Chemistry of Natural Compounds, Vol. 31, No. 1, 1995

SESQUITERPENE LACTONES FROM PLANTS OF THE FAMILY ASTERACEAE IN THE KAZAKHSTAN FLORA AND THEIR BIOLOGICAL ACTIVITY

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UDC 547.314

The results are generalized of the results of a study of 109 species of plants of the family Compositae (Asteraceae) growing on the territory of Kazakhstan. The promising nature is shown of the search for biologically active sesquiterpene γ-lactones among them. Fourteen new compounds of this series belonging to the germacranolides, eudesmanolides, guaianolides, and pseudogermanolides have been isolated and characterized by various methods. The results are given of biological tests of the lactones isolated and their derivatives. The antifeedant, antitumoral, antiviral, and growth-regulating activities of the sesquiterpene lactones are reported.

Plants of the family Asteraceae (Compositae) are the main source of sesquiterpene γ-lactones — an important group of natural isoprenoids numbering more than 2000 compounds at the present time [1, 2].

A study of the biological properties of these substances has revealed their antifeedant, repellent, antibacterial, fungicidal, growth-regulating, antimutagenic, and antitumoral activities, which permits the sesquiterpene lactones to be regarded as a source of new ecologically pure protective agents and promising pharmacological preparations [3]. An important role of sesquiterpene lactones has been revealed as chemotaxonomic markers that are convenient for solving complex and intricate problems of the systematics and phylogeny of the taxons of the Asteraceae family [1].

The search for plant raw material rich in the sesquiterpenes and the creation of a base for subsequent synthetic investigation directed to obtaining highly biologically active derivatives of these cyclic terpenes may serve as a foundation for the solution of individual aspects of the fundamental problem of the structure—activity relationship.

The determination of the complete structure of the new sesquiterpene lactones is important for the development of many questions of the stereochemistry of cyclic systems and for modern conformational analysis, and also for the biogenetic relationship between individual classes of natural compounds.

Although by their number the representatives of the Asteracae family occupy the first place in the natural flora of Kazakhstan (about 1000 species), they have been studied chemically to an inadequate extent. Many endemic species of plants of the region have hardly been investigated for the presence of sesquiterpene lactones. We have examined 109 species of plants of the family Asteraceae growing on the territory of Kazakhstan for the presence of compounds of this series.

Total preparations (resins) from plant samples were evaluated chromatographically (TLC, plates with a fixed layer of SiO_2 of the Silufol brand) and spectrally (IR spectrum in the 1600-1800 cm^{-1} region), and also by standard qualitative tests for terpenoids and γ-lactones. The biological activity of the total extractive substances isolated was tested simultaneously. Positive tests for the presence of γ-lactones were found for 84 species of plants. However, only 28 species the extractive substances of which revealed biological activity were promising for subsequent, more profound study. The raw materials basis of the plant species studied served as an additional criterion for the final choice of objects of investigation. As a result of this approach we have isolated and characterized 48 sesquiterpene lactones, including 14 new compounds belonging to four structural types — germacranolides, eudesmanolides, guaianolides, and pseudoguaianolides. This conclusion was made on the basis of a study of the individual substances using modern chromatographic and spectral methods, including x-ray structural

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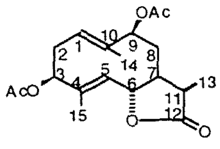
Institute of Organic Synthesis and Coal Chemistry, National Academy of Sciences of the Republic of Kazakhstan, Karaganda. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 29-36 January-February, 1995. Original article submitted October 7, 1994.

0009-3130/95/3101-0021$12.50 ©1996 Plenum Publishing Corporation

investigation (XSI) and, where this was necessary, chemical experiments. The same approach has been used to identify known sesquiterpenes using authentic samples in individual cases.

Sesquiterpene Lactones of Plants of the Genus *Achillea* L. As a result of a chemical study of nine species of this genus growing on Kazakhstan territory we isolated ten lactones belonging to the guaianolide and germacranolide types. New sesquiterpenes were detected: achimicrin (**1**), anobin (**2**), and anolide (**3**). At the same time, we isolated for the first time 8-hydroxyachillin (grossmizin) from *A. millefolium* L., achillin, artelesin, and grossmizin from *A. micrantha* Willd., and estafiatin and hanphyllin from *A. nobilis* L.



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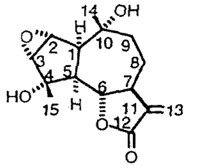

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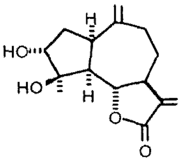

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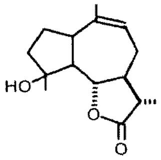
The structure of achimicrin as 3β,9β-diacetoxy-6β,7α,11α(H)-germacra-4E,1(10)E-diene-6,12-olide (**1**) was determined on the basis of HNMR spectra, including the technique of ^{1}H—^{1}H and ^{1}H—^{13}C two-dimensional spectroscopy taking into account existing information for related structures — herbolide A [4] and syntenin [5].

According to the IR and PMR spectra of anobin (**2**), its molecule contains two methyl groups adjacent to hydroxy groups, and an epoxide group and a conjugated exomethylene group of a γ-lactone condensed with a hydroazulene skeleton. The nature of the splitting of the signals of the protons of the lactone fragment showed its localization at C6—C7, while the magnitude $^{3}J_{5,6}$= 12.4 Hz showed the mutual *trans*-arrangement of the corresponding protons. On the basis of the results of a comparison of other spectral characteristics of anobin with those for the related guainolides canin and rupin A [6, 7], and taking into account the results of some chemical transformations, it was concluded that this lactone has the structure of 2α,3α-epoxy-4α,10α-dihydroxy-5,7α,6β(H)-guaia-11(13)-en-6,12-olide (**2**).

The main characteristics of the structure of anolide (**3**) were originally deduced by an analysis of its ^{1}H and ^{13}C NMR spectra and were established definitively as the result of the XSI of its molecule. According to all these results, anolide has the structure and absolute configuration of 3(R),4(R)-dihydroxy-1(R),5(S),6(S),7(S)-guaia-10(14),11(13)-dien-6,12-olide (**3**).

Sesquiterpene Lactones of Plants of the Genus *Artemisia* L. An investigation of two species of wormwood led to the isolation of eight new sesquiterpene lactones. Simultaneously, ten previously known lactones, including α-santonin (in four species), austricin (in three species), artemin, and gracilin (in two species) were detected for the first time in these plants.



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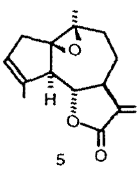

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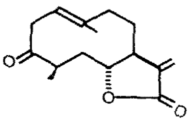

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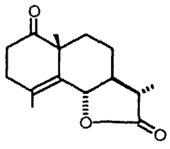
Among the new compounds isolated from Austrian wormwood *Ar. austriaca* Jacq., the guaianolide artausin (**4**) was identified on the basis of the results of physicochemical investigations. So far as concerns the lactone arglabin (**5**) isolated from *A. flabella* Kar. et Kir., its structure as 1(R),10(S)-epoxy-5(S),6(S),7(S)-guaia-3,11(13)-dien-6,12-olide followed unambiguously from the result of selective hydrogenation of the disubstituted C=C bond to the known guaianolide arborescin and was additionally confirmed by comparing the spectral properties of (**5**) and the related arborescin [8] and *iso*-epoxyestafiatin [9]. Analogously, argolide (**6**), isolated from the same source, was identified as 3-oxo-4(R),6(R),7(S)-germacra-1(10)E,11(13)-dien-6,12-olide by conversion into its dihydro derivatives — the known germacranolide ketopelenolide B [10] and also by the XSI of the new lactone.

The structures of the new sesquiterpene lactones gracilin (**7**) and argracin (**8**) isolated from *Ar. gracilescens* Krasch. et Iljin. were established with the use of the same methodology. The first of them proved to be 1-oxo-6,11β,7α(H)-eudesm-4-en-6,12-olide (**7**), as followed from the XSI of the corresponding ketoxime. In the second case, it was necessary to correlate the PMR spectra of (**8**) and the related herbolide A [4] and haagenolide acetate [11] to obtain reliable evidence in favor of the structure as 9β-acetoxy-6β,7α(H)-germacra-4,10,11-trien-6,12-olide (**8**).

The previously unknown arlatin (9) isolated from *Ar. latifolia* Ldb. is 1(R),10(R)-dihydroxy-5(R),6(S),11(S)-guai-3-en-6,12-olide. This conclusion followed mainly from a comparison of its PMR spectrum with that of arborescin and was confirmed by the catalyzed (CO_2H_2) hydrolysis of the latter to the ditertiary glycol (**9**), which is difficult to acetylate under mild conditions.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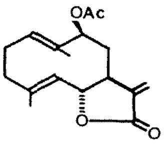
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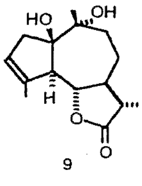
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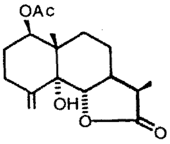
The structure and some fine stereochemical features of the molecule of the previously undescribed nitrosin (**10**) from *Ar. nitrosa* Web. ex Stechm. were revealed by its SSI, which enabled it to be described as 1β-acetoxy-5α-hydroxy-6β,1,11α(H)-*trans,trans*-eudesm-4-en-6,12-olide (10).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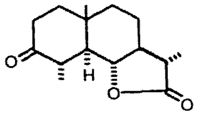
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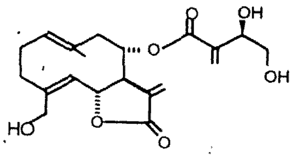
A combination of spectral characteristics (^1H and ^{13}C NMR, MS) and the results of some chemical transformations (dehydrogenation, formation of a ketoxime) permitted the proposal for the new sesquiterpene lactone artepaulin from *Ar. pauciflora* Web. the structure of 3-oxo-5,7α,4,6,11β(H)-eudesman-6,12-olide (**11**). This was confirmed by independent synthesis through the $HClO_4$-catalyzed equilibration of the product of the exhaustive catalytic hydrogenation of α-santonin.

Sesquiterpene Lactones of Plants of the Genus *Centaurea* L. In a study of plants of the centaurea genus (*Centaurea* L.), six species were chosen as promising sources of biologically active sesquiterpene lactones. Of these, *C. pseudomaculosa* Dobrocz was determined as a source of cnicin (**12**), while as a minor component this plant contained another germacranolide, salonitenolide (**13**) [12].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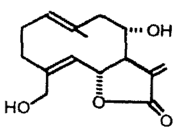
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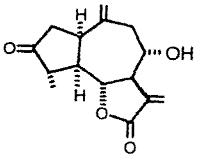
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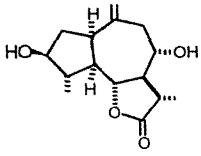
The XSI of (**12**) showed the (R)- configuration of the single chiral center in the side-chain of this molecule and the chair-chair conformation of its ten-membered carbocycle in the crystalline state that is characteristic for nonlinear annelated (E,E)-germacranolides. In addition, the secondary alcohol (**13**) corresponding to the ester (**12**) was subjected to XSI.

We have isolated the guaianolide grosshemin (**14**), which is widely distributed in plants of the tribe Cynareae Less., from Ruthenian centaurea (*C. ruthenica* Lam.), [13] and also from *Chartolepis intermedia* Boiss. and *Grossheimia macrocephala* (Muss.-Puschk.) Sosn. et Takht. XSI carried out for (**14**) permitted an evaluation of some fine features of the structures of this molecule and, in particular, an explanation of the multiplicity of signal of the exomethylene protons observed in the PMR sprctrum of (**14**), which is not characteristic for, for example, the related cumambrin, rupin A and other 8-hydroxyguaianolides [14], as the result of a shortened nonvalent contact between the oxygen atom of the secondary hydroxyl and the *syn*-hydrogen atom of the $C=CH_2$ group (2,73 Å).

Sesquiterpene Lactones of *Crepis tectorum* L. From narrowleaf hawk's-beard, *Crepis tectorum*, we have isolated and identified isolipidiol (**15**) and 8-epideacylcynaropicrin (**16**), this being the first time that they have been detected in this species. The XSI of the new lactone (**16**) showed that it is 1(R),3(S),5(R),6(R),7(R),8(R)-1,3-dihydroxyguaia-4(15),10(14),11(13)-trien-6,12-olide.

A study by the XSI method of the structure and conformation of the glycosidic guanolide crepidiaside A (**17**) from *Crepis multicaulis* Ldb. showed that the five-membered ring of the carbon skeleton is planar: the C1-C5 atoms are present in one plane. The conformation of the seven-membered ring is that of a distorted 7α,1,10β chair, and the lactone ring assumes the conformation of a 6β,7α-half-chair. The glycosidic residue has the form of a considerably distorted chair.



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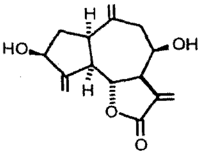

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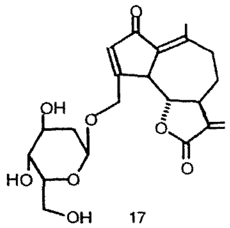

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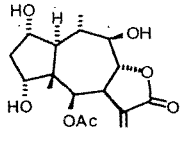
Sesquiterpene Lactones of *Gaillardia grandiflora* Hort. A study of *Gaillardia grandiflora* led to the isolation of the new sesquiterpene lactone gaigranin (**18**) and the known pseudoguanolide spathulin (**19**). The XSI of the molecule of the latter and a comparison of its NMR spectrum with the spectra of gaigranin and the products of its exhaustive O-acetylation, which proved to be identical compounds, and, finally, an analysis of the two-dimensional PMR spectrum of (**18**) enabled this lactone to be regarded as 6(R)-acetoxy-2(S),4(R),9(R)-trihydroxy-1(S),7(S),8(R),10(R)-pseudoguaia-11(13)-en-8,12-olide.



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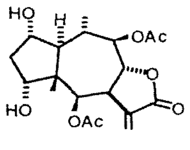

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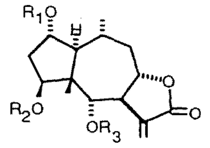
Sesquiterpene Lactones of Plants of the Genus *Inula* L. From the five species of *Inula* studied we isolated and identified seven sesquiterpene lactones of the pseudoguaiane and eudesmane structural types, all having linear structures, i.e., the lactone ring was located at C7—C8. The main lactone-containing sources among the plant of this species were found to be British inula, *I. britannica* L., and Caspian inula, *I. caspica* Blume, in which the pseudoguaianolides inuchinenolide C (**20**) and britanin (**21**) and the eudesmanolides 2α-senecioyloxy-3β-hydroxyisoalantolactone (**22**) and pulchellin C (**23**) were found [15].

The XSI of the molecules of these four lactones enabled us to refine their spatial structures and, in particular, to determine the configurations of the methyl, ester, and hydroxy groups and also the nature of the ring linkages. For (**20**) we propose the structure of 2(S),4(S)-diacetoxy-6(S)-hydroxy-1(S),5(R),7(R),8(S),10(R)-pseudoguaia-11(13)-en-8,12-olide and for (**21**) 2(S),6(S)-diacetoxy-4(S)-hydroxy-1(S),5(S),7(R),8(S),10(R)-pseudoguaia-11(13)-en-8,12-olide. The eudesmanolides (**22**) and (**23**) have the structures of 3(R)-hydroxy-2(R)-senecioyloxy-5(R),7(R),8(R),10(S)-eudesma-4(15),11(13)-dien-8,12-olide, and 2(R),3(R)-dihydroxy-5(R),7(R),8(R),10(S)-eudesma-4(15),11(13)-dien-8,12-olide, respectively.



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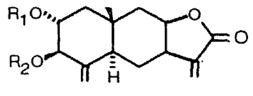
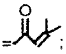
21: R_1=R_3=Ac; R_2=H



22: R_1=; R_2=H
23: R_1=R_2=H

Thus, as the result of a chemical investigation of 28 species of plants of the family Asteraceae growing on the territory of Kazakhstan, a large number of previously undescribed sesquiterpene lactones of the above-mentioned four structural types have been isolated and characterized. The overwhelming majority of them proved to be α-methylene-γ-butenolides with nonlinear *trans*-annelation relative to the mono- or bicyclic ten-carbon skeleton; in the case of pseudoguaianolides this fragment is oriented predominately to the C6—C7 bond.

A number of sesquiterpene lactones have become preparatively accessible as the result of structural chemical investigation. As renewable chemical material, they are of particular interest for finding new biologically active substances. Furthermore, they serve as convenient objects for the study of some stereochemically controlled reactions and biogenetically similar transformations [16]. In this connection we have performed the synthesis of a large number of various derivatives of a series of readily accessible sesquiterpene lactones. The majority of them have been subjected to biological evaluation with the aim of finding substances of practical significance.

Thus, the bioscreening of 14 sesquiterpene lactones for herbicidal and growth-regulating activity has shown that the presence of an α-methylene-γ-lactone function leads to pronounced growth-inhibiting action, while the introduction of a chlorine atom, a hydroxy group, and conjugated or isolated double bonds into the molecule of compounds of this series appreciably increases their activity. A study of photosensitizing action and also of the influence of sesquiterpene lactones in the biosynthesis of porphyrins has enabled us to conclude that substances of this type are promising in the scheme of seeking new photosensitizers for green plants.

A determination of the repellent and antifeedant action of 29 sesquiterpene lactones and their derivatives revealed their high bioactivity in relation to the glasshouse whitefly, the yellow mealworm beetle, and other pests of grain stores — adult individuals and larvae of *Tribolium confusum*, *Trogoderma granarium*, and *Sitophilus granarius*.

Among the fungicidally active lactones studied, a well-defined antifungal action is possessed by cnicin (**12**) from which the preparation Tsepseudin is manufactured for use as an ecologically pure seed dressing for cereal crops and a replacement for imported fungicides for covered soil.

In the determination of the antiviral activity of 30 sesquiterpene lactones it was established that the compounds tested protect cells from damage due to the immunodeficiency virus, lower the reproduction of hemorrhagic fever with a renal syndrome by 95-98%, and possess a 100% inhibiting action on the A influenza virus.

The results of a study of 43 sesquiterpene lactones for antitumoral activity in relation to transplantable strains has shown that the presence in their molecule of such alkylating centers as an α-methylene-γ-lactone, an α,β-unsaturated ketone group, and an epoxide ring, and also hydroxy and halogen functions, enables the growth of tumor strains to be inhibited. Five medicinal forms of the new antitumoral preparation Arglabin (lyophilized form, tablets, 2% aqueous solution, salve, suppositories) have been developed from the results of the first stage of the clinical trials of which the Pharmaceutical Committee of the Ministry of Health of the Republic of Kazakhstan has given approval for the second phase of clinical approbation in order to determine the regime of treatment and the optimum therapeutic dose to be used in various localizations of tumors.

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