PAISIY HILENDARSKI UNIVERSITY OF PLOVDIV
FACULTY OF BIOLOGY
DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Associate professor Dr. PETKO IVANOV BOZOV

Clerodane diterpenoids from species of the Lamiaceae family

ABSTRACT

ON

DISSERTATION

to acquire the scientific degree

"Doctor of Science"

Field of higher education: 4. Natural sciences, mathematics and informatics
Professional field: 4.2. Chemical Sciences (Organic Chemistry)

PLOVDIV, 2021

The dissertation was discussed and proposed for public defense at an extended meeting of the Department of Biochemistry and Microbiology at the Faculty of Biology of Plovdiv University "Paisii Hilendarski" on 12.02.2021.

The dissertation contains 290 pages and includes: 3 diagrams, 33 tables, 137 figures, 276 cited titles, of which four in Bulgarian, 23 appendices.

Scientific jury:
Prof. D.Sc. Georgi Yankov Papanov
Prof. D.Sc. Panteley Petrov Denev
Prof. Dr. Petko Nedyalkov Denev
Prof. Dr. Nikolai Georgiev Vasilev
Prof. Dr. Ilian Ivanov Ivanov
Prof. Dr. Velizar Kostadinov Gochev
Assoc. Prof. Dr. Plamen Angelov Angelov

The defense of the dissertation will take place on 11.05.2021…… am in auditorium of the Rectorate at Plovdiv University "Paisii Hilendarski", 24 Tsar Asen Str.

The materials on the defense are available to those interested in the University Library, Rectorate, 24 Tsar Asen Str.

INTRODUCTION

The species of the family Lamiaceae are distributed in all climatic zones and altitudes of the earth. They are not found only in the tundra region and in the icy deserts of the Arctic and Antarctic. They inhabit dry and moist soils, they are also found in swampy and swampy places, but there are no aquatic plants between them.

To this family belong 200 genera with over 3500 species. Of these, about 135 species belonging to 30 genera have been identified in Bulgaria. For centuries, plants have been used around the world as a remedy in folk medicine, perfumery, cosmetics and the food industry.

The different species of the genera *Scutellaria* (Prevara), *Teucrium* (Podubiche), *Ajuga* (Srestniche) and *Salvia* (Kakula, Horse basil) from the Lamiaceae family are a rich source of diterpene compounds with clerodane skeleton, which are characterized by diverse biological activity - antifungal , insecticidal, antiulcer, cytotoxic, antipyretic, analgesic and others. They are also characterized as potent antifidants, inhibiting the feeding and development of larvae of insect pests (*Leptinotarsa decemlineata* Say, *Spodoptera littoralis*, *Spodoptera exempta*) causing economically significant damage to crops.

Representatives of the cosmopolitan genus *Scutellaria* L (Skullcaps) (about 360 species), known as scammers and skullcaps, grow in various climatic zones around the world except South Africa. Most of them are distributed in Asia. A distinctive feature of the species of the genus *Scutellaria* is a petal with an upper lip with two shallow teeth with a thyroid, larger or equal to it, growth (scutellum). Plants of this genus have been widely used in herbal medicine for thousands of years. Extracts from the roots and their aboveground parts in the form of a decoction are still used in Chinese folk medicine as an effective remedy against staphylococci, cholera, dysentery, pneumonia and others. Pharmacological studies have confirmed that extracts or individual compounds of *Scutellaria* have cytotoxic, hepatoprotective, antioxidant, antiinflammatory, anticonvulsant, antibacterial and antimicrobial effects. Followers of the Anglo-American School of Herbal Medicine, called physiomedicals, were the first to use a decoction of deception with a calming effect on the nervous system and use them to treat hysteria, epileptic convulsions and serious mental illnesses such as schizophrenia. Some of the most active *neo*-clerodane diterpenes isolated from members of the genus *Scutellaria*, clerodin (**1**), jodrelin A (**11**), jodrelin B (**12**), skutalbin A (**9**) and skutekiprol B (**57**) exhibit antifidant, antifungal, cytotoxic, antimicrobial effect. Jodrelin B is the most powerful antifidant known so far.

Isolated clerodans from *Salvia divinorum* are naturally occurring, nitrogen-free substances with potential and selective agonist activity against β-opioid receptors. Among them, salvinorin has the greatest psychoactive effect.

The species of the genus *Teucrium*, about 360, are honey-bearing and have medical applications. Paws and decoctions are used in the treatment of open wounds, pain in the stomach and intestines, disorder and others. *T. polium* is used in Mediterranean countries in the treatment of abdominal pain, stomach disorders, rheumatism, diabetes. In the studies of alcoholic and aqueous extracts of white podubiche, antimicrobial, hypoglycemic and antioxidant properties have been established. Stankovic and co-

authors demonstrated cytotoxic activity of seven members of the genus *Teucrium* against carcinogenic cells.

T. capitatum is taxonomically close to *T. polium* subsp. *polium*. Both species contain the *neo*-clerodane diterpenoids capitatin and auropolin, but more clerodane compounds have been found in *T. capitatum* that have not been identified in *T. polium* subsp. *polium*. Bruno et al., examining in 2003 an authentic sample of *T. polium* subsp. *polium* prove that the isolated diterpenoids are radically different from the *neo*-clerodanes found in all taxa designated *T. polium*. They recommend reconsidering the identification of plant material in studies investigating the diterpene composition of this species.

In 1973, phytochemical studies of various species of the Lamiaceae family began at the Department of Organic Chemistry of the Paisii Hilendarski University of Plovdiv. A large number of bicyclic diterpenoids of the *neo*-clerodane and *nor*-clerodane type and pentacyclic triterpenoids with ursan and olean skeletons have been isolated and characterized. Plants of the genera *Ajuga*, *Lavandula*, *Teucrium* of the family Lamiaceae have been relatively fully studied in Bulgaria, while the representatives of the genus *Scutellaria* have not been the subject of phytochemical studies until 1992.

Malakov and co-authors published, from 1978 to 1983, the results of the isolated and structurally characterized eleven clerodane diterpenoids of the species *Teucrium polium* subspecies *polium*. In volume IX of Flora of Bulgaria, 1989 the taxonomy of plants of the genus Lamiaceae is updated. It states that the species *Teucrium polium* subspecies *polium* is not found in Bulgaria, but *Teucrium polium* subspecies *capitatum* and *Teucrium polium* subspecies *vincentinum* are widespread.

The subject of research in the present paper are the clerodane diterpenoids in the Bulgarian species of the genus *Scutellaria*, *Teucrium polium* subsp. *vincentinum* L. (Rouy) D. Wood, *Teucrium scordium* subsp. *scordioides* (Schreb.) Maire et Petitmengin, *Salvia splendens* Ker.-Gawl. and *Salvia nemorosa* L. Diterpenes attract the interest of the chemists in their intensive research with the great variety of chemical structures, the presence of different functional groups in their molecules, and the problems that arise in proving the structure, location of substitutes and stereochemistry of the chiral centers. These compounds also exhibit a variety of biological activity. *Neo*-clerodanes are natural biodegradable substances, with low environmental impact, potential antifidants with the possibility of application as an alternative to chemical synthetic and highly toxic pesticides in the control of pests on agricultural crops.

LITERATURE REVIEW

The review is based on 276 literature sources, 4 of which are in Cyrillic and is structured in three subsections: **I**. Composition, structure, classification and biosynthesis of terpenes; **II**. Examination of the isolated from species of the genus *Scutellaria*, *Teucrium polium* and *T. scordium* clerodane diterpenoids and polyphenols and of the biological activities they exhibit; **III**. Summary of the chemical composition of the essential oil obtained in the countries of the Balkan Peninsula from the same species as the plant species distilled in the present work; **IV**. Results of phytochemical

study of *Scutellaria alpina* and *Ajuga salicifolia*, which are part of the author's dissertation for the acquisition of ONS "Doctor".

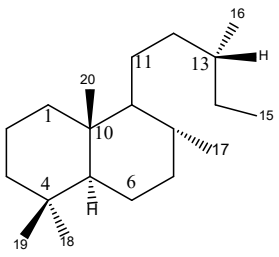
The composition, structure, classification and biosynthesis of terpenes are presented, with emphasis on bicyclic and polycyclic diterpenoids. The classification and nomenclature of diterpenoids isolated from the genus *Scutellaria* are considered in detail. The Clerodane skeleton is divided into two main fragments: the C-1-C-10 decalin ring and the C-11 – C-16 side chain. The substructures in the decalin fragment of the molecule are systematized on the basis of the oxidation number of C-18, and in the C-11–C-16 fragment of the carbon skeleton the substructures are grouped on the basis of the formed cycles.

A list of the names and formulas of isolated clerodane diterpenoids of the species of the genus *Scutellaria* and of the species *Teucrium polium* and *T. scordium* is given in Annex 1 as well as of the isolated polyphenols of the species of the genus *Scutellaria*.

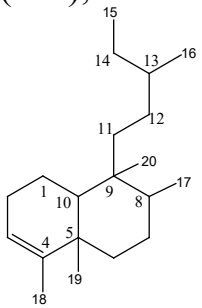
The chemical composition of the essential oil of Ajuga laxmanii Benth, Salvia amplexicaulis Lam is considered. and Stachys cretica subsp. bulgarica Rech. Fil distributed on the Balkan Peninsula. A review of the biological activities of extracts of different species of the genus *Scutellaria*, *T. polium* and *T. scordium*, essential oils of *Ajuga laxmanii*, *Salvia amplexicaulis* and *Stachys cretica* L. subsp. *bulgarica* rech. fil. The activities of individual compounds - of polyphenols and especially of *neo*-clerodane diterpenoids and *neo*-clerodane diterpene alkaloids - are examined in more depth.

After analyzing the summarized data from the literature, the conclusions are made:

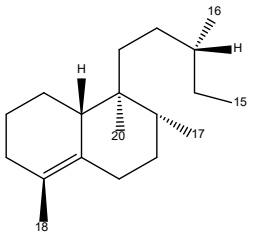
1. Of the 14 major skeletons of cyclic diterpenoids classified by Rowe and co-authors in the genera *Scutellaria* and *Ajuga*, compounds with the bicyclic clerodane skeleton are found. Clerodan diterpenoides are considered to be compounds with a rearranged Labdan skeleton due to migration of the CH_3-19α methyl group from C-4 to C-5. The 19-*nor*-clerodane skeleton is a variant of the *neo*-clerodane skeleton. It is obtained by eliminating the methyl group and forming a double bond $\Delta^{4,5}$. Of the 286 diterpenes isolated from species of the genus *Scutellaria*, only three have 19-*nor*-clerodane skeleton, scutefolid B1 (**249**), scutefolid B2 (**250**) and scutefolid C (**251**). Of



labdane skeleton



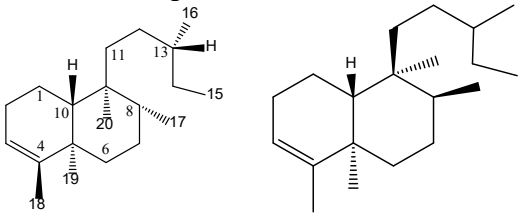
clerodane skeleton



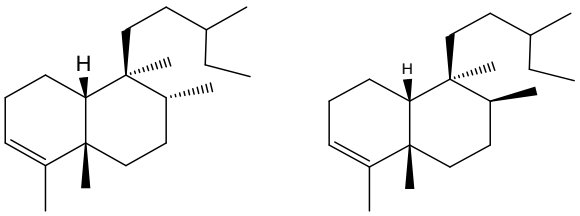
19-*nor*-clerodane skeleton

the remaining 283 most compounds have a *neo*-clerodane backbone (TC type). An exception is observed at a series of diterpenoids isolated from the Asian species *S. rivularis*, *S. barbata*, *S. coleifolia* and *S. repens*, which have a skeleton of the SS type. Only *neo*-clerodane diterpenoids have been found in the genus *Ajuga*. Diterpenoids with both *neo*-clerodane and 19-*nor*-clerodane skeletons have been shown in *Teucrium polium* and *T. scordium*.

trans-клероданови скелети



cis–клероданови скелети



TC - type TT - type CC - type CT - type

A furan ring is present in all isolated compounds. One or two lactone rings are present in many clerodanes. An oxirane, oxetane, oxolane, oxane, hemiacetal or acetal ring is present in some representatives. Montanin A (**309**) is the only clerodane diterpene that has two furan nuclei. *Neo*-clerodanes with both TC and CC skeletons were isolated. In two diterpenoids, teuchamahedrin A (**357**) and teuvincentin C (322), the methyl groups CH_3-17 and CH_3-20 are trans linked.

2. The hydroxyl groups in the clerodanes isolated from the genus *Teucrium* are esterified only with acetic acid, while the diterpene compounds proven in the genera *Scutellaria* and *Ajuga* have a variety of acyl groups: acetyl, 2-methylbutanoyl, 2-methylpropanoyl, (E) -2-methyl-2-butenoyl, trans-cinnamoyl, cis-cinnamoyl, benzoyl, nicotinoyl, senecyoyl and 3-hydroxybutanoyl.

3. Extracts of species of the genus *Scutellaria* from ancient times are used in folk medicine in America, Russia, Asian countries for the prevention and treatment of a wide range of diseases. For example: *1*. *S. rivularis* extract is used in Thailand and Japan to treat tumors, hepatitis and liver cirrhosis. *2*. The Mexican species *S. guatemalensis* is used in local herbal therapy as a medicinal plant for the treatment of psychosomatic illnesses and certain gastrointestinal disorders. *3*. *S. albida.* ssp. *albida*, herbaceous perennial plant distributed from northern Italy to the Balkan and Crimean peninsulas, is used in folk medicine for spasms, sweating and fever. *4*. In the Pharmacopoeia and the National Recipe Book of the United States, air-dried aerial parts of *S. lateriflora* (known as skullcap) have been reported as a sedative tonic for nerves and dealing with neuralgia and excruciating anxiety. They are also an antispasmodic, a drug for the treatment of epilepsy, a means of relieving the symptoms of addiction to barbiturates and tranquilizers. In Canada, the drug skullcap is usually sold as a tea in health food stores, but can also be found as a sedative or in combination with other herbs such as valerian and lemon balm in sleeping pills. *5*. In China, Korea and India, S. indica is used as an analgesic, to detoxify and to stimulate blood circulation. The leaves of S. scandens growing in Nepal are used to treat wounds and swelling from insect bites. Secondary metabolites of S. amoena are used as agents with diuretic and analgesic properties. *6*. *Scutellaria barbata*, native to Korea and South China, is a popular herb in traditional folk medicine and is listed in the Chinese Pharmacopoeia under the name "Ban-Zhi Lian". The therapeutic history of *Scutellaria barbata* spans more than a thousand years. Extracts of the plant show a wide range of antitumor activity against human gynecological tumor, leukemic and cancer cells of the colon, liver and lung tumor cells, those on the skin and others. The herbal material from *S. barbata* is one of the important ingredients in Chinese traditional recipes for

the treatment of sore throats and tumors, swelling and hemorrhoids, cancer, inflammation of the urinary system. The drug has a slightly bitter taste, with a cool and soothing effect, with a beneficial effect on the activity of the liver and lungs and stomach. It is also used to treat venomous snake bites. In the Pharmacopoeia of China, the dried aerial parts of the plant are registered as a remedy. Extracts of *S. barbata* have shown an in vivo inhibitory effect on the growth of a series of cancer cells. The drug is used to treat tumors of the digestive system, liver inflammation, breast cancer and malignancies of the epidermis. Sixty-two percent of patients with hepatocellular carcinoma of the liver (hepatoma) were completely cured by treatment with *S. barbata*.

4. Polyphenolic compounds isolated from species of the genus *Scutellaria* contain methoxy or hydroxyl groups in the aromatic rings. A number of biological properties of the substances have been tested and antibacterial, antitumor, anti-oxidant effect and other activities have been established. Verbascoside, isolated and identified in *S. altissima*, has a pronounced therapeutic effect. Its anti-inflammatory, antiviral, anti-bacterial, antioxidant and cytotoxic activity has been proven.

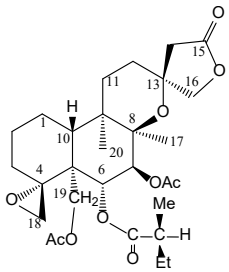
5. *Neo*-clerodane diterpenoids are biologically active substances manifesting antimicrobial,
antifungal, insecticidal and other properties. They are also characterized as powerful antifidants, inhibiting the nutrition and development of larvae of pests. Jodrelin B (**12**) isolated in 1989 from *S. woronowii* is the most powerful antifidant known.

6. *Scutellaria barbata* is especially rich in diterpenes. 73 *neo*-clerodane diterpene alkaloids and 45 *neo*-clerodane diterpenoids were isolated from this plant, four of which have a clerodane skeleton with CC binding of the methyl groups CH_3-18 / CH_3-19 and CH_3-17 / CH_3-20. For other compounds, the skeleton is of the TC type. Apart from the *neo*-clerodane diterpene alkaloids proven in *S. barbata*, scotestrigilosin A (**237**) is the only nitrogen-containing diterpenoid isolated from *S. strigillosa*. Many of the isolated *S. barbata* clerodanes exhibit strong cytotoxic activity with IC_{50} values ranging from 2.5–8.0 to 17.9–35.7 μM against a series of carcinoma cell lines from human tumors.

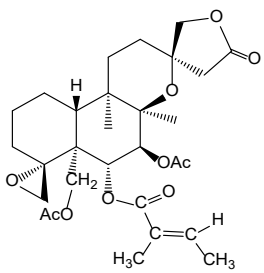
Results of a phytochemical study of Scutellaria alpina and Ajuga salicifolia for the presence of neo-clerodan diterpenoids involved in a procedure for the acquisition of ONS "Doctor" on the topic: "Di- and triterpenoids in members of the Lamiaceae family and their biological activity"

Phytochemical studies of plants of the genus *Scutellaria* in Bulgaria began in 1991 in the laboratory of Bioorganic Chemistry at the Department of Organic Chemistry at PU "Paisii Hilendarski", with the study of *neo*-clerodane diterpenoids in *Scutellaria alpina*. In 1993, Bozov and co-authors published data on the first isolated clerodane, scutalpin A (**384**). The following year, Bozov in another team characterized two more diterpenoids from this plant, scutalpin E (**385**) and scutalpin F (**386**). All three compounds have, in the C 11 – C 16 substructure, a saturated γ-lactone ring 13-spiro-linked to an oxane ring between C-8 and C-13. The absolute stereochemistry of scutalpin A and the absolute configuration of the asymmetric carbon atom C-2' were determined by X-ray diffraction analysis. Scutalpin A is the first 8β, 13S-epoxy-*neo*-

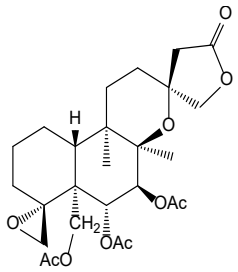
clerodane-15,16-olide found in a species of the genus *Scutellaria* growing in Europe, although these structural characteristics have been demonstrated in *neo*-clerodanes isolated from species of the genus *Scutellaria* from the Far East - Scutellaria hematochlora, *Scutellaria rivularis*.



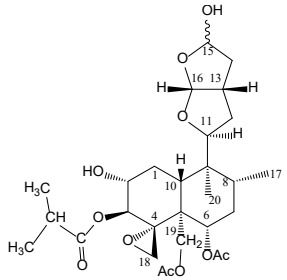
384



385



386



387

Simultaneously with this study, a plant extract of *Ajuga salicifolia* (L.) Shreb was developed. The only isolated diterpenoid is a C-15 epimeric mixture of 15R, 15S-14,15-dihydro-15-hydroxy ajugapyrin A (**387**). A characteristic structural fragment of 387 is the presence of a hexahydro furofuran ring concluded between C-11-C-16, which is common in clerodane compounds isolated from members of the genus *Ajuga*.

The antifidant activity of extracts of *Scutellaria alpina*, *Scutellaria galericulata* and the individual substances *scutalpin* A, 14,15-dihydrojodrelin T and ayugahin A against Colorado potato beetle larvae was tested. *Neo*-clerodane diterpenoids have been shown to have potent antifidant activity, with higher activity than plant extracts.

PURPOSE AND TASKS

The aim of the present study is the isolation of clerodane diterpenoids from species of the genera *Scutellaria*, *Teucrium* and *Salvia*. Study of the structure, stereochemistry and antifidance activity of the compounds, testing of the cytotoxic and antimicrobial action of selected diterpenoids.

To achieve the set goal the tasks were performed:

1) collection of aboveground parts of the species for research, their determination and preparation of the raw material for exhaustive extraction of the organic compounds;
2) obtaining a bitter fraction which contains mainly clerodane diterpenoids;
3) chromatographic separation of the bitter fraction to isolate individual diterpenes;
4) determination of the physico-chemical parameters of the compounds
5) spectral characterization of diterpenoids (IRS, 1D and 2D NMR, Mass spectrometry) in order to prove the structure and stereochemistry of substances;
6) testing of the antifidant activity of plant extracts and of individual clerodane diterpenoids, establishment of the interrelation structure-antifidant activity;
7) testing the antimicrobial and cytotoxic activity of selected clerodane diterpens.

MATERIALS AND METHODS

1. Plant starting material for scientific research

The present dissertation is a continuation of the phytochemical study of the *neo*-clerodane diterpenoid fraction of Scutellaria alpina and *Ajuga salicifolia* (Lamiaceae), which is part of the work to obtain the scientific and educational degree "Doctor". The subject of the study are unexplored and poorly studied species and subspecies of five genera: *Scutellaria*, *Teucrium*, *Salvia*, *Ajuga*, *Stachys*. All studied plants are wild, widespread or less common in different regions of the country. The plant material, consisting of the above-ground parts of the plants, was collected during flowering from natural habitats throughout Bulgaria and was determined in the departments of Botany at the Paisii Hilendarski University of Plovdiv, at the Agricultural University - Plovdiv and at the Institute of Botany at the Bulgarian Academy of Sciences - Sofia. The plant species were collected and studied: *Scutellaria alpina* (L) (Alpine Scam), *Scutellaria orientalis* (L) (Perestolistna, Eastern Scam), *S. albida* (L) (Whitish Scam), *S. altissima* (L) (High-stem Scam) , *S. galericulata* (L), *S. hastifolia* (L), *S. velenovskyi* Rech. Phil. (Rhodope deception), *S. columnae* All. (Purple fraud), *Teucrium polium* subsp. *vincentinum* L. (Rouy) D. Wood (White podubiche), *T. scordium* subsp. *scordioides* (Schreb.) Maire et Petitmengin (Garlic podubiche), *Salvia splendens* Ker.-Gawl. (Flame, Fire), *S. nemorosa* L. (Forest pupa), *S. amplexicaulis* LAM. (Stem-covering cacula), *A. laxmanii* (L.) Benth. (Laxman's counterpart), *Stachys cretica* L. subsp. *bulgarica* rech. fil. (Cretan purgatory).

2. **Methods for isolation and separation of biologically active substances**

2.1. Isolation of clerodane diterpenes

Clerodanes are found mainly in the leaves and stems of plants. To extract them, the plant material is dried in the shade, ground finely and extracted thoroughly with a suitable organic solvent (acetone, methanol, diethyl ether, etc.), the selection of which depends on the stability and polarity of the substances. After filtration, the extract was concentrated in vacuo to a temperature not exceeding 45 °C, diluted with water (1: 1) and left in a refrigerator at 4 °C for 24 hours. Under these conditions, chlorophyll, tannins, waxes and other substances present in large quantities precipitate, and terpene compounds remain in the aqueous-acetone solution. The precipitate was filtered off and the filtrate was extracted with chloroform until complete extraction of the diterpenes (TLC negative sample). The dark green precipitate was redissolved in acetone, diluted 1: 1 with water and left at 4 °C. This operation is repeated until the complete extraction of terpenoids. The combined chloroform extracts were dried over anhydrous Na_2SO_4 and, after filtration, distilled in vacuo. The distillation residue is a yellow-brown resinous product called the bitter fraction due to the highly bitter taste of the clerodane diterpenoids. The bitter fraction was subjected to chromatographic separation and recrystallization to give pure substances. The methods used are column chromatography (CX), preparative thin layer chromatography (PTH) and preparative high performance liquid chromatography (HPLC) on various adsorbents - silica gel, alumina and others. The fission process and the purity of the compounds were

monitored by thin layer chromatography (TLC). Organic solvents such as chloroform, methylene chloride, petroleum ether, methanol, diethyl ether, ethyl acetate and mixtures thereof are used as eluent.

2.2. Obtaining essential oil

The plant material is dried in the shade and the moisture content of the drug is determined by heating to 105 °C. The oil was distilled using laboratory glassware from the British Pharmacopoeia, dried over anhydrous sodium sulphate and stored in tightly closed dark violets at 4 ° C until analysis. The compounds in the essential oils are identified by comparing their retention times, gas chromatograph separation, with the literature data for standard essential oils and the recorded Mass spectra. Calculate the amount of components in percent.

2.3. Study of the total flavonoid content

Chromatographic methods are widely used for the analysis of biologically active substances. Aboveground parts of Scutellaria altissima were dried at 35 °C and sprayed. Prepare 2% solutions of ground plant material in distilled water, 70% ethanol, 96% ethanol and methanol. The extraction was performed at room temperature 25 °C for 24 hours. The combined extracts were filtered through a microfilter (0.25 μm) and injected into the HPLC system. After elution with a gradient of methanol, acetonitrile and water and recording ultraviolet and visible absorption spectra, the flavonoids are quantified. The extracts were obtained in the Laboratory of Phytochemistry at the Medical College Plovdiv. The analyzes were performed in the Department of Bioorganic Chemistry at the Faculty of Pharmacy of the Medical University, Plovdiv.

3. Spectral methods for determining the structure and stereochemistry of compounds

3.1. Determination of the elemental composition of organic compounds

In order to compile the molecular formula of substances, it is necessary to know their elemental composition, for the determination of which the methods of qualitative and quantitative elemental analysis are applied. *Neo*-clerodan compounds are made up of atoms of chemical elements, carbon, hydrogen and oxygen. To find the molecular formula of clerodanes, the percentage of carbon and hydrogen in their molecules is determined. In modern organic analysis with high-resolution mass spectrometers, the molecular weight of the compounds is determined to the fourth decimal place. In this way we can determine the molecular formula of the substance.

3.2. Infrared spectroscopy (ICS)

In structural studies of organic compounds, the absorption spectra in the middle IR region determined by electromagnetic oscillations with a frequency of 4000 to 400 cm^{-1} are of major importance. Using IR spectrometry, various functional groups, some fragments of the carbon skeleton, as well as its identity are determined by comparing its spectrum with those of known organic compounds. IR spectra were recorded in KBr tablets for crystalline substances and in a capillary layer between two KBr tablets for

oily, on a Perkin-Elmer 1750 FT-IR spectrometer from 4000 cm^{-1} to 450 cm^{-1} at a resolution of 4 cm^{-1} with 9 scans and the Vertex 70 spectrometer from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1} with 25 scans and the Bruker Tensor 27 spectrometer from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1} with 70 scans. The measurements were performed in the Department of Analytical Chemistry at the Faculty of Chemistry of Paisii Hilendarski University of Plovdiv and in the Laboratory of Vibration Spectroscopy at the IOH at BAS, Sofia.

3.3. Mass spectrometry (MS)

Mass spectra were measured on a Hewlett Packard 6890 GC System Plus / 5973 MSD spectrometer and on an ACQUITY UPLC® (Waters Corporation, Milford, MA) with a Q-Tof Premier™ mass spectrometer with a detector (Waters Corporation) in the range 50-1500 Da. Dissolve 0.2 mg of the substances in 100 μL MeOH, add 10 μL HCOOH and dilute the solution to 1 mL. The samples are injected directly into the ionization chamber, where they are bombarded with an electron flux of 10 to 70 eV under positive and negative ionization conditions.

The measurements were performed at the Faculty of Biology of the University of Plovdiv "Paisii Hilendarski" and Department of Biological Chemistry and Molecular Biology, at the Institut de Química Avançada de Catalunya, CSIC, J. Girona, Barcelona, Spain.

The use of high-resolution mass spectrometry, in which the mass number of the molecular ion and / or fragment ions is determined with great accuracy (a few decimal places), allows to calculate the gross formula of the compound. MS provides information on available functional groups in the molecule (hydroxyl, ester) and on some characteristic fragments of the hydrocarbon skeleton.

3.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR (600.130 MHz, 500 MHz, 400.13 MHz and 250.13 MHz) and ^{13}C NMR (150.903 MHz, 125 MHz, 100.61 and 62.91 MHz) spectra were measured on a Bruker Avance II + 600 spectrometer, Bruker Avance 500 spectrometer, Mercury 400 spectrometer (Varian, Zug, Switzerland) and Bruker DRX-250 spectrometer in $CDCl_3$ or (CD_3) 2CO under standard 1D and 2D serial pulse conditions. Dissolve a quantity of the substances in 0.6 mL of deuterated chloroform or acetone to provide a 0.05 M concentration to record ^{13}C NMR spectra and a 0.005 M concentration in ^{1}H NMR spectra. TMS was used as an internal standard. The chemical shifts (δ) are expressed in ppm and the spin interaction constants (J) in Hertz. The measurements were performed at the Institute of Organic Chemistry with a center in Phytochemistry at the Bulgarian Academy of Sciences; in the Department of Biological Chemistry and Molecular Biology, at the Institut de Química Avançada de Catalunya, CSIC, J. Girona, Barcelona, Spain and in the Department of Pharmacognosy at the University of Szeged, Hungary.

3.4.1. Proton nuclear magnetic resonance (^{1}H NMR)

Neo-clerodanes contain from 22 to 44 hydrogen atoms, the nuclei of which give signals in a wide range from 0.8 to 11 ppm. in the ^{1}H NMR spectrum. In the ^{1}H NMR

spectra of the neo-clerodan diterpenoids, with trans binding of rings A and B in the decalin substructure of the TC type molecule, there are characteristic signals for the protons: H-10, which appears as a double doublet in the range 2.70 - 1.7 ppm with a value of the spin-spin interaction constant J of about 10 and 4 Hz; methyl protons H_3-17 are observed as a doublet at about 0.90 ppm with a J constant of 5.5 to 6.7 Hz; methyl protons H_3-20 resonate as a singlet in the range of 0.90 to 1.25 ppm.

3.4.2. Carbon magnetic resonance imaging (^{13}C - NMR)

In determining the structure and stereochemistry of organic compounds ^{13}C - NMR spectroscopy allows for direct study of the carbon skeleton and identification of functional groups containing carbon atoms. For example, in the γ-lactone ring, the resonant signal for the carbonyl carbon atom appears in the range 172-178 ppm. The resonant signals for the carbon atoms at the double bond are observed in the range of 120-150 ppm, and that for the carbonyl carbon atom in the acetate group at 170 ppm. The resonant signal for carbon from the ketone group appears at 210 ppm, and that for carbon bound to an oxygen atom - in the range of 55-85 ppm. The location of the hydroxyl groups can be determined by the change in the chemical displacement of the corresponding carbon atom after acetylation with Ac_2O / Py.

3.4.3. DEPT (Distortionless Enhancement by Polarisation Transfer)

The DEPT 135 method is a variant of ^{13}C-NMR in which resonances are obtained signals for primary, secondary and tertiary C-atoms. In this method, the phases for the resonant signals for the CH_2 groups are opposite to the phases for the resonant signals for the CH and CH_3 groups, the quaternary and unprotonated carbons do not appear in the spectrum. By comparing the normally recorded ^{13}C-NMR spectrum with this taken after the application of the DEPT method, the signals for unprotonated C-atoms are determined and information is obtained for all CH_n-fragments (n = 1, 2 or 3).

3.4.4. Two-dimensional NMR spectra

^{1}H-^{1}H COSY (Correlated Spectroscopy) spectroscopy. In two-dimensional ^{1}H-^{1}H COSY experiments, known as homonuclear correlation, the chemical displacements of protons are represented on the abscissa and coordinate. In both halves of the spectrum, where there is a correlation between the chemical signals, a contour appears. A graph with the same spectrum is formed for each of the two axes and a diagonal of the contours of the ordinary spectrum. The extradiagonal contours are interpreted. The values of the spin interaction constants cannot be determined by this method, but from the fact that the two resonances are connected information is obtained about the spin spin interaction between the protons.

HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond connectivity). In these experiments, a correlation is made between two different nuclei. There is no diagonal element in the spectrum, and the correlation is indicated at the point of intersection of the two signals with contour lines as in a topographic map. HSQC spectra show the correlation through a single bond of directly bonded hydrogen and carbon atoms. The correlation contours for methylene protons

are two at one level (except in the case of isochronous nuclei) and are differently colored from the methine and methyl protons, which correlate with one contour spot. In interpreting the HSQC spectra, all H-atoms refer to the corresponding C-atoms.

The HMBC spectrum provides information about H- and C-atoms that interact through 2, 3, 4 to 5 bonds. It does not distinguish methylene protons from methine and methyl protons. Often of the pair of methylene protons, only one heterocolores with a given carbon atom. This method also determines the locations of unprotonated carbon atoms.

NOE (Overhauser nuclear effect). It compares the ^{1}H - NMR spectrum, which was taken under normal conditions, with that taken under selective ^{1}H irradiation. If in the last spectrum there is one intense negative peak at the irradiated frequency and several less intense positive resonance signals, then the latter correspond to the protons with increased intensity caused by NNP. Thus, by precisely integrating the resonant signals, it is established which of the protons are spatially close and a distant spin-spin interaction between them is observed. For resonant signals of protons that are not close in space, the Overhauser effect is not observed and have the same intensity in both spectra. NEE is used in proving the stereochemistry of organic compounds.

NOESY (Nuclear Overhauser effect spectroscopy). NOESY correlation peaks connect only resonances from nuclei that are spatially close, not connected nuclei through chemical bonds. The correlation between the signals depends on the distance at which the protons are located, and normally a signal is observed only if the distance between them is less than 5 Å. The NOESY experiment is an important means of establishing the stereochemistry of the molecule in solution, in contrast to the X-ray diffraction analysis of a single crystal used to elucidate stereochemistry in the solid state.

4. Testing of biological activities

4.1. Antifidant activity

The biological tests were performed in petri dishes (15 x 85 mm) with coated bottoms filter paper and use of potato discs with an area of 2 cm^2 according to the methodology described by Belles and co-authors. Dissolve 3.3 mg of the substances in 1 mL of acetone, which provides a dose of 1000 ppm (33 μg / cm^2) when applying 10 μL of the solution to 1 cm^2 of the potato discs. When the solution is diluted, the corresponding concentrations of the working solutions used in the bioassays are obtained. The upper surface of the disks was treated with 20 μL acetone solutions of the test compounds with a microsyringe and after evaporation of the solvent the treated disks (TD) were obtained. Control disks (CDs) were prepared analogously to pure solvent. Scutekiprol A and clerodin, which are known for their powerful antifidant action, are used as standard. The activity is tested against larvae of the Colorado potato beetle (Leptinotarsa decemlineata Say), II - IV stage of development, collected from potato fields in Parvenets, Plovdiv region. The analyzes are performed under optional feeding conditions. Four treated and four control discs are placed in each petri dish. Newly hatched larvae in the fourth stage of development, left without food for 5 hours, are placed eight in each petri dish to feed. At regular intervals of 20 minutes for 4-6

hours, the consumed areas of the treated discs (CTD) and those of the control discs (CCD) were measured. The antifidant activity was reported by the feeding ratio FR, calculated as the ratio between the area consumed by the treated discs and the area consumed by the control discs according to the formula:

$$FR = CTD/CCD.$$

To obtain comparable results, the FR_{50} value, reported as FR when consuming 50% of the control discs (CCD_{50}), was reported. Bioassays are performed at the same temperature, humidity and the same intensity of sunlight. Testing is stopped after consuming 75% of the area of the control potato disks in each petri dish, calculating FR_{75}.

4.2. Antimicrobial activity

The antimicrobial effects of the test compounds were tested against Gram-positive bacteria Bacillus cereus (food isolate), three strains of Staphylococcus aureus (ATCC 6538, ATCC 1805 and one food isolate), Streptococcus pyogenes (ATCC 12344) and Listeria monoCCtogenes food isolate) as well as Gram-negative bacteria: three strains of Escherichia coli (ATCC 25922, ATCC 3397 and one clinical isolate), three strains of Salmonella abony (ATCC 6017, ATCC 6017 and one clinical isolate), three strains of Pseudomonas aeruginosa (ATCC 27853, one clinical isolate and one spoiled food isolate), P. fluorescens (food isolate) and Aeromonas hydrophila (food isolate), identified and systematized as published in a study by Girova et al. An additional antimicrobial study was performed against three strains of Candida albicans (ATCC 10231, ATCC 90030 and one clinical isolate). Bacterial strains were isolated from spoiled chilled food as described by Delaquis.

The analyzes were performed in the Department of Microbiology at the University of Plovdiv.

4.3. Cytotoxic activity.

Colorimetric MTT (tetrazolium) analysis. Testing for cytotoxic activity of diterpenes isolated from members of the genus Scutellaria from the Lamiaceae family was performed according to the methodology developed by Tim et al. The tetrazolium salt MTT [3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide], (Sigma Catalog № M2128) was dissolved in PBS at a ratio of 5 mg /mL and filtered to sterilize and removing the small amount of insoluble residue in some MTT batches.

Cells ($0.5 \times 10^4 \div 1 \times 10^4$) were seeded in 125$\mu$L medium in 96-well plates. It is added from the working solution of the substances to the nutrient medium of the cells so that the final concentration is 300 ÷ 150, 75, 37.5, 19 μM. The diterpenoid is expected to act on the cells for 72 hours. Discard the old nutrient medium together with the substance and add a new medium in which the MTT is dissolved. The color of the reduced MTT to furmasan crystals changes from yellow to purple. The plates were incubated at 37 °C for 4 hours. Discard the medium + MTT and dissolve the resulting furmasan purple crystals with a solution of 5% formic acid in isopropanol.

Where there are more living cells, there are more purple crystals and the intensity of the purple color is greater. Wait a few minutes at room temperature to ensure that all crystals are dissolved. The intensity of the purple staining was measured with a Dynatech MR580 Microelisa reader using a wavelength of 570 nm, a reference wavelength of 630 nm and a calibration setting of 1.99 (or 1.00 if the samples are strongly stained). Plaques are usually detected within 1 hour after the addition of isopropanol, taking into account the MTT index. The data are processed on a program (Graph Pad Prism), which calculates the IC50 - the concentration of substances and records the results in tabular form. Clean water is used for control. Four tests are performed for each concentration. The analyzes were performed at the Institute of Molecular Biology at the Bulgarian Academy of Sciences - Sofia.

Cell lines: Two cell lines were selected for screening for in vitro cytotoxic activity: carcinogenic cells from lung tumors, designated H1299, and normal cell lines, HUVEC (umbilical cord cells). Cell lines were obtained from BPS Bioscience. The experiments were performed with ready-made cells maintained in laboratory conditions under artificial environment. Purchased cells are maintained in an artificial environment (DMEM - HUVEC; RPMI - H1299) and are frequently screened.

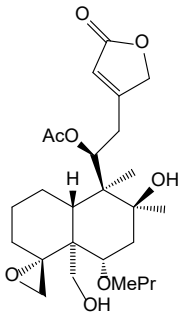
Working solutions: Samples of the test solutions were prepared by dissolving 1 mg of diterpenoids in 50 μL DMSO (dimethyl sulfoxide). To prepare the test solutions, two or three μL of the solution of the compounds in DMSO were diluted with 1 mL of culture medium so that the concentration of DMSO in the working solutions became 0.2 ÷ 0.3%. For biotests, so much of the working solutions is taken that, after further dilution in the culture medium, concentrations of 300, 150, 75, 37.5 and 19 μM are obtained in 125 μL.

RESULTS AND DISCUSSION

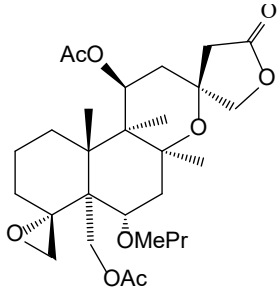
1. Determination of the structure and stereochemistry of isolated biologically active substances of species of the genus Scutellaria, Teucrium and Salvia

1.1. Phytochemical study of species of the genus *Scutellaria* for the presence of *neo*-clerodan diterpenoids

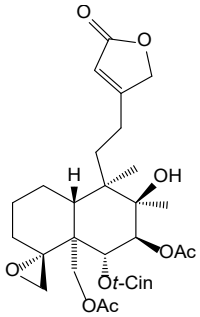
As a result of the analysis of the acetone extract obtained from the aboveground parts of *Scutellaria orientalis* subsp *pinnatifida*, the known *neo*-clerodane diterpenoids, scutorientalin B (**71**) and scutorientalin D (**162**) together with a new neo-clerodan, scutorientalin E (**388**), whose structure, 7β, 19-diacetoxy-6α (E) -cinamoyloxy-4α, 18-epoxy-8β-hydroxy -neo-cleroda-13-ene-15,16-olide, was determined by spectral studies and comparison of data with related compounds. Based on the elemental analysis, molecular formula C33H40010 was determined for **388**. Its IR spectrum shows bands for the hydroxyl group (3458 cm^{-1}), α, β-unsaturated γ-lactone (1780 and 1173 cm^{-1}) and ester groups (1746 and 1240 cm^{-1})



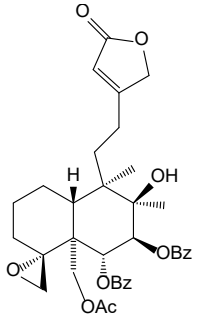
71



162



388



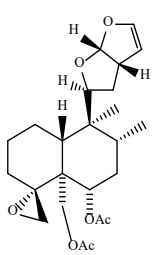
65

The ^{1}H NMR and ^{13}C NMR data of **388** (Table 1) are almost identical to those of scutalpine L (**65**) isolated from de la Torre and co-authors from *Scutellaria alpina*. The observed differences are in agreement with the (E) cinnamic acid ester present in **388**, δ_H 6.32 d, H-2'; 7.58 d, J = 16 Hz, H-3'; 7.51 m, 2H-5', 9' ; 7.38 m, 3H-6 ',7',8 '; δ_C 165.5 s C-1'; 117.5 d, C-2 '; 146.0 d, C-3'; 134.3 s, C-4'; 128.3 d, C-5 'and C-9', 128.9 d, C-6 'and C-8' and 130.4 d, C-7 ') an an acetate group at C-6 and C-9 instead of the two benzoate groups represented in **65**. In ^{1}H- and ^{13}C NMR spectra of **388** were present signals for two acetate groups at δ_H 1.99 s and 2.08 s / δ_C 169.9 s and 170.8 s and for two doublets corresponding to the axial protons at C-6β and C-7α ($\delta_{H\text{-}6\beta}$ 5.30 d ; $\delta_{H\text{-}7\alpha}$ 5.49 d, $J_{6\beta,7\alpha}$ = 10.0 Hz). The position of the (E) cinnamon ester in scutorientalin E is determined by the heterocorrelations in the HMBC spectrum between the carbonyl carbon of the cinnamon acyl residue (δ_C 165.5) and the H-6β (δ_H 5.30) proton, while the carbonyl carbons of the two acetate groups.9 (δ_C) 170.8 s) correlate with H-7α (δ_H 5.49) and H2-19 (δ_H 4.71) protons, so that the binding site of (E) cinnamon ester is at C-6 and of the two acetate groups at C-7 and C -19. The presence of cinnamon function is confirmed by the intense peak at m/z 131 in the mass spectrum of scutorientalin E. The tertiary hydroxyl group is localized at C-8 based on the shrinkage of the usual doublet for Me 17 protons in singlet and their paramagnetic displacement at δ_H 1.15 s and δ_C 21.3 q (C-17). Also in the HMBC spectrum there is a correlation from Me-17 to unprotonated oxygenated C-8 (78.6, C). The relative configuration of **388** was determined by the NOESY interactions of the axial 6β proton with H-10β, HB-18 and the acetoxy group at C-7β. On the other hand, Me-20 protons homo-correlated in the NOESY experiment with H-7α, Me-17 and H_2-19. From these correlations, it was concluded that scutorientalin E possessed the same stereochemistry as scutalpin L. From all the data described for scutorientalin E, structure **388** was assined. The absolute configuration of **388** has not been proven, but from a biogenetic point of view it can be concluded that the compound belongs to the neo-clerodan series of diterpenoids isolated from members of the genus *Scutellaria*.

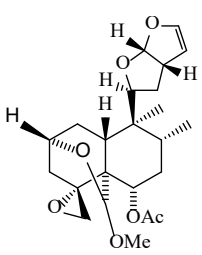
Continuing the search for insect-antifidants of plant origin in 2000, the biologically active fraction of *Scutellaria albida* was developed. Five *neo*-clerodane diterpenoids were obtained, scutalbin D (= scupolin I, **59**), scutalbin A (**9**), scutekiprol A (**42**), scutecyprol B (**57**), scutaltisin (= scutalbin C, **52**). The known compounds scutalbin A, scutekiprol A, scutekiprol B and scutaltisin were identified by comparing their physical constants (mp, R_f - value, $[\alpha]_D^{22}$), ICS and ^{1}H NMR data with authentic samples. For scutalbin D (**59**) the molecular formula $C_{24}H_{36}O_8$ was determined on the basis of quantitative elemental analysis and the molecular peak in the mass spectrum at m/z 452

[M]$^+$. Data from ^{1}H and ^{13}C NMR spectra revealed the presence in the structure of **59** of two methoxy groups (δ_H 3.33 s, 3H / δ_C 54.7 and δ_H 3.49 s, 3H / δ_C 55.2), acetoxy group (δ_H 2.10 s, 3H / δ_C 170.4 s) and the characteristic signals for a decalin system (Me-17 at δ_H 0.90 d, J = 6.1 Hz / δ_C 16.6 q; Me-20 at δ_H 1.10 s / δ_C 14.1 q) with 4α, 18 oxirane ring (δ_H 2.93 and 2.35 d, J = 4.4 Hz; δ_C 48.9 CH_2) and hexahydro furofuran nucleus in the side C-11 - C-16 chain (δ_H 3.99 dd, J = 11.5, 4.7 Hz, H-11 / δ_C 48.9 CH; δ_H 2.96 m, H-13 / δ_C 40.9 CH; δ_H 5.70 d, J = 5.4 Hz, H-16 / δ_C 104.9 CH). The two methoxy groups are assigned to the carbon atoms C-15 and C 19, forming two acetal rings with characteristic signals at δ_H 5.10 s / δ_C 100.2 and 5.11 br d, J_1 = 3.5; J_2 <1.0 / δ_C 144.1, which is almost identical to the NMR data reported by Ohno in 1996, for compound **60** (no trivial name) with two ethoxy groups isolated from *S. discolor* growing in Nepal. The relative configuration of scutalbin D has been demonstrated by NOE experiments. Irradiation of the signal at δ_H 3.99 dd (H-11α) caused a positive NOE increase in the signal at δ_H 0.90 d (Me-17) (7.2%) and 1.0 s (Me-20) (8.0%), which indicates the α orientation of these groups. In addition, a noticeable NOE increase was observed between H-19 and Me-20, which revealed the β position of the methoxy group in C-19. The chemical shifts of H-15 in the ^{1}H NMR spectrum, of C-15 in the ^{13}C NMR spectrum and the NOE results indicate the β orientation of the other methoxy group (MeO-15). From the analysis of the spectral data, structure **59** was assigned to scutalbin D. After proving the structure of the isolated diterpene, it was established that it was isolated in 1997, from distributed in Spain species *S. polyodon*, by de la Torre and co-authors, with the trivial name scupolin I.The phytochemical study of the species of the genus Scutellaria continued with the study of *S. altissima* in the period from 2011 to 2014. Of this type, scutaltisin (= scutalbin C, **52**) isolated by Malakov and co-authors in 1996 from S. altissima collected around Asenovgrad and scutalsin (**55**) isolated in 1993 by Bruno and co-authors of *S. altissima* growing in Italy.

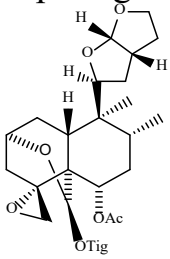
From plant material collected over the village of Balkanets, four *neo*-clerodane diterpenoids, clerodine (**1**), have been identified (isolated from *S. violacea*, common in England in 1991). (Cole et al.), scopolin H (**10**) (isolated from the Spanish species *S. polyodon* in 1997 by de la Torre et al.), scutecyprin (**25**) (isolated from *S. cypria elatior*, growing in Italy, in 1993 by Bruno et al.) As well as scutecyprol A (**42**) (isolated from another Italian subspecies, *S. cypria* var. *Cypria*, by Bruno et al., 1996). Isolated compounds **1**, **10** and **42** were identified by comparing their melting temperatures and



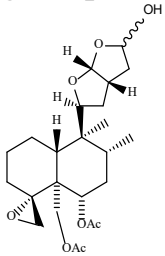
10



25



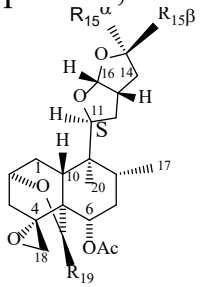
42

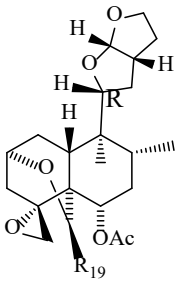


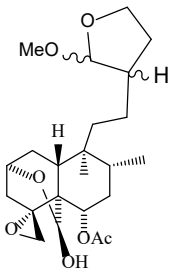
1

IR spectra with the literature data of authentic samples. Scutequiprine was first obtained in the crystalline state and its melting point was determined, which has not been published in the literature so far. The compound was identified after analysis of the 1D and 2D NMR spectra and its molecular formula **25** was formulated. After determining the structure and stereochemistry of **25**, it was found that due to 2D

experiments not performed, the data published by Bruno ^{1}H and ^{13}C NMR lack references to the signals of protons 1*α*, 1*β*, 3*β*, 7*α*, 7*β*, 8*β*, 10*β*, 12*α*, 12*β*, 14*α* and 14*β*; The signals for the carbon atoms C-5/C-9 and C-7/C-12/C-14 are not unambiguously referred to. As a result of our research, the literature has been enriched with these data for scutecyprin. A complete set of NMR spectra was recorded on a Bruker Avance II + spectrometer operating at 600.130 MHz for ^{1}H NMR spectra and at 150.903 MHz for ^{13}C NMR spectra. After careful analysis of the 2D experiments, the signal of the carbon atom C-14 was unambiguously assigned, which Bruno and co-authors did not distinguish from the signals for C-7 and C-12. ^{1}H NMR signals were also taken for all eleven protons, for which there is no data in the literature.







	R_{19}	$R_{15}\beta$	$R_{15}\alpha$
scutecolumnin C (**23**):	OH	H	H
scutaltisin B (**392**):	MeO	H	H
scutaltisin A (**52**):	OH	H,OH (15R + 15S)	
scutaltisin A (**55**):	MePrO	H,OH (15R + 15S)	
scutaltisin D (**394**):	MeO	H,OH (15R + 15S)	
scutaltisin E (**395**):	OH	MeO	H
scutaltisin F (**396**):	OH	H	MeO
scupoline I (**59**):	MeO	MeO	H

	R_{19}
11-*enu*- scutecolumnin C (**28**):	OH
scutaltisin C (**393**):	MeO

scutaltisin G (**397**)

Figure 1: Isolated *neo*-clerodane diterpenoids from *S. altissima*

Simultaneously with the described study, the bitter fraction extracted from *S. altissima* collected in the village of Bachkovo was developed. Ten *neo*-clerodane diterpenoids, scutaltisins A-G (**52**, **392** - **397**), scutecolumnin C (**23**), 11-epi-scutecolumnin C (**28**) and scupoline I (**59**) were isolated. Scutaltisin, isolated from Malakov and co-authors, is referred to as scutaltisin A (**52**). Scutaltisins B-G were found to be new compounds, and diterpenes **23**, **28**, and **59** were first demonstrated in this plant species.

The isolated resinous bitter fraction was chromatographed on a glass column on silica gel with a solvent mixture of petroleum ether / EtOAc as eluent. The combined eluates were combined into 5 fractions based on TLC results: A, B, C, D and E. Fraction B was rechromatographed on CC to give subfractions B1 (**392** + **393**); raw product B2 and B3 (flasks containing 25 mg of a very complex mixture of more polar compounds, not studied additionally). Recrystallization from acetone on B2 gave 20 mg of crude crystalline mass and 59.5 mg of residual substance (B2r). Recrystallization of the crystalline mass provided a homogeneous TLC substance, B2 (394, 16 mg). The residual material was partitioned by preparative TLC with a 98: 2 mixture of CH2Cl2 / MeOH solvents as eluent to give 2 mg of 394 and two mixtures of diterpenes B2r1 (29.3 mg) and B2r2 (11.2 mg).

In the infrared spectrum of B1 there are bands for oxirane ring (3061 cm^{-1}) and acetate group (1724 and 1262 cm^{-1}). Duplicate signals were observed in the ^1H NMR spectrum of B1 at δ_H 5.54 and 5.63 (d, H-16); 2,008 and 2,010 (s, AcO), which show the presence of a 1: 1 mixture of two basic clerodane diterpenes, together with other minor components. The ^1H and ^{13}C NMR spectra and spectral characteristics of B1 and those published by Malakov et al. for "Indivisible, with different solvents and recrystallization, 3: 7 mixture of scotecolumnin C (**23**) and its C-11 epimer (11-epi-scotecolumnin C, **28**) isolated from *S. columnae* var. *columnae*, show great affinity. Additionally, the presence of methoxy groups (δ_H 3.487 and 3.494) and the chemical shift for H-19 (δ_H 5.097 and 5.123, versus 5.72 and 5.73 of related structures **23** and **28**, similar to published values for 19-O-methyl ether derivatives such as scupolin H (**10**) and scupolin I (**59**), both with δ_H 5.11), confirmed the reference of B1 as a mixture of 19-O-methyl ethers of scutecolumnin C (**392**) and 11-*epi*-scutecolumnin C (**393**).

One wide and one minor follow-up peak, with retention times of 16.05 and 18.45 min, were observed by analytical high performance liquid chromatography of fraction B1. NMR analyzes of the fractions collected in the range of 14-17 min by preparative HPLC of B1 revealed a partial separation of the two compounds, named scutaltisin B (**392**) and scutaltisin C (**393**), (**392** moves faster than the other minor representative), allowing separate spectral ratios, and the subsequent peak was identified as scupolin I. The signals for protons from the decalin nucleus, in the ^1H NMR spectra of the two C-11 epimers **392** and **393**, are almost completely identical. A noticeable deviation of 0.15 ppm is observed only for methyl protons H_3-17. In the 11S epimer the scutaltisin B methyl group resonates at δ_H 0.90 (d, 3H, J = 6.2 Hz, H_3-17), while in the 11R epimer the scutaltisin C this signal occurs at δ_H 1.05 (d, 3H, J = 6.5 Hz, H_3- 17). In the hexahydro furofuran ring, the largest difference is in the chemical displacement of the proton at the chiral epimeric center C-11. In diterpene **392** the H-11α signal resonates at δ_H 4.07 (dd, 1H, J = 11.1 Hz, J = 5.7 Hz) while in epimeric clerodane **393** it is displaced in the strong field by 0.42 ppm at δ_H 3.64 (dd, 1H, J = 11.8 Hz, J = 4.9 Hz, H-11α). Another noticeable deviation in the spectrum was recorded for the chemical displacement of methylene protons at C-15 carbon. In compound **392** these protons were observed as a multiplet at δ_H 3.88. The methylene protons H_2-15 in epimer **393** are well separated into t and ddd. The signal for one proton is shifted by 0.09 ppm in the weak field at δ_H 3.97 (t, 1H, J = 8.3 Hz, H-15B), and the signal for the other proton is shifted by 0.08 ppm in the strong field at δ_H 3.80 (ddd, 1H, J = 13.4 Hz, J = 8.5 Hz, J = 4.8 Hz, H-15A).

In the IR spectrum of fraction B2, intense absorption bands were observed at 3380 cm^{-1} for the hydroxyl group and at 1730 and 1258 cm^{-1} showing the presence of acetate function in the molecule. Again, MeO and duplicate signals were observed in the ^1H NMR spectrum. However, the data are very close to those reported for scutaltisin (**52**). Based on ^1H and ^{13}C NMR spectral data, a conclusion was made for the presence of a C-15 epimeric mixture of scutaltisin 19-O-methyl ether (named scutaltisin D, **394**), based on the observed differences for the hemiacetal proton H-19 as the signals already described above (δ_C 5.101 and 5.112 vs. 5.72 and 5.73) and two MeO signals (δ_C 3.494, 3.496).

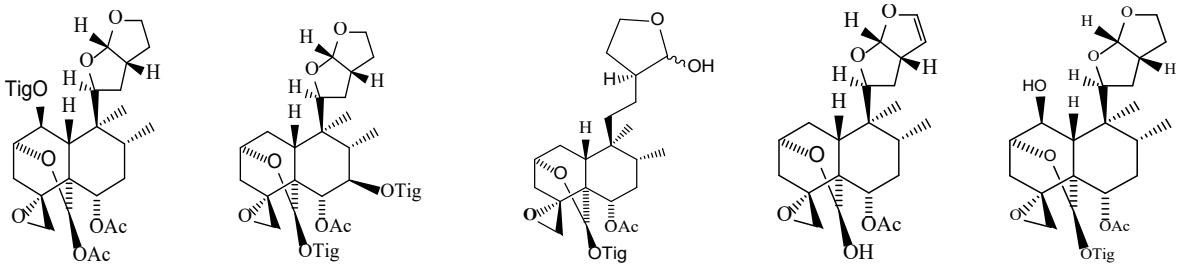
Analytical HPLC analysis of the B2r1 subfraction showed five main peaks. Preparative HPLC of the substances from the first peak resulted in partial separation of the epimeric mixture of 11-epi-scutecolumnin C (**23**) and scutecolumnin C (**28**). The structures were confirmed by IR and ^{1}H NMR spectra. From the second and third peaks are isolated two more new compounds, 15*β*-methoxyscutecolumnin C (with the trivial name scutaltisin E, **395**) and 15*α*-methoxyscutecolumnin C (= scutaltisin F, **396**). For both substances, the molecular formula $C_{23}H_{34}O_8$ was determined based on the pseudomolecular positive ion in HR-ESIMS at m/z 461.2135 $[M + Na]^+$ for **395** and 461.2164 for **396**; the calculated molecular weight for $C_{23}H_{34}O_8Na$ is 461.2151.

The diterpenes were characterized on the basis of the ^{1}H NMR spectra and the comparison of the relative spectral characteristics with the published values and the established rules for reference of the correct configuration at C-15 of 15-methoxyhexahydro furofuran *neo*-clerodane diterpenoids. Although MeOH was used in TLC / HPLC procedures in the chromatographic separation of the bitter fraction, an artifact from the tetrahydro furofuran precursor scutalbin A, which was not detected in the mixture, was excluded because no change in the spots was observed when chromatographing the substances. of TLC.

In the ^{1}H NMR spectrum of the fourth peak, the presence of signals from two OMe and one OAc groups (δ_H 3.34, 3.49 and 2.06), one doublet related to H-16 (δ_H 4.64, J = 1.8 Hz) and the specific multiple cleavage were detected for H_2-15 15 protons (H -15A: δ_H 3.86, td; H-15B: δ_H 3.93, td). ^{1}H NMR data correspond to structure **397** (named scutaltisin G) derived from the scaltaltisin A decalin nucleus and an open side chain as reported in the literature for scuterpenoside A1-A4 (**158-161**).

Seventeen compounds were isolated and spectrally characterized from *Scutellaria altissima*. Of these, 14 are *neo*-chlerodane diterpenoids: clerodin (**1**), scupolin H (**10**), scuteqiprine (**25**), scutecyprol A (**42**), scupoline I (**59**), 11-epi-scutecolumnin C (**28**), scutaltisin ≡ scutaltisin A (= scutalbin C, **52**) and scutecolumnin C (**23**) were proven for the first time in this species, and the other six clerodanes, scutaltisin B (**392**), scutaltisin C (**393**), scutaltisin D (**394**), scutaltisin E (**395**) scutaltisin F (**396**) and scutaltisin G (**397**) are new natural organic compounds. All diterpenes contain a 4α,18 epoxy ring spiro-linked to the decalin core. With the exception of clerodin and scutecyprol A, a 2α,19 hemiacetal or acetal ring is present in the molecules of the compounds. In ten of the diterpenoids a hexahydrofurofuran ring is formed in the C 11 – C-16 substructure, in two diterpenoids the ring is tetrahydro furofuran, due to the presence of a double bond between C-14 and C-15 and in one diterpenoid a lactol ring enclosing the C -13 to C-16. The other three compounds, globularin (**391**), *β*-sitosterol (**390a**) and stigmasterol (**390c**) were also identified for the first time in *S. altissima*

The research of *Scutellaria galericulata* began in 2012 with the collection of plant material from the habitat plant near the town of Lovech. The bitter resin obtained as described was separated into five homogeneous fractions by chromatography on a column packed with silica gel eluting with a 3: 2 ÷ 1: 1 hexane / EtOAC mixture. After recrystallization from acetone, five crystalline *neo*-clerodane diterpenoids, 18 mg of 14,15-dihydrogodrelin T (**22**), 10 mg of scutegalin A (**27**), 15 mg of scutegalin D.



22 **27** **155** **9** **398**

Figure 2: Isolated *neo*-clerodane diterpenoids from *S. galericulata*

(**155**), 16 mg of scutalbin A (**9**) were separated and 15 mg of ajugapyrin A (**398** ⟹ neoajugapyrin A, **399**). The compounds were identified by comparing their melting points and IR spectra with data published in the literature for these substances. Ajugapyrin A (**398**) has been proven by Boneva and co-authors in the Bulgarian species *Ajuga pyramidalis*.

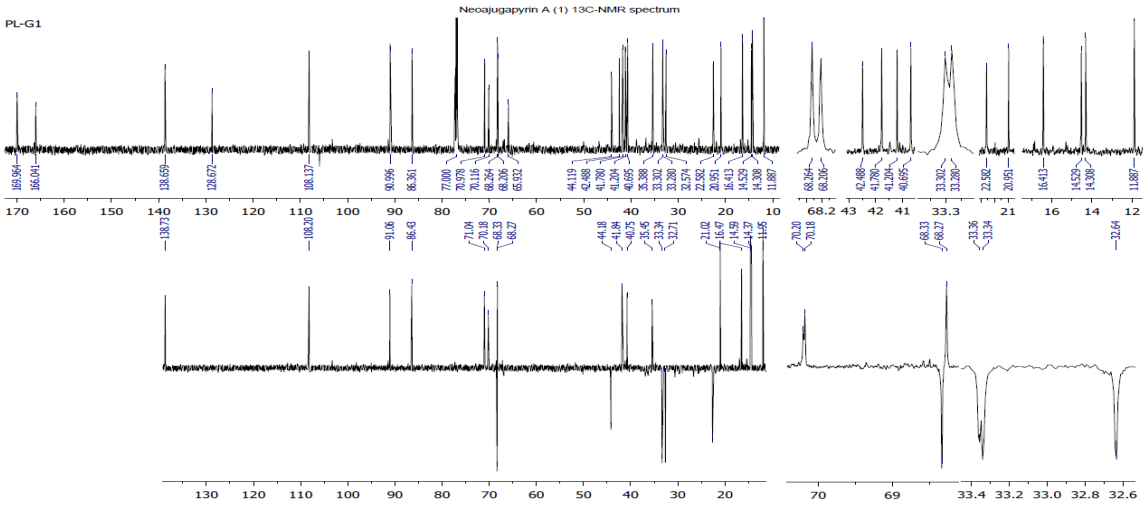


Fig. 3: ^{13}C NMR and DEPT 135 spectrum of ajugapyrin A (**398** ⟹**399**)

In the present work, diterpene **398** was first isolated as a genus *Scutellaria*, which is evidence of the chemotaxonomic proximity of the two genera. The ^{1}H NMR spectrum of **398** recorded at 250 MHz is completely identical (in terms of chemical shifts and

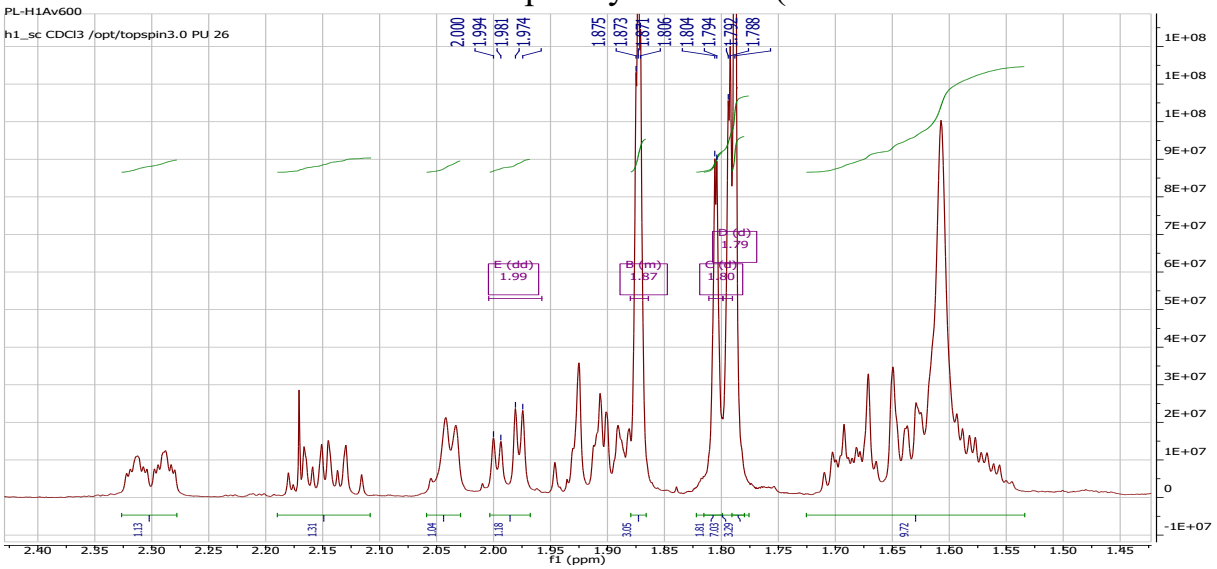
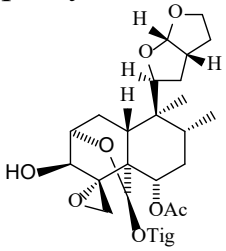
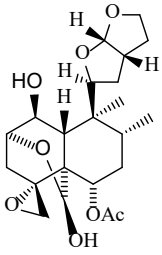


Fig. 4. ^{1}H NMR spectrum of ajugapyrin A (**398** ⟹399) with extended region for proton H-10

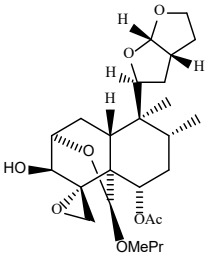
signal shape) to the spectrum of ajugapyrin A taken by Boneva under the same conditions and of the same apparatus. Additionally, the spectrum measured at 600 MHz makes it possible to adjust the ratios of some signals and the values of some *J* constants. ^{13}C NMR data for ajugapyrin A are not available in the literature. To facilitate the identification of **398** in subsequent isolations, ^{13}C NMR spectra were recorded. It contains 27carbon signals and the DEPT 135 spectrum reveals 21 resonances, 6 of them for methylene CH_2 groups, 5 for methyl CH_3 groups, 10 for methine CH groups and 6 signals for unprotonated C-atoms. Some of the signals, from tiglic acid and the perhydro-furofuran ring, are related to the interpretation of the two spectra and these



399



400



26

ratios are in agreement with the data published by Bruno for related compounds. In the high-resolution ^{1}H NMR spectrum of **398**, the H-10 signal at δ_H 1.99 appears as dd (Fig. 4) while Boneva carries the signal for H-10 as d at δ_H 1.97. Multiple signal cleavage indicates the presence of two adjacent hydrogen atoms in C-1. The question arises as to the exact location of the hydroxyl group. 2D NMR experiments were performed to fully capture the ^{13}C NMR spectrum and to elucidate the exact structure of ajugapirin A. For this purpose, a larger amount of diterpene ajugapirin A was isolated from 2.8 kg of dry material from above ground parts of *S. galericulata*, collected near the city of Pleven. From it, scutecolumnin C (**23**), 53 mg of augapyrin A (= neoajugapyrin A **399**) and two new compounds, scutegalerin A (real structure **398**) and scutegalerin B (**400**), were described. The major diterpenoid, neoajugapyrin A (**399**), to which the 3*β*-hydroxyscutecyprin structure corresponds, was isolated from Boneva from *Ajuga pyramidalis* and named ajugapyrin A, but was reported as 1*β*-hydroxy scutecyprine (**398**). It was found that the previous proposed structure is not correct. From the data of the elemental analysis and the counted 27 carbon atoms in ^{13}C NMR spectrum for the compound, the molecular formula $C_{27}H_{38}O_9$ was composed. Boneva published the wrong formula $C_{17}H_{38}O_4$, probably due to a copy/paste error. ^{1}H,

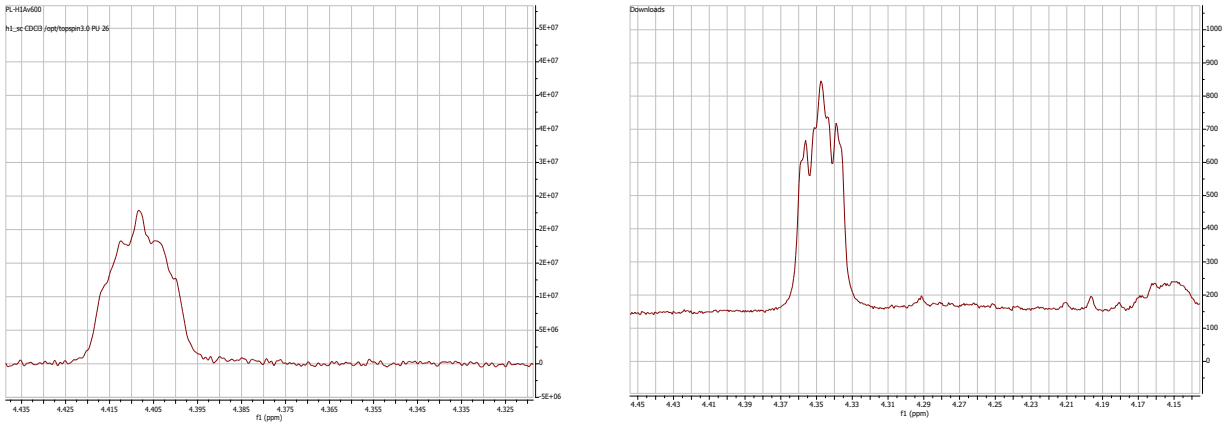
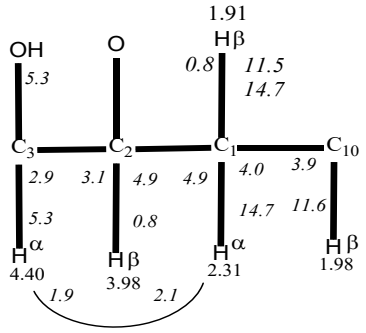


Figure 5: Contraction at δ_H 4.40, in the ^{1}H NMR spectrum of **399** after exchange with D_2O

^{13}C and DEPT 135 NMR data for hexahydro furofuran function, tigline and acetate ester, as well as for atoms C-6 to C-10 in ring B of **399**, are very close to those for the related system scetecyprine (**25**). The multiplet at δ_H 4.40 is simplified after exchange with D_2O (Figure 5), which determines the exchange of signal ratios for protons 3*α*/2*β* published by Boneva for ajugapyrin A. Detailed analysis of the HSQC and HMBC spectra for C-1 to C-5 unequivocally established the C-2-OC-19 bridge: δ_H 3.98 for H-2 [HMBC correlations with C-19, C 10 and reciprocally from H-19 to C -2], while H-3*α* (δ_H 4.40 / δ_C 70.1) shows only the reciprocal H-18 to C-3 correlation.

The spin system in ring A (H-10- [H-1*α*-H-1*β*] -H-2β-H-3*α*) is elucidated by the strong ^1H-^1H-COSY cross signals and is summarized in Figure 6. It is worthwhile to mention the strong ^1H-^1H COSY correlation and the relatively distant 4*J* constant between the H-3*α* and H-1*α* signals, which is a consequence of the flat zig-zag (W) location in the skeleton of the compound. This arrangement and distant spin-spin interaction would not be observed if the hydroxyl group is in the 3*α* position, while a 1*β* / 3*β* NOE interaction can be expected.



Фиг. 6: Спинова система в пръстен А на **399** (H-10–[H-1*α*–H-1β]–H-2*β*–H-3*α*; *J* в курсив)

In addition, the broad doublet at δ_H 2.55 relative to H-3*α* in scotequiprine (**25**) was not observed in the spectrum of **399**. These ratios, together with the signal form for H-10 (dd), are more likely to indicate the presence of an oxygen substitute in C-3 than C-1. Assumption demonstrated by careful analysis of two-dimensional NMR spectra. Sur-prisingly, ^1H NMR spectral data for the second isolated compound point to the true 1β-hydroxy scutecyprine structure (**398**). Again, two H-C-O signals are part of ring A, but now one is located at δ_H 4.38 and is associated with a δ_H 1.77 doublet (*J* = 2.9 Hz), indicating the HC (1) -HC (10) relationship. In addition, the putative HC (1) -O signal is associated with the second H-C-O multiplet (δH 4.11, dq cozy), partially overlapping with HC (11) -O (δH 4.09).The four cross peaks, in the COSY spectrum,

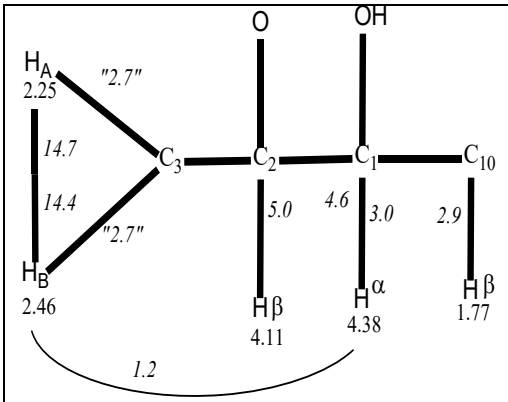


Fig. 7: Spin system in ring A of **398** (H-10—H-1*α*—H-2*β*—[H-3A–H-3B *J* in italics)

of the signal resonating at δ_H 4.11 (H-2*β*) with the signals at δ_H 2.46 and 2.25 (H_{2-3}) and of the signal at δ_H 4.09 (H-11) with the signals 1.65 and 1.97 (H_2- 12) are divided into pairs in the HSQC spectrum by correlation with δ_C 30.9 (first two) and δ_C 33.6 (last two). Therefore, they can be defined as C-3 and C 12. In addition, a correlation of the low-field de-shielded signal for H-1*α* (δ_H 4.38) with the signal for H-2*β* (δ_H 4.11) was observed.

Additional evidence in support of stereochemistry was obtained from the NOESY results: HC-(6) correlation with H_B-C (18), HC-(8) and HC-(10) determined the coefficient *β*-position of these atoms, and the interaction of HC (1) with HC (20) (oriented on the *α* side of the skeleton) reveals the *β*-orientation of the substituent at C-1.

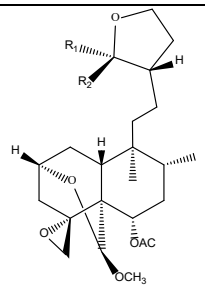
The chemical shifts and binding constants presented in were corrected in turn for those of compound **398**. The isolated 1β-hydroxy scʁtecyprin is now called scutegalerin A (**398**).

The ^{1}H NMR spectrum of the third (minor) isolated compound showed most of the data on the structural characteristics of **398**. A noticeable difference is that the signals of the crucible substituent are not available, while a change is observed for the hydroxyl group as a substituent in C-19 [HC (19): δ_H 5.61 vs. δ_H 6.68 ppm]. The compound is characterized as 1-β-hydroxy-scutecoluminin C and is named scutegalerin B (**400**).

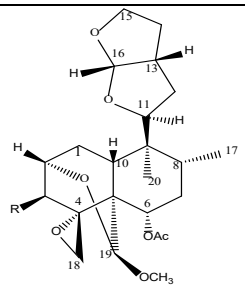
Bands for hydroxyl (3444 cm^{-1}) and acetate (1729, 1250 and 1093 cm^{-1}) groups were observed in the IR spectrum of the fourth isolated *neo*-clerodane diterpenoid. A complete agreement was found between the 1D and 2D NMR spectral data of the substance and the values for scutecolumnin C (**23**) reported in the literature. Clerodane **23** has been reported for the first time in this form.

The phytochemical study of *S. galericulata* L. continues with the search for minor neo-clerodan diterpenes. The bitter fraction obtained by developing 5.2 kg of dried and finely powdered aboveground parts of the plant by the described method was divided into five fractions, **I** (150 mg), **II** (450 mg), **III** (140 mg), **IV** (83 mg). and V (250 mg). Four non-isolated neo-chlorodene diterpenoids, in the previous two studies of this species, were demonstrated by fractions **II** and **IV**. After re-chromatography of **II**, eluting with CH_2Cl_2, two subfractions (**IIa** and **IIb**) were obtained, each of which showed a TLC-homogeneous spot. 36 mg of scutaltisin B (**392**) were isolated from subfraction **IIa**. From **IIb**, 15.3 mg of a 1: 2 indivisible (by TLC using various solvents and mixtures of solvents or by crystallization) mixture of C 16 epimers, scutegalerin C (**401**) and scotegalerin D (**402**) were obtained. Preparative TLC of fraction **IV** (EtOAc, x 2) afforded 8 mg of a third novel diterpene, scutegalerin E (**403**). Recrystallization from acetone provided 6 mg of pure product **403**.

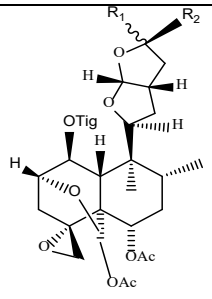
The IR spectrum of scutaltisin B (**392**) indicates the presence of absorption bands for the acetate group (1732 and 1250 cm^{-1}) and a lack of absorption for hydroxyl function and double bonding. Comparing the ^{1}H NMR spectrum of scutaltisin B with the proton spectra of isolated diterpenes from *Scutellaria altissima* (Bozov and Coll, 2015), it was found that **392** is the known 11S isomer previously obtained as an inseparable mixture with the 11R epimer, scutaltisin C. In the previous study, some of the ^{1}H and ^{13}C NMR signals were not uniquely assigned to each epimer due to the same quantitative ratio of the two diterpenoids in the mixture. Now, scutaltisin B has been isolated as a pure



	R_1	R_2
scutegalerin C (**401**):	OMe	H
scutegalerin D (**402**):	H	OMe



	R
scutaltisin B (**392**):	H
scutegalerin E (**403**):	OH



	R_1	R_2
scutegalerin F (**404**):	H	OH
scutegalerin G (**405**):	OH	H

substance and all signals in the ^{1}H and ^{13}C NMR spectra have been uniquely mapped using DEPT, HSQC, COSY and HMBC experiments. The updated ^{1}H- and ^{13}C NMR data show that in **392**, the signals at δ_C 33.45 and 33.30 are due to C-7 and C-12, respectively, and that the previously given values of δ_C 33.38 / 33.55 are incorrect. C-7 and from δ_C 34.2 / 32.4 for C-12. The chemical shift for C-9 at δ_C 41.3 was also corrected (42.47 / 41.67, previously published).

For the second isolated TCX-homogeneous substance, molecular formula $C_{24}H_{38}O_7$ was formulated, which corresponds to the negative molecular ion $[M-1]^-$ in HR-ESIMS at m/z 437.2508 (calculated for $C_{24}H_{37}O_7$, 437.2529). The peaks at m/z 407,378 and 347 are due to the fragments [M - CH3O]$^-$, [M - CH3COOH]$^-$ and [M - CH3COOH - CH3O]$^-$, which correspond to the loss of methoxy group, acetic acid and respectively methoxy group and acetic acid. Bands for the acetate group (1732 and 1250 cm^{-1}) and no absorption for the hydroxyl group were observed in the IR spectrum.

^{1}H and ^{13}C NMR spectra revealed the presence of two structurally very similar *neo*-clerodan diterpenes in a ratio of approximately 1: 2 (in the following discussion, the area of proton signals is given in parentheses so as to add 1.0 for both compounds), called scutegalerin C (**401**) and scutegalerin D (**402**). The characteristic signals for two 4α,18-epoxy-*neo*-clerodan skeletons at δ_H 0.79 s / 0.80 s (Me-20), 0.787 d / 0.791 d (Me-17), 2.33 d are easily distinguished. (1H, C-18A) and 2.92 d (0.33H, C-18B) / 2.90 d (0.67 H, C-18B). Acetate group signals at δ_H 1.97, two singles for C-15 methoxy groups at δ_H 3.451 / 3.450 (3.1 H), 3.33 (1.08 H) and 3.35 (2.07 H) were observed in the spectrum. Signals at δ_H 3.45 were assigned to methoxy groups at C-19 based on HMBC correlations with δ_C 100.3 (C-19). The two C-16 methoxils show a greater difference in the value of their chemical displacement because they are closer to the epimeric center.

The above discussion together with some other duplicate signals [δ_H 4.73 / 4.59 (d, H-16*β*); 5.05 / 5.04 (s, H-19*α*)], show an identical decalin nucleus for the compounds of the mixture, **401** and **402** with scpulines H and I [H-2*β* (δ_H 4.12dt / 4.11 dt vs. 4.104 m / 4.08 m), H- 3*α* (δ_H 2.50 dt / 2.51 dt, vs. 2.53dt / 2.54 dt), H-6*β* (4.58 dd / 4.56 dd vs. 4.62 dd / 4.62 dd), Me-17 (δ_H 0.787d /0.791 d vs. 0.89 d / 0.90 d), H2-18 (δH 2.33 d and 2.92 d / 2.90 d vs. 2.37 d / 2.35 d and 2.97 d / 2.95 d), H-19*α* (5.05 s / 5.04 s vs. 5.11 s / 5.11 s)]. The H-19*α* signals at 5.05 / 5.04 have overlapping ^{1}H-^{13}C HMBC cross peaks with δ_C 68.4 / 68.3 (C-6), 66.6 /66.5 (C-2), 60.7 (C-4), 55.2 -OCH_3), 49.8 / 49.7 (C-18, weak correlation, ^{4}J), 36.81 / 36.77 (C-3, very weak correlation, ^{4}J).

Proton H-2*β* at δ_H 4.12 / 4.11 shows HMBC cross peaks with δ_C 100.3 / 100.2 (C-19), 60.7 (C-4) and 41.0 / 41.1 (C-10) and COSY correlations with δ_H 2.50 / 2.51 3α) and 1.61 (H-3*β*). The six-membered side chain (C-11 – C-16 atoms) contains a lactol ring between C-15-C-16, as reported by Kizu and co-authors for A_1-A_4 sc$_o$terepinosides. A weak HMBC correlation was observed in compound **401** of H-13 (δ_H 1.85 / .92) with the carbon atoms C-16 (δ_C 104.6), C-14 (29.4), C-12 (δ_C 21.8) and C-11 (δ_C). 36.2) and in diterpene **402** with C-16 (δ_C 109.8), C-15 (δ_C 66.5), C-14 (δ_C 30.6), C-12 (δ_C 25.9) and C-11 (δ_C 35.6). Chemical shifts for methylene protons 12a/12b in **401** occur at δ_H 1.10 / 1.41, while in **402** they are displaced in the strong field, at 0.98 / 1.35. The signal of the isochronous protons H_2-11 appears at δ_H 1.21 in both compounds. These ratios

are supported by the HMBC correlations of H_2-11 with C-9 (δ_H 38.84 / 38.77) and ^{1}H-^{1}H COSY cross peaks with H_2-12 at δ_H 0.98 / 1.35 for **402**.

Correlations from H-12a (δ_H 0.98) to C-16 (δ_C 104.6 / 109.8), C-13 (δ_C 44.5 / 45.9), C-11 (δ_C 36.2 / 35.6) and C-14 (δ_C) were observed in the HMBC spectrum 29.4 / 30.6). The ^{1}H-^{1}H COSY spectrum showed a correlation of H-12a (δ_H 1.10 / 0.98) with H-13 (δ_H 1.85 / 1.92) and H_2-11 (δ_H 1.21). A remarkably large deviation of 4.12 ppm was observed in the C-12 signal value in both epimers, but such a deviation was consistent with scuterpinosides A_1 - A_4. values. H-13β in both epimers showed a ^{1}H-^{1}H COSY correlation with H-14α and H-16β. Diterpenoids **401** and **402** differ from each other in the configuration of the asymmetric carbon atom C-16. In the 16R epimer (**401**) this carbon appears at δ_C 104.6, and in the 16S epimer (**402**) at δ_C 109.8, giving a chemical deviation of 5.2 ppm. This deviation is very similar to that shown for scuterepinosides A_3/A_1 ($\delta_{C\text{-}16}$ 104.9 / 110.2) and scuterepinosides A_4 / A_2 (δ_C-16 105.0 / 110.3). H-16β in clerodan **401** resonates at δ_H 4.73 ($^3J_{H\text{-}16\beta,H\text{-}13\beta}$ = 4.5 Hz), and the signal for H-16β in **402** is shifted to strong field at 4.59 ($^3J_{H\text{-}16\beta,\ H\text{-}13\beta}$ = 1.7 Hz). In accordance with the Karplus equation, the higher value of the ^3J - coupling constant in **401** is determined by the dihedral torsion angle between H-16β – C-16 – C-13 – H-13β of about 0°. The lower value of the ^3J constant in **402** (1.7 Hz) is in agreement with the dihedral torsion angle between H-16α – C-16 – C-13 – H-13β close to 120°. The constants measured for scoterepinosides A_1 - A_4 were 4.0 Hz for the 16R and 1.5 Hz for the 16S isomer on average.

^{1}H and ^{13}C NMR spectra showed characteristic signals for a *neo*-clerodane structure (Me-17 at δ_H 0.88 d, J = 6.0 Hz; Me-20 at δ_H 1.11 s), intercepting 4α,18-oxirane ring (H-18A at δ_H 2.82 d and H-18B at δ_H 3.01 d with binding constant J_{gem} = 4.6 Hz), 2α,19-acetal moiety (δ_H-19A 5.05 s; $\delta_{H\text{-}2\beta}$ 3.89 br dd; $\delta_{C\text{-}19}$ 99.8) and C-11 – C- 16 hexahydro furofuran side chain [$\delta_{H\text{-}11}$ 4.08 dd, J = 11.0, 5.6 $\delta_{H\text{-}13}$ 2.83; $\delta_{H2\text{-}15}$ ca. 3,857 / 3,866 ddd; $\delta_{H\text{-}16}$ 5.62 d, J = 5.1; $\delta_{C\text{-}16}$ 108.1]. In addition, NMR data (Table 6) revealed the presence of acetate (δ_H 2.0 s, 3H; δ_C 170.4 and 21.2), methoxy δ_H 3.47 s, 3H; δ_C 55.2) and hydroxyl groups (at C-3 (δ_C 70.1). The chemical shifts of H-19α at δ_H 5.05 s and C-19 at δ_C 99.8 are in agreement with structures **401** and **402** (δ_H 5.04 and 5.05; δ_C 100.3 and 100.2). These signals are similar to the reported values for 19-O-methyl ether derivatives as published by de la Torre and co-authors for scupolins H and I, both 5.11 / 100.3. Placing the hydroxyl group in position 3β is consistent with the chemical shifts of H 18A (δ_H 2.82) as in neoajugapyrin A (**399**), scupolin G (**26**) and in scupolin J (**15**) and K (**58**), all at δ_H 2.88.

The H-18A proton resonates at δ_H 2.44 in scutecyprin (**25**), which is devoid of substituents at C-1 and C-3. In diterpenes with a C-1 substituent, the H-18A signal appears in a region close to that of scotequiprine, such as δ_H 2.46 in 14,15-dihydrojodrelin T (**22**) and δ_H 2.51 in scutegalerin A (**398**). The signal for the 3α-proton, which appears in scutecyprine at δ_H 2.55 dt, is simplified and shifted in the weak field at **403** to δ_H 4.39 br s. In the ^{13}C NMR spectrum, the C-4 carbon signal is shifted in the weak field by 5.5 ppm compared to that in scotequiprine, while the C-18 signal (which is in the γ position relative to the hydroxyl group) is shifted in the strong field by 6.3 ppm.

Derivatives with C-1 substituents, such as 14,15-dihydrojodrelin T (**22**), show a significant shift in the weak field (compared to scutecyprine) for carbon C-10 by about 7.5 ppm. Finally, the ^{1}H and ^{13}C NMR spectra of scutegalerin E were almost identical to those of neoajugapyrin A. Notable differences in the spectra of the two compounds are the presence of methoxy signals instead of those of the crucible group, as well as the chemical shift for the methoxy group at δ_H 3.47 and δ_C 55.3, the H-19 singlet at δ_H 5.05 vs. 6.76 s ($\Delta\delta$-1.71) and the offset in the weak field signal of the acetal carbon C-19 at δ_C 99.8 against 91.0 ($\Delta\delta$ + 8.8).

After chromatography with preparative TLC on the mother liquors from the recrystallization of scutegalerin E (**403**), a substance giving a homogeneous TLC stain was obtained. In the recorded IR spectrum, absorption bands of carbonyl (1734, 1712 and 1269 cm^{-1}) and hydroxyl groups (3422 cm^{-1}) were observed. No absorption for double bond. From the positive pseudo-molecular ion peak $[M + Na]^+$ in the HR-ESIMS spectrum of the isolated substance at m/z 587.2460, the molecular formula $C_{29}H_{40}O_{11}$ was composed. The calculated molecular weight for $C_{29}H_{40}O_{11}Na$ is 587.2438.

^{1}H- and ^{13}C NMR data revealed the presence in the molecule of a decalin nucleus bearing two acetate (δ_C 170.0 / δ_H 2.14 and δ_C 169.8 / δ_H 1.96) and tiglate groups (δ_C 166.5 / δ_H 6.81 qq). The characteristic signals for the oxirane ring between C-4 and C-18 [H_2-18 (δ_H 2.45 d and 3.05 d)] and 2α, 19 hemiacetal [H-2β (δ 4.41 m), H-19α 56.69)] are also easily observed. Although the substance gave a homogeneous TLC spot, eluting with different solvents, the ^{1}H NMR spectrum showed the presence of some well-distinguished duplicate signals [δ_H 5.50 and 5.52 (d, H-16); 5.56 and 5.46 (m, H-15); 3.06 and 2.86 (m, H-13); 3.98 and 4.55 (dd, H-13)], indicating the presence of an approximately 2: 3 mixture of two 15 epimeric *neo*-clerodane derivatives called scutegalirin F (**404**) and G (**405**).

Trans binding between the A / B rings in the decalin nucleus of the molecule is established by the signals in the ^{1}H NMR spectrum for the methyl protons CH_3-17 (δ_H 0.89 d and δ_H 0.92 d) and CH_3-20 (δ_H 1.25 s), the doublet at δ_H 2.04 (H-10) and singles at δ_H 6.69 (H-19). These conclusions are confirmed by the observed NOESY correlations H-18B / H-10, H 18A / H-2eq, H-19α / CH3-20, CH_3-17 / CH_3-20, CH_3-17 / H-11α and CH_3 20 / H 11α (Fig. 8).

In addition, hexahydro furo [2,3-b] furan ring signals were observed in the ^{1}H NMR spectrum in the six-membered C-11 – C-16 side chain [characteristic multiplet for the H-13β proton resonating in the δ_H 3.10 – 2.80 region and a doublet at δ_H 5.50 (H-16)] having a hydroxyl substituent at C-15 (δ_H 3.49 br s). All signal ratios in the ^{1}H and ^{13}C NMR spectra are in accordance with the 2D experiments - HSQC, HMBC, ^{1}H-^{1}H COZY. The ^{1}H and ^{13}C NMR spectral characteristics (Tables 7 and 8) for the atoms in the decalin substructure are almost identical to those reported for 14,15-dihydrojodrelin T (**22**). The maximum deviation for ^{13}C signals is 0.3 ppm and for ^{1}H signals is 0.03 ppm. Rodríguez and co-authors assigned the signal at δ_C 66.7 for carbon C-1 and that at 69.6 for C-2 in compound **22**, noting that they could be exchanged. The correlations, in the HSQC spectrum of scutegalirins F and G, of the signals at δ_H 5.52 with δ_C 69.8 and at 4.40 with 66.9 are grounds to unambiguously accept the signal at δ_C 69.8 for

carbon C-1 and that at δ_C 66.9 for C-2. On the other hand, the spectral data for the hexahydro furofune ring are very similar to those reported for the epimeric diterpenes 15R- and 15S-scutecyprol A. The maximum deviation of 1.4 ppm for the ^{13}C signals in these substructures is for C-11 and for 1-H signals it is 0.03 ppm for H-16. The main differences in spectral data of the epimers **404** and **405** are in the values of the signals from the hexahydro furofurane part. In the ^{13}C NMR spectrum, the largest difference is for the C-16 carbon atom. Its signal in **404** appears at δ_C 108.5, while in **405** this value is 110.3. In ^{1}H NMR spectrum the largest difference is for $H_{11\alpha}$ the chemical shift from 3.98 ppm in **404** to 4.55 ppm in **405**. These values are in agreement with the data for the epimeric 15R- and 15S-scutecyprol A.

The established configuration of the C-15 epimeric center in compounds **404** and **405** is confirmed by the correlations in the NOESY spectrum between $H_{15\alpha}$ and $H_{11\alpha}$ in epimer **404** and between $H_{15\alpha}$ and $H_{13\beta}$ in **405**.

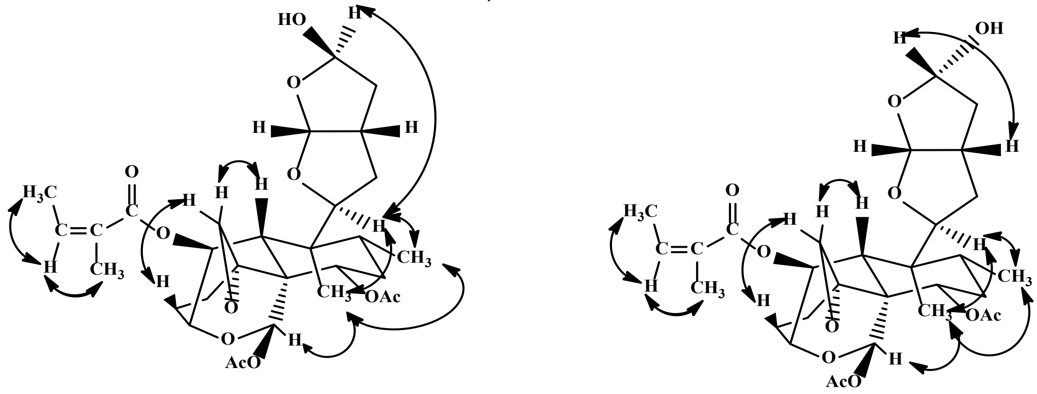
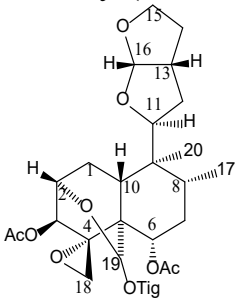


Fig. 8: Key $^{1}H\leftrightarrow^{1}H$ NOESY correlations of scotegalirins F/G **404** и **405**

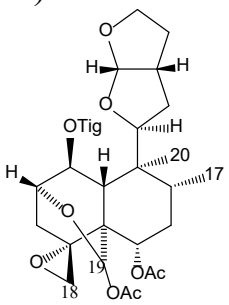
From *Scutellaria galericulata* 14 *neo*-clerodane diterpenoids were isolated and spectrally characterized. Three compounds, 14,15-dihydrojodrelin T (**22**), scutegalin A (**27**) and scutegalin D (**155**) are known for this plant. Scutalbin A (**9**), scutecolumnin C (**23**) and scutaltisin B (**392**) were obtained for the first time from this plant species. Ajugapyrin A (**398** neoajugapyrin A, **399**), first found in Ajuga pyramidalis, is a new structure for both *Scutellaria galericulata* and the entire genus *Scutellaria*. The other 7 neo-chlerodane diterpenoids, scutegalerin A (real structure **398**), scutegalerin B (**400**), scutegalerin C (**401**), scutegalerin D (**402**), scutegalerin E (**403**), scutegalerin F (404) and scutegalerin G (**405**) are new structures for science. All compounds contain 4α,18 epoxy and 2α, 19 hemiacetal or acetal ring in the decalin core. In 10 of the diterpenoids a hexahydro furofuran ring is formed in the C-11 - C 16 substructure, in one diterpenoid the ring is tetrahydro furofuran, and in three diterpenoids a lactol ring is formed between the C-15 / C-16 atoms.

From the aboveground parts of *Scutellaria velenovskyi* Rech. fil, collected in 2016 in the Mezek region, two *neo*-clerodane diterpenoids, the known 14,15-dihydrogodrelin T (**22**) and the new compound scutevelin A (**406**), were isolated. Both compounds have very similar R_f TLC values. The IR spectra of the two compounds, which are almost identical, reveal the presence of acetoxy groups characterized by absorption bands at 1740 cm^{-1} and a tiglate ester identified by the strong absorption for carbonyl function at 1715 cm^{-1} in combination with the intense band for conjugated double bond at 1653 cm^{-1}.

A molecular formula $C_{29}H_{40}O_{10}$ was formulated for both substances, which corresponds to the spectra of the positive pseudo-molecular ion peaks observed in HR-ESIMS $[M + Na]^+$ at m/z 571.2542 for compound **406** and 571.2515 for **22**, respectively (calculated for $C_{29}H_{40}O_{10}Na$: 571.2519).



406



22

Consistent with the observed absorptions in the infrared spectra for the hydroxyl and ester groups, the mass spectrum shows fragment ions at m/z 489, 449 and 389 corresponding to the loss of acetoxy, tigloyloxy group or both, respectively. A noticeable difference in the spectra of the two compounds is that in the spectrum of **406** the most intense fragment ion is at m/z 449, while in **22** the signal is most intense at m/z 389. The presence of said substituents in the molecules of **406** and **22** is confirmed by the typical signals corresponding to tigloyloxy and acetyloxy residues observed in the ^1H and ^{13}C NMR spectra. The signal shifted in the weak field at δ_H 7.07 / 6.81 (1H, qq, J = 7.1, 1.1 Hz /7.3, 1.8 Hz, H-3'), the signals for methyl groups at δ_H 1.80 / 182 (3H, br d, J = 7.6 Hz, H_3-4'), 1.88 / 1.81 (3H, br s, H_3-5') and carbon signals at δ_C 166.0 / 166.34 (C = O), 128.50 / 128.14 (C-2'), 137.8 / 137.77 (C-3') indicate the presence of a tigloyl function (Table 9). Signals for two acetate groups appear at δ_H 2.05 / δ_C 169.48 and δ_H 1.78 / δ_C 169.98 for compound **406** and at δ_H 2.14 / δ_C 169.9 and δ_H 1.97 / δ_C 169.8 for compound **22**. In addition, signals for three geminal protons with ester groups present at δ_H 4.64 (1H, dd, J = 11.1, 4.6 Hz), 5.38 (1H, t, J = 2.2 Hz) and 6.68 (1H, s) in the ^1H spectrum of **406** and at δ_H 4.65 (1H, dd, J = 11.1, 4.6 Hz), 5.36 (1H, t, J = 2.2 Hz) and 6.77 (1H, s) in the spectrum of **22**. The characteristic signals for the two 4α,18-epoxy-*neo*-clerodane skeletons are easily distinguished at δ_H 1.14 s / 1.24 s (Me-20), 0.89 d / 0.89 d (Me 17). ^1H and ^{13}C NMR spectral data of 14,15-dihydrogodrelin T (**22**) coincide in all respects with those of the authentic sample and with the data reported in the literature. The ^1H NMR spectrum of scutevelin A showed all the structural features characteristic of **22** with the expected differences for the signals corresponding to ring A in the decalin nucleus and the C-4 / C-18 oxirane fragment. For example, signals from the AB quartet corresponding to the two C-18 hydrogen atoms were observed at **22** at δ_H 2.45, (1H, d, J = 4.6 Hz, H-18A) and 3.05 (1H, d, J = 4.7 Hz, H-18B) shifted to **406** at δ_H 2.88 (1H, d, J = 4.3 Hz, H-18A) and 2.91 (1H, d, J = 4.3 Hz, H-18B). Such signal collection for protons 18A and 18B is characteristic of compounds that have an electronegative substituent at the third position. Another deviation of the signals in the ^1H NMR spectra of **406** and **22** is the C-5 / C-19 ether bond (δ_H 4.07 / 4.42, H-2β m) displaced by 0.35 ppm in the weak field.

The signals from the hexahydro furofurane substructure and from ring B of the decalin core coincide completely. The measured ^{13}C NMR spectra of the two

compounds showed 29 signals and the DEPT experiment revealed 23 resonances for six methyl, six methylene, ten methine (one of which is olefinic) and seven unprotonated carbon atoms (one olefinic and three carbonyl). Based on the discussed data, it was assumed that compound **406** is a positional isomer of **22** with a *β*-ester group at carbon C-3 instead of C-1. This assumption is confirmed by comparing their ^{13}C NMR spectra with those of scutecyprine, compound without substituents in ring A, as described in characterizing the structure and stereochemistry of neoajugapyrin A (p. 27). In the ^{13}C NMR spectrum of **406**, the signal for the C-4 carbon atom is shifted in the weak field by 2.89 ppm, while that for C-18 is shifted in the strong field by 5.65 ppm. In the ^{13}C NMR spectrum of **22**, the value of these signals did not change, but the chemical shift of carbon C-10 shifted to the weak field by 7.42 ppm. The assumption that C-3 is an oxygenated methine carbon atom is consistent with the observed correlations: in the HSQC spectrum, the triplet at δ_H 5.38 ppm (H-3*α*) interacts with the signal at δ_C 71.69; in the HMBC spectrum, the signal at δ_H 5.38 hetero-correlated with δ_C 63.5 (C-4); in the ^{1}H-^{1}H COZY spectrum, correlations from H-3*α* to protons H-1*α*, H-2*β* and H-18B were observed.

Finally, the site of attachment of the tigloyloxy and the two acetoxy groups to the *neo*-clerodane backbone in **406** must be unambiguously established. The resonance for the carbonyl group at δC 169.95 (C-3′) and the weak-field resonating doublet of doublets at δ_H 4.64 (*J* = 11.1, 4.6; H-6*β*) relative to the methine proton at the oxygenated carbon atom are very common structural characteristics for clerodane diterpenoids with acetate substituent at C-6 at the *α*-position, as at 14,15-dihydrojodrelin T. This conclusion is also supported by the HMBC correlations from H-6 to C-4 (δ_C 63.49), C-5 (δ_C 42.51), C-7 (δ_C 33.40), C-19 (δ_C 90.70), C = O (δ_C 169.95) and ^{1}H-^{1}H COSY correlations of H-6 with H-7α and H-7β. The binding site of the tiglate group is C-19, determined based on the HMBC correlation of H-19 with C-1'. The cross-signals of H-1 with H-11, H$_3$-17, H$_3$-20 and H-19 with H$_3$-20 observed in the NOESY spectrum show that these protons lie in one plane and are *α*-oriented. Accordingly, the NOESY correlations of H-6 with H-8 and H-10 reveal its *β*-position.

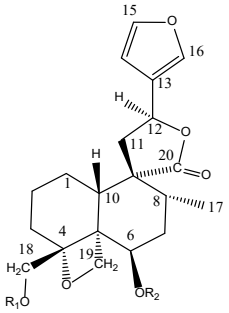
1.1.1. Summary of the results of the phytochemical study of the species of the genus *Scutellaria*

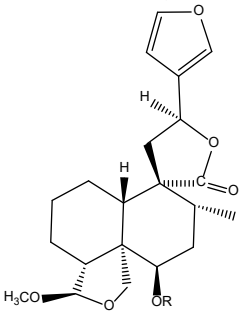
Of the species of the genus *Scutellaria*, 41 secondary metabolites were isolated, of which two sterols, two iridoids glycosidically bound to glucose, two cleroindicines, and 35 *neo*-clerodane diterpenoids. With new structures for science are 15 diterpenoids. Neoajugapyrin A is a new diterpene for the genus *Scutellaria*. Ten compounds are new to the plant species from which they were isolated. In the decalin nucleus of all clerodanes there is a spiro-linked 4α,18-oxirane ring, and in **27** of the diterpenoids there is a 2α,19 oxygen bridge, in which a hemiacetal is formed (in seven compounds: **23**, **28**, **52**, **395 - 397**, **400**) or acetal (in twenty compounds: **10**, **22**, **25**, **27**, **55**, **57**, **59**, **155**, **392-395**, **397**, **399**, **401-406**). For 25 of the compounds, tetrahydro (**1**, **9**, **10**) or hexahydro (**22**, **23**, **25**, **27**, **28**, **42**, **52**, **55**, **57**, **59**, **392 - 396**, **398 - 400**, **403 - 406**) furofuran ring is formed in the side C-11 – C-16 chain. In four of the diterpenoids (**162**, **384 - 386**), between the carbon atoms C-15 – C-16, a γ-lactone is concluded,

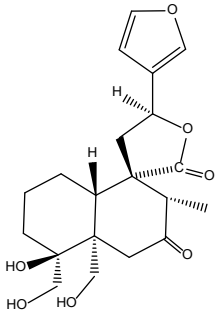
spiro-bound at C-13 with an oxane ring enclosing the atoms from C-8÷C-13. Two *neo*-clerodane diterpenoids (**71**, **388**) showed *α*,*β*-unsaturated C-15 – C-16 *γ*-lactone, and four lactone ring, one semi-acetal (**155**) and three acetal (**397**, **401**, **402**).

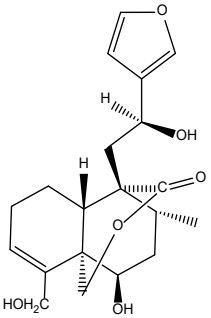
1.2. Phytochemical study of *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood and *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin. for the presence of *neo*-clerodane diterpenoids.

The bitter fraction obtained from the acetone extract of *Teucrium polium* subsp. *vincentinum* L., is divided by CC into four subfractions (**I - IV**). After further separation of **I** with TLC, compound **409**, called polivincin A, was obtained, for which the molecular formula $C_{24}H_{30}O_8$ was determined based on the pseudomolecular positive ion peak, in its HR-ESIMS, at m/z 469.1844 $[M + Na]^+$,
(calculated for $C_{24}H_{30}O_8Na$: 469.1838).









	R_1	R_2		R		
polivincin A (**409**):	Ac	Ac	polivincin B (**410**):	Ac	polivincin C (**411**)	teulamifin B (**289**)
montanin D (**312**):	H	H	teupolin XII (**317**):	H		

Absorptions for furan ring (1508, 875 cm^{-1}), *γ*-lactone and acetate ester group (broad band at 1743 cm^{-1} and second band at 1246 cm^{-1}) are present in the IR spectrum of polivincin A. The ^{13}C NMR spectrum showed the presence of twenty-four carbon atoms, and the DEPT experiment identified three methyl, seven methylene, seven methine (three for olefinic double bonds) and seven unprotonated carbon atoms, three of which are for carbonyl groups at δ_C 171.0 and 170.0 (acetates) and 177.6 (γ-lactone). The presence of the furan ring in the structure of the molecule is confirmed by the signals at δ_C 125.0 (C-13), 108.1/δ_H 6.39 (dd, CH-14); 144.2/7.44 (t, CH-15) and 139.6/7.45 (m, CH-16) in ^{13}C and ^{1}H NMR spectra. The assignment of the olefin protons to the corresponding carbon atoms is in agreement with the data from the HSQC spectrum and the observed HMBC correlations from H-14 to C-15 and C-16, from H-15 to C-13 and from H-16 to C-14 and C-15. Additional correlations are presented in the HMBC spectrum from the H-12 proton de-screened at δ_H 5.40 (bound to carbon at δ_C 72.2) to C-13 carbon and from the H*β*-11 proton resonating at δ_H 2.33, which showed a cross peak in the HSQC spectrum with carbon at δ_C 41.5, to carbon atoms 9 (δ_C 51.9), 10 (δ_C 38.8) and 12 (δ_C 72.2). The proton H*α*-11 (δ_H 2.48) shows cross peaks with the signal at δ_C 177.7 (carbonyl C-20) and C-9. In the COSY experiment, the olefin proton at δ_H 7.44 bound to carbon at δ_H 144.2 (C-15) correlated with the methine proton at δ_H 6.39, which has a cross peak with carbon at δ_C 108.1 (C-14) in the HSQC experiment. Another methine proton, resonating at δ_H 5.40 and bound to oxygenated carbon C-12 (δ_C 72.2), correlates with the two doublets of doublets at δ_H

2.47 (Hα-11) and 2.33 (Hβ-11). The determination of the furan moiety at the 12β position is consistent with the shown cross-peaks in the NOESY spectrum of Me-17 (δ_H 0.94) with H-14 (δ_H 6.39) and H-16 (δ_H 7.45) and H-1α (δ_H 2.19) with H-12α (δ_H 5.40). The H-6 proton (δ_H 5.68 dd) homo-correlated, in the ^{1}H-^{1}H COSY experiment, with the two H_2-7 protons (δ_H 2.12 and 1.85) and interacted in the HSQC experiment with the carbon atom C-6 (δ_C 73.0), from which was established the geminal position of H-6 with acetate group. In the recorded HMBC spectrum, the methylene protons H_2-18 heteroly-correlated with the carbon atoms, C-3 (δ_C 29.7), C-4 (δ_C 86.2), C-5 (δ_C 46.7) and with the carbonyl carbon atom C-18^1 (δ_C 170.0). The methyl groups in both acetates refer to the corresponding carbonyl carbon of the HMBC correlations, δ_H 2.06/δ_C 171.0 and δ_H 2.10/δ_C 170.0. The positions of the two acetoxy groups were determined at C-6 and C-18 from HMBC correlations from H 7α to C-6^1 (δ_C 171.0, C = O), from H-18A to C-18^1 (δ_C 170.0, C = O) and H-18B to C-18^1 (δ_C 170.0, C = O).

Placing the C-6 acetoxy group in the β-position and the H-6 in the α-position is consistent with the small value of 3.7 Hz and 2.1 Hz for the dd binding constants in the ^{1}H NMR spectrum at δ_H 5.68, determining the equatorial methine proton H-6 (Table 12). This is supported by the interaction of H-6 observed in the NOESY experiment with the weakly displaced doubledublet at δ_H 4.16, which is related to one of the methylene protons (HB-19) of oxygenated C-19 (δ_C 71.7). Protons HA-19 and HB-19 HMBC heterocolor to C-4, C-5, C-6 and C-10 (δ_C 38.8) and additionally show NOESY interaction, with H-2α and H-7α, respectively. The last oxygenated, unprotonated carbon atom at δ_C 86.2 (C-4) participates in the formation of an oxetane ring in the molecule. This reference is supported by said hetero-correlations to carbon C-4 in the HMBC spectrum of HA-18, HB-18 and HB-19. ^{1}H NMR data of **409** are very close to those published by Malakov et al. For a product obtained after acetylation of montanin D (**312**) isolated from *T. montanum*. The cited data are: δ_H 2.10 and 2.05 (each 3H, s) / vs. 2.10 and 2.06 in **409** for the acetate groups; 4.05 (H_2-18) / vs. 4.06 and 4.10; 5.62 (1H, t, J = 4.0 Hz, H-6α) / vs 5.68 (1H, dd, J = 3.7, 2.1 Hz) and AB type quartet at δ_H 5.08 and 4.35 (1H each, J = 7.5 Hz, H_2-19) / vs. 4.77 (1H d, J = 8.0 Hz, HA-19) and 4.16 (1H d, J = 8.0 Hz, HB-19). The differences in the data are probably due to the resolution of the spectrometers used to measure 1H NMR spectra operating at 600.130 MHz for **409** and the cited 80, 100 and 220 MHz by Malakov for the hemi synthetic product. It is also not clear in what solvent the samples for recording the analyzes were dissolved. Cited by Malakov are $CDCl_3$ and C_5D_5N together for all measurements, without specifying the conditions for the individual compounds.

HR-ESIMS of a compound with the trivial name polyvincin B (**410**) derived from subfraction **II** showed a pseudo-molecular positive ion peak at m/z 441.1861 [M + Na]$^+$, on the basis of which the molecular formula $C_{23}H_{30}O_7$ was calculated (calculated for $C_{23}H_{30}O_7Na$: 441.1889). Absorptions corresponding to a furan ring (3089, 1506, 1083 and 875 cm^{-1}), γ-lactone (1762 and 1181 cm^{-1}) and acetate (1737 and 1246 cm^{-1}) were observed in the IR spectrum. Twenty-three carbon atoms were counted in the ^{13}C NMR spectrum, and the DEPT experiment identified three methyl, six methylene, nine methine (three from furan double bonds) and five unprotonated carbon atoms, two of which are for carbonyl groups at δ_C 177.0 (γ-lactone) and 170.0 (acetate). The putative

furan residue in the molecule is confirmed by the signals, in the ^{1}H NMR spectrum of **410**, for aromatic protons at δ_H 7.44 (brs, H-16), 7.43 (brd, H-15) and 6.38 (brs H-14).

The observed triplet at δ_H 5.33/δ_C 71.7, related to the H-12 proton, is a characteristic signal for *neo*-clerodan diterpenoids having a furan ring (in the side chain C 11÷ C-16) and γ-lactone including carbon atoms C-9, C-11, C 12 and C-20 (δ_C 177.0). These functional groups were confirmed by comparing the 1D and 2D NMR spectral characteristics of diterpenoid **410** with published data on related compounds (Table 11). The olefin methine protons H-14, H-15, H-16 and H-12 sp3 the methine proton are assigned to the corresponding carbon atoms resonating at δ_C 108.1, 144.1, 139.5 and 71.7 based on the results of the HSQC spectrum. The numerous correlations in the ^{1}H - ^{13}C HMBC spectrum from H-14 to C-13, C-15, C-16, from H-15 to C-13, C-16, from H-16 to C-13, C-14, C-15, from H-11a to C-9, C-10, C-12, C-13, C-20 and from H-11β to C-8, C-9, C-10, C-12, C-13 confirms the above C– H bonds and C-atom attitudes, including the position of the unprotonated C-13 and C-20 carbon atoms. In the COSY experiment, ^{1}H-^{1}H correlations were observed between the protons H-14 / H-15, H-14 / H-16, H-15 / H-16 and between the two methylene protons H_2-11 with H-12. For the chiral center C-12, the S configuration was determined based on the observed NOESY interactions between the doublets for the Me-17 group (3H, δ_H 0.95) and H-14 (δ_H 6.38) and H-16 (δ_H 7.44) from the furan ring. The trans binding of the cyclohexane rings in the decalin nucleus of polcvincin B is determined by the characteristic signals in the ^{1}H NMR spectrum at δ_H 2.15 for the methine proton H-10 (1H dd, J = 12.0, 6.0 Hz), the doublet at δ_H 0.95 for Me-17 (3H d, J = 6.7 Hz) and NOESY correlations of the methylene proton HA-19 with protons 1α, 2α, 3α and of HB-19 with 6α and 7α.

Signals for geminal protons with oxygen atoms resonating in the high-frequency region of the spectrum are present in the ^{1}H and ^{13}C spectra for methine proton at δ_H 5.36 (1H t, J = 2.8 Hz, H-6α) / δ_C 73.2 (CH) and methine proton at δ_H 4.46 (1H brs, H-18α) / δ_C 108.7 (CH). The latter proton hetero-correlated in the HMBC spectrum with C-3 (δ_C 26.3), C-4 (δ_C 47.1), C-19 (δ_C 70.2) and 18^{1} (δ_C 54.4). Resonances for methylene protons, also geminal with an oxygen atom, at δ_H 4.14 (1H d, J = 10.6 Hz, HA-19) and 3.96 (1H d, J = 10.7 Hz, HB-19) show HMBC cross-signals with C-4 , C-6 (δ_C 73.2) and C-18 (δ_C 108.7). The signal for methyl protons at δ_H 3.25 (3H s, MeO 18^{1}) bound to carbon at δ_C 54.4 correlates in the HMBC spectrum with δ_C 108.7 (C-18). For the protons discussed above, ^{1}H-^{1}H COSY correlations were observed: HA-19/HB-19, H-6α/H-7α (δ_H 2.24), H6α/H-7β (δ_H 1.87). The considered data, together with the characteristic methine proton signal at δ_H 2.18 (dd, J = 12.6, 3.2 Hz, H-4) reveal the presence in the structure of an acetal ring formed from C-18 to carbon C-19 hydroxymethyl and methoxy group. The methoxy group was attached at C-18 in the endo position relative to ring B of the decalin nucleus, based on the cross peak in the NOESY spectrum between H-18A/H-3α and MeO-18^{1}/H-6α. NOESY interaction of proton H-10 with H-1, H-2, H-4, H 8 and H-11 protons shows that they are all co-facial and β oriented. The acetoxy group resonating at δ_H 2.08 (3H s) /170.0 (C) and 21.4 (CH_3) was placed at the 6β position in accordance with the small value of the couple constants of the geminal equatorial 6α proton, as well as the NOESY interactions

between H 6*α*/7α, H-6*α*/Me-18[1] and H-6*α* / 19A. In addition, a methine proton multiplet at δ_H 1.78 was observed in the strong field of the spectrum, which is bound to carbon at δ_C 33.2 (C-8), correlating in the ^{1}H-^{1}H COSY spectrum with H-7*α*, H-7*β* and Me- 17. Signals for a total of seven overlapping protons were found in the range of 1.38÷1.79 ppm. The spectral data of polivincin B are very close to those of teopolin XII (**317**) isolated from *Teucrium polium* L. subsp. *polium* by Fiorentino et al. The observed differences are for the weakly shifted signal in the ^{1}H NMR spectrum for proton H-6*α*, from δ_H 4.23 in **317** to δ_H 5.36 in the spectrum of **410**, except for the additional signals in the ^{1}H and ^{13}C NMR spectra of **410** for the acetoxy group at δ_H 2.08/δ_C 170.0 (CO) and 21.4 (CH_3).

For the third new *neo*-clerodane diterpenoid, polivincin C (**411**), isolated from fraction **III**, the molecular formula $C_{20}H_{26}O_7$ of the negative molecular ion peak $[M-H]^-$ was established in the HR-ESIMS spectrum at m/z 377.1599 (calculated for $C_{20}H_{25}O_7$: 377.1600). Absorptions for hydroxyl (3439 cm^{-1}), furan (1506, 875 cm^{-1}), *γ*-lactone and carbonyl (1759 cm^{-1}, broadband) functions were observed in the IR spectrum. Twenty carbon atoms were counted in the ^{13}C NMR spectrum, and the DEPT experiment identified one methyl, seven methylenes, six methines, and six unprotonated carbon atoms, two of which were for carbonyl groups at δ_C 211.0 (ketone) and 177.9 (*γ*-lacton). The signals at δ_C 125.0 (C-13), δ_C 108.2 / δH6.39 br s (H-14), δ_C 144.1 / δ_H 7.43 dd (H-15) and δ_C 139.6 / δ_H 7.45 br s (CH-16), indicate the presence of a furan ring in the structure of the molecule. The attachment of furan protons to the corresponding carbons is in agreement with the HSQC spectrum and the observed HMBC correlations from H-14 to C-16, from H-15 to C-16 and from H-16 to C-13 and C-14. The HMBC spectrum presents additional correlations to carbon C-13 from the weak field chemical shift at δ_H 5.38, proton H-12 and from the resonant at δ_H 2.32 proton H*β*-11, which shows a cross peak in the HSQC spectrum with carbon at δ_C 41.7. Two protons, H*β*-11 (δ_H 2.32) and H-8 (δ_H 2.07) show a cross peak with the signal at δ_C 177.9 (carbonyl C-20). In the COSY experiment, the furan proton at δ_H 7.43, which is bound to carbon at δ_C 144.1 (C-15), correlates with the methine proton at δ_H 6.39, which shows a cross peak with carbon at δ_C 108.2 (C-14) in the HSQC experiment. Another methine proton, resonating at δ_H 5.38 and bound to oxygenated carbon C-12 (δ_C 72.2), correlates with the two doublets of doublets at δ_H 2.47 (H*α*-11) and 2.32 (H*β*-11). The furan ring occupies the 12*β* position in accordance with the crossed peaks of Me-17 (δ_H 0.94) with H-14 and H-16 presented in the NOESY spectrum, as well as between the protons H-11*α*/12α, H-11*α*/ H-1*α* (δ_H 2.13) and 12*α*/H-1*α*. The last proton (H-1*α*) shows NOESY interaction with signals at δ_H 4.04 (Ha-19) and 4.74 (Hb-19), which indicates its *α*-orientation. The other four signals for oxygenated carbon atoms, two unprotonated and two methylene, are related to the carbon atoms in the decalin nucleus: C-4 (δ_C 88.2, C), C-7 (δ_C 211.0, CO), C-18 (δ_C 66.6, CH_2) and C-19 (δ_C 71.8, CH_2). The ratios of the signals (Table 12) are consistent with 2D spectra.

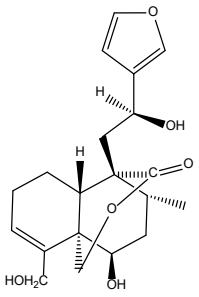
In the HMBC spectrum, cross peaks were observed from the doublets at δ_H 3.37 and 4.14, which are for hydrogen atoms bound to carbon resonating in the HSQC experiment at δ_C 66.6 (C-18), to δ_C 88.2 (C-4), 47.6 (C 5). and to δ_C 30.1 (C-3), C-4, C-5. The doublets at δ_H 4.04 and 4.74, which show in the HSQC spectrum a bond with

carbon resonating at δ_C 71.8 (C-19), have HMBC correlations, in turn to C-4, C-5 and to C-9 (δ_H 69.6). The signal for the ketone functional group at δ_C 211.0 is related to C-7 based on the observed HMBC correlations from the singlet for two protons at δ_H 2.63 (CH_2-6) to δ_C 211.0 (C-7), 30.1 (C-3), 69.6 (C-9) and from doublets of doublets at δ_C 2.17 (H-10) to δ_C 53.7 (C-6) and to 211.0 (C-7). Also, the usual multiplet for H-8, in diterpenoids with unsubstituted carbon C-7, was reduced to a quartet at δ_H 2.07. This simplified signal (H-8) shows an interaction in the ^{1}H-^{1}H COSY spectrum, with doublets for three protons at δ_H 0.94 (Me-17) and HMBC cross-peak to the carbonyl atom at δ_C 177.9 (C-20). On the other hand, Me-17 protons hetero-correlate to δ_C 32.1 (C-8) and 53.7 (C-6). The interaction between the HA-18 and HA-19 protons observed in the NOESY experiments confirmed the *α*-orientation of the H-18 hydroxy methylene group and the *β*-orientation of the tertiary hydroxyl group at C-4, respectively. Other observed NOESY interactions are H-3*α* (δ_H 1.58) /H-1α (δ_H 2.13), H-3α/H-2α (δ_H 2.14), 10*β* (δ_H 2.16)/1*β* (δ_H 1.80) and 10*β*/2*β* (δ_H 1.72), which show the orientation of the respective hydrogen atoms.

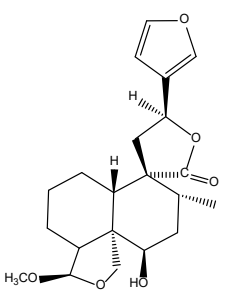
From fraction **III**, a second compound was isolated identical in all respects to the *neo*-clerodane diterpenoid teulamyfin B (**289**) isolated in 1988 by Malakov and co-authors from *Teucrium lamifolium*.

The spectral data for the *neo*-clerodane diterpenoid prouved in the last fraction **IV** are exactly the same as the published data for the isolated from *Teucrium polium* L. subsp. *polium* in 2011 by Fiorentino et al *neo*-clerodane diterpenoid, teupolin XII (**317**).

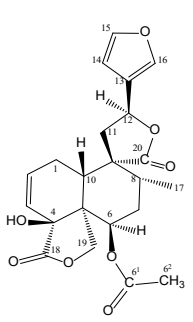
The phytochemical study of the genus *Teucrium* continued with the last unexamined representative for the presence of diterpenoids - *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin. In a qualitative TLC analysis of the bitter fraction obtained from the acetone extract of plant material from the plant, two groups of compounds (**I** and **II**) clearly distinguishable in polarity were observed. Six clerodane diterpenoids were isolated from the bitter fraction after chromatographic separation of a column packed with silica gel and eluting with petroleum ether (12 L) and a subsequent mixture of solvents CH_2Cl_2 – CH_3OH with increasing polarity with a gradient from 10:0 to 9.7:0.3. Three fraction of furoclerodene dilactones **412** - **414** with trivial names, 6-acetylteucrine F (**412**), teucrin E acetate (**413**) and 3*α*-acetoxy-teukvin (**414**) were isolated from fraction **I** by elution with a mixture of CH_2Cl_2 - CH_3OH with a gradient from 10:0 to 9.9:0.1.



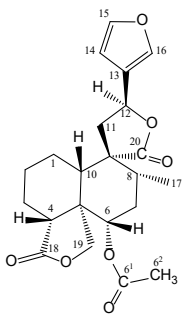
(**289**)



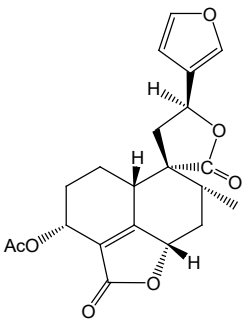
(**317**)



(**412**)



(**413**)



(**414**)

Although compounds **412** and **413** were prepared earlier, semi-synthetically by acetylation with acetic anhydride/pyridine of the corresponding hydroxy derivatives isolated from *Teucrium hamaedris*, in the present study they were obtained for the first time of plant origin. For compound **412** obtained by acetylation of teucrine F, all signals in the ^{1}H and ^{13}C NMR spectra were determined. Only the ratios for C-1 and C-2 have been adjusted. Compound **413** was only reported with ^{1}H NMR data. In order to facilitate the identification of the substances during subsequent isolation, the fully included ^{13}C NMR data are included in the work.

Based on the pseudo-molecular positive ion peak at m/z 439.1367 $[M + Na]^+$ in HR-ESIMS for compound **412**, molecular formula $C_{22}H_{24}O_8$ (calculated for $C_{22}H_{24}O_8Na$: 439.1369) was prepared showing 11 degrees of unsaturation. Absorption bands corresponding to a furan ring (3146, 1505, 875 cm^{-1}), lactone and acetate function (1761 broad bands 1178 and 1239 cm^{-1}), and a hydroxyl group (3437 cm^{-1}) were observed in the IR spectrum of the compound. Signals for 22 carbon atoms were present in the ^{13}C NMR spectrum, and the DEPT experiment identified two methyl (one for the acetate group), four methylene (one oxygenated, CH_2-19), nine methine (including three aromatics, two olefins, two oxidized) and seven unprotonated carbon atoms (classified as two quaternary, one olefinic, one oxygenated and three for carbonyl groups - one of acetate and two of γ-lactone rings. Spectral NMR data (Table 13) confirm the presence of fused diterpene in the molecule ring detected by signals in the ^{1}H and ^{13}C NMR spectra at δ_C 124.6 (C-13), 108.0/δ_H 6.41 (dd, CH-14), 144.3/7.46 (t, CH-15) and 139.6/7.47 (m, CH-16). The assignment of aromatic olefin protons to the corresponding carbon atoms is in agreement with the data from the HSQC spectrum and the observed HMBC correlations from H 14 to C-15 and C-16, from H-15 to C-13 and from H-16 to C-14 and C-15.

In the HMBC spectrum, additional correlations were observed from the signal at δ_H 5.42 t (proton H-12, which hetero-correlated in the HSQC spectrum with a carbon atom resonating at δ_C 72.1) to the carbons C-13, C-14, C-16 and from the signals at δ_H 2.57 and 2.47 (for methylene protons H_2-11, which show cross peaks in the HSQC spectrum with the carbon signal at δ_C 42.5) to C-13. In the COSY experiment, correlations were observed between H-14/H-15, H-12/H-11α, H-12/H-11β. Signals for two γ-lactone rings between C-20–C-12 and C-18–C-19 are presented in the ^{1}H and ^{13}C NMR spectra: for carbonyl groups at δ_C 177.5 (C-20) and 176.0 (C-18), for oxygenated methine and methylene groups at δ_C 72.1/δ_H 5.42 t (CH-12) and δ_C 69.0/δ_H 4.54 d and 4.12 d (CH_2-19). These conclusions are in agreement with the hetero-correlations observed in the HMBC spectrum. For C-20–C-12 γ-lactone ring, from H-11β (δ_H 2.47 dd) to C-12, from H-11α (δ_H 2.57 dd) to C-12 and C-20 (δ_C 177.5), from H -12 to C-11, from H-10β (δ_H 2.61 ov) to C-20, from the methine proton H-8β (2.13 m) to C-20 and from the two methylene protons H_2-1 (2.08 ov and 2.61 ov) to C -20. For C-18 - C-19 γ-lactone ring, from H-1β (δ_H 2.61 ov) to C-4 (δ_C 75.9) and C-5 (δ_C 47.8), from H 7β (δ_H 1.69 dt) to C-5, from H-10β to C-4 and C-5, from H-19A (δ_H 4.54, d) to C-4 and C-18 (δ_C 176.0) and from H-19B (δ_H 4.12, d) to C-18. In the COSY spectrum, cross peaks between H-11α/ H-12β are observed. The binding of the acetyloxy group to C-6 was established from the HMBC correlations from H-6α (δ_H 5.41, dd) to 6^1 (δ_C 170.2,

C = O) and from 6^2 (δ_H 2.04, 3H) to C-6 (δ^C 68.0). Placing the acetyl ester at the *β*-position and H-6 at the *α*-position agrees with the small value of 4.2 and 2.0 Hz for the couple constants of dd at δ_H 5.41, in the ^{1}H NMR spectrum, due to the methine proton H-6. This conclusion is confirmed by the observed interactions in the NOESY experiment of H-6 with the displaced in weak field doublet at δ_H 4.54, which was assigned to one (H-19A) of the methylene protons of oxidized C-19 (δ_C 69.0). The two olefinic carbons resonating at δ_C 129.9 and 125.6 are methine atoms. This fact determines the possibility of the double bond being formed between C-1 – C-2 or between C-2 – C-3. Based on the multiplicity and value of the coupling constants (Table 13) of the H-3 signal (dq, J = 9.7, 1.3, <1.0), we concluded that the Δ^2 olefinic bond is represented in the decalin ring of compound **412**. In the case in the presence of a Δ^1 double bond, the H-1 signal, which is equivalent to H-3 in compounds with a Δ^2 olefinic bond, will be more complex due to the presence of an additional neighboring proton (H-10). This conclusion is confirmed by the strong HMBC correlations from H-1*β* to C-9 (δ_C 51.6), C-20 and C-11. Also, the interaction between H-1*β*/H-12*α* is presented in the NOESY spectrum. The last oxidized unprotonated carbon atom at δ_C 75.9 was assigned to C-4 based on HMBC hetero-correlations to C-4 from H-1*β*, H-10*β* and H-19A. The relative configuration of **412** was determined by the homo-correlations H-1*β*/H-12*α*, H-11*α*/H-12*α*, H-11*β*/H-14, H-11*β*/H-16, Me-17/H-11*β*, Me-17/H-14 and Me-17/H-16 observed in the NOESY spectrum, indicating that the furan ring is in the *β*-configuration. Additionally, the correlations of H-6 with H-7*α*, H-19A, H-1*α* with H-19B, and H-7*α* with H-19A show their co-relative affinity and are referred to the α-position. In the HR-ESIMS spectrum of compound **413**, trivially named teucrine E acetate, a pseudo-molecular positive ion peak was recorded at m/z 403.1757 $[M + H]^+$, which determines the molecular formula $C_{22}H_{26}O_7$, (calculated for $C_{22}H_{26}O_7H$: 403.1753). Compared to compound **412**, the molecular formula of teucrine E acetate contains two more hydrogen atoms, respectively 10 degrees of unsaturation, and one less oxygen atom. In the IR spectrum of **413**, absorptions were observed revealing the presence in the molecule of a furan ring (3147, 1506, 1113 and 875 cm^{-1}), a lactone and an acetate group (1764 broad bands, 1180 and 1240 cm^{-1}) and no absorption bands for olefinic double bond and hydroxyl group.

Signals for 22 carbon atoms are present in the ^{13}C NMR spectrum, and the DEPT experiment identified two methyl, six methylene, eight methine (three from furan double bonds) and six unprotonated carbon atoms, two of which are quaternary, one is for olefinic carbon, the last three for carbon of carbonyl groups (at δ_C 176.5 and 178.5 for *γ*-lactones and 170.8 for acetate) (Table 14). ^{1}H and ^{13}C NMR spectral data for **413** are very similar to those of diterpenoid **412**. The small differences are in the absence of olefinic double bond and hydroxyl group - characteristic signals in the NMR spectra of **412** for two olefin methine carbons at δ_C 129.9, CH/δH 6.10 ddd and δ_C 125.6, CH/δ_H 5.55 dq and for an unprotonated carbon atom at δ_C 75.9 (C-4) do not appear in the NMR spectra of **413**. Instead, characteristic signals were observed for the C-4/H*β* methine group at δ_C 46.01, CH/δ_H 2.18 br s. The relationships made are consistent with the data from the HSQC spectrum. H-6*β*/8β and H-6*β*/10*β*. The furan ring is placed in the *β*-position according to the correlations of H-12*α* with H-1*β* and H-11*α* observed in the

NOESY spectrum, of H-11*β* with H-14 and H-16 and of Me-17 with H-11*β*, H -14 and H-16. The interactions of hydrogen H-6 with H-4, H-8 and H-10 show that these protons lie in one plane and are *β*-oriented. On the other hand, the interactions of H-7*α* with H-19a, H3-6^2 and H_3-17 and of H-19b with H-2*α* emphasize their *α*-position. Based on the considered spectral data for teucrin E acetate, structure **413** was assigned.

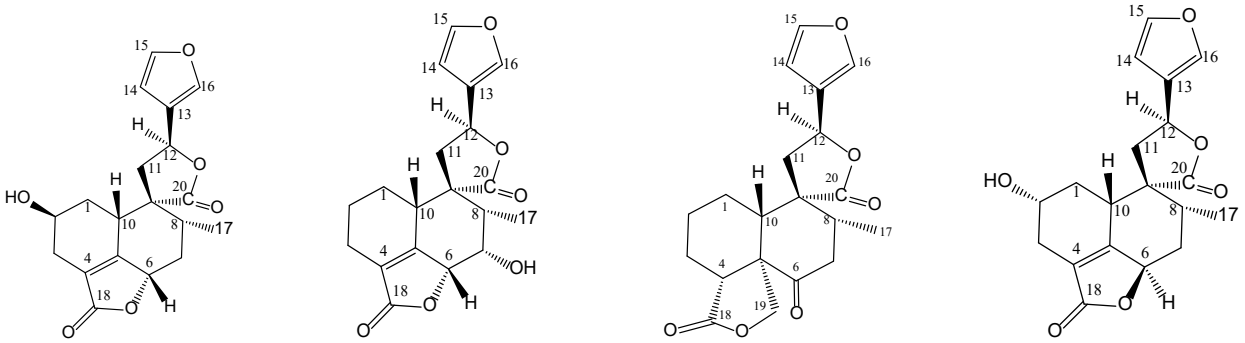
For the third isolated diterpene, from *Teucrium scordium* L. subsp. *scordioides*, 3α-acetoxy-teucvin (**414**) established molecular formula $C_{21}H_{22}O_7$ from the pseudo-molecular positive ion peak, in its HR-ESIMS spectrum, at m/z 403.1757 $[M + Na]^+$, (calculated for $C_{21}H_{22}O_7Na$: 403.1753). The molecular formula shows 11 degrees of unsaturation. The odd number of carbon atoms suggests a structure with a 19-*nor*-clerodane backbone or the presence of a methoxy group in the molecule. In the IR spectrum of **414**, absorbances were observed revealing the presence in the molecule of furan (3146, 1506, 1069, 875 cm^{-1}) and lactone ring and acetate group (1761 broad band, 1178 and 1240 cm^{-1}). In the ^{13}C NMR spectrum, signals were presented for 22 carbon atoms, which were classified by the DEPT experiment into two methyl, four methylene, eight methine (three of which are aromatic and three oxidized) and seven unprotonated (including three carbonyl at δ_C of 174.9 *γ*-lactone, 170.7 of *α,β*-unsaturated γ-lactone, 170.5 of acetate, three olefins at δ_C 124.1, 124.7, 167.5 and one quaternary at δ_C 53.6) carbon atoms. No characteristic methoxy group signals were observed in the ^1H and ^{13}C NMR spectra. The spectral data described identify compound **414** as a diterpenoid with a 19-*nor*-furocleroda-dilactone backbone with one acetyloxy substituent. The spectral data for the C-11 - C-16 substructure are very close to that for the diterpenes **412** and **413**. The signals at δ_C 124.7 and 167.5, for unprotonated olefinic carbon atoms, refer to C-4 and C-5. They are part of *α,β*-unsaturated *γ*-lactone concluded between C-18 and C-6. Further correlations up to C-6 of the methylene protons H_2-1 and of the methyl protons H_3-17 are presented in the spectrum. The binding of the oxygen atom to C-6 by an *α*-bond was established by the NOESY correlation of H-6*β* with H-10*β*.

The location of the acetyloxy group at C-3 was determined based on the ^1H-^1H COSY heterocorrelations of the geminal acetate group proton H-3*β* (δ_H 5.601) with H-6*β* (δ_H 4.815) and with H-10*β* (δ_H 2.685). This conclusion is supported by the HMBC correlations from H-1*α* (δ_H 2.13) to C-3 (δ_C 60.39) and from H-2*α* (δ_H 2.16) to the methyl carbon of the acetyloxy group C-3^2 (δ_C 21.17). The beta orientation of the substituent is determined by the low value of the coupling constants of the signal (br s) for the equatorial H-3 when it is connected to the adjacent protons H-2*α* and H-2*β*. This assumption is also confirmed by the interaction in the NOESY spectrum of H-3 with H-2*α* and H-2*β* indicating that H-3 is in the equatorial position, in this case *β*-oriented. NOESY interactions between protons H-6/H-8, H-6/H-10 and H-8/H-10 define protons H-6, H-8 and H-10 as coplanar and *β*-oriented. Compound **414** was determined as a 3*α*-acetyl derivative of teuquin (**355**) isolated from Fujita and Uchida from *Teucrium viscidurn* var. *miquelianum*.

Elution of fraction **II** with a mixture of CH_2Cl_2 - CH_3OH with a gradient from 9.8:0.2 to 9.7:0.3 isolated two homogeneous TLC subfractions **IIa** and **IIb**. After recrystallization from acetone, 12 mg of a mixture of the positional isomers scordidesin

(**415**) / teucrine A (**356**) and from substance **IIb** 16 mg of 6-ketoteuscordin (**361**) were obtained.

From the observed positive pseudo-molecular ion peak at m/z 367.1171 $[M + Na]^+$ in HR-ESIMS for subfraction **IIa**, the molecular formula $C_{19}H_{20}O_6$ (calculated for $C_{19}H_{20}O_6Na$: 367.1158). The odd number of carbon atoms in the molecule of substance **IIa** indicates the presence of a compound with a 19-*nor*-clerodane skeleton. The observed absorption peaks in IRS of IIA indicate the presence of a furan ring (1505, 1076 and 874 cm^{-1}), lactone rings (1746 shroud and 1180 cm^{-1}) and 3435 cm^{-1} (hydroxyl group). In ^1H and ^{13}C NMR spectra of substance I, different binary signals



скордидесин А (**415**) теукрин А (**356**) 6-кетотеускордин (**361**) теукрин H_4 (**345**)

were present for all hydrogen and carbon atoms, indicating the presence of a nearly 7:8 indivisible mixture of two structurally similar 19-nor-clerodane derivatives (**415**/**356**). ^1H and ^{13}C NMR data for compound **356** are identical in all respects to those of the previously diterpenoid teucrin A. In a direct comparison of ^1H and ^{13}C NMR curves of compound **356** with those published by Elmastas et al. for teucrine A show their obvious similarity. In this way the signals for teucrine A are switched off and the other signals are interpreted individually for the other component (**415**) of the mixture **I**. The same molecular formula was formulated for diterpene **415** based on both HR-ESIMS - registered under positive and negative conditions. The ^{13}C NMR spectrum revealed the presence of 19 carbon atoms, and the DEPT experiment found one methyl (δ_C 16.5, CH_3-17), four methylene, eight methine [three of which were aromatic at δ_C 140.4 (C-16), 144.4 (C-15).), 108.5 (C-14), five oxidized - at δ_C 58.2 (C-2), 77.7 (C-6), 71.7 (C-12), 144.4 (C-15) and 140.4 (C-16)] and six unprotonated [including two carbonyl, at δ_C 175.6 for γ-lactone (C-20) and 171.6 for α,β-unsaturated γ-lactone (C-18); two olefins at δ_C 126.7 (C-4) and 166.1 (C-5), one aromatic at δ_C 125.5 (C-13) and one quaternary at δ_C 53.6 (C-9)] carbon atoms. The assignment of the hydrogen atoms to the corresponding carbon atoms is in agreement with the results of the HSQC experiment. The presence of furan in the molecule was confirmed by the correlations between the signals at δ_C 108.5/δ_H 6.55 dd (CH-14), 144.4/7.62 t (CH-15) and 140.4/ 7.72 dt (CH-16). The correctness of the ratios was confirmed by the observed HMBC correlations from H-14 to C-13, C-15 and C-16, from H-15 to C-13 (125.5) and from H-16 to C-14 and C-15. Other HMBC correlations in the spectrum range from the H-12 signal (δ_H 5.62 t) to the C-13, C-14, C-16 carbons and from the H_2-11 methylene proton signals (δ_H 2.78 and 2.85) to C-13. Interactions between H-14/H-15, H-14/H-16 and H-11A/H-12 were observed in the COSY experiment. The presence of two γ-

lactone rings formed between C-20 and C-12, as well as between C-18 and C-6, was confirmed by specific signals in the ^{1}H and ^{13}C NMR spectra - for carbonyl groups at δ_C 175.6 (C- 20) and 171.6 (C-18) and for oxidized methine groups at δ_C 71.7/δ_H 5.62 t (CH-12) and at δ_C 77.7/δ_H 4.88 m (CH-6). The protons are assigned to the corresponding carbons by analyzing the results of the HSQC spectrum. For the C-20 - C-12 γ-lactone ring, HMBC hetero-correlations from H -11A (δ_H 2.78 ov m) to C-12 and C-20, from H-12 to C-11 and from the methine proton H-8β (2.12 ov m) to C-20 are presented. For C-18 - C-6 α,β-unsaturated γ-lactone ring, from H-3β (δ_H 2.04) to C-4 (δ_C 126.7) and C-5 (δ_C 166.1), from methylene protons H_2-7 (δ_H 2.25 and 2.18) to C-5 and C-6, from H-8β to C-5 and C-6 and from methyl protons H_3-17 (δ_H 1.09) to C-6. The orientation of the furan ring in the β-position is determined by the NOESY interactions of the methyl protons H_3-17 with H-14 and H-16 and of H-12 with H-11A. The observed NOESY correlations of H-6β with H-8β and H-10β show the α bond between C-6 and the oxygen atom of the lactone ring. ^{1}H and ^{13}C NMR spectral data of **415** are similar to those of teucrine A (**356**). A significant difference was observed in the ^{1}H NMR spectrum for the H-6 signal (δ_H 4.93 ddd) at **356**, which was shifted to the strong field at **415** at 4.88 and had a more complex multiplet structure due to the presence of an additional neighboring proton (H-7α).

The H-7β signal at δ_H 4.09 ddd in compound **356** was not observed among the diterpene **415** signals. Instead, it appeared shifted in the weak field br s at δ_H 4.42. The hydroxyl group in **415** is attached to the oxidized C-2 (δ_C 58.1). This view is in agreement with the observed HMBC correlations - from H-1β (δ_H 1.95) to C-2 and C-10, from H-3α to C-2, from COSY correlations between H-1β/ H-2α and from NOESY interactions between H-1α/H-2α, H-1β/H-2α and H-2α/H-3α. NOESY interactions of H-2α with axial protons 2α and 3α emphasize the equatorial position of the H-2α proton. The ^{1}H and ^{13}C NMR spectral data of **415** are very close to those previously reported for teucrin H_4 (**345**). A noticeable difference was observed for the H-6 signal which appeared at **345** at δ_H 5.98 and at diterpene **415** was displaced by 1.10 ppm in the strong field at δ_H 4.88. The orientation of H-6 at the β-position at **415** is confirmed by the observed NOESY correlations of H-6 with H-8β and H-10β. Another difference, between the two compounds, was found for the methine group CH-2 and for the adjacent CH_2-1 and CH_2-3 methylene groups. In compound **345** these signals were reported at δ_C 68.6 (CH-2)/δ_H 4.44 dddd ($J_{1\alpha\ (ax),\ 2\beta\ (ax)}$ = 10.4, $J_{1\beta\ (eq),\ 2\beta\ (ax)}$ = 3.7, $J_{2\beta\ (ax),\ 3\alpha\ (ax)}$ = 9.5, $J_{2\beta\ (ax),3\beta\ (eq)}$ = 6.5) for the axial proton H-2β. The cited signals for the adjacent methylene groups are at δ_C 34.3 (C-1)/δ_H 1.96 dddd ($J_{1\alpha,1\beta}$ = 11.8, $J_{1\alpha,10\beta}$ = 10.1, H-1α), δ_H 2.76 dt ($J_{1\beta,10\beta}$ = 3.7, H-1β) and δC 30.4 (C-3) / δH 2.39 dddd (J3α, 3β = 17.9, H-3α), δH 3.00 br dd (H-3β). In compound **415**, the proton H-2 signal (δ_H 4.42 br s) shrinks compared to dddd at δ_H 4.44 in teucrine H_4 (**345**). The small value of the spin-spin interaction constants for hydrogen H-2 in **415** requires the proton to be in the equatorial position, in this case α-oriented. This finding is confirmed by NOESY interactions of H-2α with H-1α and H-3α, indicating that these protons lie in one plane and are α-oriented. Such interactions are not possible if H-2 is in the axial position, in this case β-oriented. Also, no interaction was observed in the NOESY spectrum

between H-2 and H-10β, as would be expected if H-2 were in the beta (axial) position. Based on all the data described for scordidesin A, structure **415** was assigned.

The recorded ^{1}H and ^{13}C NMR spectra revealed substance **II** as a single compound (**361**). Based on the positive ion peak at m / z 381.1308 $[M + Na]^{+}$ in HR-ESIMS for compound **361**, molecular formula $C_{20}H_{22}O_6$ (calculated for $C_{20}H_{22}O_6Na$: 381.1314) was established indicating 10 degrees of hydrogen deficiency. Absorptions for the furan ring (1504 and 875 cm^{-1}), lactone (1786, 1760, 1182 cm^{-1}) and hydroxyl group (3436 cm^{-1}) were observed in the IR spectrum. Twenty carbon atoms were represented in the ^{13}C NMR spectrum, and the DEPT experiment identified one methyl, six methylene (one oxidized, CH_2-19), seven methine (including three aromatic, three oxidized), and six unprotonated (including two quaternary, one aromatic). and three for carbonyl groups, one for ketone and two for γ-lactones) carbon atoms. The furan ring in the molecule is confirmed by the signals at δ_C 124.5 (C-13), δ_C 107.8/δ_H 6.40 (br s, CH-14), δ_C 144.5/δ_H 7.47 (br s, CH-15) and δ_C 139.7/δ_H 7.48 (br s, CH-16) in ^{13}C and ^{1}H NMR spectra. The assignment of hydrogen atoms to the corresponding carbon atoms agrees with the data from the HSQC spectrum and from the correlations in the HMBC spectrum from H- 14 to C-15 and C-16, from H-15 to C-13 and from H-16 to C-14 and C-15. Additional correlations were observed from signals at δ_H 5.44 t (H 12) to carbons C-13, C-14, C-16 and from signals for methylene protons at δ_H 2.54 and 2.46 (CH_2-11) to C-13. Homo-correlations between H-14/H-15, H-12/H-11A, H-12/H-11B are present in the COSY spectrum.

The presence of two γ-lactone rings C-20 – C-12 as well as C-18 – C-19 is confirmed by the specific signals in the ^{1}H and ^{13}C NMR spectra: for the carbonyl functions the signals are at δ_C 177.4 (C-20) and 176.8 (C-18), for oxidized methine groups at δ_C 72.1/δ_H 5.44 t (CH-12) and oxidized methylene groups at δ_C 69.3/δ_H 4.80 d and 4.49 d (CH_2-19). These ratios are in agreement with the presented HMBC correlations: for the C-20 – C-12 γ-lactone ring, from H_2-11 (δ_H 2.46 br t) to C-12 and C-13, from H-12 to C-11 (δ_C 41.0), C-13, C-14 and C-16, from H 10β (δ_H 2.86 dd) to C-20, as well as for C-18 - C-19 γ-lactone ring, from H-7β (δ_H 2.39 dd) to C-5 (δ_C 55.4), from H-19A (δ_H 4.80, d) to C-18 and from H-19B (δ_H 4.49, d) to C-4 (δ_C 49.2). In the COSY spectrum, correlations are observed between H_2-11/H-12, H_2-11/H-8, H-2β/H-4, H-3α/H-4. The ketone group (δ_C 208.1) is assigned to C-6 based on the weak field shift of the signals for methylene protons H_2-7 at δ_H 3.41 t and δ_H 2.39 dd, which have a simpler multiplet structure than the usual dt and ddd in diterpenoids with a hydroxyl group at at C-6, due to the lack of adjacent protons at C-6. This conclusion is confirmed by the observed HMBC correlations to C-6 of the protons H-7α, H-7β, H-19A and H-19B. The stereochemistry of **361** was established by the NOESY experiment. The correlations between between H-12/1α, H-12/1β, Me-17/H-14, Me-17/H-16, indicate the β-configuration of the furan ring. The observed interactions of H-4 with H-10 and of H-8 with H-10 show their coplanarity and were carried to the β-position. NOESY correlations of H-1α with H-19A, of H-7α with H_3-17 and H-19B confirm the α-orientation of these protons.

The ^{1}H spectral data of compound **361** were found to be identical to those previously reported by Papanov and Malakov for 6-ketoteuscordin. ^{13}C NMR data of 6-

ketoteuscordin have not been published to date, so they are included in Table 16 to facilitate the identification of this diterpene in subsequent isolations.

1.2.1. Summary of the results of the phytochemical study of *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood and *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin.

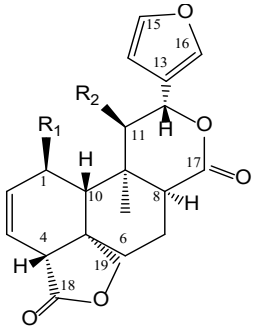
From the acetone extracts obtained from the aboveground parts of the two species of *Teucrium* were isolated 11 furoclerodane lactonediterpenoids, the new polivincins A-C (**409** - **411**), 6-acetylteucrine F (**412**), teucrine E acetate (**413**), 3α-acetoxy-4 scordidesin A (**415**) and the known teulamifin B (**289**), teupolin XII (**317**), teucrin A (**356**) and 6-ketoteuscordin (**361**). Clerodane teulamifin B was first time detected in *Teucrium polium*. Carbon spectrum of 6-ketotheuscordin has been recorded and carried on, the data of which have not been published in the literature. The structure and stereochemistry of the substances was established by in-depth spectroscopic examination. Eight of the substances have a *neo*-clerodan skeleton, 3α-acetoxy-teucvin, scordidesin A and teucrine A have a *nor*-clerodane skeleton.

No presence in the plant material of *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood of capitatin and auropolin. All five diterpenoids now isolated are different from all 11 clerodanes published by Malakov as constituents of the diterpene fraction of the species *Teucrium polium* subsp. "*polium*". The assumption that Malakov and co-authors studied *Teucrium polium* subsp. *capitatum* was confirmed.

Compounds isolated from the genus *Teucrium* are characterized by the presence of a furan ring in the molecule. It does not contain the furofuran system, which is characteristic of the species of the genus *Scutellaria* and the genus *Ajuga*. Only acetates are found in diterpenoids isolated from the genus *Teucrium*, while different ester groups are found in the *neo*-clerodanes detected in the genus *Scutellaria* and the genus *Ajuga*.

1.3. Phytochemical study of species of the genus *Salvia* for the presence of diterpenoids

Upon chromatography of the bitter fraction obtained from *Salvia splendens* Ker.-Gawl. collected in Plovdiv, on a column filled with silica gel eluting with a mixture of hexane/$CHCl_3$ (gradient from 10:2 to 2:1), four furo *neo*-cleroda dilactone diterpenoids - salviarin (**416**), splenolid A (**417**), splenolid B (**418**) and splendidin (**419**) were obtained.



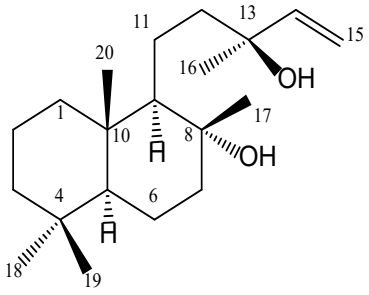
salviarin (**416**): $R^1 = R^2 = H$
splenolid A (**417**): $R^1 = OH$, $R^2 = H$
splenolid B (**418**): $R^1 = H$, $R^2 = OAc$
splendidin (**419**): $R^1 = R^2 = OAc$

The structure of the compounds was demonstrated by comparing the obtained spectral data with those published in the literature for these terpenoids. The IR spectra of the compounds are consistent with the presence of γ lactone (1780-1754 cm^{-1}), δ-lactone (1736-1714 cm^{-1}), ester groups for compounds **418** and **419** (1736 br, 1729 br, 1289, 1228 cm^{-1}) and an olefinic double bond (1653 - 1601 cm^{-1}). Spectral ^{1}H and ^{13}C NMR data for diterpenes carried out using 2D experiments - HSQC, ^{1}H-^{1}H COSY and HMBC - confirmed the structure and relative stereochemistry of the compounds.

The ^{1}H and ^{13}C NMR spectra of salviarin, splenolid B and splendidin are very similar. The presence in the spectrum of signal for only one methyl group (singlet in the range of δ_H 0.97÷1.02, CH_3-20) suggests that the carbon atoms C-18, C-19 and C-20 are oxidized and are included in the composition of γ- and a δ-lactone ring. Furocleroda dilactone skeleton with a double bond between C-2 - C-3, in ring A of the decalin nucleus, is easily detected by the observed signals in the ^{1}H and ^{13}C NMR spectra at δ_H 5.83÷5.96 (1H dddd, H-2) / δC 129.2 ÷ 129.6 (C-2) and δ_H 5.58 ÷ 5.68 (1H ddd, H-3) / δ_C 121.8 ÷ 122.9 (C-3) for the double bond; at δ_H 6.34 ÷ 6.36 (1H dd, H-14) / δ_C 108.7 ÷ 108.9 (C-14), at δ_H 7.35 ÷ 7.36 (1H t, H-15) / δ_C 144.2 ÷ 144.7 (C-15), at δ_H 7.40 ÷ 7.43 (1H dd, H-16) / δ_C 140.0 ÷ 142.1 (C-16) and at δ_C 121.9 ÷ 125.2 (C 13) for the furan ring; at δ_H 2.73 ÷ 2.82 (1H dddd, H-4β), 4.20 ÷ 4.32 (1H d, 19A), 4.09 ÷ 4.14 (1H dd, 19B) / δ_C 51.8 ÷ 53.3 (C-4), 41.8 ÷ 43.4 (C- 5), 174.4 ÷ 175.8 (C-18) and δ_C 70.4 ÷ 70.9 (C 19) for γ-lactone and at δ_H 5.22 ÷ 5.83 (1H, dd, H-12β), 2.41 ÷ 2.74 (1H ddd, H-8β) / δ_C 49.5 ÷ 50.6 (C-8), δ_C 169.5 ÷ 171.8 (C-17) and δ_C 70.4 ÷ 71.9 (C-12) for δ-lactone.

The compounds differ in the substituents on the carbon atoms C-1 and C-11. In the ^{1}H and ^{13}C NMR spectra of **416** there are no signals for the acetate group and no signal in the weak field for a geminal proton of hydroxyl or acetate group is observed. In the proton spectrum of the splenolid A, the geminal proton H-1α resonates at δ_H 4.29 (1H dddd, J = 2.4, 2.0, 2.1, 10.0) and a shifted in weakly field signal for an oxygen-bound carbon atom at δ_C 66.15 is observed (C-1). In the ^{1}H NMR spectrum of splenolid B (**418**) there is a singlet for three protons at δ_H 1.94, corresponding to the resonance for methyl protons from the acetate group and offset in the weak field signal for the geminal to acetate proton at δ_H 5.14 (1H d, J = 10.8 , H-11). Respectively, additional signals appear in the ^{13}C NMR spectrum at δ_C 169.4 (***C*** = O) and 21.2 (***C***H_3).

In the ^{1}H NMR spectrum of splendidin (**419**) there are two singles, each for three protons, at δ_H 2.00 and δ_H 1.86 corresponding to two acetate groups in the molecule and respectively the signals in the weak field for two protons at δ_H 5.44 (1H dddd, J = 2.6, 1.3, 2.6, 9.3, H-1) and δ_H 5.33 (1H d, J = 11.2). Additional signals were observed in the ^{13}C NMR spectrum at δ_C 170.4 (***C*** = O) and 22.2 (CH_3) and at δ_C 170.1 (***C*** = O) and 20.4 (CH_3). Isolated from *Salvia splendens* Ker.-Gawl. compounds have not been isolated from Bulgarian plant species so far.



sclareol (**420**)

Two known compounds, *β*-sitosterol and the labdan diterpenoid sclareol (**420**), have been isolated from the aboveground parts of *Salvia nemorosa* L. For compound **420**, molecular formula $C_{20}H_{36}O_2$ corresponding to the positive pseudo-molecular ion peak in HR-ESIMS at m/z 331.2616 $[M + Na]^+$, (calculated for $C_{20}H_{36}O_2Na$, 331.2613) was prepared. The IR spectrum shows absorptions for hydroxyl groups at 3430 cm^{-1} and for olefinic double bond at 1642 cm^{-1}. There are no spectral bands for the carbonyl functional group which is confirmed by the ^1H NMR spectrum. ^1H broadband-decoupled ^{13}C NMR and DEPT-135 spectra of **420** revealed signals for 20 and 16 carbon atoms, respectively, 5 for CH_3, 8 for CH_2, 3 for CH groups and 4 for unprotonated C atoms, which reveals the diterpene nature of the substance. One CH_2 group resonates at δ_C 111.2 (C-15) and one olefin methine CH at δ_C 145.9 (C-14), from which follows the presence in the structure of the vinyl group compound in the C-11 – C-16 carbon chain side. The latter group was confirmed by the ^1H-^1H COSY signal correlations at δ_H 5.93 (H-14) with δ_H 5.22 (H_{trans} -15) and with δ_H 5.02 (H_{cis}-15). In addition, the multiple signal structure at δ_H 5.93 (1H, dd) indicates that the vinyl group is attached to an unprotonated carbon atom C-13. From this conclusion and from the resonance of C-13 in the weak field at δ_C 73.6, it follows that it is associated with a tertiary hydroxyl group. The three olefin protons hetero-correlirat in the HMBC spectrum with δ_C 73.6 (C-13). The signal at δ_H 5.93 (H-14) additionally showed two strong HMBC correlations with δ_C 44.9 (C-12) and δ_C 27.2 (CH_3-16). The signals at δ_C 73.6 and δ_C 74.8 are due to tertiary carbon atoms attached to a hydroxyl group, which is consistent with the two oxygen atoms "O_2" in the molecular formula. Only singlets for the five methyl groups were observed in the ^1H NMR spectrum, indicating that they were attached to unprotonated carbon atoms. Three singlets, for quaternary carbon atoms, appear at δ_H 0.78, 0.78, 0.85 and two are displaced in the weak field, at δ_H 1.15 (H_3-17) and 1.27 (H_3-16), due to their geminal OH groups. The latter signal shows three strong HMBC correlations to the signals at δ_C 145.9 (C-14), 73.6 (C-13) and 44.9 (C-12), as well as very weak to δ_C 19.1 (C-11) and δ_C 111.2 (C-15). All mentioned interactions confirm the accepted C-13 position of one of the hydroxyl groups. Another methyl signal at δ_H 1.15 shows three strong HMBC correlations up to δ_C 74.8 (C-8), 61.5 (C-9) and 44.3 (C-7), which unambiguously determine the position of the second hydroxyl group at C-8 and the signal at δ_C 1.15 for methyl protons H_3-17. The assignment of all proton signals to the corresponding carbon atoms is in accordance with the HSQC experiment. The chemical shifts for unprotonated de-shielded carbon atoms at δ_C 74.8 (C-8) and 73.6 (C-13) are typical of tertiary carbon atoms attached to an oxygen atom. Based on all 1D and 2D spectral data, the structure of sclareol (**420**) was assigned to the isolated compound. The proposed formula for **420** is confirmed by comparing its spectral characteristics with the published literature data on sclareol.

The acetone extract from aboveground parts of *S. amplexicaulis* LAM was studied. for the presence of diterpenoids. The extract was developed as described in the usual manner. No diterpenoids were detected.

2. Testing of the antifidant activity of extracts of species of the genus *Scutellaria* and of *neo*-clerodane diterpenoids against larvae of *Leptinotarsa decemlineata* Say

Inhibition of feeding and inhibition of the development of the larvae of *Leptinotarsa decemlineata* (Say) from extracts of eight species of the genus *Scutellaria* were studied. The treatment of potato leaves with small amounts of extracts (concentration 10000, 1000 and 100 ppm, which is a dose of 333.33 μg/cm^2, 33.33 μg/cm^2 and 3.33 μg/cm^2) showed from good to very good activity. The antifidant action of the extracts is attributed to the *neo*-clerodane diterpenoids contained in them. Kojima and Kato emphasize that the presence in the molecule of the substructures tetra- or hexahydro furofuran, in the side C-11 – C-16 chain, is a prerequisite for significant activity. Other authors attribute the activity of these substances to the decalin ring containing in them a C-4 – C-18 spiro-linked oxirane ring and two acetate groups at C-6 and C 19. Of the studied plants *S. hastifolia* and *S. orientalis*, no compounds were found to contain a tetra- or hexahydro furofurane moiety, while in other species diterpenes with such substructures were present.

Table 1. Nutritional ratios (FR) of the tested extracts

extracts	Dose (ppm)	N	FR_{50} ± SE	FR_{75} ± SE
S. alpina	10 000	6	0.13 ± 0.01	0.19 ± 0.02
	1 000	6	0.16 ± 0.02	0.21 ± 0.02
	1 00	5	0.30 ± 0.06	0.33 ± 0.05
S. galericulata	10 000	6	0.08 ± 0.01	0.12 ± 0.02
	1 000	5	0.36 ± 0.07	0.38 ± 0.01
	100	5	0.56 ± 0.02	0.60 ± 0.03
S. altissima	10 000	6	0.08 ± 0.01	0.10 ± 0.03
	1 000	5	0.32 ± 0.03	0.34 ± 0.03
	100	5	0.43 ± 0.04	0.49 ± 0.02
S. albida	10 000	6	0.04 ± 0.01	0.09 ± 0.01
	1 000	5	0.10 ± 0.05	0.17 ± 0.08
	100	5	0.22 ± 0.03	0.30 ± 0.04
S. columnae	10 000	5	0.19 ± 0.02	0.27 ± 0.04
	1 000	4	0.47 ± 0.05	0.52 ± 0.02
	100	4	0.55 ± 0.04	0.57 ± 0.02
S. velenovskyi	10 000	4	0.21 ± 0.03	0.31 ± 0.05
	1 000	4	0.41 ± 0.03	0.46 ± 0.04
	100	4	0.59 ± 0.06	0.63 ± 0.04
S. orientalis	10 000	3	0.28 ± 0.03	0.36 ± 0.05
	1 000	3	0.39 ± 0.01	0.51 ± 0.02
	100	3	0.63 ± 0.04	0.68 ± 0.04
S. hastifolia	10 000	3	0.36 ± 0.04	0.41 ± 0.06
	1 000	3	0.49 ± 0.02	0.56 ± 0.04
	100	3	0.68 ± 0.03	0.75 ± 0.05

N - number of repetitions, SE - standard error, FR = CTD / CCD x 100%, (CTD and CCD represent the consumed areas of the treated and control disks)

The results of the bioassay for antifidant activity (Table 1) of the eight plant extracts against the larvae of *Leptinotarsa decemlineata* show that most of them show good to very good action. As can be seen from the table, a low antifidanating effect was found for the extract of *S. hastifolia* (FR_{50} = 0.36 at a dose of 10000 ppm), due to the absence of *neo*-clerodanes in this form. The greatest inhibitory effect on larval feeding was found with *S. albida extract* (FR50 = 0.04), and a significant effect was observed at a concentration of 100 ppm (FR_{50} = 0.22). The extracts of *S. altissima* (FR_{50} = 0.08) and *S. galericulata* (FR_{50} = 0.08) have very good activity, followed by *S. alpina* (FR_{50} = 0.13). Extracts of *S. columnae* (FR_{50} = 0.19) and *S. velenovskyi* (FR_{50} = 0.21) showed a moderate effect. Interestingly, *S. alpina* extract did not show complete inhibition of nutrition at 10000 ppm, but retained high activity at 1000 and 100 ppm. This is explained by the higher amount of *neo*-clerodane diterpenoids in this form. The results achieved in this study are a good justification for conducting phytochemical studies of members of the genus *Scutellaria* in search of new *neo*-clerodane insect antifidants.

The antifidant effect, under dietary conditions of choice, of seven natural *neo*-clerodane diterpenes isolated from *Scutellaria alpina* (scutalpin A, **384**; scutalpin E,

Table 2. Nutritional factors of *neo*-clerodanes isolated from Scutellaria alpina and *Salvia splendens*

Compound	Dose (ppm)	N	FR_{50} ± SE	FR_{75} ± SE
	1000	5	0.02 ± 0.01	0.03 ± 0.01
42	300	5	0.03 ± 0.05	0.04 ± 0.04
	30	6	0.05 ± 0.06	0.07 ± 0.03
	1000	5	0.02 ± 0.01	0.03 ± 0.01
43	300	5	0.03 ± 0.05	0.04 ± 0.04
	30	6	0.05 ± 0.06	0.07 ± 0.03
	1000	5	0.03 ± 0.01	0.04 ± 0.01
384	300	5	0.05 ± 0.04	0.05 ± 0.02
	30	5	0.07 ± 0.02	0.04 ± 0.02
	1000	5	0.04 ± 0.02	0.05 ± 0.02
385	300	5	0.05 ± 0.03	0.06 ± 0.03
	30	5	0.07 ± 0.06	0.08 ± 0.03
	1000	5	0.08 ± 0.06	0.11 ± 0.05
386	300	5	0.14 ± 0.21	0.19 ± 0.03
	30	5	0.24 ± 0.01	0.32 ± 0.00
	1000	5	0.09 ± 0.03	0.11 ± 0.02
416	300	5	0.12 ± 0.03	0.16 ± 0.02
	30	5	0.20 ± 0.08	0.23 ± 0.01
	1000	5	0.07 ± 0.04	0.10 ± 0.03
417	300	5	0.09 ± 0.02	0.13 ± 0.05
	30	5	0.16 ± 0.05	0.21 ± 0.05
	1000	5	0.08 ± 0.05	0.10 ± 0.05
418	300	5	0.10 ± 0.01	0.15 ± 0.03
	30	5	0.18 ± 0.01	0.21 ± 0.05
	1000	5	0.03 ± 0.01	0.04 ± 0.01
419	300	5	0.07 ± 0.03	0.10 ± 0.03
	30	6	0.10 ± 0.06	0.12 ± 0.05
acetone		6	0.50 ± 0.01	0.75 ± 0.01

385; scutalpin F, **386**; scutalpin O, **43**) and *Salvia splendens* (salviarin, **416**; splenolid B, **418**; splendidin, **419**) was studied (Table 2). Scutecyprol A (**42**) was used as a standard - a compound known for its high antifidant activity. Of the compounds studied, scutalpin O (**43**) has a very similar structure to the standard scutecyprol A (**42**). The only difference is the replacement of the acetate group in C-19 with methylbutyrate. The other 6 compounds belong to two structurally distinct groups: furo-*neo*-clerodane dilactone derivatives (**416**, **418**, **419**) and *neo*-clerodane diterpenes with a 13-spiro-γ-lactone ring linked by a $\beta \longrightarrow \alpha$ bond to a perhydro-pyranose ring (**384 - 386**).

All tested natural diterpenoids (Table 3) show very good inhibition of the larvae of *Leptinotarsa decemlineata* Say to feed at a dose of 1000 ppm (≈33 μg/cm^2). Compound (**43**) showed the strongest action of all the test substances and maintained a high activity at 30 ppm, which is due to the presence of the characteristic structural

Таблица 3. Коефициенти на хранене на *нео*-клероданови дитерпени изолирани от *Scutellaria galericulata*

Съединение	**Доза (ppm)**	**N**	**FR_{50} ± SE**	**FR_{75} ± SE**
14,15- dihydrojodrelin T (**22**)	1000	3	0.03 ± 0.00	0.04 ± 0.01
	100	5	0.12 ± 0.04	0.18 ± 0.06
	10	5	0.37 ± 0.10	0.37 ± 0.10
neo-ajugapirin A (**399**)	1000	3	0.05 ± 0.01	0.07 ± 0.04
	100	5	0.14 ± 0.09	0.21 ± 0.11
	10	5	0.44 ± 0.11	0.49 ± 0.14
scutegalerin A (**398**)	1000	3	0.04 ± 0.02	0.05 ± 0.01
	100	5	0.14 ± 0.07	0.24 ± 0.09
	10	5	0.42 ± 0.13	0.52 ± 0.16
scutegalerin B (**400**)	1000	3	0.11 ± 0.04	0.17 ± 0.07
	100	5	0.26 ± 0.08	0.33 ± 0.13
	10	5	0.63 ± 0.17	0.74 ± 0.17
scutegalerin C (**401**)	1000	5	0.30 ± 0.07	0.40 ± 0.09
	100	3	0.59 ± 0.10	0.67 ± 0.17
scutegalerin D (**402**)	10	3	0.88 + 0.19	0.99 + 0.22
scutegalerin E (**403**)	1000	3	0.14 ± 0.04	0.17 ± 0.05
	100	3	0.32 ± 0.12	0.38 ± 0.10
	10	5	0.76 ± 0.16	0.89 ± 0.18
scutecolumnin C (**23**)	1000	3	0.14 ± 0.03	0.20 ± 0.03
	100	3	0.25 + 0.10	0.29 + 0.12
	10	5	0.59 ± 0.14	0.43 ± 0.11
scutegalin A (**27**)	1000	3	0.09 ± 0.02	0.10 ± 0.05
	100	3	0.17 ± 0.10	0.22 ± 0.16
	10	5	0.47 ± 0.12	0.65 ± 0.18
scutegalin D (**155**)	1000	5	0.28 ± 0.06	0.32 ± 0.07
	100	3	0.46 ± 0.15	0.51 ± 0.19
	10	3	0.80 + 0.17	0.88 + 0.18
scutalbin A (**9**)	1000	3	0.07 ± 0.02	0.09 ± 0.05
	100	3	0.16 ± 0.10	0.18 ± 0.13
	10	5	0.45 ± 0.12	0.53 ± 0.16

fragments as in the standard compound **42**. The different antifidant activity in the two groups of compounds **384** - **386** and **416**, **418**, **418**, **419** is related to their substitutes because the substances in them have the same carbon skeletons. In the group of compounds **416**, **418** and **419**, the substitution of one or two hydrogens in C-1 and C-11 with an acetoxy group leads to higher activity. In the group of compounds **384-386** the activity decreases when the 2-methylbutyrate group at C-6 in diterpene **384** is replaced by an (E) -2-methyl-2-butenoyloxy group in clerodane **385** and increases when it is replaced by an acetoxy group at **386**, respectively.

The two different basic structures (series **416**, **418**, **419** and **384** - **386**) show similar feeding coefficients. In the first series the activity of splendidine is the highest (**416**), and in the second of scutalpin F (**386**). The achieved results show that *neo*-clerodanes with hexahydro furofuran ring in the C-11 – C-16 side chain and with a decalin ring in the C-1 – C-10 substructure bearing 4α,18-oxirane and two ester groups at C-6 and C-19. The activity of diterpenes with *α,β*-unsaturated lactone or 13-spiro-bonded *γ*-lactone ring is also high. 18 – C-19 in the decalin nucleus. Due to insufficient data, the influence of the C-13 configuration in compounds **384** and **386** relative to **385** cannot be determined.

Eleven natural *neo*-clerodane diterpenoids isolated from *Scutellaria galericulata* were tested for insect-antifidant activity against larvae of *Leptinotarsa decemlineata* Say in optional feeding experiments. Nine of the compounds are in the individual state - scutalbin A (**9**), 14,15-dihydrojodrelin T (**22**), scutecolumnin C (**23**), scutegalin A (**27**), scutegalin D (**155**), scutegalerin A (**398**), *neo*-ajugapirin A (**399**), scutegalerin B (**400**) and scutegalerin E (**403**), and two form an epimeric mixture at C-16, scutegalerin C (**401**) and scutegalerin D (**402**). All *neo*-clerodane diterpenoids tested have 2*α*,19-hemiacetal or acetal function in the decalin ring, C-4 − C-18 spiro epoxide and carbon acetate group C-6. The C-11 − C-16 substructure of seven clerodanes contains a hexahydro furo [2,3-b] furan ring, and in compound **9**, the ring is tetrahydro furfuran. Diterpenes **155**, **401** and **402** contain a hemiacetal or acetal between C-15 and C-16. The individual compounds differ from each other by the change of the substituent in C-19, hydroxy (OH), acetoxy (OAc), methoxy (OMe), E-2-methyl-2-butenoyloxy (OTig) group. An additional substituent in some diterpenes is present in C-1, C-3 and C-7. Compounds **398** and 399 are positional isomers, while **401** and **402** are epimers at C-16. Data on antifidant activity (Table 3) show that most of the tested natural diterpenoids act as potent repressors of Colorado potato beetle larvae ($FR_{50} \approx 0.1$) at a dose of 1000 ppm (33 *μ*g/cm2), with the exception of compounds **155**, **401** and **402**. The low activity of these three clerodanes is due to the lack of a furofuran C-11 − C-16 substructure in the main clerodan skeleton. Some of the substances retain strong activity at a dose of 100 ppm (3.3 $\mu g/cm^2$). Of the positional structural isomers, scutegalerin A (**398**) and *neo*-ayugapirin A (**399**), the former showed greater activity. Compound **398** has the strongest activity among the tested *neo*-clerodane diterpenoids after 14,15-dihydrojodrelin T (**22**), from which it differs by the substituent at C-1, a hydroxyl instead of a tiglate ester group.

The insect-antifidant activity of fourteen *neo*-clerodane diterpenoids isolated from *S. altissima*: clerodin (**1**), scupolin H (**10**), scutecyprol A (**42**), scutecolumnin C (**23**), 11-

epi-scutecolumnin C (**28**), scutaltisin A (scutalbin C, **52**), scutaltisin B (**392**), scutaltisin C (**393**), scutaltisin D (**394**), scutaltisin E (**395**), scutaltisin F (**396**), scutaltisin G (**397**), scupolin I (**59**), and scutecyprin (**25**) was tested against *Leptinotarsa decemlineata* Say. larvae. The use of discs of potato leaves treated with small amounts of the compounds (dose of 1000, 100, 10 ppm) leads to good and very good antifidant activity (Table 4).

Table 4. Nutritional factors of *neo*-clerodane diterpenes isolated from *S. altissima*

Compound	Dose (ppm)	N	$FR_{50} \pm SE$	$FR_{75} \pm SE$
clerodin (**1**)	1000	7	0.03 ± 0.02	0.05 ± 0.02
	100	7	0.11 ± 0.03	0.17 ± 0.05
	10	5	0.33 ± 0.10	0.39 ± 0.08
scupolin H (**10**)	1000	5	0.11 ± 0.02	0.15 ± 0.03
	100	5	0.26 ± 0.03	0.32 ± 0.05
	10	5	0.39 ± 0.08	0.48 ± 0.09
scutecyprol A (**42**)	1000	6	0.09 ± 0.01	0.14 ± 0.02
	100	5	0.18 ± 0.03	0.25 ± 0.05
	10	5	0.34 ± 0.06	0.44 ± 0.07
scutecolumnin C (**23**)	1000	5	0.11 ± 0.06	0.16 ± 0.05
	100	5	0.27 ± 0.08	0.34 ± 0.10
	10	5	0.53 ± 0.11	0.71 ± 0.09
11-*enu*-scutecolumnin C (**28**)	1000	7	0.05 ± 0.02	0.09 ± 0.01
	100	7	0.13 ± 0.07	0.21 ± 0.08
	10	6	0.38 ± 0.04	0.46 ± 0.06
scutaltisin A (**52**)	1000	7	0.08 ± 0.03	0.12 ± 0.04
	100	6	0.22 ± 0.07	0.29 ± 0.11
	10	6	0.33 ± 0.14	0.41 ± 0.10
scutaltisin B (**392**)	1000	7	0.07 ± 0.04	0.11 ± 0.06
	100	6	0.27 ± 0.10	0.30 ± 0.08
	10	6	0.48 ± 0.11	0.69 ± 0.12
scutaltisin C (**393**)	1000	5	0.14 ± 0.04	0.17 ± 0.06
	100	5	0.32 ± 0.10	0.38 ± 0.08
	10	5	0.66 ± 0.11	0.81 ± 0.12
scutaltisin D (**394**)	1000	5	0.10 ± 0.02	0.13 ± 0.07
	100	5	0.18 ± 0.10	0.22 ± 0.13
	10	5	0.40 ± 0.12	0.52 ± 0.14
scutaltisin E (**395**)	1000	6	0.09 ± 0.01	0.11 ± 0.03
	100	6	0.15 ± 0.09	0.23 ± 0.04
	10	5	0.40 ± 0.08	0.59 ± 0.10
scutaltisin F (**396**)	1000	6	0.09 ± 0.01	0.12 ± 0.03
	100	5	0.16 ± 0.06	0.20 ± 0.10
	10	5	0.42 ± 0.10	0.61 ± 0.11
scutaltisin G (**397**)	1000	5	0.32 ± 0.04	0.42 ± 0.08
	100	5	0.50 ± 0.05	0.67 ± 0.11
	10	5	0.81 ± 0.10	0.98 ± 0.12
scutecyprin (**25**)	1000	7	0.06 ± 0.01	0.09 ± 0.03
	100	7	0.13 ± 0.03	0.19 ± 0.05
	10	6	0.36 ± 0.09	0.41 ± 0.08
scupolin I (**59**)	1000	5	0.13 ± 0.04	0.17 ± 0.09
	100	5	0.19 ± 0.10	0.24 ± 0.13
	10	5	0.44 ± 0.12	0.54 ± 0.14

Clerodin (**1**), scutecyiprine (**25**) and 11-epi-scutecolumnin C (**28**) showed strong inhibition of larval nutrition at a dose of 1000 ppm and showed significant antifidant activity at a dose of 100 ppm. All fourteen *neo*-clerodane diterpenoids tested had C-4 –

C-18 spiroepoxide and an acetate group at the α-position at C-6 in the decalin ring. Twelve of them present 2α,19-hemiacetal or acetal function, which is not a structural feature only in scutecyprol A (**42**) and in clerodin (**1**). The six atoms of the C-11 – C-16 side chain form very common in clerodanes isolated from *Scutellaria* species, the tetra- or hexahydro furo-[2,3-b]-furan ring, with the exception of scutaltisin G (**397**), in which a lactol ring concluded between C-15 – C-16 is present, as reported for scoterepinosides A_1-A_4 (**158-161**).

The carbon atom C-11 assumes the unusual R-configuration in clerodanes 11-epi-scutecolumnin C (**28**) and scutaltisin C (**393**). With the exception of these two compounds, only in 11-epi-scutecyprine (**29**), isolated from Malakov and Papanov, C-11 has an R-configuration. Scutaltisin E (**395**) and scutaltisin F (**396**) are C-15 epimers, while the pairs of compounds scutecolumnin C (**23**) / 11-epi-scutecolumnin C (**28**) and scutaltisin B (**392**) / scutaltisin C (**393**) are C- 11 epimers.

The results of the biological tests (4) show that the tested diterpenoids show very good to excellent (FR_{50} <0.1) antifidant activity against *L. decemlineata* larvae at a dose of 1000 ppm (~ 33 μg/cm2), with the exception of scutaltisin G (**397**). The weak suppression of larval feeding by compound **397** can be attributed to the absence of the furofuran substructure in the C-11 – C-16 fragment of the main clerodane skeleton. Biological tests show a difference in the results for the epimeric pairs at C-11 and at C-15. Isomers **28** and **392** with C-11 R-configuration exhibit greater activity than isomers with C-11 S-configuration (**23** and **393**), while compounds with different stereochemistry at C-15 (**395** and **396**) show similar activity. It can be concluded that the stereochemistry of C-15 is a structural feature of the molecule that does not affect biological activity, while the epimers of C-11 show a marked difference in their antifidant action. It can also be seen that the antifidant action decreases in substances with a methoxyl instead of a hydroxyl or ester group in C-15 or C-19.

The antifidant activity of ten diterpenoids isolated from two species of the genus *Teucrium* - *T. polium* and *T. scordium* was tested (Table 5). Scordidesin A (**415**), teucrin A (**356**), 3α-acetoxy-teucvin (**414**) have a *nor*-clerodane skeleton, and the other compounds are *neo*-clerodane diterpenoids. All tested clerodanes contain a furan ring in the C-11 - C-16 side chain. In teulaumifin B (**289**) δ-lactone is formed, in which C-5, C-9, C-10, C-19 and C-20 carbon atoms participate. In all other tested terpenoids, γ-lactone is present, including the carbon atoms C-9, C-11, C-12 and C-20. In polivincin B (**410**) and teupolin XII (**317**) an acetal is concluded between C-18 - C-19.

An oxetane ring is formed in polyvincin A (**409**), comprising the carbons C-4, C-5 and C-19. In the clerodane ring of the compounds scordidesin A (**415**), teucrin A (**356**) and 3α-acetoxy-teuquin (**414**) α,β-unsaturated C-18 - C-6 γ-lactone is present, and in 6-keto-teuscordin (**361**), 6-acetylteucrine F (**412**) and teucrin E acetate (**413**) saturated C-18 – C-19 **γ**-lactone. The rare $\Delta^{2,3}$ double bond is present in clerodan **412**. As shown in Table 21, all tested diterpenoids showed good inhibition of feeding on the larvae of *Leptinotarsa decemlineata* Say. with FR_{50} < 0.3 at a dose of 1000 ppm (~ 33 μg / cm^2) with the exception of compounds 289 and 411. In *neo*-clerodane **289** C-20 - C-12 γ-lactone is replaced by C-19 - C-20 δ-lactone. Both *neo*-clerodanes lack a second lactone ring as well as an acetal or oxetane ring.

Table 5. Nutritional factors of clerodan diterpenoids from *T. polium* and *T. scordium*

Съединение	Доза (ppm)	N	FR_{50} ± SE	FR_{75} ± SE
polyvincin A (**409**)	1000	3	0.15 ± 0.04	0.20 ± 0.05
	100	5	0.22 ± 0.04	0.28 ± 0.03
	10	5	0.31 ± 0.09	0.42 ± 0.10
polyvincin B (**410**)	1000	3	0.23 ± 0.03	0.32 ± 0.06
	100	5	0.31 ± 0.08	0.43 ± 0.11
	10	5	0.46 ± 0.10	0.49 ± 0.12
polyvincin C (**411**)	1000	3	0.32 ± 0.04	0.39 ± 0.07
	100	5	0.37 ± 0.07	0.44 ± 0.03
	10	5	0.43 ± 0.12	0.47 ± 0.07
teulaumifin B (**289**)	1000	3	0.31 ± 0.05	0.37 ± 0.04
	100	5	0.37 ± 0.07	0.46 ± 0.07
	10	5	0.43 ± 0.10	0.49 ± 0.11
teupolin XII (**317**)	1000	3	0.25 ± 0.06	0.29 ± 0.08
	100	5	0.30 ± 0.11	0.42 ± 0.10
	10	5	0.38 ± 0.13	0.49 ± 0.12
6-acetylteucrine F (**412**)	1000	3	0.15 ± 0.04	0.23 ± 0.05
	100	5	0.21 ± 0.04	0.29 ± 0.03
	10	5	0.30 ± 0.09	0.37 ± 0.10
teucrin E acetate (**413**)	1000	3	0.16 ± 0.03	0.24 ± 0.06
	100	5	0.22 ± 0.08	0.31 ± 0.11
	10	5	0.32 ± 0.10	0.39 ± 0.12
3α-acetoxy-teucvin (**414**)	1000	3	0.10 ± 0.04	0.18 ± 0.07
	100	5	0.17 ± 0.07	0.21 ± 0.03
	10	5	0.26 ± 0.12	0.37 ± 0.07
scordidesin A (**415**) / teucrin A (**356**)	1000	3	0.12 ± 0.05	0.18 ± 0.04
	100	5	0.19 ± 0.07	0.25 ± 0.07
	10	5	0.28 ± 0.10	0.29 ± 0.11
6-keto-teuscordin (**361**)	1000	3	0.13 ± 0.06	0.20 ± 0.08
	100	5	0.20 ± 0.11	0.27 ± 0.10
	10	5	0.29 ± 0.13	0.35 ± 0.12

The inhibition of feeding and the development of the larvae of *Leptinotarsa decemlineata* (Say) from extracts of eight species of the genus *Scutellaria* and 43 *neo*-clerodane diterpenoids isolated from species of the genus *Scutellaria* - 30 clerodanея, Salvia splendens - 3 clerodanея and representatives of the genus Teucrium - 10 clerodanes. The last 13 compounds are furo-*neo*-clerodane lactones. All tested diterpenes, of species of the genus *Scutellaria*, contained 4α,18 *spiro*-linked oxirane ring. A 2α,19 hemiacetal or acetal ring is formed in 23 clerodans. The compounds differ in the substituents in the clerodane nucleus in C-1, C-3, C-6, C-7 and C-19 and in the substructures in the C-11 -C-16 side chain. In three *neo*-clerodans (**1**, **9**, **10**) a tetrahydro furofuran ring is present, in 21 hexahydro furofuran rings, in three diterpenes (**384**, **385**, **386**) a *spiro*-linked *γ*-lactone is formed (**384** and **386** with 13S and **385** with 13R configuration), and in four (**155**, **397**, **401**, **402**) lactone ring - hemiacetal in clerodane **155** and acetal in compounds **397**, **401** and **402**.

The obtained results confirm the thesis of Kojima and Kato that the presence in the diterpene structure of a decalin nucleus bearing 4*α*,18 *spiro*-bound epoxide and ester

groups in C-6 and C-19 is a prerequisite for potent action as an insect antifidant. On the other hand, a very good antifidant effect with an FR_{50} value of 0.03 was observed for the diterpenoids scutalpin A (**384**) and splendidin (**419**). In C-11 - C-16 the substructure of **384** is formed *γ*-lactone *spiro*-linked at C-13 with C-8 – C 13 oxane ring, and in **419** a furan ring is concluded between C-15 – C 16, a *δ*-lactone ring between C-12 – C 17 and in addition C-18 – C-19 is formed in the decalin nucleus instead of an oxirane ring and an acetate group. The activity of clerodanes isolated from members of the genus *Teucrium* is lower than that of compounds isolated from the genera *Scutellaria* and *Salvia*. The decrease in activity is due to the replacement of furofuran function or 13-*spiro*-linked *γ*-lactone by a furan nucleus in clerodanes isolated from the genus *Teucrium*.

3.3. Testing the antimicrobial activity of clerodane diterpenoids against pathogenic and hygienic indicator microorganisms

The antimicrobial activity of twenty-two clerodane diterpens isolated from acetone extracts from the aerial parts of species of the genus *Scutellaria*, *Salvia* and *Teucrium* (Lamiaceae) have been studied against nineteen strains belonging to eleven different species of pathogenic bacteria *Listeria monocytogenes*, *Proteus vulgari*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella abony* и *Staphylococcus aureus*, as well as against two strains of yeast belonging to *Candida albicans* species.

Table 6: Antimicrobial activity of clerodan diterpenes against pathogenic and hygienic indicator microorganisms

Test microorganism	Source	MIC, μg/mL								
		384	**385**	**386**	**416**	**417**	**418**	**419**	**356/315**	**445**
B. cereus	Minced meat	50	100	100	200	200	200	200	-	-
S. aureus	ATCC 6538	25	50	50	100	100	200	200	-	-
S. aureus	ATCC 1805	-	-	-	-	-	-	-	500	500
Streptococcus pyogenes	ATCC 12344	-	-	-	-	-	-	-	250	250
L monocytogenes	ATCC 8632	-	-	-	-	-	-	-	500	500
E. coli	ATCC 3397	-	-	-	-	-	-	-	500	500
S.aureus	Pork fillet	25	50	50	100	100	200	200	-	-
L.monocytogenes	Chicken breasts	100	200	200	200	200	200	200	-	-
A.hydrophila	Fish	100	200	200	200	400	400	400	-	-
E.coli	ATCC 25922	200	400	400	400	400	800	800	-	-
E.coli	Clinical isolate	200	400	400	400	400	800	800	-	-
S. abony	ATCC 6017	200	400	400	400	400	800	800	500	500
S. abony	Clinical isolate	200	400	400	400	400	800	800	-	-
P.aeruginosa	ATCC 27853	400	800	800	800	800	800	800	-	-
P.aeruginosa	Clinical isolate	400	800	800	800	800	800	800	-	-
P.aeruginosa	Minced meat	400	800	800	800	800	800	800	-	-
P.fluorescens	Chicken breasts	400	800	800	800	800	800	800	-	-
C.albicans	ATCC10231	100	200	200	200	200	400	400	1000	1000
C. glabrata	ATCC 90030	-	-	-	-	-	-	-	1000	1000
C.albicans	Clinical isolate	100	200	200	200	200	400	400	-	-

Three of the tested compounds, scutalpin A (**384**), scutalpin E (**385**), and scutalpin F (**386**), showed moderate antimicrobial activity (Table 6) against the microbial strains used in the tests. Diterpenes containing a furan nucleus, **345**, **356**, **415**, **416**, **417**, **418**, **419**, show little activity. The remaining compounds are inactive within the study concentration limits. Among all tested compounds, the highest antimicrobial activity was found for scutalpin A against *Staphylococcus aureus* (MIC 25 μg/mL). Neo-clerodan diterpenoids containing tetra- or hexahydro furo-[2,3-b] -furan in the C-11 – C-16 side chain do not show antibacterial and anticandidal activity. With the best activity of the tested compounds are clerodanes containing spiro-linked at C-13 γ-lactone and oxane rings.

Based on the obtained results, it can be concluded that different types of biological activities are due to different chemical functions in the structure of the compounds and therefore there is no correlation between antifidant, antimicrobial and antifungal activity of the same test compound.

As can be seen from Table 6 among the antimicrobial active compounds, scutalpin A is characterized by the strongest antibacterial and anticandidal activity, followed by scutalpin E and scutalpin F, salviarin and splenolid A, splenolid B and splendidin. It is very likely that the spiro-linked γ-lactone is responsible for the higher antimicrobial activity of compounds **384-386** compared to compounds 416-419 containing a furan ring. Compounds **384-386** differ in the substituent at C-6. The most active substance, scutalpin A, which contains methyl butyrate at C-6, shows higher activity compared to scutalpin E and scutalpin F, which contain tiglate and acetate esters, respectively. Compounds **416-419** are characterized by almost identical antimicrobial activity, which means that the type of substituents at C-1 and C-11 does not appear to affect the antimicrobial activity of these compounds.

3.4. Testing the cytotoxic activity of isolated *neo*-clerodan diterpenoids against carcinogenic lung tumor cells (H1299) and normal umbilical cord cells (HUVEC)

Twelve natural *neo*-clerodane diterpenoids, 14,15-dihydrojodrelin T (**22**), *neo*-ajugapirin A (**399**), scutegalerin A (**398**), scutecoluminin C (**23**) and scutegalin D (**155**) isolated from *Scutellaria galericulata* L .; scutecyprol A (**42**), scupolin H (**10**), clerodin (**1**) and scutaltisin G (**397**) obtained from *Scutellaria altissima* L.; scutalpin A (**384**), scutalpin E (**385**) and scutalpin F (**386**) isolated from *S. alpina*; were tested for cytotoxicity against two cell lines, carcinogenic cells from human lung tumors designated H1299 and normal umbilical cord cells (HUVEC) using MTT (3-/4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) analysis. In accordance with the purpose of the study, the compounds are divided into two features: ***1***. according to the functional substituents in the decalin nucleus into two groups, **A** - containing 2α,19 hemiacetal or acetal and **B** - deprived of 2α,19 oxygen bridge; ***2***. on the basis of the presented C-11 – C-16 substructures, the clerodanes are divided into four groups **I-IV**. The assignment of diterpenes to groups **I-III** is the same as that made in the analysis of antimicrobial activity. In compounds **155** and **397** forming group **IV**, a C-15 – C 16 lactol ring is formed. The individual compounds also differ from each other in the substituents at positions C-1, C-3, C-6, C-7, C-16 and C-19.

The cytotoxic activity of *neo*-clerodane diterpenoids was assessed as the IC_{50} value (Table 7). Three compounds, scutalpins A, E and F, showed mild to moderate cytotoxicity against both cell lines. Among all tested compounds, the highest activity was found for scutalpin A (**384**), with IC_{50} values of 21.35 and 23.9. The remaining compounds are inactive within the study concentration limits. In the molecules of the three compounds exhibiting a cytotoxic effect, 13-spiro-linked *γ*-lactone with a C-8 – C-13 oxane ring is present. Diterpenoids differ in C-13 stereochemistry and C-6 carbon substitute.

Table 7. Cytotoxic activity of *neo*-clerodanes against H1299 and HUVEC cell lines

Compound	IC50 values		Compound	IC50 values	
	H1299	HUVEC		H1299	HUVEC
22	242.21	244.43	**1**	236.35	236.84
399	288.65	287.59	**384**	21.35	23.89
398	452.01	458.00	**386**	26.62	31.28
23	667.44	665.79	**385**	34.24	32.48
42	335.76	365.32	**155**	888.35	883.47
10	578.71	574.55	**397**	892.02	892.22

The most active of these, scutalpine A (**384**), contains 2-methylbutyrate, while less active scutalpin F (**386**) (with IC_{50} values of 26.62 and 31.28) and scutalpin E (**385**) (with IC_{50} values of 34.24 and 32.48) 2-methylbutyrate is replaced by acetate and with (E) 2-methyl-2-butenolate) ester. It is not certain that only the change in the function of 2-methylbutyrate with tiglate causes a greater decrease in the activity of clerodane **385** than in **386**, since the configuration of the asymmetric center C-13 in diterpene **386** is S, as in the most active compound **364**, while in the *neo*-clerodane diterpenoid **365** the configuration of the chiral center C-13 is R.

4. Isolation and characterization of other secondary metabolites

4.1. Determination of the chemical composition of the essential oil of *Ajuga laxmanii* Benth, *Salvia amplexicaulis* Lam., and *Stachys cretica* subsp. *bulgarica* Rech. fil.

The chemical composition of the essential oils from Bulgarian plants of the Lamiaceae family *Salvia amplexicaulis* Lam., *Ajuga laxmanii* Benth was analyzed. and *Stachys cretica* subsp. *bulgarica* Rech. fil. using GC and GC / MS.

Aboveground parts of *Salvia amplexicaulis* Lam were examined. and *Ajuga laxmanii* Benth. The detected moisture in the drug from *S. amplexicaulis* is 11.80 %, and for *A. laxmanii* - 10.99%. The yield of essential oil, % in absolute dry mass is 0.08 % and 0.02 %, respectively. Of the chemical constituents of *S. amplexicaulis* oil, 26 components were identified, representing 85.5% of the total content. Of these, 22 are in concentrations above 1% and the other 4 components are in concentrations below 1%. The components of the oil in an amount exceeding 3% are, *γ*-murolene (29.20%); nonadecane (5.58%), heneicosan (5.48%), spatulenol (5.14%), aromadendrene oxide-2 (4.09%) and octadecane (3.57%). From A. Laxmanii, 27 compounds were identified, representing 86.32% of the total oil content, 18 of them in concentrations above 1%,

and the remaining 9 in concentrations below 1%. The main ingredients (over 3%) of the oil are eicosan (11.21%), phytol (7.25%), hexahydro farnesylacetone (7.34%), octadecane (7.02%), henicosan (6.57%), tetracosan (4.97%), E-2 -hexenyl benzoate (4.09%), heptacosan (3.94%), tricosan (3.71%), pentacosan (3.63%), caryophyllene oxide (3.61%), 7-isopropyl-1,1,4a-trimethyl-1,2,3, 4,4a, 9,10,10a-octahydro phenanthrene (3.54%) and docosane (3.15%). The results differ from the data for these species of the genus *Salvia* from Serbia and from the genus *Ajuga* from Turkey. The difference in the chemical composition of the conducted research and the published data is probably due to the soil-climatic differences of the environment in which the plant grew, as well as to the differences in the equipment and the conditions of the analysis.

Sesquiterpene hydrocarbons (48.01%) are the dominant group in *S. amplexicaulis* oil, followed by hydrocarbons (30.11%), oxygenated sesquiterpenes (18.01%), phenyl propanoids (2.92%) and oxygen derivatives of hydrocarbons (0.95%).

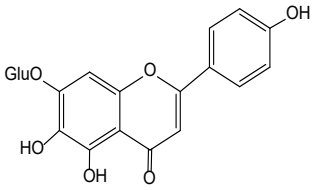
Hydrocarbons (55.34%) are the dominant group in *Ajuga laxmanii* oil, followed by oxygen-containing sesquiterpenes (15.23%), phenyl propanoids (8.84%), diterpenes (8.40%), oxygen derivatives of hydrocarbons (5.32%), sesquiterpenes. (4.02%) and oxygenated monoterpene (2.85%).

Acetone extracts of *Salvia amplexicaulis* Lam. and *Ajuga laxmanii* Benth. have also been tested for di- and triterpenoids, but have not been identified. The chemical composition of the essential oil from the aboveground parts of *Stachys cretica* subsp. *bulgarica* Rech. fil (Lamiaceae), an endemic Bulgarian species, using GC / MS. The humidity of the drug is 64.83%. The yield of essential oil,% in the absolutely dry mass is 0.04%. 20 components were identified, which are 89.20% of the total content of compounds in the oil. Five of them are in concentrations above 1% and the other 15 are in concentrations below 1%. The main compounds, with a higher concentration of 3%, are geranyllinalol (66.36%), germacrene D (9.01%) and geranylgeranyl acetate (4.88%). The difference in the chemical composition of the studied oil and the published literature data is most likely due to differences in soil-climatic conditions and chemotaxonomic features. Total oxidized monoterpenes represent the highest percentage of the components of the essential oil, constituting 71.24% in the oil. The acetone extract from the aboveground parts of the plant was examined for the presence of diterpenoids, but no such substances were found. The acetone extract from aboveground parts of the species *Stachys leucoglosa* Griseb was studied. for the presence of diterpenoids. No diterpenoids were found in this species of *Stachys*. Qualitative reactions define flavones as characteristic secondary metabolites in species of the genus *Stachys*.

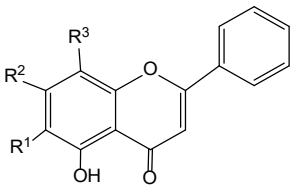
From *A. Laxmanii* 27 compounds were identified, representing 86.32% of the total oil content. Hydrocarbons (55.34%) are the dominant group in *A. laxmanii* oil. 20 components were identified, which are 89.20% of the total content of compounds in the oil of *Stachys cretica* subsp. *bulgarica*. Oxidized monoterpenes represent the highest percentage of the components of the essential oil, constituting 71.24% in the oil.

4.2. Quantitative determination of flavonoids in Sculellaria altissima

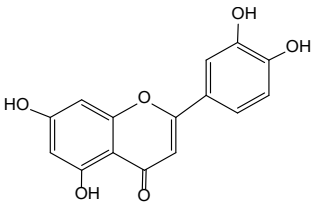
The quantitative content of polyphenols in extracts of *Sculellaria altissima* collected in the region of Mezek, Bulgaria, was investigated by matrix high-performance liquid chromatography with gradient elution and diode registration of substances. Quantitative content of flavonoids, scutelarin (**370**), baicalin (**372**), baicalein (**373**), vogonoside (**374**), vogonin (**375**), chrisin (**376**), luteolin (**368**) and verbascoside (**377**) were determined for *Sculellaria altissima*.



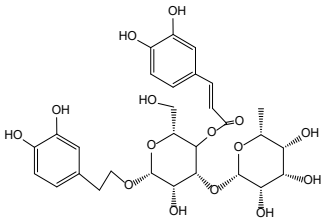
370



	R^1	R^2	R^3
372:	OH	OGlu	H
373:	OH	OH	H
374:	H	OGlu	OCH_3
375:	H	OH	OCH_3
376:	H	OH	H



368



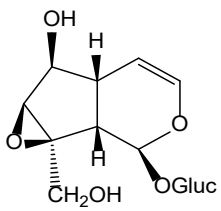
377

So far, the flavonoid composition of different species of the genus *Scutellaria* has been studied and the presence of baicalin, baicalein, scutelarin and chrisin in wild *Scutellaria galericulata*, baicalin, baicalein and vogonin in tinctures obtained from *Scutellaria lateriflora* and *Scutellaria baicalensis* has been proven, as well as baicalin in roots of *Scutellaria barbata*. Grzegorczyk- Karolak et al. have determined, in addition to the baikalin and vogonoside characteristic of this genus, also luteolin and verbascoside in *Scutellaria altissima*.

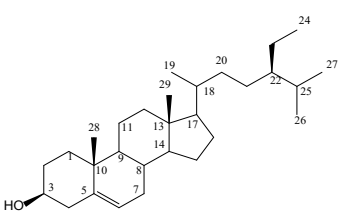
4.3. Isolation and characterization of sterols, glucoside-bound iridoids and cleroindicines

A glucoside-bound iridoid was isolated from the biologically active fraction of *Scutellaria albida*, the signals in the ^{1}H MP spectrum of which completely coincide with the data published in the catalpol literature (**389**).

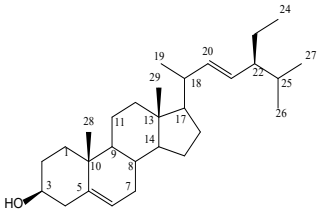
From plant material from *Scutellaria altissima*, collected over the village of Balkanets, the *β* sitosterol (**390a**), stigmasterol (**390b**) and the glucoside-related iridoid - globularin (**391**), widespread in the plant world, were isolated. The two sterols were isolated as a 1: 1 mixture. After further chromatographic separation, *β* sitosterol was obtained individually. The substances are characterized by analysis of spectral data.



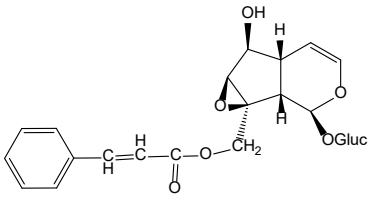
389



390a

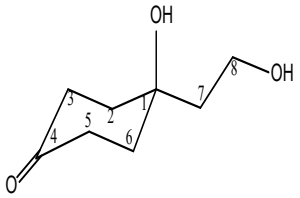


390b

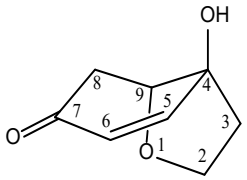


391

Continuing the search for *neo*-clerodan antifidants, *Scutellaria hastifolia* L was studied. Two oily substances were isolated from the acetone extract obtained from the aboveground parts of the plant and developed in the usual way. After analyzing the spectral data, the compounds were identified as cleroindicin B (**407**) and cleroindicin F (**408**). EIMS, 1D and 2D NMR spectra of substances **407** and **408** are included in Annex 23. Cleroindicins B and F are new compounds for both the species *Scutellaria hastifolia* L. and the genus *Scutellaria* and the family Lamiaceae.



407



408

CONCLUSIONS AND CONTRIBUTIONS OF THE DISSERTATION WORK
CONCLUSIONS

I. *In isolating and characterizing the structure and stereochemistry of clerodane diterpenoids:*

1. It has been established that in the species of the genus *Scutellaria* growing on the territory of Bulgaria, only diterpenoids with trans *neo*-clerodane skeleton of the TC type with 4α, 18 spiro bonded epoxy ring and α oriented ester function in C-6 are biogenetically synthesized. Compared to most of the *neo*-clerodanes identified in Asian *Scutellaria* species, C-19 is always oxidized. There are no substituents for C-10, C-17 and C-20 atoms.
2. In all isolated diterpenoids of *S. galericulata* and *S. altissima*, it is contained 2α/19 semi-acetal or acetal ring.
3. Scutorientalin E is the first *neo*-clerodane diterpenoid isolated from a genus *Scutellaria*, which contains cinnamon ester as a substitute.
4. Scutaltisin C is the third *neo*-clerodane proven so far with the unusual C-11R configuration.
5. Neoajugapyrin A is the only *neo*-clerodane diterpenoid with a 2α, 19 acetal or hemiacetal ring in the molecule isolated from a genus *Ajuga*. Isolation of this clerodane from *Ajuga pyramidalis* L. and *S. galericulata* L. confirms the chemotaxonomic association of the two genera. *Neo*-clerodane diterpenoids containing C-13 – C-16 furofuran ring are common in the species of both genera.
6. In species of the genus *Teucrium* and the genus *Salvia*, clerodanes with furofuran ring. In the diterpenes of these two genera, a furan ring is characteristic C-13– C-16 side chain.
7. Diterpenes detected in the genus *Salvia* contain only acetate ester groups like those of the genus *Teucrium*, in contrast to the *neo*-clerodane diterpenes common in the genus *Scutellaria* and the genus *Ajuga*, which contain a variety of ester functions.

8. Compounds such as 3α-acetoxy-teucvin with trans-furoclero-10β-18,6α; 20,12-dilactone structure have not been demonstrated to date in *T. scordium* L. subsp. *scordium*. Synthesized in *T. scordium* L. subsp. *scordioides* and *T. scordium* L. subsp. *scordium* furoclerodane compounds with trans-10β-18,19, 20,12-dilactone functions shows the chemotaxonomic proximity of the two subspecies.
9. Diterpenoids are not biosynthesized in the Bulgarian species *S. hastifolia* (L), *Salvia amplexicaulis* LAM, *Stachys. cretica* L. subsp. *bulgarica* rech. fil.

II. *Conclusions on the relationship structure / antifidant antifidant activity of clerodane diterpenoids:*

1. The strongest inhibitory effect on the feeding of Colorado potato beetle larvae was found in the extract of *S. albida*. The extracts of *S. altissima*, *S. galericulata* and *S. alpina* have very good activity. *S. alpina* extract did not show complete inhibition of nutrition at a dose of 10 000 ppm, but retained high activity at 1 000 and 100 ppm. This is explained by the higher amount of neo-clerodane diterpenoids in this form. *S. hastifolia* extract has no antifidant effect due to the absence of *neo*-clerodane diterpenoids.
2. *Neo*-clerodane diterpenoids, which contain a lactone ring in the decalin nucleus, show strong activity. The antifidant activity of the compounds, scutalpins A and O, neoajugapirin A and scutegalerin A is similar to that of the standard compound 14,15-dihydrojodrelin T. Diterpenoids containing a furan ring in the C 11 – C-16 substructure show less activity. Replacement of the tetrahydro furofuran ring with a lactol ring in scutegalerin C, scutegalerin D and scutegalin D causes a drastic reduction in antifidant activity.
3. The antifidant activity of neo-clerodan diterpenoids decreases upon replacement of the ester groups at C-1, C-3, C-7 and C-19 in the decalin ring of neo-clerodans, with a hydroxyl and methoxy group, and increases when there is a double bond between C-14 and C 15.
4. The antifidant activity of furo-neo-clerodan dilactones, salviarin, splenolide A, splenolide B and splendidine isolated from Salvia splendens increases in the presence of an acetoxy substituent at C-1 or C-11. Splendidine has the highest activity, in the molecule of which there are two acetate ester groups.
5. The diterpenoids, 11-epi-skutecolumnin C and scutaltisin C, which have the R-configuration of C-11 show stronger activity than the respective epimers scutecolumnin C and scutaltisin B, which have the 11S configuration. The C-15 epimers, scutaltisin E and scutaltisin F, show no difference in activity.

III. *Conclusions on the antimicrobial and cytotoxic activity of clerodane diterpenoids:*

1. Diterpenoids containing tetra- or hexahydro furo-[2,3-b]-furan at C-9 do not exhibit antibacterial and anticandidal activity, those containing a furan ring or α,β-unsaturated γ-lactone in the C-11 – C-16 substructure exhibit weak antibacterial and anticandidal activity. In diterpenes with the best antimicrobial activity,

scutalpins A, E and F, a 13-spiro-linked γ lactone with a C-8 – C-13 oxane ring was formed.

2. The most sensitive strains to scutalpine A, which has the strongest antibacterial and anticandidal action, belong to the species *Staphylococcus aureus* (MIC 25 μg /mL), and the most resistant strains belong to the genus *Pseudomonas* (MIC 400 μg/mL). In general, gram-positive bacteria are more sensitive compared to gram-negative bacteria. The resistance of both types of yeast are comparable to those of gram-positive bacteria.
3. Cytotoxicity is shown by clerodans with 13-spiro-linked γ-lactone in C-11 – C-16 the side chain with C-8 – C-13 oxane ring. Scutalpin A is most active with IC_{50} - 21.35 μM against H1299 carcinogenic cells and 23.9 μM against normal HUVEC cells. Its activity is higher than that published by Kurimoto for diterpenoids with the same skeleton - scкtefolides G_1, G_2, O_1, O_2, Q (IC_{50} from 36.2 to 82.5 μM) isolated from *S. coleifolia* (Japan).

IV. *By isolating and identifying other natural organic compounds*

1. It has been shown that the yield of essential oil (% in absolute dry matter), in the species *S. amplexicaulis* Lam., *A. laxmanii* Benth. and *S. cretica* subsp. *bulgarica* Rech. fil. from the family Lamiaceae is 0.08%, 0.02%, respectively. and 0.04%, therefore they are poor in essential oil. Sesquiterpene hydrocarbons (48.01%) were found to be dominant compounds in *S. amplexicaulis* Lam essential oil, and higher fatty hydrocarbons (55.34%) were dominant in *A. laxmanii* Benth oil. Oxygen-containing monoterpenes are the main terpenoids (71.24%) in the essential oil of *S. cretica* subsp. *bulgarian*.
2. The flavonoid composition of *S. altissima* L. growing in Bulgaria does not differ from that published for the same species in Poland. For the first time, *β*-sitosterol, stigmasterol and the glucoside-bound iridoid globularin were isolated from this species. The glucoside-bound iridoid globularin isolated from *S. albida* L. is a novel compound of the species.
3. Cleroindicin B and cleroindicin F isolated from *S. hastifolia* are new substances for both the genus *Scutellaria* and the family Lamiaceae.

ORIGINAL SCIENTIFIC CONTRIBUTIONS

I. By isolating clerodane diterpenoids:

1. A phytochemical analysis was performed for the presence of clerodane diterpenoids in 15 Bulgarian plant species of 5 genera of the Lamiaceae family (8 from *Scutellaria*, 3 from *Salvia*, 2 from *Teucrium*, 1 from *Ajuga* and 1 from *Stahys*). 48 diterpenoids were isolated and spectrally characterized. One of them has a labdane skeleton (sclareol), three have a 19-*nor*-clerodane skeleton, and the other 44 are *neo*-clerodane diterpenoids. There are 22 diterpenes with new structures for science: two with a 19-*nor*-clerodane skeleton and twenty with a

neo-clerodane skeleton. Another 13 diterpenoids were detected for the first time in the studied species.

2. The structure of ajugapirin A (reported as 1*β*-hydroxyscutecyprine) to 3*β*-hydroxyscutecy- prine (neoajugapyrine A) was corrected. The real structure 1β of hydroxyscutecyprine, with the trivial name scutegalerin A, was isolated and spectrally characterized.
3. The presence of epimeric pairs has been demonstrated in 11 clerodane diterpenoids. In five of them are characterized spectrally and both isomers: 15R,15S-14,15-dihydro-15-hydroxyajugahyn A; 15R,15S-scutecyprol A; 15R,15S-scutecyprol B; 15R,15S-scutaltisin A; 15R,15S-scutaltisine D; 15R,15S-scutaltisin G; scutecolumnin C/11-epi-scotecolumnin C; scutaltisin B/scutaltisin C; scutaltisin E/scutaltisin F; scutegalerin C/scutegalerin D; scutegalerin F/scutegalerin G.
4. *Neo*-clerodan (scutaltisin C) with the unusual C-11R configuration was isolated. For the first time, the epimeric mixture of scutecolumnin C (with 11S configuration) and 11 epi-scutecolumnin C (with 11R configuration), previously published as "inseparable with different solvents and recrystallization mixture in the ratio of epimers 3: 7" was partially separated.

II. *In proving the structure of isolated clerodanes and the assignment of signals in ^{1}H and C ^{13}NMR spectra*:

1. Scutecyprine is obtained in the crystalline state. Its melting point is determined. The literature is enriched with a full set of 1D and 2D NMR spectra. The signals in the ^{1}H NMR spectrum for the protons 1*α*, 1*β*, 3*β*, 7*α*, 7*β*, 8*β*, 10*β*, 12*α*, 12*β*, 14*α*, 14*β* and in the 13C NMR spectrum for C-5/C-9 and C-7/C-12/C 14.
2. The signals for the C-1 and C-2 atoms in the ^{13}C-NMR spectrum of scutegalerins F and G have been corrected. In the literature, these signals have been exchanged into compounds with the same fragments in the decalin substructure.
3. The published structure of ajugapirin A (1*β*-hydroxyscutecyprin) has been corrected to 3*β*-hydroxyscutecyprin with the trivial name neoajugapirin A. Based on the values of the *J* constants and the multiplicity of the signals, the ratios of the signals at δ_H 1.81 for Me-4′ and δ_H 1.87 for Me-5′ were corrected in neoajugapyrine A, which are exchanged in ajugapyrin A.
4. The published signals at δ_H 3.98/4.40 for protons 3*α*/2*β* in clerodanes with OH-group at C-3, such as scupolins G, H and I, ajugapyrine A and others, have been corrected. After exchange with D_2O, the multiplet at δ_H 4.40 was observed as t, on the basis of which these signals were exchanged: in neoajugapyrin A the signal at δ_H 4.40 was referred to H-3*α* and that at δ_H 3.98 to H-2*β*.
5. All isolated diterpenes underwent complete signal transfer in ^{13}C-NMR spectra. Missing in the literature ^{13}C NMR data for ajugapirin A (neoajugapyrin A) as well as for 6- ketoteuscordin.

6. The stereochemistry of all asymmetric carbon atoms in the isolated compounds has been demonstrated by interpretation of the couple *J* constants and the NOESY studies.

CONTRIBUTIONAL CONTRIBUTIONS

1. The antifidant activity of extracts of 8 species of the genus *Scutellaria* and of 43 clerodane diterpenoids isolated from *S. alpina*, *S. galericulata*, *S. altissima*, *S. splendens*, T. polium and *T. scordium* against Colorado potato beetle larvae was tested.
2. The antifidant activity of *neo*-clerodane diterpenoids with an unusual R-configuration of C-11 was studied for the first time.
3. The cytotoxic activity of 12 *neo*-clerodane diterpenoids isolated from members of the genus *Scutellaria* was tested against two cell lines: carcinogenic human lung tumor cells labeled H1299 and normal umbilical cord cells (HUVEC).
4. The antimicrobial activity of 22 clerodane diterpenoids against nineteen strains belonging to eleven different types of pathogenic and hygienic indicator microorganisms in food products and against two strains of yeast was tested.

CONTRIBUTIONS TO THE ISOLATION OF OTHER ORGANIC COMPOUNDS

1. The essential oil of three plant species was extracted by hydrodistillation: in *S. amplexicaulis* Lam. 26 natural organic compounds (85.5% of the total content of compounds in the oil) were identified in A. laxmanii Benth. 27 (86.32% of the total oil content), and in S. cretica subsp. bulgarica Rech. fil. 20 (89.20% of the total oil content).
2. From *S. altissim*a quantitatively identified 8 polyphenolic compounds - 7 flavonoids (scutelarin, baicalin, vogonin, vogonoside, baicalein luteolin, chrysin) and caffeoylphenylethanoid glycoside - verbascoside.
3 Two sterols, twu glucoside-linked iridoids, two cleroindicine were isolated and characterized.

AFFIRMATIVE CONTRIBUTIONS

I. *Regarding the structure / antifidant activity relationship:*

1. The hypothesis of the Japanese researchers Kojima and Kato that the presence in the clerodane structure of spiro epoxide in C-4α,18 and two ester groups in C-6 and C-19 together with a hexahydro- or tetrahydro furofuran ring in C- 9, are a prerequisite for high antifidant activity.
2. The excellent activity of compounds in which the furofuran system is replaced by a γ-lacton ring spiro-linked at C-13 with an oxane ring concluded between C-8-C-13 was confirmed, as established by the Italian chemists Raccuglia et al. when testing the antifidant action of hastifolins A-G isolated from *S. hastifolia*.

II. ***Regarding the structure / cytotoxic activity relationship:***

1. All three *neo*-clerodane diterpenoids showing cytotoxicity, scutalpins A, E and F have a γ-lactone ring in their molecule, 13-spiro bound to an oxane ring concluded between C-8 and C-13, also as published for a series diterpenoids isolated from Asian species of the genus *Scutellaria* - *coleifolia*, *rivularis*, *caerulea*, *formosana*.

LIST
of the scientific works of the author on the topic of the dissertation did not participate in the procedure for acquiring the qualification "Doctor"

I. Scientific publications peer-reviewed in connection with the academic position of "Associate Professor" (2015):

I.A. ***Publications in scientific journals, referenced and indexed in world-famous databases of scientific information (ISI Web of Knowledge and/or SCOPUS)***

1. Malakov P., **Bozov P.** and Papanov G., (1997). A neo-clerodane diterpenoid from S*cutellaria orientalis* subsp. *pinnatifida*, Phytochemistry, vol. 46, № 3, pp. 587 – 589. **Q-1; IF – 1.165**
2. **Bozov P.**, Penchev P. and Coll J., (2014). Neo-Clerodane Diterpenoids from *Scutellaria galericulata*, Natural Product Communications, v. 9, (3), pp. 347-350. **Q-2; IF – 1.082**
3. **Bozov P.**, Vasileva T. and Iliev I., (2014). Structure and antifeedant activity relationship of neo-clerodane diterpens against Colorado potato beetle larvae, Chemistry of Natural Compounds, v. 50 (4), pp. 762-764. **Q-3; IF – 0.5**
4. **Bozov P.**, Penchev Pl., Vasileva T. and Iliev I., (2014). Diterpenoids from *Scutellaria galericulata*, Chemistry of Natural Compounds., V.49, № 3, pp. 479-480. **Q-3; IF – 0.5**
5. Yoana P. Georgieva, Rumen D. Mladenov and **Petko I. Bozov**, (2014). Chemical constituents of *Scutellaria altissima*, Chemistry of Natural Compounds, v. 50, № 6, pp. 1146-1147. **Q-3; IF – 0.5**
6. Plamen N. Penchev, Stefka R. Nachkova, Tonka A. Vasileva and **Petko I. Bozov**, (2014), ^{1}H and ^{13}C NMR Analysis of the *neo*-Clerodane Diterpenoid Scutecyprin, Natural Product Communications, v. 9, (8), pp. 1065-1068. **Q-2; IF: 1.08**
7. **Petko I. Bozov** and Josep Coll, (2015). *neo*-Clerodane Diterpenoids from *Scutellaria altissima*, Natural Product Communications, v. 10, (1), pp. 13-16. **Q-2; IF – 1.001**

I.B. ***Publication in a referenced edition, cited in a scientific publication, referenced and indexed in world-famous databases with scientific information (ISI Web of Knowledge and/or SCOPUS)***

8. **Petko I. Bozov**, Katia H. Nicolova Veselin P. Bivolarski Tonka A. Vasileva, (2014). Antifeedant activity of neo-clerodane diterpenoids from *Scutellaria*

galericulata agains Colorado potato beetle larvae, J. BioSci Biotech., SE/ONLINE.

Цирана в:

Raju Sripathi and Subban Ravi, (2017). Ethnopharmacology, Phytoconstituents, Essential Oil Composition and Biological Activities of the genus scutellaria, J. Pharm. Sci. & Res. Vol. 9, (3), 275-287. **IF - 0.3**

https://www.jpsr.pharmainfo.in/Documents/Volumes/vol9Issue03/jpsr09031703.pdf

I.C. ***Publications in refereed editions***

9. Malakov P., **Bozov P**. and Papanov G., (2001). Chemical constituents of the aerial parts of *Scutellaria albida*, Annuaire de L`Universite de Sofia "St. KlimentOhridski", Faculte de Chimie, vol. 91, pp. 133-137
10. Merkova S., **Bozov P**. and Iliev I., (2011). Chemical constituents of the aerial parts of Salvia splendenes, Annuaire de L`Universite de Sofia "St. KlimentOhridski", Faculte de Chimie, vol. 102/103, pp. 279-284.
11. **Bozov P.**, (2012). Antifeedant activity of stem extracts from species of genus *Scutellaria* L. Against Colorado potato beetle larvae, Annuaire de L`Universite de Sofia "St. KlimentOhridski", Faculte de Chimie, vol. 104, pp. 125-130.
12. **P.I. Bozov**, Y. P. Georgieva, R.D. Mladenov (2014) Diterpenoids wth *neo*-Clerodane skeleton of *Scutellaria altissima*, Annual of Sofia University "Str. Kliment Ohridski" Faculty of Chemistry and Pharmacy, 106, 53-59.

II. Scientific publications, other than those presented in the procedures for obtaining the "Doctor" degree and for holding the academic position of "Associate Professor":

IIA ***Publications in scientific journals, referenced and indexed in world-famous databases of scientific information (ISI Web of Knowledge and / or SCOPUS)***

13. **P. Bozov**, T. Girova, N. Prisadova, Y. Hristova, V. Gochev, (2015). Antimicrobial Activity of neo-Clerodane Diterpenoids isolated from Lamiaceae Species against Pathogenic and Food Spoilage Microorganisms, Natural Product Communications, 10 (11), 1797-1800. **Q-2; IF – 1.001**
14. Plamen N. Penchev, Josep Coll, Katia H. Nicolova, Ilia N. Iliev and **Petko I. Bozov**, (2016). Minor diterpenoids from *Scutellaria galericulata*, Phytochemistry letters, 15, 103-107. **Q-2; IF - 1.542**
15. **Petko I Bozov**, Yoana P Georgieva, (2017). Antifeedant Activity of *Neo*-clerodane Diterpenoids from *Scutellaria altissima* against Colorado Potato Beetle Larvae, Natural Product Communications, 12, (3), 327-328. **Q-2; IF - 0.852**
16. Katia H. Nicolova1, Norbert Kusz, Judit Hohmann, Mladen M. Naydenov, **Petko I. Bozov**, (2018). Two new *neo*-Clerodane Diterpenes from *Scutellaria galericulata*, Chemistry of Natural Compounds, v. 54, No 1, pp. 77-80. **Q-3; IF - 0.567**

17. K. H. Nikolova, I. T. Stoykov and **P. I. Bozov**, (2018). Responsible Structural Features for Cytotoxic, and other kind activity of *neo*-clerodane diterpenes from genus *Scutellaria*, Bulgarian Chemical Communications, v. 50, Special Issue C, pp. 7 – 13. **Q-4; IF - 0.349**
18. **Petko I. Bozov** and Plamen N. Penchev, (2019). *Neo*-clerodane diterpenoids from *Teucrium polium* subsp. *vincentinum* (rouy) D. Wood, Phytochemistry Letters, 31, 237-241.

Q-2; IF -1.338

19 **P. I. Bozov**, P. N. Penchev, (2019). *Neo*-Clerodane Diterpenoids from *Scutellaria velenovskyi* Rech. fil., Bulgarian Chemical Communications, Volume 51, Special issue D pp. 103 – 10 **Q-4; IF - 0.640**

20. Y. Georgieva, M. Katsarova, K. Gercheva, **P. Bozov**, S. Dimitrova, (2019). HPLC analysis of flavonoids from *Scutellaria altissima*, Bulgarian Chemical Communications, v. 51, Special issue D, pp. 119 – 123. **Q-4; IF - 0.640**
21. **Petko I. Bozov**, Plamen N. Penchev, Yoana P. Georgieva and Velizar Gochev, (2020). *Neo*-clerodane diterpenoids from *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin, Bulgarian Chemical Communications, v. 52, issue 4, pp. 453-459. **Q-4; IF - 0.640**
22. **P. I. Bozov** (2020) Clerodane diterpenoids, isolated from Bulgarian species of genus *Teucrium* (Lamiaceae), Bul. Chem. Communications., v. 52 (2) pp. 250-258. **Q-4; IF – 0.640**
23. **Petko I. Bozov**, Plamen N. Penchev, Tania D. Girova and Velizar K. Gochev, (2020). Diterpenoid constituents of *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin, Natural Product Communications, 15 (9):1–6.

Q-2; IF - 0.580

II.B. *Publications in refereed editions*

24. **P.I. Bozov**, T. Atanasova, P. Merdzhanov, A. Stoyanova, (2016). Chemical composition of the essential oils of *Salvia amplexicaulis* lam. and *Ajuga laxmanii* benth. from Bulgaria., Journal of Food and Packaging Science, Technique and Technologies, 10, 14-17.
25. Stanka Damyanova, Albena Stoyanova, Teodora Atanasova, **Petko Bozov**, (2016). Biotechnologies and food technologies, Volume 55, book 10.2, 71-74.
26. **P. I. Bozov**, (2016) *Neo*-Clerodane Diterpenoids From Species Of Genus *Scutellaria* (Lamiaceae), Annual of Sofia University "st. Kliment Ohridski" Faculty of Chemistry and Pharmacy, 107/108, 41-55.
27. P. N. Penchev, M. M. Naydenov, **P. I. Bozov**, (2016). Constituents of *Salvia nemorosa*., Annual of Sofia University "st. Kliment Ohridski" Faculty of Chemistry and Pharmacy, 107/108, 1-6.

II.C. *Conference publications*

28. Y. Georgieva, M. Katsarova, S. Dimitrova, **P. Bozov**, (1-3 June, 2018). Химичен състав и биологична активност на растения от род *Scutellaria* L. – Мини

обзор, National Scientific Conference "15 Years of Pharmacy in Medical University - Plovdiv", 267-275.

Participation in international and national scientific forums in connection with the dissertation

International scientific forums

1. **P.I. Bozov**, T. Atanasova, P. Merdzhanov, A. Stoyanova, Chemical composition of the essential oils of *Salvia amplexicaulis* lam. and *Ajuga laxmanii* benth. from Bulgaria, Development of the Science, Technologies and Techniques for the Manufacture, Packaging, Labeling, Storage and Distribution of Foods, 3-4 June, 2016, Burgas, Bulgaria.
2. Katya Nikolova, **Petko Bozov**, Thematic area 2: „Food Chemistry, Microbiology, Biotechnology and Safety" entitled: „Isolation and identification of *Scutellaria* diterpenoids and testing on vegetable culture against Colorado larvae /Leptinotarsa decemlineata (Say)/" (доклад), 65th Anniversary Scientific Conference with International Participation, „Food Sciences, Equipment and Technology - 2018", University of food Technologies - Plovdiv: 11nt – 13th October, 2018, Plovdiv, Bulgaria.
3. Katya H. Nikolova, I. T. Stoykov and **P. I. Bozov**, „Responsible structural features forcytotoxic and other kinds activity of neo-clerodane diterpenes from genus *Scutellaria*" (постер), First International Conference on Bio-antioxidants (ICBA 2017): „Natural Bio-antioxidants - as a base for new synthetic drugs and food additives/supplements": 25 – 29, June, 2017, Sofia, Bulgaria.
4. Katya Nikolova, I. T. Stoykov and **Petko Bozov**, „Two new neo-clerodane diterpenes isolated from *Scutellaria galericulata* and cytotoxic activities of diterpenes from different Scutelaria genus" (постер), 4-th Balkan Scientific Conference on Biology: 1st - 3rd November, 2017, Plovdiv, Bulgaria.
3. S. Dimitrova, Y. Georgieva, M. Katsarova1, K. Gercheva, P. Denev, **P. Bozov**, Study on the flavonoid composition and antioxidant activity of four *Scutellaria* species from the region of South Bulgaria, 4-th Word Congress & Expo on Pharmaceutics and Drag Delivery Systems, March, 25-26, 2019 Milan, Italy.

National scientific forums

1. **Bozov P**., Stoyanov Pl., Mladenov R., Vasileva T., (2011), Antifeedant activity of natural *neo*-clerodanes, isolated from *Scutellaria alpina* и *Salvia splendens* Ker.-Gawl. and four synthetic derivatives against Colorado Potato Beetle Larvae (Report). Scientific Seminar "Biological Sciences for a Better Future" (50 years of PU), October 28-29, 2011, Pamporovo.
2. K. Nikolova and **P. Bozov**, Diterpenes from *Scutellaria galericulata* and their biological activity (report), XVI National Conference on Chemistry for students

and PhD students 2017, Sofia University“ St. Kliment Ohridski ”, May 17 - 19, Sofia, Bulgaria.

3. Stanka Damyanova, Albena Stoyanova, Teodora Atanasova, **Petko Bozov**, Chemical composition of essential oil from *Stachys cretica* subsp. *bulgarica* Rech. fil., 56th Scientific Conference RU & SU ’17 “Industry 4.0 and the Conventional Business Models” University of Ruse “Angel Kanchev”, РУ & СУ ’17, 27-28.10.2017.
4. Y. Georgieva, M. Katsarova, K. Gercheva, **P. Bozov**, S. Dimitrova, “HPLC Analysis of Flavonoids from *Scutellaria altissima*” 11th Chemistry Conference Plovdiv (11cc), 11 -13 October 2018.
5. **Petko I. Bozov**, Plamen N. Penchev, Two cleroindicins from *Scutellaria hastifolia* – first time isolated from Lamiaceae family species, 10th Chemistry Conference Plovdiv (10cc), 09 -11 October 2016.
6. Plamen N. Penchev, **Petko I. Bozov**, *Neo*-Clerodane Diterpenoids from *Scutellaria velenovskyi* Rech. fil., 11th Chemistry Conference Plovdiv (11cc), 11 -13 October 2018.
7. J. Georgieva, M. Katsarova, S. Dimitrova, **P. Bozov**, Poster - Chemical composition and biological activity of plants of the genus *Scutellaria* L. National scientific conference "15 years of pharmacy at the Medical University - Plovdiv" - 01-03 June 2018, Devin.

According to Scopus, the publications have been cited 139 times. The unused citations in other procedures are 73. The author of the dissertation has a H-index - 7.

The dissertation includes the work of:

my graduates: Katya Nikolova, Pavlina Sredkova, Tihomira Dineva, Stella Pamukova, Joana Georgieva;
my PhD students: Dr. Katya Nikolova, PhD student Joanna Georgieva.

ACKNOWLEDGMENTS

Thank you very much to all who have supported and helped me in the development and implementation of this dissertation:

- To Prof. Georgi Papanov and Assoc. Prof. Petar Malakov, who introduced me to the chemistry of terpenes.
- To all colleagues with whom we have worked together or who have guided me with constructive suggestions, advice or constructive criticism.
- I thank my family for the understanding, patience and support I have always received.
- This work was carried out with the active participation of Prof. Plamen Penchev