setacea* Waldst. & Kit., 20 sesquiterpene γ-lactones have been detected, including an unusual guaianolide with a peroxide bridge (Todorova et al., 2000; Xue et al., 2025; Zitterl-Eglseer et al., 1991).

Another currently investigated plant of this genus is *Achillea alpina* L. of section Ptarmica (Mill.) Koch, series Lingulatae Botsch., from which 13 new sesquiterpene lactones have been isolated – 5 germacranolides, 6 guaianolides, and 2 dimeric guaianolides (Xue et al., 2022; Xue et al., 2023). Among them, a germacranolide and a guaianolide exhibit antidiabetic activity, while a 1,10-seco-guaianolide and a dimeric 1,10-seco-guaianolide show comparatively high hypoglycemic activity (Xue et al., 2022; Xue et al., 2023). From *Achillea arabica* Kotschy of section Filipendulinae (DC.) Afan., a new guaianolide, edremitin, has been isolated (Hanalp et al., 2022). From *Achillea ligustica* All. of section Achillea a new guaianolide, 3α-chloro-4β,10β-dihydroxy1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide, has been isolated (Mahmoud et al., 2012). By methanol extraction of the aerial part of *Achillea falcata* L. of section Babounya (DC.) Boiss., three known guaianolides have been isolated, exhibiting comparatively high cytotoxicity towards CT-116 colorectal cancer cells (Tohme et al., 2013).

The analysis of the literature and patent material for 2015–2025 on biologically active sesquiterpene lactones of plants of the genus *Achillea* L. testifies to the high potential of the above-mentioned taxon for the development of new antitumor and antiparasitic preparations.

The aim of this study was to search for potential sources of original medicinal agents based on biologically active sesquiterpene lactones from plants of the genus *Achillea* L.

Materials and methods

Plant material samples of *Achillea* L. species for chemical study were collected during the budding and flowering stages in different floristic regions of Kazakhstan, including Almaty, East Kazakhstan, Zhambyl, Ulytau, Karaganda, Turkistan, and Pavlodar regions. The collected herbarium specimens were identified and deposited in the herbarium of the Botanical Garden of the Research and Production Center "Phytochemistry" (Karaganda, Kazakhstan) (Table 1).

Table 1

Sites of raw material collection of the genus Achillea L. on the

Kazakhstan territory

№	Plant species	Collection site
	Section Filipendulinae (DC.) Afan.,	
1	*Achillea filipendulina* Lam.	Zhambyl region
	Section Micranthae Klok. & Krytzka	
2	*Achillea arabica* Kotschy	Turkistan region
3	*Achillea micrantha* Willd.	Ulytau region, Sarysu river valley
	Section Nobilia Klok. & Krytzka	
4	*Achillea nobilis* L.	Karaganda region, vicinity of Egindybulak village
	Section Millefolium (Mill.) Koch	
5	*Achillea asiatica* Serg.	Botanical Garden collection of JSC "RPC "Phytochemistry"
6	*Achillea inundata* Kondr.	Botanical Garden collection of JSC "RPC "Phytochemistry"
7	*Achillea karatavica* Kamelin	Almaty region
8	*Achillea millefolium* L.	Karaganda region, Karkaraly mountains
9	*Achillea setacea* Waldst. & Kit.	Pavlodar region, Bayanaul mountains
10	*Achillea tianschanica* Kupr. & Kulemin	Turkistan region, Sairam-Ugam National Park, slope and floodplain of the Sairamsu river
	Section Ptarmica (Mill.) Koch	
11	*Achillea impatiens* L.	Karaganda region, Kyzylarai mountains
12	*Achillea ledebourii* Heimerl	East Kazakhstan region, vicinity of Ridder town
13	*Achillea salicifolia* Bess.	Karaganda region, Abay district

Introduction and cultivation studies of *Achillea asiatica* Serg. and *Achillea inundata* Kondr. were carried out at the introduction plot of the Botanical Garden of JSC "Research and Production Center Phytochemistry" (Karaganda, Republic of Kazakhstan). Herbarium collections from this plot are deposited in the Herbarium Fund of JSC "Research and Production Center Phytochemistry".

To screen plants for the presence of sesquiterpene lactones, total extractive substances were isolated from the plant material. The obtained extracts were analyzed by a qualitative terpenoid assay, infrared (IR) spectroscopy of the crude extract, and chromatographic techniques, including thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Total extractive substances were isolated from plant materials by carbon dioxide extraction (CO_2 extraction) using a USFE-5/2 supercritical fluid extraction system (manufactured by LLC "Goro-Engineering", Russia, under license from the Austrian company "Natex", Ternitz, Austria) at the following conditions (applied to all plant samples): pressure 160 bar, temperature 60°C, and extraction time 240 min. The resulting extracts were subsequently subjected to qualitative tests for terpenoids. Upon addition of a vanillin solution in sulfuric acid to the powdered extractive fraction, the mixture developed a red-violet coloration, indicating the presence of terpenoid compounds (Harborne, 1998). In samples of extracts that gave a positive reaction for terpenoids, the presence of sesquiterpene lactones was confirmed by IR spectroscopy through the absorption band characteristic of the γ-lactone carbonyl in the 1740–1800 cm^{-1} region. The IR spectrum was recorded on an Avatar 360 ESP spectrometer (Thermo Nicolet, USA) in KBr pellets. The extracts were analyzed by HPLC on a Hewlett Packard Agilent 1100 Series liquid chromatograph (USA) under isocratic conditions using a mobile phase of acetonitrile–acetic acid (50:50, v/v) at a flow rate of 0.5 $mL \cdot min^{-1}$. A stainless steel column (150 mm × 4.6 mm) packed with Zorbax SB-C18 sorbent (5 μm particle size) was used at a column temperature of 20°C. The injection volume was 20 μL. Detection was performed with a UV detector at wavelengths of 254 and 289 nm. The components were identified by comparison of their retention times with reference samples of sesquiterpenoids, the corresponding information on their content was obtained. Individual sesquiterpene lactones were isolated from the total extractive substances by liquid column chromatography on silica gel KSKG (macroporous coarse-grained granular silica gel) (Qingdao Shuoyuan Silica Gel Technology Co., Ltd.,

China) at a sample-to-sorbent ratio of 1:20. The column was eluted with benzene, benzene–diethyl ether mixtures (4:1, 1:1), diethyl ether, diethyl ether–ethyl acetate mixtures (4:1, 1:1), ethyl acetate, or petroleum ether–ethyl acetate mixtures with increasing content of the latter. The purity of the isolated compounds was monitored by thin-layer chromatography (TLC) and HPLC data.

For TLC, Silufol TLCP-AI A-UF plates manufactured by Imid (Krasnodar, Russia) were used with a benzene–ethanol (9:1) solvent system. The substances were visualized by spraying the plates with a 1% solution of vanillin in H_2SO_4 and a saturated solution of $KMnO_4$. Reference standards of the compounds were obtained from the Republican Bank of Biologically Active Compounds (Karaganda, Republic of Kazakhstan) (State Pharmacopoeia of the Republic of Kazakhstan, 2014).

HPLC was performed on an Agilent 1100 Series liquid chromatograph (USA) equipped with a Quat-Pump G1311A pump, a Rheodyne injection valve with a 20 μL loop, a G1322A column holder, and a G1314A ultraviolet (UV) detector. All analyses used a Zorbax SB-C18 column (15 cm × 0.46 cm, 5 μm particles; Agilent, Santa Clara, CA, USA) and the following solvents: methanol, acetonitrile, and water (analytical-grade reagents of purity ≥ 99.9%, supplied by Sigma-Aldrich (Merck-Millipore, USA). The maximum UV absorption at 205 nm was processed by ChemStation software (Agilent Technologies, USA).

Antiparasitic activity against extracellular forms was assessed using a standardized microplate assay. Parasite suspensions were adjusted to the appropriate density and distributed into 96-well plates. *Leishmania amazonensis* promastigotes in the stationary phase were diluted to 5×10^5 cells/well in phenol red-free RPMI medium. *Trypanosoma brucei brucei* bloodstream forms were diluted to 3.5×10^4 parasites/mL, and 200 μL aliquots were added per well. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and further diluted in the corresponding culture medium to obtain final concentrations of 1, 10, and 50 μg/mL for *Leishmania amazonensis*, and 0.1, 1, and 10 μg/mL for *Trypanosoma brucei brucei*, in duplicate wells. The final DMSO concentration did not affect parasite viability and was used as a negative control. Following incubation (48 h at 24°C for *Leishmania amazonensis*; 72 h at 37 °C, 5% CO_2 for *Trypanosoma brucei brucei*), parasite viability was quantified: for promastigotes, 50 μL of XTT was added and incubated for 4 h at 24 °C, with absorbance measured at 450 nm using a microplate reader (Tecan); for bloodstream forms, parasites were counted directly using a Beckman Z2 Coulter counter. The percentage of inhibition was calculated for each compound at each concentration (Laurella et al., 2023).

Trypanocidal activity against intracellular forms of *Trypanosoma cruzi* was evaluated using a cell-based infection assay. Vero cells (5×10^3 per well) were infected with tissue culture-derived trypomastigotes of the *Trypanosoma cruzi* Tulahuen strain expressing β-galactosidase (MOI: 10). Following overnight incubation at 37°C, 5% CO_2, non-internalized parasites were removed by washing, and infected cells were incubated with test compounds at 10 μg/mL. After 5 days, cells were lysed with 1% Nonidet P-40, and chlorophenol red-β-D-galactopyranoside (CPRG) (100 μM) was added as a β-galactosidase substrate. The plates were incubated for 4 h at 37°C, and β-galactosidase activity was determined by measuring absorbance at 570 nm. The percentage of inhibition was calculated according to the following formula: 100 − {[(absorbance of treated infected cells) / (absorbance of untreated infected cells)] × 100} (Laurella et al., 2023).

Cytotoxicity was determined by the MTT method. Vero cells were seeded in flat-bottom 96-well plates and cultivated at 37°C in an atmosphere of 5% CO_2 in the presence of the sesquiterpene lactones of plants of the genus *Achillea* L. at a concentration of 10 μg/mL or DMSO as a viability control for 48 h. MTT was then added at a final concentration of 0.6 μg/mL, and after 2 h of incubation at 37°C, the formazan crystals were dissolved in 100 μL of isopropanol. Absorbance was measured at 595 nm in a microplate reader (Rayto RT-6000). Results were expressed as the percentage of cell viability: {(absorbance in the presence of compound) / (absorbance in the absence of compound)} × 100 (Borgo et al., 2023).

The *in vivo* anthelmintic activity of leucomisin (18) was evaluated at the Kazakh Scientific Research Veterinary Institute (Almaty, Kazakhstan) in accordance with methodological guidelines for determining the antiparasitic effects of pharmacological substances (Adekenov, 2023; Guidelines for the Experimental (Preclinical) Investigation of New Pharmacological Substances, 2000). Coproovoscopy of the collected feces was carried out by the Darling method using a digital microscope (Levenhuk D50 LNG, Levenhuk, Inc., USA/China). In accordance with the study protocol, fecal samples were taken from 20 sheepdogs to determine their initial helminth infestation. As a result of the coprological examination, 10 of the 20 dogs (50%) proved to be infested with various helminth species, with infestation inten-

sity (hereinafter – II) ranging from 1 to 15 eggs. Of the 10 infested dogs, eggs of *Trichuris vulpis* with II 1–15 were detected in 8 dogs (80%), and eggs of *Dipylidium caninum* in 2 dogs (20%). No other helminth species were detected in the feces of the sheepdogs by coproovoscopy. Based on the coproovoscopy results, the helminth-infested dogs (10 dogs) were divided into two experimental groups, 5 animals each. Dogs of the first group (5 animals) were administered capsules with leucomisin (18) at a dose of 150 mg (3 capsules) per 20–25 kg of body weight. In the comparison group, dogs received the preparation "CesTremForte" (LLP "Kazakh Scientific Research Veterinary Institute", Kazakhstan) at a dose of 150 mg (2 tablets) per 20–25 kg of body weight. The capsules with leucomisin (18) were administered as a single dose. Coproovoscopy of each fecal sample before and after deworming of the dogs was carried out twice. Four days after administration of the capsules with leucomisin (18), coproovoscopy of the dogs' feces was performed to determine the extens- and intens-efficacy of the test sample (18).

Statistical analysis of the results was performed using Statistica 8.0 software. The obtained data are expressed as mean ± standard error of the mean.

Ethical considerations

The study was conducted in accordance with the decree of the Minister of Health of the Republic of Kazakhstan No. KR DSM-181/2020 (November 4, 2020) and approved by the Local Bioethics Commission of the NJSC "Karaganda Medical University" (Protocol No. 18, October 21, 2025) (Order of the Minister of Healthcare of the Republic of Kazakhstan, 2020).

Results and discussion

The primary chemical screening of *Achillea* L. plant species for sesquiterpene lactones comprises qualitative reaction of total extractives for terpenoids, infrared spectroscopy and screening for biological activity. The results of the chemical evaluation and the bioscreening of the total extractive substances from *Achillea* L. species are presented in Table 2.

Table 2

Results of chemical and biological screening of plants of the genus Achillea L. for the content of sesquiterpene lactones

№	Plant species	Qualitative reaction for terpenoids	IR spectrum data of total extractive substances, cm^{-1}	Biological activity of total extractive substances
	Section Filipendulinae (DC.) Afan.			
1	*Achillea filipendulina* Lam.	-	1713	-
	Section Micranthae Klok. & Krytzka			
2	*Achillea arabica* Kotschy	+	1767, 1759, 1731	-
3	*Achillea micrantha* Willd.	+	1780, 1730, 1680, 1630	Antitumor, insecticidal, antifeedant
	Section Nobilia Klok. & Krytzka			
4	*Achillea nobilis* L.	+	1770, 1720,1650	Growth-regulating, antitumor, insecticidal
	Section Millefolium (Mill.) Koch			
5	*Achillea asiatica* Serg.	+	1775, 1715, 1650	Growth-regulating
6	*Achillea inundata* Kondr.	+	1745	-
7	*Achillea karatavica* Kamelin	-	1739, 1712	-
8	*Achillea millefolium* L.	+	1760, 1720, 1650	Fungicidal, insecticidal, antitumor, antibacterial
9	*Achillea setacea* Waldst. & Kit.	+	1770	Growth-regulating, antitumor
10	*Achillea tianschanica* Kupr. & Kulemin	+	1750, 1640,1620	-
	Section Ptarmica (Mill.) Koch			
11	*Achillea impatiens* L.	-	1722	Antitumor
12	*Achillea ledebourii* Heimerl	-	1710	Growth-regulating
13	*Achillea salicifolia* Bess.	+	1755, 1740, 1620	Antitumor

Based on the results of chemical screening, the absorption bands in the IR spectra of the total extractive substances from yarrow species in the 1740–1800 cm^{-1} region indicate the presence of sesquiterpene γ-lactones

in 9 species (69.23%) of the plants studied, which is confirmed by the qualitative reaction for terpenoids (Table 2). In addition, the total extractive substances from *Achillea millefolium* L., *Achillea micrantha* Willd., and *Achillea nobilis* L. exhibited growth-regulating, insecticidal, and antitumor activities.

According to the data of Table 2, among the thirteen plant species of the genus *Achillea* L. collected on the territory of Kazakhstan for chemical investigation, the presence of sesquiterpene lactones was established in 5 species (83.3%) of section Millefolium (Mill.) Koch, in 2 species (100%) of section Micranthae Klok. & Krytzka, and in 1 species (33.3%) of section Ptarmica (Mill.) Koch. Three species of the genus *Achillea* L. – *Achillea inundata* Kondr., *Achillea salicifolia* Besser, and *Achillea tianschanica* Kupr. & Kulemin – are promising, previously uninvestigated objects for the isolation and identification of sesquiterpene lactones.

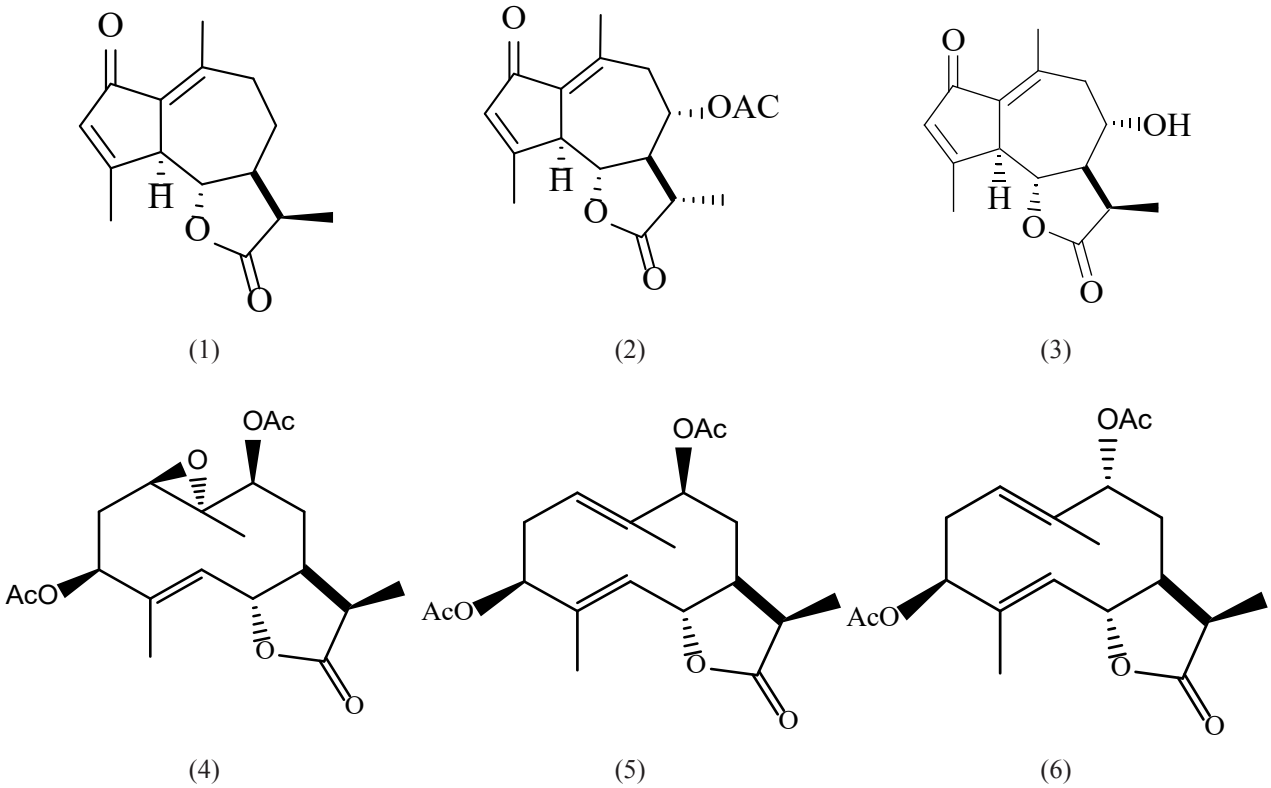
Sesquiterpene Lactones of *Achillea* L. of the Flora of Kazakhstan

Achillea micrantha Willd. (small-flowered yarrow) is a plant widely distributed in the territory of Kazakhstan; it occurs on sands and sandy soils, more rarely in steppe, pasture, and solonetzic meadows from the forest-steppe to the desert zone (Flora of Kazakhstan, 1966).

We have previously established that the aerial parts of *Achillea micrantha* Willd. contain the guaianolides achillin (1), artilesin (2), and grossmisin (3), as well as the germacranolides micranthin (4) and achimicrin (5) (Barda et al., 2021).

Figure 1

The structure of Achillea micrantha Willd sesquiterpene lactones (guaianolides achillin (1), artilesin (2), grossmisin (3), germacranolides micranthin (4), achimicrin (5) and sintenin (6)



The quantitative yield of sesquiterpene lactones is provided by the method of extraction of the raw material with hot water followed by recovery of the total substances from the aqueous extract with chloroform and chromatographic separation of the total extractive substances on a silica gel column, which gives the yield (per air-dry raw material weight) of achillin (1) – 0.05%, artilesin (2) – 0.003%, grossmisin (3) – 0.005%, micranthin (4) – 0.003%, and achimicrin (5) – 0.003% (Barda et al., 2021).

A colorless crystalline substance of composition $C_{19}H_{26}O_6$, m.p. 220–222 °C (from a diethyl ether–chloroform mixture), $[\alpha]_D^{22}$ +15.5° (c 0.42; chloroform), M^+ 350, proved to be a new sesquiterpene lactone and was named achimicrin (5).

The IR spectrum of (4) shows absorption bands

characteristic of the γ-lactone carbonyl (1770 cm^{-1}), an ester group (1725, 1250 cm^{-1}), and a double bond (1660 cm^{-1}). The ^{1}H NMR and ^{13}C NMR data and their correlation with the literature (Natiq et al., 1992) allowed the structure of a *trans,trans*-germacradienolide of the 11,13-dihydrocostunolide series to be proposed for molecule (4). Signal assignments were made on the basis of comparative analysis of two-dimensional ^{1}H–^{1}H and ^{13}C–^{1}H NMR spectra.

In the ^{1}H NMR spectrum (recorded in $CDCl_3$), a doublet at 1.24 ppm (3H, J = 7.7 Hz) reflects the presence of a secondary methyl group in molecule (4); broad singlets at 1.50 and 1.72 ppm (3H each) correspond to two methyls at double bonds; and singlets at 2.03 and 2.10 ppm (3H each) correspond to two acetoxy groups. In the ^{13}C NMR spectrum, 5 quartets, 2 triplets, 7 doublets, and 5 singlets are observed, indicating that molecule (4) contains five methyl groups, two olefinic double bonds, and three carbonyl groups.

The presence of double bonds in the carbocycle is also confirmed by the signals of olefinic protons in the ^{1}H NMR spectrum (recorded in C_5D_5N): a broad doublet at 4.06 ppm (1H, J = 9.5 Hz) and a doublet of doublets of quartets at 5.24 ppm (1H, J = 12, 4.7, and 1 Hz). The triplet at 5.05 ppm (1H, J = 9.5 Hz) was assigned to the lactone proton, while the doublets of doublets at 5.18 ppm (1H, J = 10.5 and 5.6 Hz) and at 5.12 ppm (1H, J = 10.5 and 2.7 Hz) were assigned to gem-acyl protons. Upon decoupling of the Me-10 signal, the H-1 signal – a doublet of doublets of quartets at 5.24 ppm – simplifies to a doublet of doublets, while the H-2a signal – a broad doublet of doublets of doublets at 2.56 ppm – simplifies to a doublet of doublets of doublets. Moreover, H-2a is positioned such that the C–H-2a bond is almost perpendicular to the plane of the double bond, i.e. this proton is pseudo-equatorial and has the α-configuration.

One of the acetyl groups in the achimicrin (5) molecule is located at C-3, since the doublet of doublets at 5.18 ppm assigned to H-3 has J = 10.5 and 5.6 Hz, characteristic of the H-3/H-2a and H-3/H-2b couplings, respectively. The other acetoxy group is located at C-9, as follows from the correlation of the H-9 chemical shift in achimicrin (5) with that in sintenin (6).

The signal of the gem-acyl proton H-9 – a doublet of doublets at 5.12 ppm (J = 10.5 and 2.7 Hz) – simplifies, upon irradiation of H-8, to a doublet with J = 4 Hz, which is fully consistent with the presence of an axial H-9 proton whose signal is split through coupling with axial and equatorial vicinal H-8 protons. From Dreiding models it can be seen that, in the 1,5-*trans,trans*-germacradienolide conformation of the carbocycle, i.e. when both methyl groups have a syn-orientation and point upward, the data obtained correlate with 3β- and 9β-positions of the acetoxy function. According to the studies of Randall et al., C-11 in the γ-lactone ring has the α-configuration if the C-13 chemical shift in the ^{13}C NMR spectrum is observed in the 9.5–10 ppm range (Pregosin et al., 1972). Consequently, the methyl group at C-11 in achimicrin (5) is β-oriented, since the C-13 chemical shift is a quartet at 10.80 ppm.

The proposed configuration of Me-11 is also confirmed by the H-11/H-7 coupling (J = 7.7 Hz), indicating a pseudo-equatorial position of the proton at C-11. Thus, on the basis of the data obtained, it can be concluded that achimicrin (5) is an isomer of sintenin (6) at C9-OAc and has the structure of 11*R*-3-β,9β-diacetoxy-1,5-*trans,trans*-6β,7α(H)-germacra-1(10),4(5)-dien-6,12-olide.

It has been determined that aqueous and ethanolic extracts of *Achillea micrantha* Willd. exhibit antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Astafyeva et al., 2018).

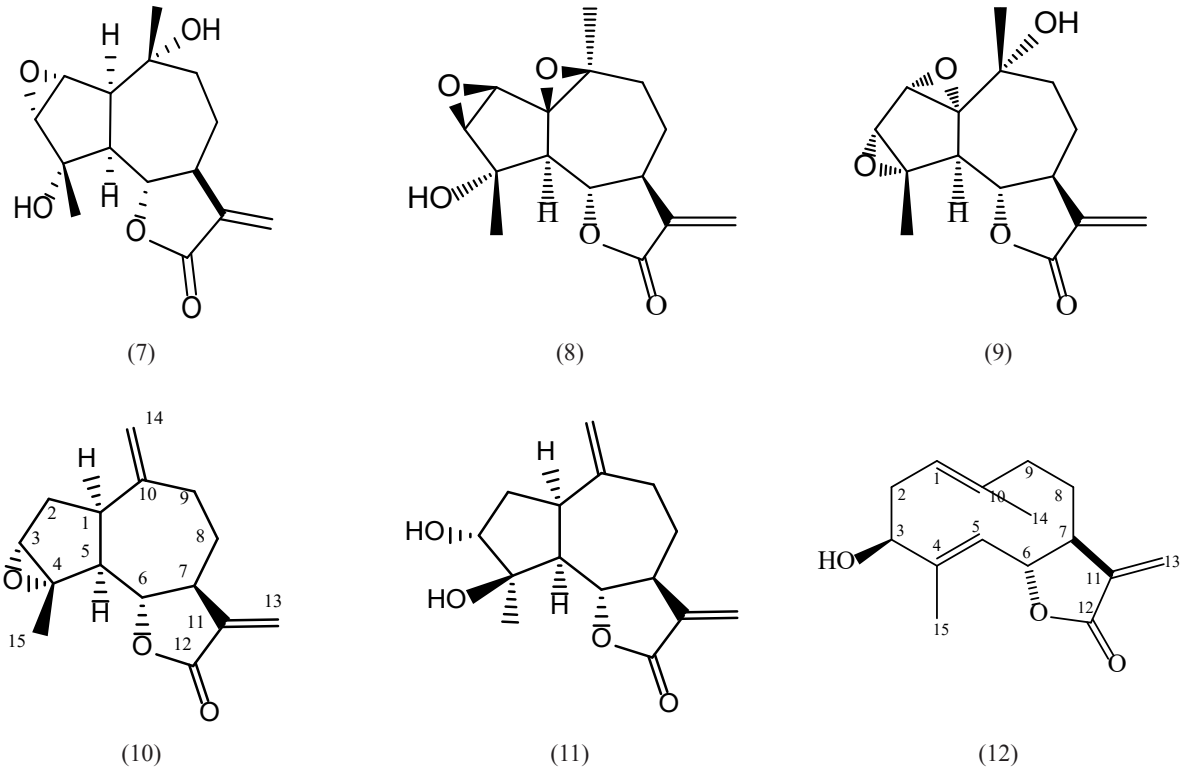
Achillea nobilis L. (noble yarrow) is a perennial plant whose range covers the steppe zone from the Western Mediterranean to Western Siberia. In the steppe part of Central Kazakhstan, it occurs on the slopes of granite hills. In certain districts of Central Kazakhstan, the exploitable reserve of noble yarrow on an area of 3916 hectares amounts to 136.6 tons (Flora of Kazakhstan, 1966).

From the aerial part of this plant, the flavonoids cosmosiin, cynaroside, and apigenin-7-glucuronide have been isolated and identified (Shevchenko et al., 2025).

From the raw material of *Achillea nobilis* L., the guaianolides anobin (7), chrysartemin A (8), canin (9), estafiatin (10), and anolide (11), as well as the germacranolide hanphyllin (12), have been isolated (Adekenov et al., 1984; Kishkentayeva et al., 2020; Turdybekov et al., 1994; Turmukhambetov et al., 1999).

Figure 2

The structure of Achillea nobilis L. sesquiterpene lactones (guaianolides anobin (7), chrysartemin A (8), canin (9), estafiatin (10), and anolide (11), germacranolide hanphyllin (12)



(7) (8) (9)

(10) (11) (12)

From the aerial part of *Achillea nobilis* L. collected at the flowering phase, by aqueous extraction followed by chromatographic separation of the isolated total extractive compounds on a silica gel column, a colorless crystalline substance of composition $C_{15}H_{20}O_5$, m.p. 175.5–177.5°C (from ethanol), M^+ 280 (mass spectrometry), was isolated. On the basis of the investigation of the physicochemical properties of the isolated substance and the comparison of the obtained constants and spectral data with the literature (Shevchenko et al., 2025), it was concluded that this compound differs from sesquiterpene lactones previously isolated from the genus *Achillea*. It proved to be a new sesquiterpene lactone and was named anobin (7).

Anobin (7) is soluble in alkalis on heating, and on acidification of the alkaline solution it precipitates out. The IR spectrum shows absorption bands characteristic of a hydroxyl group (3500 cm^{-1}), the γ-lactone carbonyl (1700 cm^{-1}), and a double bond (1660 cm^{-1}). The hydroxyl groups of anobin (7) are not acetylated by acetic anhydride in pyridine and are not oxidized by chromic anhydride, i.e. they are tertiary. In the ^{1}H NMR spectrum of anobin (7), the following signals are observed: singlets at 1.16 and 1.56 ppm (3H each) for tertiary methyls; weakly split doublets at 3.23 and 3.44 ppm (1H each, J = 2 Hz) for protons of the epoxy group; a multiplet at 3.93 ppm (1H) for H-7; a doublet of doublets at 4.38 ppm (1H, J = 12.4 and 9.5 Hz) for the lactone proton; a singlet at 4.93 ppm (2H) for the protons of the hydroxyl groups; and doublets at 5.28 and 6.10 ppm (1H each, J = 2.4 Hz) for the protons of the exomethylene group conjugated with the carbonyl. The doublet at 2.73 ppm (1H, J = 12.4 Hz) was assigned to H-5.

The fact that on dehydrogenation of anobin (7) chamazulene is formed, together with the spin–spin coupling constant of the protons of the exomethylene group of the lactone ring in the ^{1}H NMR spectrum, leads to the conclusion that anobin (7) has either a guaiane or a germacrane skeleton. Integration of the signals of the ^{1}H NMR spectrum reveals 20 hydrogen atoms. With this number of protons and the presence of an epoxide ring and two tertiary hydroxyl groups, a monocyclic (germacrane) structure is excluded for the compound under study. It can therefore be unambiguously stated that anobin (7) has a guaiane carbon skeleton.

The splitting pattern of the lactone proton signal in the ^{1}H NMR spectrum indicates that the lactone ring is fused at C6–C7, while the large coupling constant of the H-6 and H-5 protons (J = 12.4 Hz) indicates their mutual *trans*-disposition. Comparison with the ^{1}H NMR spectrum of the structurally related guaianolide canin (9) allows an α-orientation, rela-

tive to the main carbon skeleton, to be proposed for the epoxide ring and the hydroxyl groups of anobin (7).

The intensity of the molecular ion peak of anobin (7) at *m/z* 280 is low compared with the principal peaks in the mass spectrum, which is evidently due to the relative instability of the guaiane skeleton, enhanced by the presence of epoxy and hydroxyl groups. The fragments at *m/z* 216, 246, 250, and 262 reflect the initial fragmentation of the molecular ion through sequential elimination of methyls and hydroxyls. Loss of the neutral fragment CH_3OH gives the ion at *m/z* 216 and is followed by cleavage of rings A and B, which is supported by the ion at *m/z* 123. An alternative pathway leading to the ion at *m/z* 123 is the elimination from M^+ of an H_2O molecule with participation of the hydroxyl at C-4 and localization of the charge on the oxygen of the 2,3-epoxy group, which initiates cleavage of rings B and C. This process is enhanced by the presence of the OH group at C-10. The peak at *m/z* 250 can be explained because of simultaneous loss of methyls geminal to the hydroxyl groups, leading further to the ion at *m/z* 97. Simultaneous loss of OH groups from M^+ gives rise to the fragment at *m/z* 95.

Thus, on the basis of physicochemical constants, spectral data (IR, ^{1}H NMR, MS), and chemical transformations, it was concluded that anobin (7) has the structure and configuration of 2α,3α-epoxy-4α,10α-dihydroxy-5,7α(H),8β(H)-guai-11(13)-en-6,12-olide.

On further elution of the column, after the isolation of anobin (7), with benzene, with benzene–diethyl ether mixtures (4:1, 1:1), and with diethyl ether, four crystalline substances were obtained, three of which were identified as anobin (7), the guaiane-type sesquiterpene lactone estafiatin (10), and the germacranolide hanphyllin (12). Estafiatin (10) and hanphyllin (12) were isolated from noble yarrow for the first time.

A colorless crystalline substance of composition $C_{15}H_{20}O_4$, m.p. 167–169°C (from ethanol), $[\alpha]_D^{20}$ +52.9° (c 0.017; ethanol), proved to be a new sesqui-terpene lactone and was named anolide (11).

The IR spectrum of (11) shows absorption bands at 3550 (OH group), 1740 (γ-lactone carbonyl conjugated with an exomethylene group), 1670, and 1640 cm^{-1} (C=C). The UV spectrum displays a maximum at 204 nm (ε 18 521), characteristic of an exocyclic methylene in conjugation with the carbonyl of a γ-lactone ring.

The ^{13}C NMR spectra of (11) recorded under fully and partially decoupled ("off-resonance") conditions show signals of 5 sp^2-hybridized and 10 sp^3-hybridized carbon atoms, which is consistent with a guaiane carbon skeleton for anolide (11).

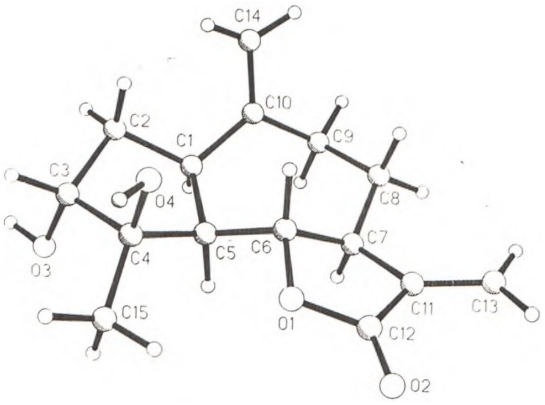
The ^{1}H NMR spectrum of (11) shows the following proton signals: a methyl group geminal to the hydroxyl group – singlet at 1.49 ppm (3H); hydroxyl protons – singlet at 2.08 ppm (2H); a gem-hydroxyl proton – doublet at 3.92 ppm (1H, J = 3.5 Hz); the lactone proton – doublet of doublets at 4.23 ppm (1H, J = 11.5 and 9.5 Hz); methylene protons – doublet at 4.92 ppm (2H, J = 3.5 Hz); and protons of the exocyclic methylene group conjugated with the γ-lactone carbonyl – doublets at 5.47 and 6.17 ppm (1H each, J = 3.5 Hz).

The multiplicity of the lactone proton signal indicates that the lactone ring is fused at the C6–C7 position, while the magnitude of J indicates its *trans*-junction relative to the main carbon skeleton.

Acetylation of the substance afforded a monoacetate of composition $C_{17}H_{22}O_5$, m.p. 182–185°C. The IR spectrum shows absorption bands at 3460 (OH group), 1740 (CO of γ-lactone), 1250 (ester group), 1665, and 1640 cm^{-1} (C=C). The ^{1}H NMR spectrum of the acetyl derivative of anolide shows signals of: a methyl group geminal to the hydroxyl group – singlet at 1.40 ppm (3H); a methyl group of the acetyl moiety – singlet at 2.02 ppm (3H); the lactone proton – triplet at 4.24 ppm (1H, J = 10 Hz); a gem-acyl proton – a signal centered at 4.95 ppm; and protons of the exocyclic methylene group conjugated with the lactone carbonyl – doublets at 5.46 and 6.17 ppm (1H each, J = 3.5 Hz). The acetylation results show that the structure of anolide (11) contains two hydroxyl groups: one secondary and the other tertiary, the latter being geminal to one of the methyl groups.

To establish the positions of the hydroxyl groups in the structure of anolide (11), as well as the relative configuration, an X-ray diffraction study of its molecule was carried out (Figure 3). It was established that ring A adopts an envelope conformation (see Figure 3). Atoms C-1, C-2, C-4, and C-5 are coplanar within 0.01 Å (torsion angle C-4, C-5, C-1, C-2 = 1.98°). Atom C-3 lies 0.09 Å below this plane. Ring B has a chair conformation. Atoms C-6, C-7, C-9, and C-10 are coplanar within 0.007 Å. Atoms C-1, C-5, and C-8 are displaced from the plane by 1.11–1.18 and 0.68 Å, respectively. The lactone ring is in an envelope conformation. Atoms C-6, C-11, and C-12 are coplanar within 0.03 Å. Atom C-7 is located 0.48 Å below the plane, while atoms C-13 and C-2 are 0.37 and 0.11 Å above it, respectively. Rings A and B are *cis*-fused, and rings B and C are *trans*-fused.

Figure 3
The structure of anolide (11) molecule



Thus, on the basis of the studies performed, it has been proved that anolide (11) has the structure and relative configuration of 3α,4β-dihydroxy-1,5,7α(H),6β(H)-guai-10(14),11(13)-dien-6,12-olide.

It was established that the quantitative yield of sesquiterpene lactones from the aerial part of *Achillea nobilis* L. is provided by aqueous extraction of the raw material: the yield of anobin (7) is 0.008% based on air-dry raw material, of estafiatin (10) – 0.01%, of hanphyllin (12) – 0.007%, and of anolide (11) – 0.01%. Whereas anobin (7) is isolated from the raw material of *Achillea nobilis* L. by both aqueous and chloroform extraction, anolide (11) is recovered only by aqueous extraction followed by chromatographic separation of the total substances on a silica gel column (Astafyeva et al., 2018).

In studying the dynamics of localization of sesquiterpene lactones in individual organs of *Achillea nobilis* L., it was established that estafiatin (10) and hanphyllin (12) are invariable components in all organs (Table 3).

Table 3
Content of sesquiterpene lactones in organs of Achillea nobilis L.

Vegetation phase	Plant organ	Raw material, g	Total extractive substances, g	Sesquiterpene lactones	Content, g	Content, % of air-dried raw material
Rosette	Leaves	2100	107.0	Estafiatin (10)	2.21	0.11
				Hanphyllin (12)	0.89	0.04
Budding	Leaves	1500	85.0	Estafiatin (10)	3.61	0.24
				Hanphyllin (12)	0.22	0.01
				Anobin (7)	0.16	0.01
	Buds	2000	81.3	Estafiatin (10)	1.76	0.09
				Hanphyllin (12)	0.08	0.01
				Anolide (11)	0.23	0.01
				Anobin (7)	0.18	0.01
Flowering	Leaves	500	42.0	Estafiatin (10)	0.72	0.14
				Anobin (7)	0.04	0.01
	Flower heads	2000	47.0	Anolide (11)	0.52	0.03

On chromatographic separation of the total substances from plants harvested in the juvenile state, on elution of the column with a benzene–diethyl ether mixture (9:1), were isolated colorless needle-like crystals of composition $C_{15}H_{18}O_3$, m.p. 102–104°C (from diethyl ether), identified as estafiatin (10). Its yield was 0.11% of the air-dry raw material weight (see Table 3). On elution of the column with a benzene – diethyl ether mixture (1:1), colorless plate-like crystals were obtained with R_f 0.76 (diethyl ether). After recrystallization from ethanol was obtained a crystalline substance of composition $C_{15}H_{20}O_3$, m.p. 190 °C (decomposition), identified as hanphyllin (12). Its yield was 0.04% of the air-dry raw material weight (see Table 3).

On chromatographic separation of the total extractive substances from leaves at the budding phase, from the fraction obtained on elution of the column

with a hexane–benzene mixture (1:1), estafiatin (10) was isolated in a yield of 0.24%. On elution of the column with benzene, hanphyllin (12) was isolated in a yield of 0.01%, and on further elution with a benzene–diethyl ether mixture (9:1), the new sesquiterpene lactone anobin (7) was obtained. Its yield was 0.011% of the air-dry raw material weight (see Table 3).

In the buds, the content of estafiatin (10) was 0.09%, of hanphyllin (12) – 0.004%, and of anobin (7) – 0.009%. On elution of the column with a benzene–diethyl ether mixture (1:1), plate-like crystals with R_f 0.53 precipitated. After recrystallization from ethanol, a colorless crystalline substance of composition $C_{15}H_{20}O_4$, m.p. 167–169 °C, was obtained, which was identified as the new sesquiterpene lactone anolide (11). The yield of anolide (11) was 0.01% based on air-dry raw material (see Table 3).

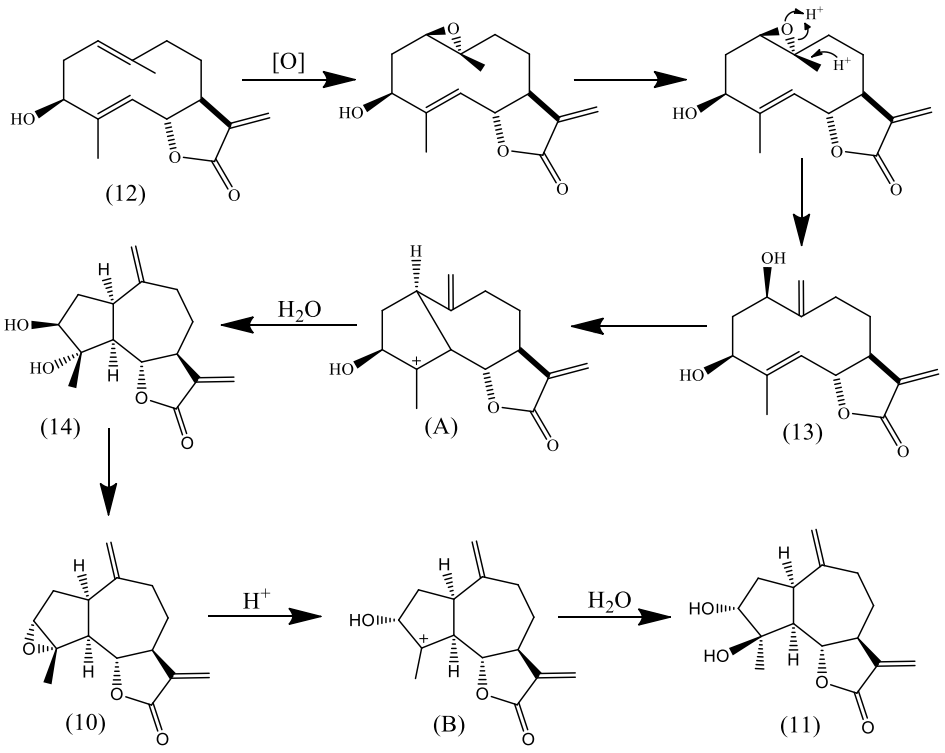
From the leaf extract at the flowering phase, on elution of the column with a benzene–diethyl ether mixture (9:1), estafiatin (10) (0.14%) was isolated, and on further elution of the column with a benzene–diethyl ether mixture (1:1), anobin (7) was isolated in a yield of 0.008% (see Table 3).

On chromatographic separation of the total extractive substances from the flower heads, crystals of anolide (11) were isolated in a yield of 0.03% (see Table 3).

The biosynthesis of the new sesquiterpene lactones anobin (7) and anolide (11) takes place at the budding and flowering phases of the plant. The data obtained on the localization of sesquiterpene lactones in noble yarrow, together with their correlation with literature data (Álvarez-Calero et al., 2021), allows a possible pathway to be proposed for the formation of the guaianolides estafiatin (10) and anolide (11) through epoxidation and successive cyclization of the germacranolide hanphyllin (12).

Since the hanphyllin (12) molecule belongs structurally to the *trans,trans*-germacradienolides, its epoxidation gives the 1β,10α-epoxy derivative; subsequent opening of the oxirane ring in this structure leads to ridentin (13). Nucleophilic attack of the 4,5-double bond at C-1 in molecule (13) brings about a 1,5-cyclization and gives rise to cation (A); hydroxylation at C-4 yields the 3β,4α-dihydroxyguaianolide (14), the subsequent dehydration of which, with elimination of C3–OH, gives the estafiatin (10) molecule. Opening of the α-epoxide ring in this molecule produces cation (B), and hydroxylation of the C-4 cationic center leads to the formation of anolide (11).

Figure 4
Proposed biosynthetic pathway of sesquiterpene lactones of Achillea nobilis L.



It is known that the biosynthesis of terpenoids proceeds via two different routes: the plastid non-mevalonate pathway, also called the methylerythritol-4-phosphate (MEP) pathway, which is responsible for the formation of monoterpenes, diterpenes, and tetraterpenes, and the cytosolic mevalonate (MVA) pathway, which mainly drives the biosynthesis of sesquiterpenes, triterpenes, and polyprenols. In both pathways, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are synthesized; these are considered universal precursors in the biosynthesis of all terpenoids. Expression analysis of the genes involved in terpenoid biosynthesis in *Achillea millefolium* L. has shown that the DXR, LIS, PAL, and CHS genes are highly expressed specifically in glandular trichomes, whereas the expression of GPPS is ubiquitous (Fathi et al., 2019).

In plants, sesquiterpenes are formed with the participation of sesquiterpene synthases and perform a variety of ecological functions in higher plants, being localized in flowers, leaves, rhizomes, and roots. In a number of species of the family Asteraceae, germacrenes play an important role in the biosynthesis of various sesquiterpene derivatives, particularly sesquiterpene lactones. The key cytosolic enzyme in the biosynthesis of sesquiterpene lactones in *Achillea millefolium* L. is germacrene-A synthase (GAS) (Pazouki et al., 2015).

Analysis of the available literature data (Kupriyanov et al., 2023; Li et al., 2023c; Strzępek-Gomółka et al., 2021) indicates that *Achillea millefolium* L. contains sesquiterpene lactones with unusual structures, namely 5 dilactones – millefolactones A–E; 3 sesquiterpene lactones of the psilostachyin type bearing, in addition to the γ-lactone ring, a δ-lactone fragment – paulitin, isopaulitin, and psilostachyin C; 9 halogen-containing guaianolides – millefolactones B_1–B_9; and a guaianolide with a peroxide bridge – α-peroxyachifolide – which is connected with the specific features of sesquiterpenoid biosynthesis in this species.

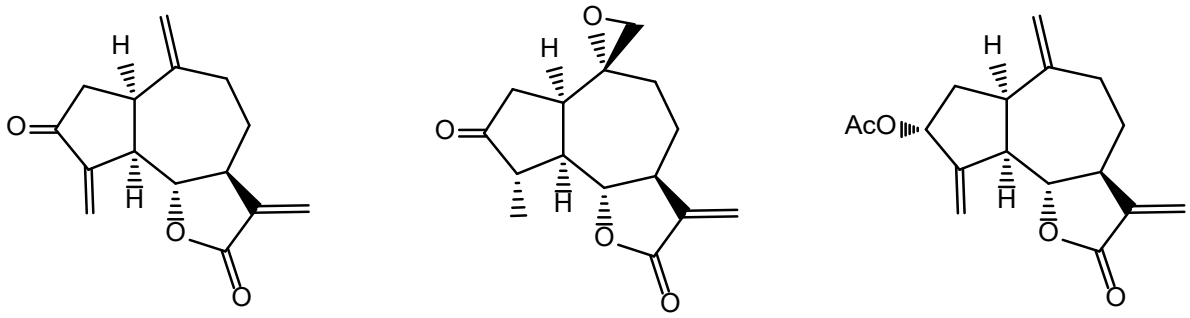
Thus, as a result of the chemical study of 13 species of *Achillea* L. growing on the territory of Kazakhstan have been isolated 11 sesquiterpene lactones belonging to the guaianolides and germacranolides (Adekenov et al., 1984; Barda et al., 2021; Dracinsky et al., 2012). New sesquiterpene lactones – achimicrin (5), anobin (7), and anolide (11) – have been isolated and studied. The following compounds have been identified for the first time: from *A. millefolium* L. – 8-hydroxyachillin (grossmisin) (3); from *A. micrantha* Willd. – achillin (1), artilesin (2), and grossmisin (3); from *A. nobilis* L. – estafiatin (10) and hanphyllin (12).

Biological activity of sesquiterpene lactones isolated from *Achillea* L. plants

Sesquiterpene lactones from plants of the genus *Achillea* L. exhibit a wide spectrum of biological activity (Nemeth & Bernath, 2008).

Among the derivatives of estafiatin (10) isolated from *Achillea nobilis* L., comparatively high antitumor activity is exhibited by 3-keto-4-methylene-estafiatin (15), 3-keto-10(14)-epoxy-estafiatin (16), and 3-aceto-4-methylene-estafiatin (17), which inhibit the growth of Pliss lymphosarcoma, Walker carcinosarcoma, sarcoma 45, alveolar liver cancer PC-1, and sarcoma 180 by 68–90%, and of P-388 lymphocytic leukemia and lymphoid leukemia (ILS by 62–104%) (Adekenov, 2022).

Figure 5
The derivatives of estafiatin (10) isolated from Achillea nobilis L. (3-keto-4-methylene-estafiatin (15), 3-keto-10(14)-epoxy-estafiatin (16), and 3-aceto-4-methylene-estafiatin (17)



(15) (16) (17)

Among the germacranolides studied for antitumor activity, comparatively high activity is exhibited by hanphyllin (12) isolated from *Achillea nobilis* L. This germacranolide inhibits the growth of transplantable tumor strains – Guerin carcinoma, Pliss lymphosarcoma, alveolar liver cancer, and Pliss lymphosarcoma resistant to rubomycin (70–89% inhibition). In comparison with the analogue with the same indication – colchamine, a preparation of plant origin – the substances under study proved to be of low toxicity: the maximum tolerated dose (MTD) in chronic experiments is 20–70 mg/kg versus 1.0 mg/kg for colchamine (Sülsen & Martino, 2018). In-depth study of the antitumor activity of 3-keto-4-methylene-estafiatin (15) and 3-keto-10(14)-epoxy-estafiatin (16) is recommended. From *Achillea nobilis* L., the guaianolides anobin (7), exhibiting antitumor activity, and anolide (11), exhibiting acaricidal and insecticidal action, have also been isolated (Adekenov et al., 1984).

In the investigation of the antiparasitic activity of sesquiterpene lactones of plants of the genus *Achillea* L. against bloodstream trypomastigotes of *Trypanosoma brucei brucei*, amastigotes of *Trypanosoma cruzi*, and promastigotes of *Leishmania amazonensis*, a comparatively high antiparasitic activity has been established for estafiatin (10) and anabasinyl-estafiatin (20).

Table 4
Activity of Achillea L. sesquiterpene lactones against Trypanosoma brucei brucei bloodstream trypomastigotes

Compound	Inhibition, % ± SD		
	10 μg/mL	1 μg/mL	0.1 μg/mL
Achillin (1)	27.45 ± 11.02	1.47 ± 0.01	NI
Grossmisin (3)	1.47 ± 0.55	NI	NI
Estafiatin (10)	99.24 ± 0.18	72.91 ± 1.83	44.33 ± 1.83
Leucomisin (18)	4.71 ± 6.20	2.77 ± 1.83	2.77 ± 0.02
Cytisinyl-estafiatin (19)	4.77 ± 3.67	NI	NI
Anabasinyl-estafiatin (20)	99.31 ± 0.52	5.36 ± 1.83	NI
Suramin	99.74 ± 0.19	99.2 ± 0.31	29.4 ± 1.83
Note: NI – No Inhibition			

Figure 6
The derivatives of estafiatin (10) isolated from Achillea L. (leucomisin (18), cytisinyl-estafiatin (19), anabasinyl-estafiatin (20)

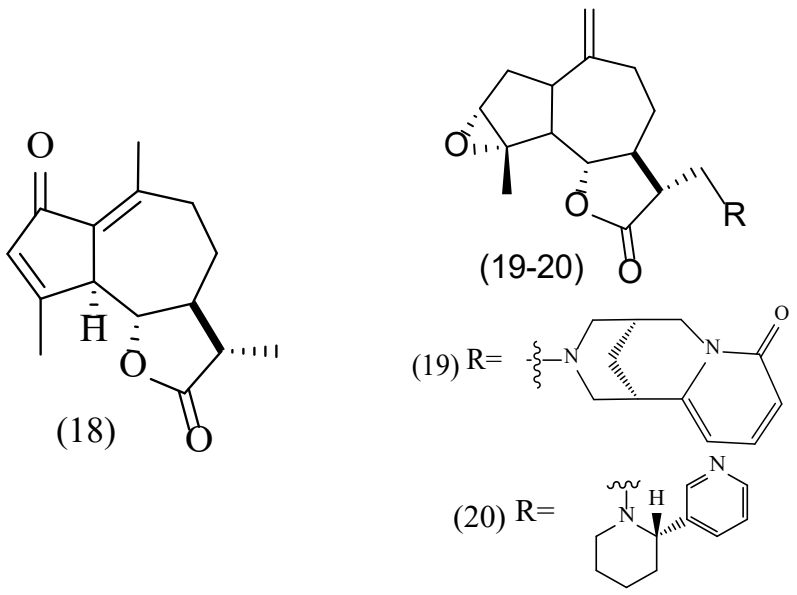


Table 5
Antiamastigote activity of Achillea L. sesquiterpene lactones against Trypanosoma cruzi at 10 μg/mL

Compound	Inhibition, %
Achillin (1)	13.63 ± 0.21
Grossmisin (3)	5.22 ± 5.35
Estafiatin (10)	97.24 ± 0.21
Leucomisin (18)	15.37 ± 3.18
Cytisinyl-estafiatin (19)	26.83 ± 0.17
Anabasinyl-estafiatin (20)	99.06 ± 0.31
Suramin	99.76 ± 0.18

Considering the comparatively high activity of *Achillea* L. sesquiterpene lactones at 10 μg/mL against *Trypanosoma cruzi* and *Trypanosoma brucei brucei*, we determined their cytotoxicity at the same concentration (Table 6).

It is important to note the comparatively high percentage of viable cells observed for the anabasinyl derivative of estafiatin (20), in comparison with the parent estafiatin (10), which indicates a decrease in the cytotoxicity of the sesquiterpene lactones as a result of Michael-type amination at position C-13.

Table 6
Cytotoxicity of Achillea L. sesquiterpene lactones against Vero cells at 10 μg/mL

Compound	Cell viability, %
Estafiatin (10)	3.15 ± 5.46
Anabasinyl-estafiatin (20)	25.72 ± 0.13
Suramin	99.2 ± 0.55

Table 7
Activity of Achillea L. sesquiterpene lactones against Leishmania amazonensis promastigotes

Compound	Inhibition, % ± SD		
	50 μg/mL	10 μg/mL	1 μg/mL
Achillin (1)	35.14 ± 7.90	30.51 ± 14.10	25.95 ± 4.76
Grossmisin (3)	40.49 ± 8.33	34.92 ± 0.03	31.84 ± 0.14
Estafiatin (10)	98.68 ± 0.28	100 ± 0.05	100 ± 0.91
Leucomisin (18)	17.87 ± 4.54	11.80 ± 4.28	8.09 ± 7.13
Cytisinyl-estafiatin (19)	22.80 ± 9.07	13.31 ± 0.01	13.52 ± 4.45
Anabasinyl-estafiatin (20)	100 ± 0.63	100 ± 1.20	17.39 ± 7.34
Amphotericin B	100.0± 0.03	100.0± 0.012	98.1± 0.53

Based on the experimental data presented above, it has been determined that Michael-type amination of estafiatin (10) at the C11=C13 exomethylene group leads to a decrease in the antiparasitic activity of its derivatives cytisinyl-estafiatin (19) and anabasinyl-estafiatin (20) against *Trypanosoma brucei brucei* and promastigotes of *Leishmania amazonensis*, as well as to a reduction in their cytotoxicity.

The guaianolide leucomisin (18) was isolated from the aerial part of *Achillea collina* Becker, *Achillea filipendulina* Lam., *Achillea millefolium* L., *Achillea santolina* L., and *Achillea sibirica* Ledeb. (Abdel-Baki et al., 2025; Glasl et al., 2002; Kaneko et al., 1971; Trendafilova et al., 2006) and determined on dogs infested with the helminths *Trichuris vulpis* and *Dipylidium caninum*. Based on the coproovoscopy results, the helminth-infested dogs (10 dogs) were divided into two experimental groups, 5 animals each. Dogs of the first group (5 animals) were administered capsules with leucomisin (18) at a dose of 150 mg (3 capsules) per 20–25 kg of body weight. In the comparison group, dogs received the preparation "CesTremForte" at a dose of 150 mg (2 tablets) per 20–25 kg of body weight. The capsules with leucomisin (18) were administered as a single dose. Coproovoscopy of each fecal sample before and after deworming of the dogs was carried out twice. Four days after administration of the capsules with leucomisin (18), coproovoscopy of the dogs' feces was performed to determine the extens- and intens-efficacy of the test sample (18). In all groups of animals, no side effects from the application of the test sample (18) were observed. As a result of the trials, no helminth eggs were detected in the dogs dewormed with capsules of leucomisin (18). Thus, the extens- and intens-efficacy of the capsules with leucomisin (18) is 100%

against the helminths *Trichuris vulpis* and *Dipylidium caninum*. The extens-efficacy and intens-efficacy of the comparison preparation "CesTremForte" are 80% and 83.33%, respectively. According to the results of the experiments, it was established that the extens-efficacy and intens-efficacy of capsules with leucomisin (18) are higher than those of the comparison preparation "CesTremForte" by 20% and 16.6%, respectively.

With a view to enhancing the water solubility of leucomisin (18) and, accordingly, the bioavailability of the pharmacologically active molecule in the body, a solid-phase synthesis was carried out on the basis of leucomisin (18) with disodium glycyrrhizinate (Adekenov et al., 2026).

Conclusion

Plants of the genus *Achillea* L. can be regarded as one of the promising taxa of the family Asteraceae as a renewable source of sesquiterpene lactones exhibiting antiparasitic and antitumor activity.

Based on the results of chemical screening of 13 species of plants of *Achillea* L. collected on the territory of Kazakhstan, the content of sesquiterpene lactones was confirmed in 9 (69.23%) of them. In the plants of section Millefolium (Mill.) Koch, the content of sesquiterpene lactones was determined in 5 species (83.3%); in plants of section Micranthae Klok. & Krytzka, in 2 species (100%); and in plants of section Ptarmica (Mill.) Koch, in 1 species (33.3%). Three species of the genus *Achillea* L. – *Achillea inundata* Kondr., *Achillea salicifolia* Besser, and *Achillea tianschanica* Kupr. & Kulemin – have been identified as promising, previously uninvestigated objects for the isolation and identification of sesquiterpene lactones.

The presence in *Achillea millefolium* L. of sesquiterpene lactones with unusual structures, namely 5 dilactones – millefolactones A–E; 3 sesquiterpene lactones of the psilostachyin type bearing, in addition to the γ-lactone ring, a δ-lactone fragment – paulitin, isopaulitin, and psilostachyin C; 9 halogen-containing guaianolides – millefolactones B_1–B_9; and a guaianolide with a peroxide bridge – α-peroxyachifolide – is connected with the specific features of the biosynthetic pathway in this species, the key cytosolic enzyme of which is germacrene-A synthase (GAS).

In Kazakhstan, the industrial reserves of the raw material of *Achillea nobilis* L., a source of the pharmacologically active estafiatin and hanphyllin, have been determined.

From *Achillea nobilis* L. and *Achillea micrantha* Willd. we have isolated 11 sesquiterpene lactones, including 3 new compounds. We have determined the physicochemical characteristics of the isolated sesquiterpene lactones and established their structures by IR, UV, ^{1}H NMR, and ^{13}C NMR spectroscopy.

The biosynthesis of sesquiterpene γ-lactones in *Achillea nobilis* L. is observed in flower heads, buds, and leaves. Their biosynthesis is carried out by specialized chloroplasts in the bracts of the buds and flower heads of the plant.

In the study of the dynamics of localization of sesquiterpene lactones in the leaves, buds, and flower heads of *Achillea nobilis* L., it has been established that estafiatin can be regarded as the main marker component for this species, since it is biosynthesized throughout almost the entire vegetation cycle, and quantitatively its content based on air-dry raw material was determined in the leaves of *Achillea nobilis* L. collected at the budding phase as 0.24%. The biosynthesis of anobin and anolide is closely linked to the development of the reproductive organs (buds and flower heads) of *Achillea nobilis* L., whereas hanphyllin is characteristic of the early developmental stages of the plant (rosette).

The quantitative yield of sesquiterpene lactones from the raw material of *Achillea micrantha* Willd. and *Achillea nobilis* L. is achieved by the methods of aqueous and supercritical fluid carbon dioxide extraction. By contrast, on chloroform extraction from the aerial part of *Achillea nobilis* L., the guaianolide anolide is not isolated, while estafiatin and hanphyllin have practically low yields and anobin is detected only in trace amounts, which indicates the comparatively low efficiency of this isolation method.

Based on the results of an *in vitro* study of sesquiterpene lactones of plants of the genus *Achillea* L., comparatively high antiparasitic activity has been determined for estafiatin and anabasinyl-estafiatin against bloodstream trypomastigotes of *Trypanosoma brucei brucei*, amastigotes of *Trypanosoma cruzi*, and promastigotes of *Leishmania amazonensis*. It has been established that Michael-type amination of estafiatin at the C11=C13 exomethylene group leads to a reduction of the antiparasitic activity of anabasinyl-estafiatin by approximately 6–13 times, and also reduces its cytotoxicity towards Vero cells by approximately 8 times.

Leucomisin, detected in 5 species of *Achillea* L., exhibits 100% extens- and intens-efficacy against the helminths *Trichuris vulpis* and *Dipylidium caninum*.

Based on leucomisin and disodium glycyrrhizinate, a solid dispersion has been synthesized whose water solubility is increased 18-fold, with a corresponding increase in the bioavailability of the pharmacologically active substance.

Estafiatin from *Achillea nobilis* L. is a renewable starting material for the synthesis of new biologically active compounds, including the compounds 3-keto-4-methylene-estafiatin and 3-keto-10(14)-epoxy-estafiatin, which possess potential antitumor activity.

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Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

Appendix A. Supplementary data

Detailed data on the extraction of raw materials and the isolation of individual compounds are provided in the Supplementary material.

Author contributions

Sergazy M. Adekenov: Conceptualization, Methodology, Project Administration, Writing – Review & Editing

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Aylina B. Syzdykova: Investigation, Writing – Original Draft, Formal Analysis

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Appendix A. Supplementary data

Extraction of raw materials and the isolation of individual sesquiterpene lactones

Sesquiterpene lactones of *Achillea micrantha* Willd. 30 kg of air-dried, ground flower heads and leaves of Achillea micrantha Willd., collected along the channel of the Sary-Su River in the Ulytau region, were infused four times with hot water (1 h; 80–85°C). The aqueous extract was extracted three times with chloroform at a ratio of 10:1. After evaporation of chloroform, 174 g of a syrupy mass of cream color was obtained, which was chromatographed on a column with silica gel of KSK grade at a ratio of total–carrier = 1:24. The column was eluted with benzene, a mixture of benzene–diethyl ether (4:1, 1:1), diethyl ether, a mixture of diethyl ether–ethyl acetate (4:1, 1:1), and ethyl acetate. A total of 220 fractions of 250–300 mL each were collected.

Upon elution of the column with a benzene–ether system (ratio 4:1), a crystalline mass precipitated, after threefold recrystallization of which from ethyl acetate 15.03 g of a colorless crystalline substance of composition $C_{15}H_{18}O_3$ with m.p. 144–145.5°C, $[\alpha]_D^{24}$ +160° (c 1.20; chloroform) was obtained. R_f 0.47 (benzene–ethanol system, 9:1). Yield 0.05% based on air-dried raw material.

IR spectrum (KBr, ν/cm^{-1}): 1776 (C=O γ-lactone), 1681 (C=O), 1634 and 1617 (C=C).

UV spectrum (EtOH), λmax/nm: 256±2 nm (cyclopentadienone).

^{1}H NMR spectrum (CD3OD+ $CDCl_3$,δ, ppm, J/Hz): 1.01 (3H, d, H-13), 1.29 (1H, dd, J8β,9β=2.5; J8β,9α =11.6 Hz, H-8β), 1.73 (1H, m., J8α,8β=13.7; J8α,9α=2.0 Hz, H-8α), 2.15 (3H, s, H-15), 2.17 (1H, d, J9α,9β=14.5; J8β,9β=2.5 Hz, H-9β), 2.19 (1H, ddd, J9α,9β=14.5 Hz, H-9α), 2.28 (3H, s, H-14), 2.33 (1H, m., J6,7=10.2; J7,11=7.2 Hz, H-7), 2.56 (1H, k, J7,11=12.2 Hz, H-11), 3.3 (1H, d, J5,6 =10.2 Hz, H-5), 3,68 (1H, t, J6,7 =10.2 Hz, H-6), 6.03 (1H, d., H-3).

^{13}C NMR spectrum (CD3OD+CDCl3, ppm): 9.5 (C-13), 19.3 (C-15), 21.0 (C-14), 23.1 (C-8), 37.1 (C-9), 38.8 (C-11), 51.3 (C-7), 52.4 (C-5), 83.9 (C-6), 131.3 (C-1), 134.9 (C-3), 151.8 (C-10), 169.7 (C-4), 178.0 (C-12), 195.3 (C-2).

According to physicochemical constants and spectral data (IR, ^{1}H NMR), the isolated compound (1) was identical to the guaianolide achillin.

Upon elution of the column with a benzene–diethyl ether system (ratio 1:1), colorless rhombic crystals precipitated, after recrystallization of which from ethyl acetate 0.9 g of a crystalline substance of composition $C_{17}H_{20}O_5$ with m.p. 183–185°C, $[\alpha]_D^{25}$ +18.8° (chloroform) was obtained. Yield 0.003% based on air-dried raw material.

Comparison of physicochemical constants and spectral data allowed identification of compound (2) as the sesquiterpene lactone artilesin.

Upon elution of the column with a benzene–diethyl ether system (ratio 1:1), a precipitate formed, after recrystallization of which from ethyl acetate 1.0 g of a colorless crystalline substance of composition $C_{19}H_{26}O_7$ with m.p. 203–205°C, Rf 0.40 (benzene–ethanol system, 9:1) was obtained. Yield 0.003% of the weight of air-dried raw material.

IR spectrum (KBr, ν_{max}, cm^{-1}): 1767 (C=O, lactone), 1732 (C=O, ester).

MS 70 eV m/z (rel. int.): 367.1757 (calcd for $C_{19}H_{27}O_7$ 367.1757 $[M^+H]^+$, 3), 366.1679 (calcd for $C_{19}H_{26}O_7$ 366.1679 $[M]^+$) (2), 308 (18), 307 (100), 264 (27), 247 (20), 139 (26), 95 (38), 43 (100).

^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.817 (1H, dd, J=11.4, 2.2 Hz, H-1), 2.416 (1H, ddd, J=-13.1, 5.7, 2.2 Hz, H-2α), 1.540 (1H, ddd, J=-13.1, 11.4, 11.3 Hz, H-2β), 5.440 (1H, dd, J=11.3, 5.7 Hz, H-3), 5.325 (1H, d, J=10.0 Hz, H-5), 4.820 (1H, dd, J=10.0, 10.0 Hz, H-6), 2.368 (1H, dddd, J=10.9, 10.0, 7.8, 1.4 Hz, H-7), 1.817 (1H, ddd, J=-15.7, 2.3, 1.4 Hz, H-8α), 1.740 (1H, ddd, J=-15.7, 10.9, 10.9 Hz, H-8β), 4.300 (1H, dd, J=10.9, 2.3 Hz, H-9), 2.735 (1H, q, J=7.8 Hz, H-11), 1.208 (3H, d, J=7.8 Hz, C13-Me), 1.252 (3H, s, C14-Me), 1.822 (3H, d, J=1.4 Hz, C15-Me), 2.097 (3H, s, OAc), 2.089 (3H, s, OAc).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 62.4 (C-1), 28.6 (C-2), 75.2 (C-3), 140.9 (C-4), 123.0 (C-5), 78.5 (C-6), 45.4 (C-7), 30.3 (C-8), 80.1 (C-9), 61.1 (C-10), 40.2 (C-11), 178.2 (C-12), 10.5 (C-13), 12.6 (C-14), 12.5 (C-15), 169.9, 169.6 (2C, OCOMe), 21.1, 20.8 (2C, OCOMe).

According to physicochemical and spectral data (IR, ^{1}H NMR, ^{13}C NMR), the isolated compound (3) was identified as the germacranolide micranthin.

From the ether fractions, a colorless crystalline substance of composition $C_{19}H_{26}O_6$, m.p. 220–222°C (from a mixture of diethyl ether–chloroform), $[\alpha]_D^{22}$ +15.5° (c 0.42; chloroform), M^+ 350, was isolated.

IR spectrum: 1770 (carbonyl of γ-lactone), 1725, 1250 (ester group), 1660 (double bond), 1300, 980, 920 cm^{-1}

^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.24 (3H, d, J=7.7 Hz, C11-Me), 1.50 (3H, br. s, C10-Me), 1.72 (3H, br. s, C4-Me), 1.84–1.89 (2H, m, H-8), 2.03 (3H, s, CH_3COO), 2.10

(3H, s, CH_3COO), 2.28 (1H, ddd, J=12.0, 12.0, 10.5 Hz, H-2), 2.3 (1H, m, H-7), 2.56 (1H, br. ddd, J=12.0, 5.6, 4.7 Hz, H-2), 2.73 (1H, q, J=7.7, H-11 Hz), 4.78 (1H, d, J=9.5 Hz, H-5), 4.92 (1H, t, J=9.5 Hz, H-6), 5.12 (1H, dd, J=10.5, 2.7 Hz, H-9), 5.18 (1H, dd, J=10.5, 5.6 Hz, H-3), 5.24 (1H, ddq, J=12.0, 4.7, 1.0 Hz, H-1).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 10.80 (q, C-13), 11.51 (q, C-14), 12.21 (q, C-15), 20.83 (q, $\underline{CH_3}COO$), 21.06 (q, $\underline{CH_3}COO$), 31.07 (t, C-2), 31.26 (t, C-8), 40.30 (d, C-11),45.67 (d, C-7), 78.40 (d, C-6), 79.26 (d, C-9), 80.51 (d, C-3), 125.56 (d, C-5), 127.78 (d, C-1), 136.33 (s, C-4), 137.25 (s, C-10), 169.63 (s, CH3$\underline{COO}$), 169.69 (s, $CH_3\underline{COO}$), 178.52 (s, C-12).

Comparison of physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR) allowed proposing for compound (4) the structure 11R-3β,9β-diacetoxy-1,5-trans,trans-6β,7α(H)-germacr-1(10),4(5)-dien-6,12-olide, which proved to be a new sesquiterpene γ-lactone and was named achimicrin.

Upon elution of the column with an ether–ethyl acetate system (ratio 4:1), plate-like crystals of composition $C_{15}H_{18}O_4 \cdot H_2O$ with m.p. 156–156.5°C, $[\alpha]_D^{20}$ +15.26° (c 1.2; ethanol), Rf 0.17 (benzene–ethanol system, 9:1) were isolated. Yield 0.005% based on air-dried raw material.

According to IR, ^{1}H NMR, and ^{13}C NMR spectral data, the isolated compound (5) is identical to the sesquiterpene lactone grossmisin.

Sesquiterpene lactones of *Achillea nobilis* L. 8 kg of the aerial part of raw material (flower heads, buds, leaves) of *Achillea nobilis* L., collected at the flowering stage in the vicinity of the village of Egindybulak, Karkaraly district, Karaganda region, were extracted twice with liquid carbon dioxide at a pressure of 160 bar, a temperature of 60°C, for 240 minutes. After twofold CO_2 extraction, 475 g of the total extractive substances were isolated in the form of a viscous mass of dark green color with a characteristic odor. The yield of the CO_2 extract was 5.94% (calculated on air-dried raw material).

The obtained total extractive substances (475 g) were treated three times with an aqueous alcoholic solution (at an alcohol:water ratio of 2:1). The ballast substances that precipitated were filtered off, and the combined filtrate was extracted three times with chloroform. The chloroform extracts were combined and evaporated under vacuum; the resulting cream-colored total extractive substances weighing 258 g were chromatographed on a column with silica gel of KSK grade at a ratio of total:carrier = 1:10. A mixture of petroleum ether with ethyl acetate with an increasing content of the latter was used as the eluent.

Upon elution of the column with a petroleum ether:ethyl acetate system (ratio 95:5), colorless needle-shaped crystals of composition $C_{15}H_{18}O_3$ with m.p. 102–104°C, $[\alpha]_D^{20}$ +312.50° (c 0.16, ethanol), and a purity of 99.5% according to HPLC data were isolated.

IR spectrum (KBr, ν_{max}, cm^{-1}): 1753 (carbonyl of γ-lactone), 1685, 1653, 1637 (C=C). In the UV spectrum (EtOH, λmax, nm), compound (7) has an absorption maximum at 203 ± 2 nm, characteristic of a conjugated system.

^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz,): 2.85-2.95 (2H, m, H-1, H-7), 1.89-1.91 (2H, m, H-2α, H-2β), 3.38 (1H, m, H-3), 2.16-2.3 (4H, m, H-5, H-8α, H-9α, H-9β), 4.2 (1H, dd, J=11.01, 8.71 Hz, H-6), 1.48-1.58 (1H, m, H-8β), 4.9 (1H, d, J=2.02 Hz, H-13α), 4.8 (1H, J=2.02 Hz, H-13β), 6.09 (1H, d, J=3.6 Hz, H-14α), 5.5 (1H, d, J=3.6 Hz, H-14β), 1.5 (3H, s, H-15).

^{13}C NMR spectrum (125.7 MHz, CD_3OD, δ, ppm): 45,27 (d, C-1), 32.75 (t, C-2), 63.57 (d, C-3), 65.96 (s, C-4), 50.89 (d, C-5), 80.83 (d, C-6), 43.6 (d, C-7), 28.84 (t, C-8), 27.86 (t, C-9), 140.32 (s, C-10), 146.74 (s, C-11), 170.44 (s, C-12). 114.10 (t, C-13), 119.18 (t, C-14), 17.55 (q, C-15).

Comparison of physicochemical constants and spectral data with the literature made it possible to identify compound (7) as the guaianolide estafiatin.

Upon further elution of the column with a hexane:ethyl acetate system (ratio 6:4), colorless plate-like crystals of composition $C_{15}H_{20}O_3$, with m.p. 189.6–191.9°C, $[\alpha]_D^{20}$ +58.6° (c 0.39 chloroform), and a purity of 99.7% according to HPLC data were isolated.

IR spectrum (KBr, νmax, cm^{-1}): 3476 (OH group), 3101, 1744 (carbonyl group of the γ-lactone ring), 1661 (α,β-unsaturated ketone conjugated with a C=C bond), 1445, 1360, 1295, 1250 (ester group), 1070, 975, 955. In the UV spectrum (EtOH, λmax, nm), compound (8) has an absorption maximum at 204 ± 2 nm, characteristic of a conjugated system.

^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 4.82 (1H, ddq, J=12.0, 3.0, 1.3 Hz, H-1), 2.42 (2H, m, H-2α, H-9α), 2.26 (1H, ddd, J=12.1, 12.1, 10.4 Hz, H-2β), 4.26 (1H, dd, J=10.4, 5.9 Hz, H-3), 4.71 (1H, d, J=10.0 Hz, H-5), 4.57 (1H, dd J=9.8, 8.5 Hz, H-6), 2.49 (1H, ddd, J=10.0, 8.7, 3.2 Hz, H-7), 1.65 (1H, ddd, J=14.5, 11.0, 9.0 Hz, H-8), 2.08 (1H, m, H-8β), 2.07 (1H, m, H-9β), 6.23 (1H, d, J=3.2 Hz, H-13α), 5.48 (1H, d, J=3.2 Hz, H-13β), 1.40 (3H, d, J=1.2 Hz, H-14), 1.69 (3H, d, J=1.5 Hz, H-15), 2.05 br. s. (OH).

^{13}C NMR spectrum (125.7 MHz, CD_3OD, δ, ppm): 124.22 (d, C-1); 35.27 (t, C-2); 78.05 (d, C-3); 142.67 (s, C-4); 125.05 (d, C-5); 81.26 (d, C-6); 50.02 (d, C-7); 28.09 (t, C-8); 40.93 (t, C-9); 137.6 (s, C-10); 139.62 (s, C-11); 170.2 (s, C-12); 119.98 (t, C-13); 16.17 (q, C-14); 11.79 (q, C-15).

According to physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR), the isolated compound (8) is identical to the germacranolide hanphyllin.

Upon further elution of the column with a hexane:ethyl acetate system (ratio 6:4), colorless plate-like crystals of composition $C_{15}H_{18}O_5$, with m.p. 249.6–250.4°C and a purity of 99.69% according to HPLC data were isolated.

IR spectrum (KBr, νmax, cm^{-1}): 3428 (OH), 1739 (carbonyl of γ-lactone), 1697, 1663 (C=C).

Comparison of physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR) made it possible to identify compound (9) as the guaiane-type sesquiterpene lactone chrysartemin A.

Upon further elution of the column with a hexane:ethyl acetate system (ratio 1:1), colorless plate-like crystals with m.p. 241–243°C and a purity of 99% according to HPLC data were isolated.

IR spectrum (KBr, νmax, cm^{-1}): 3335 (OH), 1762 (carbonyl of γ-lactone), 1667, 1642 (C=C).

^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.62 (3H, s, Me-4), 1.22 (3H, s, Me-10), 2.27 (1H, d, J=11.0 Hz, H-5), 3.28 (1H, d, J=1.5 Hz, H-2), 3.49 (1H, d, J=1.5 Hz, H-3), 4.45 (1H, q, J=11.0, 9.5 Hz, H-6), 5.36 (1H, d, J=3.0 Hz, H-13α), 6.18 (1H, d, J=3.0 Hz, H-13β), 3.94 (1H, m, H-7).

According to physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR), compound (10) is identical to the sesquiterpene lactone canin.

15 kg of air-dried flower heads and leaves of *Achillea nobilis* L., collected at the flowering stage in the vicinity of the village of Egindybulak, Karkaraly district, Karaganda region, were infused three times with hot water (80–85°C). The aqueous extracts were combined, then extracted with chloroform at a ratio of 10:1. The chloroform extracts were combined, evaporated under vacuum, and a syrupy residue (161 g) was obtained, which was chromatographed on a column with silica gel of KSK grade at a ratio of total–carrier = 1:20. Upon elution of the column with benzene, a benzene–ether mixture (9:1, 4:1, 1:1), ether, an ether–acetone mixture (4:1, 1:1), and acetone, 217 fractions of 250 mL each were obtained. The yield of anobin (11) was 0.008% of the air-dried raw material, estafiatin (7) – 0.01%, hanphyllin (8) – 0.007%, and anolide (12) – 0.01%.

From fractions 13–28 (benzene), 1.20 g of rhombic crystals were isolated, which were washed with ether and recrystallized from alcohol. A colorless crystalline substance of composition $C_{15}H_{20}O_5$ with m.p. 175.5–177.5°C (from alcohol), M^+ 280 (mass spectrometrically), was obtained. Rf 0.37 (benzene–ethanol system, 9:1).

In the IR spectrum, absorption bands characteristic of the hydroxyl group (3500 cm^{-1}), carbonyl of γ-lactone (1780 cm^{-1}), and double bond (1660 cm^{-1}) are observed.

Mass spectrum (m/z, intensity): M^+ 280 (5.36%), 278 (7.14%), 271 (7.14%), 262 (5.36%), 260 (15%), 250 (3.57%), 248 (10.71%), 246 (3.57%), 231 (17.86%), 216 (17.76%), 214 (89.28%), 212 (92.86%), 203 (17.86%), 189 (17.86%), 175 (17.86%), 173 (32.14%), 161 (25%), 151 (58.93%), 149 (65%), 135 (32.14%), 131 (23.21%), 123 (38.57%), 111 (92.86%), 109 (65.71%), 105 (42.86%), 97 (25%), 91 (76.78%), 83 (57.14%), 81 (78.57%), 79 (64.28%), 71 (60.71%), 66 (100%), 64 (33.93%), 53 (32.14%), 44 (23.21%), 32 (42.86%), 28 (42.86%), 18 (60.71%), 17 (57.14%), 15 (50%).

^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.16 (3H, s, Me-4), 1.56 (3H, s, Me-10), 2.73 (1H, d, J=12.4 Hz, H-5), 3.23 (1H, d, J=2.0 Hz, H-2), 3.44 (1H, d, J=2.0 Hz, H-3), 4.38 (1H, dd, J=12.4, 9.5 Hz, H-6), 5.28 (1H, d, J=2.4 Hz, H-13α), 6.10 (1H, d, J=2.4 Hz, H-13β), 3.93 (1H, m, H-7), 4.93 (2H, s, OH).

According to physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR), compound (11) is identical to the new guaianolide anobin.

From fraction 147–182, by repeated washing with diethyl ether and recrystallization from alcohol, 1.58 g of a colorless crystalline substance of composition $C_{15}H_{20}O_4$ with m.p. 167–169°C (alcohol), $[\alpha]_D^{20}$ +52.9° (c 0.017; ethanol), Rf 0.33 (system: benzene–ethanol, 9:1), was isolated.

IR spectrum: 3550 (OH group), 2985, 2950, 2900, 1740 (carbonyl of γ-lactone conjugated with the exomethylene group), 1665, 1640 (C=C), 1455, 1420, 1380, 1325, 1260, 1165, 1125, 1065, 995, 970 cm^{-1}.

In the UV spectrum, an absorption maximum at 204 nm (ε 18521) is observed, characterizing the exocyclic methylene conjugated with the carbonyl of the γ-lactone ring.

^{1}H NMR spectrum (multiplicity, ppm): 1.49 (3H, s); 2.08 (2H, s); 3.92 (1H, d, coupling constant 3.5 Hz); 4.23 (1H, dd, coupling constants 11.5 and 9.5 Hz); 4.92 (2H, d, coupling constant 3.5 Hz); 5.47 and 6.17 (1H each, d, coupling constant 3.5 Hz).

^{13}C NMR spectrum (multiplicity by off-resonance, ppm): 170, s; 146.1, s; 138.9, s; 120.1,

t; 112.6, t; 82.9, s; 82.6, d; 80.3, d; 52.2, d; 48.1, d; 44.1, d; 37.6, t; 35.9, t; 31.2, t; 24.2, q.

Comparison of physicochemical constants and spectral data (IR, ^{1}H NMR, ^{13}C NMR) made it possible to identify compound (12) as the guaianolide anolide.