MINISTRY OF EDUCATION AND RESEARCH
OF THE REPUBLIC OF MOLDOVA
MOLDOVA STATE UNIVERSITY

As manuscript
C.Z.U.: 547.1:577.1 (043.3)

CIOCARLAN ALEXANDRU

SYNTHESIS OF BIOLOGICALLY ACTIVE NORLABDANE DERIVATIVES WITH ADVANCED FUNCTIONALIZATION AND PHYTOCHEMICAL STUDY OF SOME LOCAL VEGETAL SOURCES

143.04 – BIOORGANIC CHEMISTRY, CHEMISTRY OF NATURAL AND PHYSIOLOGICALLY ACTIVE COMPOUNDS

Summary of doctor habilitat thesis in chimical sciences

CHISHINAU, 2024

The thesis has been elaborated in the Laboratory Chemistry of Natural and Biologically Active Compounds of the Institute of Chemistry of the Moldova State University.

Scientific advisor:
ARÎCU Aculina, doctor habilitat in chemical sciences, associate professor, Institute of Chemistry, Moldova State University.

Members of the Commission for public defence of the doctor habilitate thesis:

MACAEV Fliur	corresponding member of MAS, doctor habilitat in chemical sciences, profesor, Institute of Chemistry, Moldova State University, *chairman*;
ARÎCU Aculina	doctor habilitat in chemical sciences, associate professor, Institute of Chemistry, Moldova State University, *scientific advisor*;
BOBEICĂ Valentin	doctor habilitat in chemical sciences, professor, Moldova State University, *reviewer*;
DANAC Ramona	profesor doctor in chemical sciences, "*Alexandru Ioan Cuza*" University of Iasi, Romania, *reviewer*;
CALALB Tatiana	doctor habilitat in biological sciences, profesor, "*Nicolae Testemițanu*" State University of Medicine and Pharmacy, of the Republic of Moldova, *reviewer*;
STURZA Rodica	corresponding member of MAS, doctor habilitat in chemical sciences, profesor, Technical University of Moldova, *reviewer*;
KULCIȚKI Veaceslav	doctor habilitat in chemical sciences, associate professor, Institute of Chemistry, Moldova State University, *scientific secretary*.

The public defence of the thesis will take place on **April 25**, **2024**, **14:00**, in the meeting of the Commission for the public defense of the doctor habilitat thesis, at the Institute of Chemistry, small hall, 3 Academiei street, Chishinau, Republic of Moldova. (*http://www. usm.md*)

The doctor habilitat thesis and summary can bee found at the National Library of the Republic of Moldova, the USM library, on the web page of ANACEC (*http://www.cnaa.md/*) and on the web page of USM (*http://www.usm.md/*)

The summary was sent on March 25 2024

Scientific secretary of the Specialized scientific counsil, **KULCIȚKI Veaceslav**
Dr. hab., assoc. prof.
Scientific advisor, **ARÎCU Aculina**
Dr. hab., assoc. prof.
Author, **CIOCÂRLAN Alexandru**
Dr., assoc. prof.

CONTENTS

CONCEPTUAL FRAMEWORK OF THE RESEARCH

Actuality and importance of the topic addressed. Organic synthesis, the applied field of organic chemistry, has as its object of study a unique tool, the carbon chain, which offers unlimited possibilities for generating chemical structures. They can be synthetic compounds with desired structure and properties, or natural compounds isolated from various natural sources, i.e. created by Nature and reproduced by humans.

The contemporary level of development of organic chemistry allows rapid identification of target compounds with specific properties in natural sources and their synthesis in useful times. A conclusive example in this sense is the terpene compounds, which are characterized by a wide variety of carbon skeletons.

This thesis represents a combination of several strategies that drive modern organic synthesis. One of them can be called a biomimetic strategy, since it aims at the total synthesis or semi-synthesis of known natural compounds based on accessible raw materials, such as (-)-sclareol and (+)-larixol, which possess the basic terpene skeleton. In this case, the focus is on rational synthesis, i.e. a minimum required number of steps and efficient non-polluting reagents, factors that ultimately result in acceptable to excellent overall yields.

Another strategy used is the synthesis of new terpene compounds with advanced functionalization based on the same raw materials. In this case, it is not a simple reproduction of natural models, but the realization of ambitions to create new structures with useful properties. This strategy also includes the new direction of creating molecular hybrids that possess biological activity and are composed of the terpene and heterocyclic units. An advantage of the work lies in the frequent use, in both cases, of non-conventional and environmentally friendly synthesis methods.

Another strategy is the phytochemical studies of plant sources, which usually precede the actual chemical synthesis and provide useful information about their chemical composition, the structure of the identified components and, in general, allow the conclusion regarding the practical uses of the studied species.

Another advantage of the work is the fact that the antibacterial activity of all synthetic compounds, plant samples and products derived from them was tested *in vitro*, and the synthetic compounds with high activity and the procedures for obtaining them were patented.

Natural and synthetic terpene compounds enjoy a constant attention of researchers due to the wide spectrum of biological properties they exhibit, which makes it possible to use them in medicine, pharmaceuticals, cosmetics, agriculture and other fields of human activity. For example, currently, one of the serious problems that humanity has faced is diseases caused by fungi and bacteria. For this reason, the requirement for effective antifungal and antibacterial remedies remains constant and provides a wide field of research for chemists. Popular memory preserves information about the natural sources used for centuries to treat these ailments, many of which are currently sufficiently well studied and contain especially terpene components. On the other hand, the pharmaceutical industry continues to design new molecular structures, which in some cases become the active principles of drugs with antimicrobial properties. Obtaining molecular hybrids with heterocyclic units based on accessible terpene compounds represents a new direction in organic synthesis. In this case, the terpene units, due to their lipophilic character and natural origin, determine the biocompatibility and reduced toxicity of the synthetic products, and the heterocyclic fragments their pronounced and varied biological activities.

The purpose of the work. The main goal of this work was the development of some schemes for the synthesis of natural analogues, derivatives with an advanced degree of

functionalization and terpene-heterocyclic molecular hybrids, biologically active, by classical and non-conventional methods based on (-)-sclareol and (+)-larixol, the elucidation of the reaction mechanisms, the confirmation of the structures, the determination of the biological activity of the synthesis compounds and the realization of a complementary phytochemical study of the local plant sources of interest, the testing of the biological activity.

Research objectives.

- New and efficient syntheses of natural and biologically active norlabdane compounds, previously isolated from various natural sources, or of their precursors;
- Syntheses of new norlabdane compounds with advanced functionalization;
- Syntheses of norlabdane molecular hybrids with various heterocyclic units;
- The combined use of classical synthesis methods and non-conventional, environmentally friendly ones, such as photochemical, electrochemical and microwave-assisted transformations;
- Carrying out the phytochemical study on some local plant species of food or therapeutic importance;
- *In vitro* biological activity testing of synthetic compounds, vegetal sources and products derived from them;
- Establishing the structure-activity relationship on the example of synthetic compounds with increased activity.

Research hypothesis. It involves the directed synthesis of natural di-, tri-, tetra- and pentanorlabdane compounds, those with an advanced degree of functionalization and molecular hybrids with terpene and heterocyclic structural units, such as diazine, 1,2,4-triazole, 2-amino-1,3-thiazoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, based on labdane diterpenoids (-)-sclareol and (+)-larixol, and phytochemical characterization of some local plant sources. The synthetic compounds and plant sources mentioned can be used in the production of new therapeutic remedies.

Synthesis of research methodology and justification of chosen research methods. The research methodology includes both classical and non-conventional methods of synthesis, such as photochemical, electrochemical, microwave irradiation, but also classical methods of extraction and analysis. Melting points (m.p.) were determined with the Boetius apparatus. Specific rotation was measured with a Jasco DIP 370 polarimeter. IR absorption spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. NMR spectra were recorded on a Bruker 400 Avance III spectrometer at 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C and 40.55 MHz for ^{15}N in $CDCl_3$, tetramethylsilane (TMS) served as the internal standard. High resolution HR-ESI-MS mass spectra were recorded on AEI MS-902 mass spectrometer. Some molecular structures were confirmed by the single crystal X-ray diffraction method, using the XCALIBUR E CCD diffractometer equipped with a Mo-Ka graphite-monochromate radiation source. Microwave-assisted reactions were carried out in a quartz vessel in a single-mode reactor (800 W, STAR SYSTEM-2). The monitoring of the reaction process was carried out on an Agilent-5975C GC-MS system and with the help of thin layer chromatography (TLC) on Silufol silica gel plates. GC-MS analyzes of volatile oils were performed on an Agilent 7890 A type chromatograph coupled to an Agilent 5975C mass detector (MSD). Phenolic and triterpene acids were quantified by an HPLC-PDA method using a Thermo Finnigan Surveyor Plus HPLC system. The mineral composition of the analyzed plant species was determined by the neutron activation method on an IBR-2 reactor. The antimicrobial activity of the newly synthesized compounds was tested *in vitro* on 5 strains of fungi and 2 species of Gram-negative and Gram-positive bacteria by the method of consecutive dilutions in agar medium.

The theoretical importance and scientific innovation of the work. Based on labdane diterpenoids (-)-sclareol and (+)-larixol, the importance and possibility of transforming some waste into compounds of practical importance was confirmed, by:

- the first realization of effective, original, stereo- and regioselective syntheses of natural analogues;
- making syntheses of norlabdane compounds with advanced functionalization;
- the synthesis of a series of terpene-heterocyclic molecular hybrids with diazine, 1,2,4-triazole, 2-amino-1,3-thiazole, 1,3,4-oxadiazole and 1,3,4-thiadiazole units;

Were:

- completed the scientific information regarding the mechanisms of some reactions in the series of norlabdane compounds, including those with heterocyclic units;
- demonstrated the usefulness and efficiency of applying non-conventional synthesis methods in the chemistry of natural compounds;
- identified series of synthetic compounds with advanced antimicrobial activity;
- completed scientific information regarding the structure-activity relationship of bioactive compounds;
- phytochemical studies of some local plant sources were carried out for the first time, which confirmed the value and usefulness of local plant materials and products derived from them.

Fundamentally new results for science and practice obtained. It consists in the development of new processes for the transformation of natural labdane diterpenoids (-)-sclareol and (+)-larixol, obtained from incense sage waste and larch resin, into biologically active natural or synthetic compounds with possible applications in the pharmaceutical industry, including through environmentally friendly synthesis methods. A new direction for the synthesis of heterocyclic molecular hybrids possessing biological activity was developed. The phytochemical composition of local sources of food and therapeutic importance, such as apples and forest fruits, aromatic and hetero-oleaginous plants, was established for the first time.

Theoretical significance. The research results confirm the possibility of transforming local waste into compounds of practical importance; demonstrates the utility and efficiency of applying non-conventional synthesis methods in the chemistry of natural compounds; completes the scientific information on the mechanisms of reactions in the series of norlabdane compounds, including heterocyclic hybrids; and those related to the structure-activity relationship of synthetic compounds, the qualitative composition and usefulness of local plant materials and products derived from them.

The applicative value of the work. Antimicrobial activity of all new synthetic compounds was tested on five fungal strains and two bacterial species. Of these, 28 compounds showed good antimicrobial activity, and four of the reported compounds showed pronounced antimicrobial activity. Pharmaceutical companies may be interested in these developments, and the synthesized biologically active norlabdane derivatives may serve as active principles.

Approval of the work at national and international scientific forums. The obtained results were presented at 23 national and international conferences: International Conference dedicated to the 50th anniversary from the foundation of the Institute of Chemistry of the Moldavian Academy of Sciences May 26 – 28, 2009, Chişinău, Moldova; National Chemistry Conference, 2010, 2012, 2014, 2016 and 2018 editions, Călimănești-Căciulata, Romania (and oral communication); ZAI 2011, XXIII scientific communication session "*Progress in the science of organic and macromolecular compounds*", September 28 - October 1, 2011, Iasi, Romania; International Conference dedicated to the 55th anniversary of the Institute of Applied

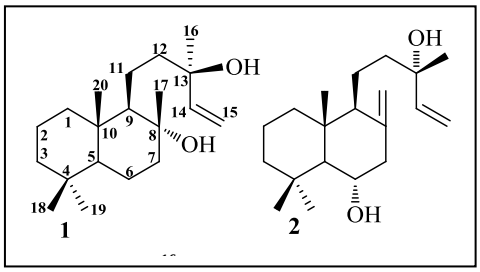
Physics of the A.S.M. Chisinau, Moldova, 2014; International Conference dedicated to the 55th anniversary of the Institute of Chemistry of the A.S.M. Chisinau, Moldova, May 28-30, 2014; 20th National Conference with International Participation "*Progress in Cryogenics and Isotope Separation*", October 23-24, 2014, Râmnicul Vâlcea, Romania; XVIII-th Conference "*Physical Methods in Coordination and Supramolecular Chemistry*", Chisinau, Moldova, October 8-9, 2015; 8th International Conference on Materials Science and Condensed Matter Physics. Chisinau, Moldova, September 12-16, 2016; "*A.I. Cuza*" University Days, Faculty of Chemistry Conference. October 27-29, 2016; 6th International Conference Ecological & Environmental Chemistry. Chisinau, Moldova, March 2-3, 2017; 20-th Romanian International Conference on Chemistry and Chemical Engineering., Poiana Brașov, Romania, September 6 – 9, 2017; 19th Central and Eastern European NMR Symposium & Bruker Users' Meeting, Timișoara, Romania, September 5-8, 2017; International Conference "*Achievements and perspectives of modern chemistry*" dedicated to the 60th anniversary of the Institute of Chemistry. Chisinau, Moldova, October 9-11, 2019; 23rd International Conference "*New Cryogenic and Isotope Technologies for Energy and Environment*" - EnergEn 2020. Băile Govora, Romania, May 25–27, 2020; Scientific seminar "*New frontiers in natural product chemistry*" 6th Edition. Chisinau, Moldova, June 4, 2021; International Conference "*Intelligent Valorisation of Agro-Industrial Wastes*", 07-08 October 2021; Conference "*Ecological and environmental chemistry 2022*" 7th Edition, Chisinau, Moldova, March 3-4, 2022.

Publications on the topic of the thesis. The research results presented in the paper have been published in 66 scientific papers, including 1 chapter in a monograph, 15 articles in impact factor journals, 9 in national journals and 37 abstracts at national and international scientific events, and 4 invention patents.

Summary of thesis chapters. The work is presented on 235 typed basic text pages and includes the sections: annotations in Romanian, English and Russian, introduction, 6 chapters, general conclusions and recommendations, bibliography of 514 references, 144 figures and 37 tables.

1. GENERAL CHARACTERISTICS OF LABDANE COMPOUNDS

Chapter I includes data on the classification, distribution, and properties of terpene compounds, especially diterpenoids. Information on the biological activity of some reference representatives from this group is presented, and more importantly, recent syntheses based on (-)-sclareol **1** and (+)-larixol **2**, natural labdane diterpenoids obtained industrially from vegetale waste and accessible as commercial raw materials for synthesis are described.



Emphasis is placed on known syntheses of highly functionalized compounds, natural analogs, and terpene-heterocyclic molecular hybrids. In this chapter, non-conventional methods of synthesis (photolytic degradation, sensitized photooxidation, anodic oxidation and microwave irradiation) are theoretically grounded, the advantages of these methods are described and their usefulness on terpene substrates is presented. Also, in the last subchapter, recent phytochemical studies on some plant sources of interest are presented.

2. CHEMICAL TRANSFORMATIONS BASED ON SOME TETRA- AND PENTANORLABDANE DERIVATIVES OF (-)-SCLAREOL

2.1. Overview

The labdane diterpenoid (-)-sclareol **1** is an abundant natural source, which is obtained industrially from the waste of Clary sage (*Salvia sclarea* L.), which remain after the distillation of the volatile oil. The chemistry of (-)-sclareol **1** has experienced an impressive development in recent years not only from a theoretical point of view, but also due to its significant practical importance in many fields of human activity, predominantly in the food, pharmaceutical and tobacco processing industries, perfumery, but also in organic synthesis.

Based on (–)-sclareol **1** and its derivative (+)-sclareolide **3**, multiple syntheses of important drimane sesquiterpenoids and some tetranorlabdane compounds were carried out via methyl bicyclohomofarnesoates **4**–**6** and methyl 7-oxo-13,14, 15,16-tetranorlabd-8-en-12-oate **7** [1-9].

In this Chapter, the syntheses of pentanorlabdane compounds with an advanced degree of functionalization based on drim-8(9)-en-7-one **8** and drim-7,8(11)-diene **9** are described, as well as those of some tetra- and pentanorlabdane derivatives with rearranged carbon skeleton, obtained by Baeyer-Villiger oxidation and Beckmann transposition reactions.

2.2. Synthesis and derivatization of methyl esters of bicyclohomofarnesenic acids by classical and non-conventional synthesis methods

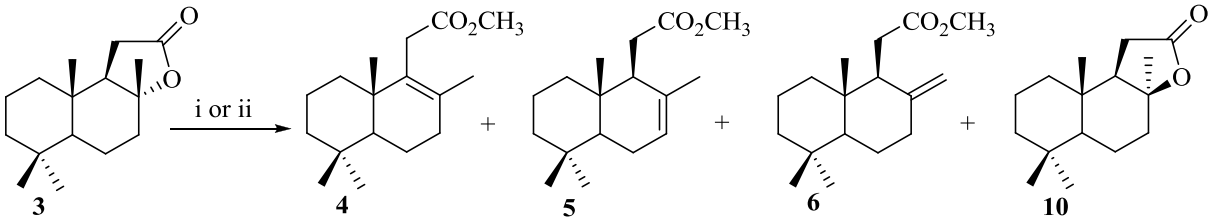
The isomeric bicyclohomofarnesene methyl esters **4**-**6** are key intermediates in the syntheses of tetra- and pentanorlabdane compounds from (-)-sclareol **1**. At the same time, these compounds are a suitable model for studies, especially those involving non-conventional synthetic methods.

The transesterification-dehydration reaction of (+)-sclareolide **3**, known as the Stoll and Hinder method, according to the authors, gives a mixture of bicyclohomofarnesenic methyl esters isomers **4** and **5**. Later it was found that in reality a mixture consisting of three methyl esters **4**-**6** in 96% yield, in a 6:3:1 ratio (according to GC-MS and NMR data), together with a small amount of isolactone **10**.

The present study [10] is dedicated to the comparative analysis of isomeric bicyclohomofarnesene methyl esters **4**-**6** methods of synthesis, using microwave (MW)

irradiation versus the Stoll and Hinder method, and their electro- and photochemical transformations.

As mentioned, previously esters **4-6** were obtained by the Stoll and Hinder method, which involves a long-term reflux of (+)-sclareolide **3** in acidified methanolic solution, which is its main drawback (Figure 2.1).



Reagents and conditions: i) H_2SO_4, MeOH, reflux, 96 hrs, 96%; ii) H_2SO_4, MeOH, MW, 30 min., 93%.

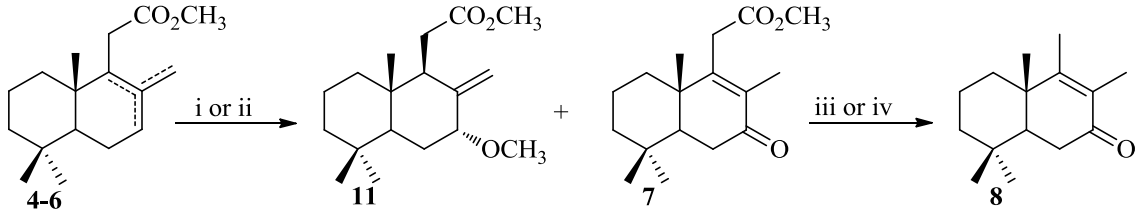
Fig. 2.1. Synthesis of bicyclohomofarnesene methyl esters **4-6** from (+)-sclareolide **3**.

Under microwave irradiation, the transesterification-dehydration reaction of (+)-sclareolide **3** proceeds in only 30 minutes (Figure 2.1), compounds **4-6** are obtained in 95% yield, in a 3:11 :5 ratio, that is different from the previously reported one.

The comparative analysis of the dynamics of the formation of esters **4-6** by both methods was carried out based on GC-MS data. According to this, after 8 hours of Stoll and Hinder reaction, the formation of the trisubstituted isomer **5** (51.5%) is favored, and the tetrasubstituted isomers **4** (36.4%) and exocyclic **6** (7.1%) are minor. After 96 hours, their ratio changes to 15:5:3, the content of isomer **4** increases (73.7%), and those of isomers **5** and **6** decrease to 24.4% and 1.53%, respectively.

According to the GC-MS analysis, after 30 minutes of MW irradiation the major isomer is the trisubstituted **5** (55.8%), followed by the exocyclic **6** (24.3%), and the tetrasubstituted **4** (14.8%) completes the series, after which starts the decomposition of the reaction products.

Initially, methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** was obtained by oxidizing the mixture of methyl esters **4-6** with potassium dichromate with in a yield of 38%. Later, the authors obtained ketoester **7** (63%) by anodic oxidation of esters **4-6** in the presence of lithium perchlorate as electrolyte, together with the minor methoxyester **11** [2]. The same authors, for the first time, obtained drim-8(9)-en-7-one **8** (drimenone) by decarboxylation of ketoester **7**, and recently, drimenone **8** was obtained by the MW assisted reaction in 92% yield (Figure 2.2), this method being more advantageous and 2 times faster.



Reagents and conditions: i) $K_2Cr_2O_7$, MeOH, reflux, 12 hrs; ii) $LiClO_4$, MeOH, ē, 3 hrs;
iii) KOH, EtOH, reflux, 3 hrs; iv) KOH, EtOH, MW, 1.5 hrs.

Fig. 2.2. Syntheses of ketoester **7** and drimenone **8**.

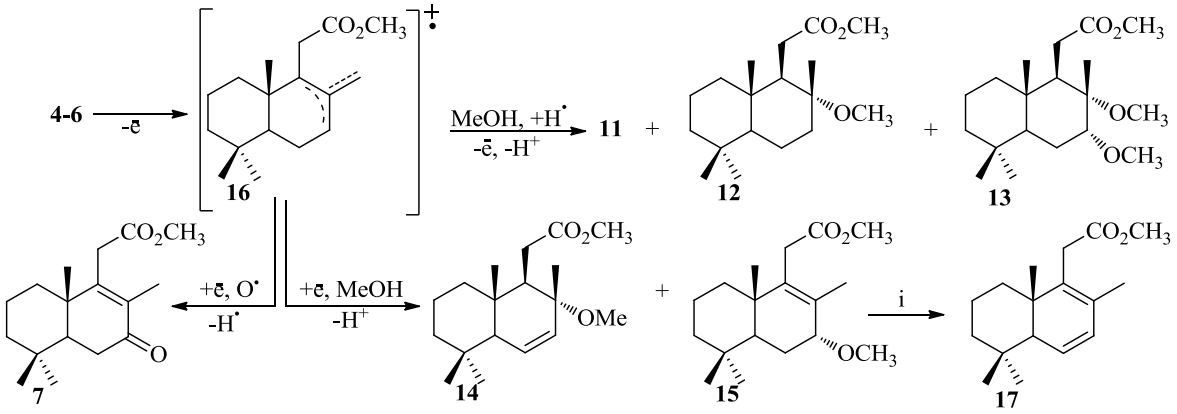
Both compounds are only synthetically prepared, since ketoester **7** is missing in natural sources, and drimenone **8** has been detected in very small amounts. Based on these compounds, until now, a large number of new synthetic compounds or natural analogues of the homodrimane (C_{16}) or drimane (C_{15}) series have been obtained [6-9, 11-13].

Chromatographic analyzes (analytical TLC and GC-MS) of the product of the anodic oxidation reaction of esters **4-6**, showed a complex mixture of compounds, the composition of which was subjected to an exhaustive study. As a result, the previously reported compounds **7** and **11** were isolated, as well as a series of new minor compounds **12-15**, their structure was demonstrated and the mechanism of their formation was proposed (Figure 2.3).

It is well known that the anodic oxidation of olefins, including terpenes, proceeds through an intermediate radical cation generated by the anodic removal of an electron from the π-olefinic electron system. Olefins with at least one allylic hydrogen undergo to the allylic substitution in which the solvent acts as a nucleophile. In this study [10], initially the mixture of methyl esters **4**–**6** loses an electron and generates the radical cation **16** as a reactive species. Further, its interaction with methanol as a nucleophile is accompanied by a series of additions/eliminations of electrons, radicals or ions and leads to the allylic substitution products **12-15** (Figure 2.3). Ketoester **7** is an allylic oxidation product of isomer **4**, with lithium perchlorate which is a strong oxidizing agent and under these conditions, its reduction occurs according to Eq. (1) with the formation of atomic oxygen.

$$LiClO_4 \xrightarrow[-\bar{e}]{} LiCl + 4O^{\bullet} \quad \text{Eq. (1)}$$

The lifetime of this highly reactive species is sufficient to attack the allylic position of radical cation **17**, causing its oxidation through several intermediate states (Figure 2.3).

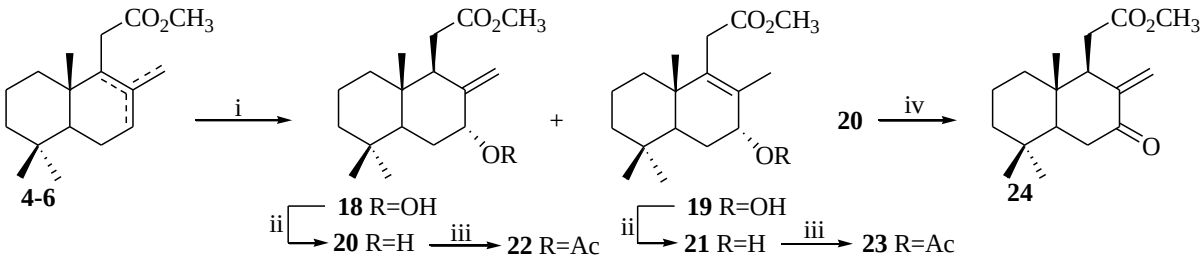


Reagents and conditions: i) H_2SO_4, THF, r.t., 6 hrs.

Fig. 2.3. Synthesis of compounds **7**, **11**, **12-15** by anodic oxydation of mixture of esters **4-6**.

In addition, a new method was developed for the synthesis of dienster **17** (86%), an important intermediate in the syntheses of polyfunctionalized homodrimane compounds from the previously unused minor compound **15**, whose content reaches ~25% (Figure 2.3) [13].

The sensitized photooxidation of methyl esters **4-6** led to the new isomeric hydroperoxides **18** and **19**, with yields of 76.9% and 9.2%, respectively, recalculated based on the conversion of methyl esters **4-6**. Next, by standard methods [8], these compounds were converted to alcohols **20** and **21** (96 and 94%), then to acetates **22** and **23** (98 and 97%). Pyridinium chlorochromate (PCC)-assisted oxidation of alcohols **20** and **21** [8] led to the known ketoesters **7** and previously undescribed **24**, both obtained in the same 98% yields (Figure 2.4).



Reagents and conditions: i) O_2, H_2tpp. hv, CCl_4, r.t.; (ii) Thiourea, MeOH, 0°C → r.t.;
iii) Py, Ac_2O, r.t.; iv) PCC, CH_2Cl_2, AcOH (gl.), r.t., molecular sieves 3 Å.

Fig. 2.4. Sensitized photooxydation of the mixture of methyl esters **4-6**.

Thus, through the syntheses based on the bicyclohomofarnesenic methyl esters **4-6** derived from (-)-sclareol **1**, which represent an accessible and suitable research model, the usefulness of combining classical methods with non-conventional ones, such as microwave irradiation, anodic oxidation and sensitized photooxidation in the optimization of key reactions of (-)-sclareol **1** chemistry, such as the Stoll-Hinder reaction, and in the synthesis of biologically active norlabdane derivatives with advanced functionalization was proved. The antifungal and antibacterial activity of the synthetic compounds described in this Chapter has been evaluated and the data are discussed together with the results in Chapter VI.

2.3. Preparation of some polyfunctional tetranorlabdane derivatives from methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate

The syntheses of tetranorlabdane derivatives are generally shorter and easier than those of the related pentanorlabdane compounds, they beeng used on an industrial scale in perfumery, cosmetics and for ennobling tobacco products. One of the most important tetranorlabane compounds, ambroxide **25** (Ambrox®) possesses an ambergris odor and can be industrially produced from (-)-sclareol **1**. It seems surprising, but there are only a few mentions of the biological activity of tetranorlabdanes, which confirm, that 13,14,15,16-tetranorlabd-7-en-12,17-dial **26** and 7-oxo-13,14,15,16-tetranorlabd-8(17)-en-12-al **27** showed significantly better antifeedant activity than the reference drimane compounds polygodial **28** and its isomer **29** (Figure 2.5).

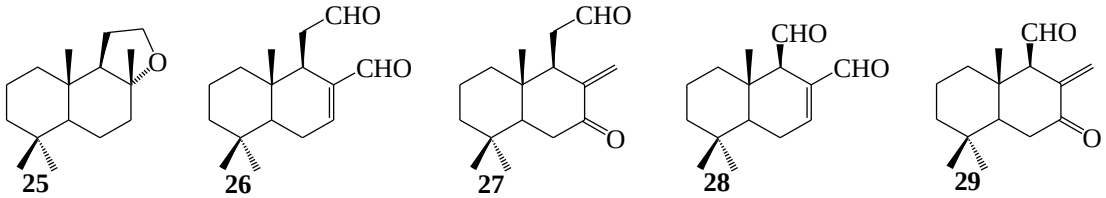


Fig. 2.5. Biologically active tetra- **25-27** and pentanorladane **28**, **29** compounds.

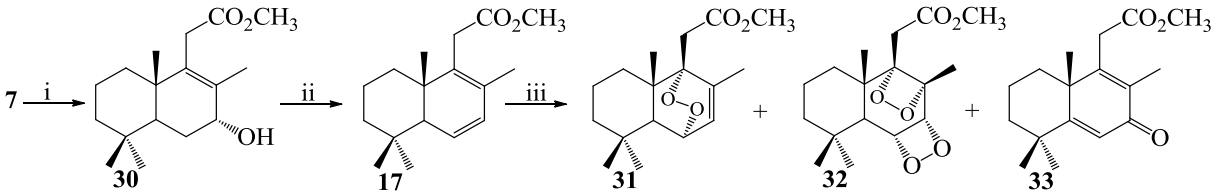
Methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** is an important intermediate in the synthesis of tetranorlabdane compounds due to the presence of the double bond, the carboxyl and carbonyl groups, which activate the positions C_6, C_{11} and C_{17} [2, 10], and to date, only a few syntheses of tetranorlabdane compounds have been performed based on it.

Next, recent results [13] in the field of (–)-sclareol **1** chemistry will be presented and new regio- and stereoselective syntheses of polyfunctional tetranorlabdane (homodrimane) compounds will be described, starting from ketoester **7**.

Reduction of compound **7** with $NaBH_4$ in the presence of $CeCl_3$•$7H_2O$ gave exclusively methyl 7α-hydroxy-13,14,15,16-tetranorlabd-8-en-12-oate **30**, and its dehydration under mild

conditions, followed by 1,4-elimination, led to methyl 13,14,15,16-tetranorlabd-6,8(9)-dien-12-oate **17**.

Sensitized photooxygenation [14] of diene **17** in the presence of *meso*-tetraphenylporphyrin (TPP) gave a mixture consisting of methyl 6*α*,9*α*-peroxy-13,14,15,16-tetranorlabd-7(8)-ene-12-oate **31**, as a result of the 1,4-cycloaddition reaction of singlet oxygen and dioxin **32** from its [2+2] addition, together with methyl 7-oxo-13,14,15,16-tetranorlabd-5,8(9)-dien-12-oate **33**, previously obtained as a result of the photooxidative dehydrogenation reaction (Figure 2.6).



Reagents and conditions: i) $NaBH_4$, $CeCl_3$•$7H_2O$, MeOH, 0.5 hrs, 93%; ii) H_2SO_4, THF, 24 hrs, 89%; iii) O_2, H_2tpp. hν, DCM, 5°C, 5 hrs, 21.0%, 7.0% and 54.0%.

Fig. 2.6. Synthesis of C_6-C_9 functionalized derivatives of ketoester **7**.

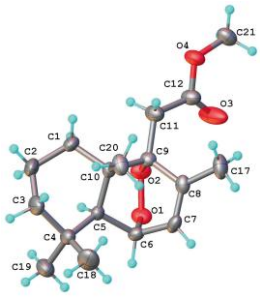
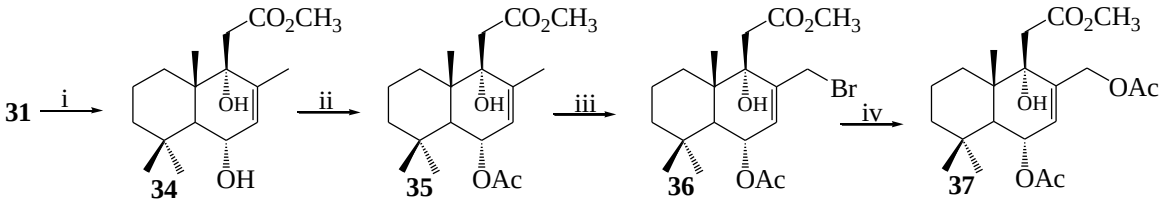


Fig. 2.7. Molecular structure of compound **31**.

The structure of compound **31** was confirmed by spectral data and additionally by X-ray diffraction (Figure 2.7). Reduction of endoperoxide **31** with thiourea led to methyl 6*α*,9*α*-dihydroxy-13,14,15,16-tetranorlabd-7(8)-en-12-oate **34**, and after acetylation of the C_6 hydroxyl group under standard conditions methyl 6*α*-acetoxy-9*α*-hydroxy-13,14,15,16-tetranorlabd-7(8)-en-12-oate **35** was obtained. Allylic bromination of the C_{17} methyl group in the molecule of acetate **35** was performed with NBS, avoiding allylic oxidation with selenium dioxide, followed by bromine substitution to form diacetate **37** (Figure 2.8).



Reagents and conditions: i) Thiourea, MeOH, 3 hrs, r.t., 96%; ii) Ac_2O, Py, 2.5 hrs, r.t., 94%; iii) NBS, CCl_4, reflux, 3 hrs, 76.0%; iv) KOAc, DMSO, 2 hrs, r.t., 88%.

Fig 2.8. Synthesis of C_{17} functionalized derivatives of ketoester **7**.

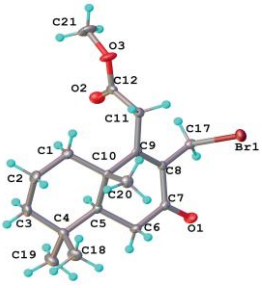
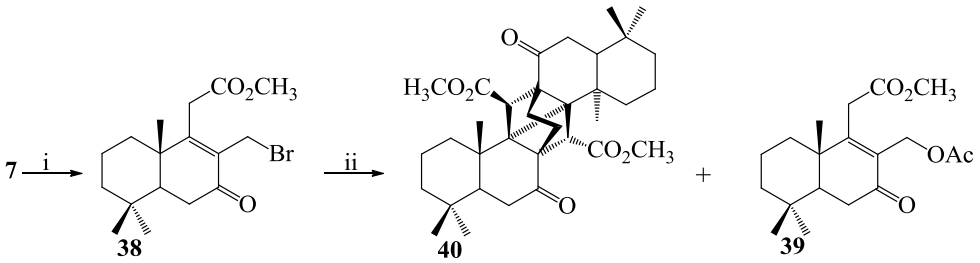


Fig. 2.9. Molecular structure of compound **38**.

Bromination of compound **7** led to methyl 7-oxo-17-bromo-13,14,15,16-tetranorlabd-8-en-12-oate **38**, the main reaction product (Figure 2.10). X-ray crystallographic analysis confirmed the structure of compound **38** (Figure 2.9). The attempted to substitute the bromine atom in the molecule of compound **38** by an acetate group, contrary to expectations, led not only to the target compound **39**, but also to the dimer **40**, an unprecedented carbon skeleton compound, which was isolated as the main reaction product (Figure 2.10).



Reagents and conditions: i) NBS, CCl_4, reflux, 2 hrs, 90%; ii) KOAc, DMSO, 2 hrs, r.t., 86.0% and 14.0%.

Fig. 2.10. Synthesis of dimer **40**.

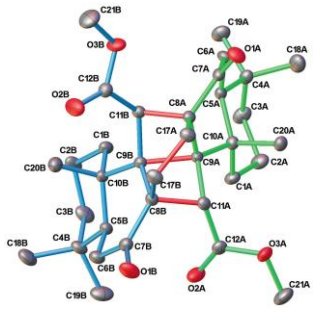


Fig. 2.11. Molecular structure of compound **40**.

The structure of compound **40** was initially established by spectroscopic analysis and confirmed by single crystal X-ray diffraction (Figure 2.11) [13]. A possible explanation for the formation of compound **40** is shown in Figure 2.12. The reaction mechanism involves the initial formation of an allylic carbocation **41** in a polar solvent (DMSO), which can follow two different reaction pathways. Through one of them, as a result of the S_{N1} reaction, acetate **39** is obtained with a moderate yield (14%). The second reaction path (Path II, Figure 2.12) proceeds with the formation of dimer **40**, as the main product of the reaction (86%).

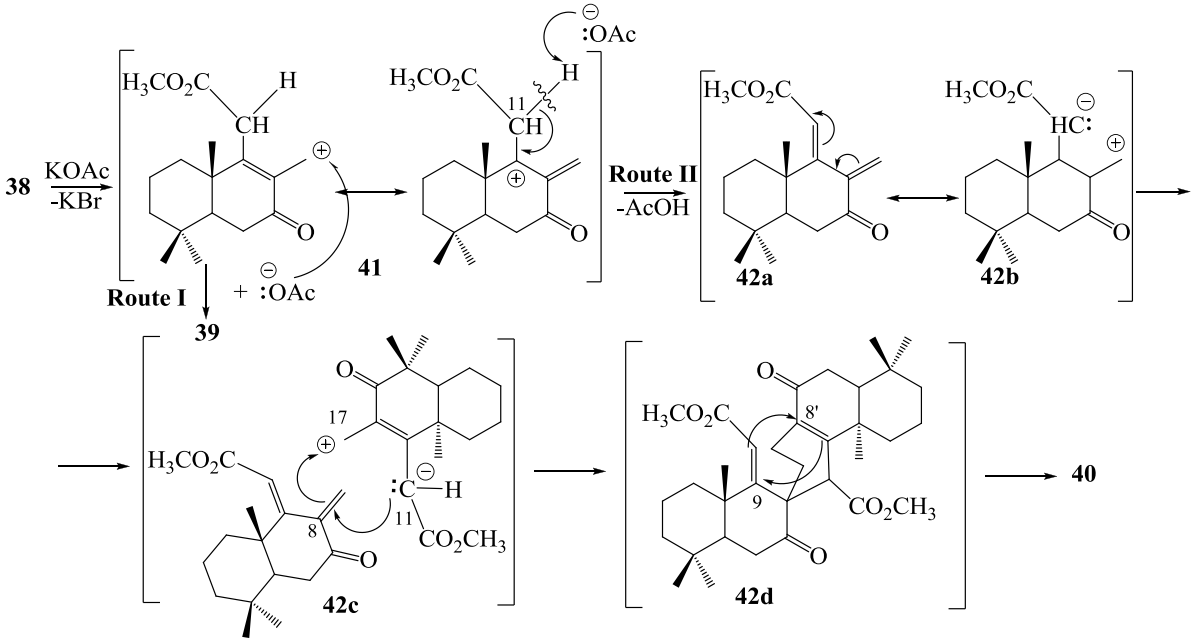
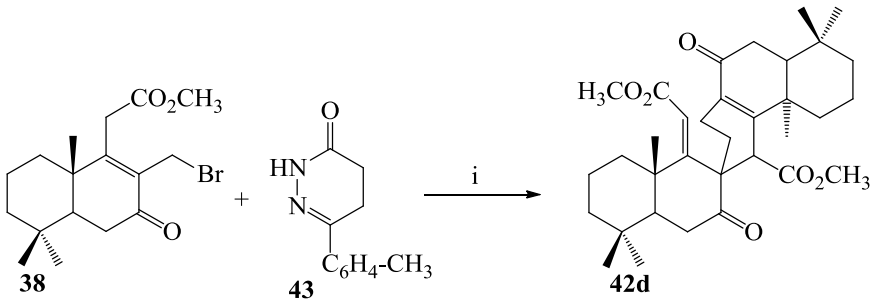


Fig. 2.12. The proposed mechanism of dimer **40** formation.

The removal of methylene protons from the position C_{11}, favored by the carbomethoxy group and the allylic carbocations **41**, proceeds easily under the action of the acetate anion. As a result, an intermediate **42** is formed, with two possible canonical structures: the conjugated diene **42a** and the other zwitterionic **42b**, and the subsequent intramolecular rearrangement of compound **42**, through the intermediate states **42c** and **42d**, leads to the formation of dimer **40**. To support the proposed reaction mechanism, it was necessary to isolate the reaction intermediate **42d**, which was later achieved when the bromide **38** was reacted with the diazine derivative **43** in the presence of K_2CO_3 in DMAA (Figure 2.13).



Reagents and conditions: i) K_2CO_3, DMAA, 48 hrs, r.t., 70.0%.

Fig. 2.13. Formation of the intermediate spirodimer **42d**.

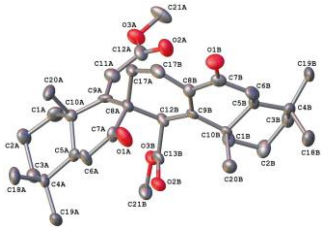


Fig. 2.14. Molecular structure of dimer **42d**.

Due to the fact that 6-(*p*-tolyl)-4,5-dihydro-3(2*H*)-pyridazinone **43**, has a saturated non-conjugated system which substantially decreases the reactivity of the *N*-H bond, the formation of the desired homodrimane-pyridazinone hybrid derivative does not occur. Instead of *N*-alkylation, a dimerization reaction occurred with the formation of the spirodimer **42d**, the structure of which was demonstrated by spectral analysis and single crystal X-ray diffraction (Figure 2.14).

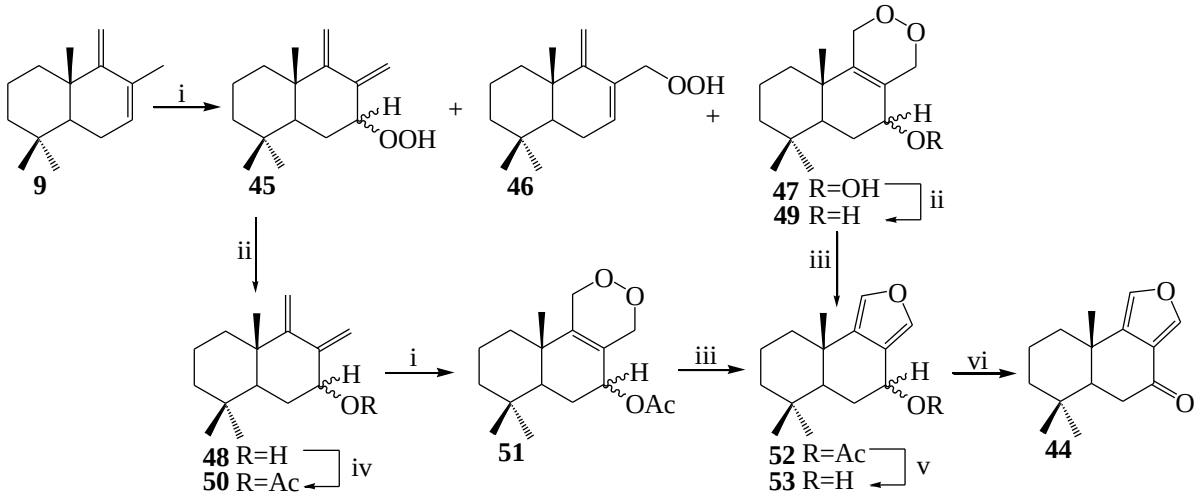
Thus, for the first time, on the basis of methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** derived from (-)-sclareol **1**, syntheses of polyfunctional tetranorlabdane derivatives and dimers with unprecedented structures were made, including by non-conventional methods, such as sensitized photooxidation, and the mechanisms of their formation was established. The antifungal and antibacterial activity of the synthesis compounds described in this Chapter has been evaluated and are discussed together with the results in Chapter VI.

2.4. Synthesis of synthetic derivatives and natural analogs with an advanced degree of functionalization from drim-8(9)-en-7-one and drim-7,9(11)-diene

This subchapter is a continuation of previous research exploring the synthetic potential of drim-7,9(11)-diene **9**, derived from drim-8(9)-en-7-one **8** [3, 6], which due to the activated C_7-C_9, C_{11} and C_{12} carbon atoms is a suitable raw material for the synthesis of polyfunctional drimane compounds.

The synthesis of (-)-7-ketoeuryfuran **44** was carried out based on drim-7,9(11)-diene **9**, which by applying a correct synthesis strategy can be a suitable starting material for obtaining C_7 functionalized drimanes [8].

Photooxygenation of diene **9** with $^1O_2$ in dichloromethane, sensitized by *meso*-tetraphenylporphyrin (H_2tpp), led to α- and β-hydroperoxides **45** (76.8%), in a 9:1 ratio according to NMR data, and an small amount of the initial diene **9** (9.7%). During the *-ene* reaction, a 1,4-cycloaddition of the singlet oxygen to the exocyclic diene **9** and rearrangements also occur, which are responsible for the formation of some minor products, such as hydroperoxide **46** (9.2%), α- and β-endoperoxides **47** obtained with a 14.0% yield, in a 3:1 ratio, a fact confirmed by the NMR analysis data (Figure 2.15).



Reagents and conditions: i) O_2, H_2tpp. DCM, hν, 5°C, 24 hrs, (77:9:14%); ii) Thiourea, MeOH, 0°C → r.t., 74% and 88%; iii) $FeSO_4$, THF, r.t., 70% and 78%; iv) Py, Ac_2O, r.t., 93%; v) KOH, EtOH, reflux, 100%; vi) PCC, DCM, siives 3A°, AcOH, r.t., 80%.

Fig. 2.15. Synthesis of (-)-7-cetoeuryfuran **44**.

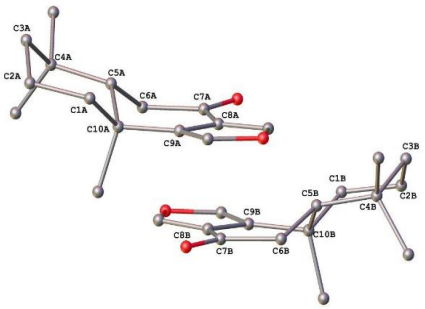
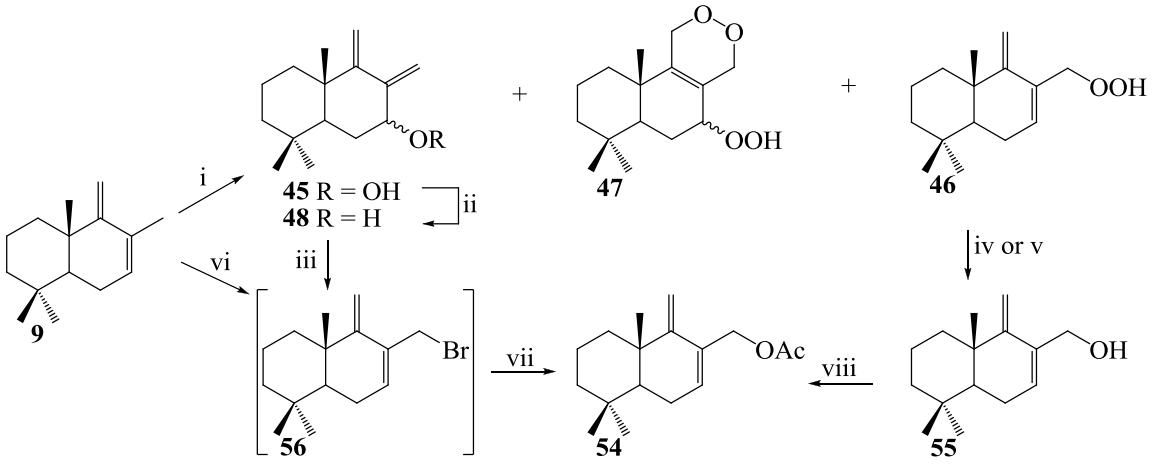


Fig. 2.16. Molecular structure of compound **44**.

Next, two alternative routes for the synthesis of (-)-7-ketoeuryfuran **44** were developed using peroxides **45** and **47** obtained by sensitized photooxygenation of diene **9**. Initially, the unstable peroxides **45** and **47** were reduced with thiourea to the corresponding alcohols **48** and **49**, with good yields. Then, endoperoxide **49** was reduced with $FeSO_4$ to 7-hydroxyeuryfuran **53** in 78% yield. After acetylation of the alcohol **48**, the resulting acetate **50** was photooxidized to acetoxyperoxide **51**, which upon reduction with $FeSO_4$ and saponification gave the same (-)-7-hydroxyeuryfuran **53**. Oxidation of 7-hydroxyeuryfuran **53** with pyridinium chlorochromate (PCC) in dichloromethane led to the known (-)-7-ketoeuryfuran **44**, which was obtained with a total yield of 23.4%, starting from the hydroperoxide **45**, and with a total yield of 59%, starting from the endoperoxide **47** (Figure 2.15). The structure of (-)-7-ketoeuryfuran **44** was confirmed by NMR spectroscopy, HRMS and single crystal X-ray analyses, its projection is shown in Figure 2.16. It should be noted that compounds **45**-**53** are described for the first time, and their structure was confirmed by spectral analyses.

Through sensitized photooxygenation reactions, bromination with *N*-bromosuccinimide (NBS) under different conditions and electrochemical transformation, a series of new functionalized derivatives of drimenone **8** and drim-7,9(11)-diene **9** were obtained. It has already been mentioned that photooxygenation of diene **9** leads to the major epimeric hydroperoxides **45**, and the minor ones **46** and **47** (Figure 2.17).



Reagents and conditions: i) O_2, H_2tpp. hν, DCM, 5°C, 24 hrs, 76.9%, 14.1% and 9.1%; ii) Thiourea, MeOH, 0°C, 24 hrs, 74%; iii) PBr_3, Et_2O/Py, -6°C, 1 hr, then r.t., 6 hrs; iv) Thiourea, MeOH, r.t., 8.5 hrs, 72%; v) $NaBH_4$, MeOH, 0°C, 1.5 hrs, 98%; vi) NBS, CCl_4, $(C_6H_5CO_2)_2$, reflux, 2 hrs; vii) KOAc, DMSO, r.t., 20 hrs, 81%; viii) Ac_2O, Py, r.t., 20 hrs, 82%.

Fig. 2.17. Alternative methods of synthesis of 12-acetoxy-drim-7,9(11)-diene **54**.

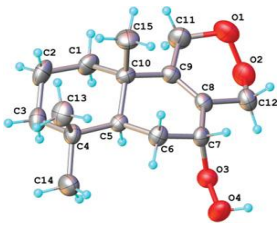


Fig. 2.18. Molecular structure of compound **47**.

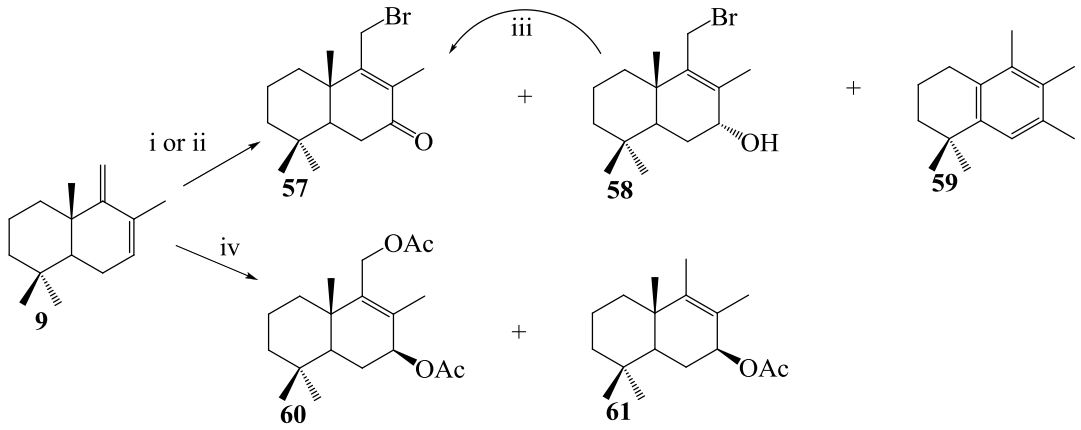
Peroxides **45-47** were used to synthesize of C_7, C_{11}, and C_{12} hydroxylated drimane compounds by reduction with the appropriate agents. The stereochemistry of the C_7-hydroperoxy- group in the molecule of major epimer **47** was proven by single crystal X-ray diffraction (Figure 2.18).

Hydroperoxides **45** and **46** were reduced with thiourea to the corresponding allylic alcohols **48** and **55** in good yields. Allylic rearrangement of alcohols **45** with PBr_3 led to allylic bromide **56**, the isolation of which was impossible due to its instability during column chromatography on SiO_2. For this reason, the reaction product was treated with KOAc, forming the known 12-acetoxidrim-7,9(11)-diene **54** in an 46% overall yield recalculated for diene **9** (Figure 2.18). Acetylation of alcohol **55** under standard conditions led to the same allylic acetate **54** in a 7.4% total yield. It should be noted that the total yield of compound **54**, obtained from diene **9** via peroxides **45** and **46**, is approximately 53.4%.

Another attempt to obtain acetate **54** was made in two steps. For this purpose, diene **9** was subjected to bromination with NBS in CCl_4, initiated with benzoyl peroxide, and the reaction product due to the instability of bromide **56**, it was treated with KOAc under the conditions previously defined, obtaining the allylic acetate **54** with a 13.8% total yield (Figure 2.17) [9].

Previously, compound **54** was used as a key intermediate in the homochiral semisynthesis of the natural drimane antibiotic pereniporin A. The structures of compounds **54** and **55** were confirmed by ^1H and ^{13}C NMR spectral analyses, and the data of compound **54** are identical to those reported in the literature.

Examples of the bromohydrins synthesis by treating unsaturated compounds with NBS in a mixture of Me_2CO/H_2O or $CHCl_3/H_2O_2$ under mild conditions have been described previously [5]. Bromination of diene **9** under the mentioned conditions led to 11-bromo-drim-8(9)-en-7-one **57**, 11-bromo-drim-8(9)-en-7α-ol **58** and the aromatic compound **59** (1,1,5,6,7-pentamethyl-1,2,3,4-tetrahydronaphthalene) (Figure 2.19).



Reagents and conditions: i) NBS, Me_2CO, H_2O, DCM, 0°C, 2 hrs, 90%; ii) NBS, $CHCl_3$, H_2O, DCM, 0°C, 1 hr, 98%; iii) PCC, DCM, 20°C, 1 hr, 89%;
iv) NaOAc, ē, AcOH gl., Ac_2O, 5°C, 2 hrs, 49% and 7%.

Fig. 2.19. Bromination and anodic oxydation of drim-7,9(11)-diene **9**.

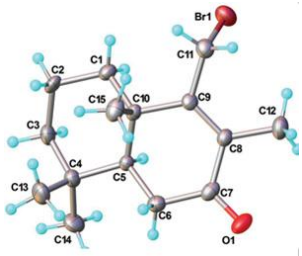


Fig. 2.20. Molecular structure of compound **57**.

Compounds **57**–**59** are new and were obtained by both procedures in comparable yields (%): **57** (47.2 and 47.0), **58** (11.6 and 11.0) and **59** (31.3 and 40.4). ^1H and ^{13}C NMR data of compounds **57-59** confirm their structure. Compound **57** can be considered a product of the selective bromination of the C_{11} methyl group from the molecule of drimenone **8**. The X-ray study reveals that compound **57** has a molecular crystal structure built from neutral entities, shown in Figure 2.20 [9].

It should be noted that compound **59** is the first and only representative of the drimane series possessing an aromatic ring B and an analog of the bicyclic diterpenoids fregenedadiol and isofregenedadiol that have been isolated from natural sources or synthesized by acid-catalyzed rearrangement reactions. The aromatic compound **59** is the product of the unstable bromohydrin **58** rearrangement during SiO_2 column chromatography, a fact proven by the separate oxidation of the crude bromination products with pyridinium chlorochromate (PCC), which led exclusively to the monobromide **57** in 89% yield (Figure 2.19).

The anodic oxidation described above is the key step in the synthesis of some homodrimane derivatives and drimenone **8** [2], but the obtained structures and the yields of the compounds depend on the reaction conditions. Some conditions were also applied to diene **9**, which was electrooxidized in a individual cell equipped with graphite electrodes, in an Ac_2O/AcOH mixture, using AcONa as the electrolyte. From the reaction mixture, the major diacetate **60** was isolated, followed by the minor monoacetate **61**, both obtained with an acceptable total yield of 60% [15].

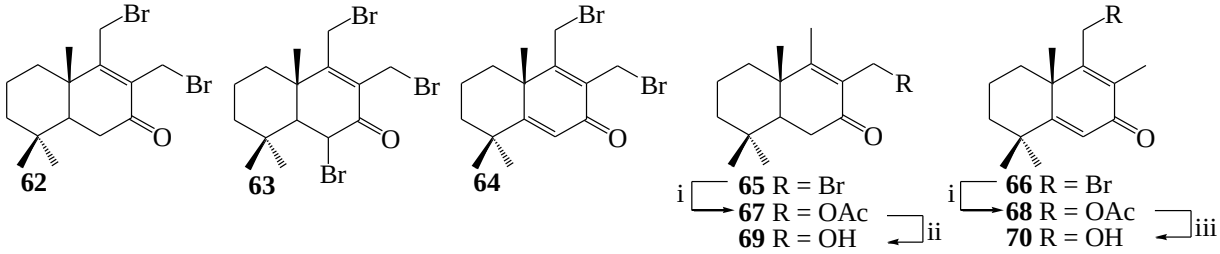
Colța M.N. et al. [2] treated drimenone **8** with variable amounts of NBS (2.5 and 3.5 equivalents), obtaining in both cases 11,12-dibromo-drim-8(9)-en-7-one **62** (90%) as major reaction product, along with two minor products: the unstable tribromoketone **63** and 11,12-dibromo-drim-5,8(9)-dien-7-one **64**, the latter resulting from the dehydrobromination of compound **63**. Since the authors [2] did not record selectivity in the bromination of C_{11} and C_{12} methyl groups, an attempt was made to establish the optimal conditions for this reaction.

According to GC-MS analysis data, selective bromination at the C_{11} and C_{12} positions occurs after 3 hours of reflux when equimolar amounts of NBS and drimenone **8** are taken

(Method A), yielding monobromides **57** (66.0%) and **65** (12.6%), and the minor dibromide **62** (5.2%) (Figures 2.19, 2.20 and 2.21).

In the case when 1.5 equiv. of NBS are taken (Method B), after 3 hours of reflux the selectivity of the reaction decreases, the total yield of monobromides **57** and **65**, being related to that of dibromide **62** as (55.9% : 44.1%), and after 5 hours the monobromoketone **66** is formed (10.2%), as a product of the compound **57** bromination at the C_6 position, followed by dehydrobromination.

The amount of 2.0 equiv. of NBS (Method C) does not give any selectivity, after 1 hour of reflux the ratio between monobromides **57**/**65** and dibromide **62** is ~6:4, and after 3 hours of reflux only dibromide **62** (98.8%) is obtained.



Reagents and conditions: i) KOAc, DMFA, 20°C, 2.5 hrs, 86%;
ii) K_2CO_3 (1%), Ar, MeOH, 20°C, 1.5 hrs, 89%; iii) K_2CO_3 (1%), Ar, MeOH, 20°C, 1.5 hrs, 94%.

Fig. 2.21. New derivatives of drimenone **8**, functionalized at positions C_6, C_{11} and C_{12}.

In conclusion, the optimal conditions for selectiv preparation of monobromides **57** and **65** are 2 hours of reflux and 1.5 equiv. of NBS (Method B), when the initial compound **8** is completelly consumed. Comparing the yields of monobromides **57** (70.2%) and **65** (11.0%) it can be concluded that the position C_{11} is more favorable for substitution than C_{12}. As a result of the bromination reaction of drimenone **8** study, three new bromides **57**, **65** and **66** were obtained, the last two being rather unstable during column chromatography. For this reason, the bromides were acetylated [9], and the resulting acetates **67** and **68** were saponified under mild conditions into the corresponding stable alcohols **69** and **70** (Figure 2.21).

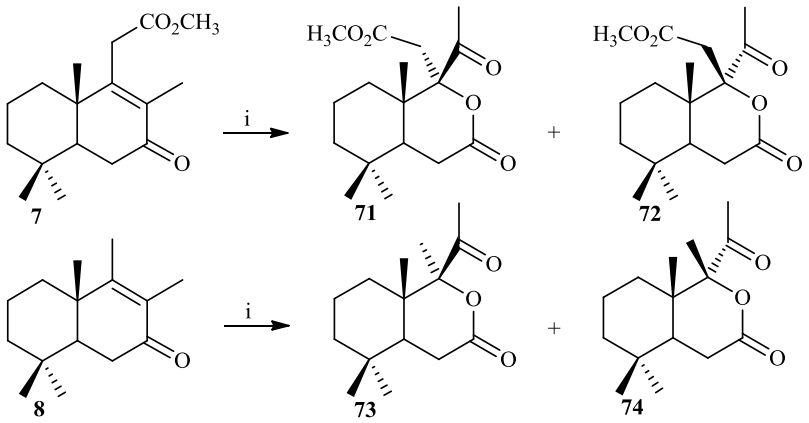
Thus, for the first time, starting from (-)-sclareol **1** derivatives, drim-8(9)-en-7-one **8** and drim-7,9(11)-diene **9**, syntheses of pentanorlabdane derivatives with advanced functionalization have been carried out and proposed new ways of natural analogues or their precursors synthesis, including through non-conventional methods, such as sensitized photooxidation and anodic oxidation, and some processes have been patented. The antifungal and antibacterial activity of the synthetized compounds described in this Chapter has been evaluated and are discussed together with the results in Chapter VI.

2.5. Tetra- and pentanorlabdane derivatives with rearranged skeleton

A large number of synthetic polyfunctional and biologically active tetra- and pentanorlabdane compounds, including nitrogen-containing compounds, have been obtained starting from (+)-sclareolide **3** via the intermediates methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** and drim-8-en-7-one **8** (drimenone) derived from it, with the involvement of functional groups in the side chain or in the B ring of the initial molecules, without modification of the carbon skeleton. Chemical transformations in the B cycle are less known. Ketoester **7** and drim-8-en-7-one **8** offer new synthetic opportunities, and this study [16] confirms the hypothesis through the series of novel tetra- and pentarnolabdane compounds with rearranged ring B obtained via Baeyer-Villiger and Beckmann reactions.

A modified Baeyer–Villiger oxidation procedure using 3-chloroperbenzoic acid (*m*-CPBA) in combination with trifluoroacetic acid (TFA) was attempted for terpene ketones **7** and **8**.

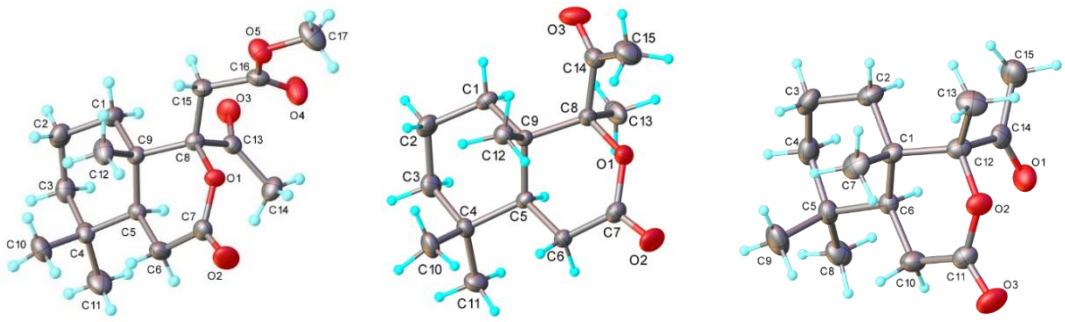
According to CSS data, the reaction mixture derived from ketoester **7** contains only one product, and that derived from drimenone **8** includes two chromatographically separable products. NMR analysis showed, that the inseparable mixture of compounds **71**/**72**, but also the individual compounds **73** and **74** are not the expected seven-membered enol lactones, as normal products of Baeyer-Villiger oxidation. Surprisingly, in both cases, after the reaction the six-membered cyclic lactones **71**/**72**, **73**, and **74**, epimers on the C_9 center, were obtained in 57% and 88% overall yields, respectively (Figure 2.22).



Reagents and conditions: i) *m*-CPBA, TFA, DCM, 0°C → r.t., 20 hrs, 57% (**71**/**72**), 33% (**73**) and 55% (**74**), in a 2:1 ratio, in both cases.

Fig. 2.22. Baeyer-Villiger oxidation of lactones **7** and **8**.

The structures of lactones **71**–**74** are confirmed by spectral analysis data, and those of compounds **72**, **73**, and **74** were definitively confirmed by single crystal X-ray diffraction (Figures 2.23a–c).



a) **b)** **c)**

Fig. 2.23. Molecular structure of compounds **72** (a), **73** (b) and **74** (c).

No such rearrangements have been reported in the literature for compounds of the norlabdane series during Baeyer-Villiger oxidation, but still the formation of compounds **71**-**74** can be explained. One of the possible pathways for the conversion of ketones **7** and **8** starts from epoxidation of the C_8-C_9 double bond to form epoxyketone **75**, followed by Baeyer-Villiger oxidation leading to epoxylactone **76** (Figure 2.24).

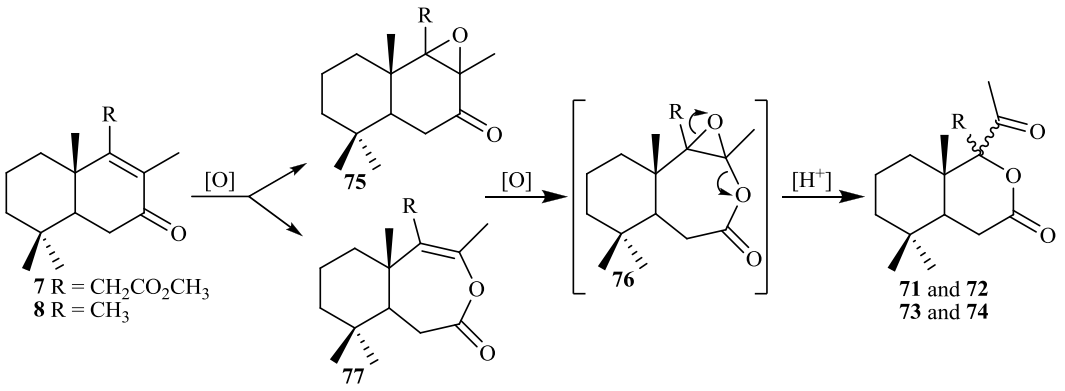
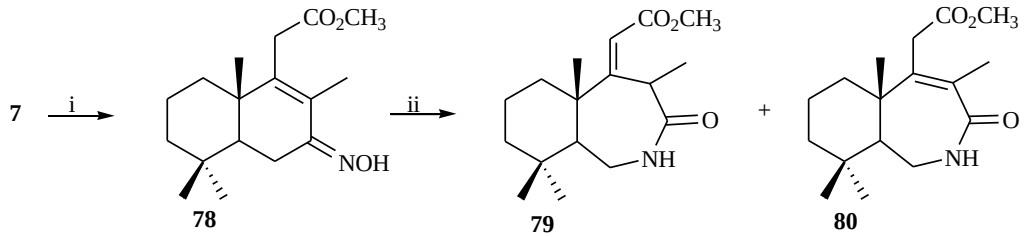


Fig. 2.24. Proposed mechanism of the Baeyer-Villiger oxidation reaction of norlabdane ketones **7** and **8**.

Another likely way to form compound **76** starts with Baeyer-Villiger oxidation of ketones **7** and **8** to form enol lactone **77**, followed by epoxidation of the double bond, and is similar to that described for cholestane-type compounds, when both processes occur as a result of the treatment of *α,β*-unsaturated ketones with peroxyacid. In our case, as a result of both oxidative processes and due to the acidic environment, a rearrangement of the intermediate seven-membered epoxylactone into more stable six-membered enantiomeric lactone mixtures **71**/**72**, **73** and **74** occurs (Figure 2.24).

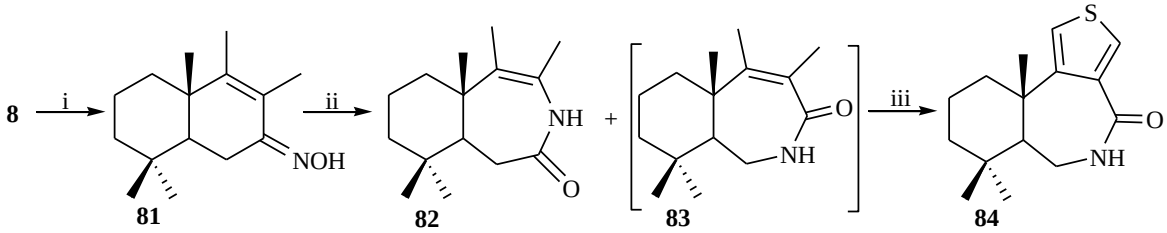
Next, the synthesis of new tetra- **79**, **80** and pentanorlabdane **82**, **84** lactams, obtained by Beckmann transposition of the corresponding oximes **78** and **81**, obtained previously [17] will be described. Treatment of ketoxime **78** with thionyl chloride led to a mixture of chromatographically separable isomeric lactams **79** and **80** (Figure 2.25).



Reagents and conditions: i) NH_2OH•HCl, EtOH, Py, 24 hrs, 96-98%;
ii) $SOCl_2$, Py, 60-65°C, 17 hrs, 55 and 19%.

Fig. 2.25. Beckmann rearrangement of tetranorlandane ketoxime **78**.

2D Homo- ($^1H/^1H$ COSY-45°, $^1H/^1H$ NOESY) and heteronuclear ($^1H/^{13}C$ HSQC, $^1H/^{15}N$ HMQC and correlation spectra $^1H/^{13}C$ HMBC, $^1H/^{15}N$ HMBC) spectra confirm the structure of compound **79**. Beckmann rearrangement of oxime **81** under identical conditions gave a mixture of lactams **82** and **84** (Figure 2.26).



Reagents and conditions: i) NH_2OH•HCl, EtOH, Py, 24 hrs, 96-98%;
ii) $SOCl_2$, Py, 50-60°C, 9 hrs, 51% and 4%.

Fig. 2.26. Beckmann rearrangement of pentanorlandane ketoxime **81**.

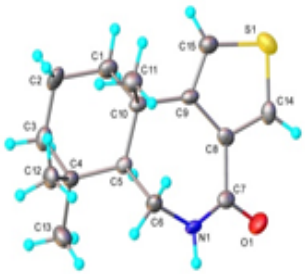


Fig. 2.27. Molecular structure of compound **84**.

Thiophenolactam **84** was isolated from the reaction product, which is the product of the interaction of intermediate lactam **83** with thionyl chloride (Figure 2.26). The presence of the *α,β*-unsaturated carbonyl group in the molecule of lactam **83** activates the methyl groups and favors their interaction with thionyl chloride. The structure of compound **84** was further confirmed by single crystal X-ray diffraction (Figure 2.27).

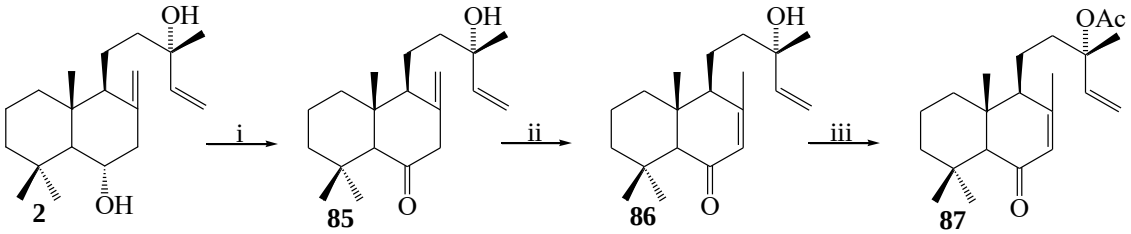
Thus, for the first time, starting from methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** and drim-8(9)-en-7-one **8** derived from (-)-sclareol **1**, through the Baeyer-Villiger and Beckmann reactions, tetra- and pentanorlabdane lactones, lactams and thiolactams with a rearranged skeleton were synthesized and the mechanisms of their formation were established. The antifungal and antibacterial activity of the synthetized compounds described in this Chapter has been evaluated and are discussed together with the results in Chapter VI.

3. CHEMICAL TRANSFORMATIONS BASED ON (+)-LARIXOL AND ITS DI- AND PENTANORLABDANE DERIVATIVES

3.1. Synthetic derivatives of (+)-larixol functionalized in the B ring

In the following, the syntheses of some derivatives with an advanced degree of functionalization of the B cycle and preservation of the side chain will be described, based on (+)-larixol **2**, by combining classical and non-conventional methods of synthesis, such as sensitized photooxidation [18].

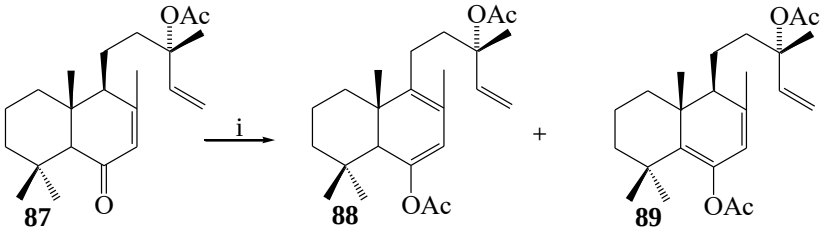
The starting compound, (+)-larixol **2**, was isolated from commercially available larch oleoresin according to the method proposed by Lagnel, B. et al. Initially, it was oxidized into the exocyclic ketone **85**, following the procedure described by the authors, which was further isomerized into the ketone **86** according to the method described by the same authors. The tertiary C_{13} hydroxyl group was protected by acetylation under standard conditions to give ketoacetate **87** (Figure 3.1).



Reagents and conditions: i) PCC, DCM, AcOH, 3Å, r.t., 75 min., 95%; ii) MeONa, MeOH, H_2O_2, r.t., 1 hr, 98%; iii) AcCl, DMA, 50 min., 5°C, then r.t., 64 hrs, 92%.

Fig. 3.1. Synthesis of labdane derivatives of (+)-larixol **2**.

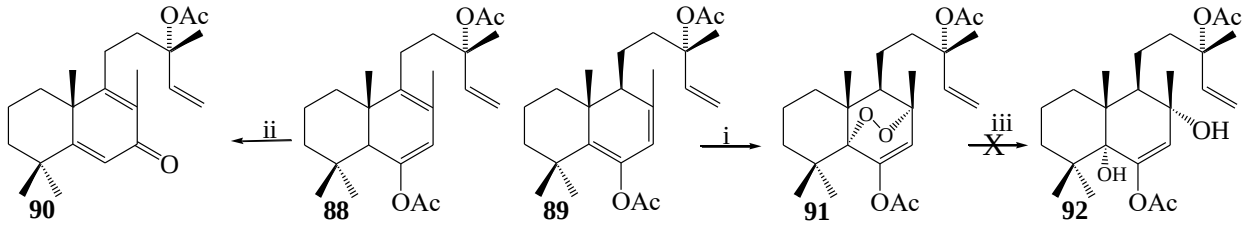
Next, the ketoacetate **87** was subjected to the enolacetylation reaction [4], obtaining the previously undescribed enolacetates **88** and **89** in 49% and 41% yields, respectively (Figure 3.2.).



Reagents and conditions: i) ≥-OAc, *p*-TsOH, N_2, 109°C, 13 hrs, 49% and 41%.

Fig. 3.2. Enolacetylation reaction of ketoacetate **87**.

Conjugated dienes **88** and **89** represent suitable substrates for sensitized photooxidation reactions with singlet oxygen, which were carried out under the conditions described in Figure 3.3.



Reagents and conditions: i) and ii) O_2, hν, H_2tpp. DCM, 12 hrs, 82% and 78%; iii) Thiourea, MeOH, 1.5 hrs.

Fig. 3.3. Sensitized photooxidation reactions of enolacetates **88** and **89**.

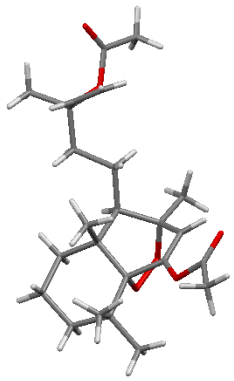


Fig. 3.4. Molecular structure of compound **91**.

Compounds **90** and **91** were isolated from the photooxidation products of compounds **88** and **89**. Dienone **90** is a product of photooxidative dehydrogenation of compound **88**, which follows the mechanism described in source [4], and endoperoxide **91** is the product of [2+4] cycloaddition of the singlet oxygen to the conjugated diene system in molecule **89**. The structure of compound **91** was also confirmed by single crystal X-ray diffraction (Figure 3.4).

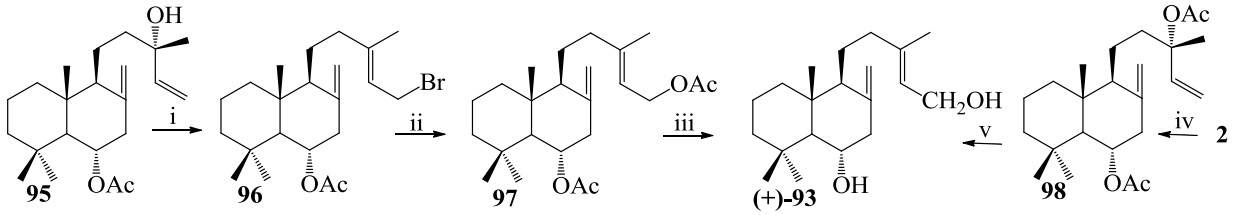
Next the endoperoxide **91** was reduced under the conditions described in Figure 3.3., but the expected derivative of (+)-larixol **2**, compound **92** decomposed during column chromatography on silica gel.

Thus, based on intermediates **85** and **86** obtained from (+)-larixol **2**, syntheses of polyfunctionalized labdane derivatives **88-91** in the B cycle, with preservation of the side chain, including by non-conventional methods, such as sensitized photooxidation, were carried out.

3.2. Synthesis of (+)-crotonadiol from 6*α*-acetoxy-14,15-*bis*-norlabd-8(17)-en-13-one

The aim of this work was the synthesis of (+)-crotonadiol **93** and its isomer **94** with the (Z)-configuration on the C_{13} center [19]. Since the *C. zambesicus* extract contains a small amount of (+)-crotonadiol **93**, its isolation was problematic. Therefore, it was decided to synthesize it and its isomer **94** from the available labdane diterpenoid (+)-larixol **2**.

The reaction of larixyl acetate **95** with PBr_3 was studied, but the product **96** was found to be unstable and decomposed during column chromatography on silica gel. Therefore, it was used without purification in the reaction with KOAc, obtaining *i*-larixol diacetate **97** in 39% yield (Figure 3.5). The structure and stereochemistry of compound **97** were proved based on spectral data and obtaining (+)-crotonadiol **93** by its saponification.



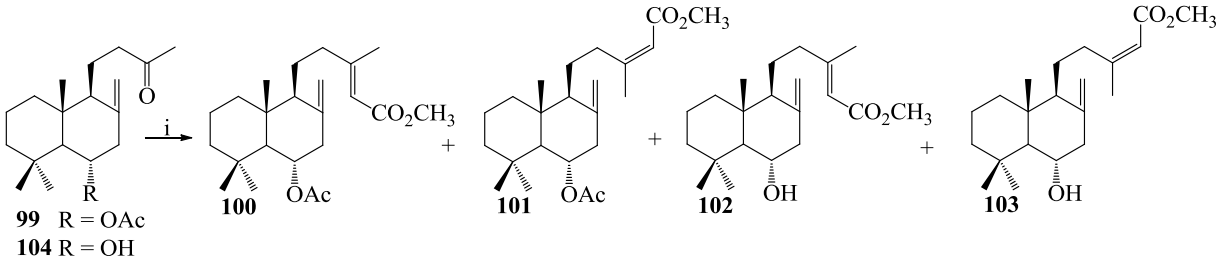
Reagents and conditions: i) PBr_3, Et_2O, r.t., 12 hrs; ii) KOAc, DMF, r.t., 12 hrs, 39%; iii) KOH, EtOH, reflux, 2 hrs, 98%; iv) AcCl, DMA, 5°C, 12 hrs, 93%; v) $PdCl_2{\cdot}(CH_3CN)_2$, THF, Ar, r.t., 12 hrs, 86%.

Fig. 3.5. Synthesis of (+)-crotonadiol **93** from larixyl acetate **95**.

Signals of the carbon atom in ^{13}C NMR spectra were assigned based on HSQC and NOESY correlation experiments. The 13*Z*-**94** isomer of (+)-**93** was not obtained in this case. Therefore, the reaction of larixol diacetate **98** with the palladium chloride diacetonitrile complex $PdCl_2{\bullet}(CH_3CN)_2$ was studied. Compound **98** was obtained by acetylation of (+)-larixol **2** with acetyl chloride in dimethylaniline (DMA), and its reaction with $PdCl_2{\bullet}(CH_3CN)_2$ proceeded in high yield (86%), obtaining *i*-larixol diacetate **97**, which was saponified under the conditions described in Figure 3.5. Its isomer (*Z*)-**94** was not obtained in this case either.

For this reason, the Wittig-Horner reaction of 6α-acetoxy-14,15-*bis*-norlabd-8(17)-en-13-one **99** with trimethylphosphonoacetate (Figure 3.6) was further studied, which gave, according to TLC, a mixture of two products in a ~1:3 ratio. These were separated by column chromatography on silica gel, but NMR analysis confirmed that each fraction was heterogeneous and contained two stereoisomers.

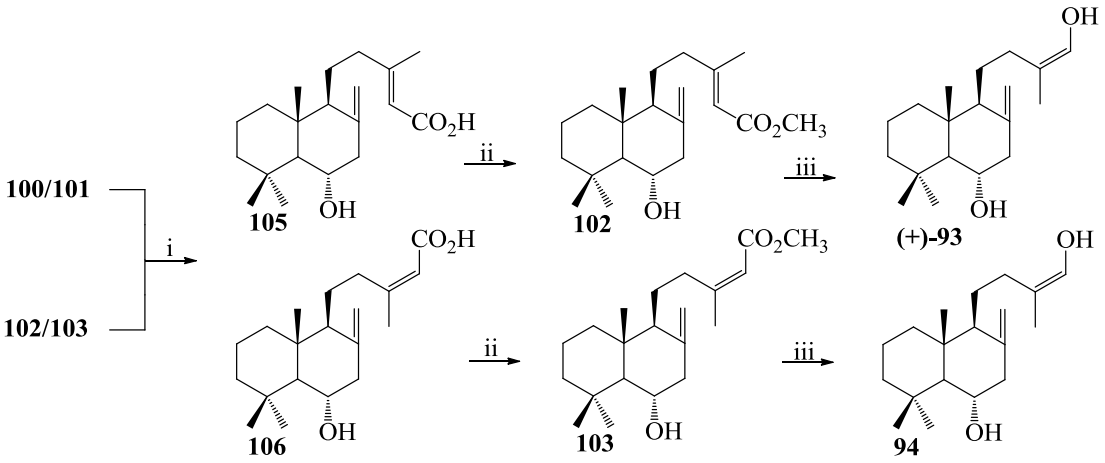
The less polar fraction, according to the spectral data, was a mixture of isomeric acetoxyesters 13(*E*)-**100** and 13(*Z*)-**101**, in a 8:2 ratio. The more polar fraction represented a mixture of hydroxyesters 13(*E*)-**102** and 13(*Z*)-**103** in a 7:2 ratio, formed as a result of the partial saponification of compound **99** into the hydroxyketone **104**, from which they were formed.



Reagents and conditions: i) $NaOCH_3$, $(CH_3O)_2P(O)CH_2CO_2CH_3$, C_6H_6, reflux, 2 hrs, **100**/**101** (23%; 8:2) and **102**/**103** (76%; 7:2).

Fig. 3.6. Wittig-Horner reaction of 6α-acetoxy-14,15-*bis*-norlabd-8(17)-en-13-one **99**.

Since esters **100**/**101** and **102**/**103** could not be separated chromatographically from the mixtures, they were saponified (Figure 3.7), in both cases yielding chromatographically separable stereoisomeric hydroxyacids **105** and **106**.



Reagents and conditions: i) KOH, EtOH, reflux, 1 hr, **105** (77% and 79%), **106** (19% and 22%); ii) $TMSCHN_2$, TFH, MeOH, r.t., 0.5 hrs, **102** (97%) and **103** (98%); iii) $LiAlH_4$, Et_2O, Ar, 0ºC, 3 hrs, (+)-**93** (86%) and **94** (80%).

Fig. 3.7. Synthesis of (+)-crotonadiol **93** and its isomer (*Z*)-**94**.

Separate methylation of the hydroxyacids 13(*E*)-**105** and 13(*Z*)-**106** led in practically quantitative yields to the pure hydroxyesters 13(*E*)-**102** and 13(*Z*)-**103**, and their reduction led in high yield to crotonadiol (+)-**93** and to its isomer (*Z*)-**94**, respectively (Figure 3.7).

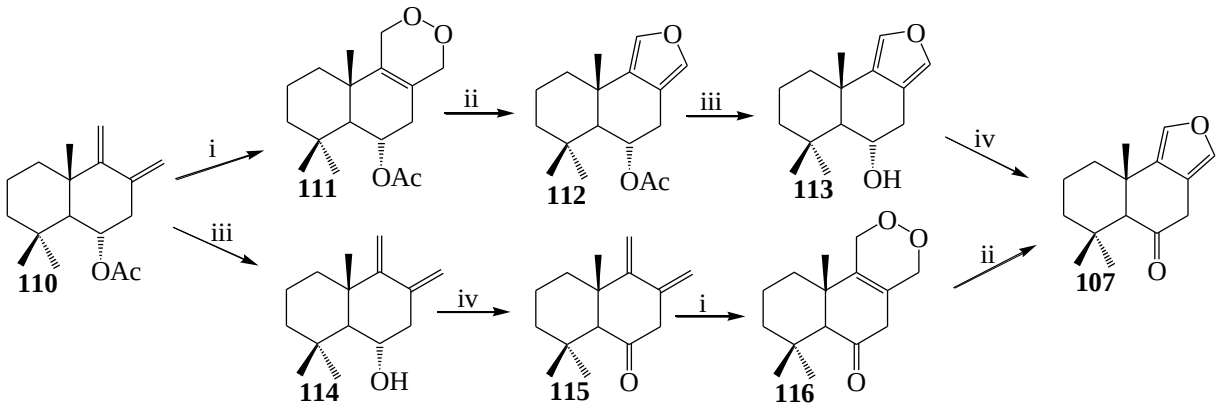
Thus, starting from (+)-larixol **2**, via 6α-acetoxy-14,15-*bis*-norlabd-8(17)-en-13-one **99** and using the Wittig-Horner reaction, it was proposed a novel method for synthesis of natural (+)-crotonadiol **93** and its isomer (Z)-**94**, obtained for the first time. Their physicochemical and spectral data confirm that both compounds are labdane diterpenoids of the normal series.

3.3. Regio- and stereoselective synthesis of some sesquiterpene natural analogues

Due to its biological activities and synthetic potential, a large number of semisyntheses and total syntheses of euryfuran, both as a racemic mixture and in optically active forms have been report.

Next, the results of the regio- and stereoselective semisynthesis of: (+)-6-ketoeuryfuran **107**, (+)-fragrolide **108** and (+)-6-ketowinterin **109** from the available labdane terpenoid (+)-larixol **2** will be reported [8], which in the form of (+)-larixyl acetate **95** is the main component of *Larix decidua* L. oleoresin.

The synthesis of (+)-6-ketoeuryfuran **107** was carried out based on 6α-acetoxidiene **110** (Figure 3.8), which was subjected to photooxidation reaction sensitized by *meso*-tetraphenylporphyrin (H_2tpp), obtaining the endoperoxide **111** in high yield. The endoperoxide **111** was reduced in 93% yield to 6α-acetoxyeuryfuran **112** by treatment with $FeSO_4$ in THF, and this was subjected to basic hydrolysis to the previously described 6α-hydroxyeuryfuran **113**. Oxidation of alcohol **113** with pyridinium chlorochromate (PCC) in dichloromethane led to (+)-6-ketoeuryfuran **107** in 68% overall yield, recalculated for diene **110** (Figure 3.8).



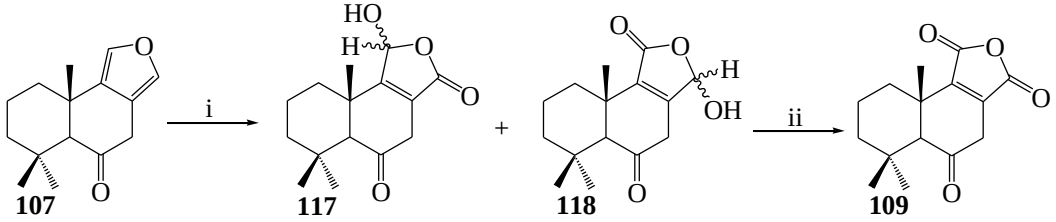
Reagents and conditions: i) O_2, H_2tpp. hν, CCl_4, r.t., 86%; ii) $FeSO_4$, THF, r.t., 93% and 91%; iii) KOH, EtOH, reflux, 94% and 99%; iv) PCC, DCM, molecular sieves 3Aº, AcOH, r.t., 90% and 93%.

Fig. 3.8. Syntheses of (+)-6-ketoeuryfuran **107**.

(+)-6-Ketoeuryfuran **107** was also obtained in 4 steps through another sequence of transformations, starting from diene **110** through intermediates **114-116** with a total yield of 72% (Figure 3.8). Acetoxydiene **110** was saponified under the conditions described above, into hydroxydiene **114** with a 99% yield, which was oxidized with pyridinium chlorochromate (PCC) to the known ketodiene **115**. Photooxidation of ketodiene **115** in the presence of *meso*-tetraphenylporphyrin (H_2tpp) led to ketoperoxide **116** in 86% yield, and reduction of ketoperoxide **116** under the conditions described above gave (+)-6-ketoeuryfuran **107** in 91% yield (Figure 3.8), whose spectral data are identical to those described in the literature.

It is known that eosin-sensitized photooxidation of (+)-euryfuran with singlet oxygen ($^1O_2$) in a mixture of *t*-BuOH and 2,6-lutidine leads to a mixture of two stereoisomeric lactols in a equal ratio. For this reason, the use of this type of photochemical transformations was attempted for the functionalization of (+)-6-ketoeuryfuran **107** at the C_{11} and C_{12} positions.

According to the TLC data, the reaction product contained two chromatographically separable products, but NMR analysis, surprisingly, showed that they consisted of the stereoisomeric lactol pairs **117** (42%) and **118** (40%) (Figure 3.9).



Reagents and conditions: i) O_2, eozin, hν, *t*-BuOH, 2,4-lutidine, 5ºC, 2 hrs, 84%; ii) PCC, DCM, sieves 3Aº, AcOH, r.t., 1 hr, 88%.

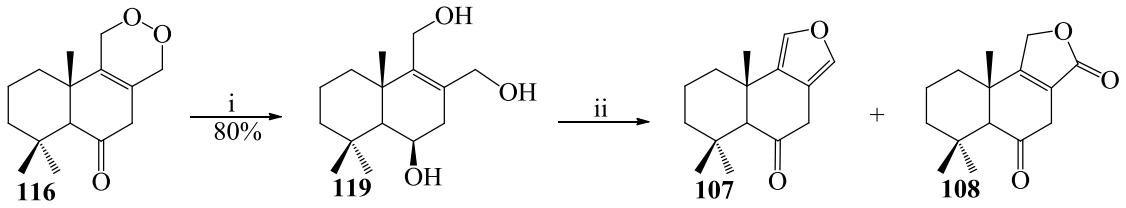
Fig. 3.9. Synthesis of (+)-6-ketowinterin **109**.

Further oxidation of the mixture of lactols **117** with pyridinium chlorochromate (PCC) led to the drymanic anhydride (+)-6-ketowinterin **109**, a relatively stable compound in 88% yield (Figure 3.9). It should be mentioned that this is the first synthesis of (+)-6-ketowinterin **109**, and the data of its spectral analysis largely correspond to those reported for natural winterin, isolated from the bark of the South American tree *Drimys winteri* or the one synthesized as a racemic mixture, or in optically pure form.

The drimane sesquiterpenoid (+)-fragrolide **108** isolated for the first time from the extract obtained from the bark of the *Cinnamosma fragrans* species exhibits multiple activities, such as

antifungal, insect-repellent, plant growth inhibitory and cytotoxic. Several syntheses of (+)-fragrolide **108** have been reported to date.

Next will be presented the data of a new synthesis of (+)-fragrolide **108** from 6-ketoperoxide **116** which was reduced to triol **119** with an 80% yield (Figure 3.10).



Reagents and conditions: i) $LiAlH_4$, Et_2O, N_2, r.t., 2.5 hrs, 80%; ii) CrO_3•Py, Py, r.t., 4.5 hrs, 50% and 33%.

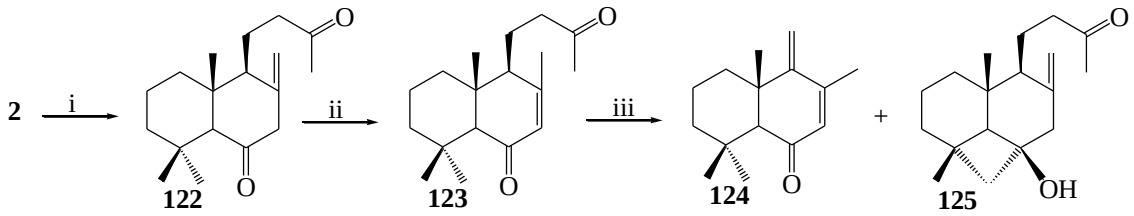
Fig. 3.10. Synthesis of (+)-fragrolide **108**.

Oxidation of triol **119** led to a mixture of three compounds, which were isolated by column chromatography on silica gel in the order: 6-ketoeuryfuran **107** (50% yield), which was previously described (Figure 3.8), crystalline (+)-fragrolide **108**, obtained in 33% yield and a mixture of lactols, derivatives of (+)-fragrolide **108**, epimers on the C_{11} center, which due to the extremely small amount were not characterized in detail. Thus, based on acetoxidiene **110**, by means of ketoperoxide **116**, a new synthesis method of (+)-fragrolide **108** was developed, with a 22.5% total yield.

The only syntheses of compounds **120** and **121** known at the time [20] were those achieved in 10 steps for (-)-albrassitriol **120** and in 15 steps for (-)-6-*epi*-albrassitriol **121**, both starting from (+)-larixol **2**, with total yields of 7.5% and 4.25%, respectively.

Next, a new five-step synthetic route of the natural drimanes, (–)-albrassitriol **120** and (-)-6-*epi*-albrassitriol **121**, from the available labdandiol (+)-larixol **2** will be described [21]. In the first step, (+)-larixol **2** was oxidized with CrO_3 in acetic acid into 14,15-*bis*-norlabd-8(17)-ene-6,13-dione **122** (48.0%), which was isomerized by treatment with sodium methoxide into 14,15-*bis*-norlabd-7-ene-6,13-dione **123** (97.0%), the total yield of these two steps being 66.0%.

Norrish II type photolytic degradation of diketone **123** leads to the formation of drim-7,9(11)-dien-6-one **124** in 67.5% yield and cyclobuto(18→6)-14,15-*bis*-norlabd-8(17)-en-6-ol-13-one **125**, as a minor product with a 17.5% yield [22]. The advantage of this photodegradation are: the simplicity of the method and the relatively good yields (Figure 3.11).

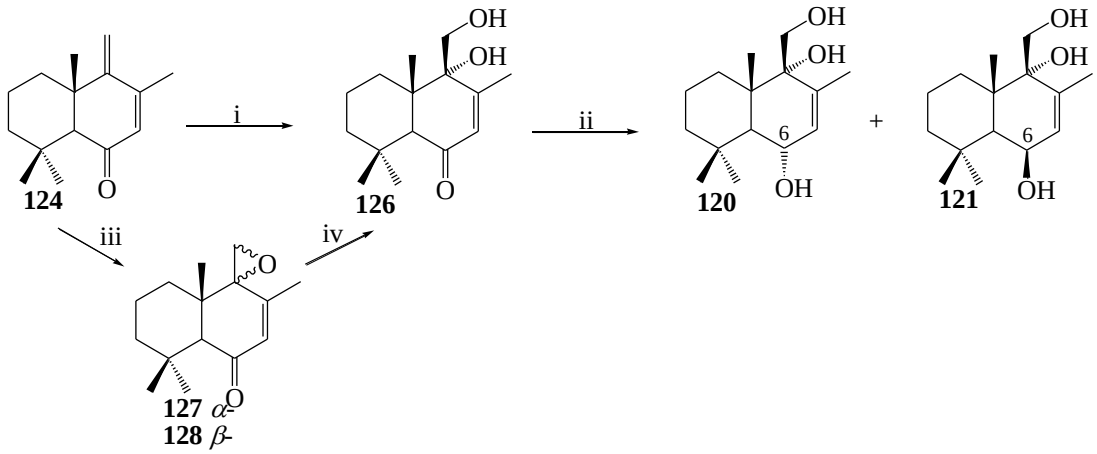


Reagents and conditions: i) CrO_3, AcOH, r.t., 2 hrs, 48%; ii) NaOMe, MeOH, r.t., 24 hrs, 97%;
iii) hν, hexane, 5°C, 3 hrs, 67.5% and 17.5%.

Fig. 3.11. Synthesis of drim-7,9(11)-dien-6-one **124** by photolitic degradation reaction.

Previously dienone **124** was converted into (-)-6-*epi*-albrassitriol **121** by selective oxidation with OsO_4 to ketodiol **126** (88.0%), followed by its stereoselective reduction with DIBAL (83.0%) [20]. Reproduction of the oxidation reaction of diene **124** with OsO_4 confirmed

its high efficiency in the preparation of ketodiol **126** with 90.0% yield (Figure 3.12). The spectral data of this compound are identical to those described in the literature.



Reagents and conditions: i) OsO_4, Py, r.t., 12 hrs, 91%; ii) $LiAlH_4$, Et_2O, N_2, 0°C, 0.5 hrs, 44% and 48%; iii) AMPF, Et_2O, r.t., 125 hrs, 82%; iv) $HClO_4$, THF, r.t., 49 hrs, 68%.

Fig 3.12. Synthesis of (-)-albrassitriol **120** and (-)-6-*epi*-albrassitriol **121**.

To avoid the use of OsO_4, an alternative two-step route for the preparation of compound **126** was developed by treating dienone **124** with monoperphthalic acid, with selective epoxidation of the C_9–C_{11} double bond, affording a mixture of inseparable diastereoisomeric epoxides **127** and **128**, in a 7:3 ratio, with a 82.0% yield (Figure 3.12).

According to the NMR analysis data, the major isomer **127** is α–oriented, and the minor isomer **128** is β-oriented. This result can be easily explained by the following: the β-part of the dienone **124** is sterically hindered by the C_{14} and C_{15} methyl groups. This configuration is also supported by the fact that the H_5 proton of isomer **127** resonates at 2.52 ppm and that of its epimer **128** resonates at 2.31 ppm. In the first case, the H_5 proton is deprotected by the epoxy group. Direct experimental NMR evidence for the $\alpha-/\beta-$ configuration of compounds **127** and **128** was obtained through nuclear Overhauser effects (NOE). Thus, the irradiation of the $H_{11}\beta$ proton from the α–oriented epoxyketone **127**, NOE induced the enhancement of the signals of the protons from the C_{13} and C_{15} methyl groups. Upon irradiation of the $H_{11}\beta$ proton from the β-oriented epoxyketone **128**, no NOE effect was observed on the signals belonging to these methyl groups, which proves the presence of the β–oriented epoxy group.

Treatment of the mixture of epoxides **127** and **128** with $HClO_4$ (30%) in THF afforded ketodiol **126** in 68.0% yield. This result can be explained by the fact that two successive S_{N2} reactions result, in which the less stable epoxide **128** isomerizes to the more stable epoxide **127** (Figure 3.13). The reaction is thermodynamically controlled and the bulkier hydroxymethyl group adopts the more stable β–equatorial configuration. An example of this type of transformation was previously published [23].

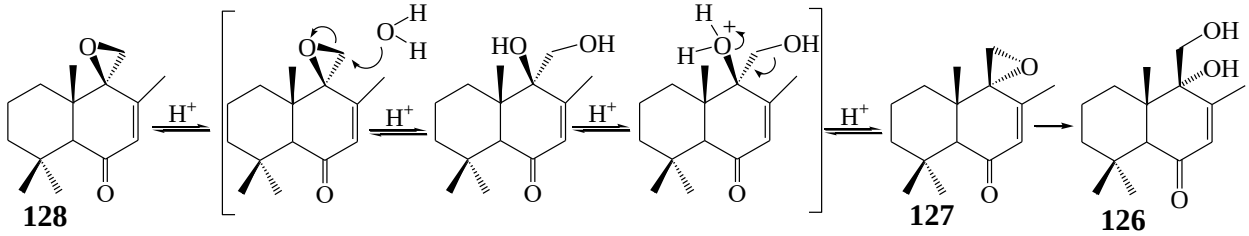


Fig. 3.13. Proposed mechanism of ketodiol **126** synthesis.

In the last step, ketodiol **126** was reduced with $LiAlH_4$, forming a mixture composed of (-)-albrassitriol **120** (44%) and (-)-6-*epi*-albrassitriol **121** (48%), separable by column chromatography on silica gel. The coupling constants between the H_5 and H_6 protons in the ^1H NMR spectra of compounds **120** (J= 9.6 Hz) confirm the equatorial configuration (α–) of the C_6 hydroxyl group and the axial orientation (β–) for (-)-6-*epi*-albrassitriol **121** (J = 4.0 Hz).

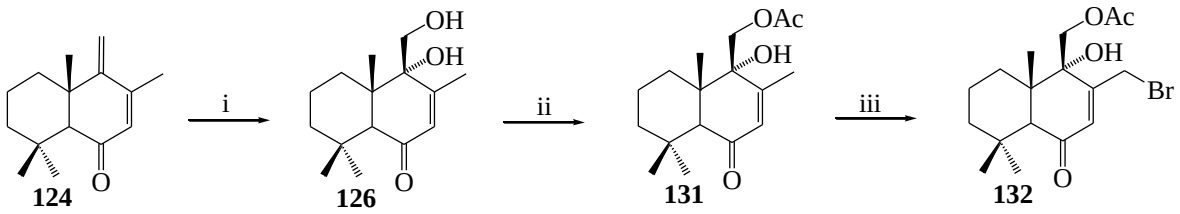
Thus, an efficient synthesis of the natural and biologically active drimane sesquiterpenoids (-)-albrassitriol **120** and (-)-6-*epi*-albrassitriol **121** was achieved, with good yields, from (+)-larixol **2** by means of the drimane diene **124**, obtained by Norrish II type photolytic degradation of 14,15-*bis*-norlabd-7-en-6,13-dione **123**, and using monoperphthalic acid, as an alternative to osmium tetraoxide [21].

Pereniporin B **129** is a plant growth inhibitor, while cinnamosmolide **130** shows antifungal activity *in vitro* against the dermatophytes *Tricophyton rubrum*, *Tricophyton mentagraphytes* and *Microsporum gypseum*. Also, both metabolites show cytotoxic activity: pereniporin B **129** against Friend leukemia cells (F5-5), and cinnamosmolide **130** against 9KB5 carcinoma in cell culture.

Several total and semi-syntheses of pereniporin B **129**, in racemic or enantiomerically pure form, are described in the literature in a different number of steps, from six with a ~11.0% total yield, to nine (~5.0 %), 19 stages (~3.0%) and up to 28 (~1.8%). For cinnamosmolide **130**, only one synthesis is known, which was carried out in nine steps and with a 14.0% total yield.

A new and efficient route for the synthesis of (-)-pereniporin B **129** and (-)-cinnamosmolide **130** from (+)-larixol **2** via the key intermediate ketodiol **126** will be described next [24].

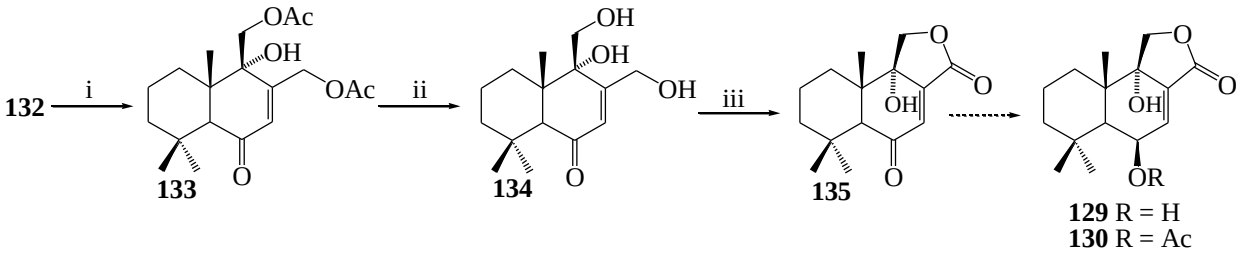
Dienone **124** may find several applications in the synthesis of new drimane compounds or natural analogues, especially through a facile functionalization of the C_{12} allylic methyl group, which was previously achieved by allylic oxidation with SeO_2. In this case, functionalization was performed by allylic bromination followed by substitution of the bromine with an acetate group (Figure 3.14). Acetylation of the primary hydroxyl group in compound **126** was performed prior to allylic bromination to prevent its unwanted oxidation. Allylic bromination of acetate **131** with *N*-bromosuccinimide (NBS) led to bromide **132** in 91.0% yield.



Reagents and conditions: i) OsO_4, Py, r.t., 12 hrs; ii) Ac_2O, Py, r.t., 12 hrs, 86%; iii) NBS, CCl_4, reflux, 9 hrs, 91%.

Fig. 3.14. Synthesis of intermediate **132**.

The subsequent substitution of the bromine atom upon treatment with KOAc led to the diacetate **133** (Figure 3.15), which after total hydrolysis led to the previously reported triol **134** [25], and the oxidation of the hydroxyl group C_{12} in its molecule, was carried out with MnO_2, proceeded selectively, followed by cyclization leading to lactone **135**.



Reagents and conditions: i) KOAc, DMSO, r.t., 1 hr, 98%; ii) K_2CO_3, MeOH, r.t., 0.5 hr, 99%; iii) MnO_2, DCM, r.t., 70 hrs, 93%.

Fig. 3.15. Formal synthesis of pereniporine B **129** and cinnamosmolide **130**.

The transformation of precursor **135** to pereniporin B **129** was reported earlier. This includes treatment of lactone **135** with DIBAL-H, followed by Fetizon oxidation of the resulting lactols and obtaining cinnamosmolide **130** by acetylation of pereniporin B **129**.

Thus, starting from (+)-larixol **2**, by means of its di- and pentanorlabdane derivatives, new and efficient methods for the synthesis of natural analogues of (+)-6-ketoeuryfuran **107**, (+)-fragrolide **108**, (+)-6-ketowinterin **109**, (-)-albrassitriol **120**, (-)-6-*epi*-albrassitriol **121**, pereniporin B **129** and cinnamosmolide **130** were developed, including by non-conventional methods such as Norrish II photolytic degradation and sensitized photooxidation under varied conditions and established reaction mechanisms.

4. TERPENO-HETEROCYCLIC MOLECULAR HYBRIDS

Hybridization of biologically active molecules is becoming a powerful tool for the discovery of new drugs with a broad spectrum of action. This offers a perspective for the development of effective and safe drugs for the treatment of diseases of the century, such as cancer, malaria, tuberculosis, AIDS, etc. Hybrid drugs can offer combined therapies in a single multifunctional agent and thus be more specific, effective and potent than conventional classical treatments. Currently this field of research is expanding rapidly and attracting researchers from all over the world. Recent advances show that biologically active molecules of molecular hybrids can produce powerful therapies [26].

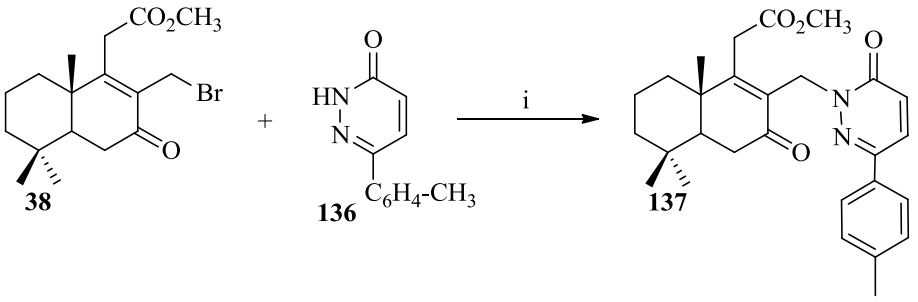
In this Chapter, the results regarding the syntheses of some molecular hybrids from the di-, tri-, tetra- and pentanorlabdane series obtained from (-)-sclareol **1** with various heteroatomic fragments or heterocyclic units will be presented.

4.1. Synthesis of tetra- and pentanorlabdane molecular hybrids with diazine units

In the following, the results of some syntheses of tetra- and pentanorlabdane molecular hybrids containing both terpene and azaheterocyclic fragments, through classical and microwave-assisted (MW) reactions, will be presented [27]. Previously, a similar study on the synthesis and structure elucidation of some homodrimane sesquiterpenoids with a diazine skeleton, which showed excellent antibacterial activity, was carried out by the authors [28, 29].

Brominated derivatives of methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** and the natural drimane sesquiterpenoid drim-8-en-7-one **8**, which can be obtained from ketoester **7** by MW-assisted method, were used as starting materials.

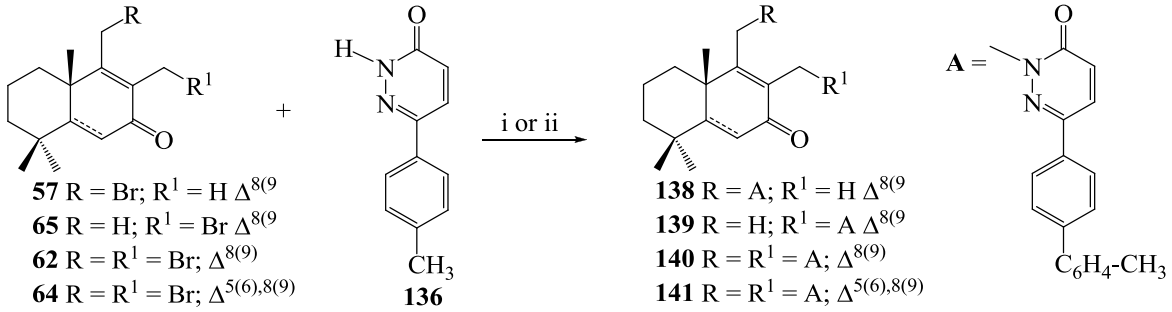
The synthesis of bromide **38** from ketoester **7** was reported in Subchapter 2.4, according to the method described [13]. Its treatment with 6-(*p*-tolyl)-3(2*H*)-pyridazinone **136** led to the molecular hybrid methyl ester of 7-oxo-12-(6-oxo-3-*p*-tolyl-6*H*-pyridazin-1-yl)-11-homodrim-8-en-16-oic acid **137** (Figure 4.1) with a 75% yield.



Reagents and conditions: i) K_2CO_3, DMAA, 48 hrs, r.t., 75.0%.

Fig 4.1. Synthesis of tetranorlabdane molecular hybrid **137**.

The initial drimane bromides **57**, **62**, **64** and **65** obtained previously [2, 9] (Subsection 2.4) were subjected to coupling reactions with 6-(*p*-tolyl)-3(2*H*)-pyridazinone **136** in *N*,*N*-dimethylacetamide (DMAA) in the presence of potassium carbonate, both under classical conditions and under microwave irradiation, obtaining molecular hybrids **138**-**141** (Figure 4.2).



Reagents and conditions: i) K_2CO_3, DMAA, r.t., 24 hrs, 34-80%;
ii) K_2CO_3, DMAA, MW, 20 min., 34-84%.

Fig 4.2. Synthesis of pentanorlabdane molecular hybrids **138**-**141**.

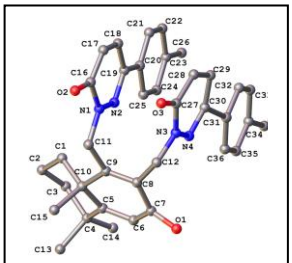


Fig. 4.3. Molecular structure of compounds **141**.

It should be noted that the MW assisted method provided higher yields and was much faster. Chromatographically inseparable bromines **57** and **65** were used in the coupling reactions as a 3:2 mixture. The monosubstituted products, 11-*p*-tolyl-pyridazonyl-drim-8(9)-en-7-one **138** and 12-*p*-tolyl-pyridazonyl-drim-8(9)-en-7-one **139**, unlike the starting bromides, were isolated by column chromatography in the same ratio, with 85% overall yields in both cases. Individual bromides **62** and **64** under the same conditions gave pure 11,12-*p*-tolyl-pyridazonyl-drim-8(9)-en-7-one **140** (80 and 84%) and pure 11,12-*p*-tolyl-pyridazonyl-drim-5(6),8(9)-dien-7-one **141** (77 and 82%). The structure of compound **141** was definitively confirmed by single crystal X-ray diffraction [27] (Figure 4.3).

Thus, for the first time, starting from the ketoester **7** and the natural drimane sesquiterpenoid drimenone **8**, a series of new molecular hybrids **138**–**141** with terpene and diazine fragments were synthesized, including by non-conventional methods, such as microwave irradiation, and their structures were confirmed by NMR spectroscopy, mass spectrometry, and single-crystal X-ray diffraction. The results of *in vitro* testing of molecular hybrids and structure-activity correlations are reported in Chapter VI, and the activity of compound **141** has been patented [12].

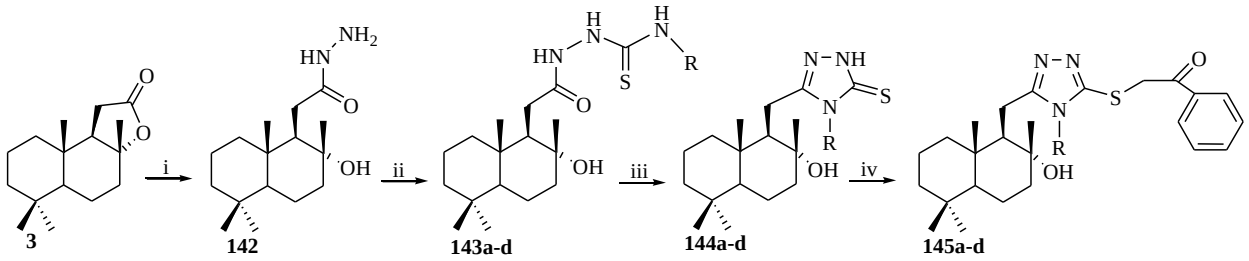
4.2. Synthesis of tetranorlabdane molecular hybrids with 1,2,4-triazole units

Triazole is a common moiety in a variety of drugs and natural products, and its substituted derivatives are privileged pharmacophores in compounds with anticancer, antimicrobial, and antiviral properties. To improve the biological properties of previously reported sesquiterpene derivatives, authors combined the homodrimane skeleton with the *N*-substituted 1,2,4-triazole unit [29], obtaining molecular hybrids that determined the increase of the antioxidant activity of cyanobacterial biomass [31].

Syntheses of new homodrimane sesquiterpenoids with 1,2,4-triazole units by means of the corresponding hydrazinecarbothioamides will be reported, their structures and biological properties will be elucidated [32].

The syntheses started with the preparation of 2-(8*α*-hydroxydriman-9-yl)acetohydrazide **142**, with a 85% yield, based on (+)-sclareolide **3** [33] (Figure 4.4). The resulting acetohydrazide **142** was treated with substituted isothiocyanates in EtOH to afford the hydrazinecarbothioamides **143a**–**d** in 83–91% yields under standard conditions. Under microwave (MW) irradiation the reaction proceeded in only 5 min, compared to the conventional approach (270–300 min), but also in this case the products **143a**–**d** were obtained with comparable yields (85–92%). The structures of compounds **143a**–**d** were confirmed by IR, 1D and 2D NMR spectroscopy as well as HRMS analyses.

Next, the hydrazinecarbothioamides **143a**–**d** were treated with aqueous NaOH solution (of 8%) [34], obtaining the tetranorlabdane molecular hybrids **144a**–**d** with 1,2,4-triazole fragments in 70–83% yields. ^{15}N NMR spectra of hybrids **144a**–**d** confirm the presence of triazole units [35].



a. R = -$CH_2CH{=}CH_2$; **b.** R = Ph; **c.** R = 4-Me-C_6H_4-; **d.** R = 3,5-Me_2-4-OH-C_6H_2-

Reagents and conditions: i) NH_2-$NH_2{\cdot}H_2O$ (95%), EtOH, reflux, 5 hrs, 85%;
ii) R-NCS, EtOH, r.t. 4-5 hrs, 83-91%, or MW, 5 min, 85-92%;
iii) NaOH (8%), 70°C, 2-3 hrs, 70-83%;
iv) 2-Bromoacetophenone, Et_3N, acetone, r.t., 2-3 hrs, 78-80%.

Figure 4.4. Synthesis of tetranorlabdane molecular hybrids **144a-d** with 1,2,4-triazole fragment.

Alkylation of compounds **144a**–**d** was accomplished with 2-bromoacetophenone [36] and led to *S*-substituted 1,2,4-triazoles **145a**–**d**. Their formation is favored by the increased nucleophilicity of the sulfur atom in the molecules of the respective 1,2,4-triazol-3-thiones **144a**–**d**, and one of the two possible tautomeric forms of compounds **144a**–**d** is an aromatic thiol, in which sulfur can readily react with 2-bromoacetophenone in the presence of Et_3N. The structures of homodrimane sesquiterpenoids **144c** and **145d** were also established by single crystal X-ray diffraction (Figures 4.5).

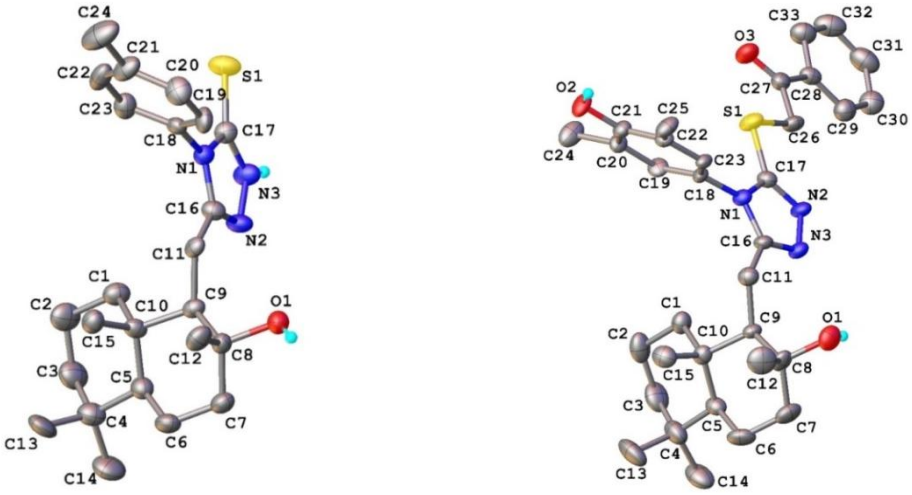
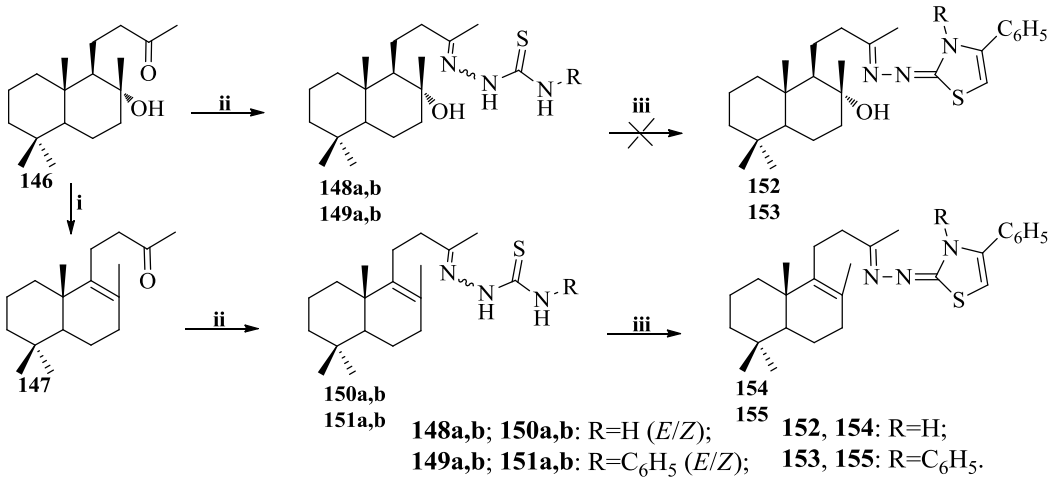


Fig. 4.5. Molecular structure of compounds **144c** and **145d**.

Thus, for the first time, based on (-)-sclareol **1**, by means of (+)-sclareolide **3**, efficient syntheses of homodrimane hybrids with hydrazinecarbothioamide fragment or 1,2,4-triazole unit were achieved. The products were obtained with high yields, by classical methods and by microwave assisted. The results of *in vitro* tests of the synthesized molecular hybrids and structure-activity correlations are reported in Chapter VI.

4.3. Di-, tri-, tetra- and pentanorlabdane molecular hybrids with 1,3-thiazole units

In addition to being important intermediates in the synthesis of compounds with 1,3-thiazole moieties, thiosemicarbazones also exhibit various pharmacological properties, including antitumor, antifungal, antibacterial, antiviral, antimalarial, etc. In the specialized literature there are few mentions about the synthesis of terpenoids with 1,3-thiazole fragments and the evaluation of their biological activity. In the following, the data related to the synthesis of dinorlabdane compounds with thiosemicarbazone fragments and 1,3-thiazole units, synthesized according to Figure 4.6 [37], will be described.



Reagents and conditions: i) $MeSO_3SiMe_3$, MeCN, r.t., 15 min., 80%;
ii) $NH_2NHCSNH_2$ or $NH_2NHCSNHC_6H_5$, EtOH, 60°C, 8-24 hrs, 60-80°C, 75-93%;
iii) $C_6H_5COCH_2Br$, EtOH, r.t., 8-14 hrs, 58-67%.

Fig. 4.6. Synthesis of dinorlabdane compounds with 1,3-tiazole fragment.

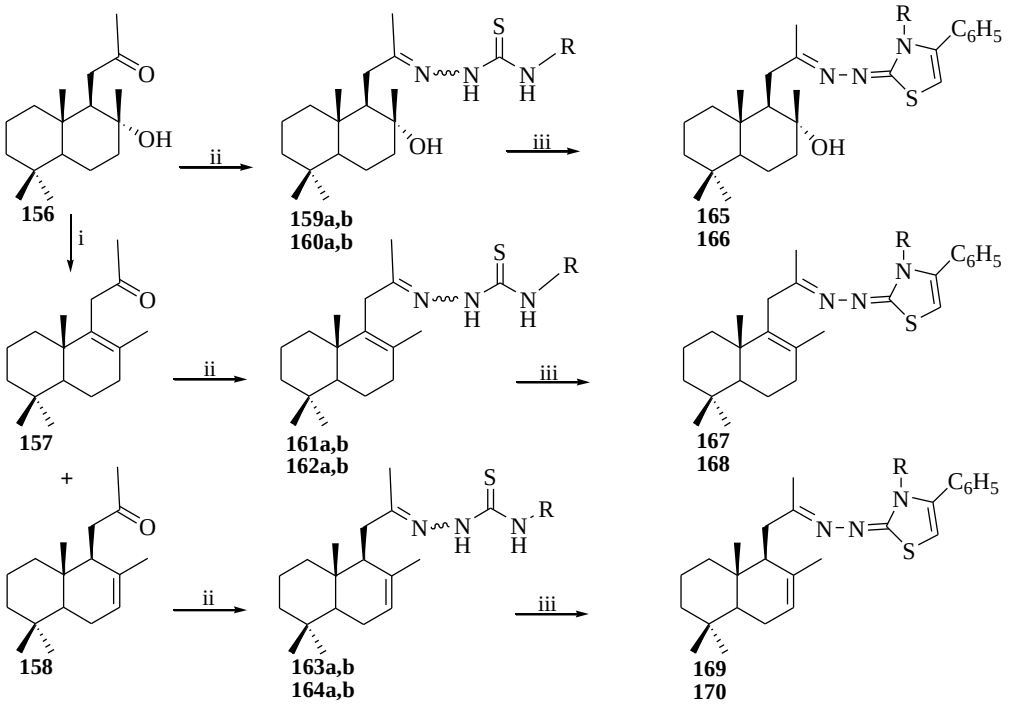
The (-)-sclareol **1** served as the raw material for the synthesis of these compounds, which, being oxidized with $KMnO_4$, produced 8*α*-hydroxy-15,16-dinorlabd-13-one **146** with a 90%

yield. As a result of the reaction of hydroxyketone **146** with trimethylsilylmethanesulfonate $MeSO_3SiMe_3$ in MeCN, 15,16-dinorlabd-8(9)-en-13-one **147** (80%) was obtained (Figure 4.6) [38].

Further, the reactions of ketones **146** and **147** with thiosemicarbazide or 4-phenylthiosemicarbazide (in a 1:1.1 molar ratio) led to the dinorlabdan compounds with thiosemicarbazone fragments **148a,b**–**151a,b** [37], all obtained as mixtures of geometric isomers.

The interaction of thiosemicarbazones **150a,b** and **151a,b** with 2-bromoacetophenone in EtOH (in 1:1 molar ratio) led to dinorlabdane compounds **154** and **155** with 1,3-thiazole moieties [37]. However, some attempts to obtain compounds **152** and **153** by heterocyclization of thiosemicarbazones **148a,b** and **149a,b** with 2-bromoacetophenone were not crowned with success.

Trinorlabdane compounds with thiosemicarbazone and 1,3-thiazole moieties were synthesized starting from (+)-sclareolide **3**, which by reaction with MeLi formed 8*α*-hydroxy-14,15,16-trinorlabd-12-one **156**, with a 65% yield. Treatment of hydroxyketone **156** with $MeSO_3SiMe_3$ in MeCN led to a mixture of chromatographically separable 14,15,16-trinorlabd-8(9)-en-13-one **157** and known 14,15,16-trinorlabd-7(8)-en-13-one **158**, (in a 4:1 ratio) with a 91% total yield [38] (Figure 4.7).



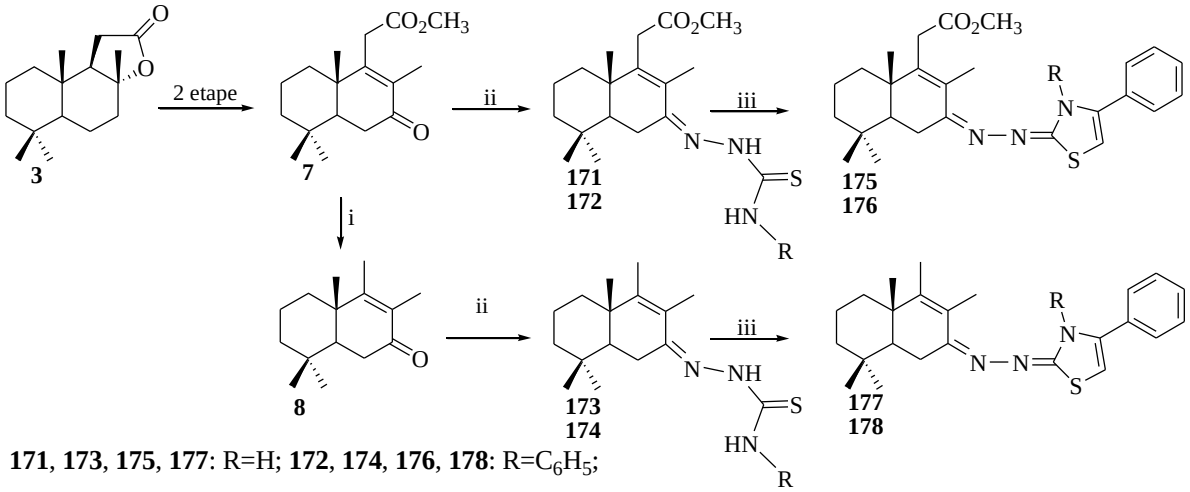
Reagents and conditions: i) $MeSO_3SiMe_3$, MeCN, r.t., 15 min., 91%;
ii) $NH_2NHCSNH_2$ or $NH_2NHCSNHC_6H_5$, EtOH, 60°C, 8-24 hrs, 60-80°C, 65-83%;
iii) $C_6H_5COCH_2Br$, EtOH, r.t., 8-24 hrs, 52-66%.

Fig. 4.7. Synthesis of trinorlabdane compounds with 1,3-tiazole fragment.

Reactions of compounds **156**–**158** with thiosemicarbazide or 4-phenylthiosemicarbazide (in a 1:1.1 molar ratio) produced trinorlabdane compounds with thiosemicarbazone moieties **159a,b**–**164a,b** [37], all obtained as mixtures of geometric isomers. The obtained thiosemicarbazones were used in the subsequent reactions without separation, because the Z-isomer during the reactions is transformed into the more stable *E*-isomer.

As a result of the reactions between thiosemicarbazones **159a,b**–**164a,b** with 2-bromoacetophenone in EtOH (in a 1:1 molar ratio) trinorlabdane compounds **165**–**170** with 1,3-thiazole fragments were formed in 52–66% yields [37].

Methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate **7** and drim-8(9)-en-7-one **8** are important intermediates in the synthesis of tetra- and pentanorlabdane compounds with thiosemicarbazonic and 1,3-thiazole fragments. These were obtained from (+)-sclareolide **3** in two and three steps, respectively, in 60% and 58% overall yields [2, 10] (Figure 4.8). Tetra- and pentanorlabdane compounds **171**–**164** with thiosemicarbazone moieties were obtained starting from ketones **7** and **8**, and thiosemicarbazide or 4-phenylthiosemicarbazide in a 1:1.1 molar ratio. Further reactions of thiosemicarbazones **171**–**174** with 2-bromoacetophenone (in a 1:1 ratio) [37] led to 1,3-thiazoles **175**–**178** in 75–83% yields. The structures of all newly synthesized compounds were confirmed by spectral methods (IR, ^{1}H, ^{13}C and ^{15}N NMR).



Reagents and conditions: i) KOH, EtOH, reflux, 3 hrs, (or MW, 1.5 hrs), 98%; ii) $NH_2NHCSNH_2$ or $NH_2NHCSNHC_6H_5$, EtOH, 60°C, 24 hrs, 71-83%; iii) $C_6H_5COCH_2Br$, EtOH, r.t., 4-6 hrs, 81-90%.

Fig. 4.8. Synthesis of tetra- and pentanorlabdane molecular hybrids with 1,3-tiazole fragment.

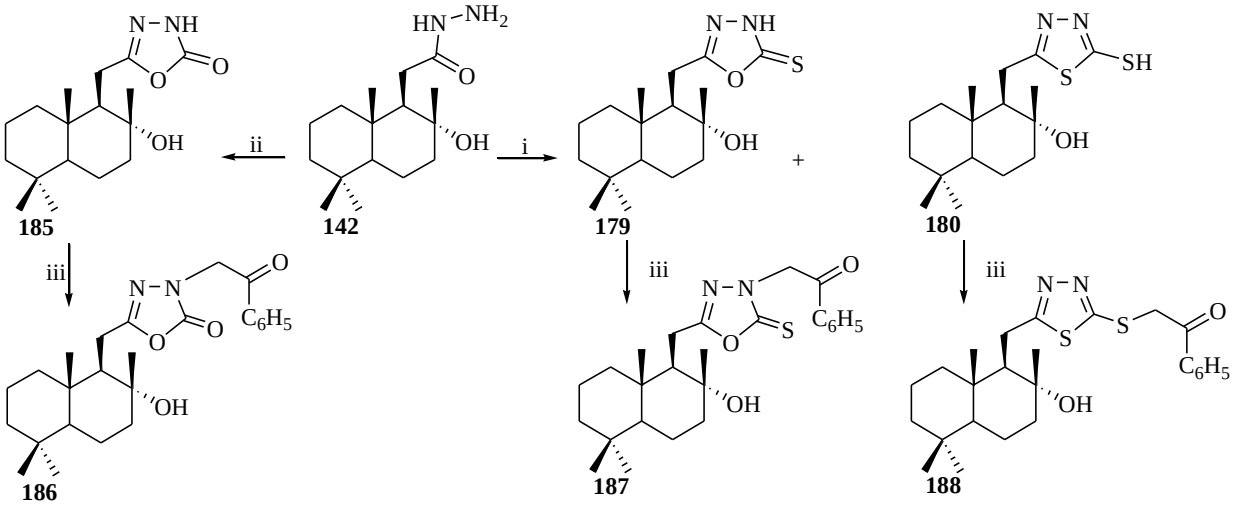
Thus, for the first time, based on (-)-sclareol **1**, by means of its di-, tri-, tetra- and pentanorlabdane derivatives, efficient syntheses of some series of molecular hybrids with thiosemicarbazone or 1,3-thiazole fragments were achieved with high yields, including by non-conventional methods, such as microwave assisted. Results of molecular hybrids *in vitro* assays and structure-activity correlations are reported in Chapter VI.

4.4. Synthesis of tetranorlabdane molecular hybrids with 1,3,4-oxadiazole and 1,3,4-thiadiazole units

Oxadiazoles and thiadiazoles are classes of heterocycles that have attracted significant interest in medicinal chemistry and exhibit a wide range of biological activities such as anti-inflammatory, anticonvulsant and antibacterial.

Next, the syntheses of new homodrimane sesquiterpenoids with 1,3,4-oxadiazole or 1,3,4-thiadiazole units will be described [39].

The synthesis of the reported compounds was carried out on the basis of 2-(8α-hydroxydriman-9-yl)acetohydrazide **142**, obtained from (+)-sclareolide **3** in one step with a 85% yield (Figure 4.9) [33].



Reagents and conditions: i) TMTD, DMF, 90 ºC, 1,5 hrs, **179** (20-86%), **180** (5-70%); ii) CDI, Et_3N, THF, 0ºC, 74%; iii) Br-acetophenone, Et_3N, acetone, 3 hrs, 80-91%.

Fig. 4.9. Synthesis of molecular hibrids with 1,3,4-oxadiazolic and 1,3,4-tiadiazolic fragments.

Next, acetohydrazide **142** was treated with varying amounts of tetramethylthiuram disulfide (TMTD) in DMF according to the known procedure. The use of 0.5 equiv. of TMTD, led only to 5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2(3*H*)-thione **179** (45%) and recovered the starting hydrazide **142** (46%). Increasing the amount of TMTD to 1 equiv. generated a mixture of 5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2(3*H*)-thione **179** (86%) and 5-(8α-hydroxydriman-11-yl)-1,3,4-thiadiazole-2-thiol **180** (5%). In the case of 1.5 equivalents of TMTD, the equilibrium shifted towards the formation of thiadiazole **179** (70%), also obtaining the minor oxadiazole **180** (20%).

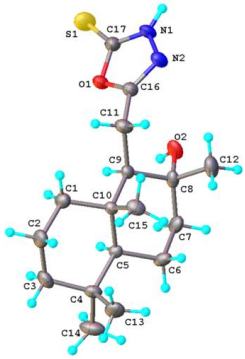


Fig. 4.10. Molecular structure of compound **179**.

Isolation of 1,3,4-thiadiazole **180** from the reaction mixture was surprising, but spectral analysis fully confirmed its structure. The structure and stereochemistry of compound **179** was definitively confirmed by single crystal X-ray diffraction (Figure 4.10).

The formation of compound **180** can be explained by the mechanism described in Figure 4.11. TMTD interacts with compounds such as acetohydrazide **142** containing the amine group, forming the *N*,*N*-dimethyldithiocarbamate ion **181**. This ion attacks the 1,3,4-oxadiazole ring in compound **179**, causing its opening. Then the nucleophilic oxygen atom in intermediate **182** attacks the carbon atom in the dithiocarbamate moiety. In the newly formed intermediate **183**, the nucleophilic sulfur atom in turn attacks the neighboring carbon atom with oxygen and completes the formation of the 1,3,4-thiadiazole ring **180** by elimination of the *N*,*N*-dimethylthiocarbamate ion **184** (Figure 4.11).

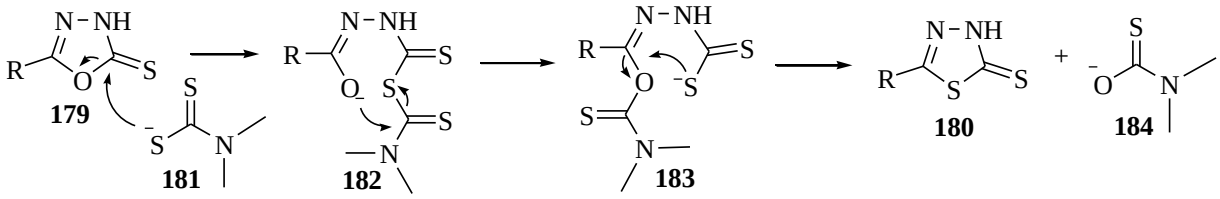
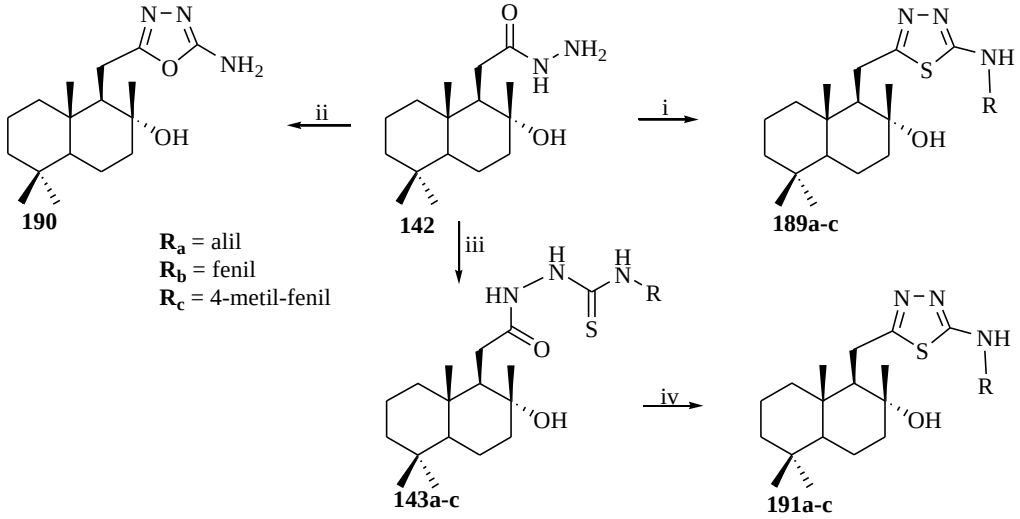


Fig. 4.11. The probable mechanism of formation of the 1,3,4-thiadiazole ring.

Acetohydrazide **142** was treated with 1,1'-carbonyldiimidazole (CDI) in the presence of Et_3N in anhydrous THF to form 5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2(3*H*)-ona **185** with a 74% yield.

Oxadiazoles **179**, **180** and thiadiazole **185** were treated with bromoacetophenone in Me_2CO in the presence of Et_3N to form 3-benzoylmethyl-5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2(3*H*)-one **186** (80%), 3-benzoylmethyl-5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2(3*H*)-thione **187** (91%) and 2-benzoylmethylsulfanyl-5-(8α-hydroxydriman-11-yl)-1,3,4-thiadiazole **188** (85%) [36] (Figure 4.9). The structure and stereochemistry of compound **188** was definitively confirmed by single crystal X-ray diffraction (Figure 4.12).

Further [39], to obtain terpene compounds with 2-amino-substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole units, tetranorlabdane hydrazide **142** was treated with isothiocyanate derivatives, without isolating the intermediate compounds. This reaction was carried out in the presence of Et_3N in H_2O, affording the substituted 2-amino-1,3,4-thiadiazoles **189a–c** in 70–78% yields (Figure 4.13).



Reagents and conditions:i) R-NCS, Et_3N, H_2O, reflux, 18 hrs, 70-78%; ii) CNBr, $NaHCO_3$, dioxane aq., 1 hr, 80%; :iii) R-NCS, EtOH, r.t., 4-5 hrs, 83-86%; iv) DCC, Me_2CO, MeOH, reflux, 5 hrs, 76-81%.

Fig. 4.13. Synthesis of homodrimane compounds with 2-amino-1,3,4-thiadiazole and 2-amino-1,3,4-oxadiazole fragments.

Next, hydrazide **142** was treated with cyanogen bromide (CNBr) in aqueous dioxane to give unsubstituted 5-(8α-hydroxydriman-11-yl)-1,3,4-oxadiazol-2-amine **190** in yield 80%

In the case, when hydrazide **142** was treated with substituted aryl isothiocyanates in EtOH, intermediate hydrazinecarbothioamides **143a**–**c** were obtained with 83–86% yields according to Figure 4.13 [32].

Treated with *N*,*N*'-dicyclohexylcarbodiimide (DCC) in a mixture of MeOH and Me_2CO, carbothioamides **143a**–**c** formed the homodrimane sesquiterpenoids with substituted 2-amino-1,3,4-oxadiazole units **191a**–**c** in 76–81% yields.

Thus, for the first time, based on (-)-sclareol **1**, by means of its tetranorlabdane derivatives, syntheses of tetranorlabdane hybrids with 1,3,4-oxadiazole and 1,3,4-thiadiazole structural units were realized. By varying the reagents and their molecular ratios, the optimal conditions of the heterocyclization reactions were established, and the mechanisms of some reactions were also explained. All synthetic compounds were tested *in vitro*, and the results of molecular hybrids tests and structure-activity correlations are reported in Chapter VI. Five compounds in these series showed antimicrobial activity, and the activity of compound **180** was patented.

5. PHYTOCHEMICAL CHARACTERIZATION OF LOCAL VEGETAL PRODUCTS

Local aromatic and medicinal plants, but also wild fruits are excellent sources for obtaining new phytotherapeutic preparations, food additives, value-added products and new semi-synthetic active substances. All investigated species are known for their biological and pharmacological properties, and often are used by the population in fresh, frozen, dried or processed into teas for the treatment of various diseases [40].

This chapter includes the data of the phytochemical study on some local plant sources, such as different varieties of apple, species of medicinal plants and wild fruits, aromatic plants such as lavender, the waste from it, but also less studied species, such as corymbflower tansy.

In the Republic of Moldova, all the mentioned sources are found in the spontaneous flora or are cultivated industrially. The novelty of the study consists in the fact that, until now, the chemical composition of these sources has been studied briefly, or is unknown.

5.1. Chemical composition of the volatile oil of lavender *Lavanda angustifolia* Mill. and corymbflower tansy *Tanacetum corymbosum* (L.) Sch. Bip.

Lavender (*L. angustifolia* Mill.; syn. L. *vera* DC, syn. *L. officinalis* Chaix ex Vil., syn. L. *spica* L.) is an evergreen semi-shrub from the family *Lamiaceae*, native to the Mediterranean region. Currently, this species is naturalized almost throughout Europe, North Africa, the United States and Australia.

A total of 41 constituents were identified in lavender volatile oil samples of local origin (Moldoveneasca 4 and Chisiniovskaia 90 varieties) by the GC-MS method [41]. It should be noted that the volatile oil with the most complex composition was obtained by the producer P1, which is the largest on the Moldovan market and operates at a modern stationary factory, and is intended for export.

The main constituents of this group, which determine the quality and authenticity of lavender volatile oil, according to the international standard, are (%): 1.8-cineole (eucalyptol) (<1.0), (*E*)-ocimene (4.0–10.0), (*Z*)-ocimene (1.5–6.0), linalool (25.0–38.0), camphor (<0.5), terpin-1-en-4-ol (2.0–6.0), α-terpineol (<1.0), linalyl acetate (25.0– 45.0) and lavandulyl acetate (>2.0) [41].

According to the GC-MS analysis data, the chemical composition of lavender volatile oil produced in the Republic of Moldova consists mainly of terpene compounds (%): monoterpenes (monoterpene hydrocarbons - 8.72–15.32; oxygenated monoterpenes - 69.00–83.83); sesquiterpenes (sesquiterpene hydrocarbons - 3.09–12.83; oxygenated sesquiterpenes - 0.19–1.26); and aliphatic compounds: alcohols - 0.13–1.01; ketones - 0.25–0.80; esters - 0.91–2.09 [41].

Analysis of extracts from lavender floral waters (LA) showed that it contains only a few hydrophilic monoterpenes (~0.3–0.5%/volume), such as 1,8-cineole (eucalyptol, 6.31%), linalool oxide (3.08%), linalool (78.05%), terpin-1-en-4-ol (1.92%) and α-terpineol (10.64%).

Corymbflower tansy (*T. corymbosum* (L.) Sch. Bip.; syn.: *Pyrethrum corymbosum* (L.) Scop., *Chrysanthemum corymbosum* L.) is a common species that grows singly or in small groups and is a perennial herbaceous plant with stalks branched. The species originates from Europe, and is currently spread throughout the Northern hemisphere (temperate Asia, North Africa and America), including the Republic of Moldova. Unfortunately, the scientific literature provides very limited information about the chemical composition of the volatile oil of *T. corymbosum*, which differs essentially from those of oils obtained from other species of the gen. *Tanacetum*.

A total of 38 constituents were identified in the samples of volatile oil obtained from fresh corymbflower tansy *T. corymbosum* plants of Moldovan origin (Sample A) and 22 in the samples obtained from dry plants (Sample B), representing 99.5% and 95.6% respectively, out of the total. Both samples contain the isomers *δ*-terpinolene (0.6%, Sample A) and *β*-terpinolene (2.0% and 1.5%), but also many previously unreported constituents.

The main constituents of the volatile oil of *T. corymbosum* are sesquiterpene hydrocarbons: (71.8% - Sample A and 62.6% - Sample B), and the results obtained show that sp. *T. corymbosum* originating from the Republic of Moldova belongs to the germacrene chemotype D (47.5% and 33.3%), which is the main constituent, followed by (*Z*)-*β*-farnesene (8.6% and 16.1%), *γ*-elemene (5.2% and 3.1 %), *β*-caryophyllene (4.2% and 6.5%) and *β*-terpinolene (2.0% and 1.5%); oxygenated derivatives: (5.9% - Sample A and 7.5% - Sample B), represented by alcohols, such as: *α*-cadinol (1.7% and 1.3%), (*E*)-muurolol (1.6% and 0.9%) and spathulenol (0.5 % and 0.7%) identified in both samples, humulan-1,6-dien-3-ol (0.4%), cubenol (0.3%) and *δ*-cadinol (0.4%) (Sample A); other oxygenated derivatives: caryophyllene oxide (0.5% and 3.3%) and 9-cedrone ketone (0.4% and 0.6%). The monoterpene fraction was identified only in Sample A and is represented by camphene (0.2%) and (*Z*)-*β*-ocimene (0.9%). The content of oxygenated diterpenoids exceeds that of monoterpenoids and includes only (*E*)-phytol (1.4% and 2.0%).

Aliphatic compounds represent the second group of constituents (19.3% and 23.5% respectively) and include: higher alkanes - heinecosan (1.0% and 6.9%), heptacosane (0.4% and 2.9%) and octacosane (0.4% and 2.6%), in especially Sample B (12.4%); higher alcohols - octadecan-1-ol (9.7% and 0.6%), 12-methyl-(*E*,*E*)-2,13-octadecadien-1-ol (0.4%) and docosanol-1 (1.2%) especially Proba A (11.3%); carbonyl compounds - nonanal (0.7%), 4-(*Z*)-4-(2,2-dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one (0.8%), octadecanal (0.2%) and tetradecanal (0.8 %, both), especially Sample A; Saturated and unsaturated fatty acids, and esters - *n*-hexadecanoic acid (2.2% and 7.1%), linoleic acid (0.2% and 1.0%), 1,7-octadecynoic acid (0.2%) and eicosanoic acid methyl ester (0.6%). The visible qualitative and quantitative variations of the constituents in Samples A and B can be explained by the chemical changes occurring in the plant material during drying and storage.

Thus, for the first time, a comparative phytochemical study of lavender volatile oil samples, obtained from seven local producers, and waste, such as floral waters"*aqua lavandula*", was carried out. Also, for the first time, the phytochemical study of the volatile oil obtained from the fresh and dried plants of corymbflower tansy, a little-studied local species, was carried out.

5.2. Quantification of triterpene oleanolic and ursolic acids in apple cultivars, lavender plants and by-products, species of medicinal plant and wild fruits.

Due to the wide spectrum of biological activities, combined with low toxicity, oleanolic **192** (AO) and ursolic **193** (AU) acids were selected as the study object of the present work (Figure 5.1).

Originally from the area between the Caspian Sea and the Black Sea, sp. apple (*Malus domestica* Borkh.) is one of the most intensively cultivated and consumed fruits worldwide, being available on the market throughout the year and representing the main source of polyphenols in the human diet [42, 43].

A significant amount of the apple harvest is processed industrially, mainly for the production of juices, resulting in a large volume of solid waste (peels, seeds and pulp), known as

"*apple pomace*" [44], which represents a valuable source of bioactive compounds and nutrients for the development of new products with nutraceutical properties.

The content of AO **192** and AU 193 in the extracts obtained from the cuticular layer of different apple varieties grown in Moldova and Romania is presented in Figure 5.2.

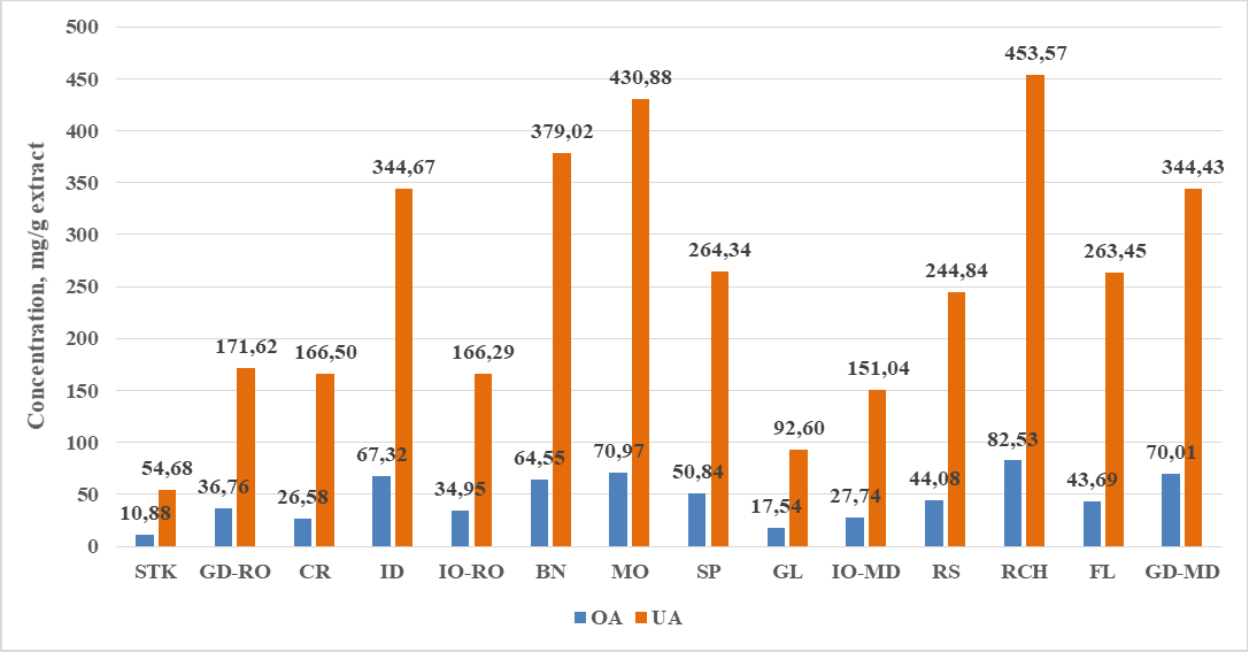


Fig 5.2 The content of oleanolic **192** and ursolic **193** acids in extracts from aplle cultivars: *Romania* – STK (Starkrimson), GD-RO (Golden Delicious), CR (Cretesc), ID (Idared), JO-RO (Jonathan); *R Moldova* – BN (Beliy Naliv), MO (Montuan), SP (Spartan), GL (Gloster), JO-MD (Jonathan), RS (Renet Simirenco), RCH (Richard), FL (Florina), GD-MD (Golden Delicious).

In extracts obtained from the cuticular layer of apples, AU **193** was quantified in higher amounts (54.68 - 453.57 mg/g of extract) compared to AO **192** (10.88–82.53 mg/g of extract), the data being consistent with those from literature. The content of AO **192** is higher in the fruits of varieties RCH (82.53 mg/g), MO (70.97 mg/g), GD-MD (70.01 mg/g), ID (67.32 mg/g) and lower in varieties STK (10.88 mg/g), CR (26.58 mg/g) and JO-MD (27.74 mg/g), and that of AU **193** is higher in the fruits of the varieties RCH (453.57 mg/g), MO (430.88 mg/g), ID (344.67 mg/g) and BN (379.02 mg/g) and lower in STK (54.68 mg/g) and GL (92.60 mg/g) cultivars. ANOVA results confirm that the content of AO **192** and AU **193** in the cuticular layer of apples differs significantly depending on the variety.

Among other sources containing acids **192** and **193**, lavender showed interest, the most cultivated voltile oil producing plant in the Republic of Moldova, and the average extraction yield of lavender plant materials (MVL) was 9.56%. Chromatographic analyzes indicated a content of AO **192** between 13.43–19.09 mg/g(extract) and that of AU **193** between 33.28–60.82 mg/g(extract), recalculated for dry MVL for AO **192** (133.11–168.57 mg/100 g) and AU **193** (329.83–537.00 mg/100 g), respectively. The experimental results showed that the summary content of AO **192** and AU **193** in MVL is ~5.0% of the dry mass, they are in a ratio of 1:2.6, which confirms that lavender is a valuable source of natural triterpene acids.

Oleanolic acid **192** and ursolic acid **193** were also quantified in lavender solid waste residue (DW) left after hydrodistillation of the volatile oil [45]. The average extraction yield was 3.90%, and the content of AO **192** between 27.48–39.37 mg/g(extract) and that of AU **193** between 80.82–135.56 mg/g(extract), recalculated for DW AO **192** (113.47–144.98 mg/100 g) and AU **193** (313.95–499.15 mg/100 g), respectively. The experimental results showed that the

summary content of AO **192** and AU **193** in MVL is ~1.0% of the dry mass, they are in a 1:3.1 ratio. It has been confirmed that fresh plants have a higher content of AO **192** and AU **193** than waste [41]. This fact can be explained by their loss and derivatization/degradation during hydrodistillation at high temperatures, and this reason seems more relevant, since the analysis of lavender floral waters (AF) confirmed that they do not contain the mentioned acids.

Another object of the phytochemical study was wild fruits and medicinal plants, in the extracts of which AO **192** and AU **193** were identified and quantified [45], their content being expressed in mg/100 g of dry plants or fruits dry/fresh forest.

Extraction yields ranged between 9.7-16.6% for medicinal plants and between 1.6-15.1% for wild fruits, with higher values for common marigold (16.6%), cornelian cherry (15.1%) and Russian olive (14.7%). A high extraction yield does not necessarily imply a high content of AO **192** and AU **193**, the natural sources also containing other compounds, such as hydroxylated derivatives and esters of acids **192** and **193**, but also other bioactive triterpenes such as uvaol, sterols, and phenolic compounds, etc.

The content of AO **192** in the analyzed extracts varied for medicinal plants in the range 0.6 - 135.0 mg/g extract and for wild fruits 4.6-165.9 mg/g extract, it being higher in the extracts from Romanian tufted thyme (~135.1 mg) and cornelian cherry (~165.9 mg), and lower in common marigold (~0.63 mg), ginger mint (~5.3 mg) and blackberry (~4.6 mg).

The content of AU **193** in extracts from medicinal plants varied (4,8-215,9 mg/g extract) and in those from wild fruits (3,9-522,8 mg/g extract), it being higher for cornelian cherry (~522.8 mg), hawthorn (~152.8 mg), Romanian tufted thyme (~215.9 mg) and bergamot mint (~111.4 mg) and less in Russian olive fruits (~3.9 mg), dog rose (~9.7 mg), in common marigold (~4.8 mg) and ginger mint (~8.8 mg).

Valuable natural sources of AO **192** and AU **193** are the extracts of Romanian tufted thyme (1142.9-1827.3 mg/100g dry mass) and bergamot mint (278.1-1092.00 mg/100g dry mass) together with those from cornelian cerry (25.0-78.9 mg/g of fresh fruit), hawthorn (6.5-10.3 mg/g of fresh fruit) and blackthorn (1.3-7.6 mg/g of fresh fruit) [46].

The data of extraction yields and quantification of AO **192** and AU **193** were subjected to hierarchical cluster analysis (HCA), the results of which are reported in the paper [46]. According to it, the group with lower content of AO **192** and AU **193** includes dog rose, blackberry, common marigold, ginger mint and Russian olive fruits, while blackthorn, hawthorn, bergamot mint and Romanian tufted thyme are found in group two, with greater content. Principal component analysis (PCA) of the quantification results of AO **192** and AU **193** in the 30 samples indicates the presence of 4 distinct groups: I includes cornelian cerry; II Romanian tufted thyme; 3rd blackberry, dog rose; and IV the inseparable species, which have the lowest content of triterpene acids [46].

Thus, for the first time, AO **192** and AU **193** were quantified in the extracts obtained from the cuticular layer of apple varieties grown in Moldova and Romania, local lavender plants, solid waste and floral waters. Also, the content of AO **192** and AU **193** was determined in the extracts obtained from 4 species of medicinal plants and 6 species of wild frutes. Analyzes showed a large variation in content from variety to variety or from species to species, but compared to bibliographic data, local sources represent valuable natural sources of AO **192** and AU **193**, which confirms the benefits of their cultivation as sources of compounds natural bioactives for human health and potential raw materials for the pharmaceutical industry [47].

5.3. Profile of phenolic compounds and flavonoids in some apple cultivars *Malus domestica* Borkh.

After the quantification of monoterpene, sesquiterpene and triterpene compounds in the sources investigated in Subchapters 5.1 and 5.2, the phytochemical study continued on phenolic acids **194-201**, flavonoids **202-205** and (*E*)-resveratrol **206** [48]. The quantification of phenolic compounds (Figure 5.3) by the UHPLC method was carried out in accordance with the analytical protocol described in the thesis.

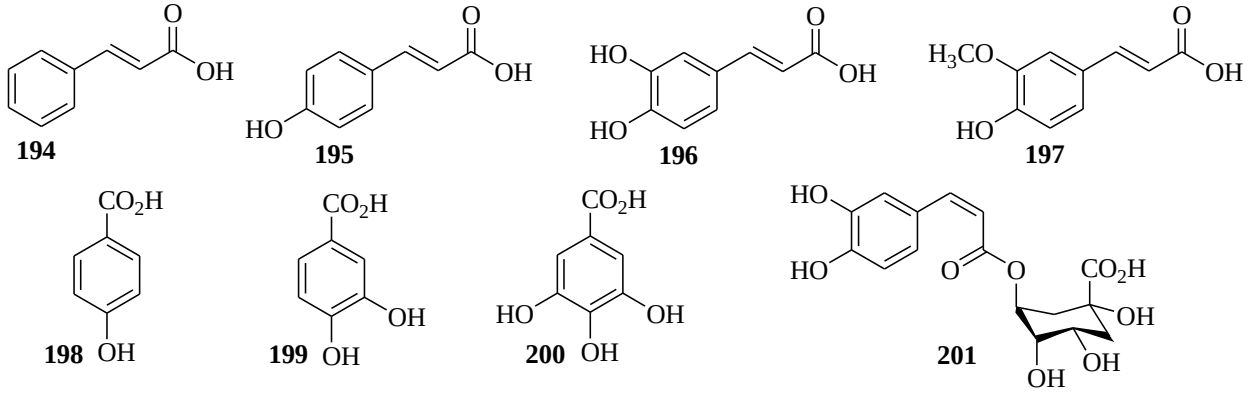


Fig. 5.3. Phenolic acids **194-201**.

Regarding the phenolic components, the generated results showed that cinnamic acid derivatives **194**, *p*-coumaric **195** (1.09–16.31 mg), caffeic **196** (9.43–47.23 mg), ferulic **197** (0.39–7.27 mg) and gallic **200** (0.49–21.74 mg) acids are the majors, with values recalculated for 100 g dry mass. Even though various studies have reported high amounts of chlorogenic acid **201** in apples, its amount in investigated apple varieties ranged from undetectable to 3.77 mg, similar to previous findings. The varieties with the highest level of phenolic acids (mg/100 g dry weight) were MO (55.03 mg), GD-MD (47.53 mg), CR (45.69 mg) and SP (44.98 mg), while the variety with the lowest level of phenolic acids was RCH (17.55 mg).

As for flavonoids (Figure 5.4), the amount of monomeric flavan-3-ols identified varied from (0.64-4.08 mg/100 g dry weight) for (+)-catechin **202** and from (n.d.-25.03 mg) for (-)-*epi*-catechin **203**, these compounds being the majority. The contents of (+)-catechin **202** and (−)-*epi*-catechin **203** in extracts from different apple cultivars also differ greatly (~1.0 and 1.81 mg) for SP to (~1.24 and 13.46 mg) for GD-RO [48].

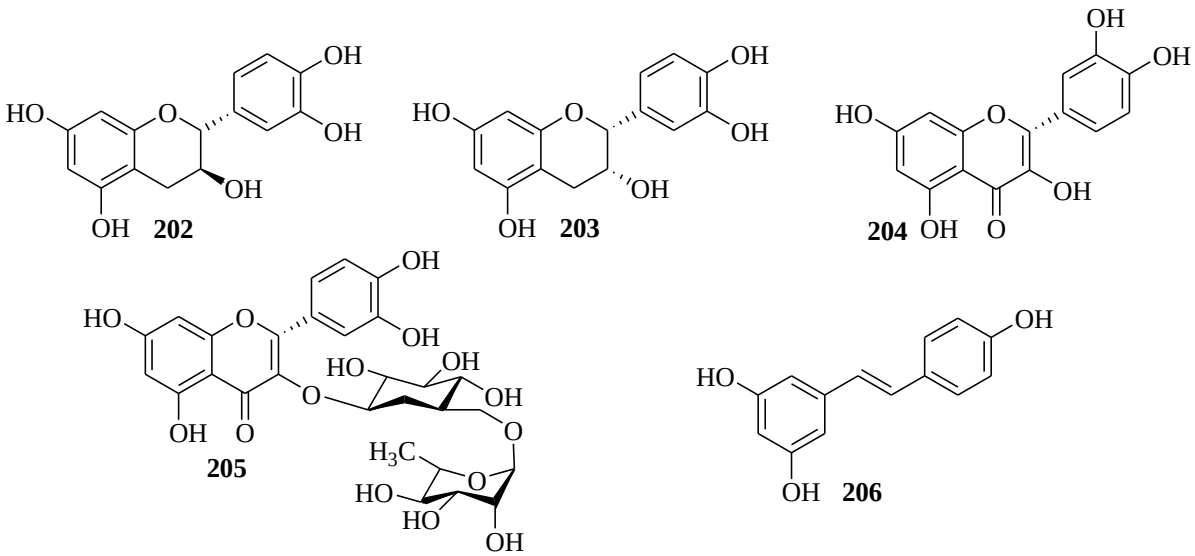


Fig. 5.4. Flavonoids **202-205** and (*E*)-resveratrol **206**.

The highest concentrations of (+)-catechin **202** and (-)-*epi*-catechin **203** (~4.1 and 25.0 mg) were identified in the STK variety, followed for (-)-*epi*-catechin **203** by FL (~19.7 mg) and RCH (~22.4 mg), at concentrations ~5 times higher than in the other varieties. The concentration of quercetin **204** quantified in the varieties studied varied between (~1.1-11.6 mg), higher for BN (~11.6 mg) and SP (9.9 mg), and lower for RS (~1.3 mg) and RCH (~1.54 mg). Rutin **205** is a minor component among the other flavonoids, with the highest concentration in the varieties BN (~3.8 mg), MO (~2.5 mg) and CR (~2.2 mg), the data being consistent with the specialized literature. (*E*)-resveratrol **206** was quantified in a large amount in cultivars BN (~4.8 mg), STK and MO (each ~3.4 mg), and the lowest amounts were detected in cultivars ID (~1.3 mg) and RS (~1.6 mg). The content of individual polyphenols is similar to other data in the literature [43, 49].

According to the results, the studied apple varieties are dominated by phenolic acids, except for the RCH variety, which is dominated by flavonoids. The highest amount of total flavonoids (mg/100 g dry weight) was determined in the variety STK (33.27 mg), while a lower amount was determined in the variety CR (6.83 mg). The FL variety shows similar amounts of phenolic acids (25.12 mg) and flavonoids (24.65 mg). The values of total phenolic compounds obtained by the Folin Ciocalteu colorimetric method varied from 240–576 mg GAE/100 g dry mass, and of total flavonoids (65–166 mg RE/100 g dry mass). STK, RCH and ID are the apple cultivars with high content of total phenolics, while MO, JO and SP are apple cultivars with high content of total flavonoids.

The results of the antioxidant capacity of the investigated apple varieties, measured by the DPPH test, showed a high variability (1277 to 2794 μmol Trolox/100 g dry mass). Extracts from varieties STK, JO, RCH and BN showed the highest antioxidant capacities, while GL, MO, GD and SP the lowest.

The data on the content of total phenolic compounds and total flavonoids obtained by the colorimetric method were higher compared to the quantification results by the UHPLC method, being consistent with those previously reported and indicating that there are more phenolic compounds, including phloretin, phloridzin, procyanidins polymeric (procyanidin B_1, procyanidin B_2), quercetin-glycosides and others, which contributed to the total content of polyphenols, but which were not quantified in this study due to the lack of the necessary standards.

ANOVA results applied to spectrophotometric data indicate that total phenols and total flavonoids were significantly different depending on the apple varieties: total phenols (STK, RCH and ID); total flavonoids (BN and MO).

Based on the data of quantitative UHPLC and UV-Vis analyzes of polyphenolic compounds, principal components analysis (PCA) was performed, the results of which are described in the paper [48]. According to it, 3,4-dihydroxybenzoic acids **199**, chlorogenic **201**, (+)-catechin **202** and (*E*)-resveratrol **206**, together with total phenolics and antioxidant capacity could suggest that polyphenolic compounds are markers of the STK variety, while that ferulic acid **197** and (-)-*epi*-catechin **203** represent the polyphenolic markers of RCH, FL and ID varieties. *p*-Coumaric **195** and gallic acids **200**, quercetin **204** and rutin **205**, characterize BN, SP and CR varieties, while (*E*)-cinnamic **194**, caffeic **196** and 4-hydroxybenzoic **198** acids are representative of RS and MO apple varieties.

Hierarchical cluster analysis (HCA) was used as an exploratory tool to evaluate the heterogeneity between different apple cultivars based on the composition of triterpene compounds, the results of which are described in the same source [48]. Its results indicate that

the cultivars with a higher potential of polyphenols (STK, JO and CR) represent a lower potential of triterpene acids, and vice versa for the cultivars MO and GD. The apple varieties BN, GD and RCH show potential for both polyphenolic and triterpene acids.

Thus, for the first time, phenolic acids **194**-**201**, flavonoids **202**-**205** and (*E*)-resveratrol **206** were quantified by various methods of analysis in extracts from apple varieties grown in Moldova and Romania. Data analysis allowed markers to be highlighted for the researched varieties and confirmed the high antioxidant value of the apple varieties compared to other studies.

5.4. Mineral composition of lavender *Lavanda angustifolia* Mill. and *Tanacetum corymbosum* (L.) Sch. Beep. plants.

Unlike terpenes, phenols or flavonoids, the mineral composition of aromatic, medicinal or volatil oil producing plants is rarely studied, which is a disadvantage when it comes to human health. For this reason, the phytochemical study continued with the analysis of the mineral composition of lavender and corymflower tansy plants by the neutron activation method (NAA) [50, 51].

36 Macro- and microelements were identified and quantified in lavender plants, of which 12 are either essential or beneficial for the human body at certain concentrations (Ca, Cl, Co, Cr, Fe, K, Mg, Mn, Na , Se, V, Zn), 6 are potentially toxic (Al, As, Ba, Rb, Sb, Sr), and the rest of the elements have no distinct biological functions [52]. Of the 36 elements determined in the organs of lavender plants, it was proven that 23 have the highest content in the leaves, listed in descending order: K>Al>Fe>Ti>Mn>Zr>Rb>Cr>Ce>La> Nd>Hf>Th>Co>As>Sc>Sm>Yb>Cs>U>Eu>Ta>Tb. 6 elements out of 36 predominate in roots, in the descending order Mg>Na>Zn>V>Ni>Sb and 5 elements in inflorescences K>Ca>SR> Ba>Br>W>Se.

In lavender stalks, the content of all elements is relatively low, except for Cl (2260 mg/kg), which is also present in inflorescences (2080 mg/kg). The content of Ba, Br, Ca, Sr in stalks and leaves was similar, while Zn is present in all parts of the plant (~31.0-96.0 mg/kg). The content of Tb in roots and stalks is comparable (~0.04 mg/kg).

Lavender plants can be characterized by the following "*chemical fingerprint*": As, Eu, Fe, La, Nd, Ta, Th, Sm, Tb, U, V, Yb, Zr. In 1999, the World Health Organization (WHO) established the maximum permissible level of As (1.0 mg/kg) in vegetalraw materials [53]. In our case, the content of As in leaves and inflorescences exceeds it (1.3 and 1.01 mg/kg). The content of Cr in all organs (4.95-12.0 mg/kg) exceeded the limit value of 2 mg/kg, which proves that lavender phytoaccumulates a variety of macro- and microelements even when grown on uncontaminated and unfertilized soils.

Unlike lavender, the mineral composition of *T. corymbosum* has not been studied. The concentrations of 21 macro- and microelements were determined in its plants. Since there are no data in the literature about the elemental composition of the fennel, the obtained results were compared with the composition of the species *T. cinerariifolium* (average value of the above-ground parts), the data obtained for the fam. *Asteraceae* and for reference plants (PR).

Elements such as Al, As, Br, Fe, Mn, Mo, Rb, SR and Zn were found to be present at low concentrations, and Co, Cs, La, Sb, Sm and Th at trace levels. Iron, Mn, Zn, Br and Mo are considered essential nutrients for the human body and become toxic only at high concentrations. The Fe content in *T. corymbosum* (85±8.5 μg/g) is higher than in *T. cinerariifolium* plants. The content of Mn in *T. corymbosum* (42±1.7 μg/g) is about 2.5 times lower than in *T. cinerariifolium*. The Zn content in *T. corymbosum* (42±1.7 μg/g) is lower than in *T.*

cinerariifolium. The content of Br (9.6±0.4 μg/g) determined in the bran is significantly lower than the tolerance limit [53].

In the analyzed plants, three rare earth elements (EPR), namely La, Sc, Sm were determined at concentrations of (~0.09, 0.03 and 0.01 μg/g). The content of As in T. corymbosum was lower than the tolerable limits (~0.7 μg/g) established by the WHO. The consumption of herbal tea significantly contributes to the intake and accumulation of metals in the human body. Elements in tea infusions can be classified into three groups: weakly, moderately and highly extractable. Ca, Co, Fe, La, Sb, Sm, Sc, SR, and Th are considered among the poorly extractable elements determined in the waste. Aluminum, As, Cr, Mg, Mn and Zn are considered moderately extractable elements. Cesium, K, Na and Rb are characterized by a high extraction capacity. Thus, corymbflower tansy can be considered as an important source of macro- and microelements.

In order to experimentally detect the "*fingerprint*" of the *T. corymbosum* species, the values obtained were compared with the normalized values of the "*reference plants*" PR, demonstrating that the values obtained for Al, As, Br, Cl, K, Mg, Mo and Sc have were higher compared to PR. According to the study [54], the higher content of the mentioned elements in the corymbflower tansy is explained by the mineral composition of the local soils, rich in dolomites, limestone, K-feldspars, etc. The content of other items was similar or lower than that in PR.

Thus, for the first time, using the analysis by the NAA method, the mineral composition of lavender plants in different organs (roots, stalks, leaves and inflorescences) and of corymbflower tansy plants was determined. The data obtained allowed the establishment of "*chemical fingerprints*" for each species, a comparative analysis with the "*reference plants* according to Market", and in the case of the corymbflower tansy with other species of the gen. *Tanacetum*.

6. BIOLOGICAL ACTIVITY OF SYNTHETIC COMPOUNDS, VEGETAL PRODUCTS AND THE STRUCTURE-ACTIVITY RELATIONSHIP

Currently, antibiotic resistance is becoming one of the main problems of modern medicine because it substantially reduces the effectiveness of antimicrobial treatments and is one of the main causes of increased patient mortality as a result of postoperative complications. One solution to this problem would be to develop new classes of antibiotics, optimizing or combining them with known bioactive compounds to provide effective remedies against multidrug-resistant strains.

In this Chapter are described the results biological activity assessments by approved, accessible and relevant methods of the antifungal and antibacterial activities of synthetic norlabdane compounds and terpene-heterocyclic hybrids obtained based on (-)-sclareol **1**, (+)-larixol **2** and larixyl acetate **95**, but also of volatile oils, secondary products, extracts obtained from local plant sources such as lavender and crymbflower tansy on the mentioned species of microorganisms. Molecular docking of the most active synthetic compounds on 4 target protein models was also performed.

6.1. Biological activity of synthetic norlabdane compounds

All the synthesized compounds were tested *in vitro* for their antifungal and antibacterial activity on pure cultures of fungi (*Aspergillus niger*, *A. flavus*, *Fusarium solani*, *Penicillium chrysogenum*, *P. frequentans*, *Alternaria alternata*) and Gram-negative bacteria (*Pseudomonas*

aeruginosa) and Gram-positive (*Bacillus polymyxa*). Out of the total number of synthesized compounds, 29 showed excellent to good or moderate antifungal and antibacterial activity.

In this Subchapter the bioactive compounds have been classified according to the carbon skeleton and the functional groups they possess. The first group includes synthetic unsaturated pentanorlabdane compounds **50**, **54**, **60** and **61** containing acetate groups, and in the case of compound **51** its combination with the peroxide group (Figure 6.1.).

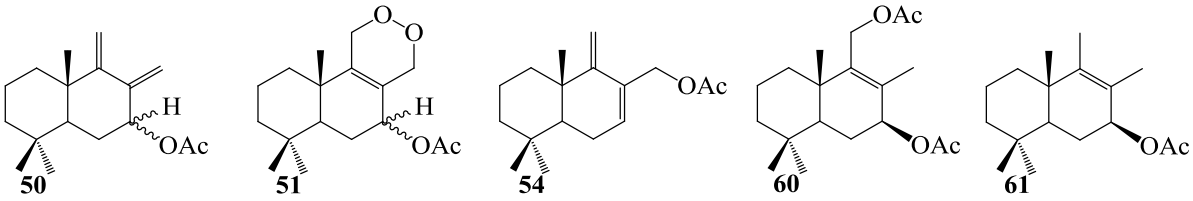


Fig. 6.1. Pentanorlabdane acetates with antimicrobial properties.

The MIC values of the compounds in the series, which range from 0.06 to 4.0 μg/mL, reveal a better antimicrobial effect of acetates **60** (MIC=3.0 μg/mL) and **61** (MIC=2.0 and 2.5 μg /mL), and for compound **50** an excellent one (MIC=0.06 and 0.125 μg/mL), compared to the reference compound (MIC=4.0 μg/mL). Acetate **51** exhibits selective antifungal activity (MIC=2.0 μg/mL), and the antimicrobial activity of acetates **51** and **54** is comparable to that of the standard. According to these data, compound **50** shows 66 times more antibacterial and 32 times more antifungal activity than the standard [8, 9].

The next group includes the unsaturated pentanorlabdane ketobromides **57**, **62** and **64**, 7-hydroxyeuryfuran **53** and the polyfunctional compound **132**. The most active compound in this group was found to be 7-hydroxyeuryfuran **53**, which exhibited excellent antimicrobial activity at MIC values of 0.9 and 1.0 μg/mL, respectively, i.e. it is ~4 times more active than the standard (MIC=4.0 μg/mL). The other compounds in the group show moderate antimicrobial activity compared to the reference compound. According to these tests, bromide **132** showed good antifungal activity (MIC of 0.85 μg/mL) compared to the reference compound Caspofungin (MIC= 0.42 μg/mL) and good antimicrobial activity (MIC=0.90 μg/mL) compared to the reference compound Kanamycin (MIC=0.50 μg/mL). It should be noted that the antifungal activity of bromide **132** is higher than that reported for cinnamosmolide **130**. Bromides **57**, **62** and **64** show moderate antibacterial activity at MIC values from 10 to 16 μg/mL (Figure 6.2.).

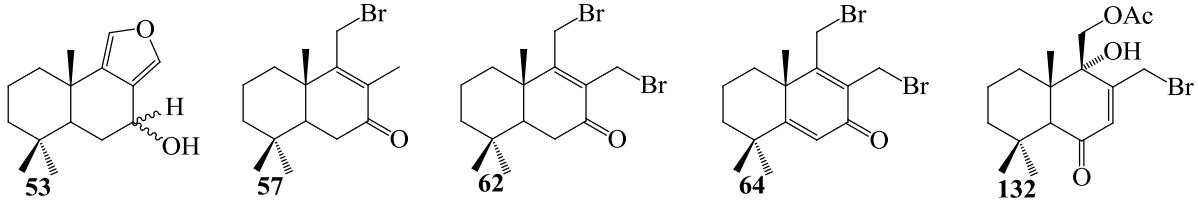


Fig. 6.2. Bromides and pentanorlabdane polyfunctional compounds with antimicrobial properties.

The synthetic bioactive compounds of the tetranorlabdane series **35**, **38**, **42d** and **71**/**72** are less numerous and include derivatives of ketoester **7**, which besides ester and carbonyl groups, also carry halogen, acetate or lactonic groups [13]. Tetranorlabdane bromide **38** shows good antifungal (MIC=1.8 μg/mL) and antibacterial (MIC=2.0 μg/mL) activity, and is followed by compounds **35** and **42d** which in this chapter show selective antifungal activity (MIC=3.5 μg/mL). The highest antifungal activity was shown by tetranorlabdane lactones **71**/**72** at

MIC=0.064 μg/mL, which exceeds it by ~62 times, and the antibacterial activity (MIC=2.0 μg/mL) by 2 times that of the reference compound (MIC=4.0 μg/mL) (Figure 6.3.).

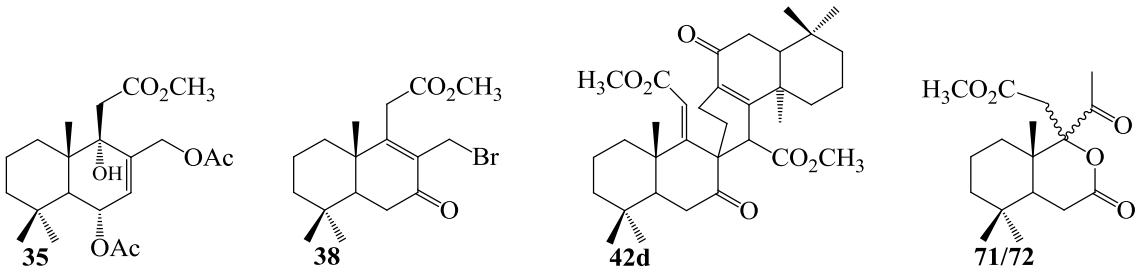


Fig. 6.3. Tetranorlabdane compounds with antimicrobial properties.

Next, the results of the evaluation of the antimicrobial activity of dinorlabdane compounds **149a,b**, trinorlabdane **159a,b** and tetranorlabdane **171** with unsubstituted and substituted thiosemicarbazone units, and hydrazinecarbothioamides **143c** and **143d** will be presented [32, 37]. Compound **159a,b** shows good antimicrobial and antibacterial activity (MIC=0.19 and 3.0 μg/mL), and compound **149a,b** comparable to the standard (MIC=0.25 and 4.0 μg/mL). The antifungal activity of trinorlabdane thiosemicarbazones **159a,b** has been patented [55]. Compound **171** exhibits selective antibacterial activity at MIC=0.125 μg/mL, which is 24 times higher compared to the standard (MIC=4.0 μg/mL). The highest antimicrobial activity was shown by compound **143c** at MIC=0.125 and 0.064 μg/mL, that is, 2 and 62 times higher than the standard (MIC=0.25 and 4.0 μg/mL) (Figure 6.4.).

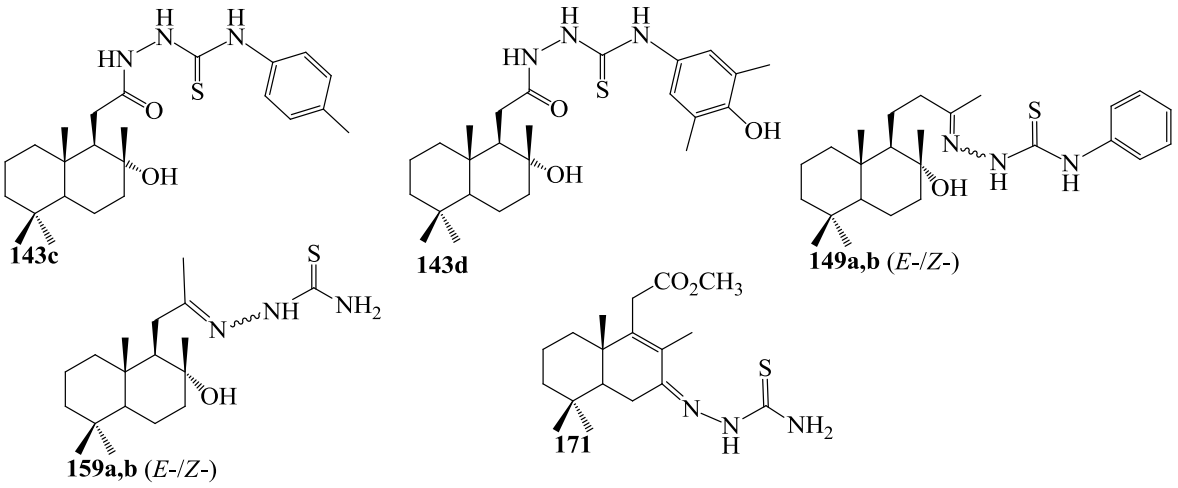


Fig. 6.4. Biologically active norlabdane compounds with thiosemicarbazone units.

The new molecular hybrids, synthesized on the basis of tetra- and pentanorlabdane intermediates, were also subjected to a preliminary screening of antifungal and antibacterial activity *in vitro*, using the same method applied to the series described above. Among them, we can mention the hybrids with diazine units **139** and **141** [10], and those with unsubstituted oxadiazole structural fragments **179** and substituted **186**, **187** and **191b** [39]. Diazine **141** exhibits antifungal activity at the MIC value= 5 x 10^{-3} μg/mL, being 50 times more active than the reference compound Caspofungin (0.25 μg/mL). Its antibacterial activity is manifested at MIC=3.2 x 10^{-2} μg/mL, being approximately 90 times higher than that of the reference compound Kanamycin (MIC=3.0 μg/mL). Compound **139**, which carries a Diazine unit at the C_{12} position, but does not possess a quinone moiety, exhibits moderate activity (MIC=15 x 10^{-1} μg/mL), but which is much lower than that of the reference compound Caspofungin (MIC=0.25

μg/mL) [10]. The antifungal and antibacterial properties of the disubstituted pyridazinone pentanorlabdane compound **141** have been patented [12]. Oxadiazole **186** shows selective antibacterial activity at MIC = 0.5 μg/mL, and the other compounds of this series **179**, **187** and **191b** show moderate antimicrobial activity at MIC from 0.75 to 11.0 μg/mL (Figures 6.5. and 6.6.).

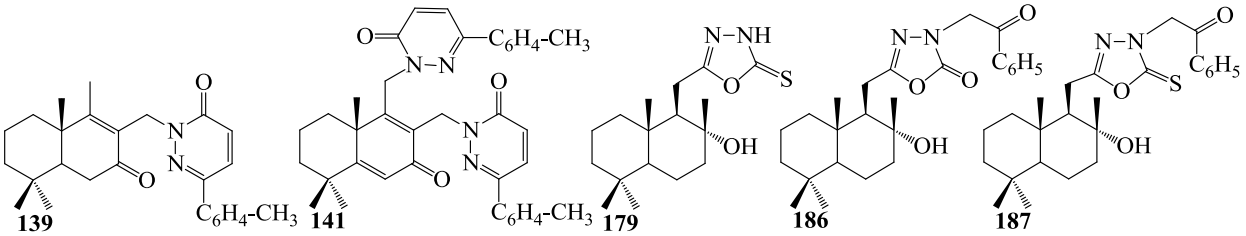


Fig. 6.5. Diazine and oxadiazole hybrids with antimicrobial properties.

Another series of molecular hybrids that have shown antimicrobial activity include triazole **144d**, thiadiazoles **180**, and **189a**. The values of the minimum inhibitory concentrations obtained show that compounds **144d** and **180** possess excellent antifungal and non-selective antibacterial activity at (MIC=0.094 and 0.047 μg/mL, respectively) and (MIC=0.032 and 0.25 μg/mL, respectively), i.e. of 2.6 and 64 times, and 7.8 and 32 times higher than the standards, respectively. The values of the minimum inhibitory concentration reveal the fact that compound **189a** possesses selective antibacterial activity at MIC=0.5 μg/mL, respectively, i.e. 6 times more pronounced than the reference compound Kanamycin. The antifungal and antibacterial properties of tetranorlabdane 1,3,4-thiadiazole **180** have been patented [56] (Figure 6.6.).

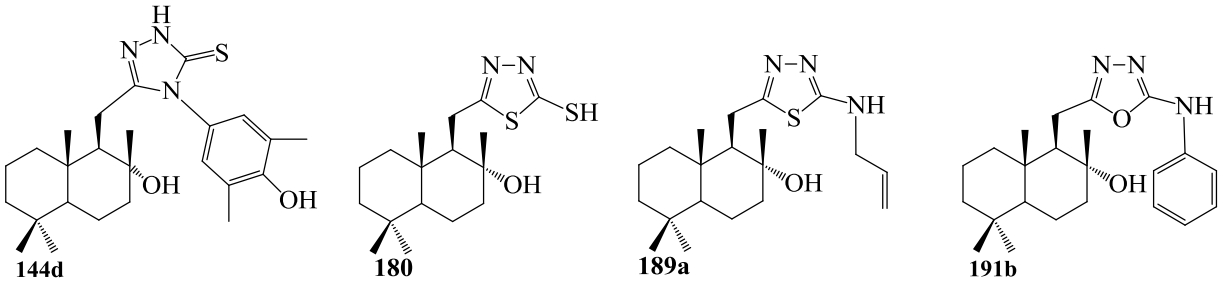


Fig. 6.6. Oxadiazole, thiadiazole and triazole hybrids with antimicrobial properties.

Thus, following *in vitro* microbiological testing of synthetic norlabdane compounds, including molecular hybrids, a series of compounds was selected that showed excellent to good antifungal and antibacterial activity. Also, two compounds showed moderate cytotoxic activity against three cancer cell lines.

6.2. Biological activity of vegetal products

Currently, from the natural sources used to obtain new antimicrobial remedies, special attention is paid to natural products, among which is lavender volatile oil, which exhibits anti-inflammatory, antioxidant, sedative, cytotoxic, analgesic, antimicrobial and anticonvulsant properties.

The therapeutic effects of *L. angustifolia* are mainly determined by mono- and sesquiterpenes, but also by other secondary metabolites such as oleanolic acid **192** and ursolic acid **193**, together with other pentacyclic triterpenes. There is, however, little information on the composition and antimicrobial activity of lavender floral water, plant extracts, and solid waste, mainly referring to lavender hydrosol, which is a synthetic product.

This Subchapter presents the data of *in vitro* antimicrobial activity testing of volatile oil samples, floral waters [41], extracts from solid waste and lavender plants, which were performed on the fungal species and bacterial strains mentioned below, using previously reported methods [32, 57].

It should be noted that the literature lacks information about any antimicrobial effects of volatile lavender oil on the species *Erwinia carotovora*, *E. amylovora* and *Candida utilis*. Volatile lavender oil of Moldovan origin, which composition is described in Chapter V, showed good antibacterial activity, both against non-pathogenic Gram-positive/Gram-negative bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) at MIC=300 μg/mL, and and good antifungal activity against phytopathogenic bacteria (*Xanthomonas campestris*, *E. amylovora*, *E. carotovora*) and *C. utilis* fungi at MIC=150–300 μg /mL. Its activity is lower compared to coriander volatile oil, but comparable to that of Clary sage and lavender, and higher than that of dill volatile oil [41].

The most pronounced antifungal and antibacterial activity was observed for floral water (MIC=0.08 and 0.125 μg/mL). Good antifungal and antibacterial activities were also found for solid waste extracts (MIC=0.50 and 4.0 μg/mL), and extracts from lavender plants showed moderate antifungal and antibacterial activity (MIC=0.75 and 6.0 μg/mL).

Due to the properties mentioned above, *Tanacetum* species are also used in the production of cosmetics, insecticides, conditioners, dyes, food preservatives, odorants and other herbal remedies. The *in vitro* evaluation of the ethanolic extract of dried corymbwlower tansy plants of Moldovan origin revealed a high antibacterial activity, both against non-pathogenic Gram-positive/Gram-negative bacteria (*B. subtilis* and *P. fluorescens*) and against phytopathogenic ones (*X. campestris*, *E. amylovora*, *E. carotovora*) in the range of MIC=300-600 μg/mL. Its antifungal properties against strains of *Candida utilis* and *Saccharomyces cerevisiae* are in the range of MIC=150-300 μg/mL.

Thus, *in vitro* microbiological testing confirmed the high antimicrobial activity of lavender floral water, the good activity of the volatile oil from local lavender and the moderate activity of extracts from lavender dried waste, lavender and corymbflower tansy plants.

6.3. Structure-biological activity relationship of norlabdane derivatives

In this Chapter, the relationship between the structure and the antimicrobial activity of the 8 synthetic compounds that showed excellent activity against microorganisms, compared to other tested substances, will be analyzed. This analysis will be based on the original studies in which these data were published. According to the MIC values obtained, compounds **44** and **49** are inactive. By comparison with compounds **50**, **51**, **53**, **54**, **60** and **61**, it can be deduced that both the furanic and endoperoxidic fragments do not determine this type of biological activity [8, 9]. In this case, the activity of the tested compounds **44**, **50**, **51**, **54**, **60** and **61**, is strongly influenced by the functional groups linked to the C_7 atom, in the case of compound **53** by the hydroxyl group (Figures 6.7 and 6.8).

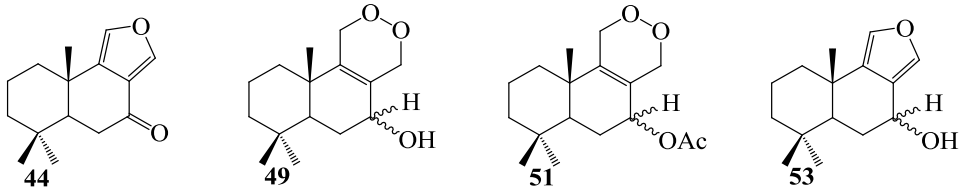


Fig. 6.7. Inactive pentanorlabdane compounds **44** and **49** versus active **51** and **53**.

The presence of hydrogen bond donor C_7 hydroxyl groups or hydrogen bond acceptor C_7 acetate groups is not very selective. In contrast, the presence of a carbonyl group at the C_7

position has a strong negative effect on antimicrobial activity. In fact, sp^2 hybridization at C_7 flattens ring B as shown in the X-ray projection of compound **44** (Figure 2.16).

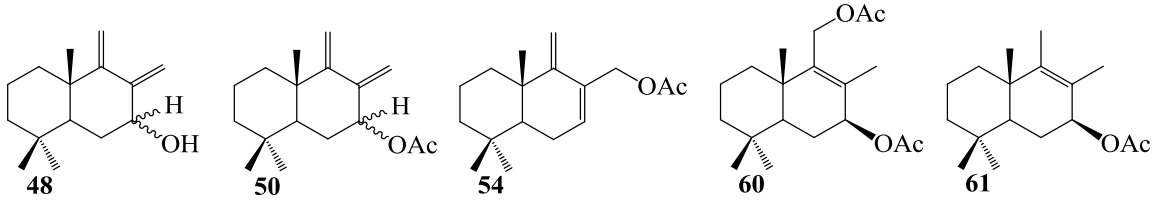


Fig. 6.8. Amplification of antimicrobial activity in the pentanorlabdane acetate series.

Compound **48** did not show any effect on the tested fungi and bacteria, since neither the diene fragment nor the hydroxy group contribute to the development of such biological activity. Compound **54**, besides its synthetic importance showed good antifungal and antimicrobial activity at MIC=4.0 μg/mL. In contrast, the presence of the C_7-OAc group improves the bioactivity of compound **60** at MIC=3.0 μg/mL. This fact is also confirmed by the MIC values of 2.0 and 4.0 μg/mL determined for compound **51**, and especially of compound **61** unsubstituted in the C_{11} position, which showed better biological activity at MIC values of 2.0 μg/mL for the species of fungi and 2.5 μg/mL for bacteria. According to the data obtained, it can be deduced that the simultaneous presence of a drimane skeleton oxidized at C_7 and a chair conformation of the B ring gives compound **50** the most pronounced potential biological activity (MIC=0.06 and 0.125 μg/mL) (Figure 6.8.).

Drimenone **8**, which contains an enonic moiety in the molecule, did not show biological activity as a result of its suppression by the C_7-carbonyl group. This assumption is supported by the MIC values of sesquiterpene bromides **57**, **62** and **64**, which possess both moderate antifungal and antibacterial activities (Figure 6.9.).

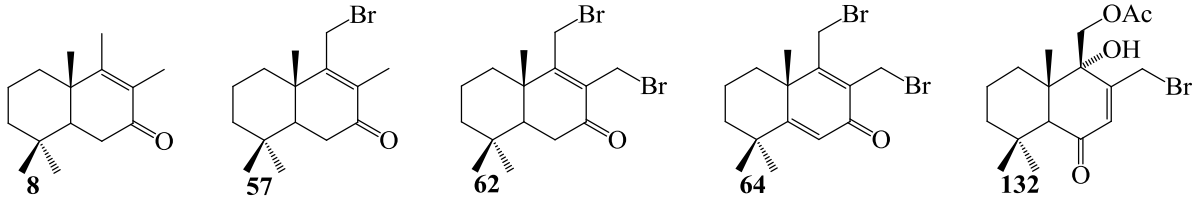


Fig. 6.9. Enhancement of the antimicrobial activity of brominated derivatives of drimenone **8**.

Additional substitution at the C_{11} position makes compound **57** active (MIC=15.0 and 16.0 μg/mL), and double substitution at C_{11} and C_{12} increases the activity of compound **62** to MIC=12.0 and 12.5 μg/mL. The complete functionalization of ring B in the molecule of compound **64**, which means two bromine atoms linked by a α,α'-dienone system, gives the best activity in this series (MIC=10.0 and 11.0 μg/mL). Compound **132** derives from (+)-larixol **2** and has certain similarities with acetates **54** and **60**, and bromide **64**, but differs from bromides **57**, **62** and **64** by locating the carbonyl group at the C_6 position, and this fact determines its high activity at MIC= 0.85 and 0.90 μg/mL values (Figure 6.9.).

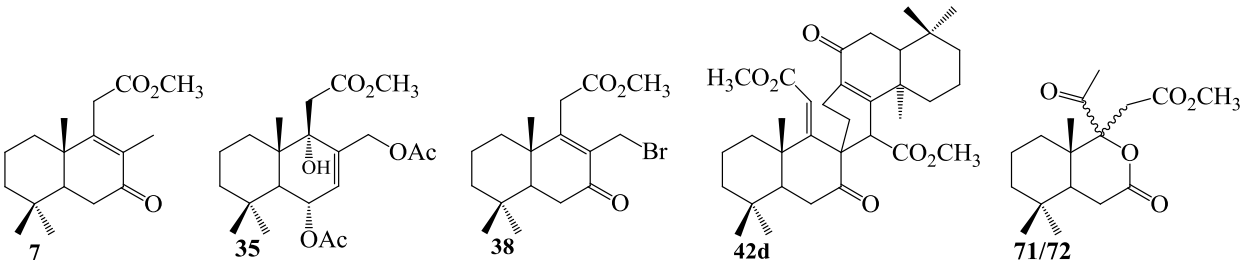
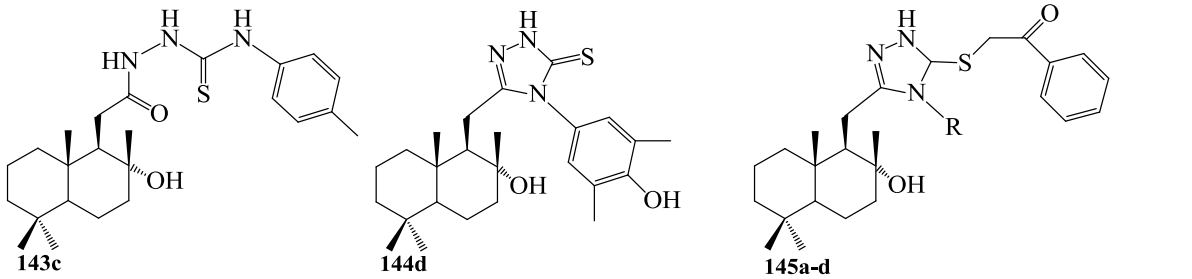


Fig. 6.10. Amplification of antimicrobial activity in the series of ketoester **7** derivatives.

According to the antimicrobial activity evaluation data, tetranorlabdane lactones **71** and **72** showed antifungal activity higher than the reference compound Caspofungin at MIC=6.4•10^{-2} μg/mL and antibacterial activity 2 times higher than that of the compound reference Kanamycin at MIC=2.0 μg/mL. The activity of compounds **71** and **72** is due to the coexistence of lactonic, >C=O and ester groups (Figure 6.10.).

Most likely, the improved antifungal and antibacterial activities of compounds **143c** and **144d** are determined by the presence of amide, thioamide and thiocarbonyl groups. Consequently, the *S*-substituted homodrimanes **145a-d** are inactive, thus confirming the important contribution of the free thiocarbonyl group in the biological activity of the investigated compounds (Figure 6.11.).



a R= -$CH_2CH=CH_2$; **b** R= Ph;
c R=4-Me-C_6H_4; **d** R= 3,5-Me_2-4-HOC_6H_2

Fig. 6.11. Active **143c**, **144d** and inactive **145a-d** sulfur containing tetranorlabdane derivatives.

Remarkably, compound **141**, which contains a combined skeleton of one analog-quinone unit and two diazine units, has extremely high antibacterial and antifungal activity (MIC=0.005 and 0.032 μg/mL). The above data suggest that the presence of the quinone unit in the skeleton and the substitution of the bromine atom in the molecule of compound **139** by the Diazine unit, as in compound **141**, favors the antibacterial and antifungal activity. These data also suggest that the position of a diazine unit in the drimane backbone is crucial, particularly at the C_{12} position, since compound **139** is still active without the C_{11} diazine unit (Figure 6.12.).

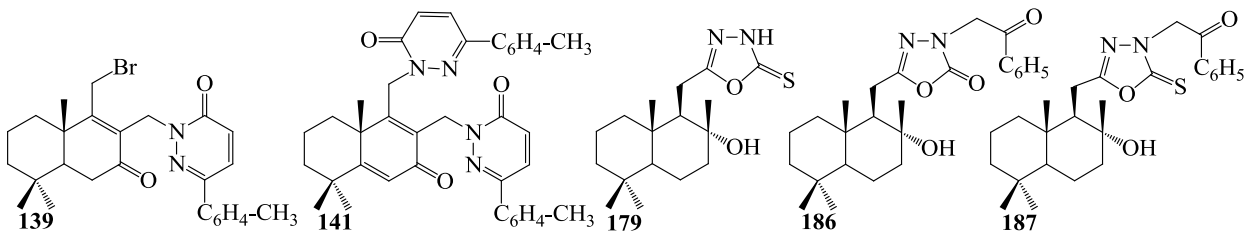


Fig. 6.12. Biologically active terpene-azaheterocyclic **139**, **141**, 1,3,4-oxadiazole **179**, **186** and **187** hybrid compounds.

Compounds **180** and **189a**, which possess the 1,3,4-thiadiazole moiety in the molecule, are more active compared to compounds **179**, **186**, **187** and **191b**, which possess a 1,3,4-

oxadiazole moiety. From the series of homodrimane sesquiterpenoids with 1,3,4-thiadiazole units reported, compound **180** possessing a mercapto (–SH) group at the position 2-, is much more active compared to the others. The presence of the triazole fragment in molecule **144d** makes this compound exhibit an activity comparable to that of thiadiazole **180** (Figure 6.13.).

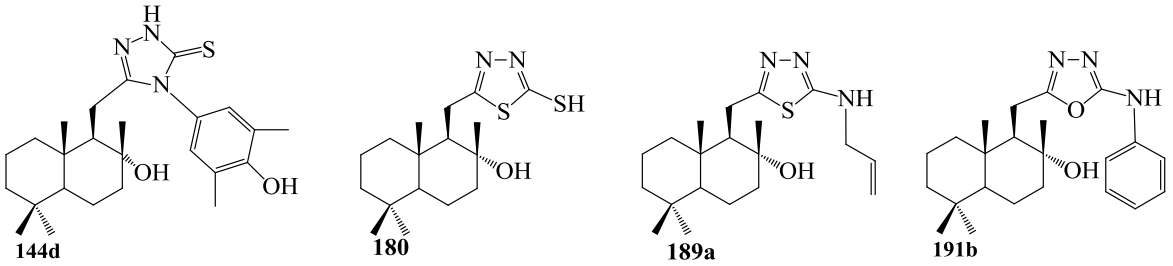


Fig. 6.13. Triazole **144d**, thiadiazole **180** and **189a**, and oxadiazole **191b** hybrid compounds.

With reference to the pronounced antimicrobial activity of the ethanolic extract of *T. corymbosum* plants, this can be explained by the significant content of flavonoids and especially sesquiterpene lactones.

In order to explain the mechanisms of inhibition of microorganisms through the ligand-receptor intermolecular interaction that define the antimicrobial activity, a comparative molecular docking study of the 8 synthetic compounds with pronounced activity on 4 protein models was carried out: DNA gyrase from *Escherichia coli* (1KZN), Fabz from *P. aeruginosa* (1U1Z), dihydrofolate reductase from *C. albicans* (3QLS) and MurB from *E. coli* (2Q85).

The binding energies of the researched compounds (Table 6.1) are similar to those of the standards used, and in many cases even exceed them, demonstrating the fact that they bind efficiently with the target enzymes contributing to their inhibition. The results of computational calculations demonstrate the fact that most of the compounds have a higher binding affinity to 1U1Z, suggesting the idea that the interruption of fatty acid biosynthesis would be the most likely way of action of the investigated compounds on pathogens. Compound **141** and Caspofungin showed the highest binding affinity to the 3QLS structure, while **53** and **180** bound most strongly to the 2Q85 structure. Kanamycin demonstrated similar binding energy values to the 3QLS and 2Q85 structures, namely -8.0 and -8.1 kcal/mol, respectively.

Tabel 6.1. Binding energy of investigated compounds to target enzymes and experimental MIC values (μg/mL).

Studied compounds	Calculated binding energy (kcal/mol)				
	E. coli ADN gyrase, 1KZN	*P. aeruginosa* FabZ, 1U1Z	*C. albicans* dihydrofolate reductase, 3QLS	*E. coli* MurB, 2Q85	MIC (μg/mL) – according to the tables above
50	-6.8	-7.7	-6.9	-6.7	0.06-0.125
53	-7.5	-7.7	-7.3	-8.1	0.9-1.0
71	-6.6	-7.7	-6.7	-7.1	0.064-2.0
72	-6.2	-7.7	-6.4	-6.4	0.064-2.0
141	-8.9	-8.8	-10.2	-8.4	0.005-0.032
143c	-7.6	-9.7	-8.7	-7.4	0.064-0.125
144d	-8.0	-10.0	-8.5	-7.7	0.047-0.094
180	-6.1	-6.3	-6.9	-7.3	0.032-0.094
Caspofungin	-6.1	-7.3	-7.7	-6.8	0.2-4.0
Kanamycin	-6.8	-8.0	-7.6	-8.1	

For *E. coli* DNA gyrase (1KZN), the activity sequence of the investigated compounds according to the binding energy with the bacterial structure is as follows: **141** > **144d** > **143c** ≈ **53** > **50** = Kanamycin > **71** > **72** ≈ **180** = Caspofungin. The detailed visualization of the obtained results shows that all the investigated compounds occupy the protein pocket in the same way as the standards used, Caspofungin and Kanamycin (Figure 6.14.).

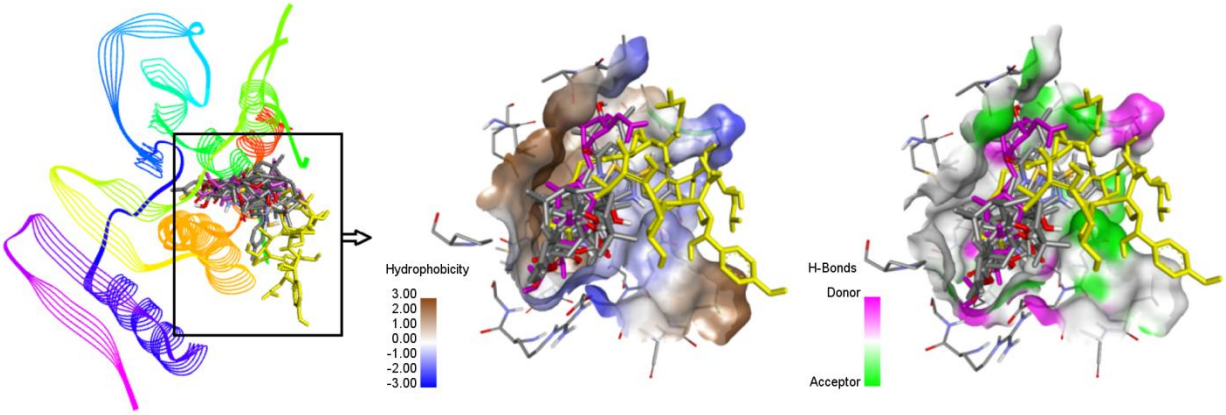


Fig. 6.14. The positions of the highest scoring conformations of all investigated compounds in the protein pocket of 1KZN (Caspofungin - yellow, Kanamycin - violet).

For *P. aeruginosa* FabZ (1U1Z), the activity sequence of the investigated compounds according to the binding energy with the structure of the bacterium is as follows: **144d** > **143c** > **141** > Kanamycin > **53** = **50** = **71**/**72** > Caspofungin > **180**. The detailed view of the obtained results shows, that most of the investigated compounds occupy the protein pocket in the same way as Kanamycin (Figure 6.15). Compound **141** is placed in the same pocket as Caspofungin, and compound **180** binds close to Caspofungin, but in a different pocket, the two locations having different physicochemical properties (Figure 6.15).

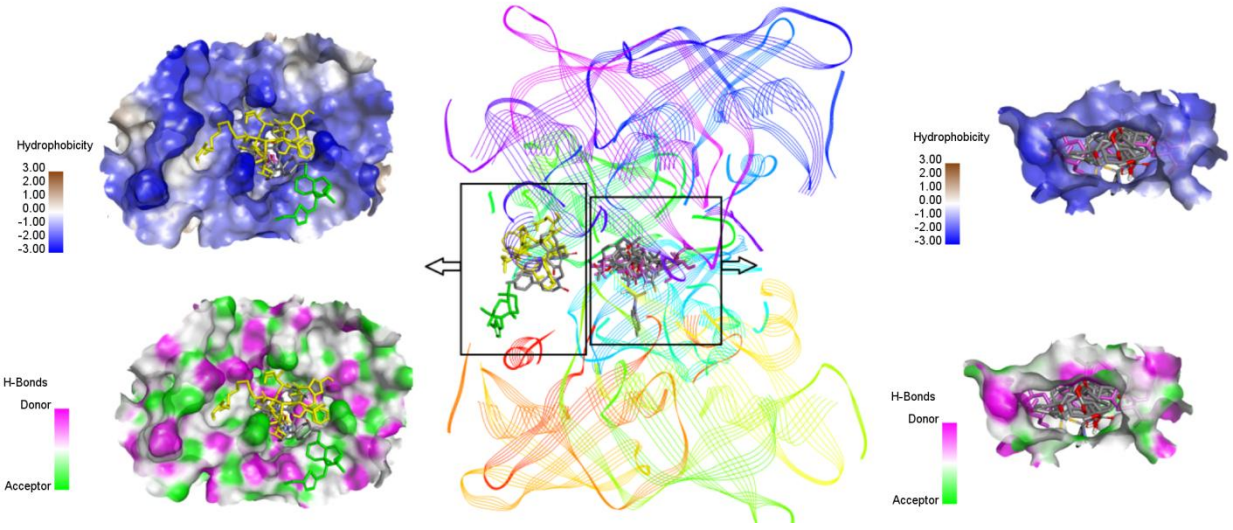


Fig. 6.15. The positions of the highest scoring conformations of all investigated compounds in the protein pocket of 1U1Z (Caspofungin - yellow, Kanamycin - violet, **141** - gray, **180** - green).

For *C. albicans* dihydrofolate reductase (3QLS), the activity sequence of the investigated compounds according to the binding energy with the bacterial structure is as follows: **71** > Kanamycin > **72** > **143c** ≈ **144d** > **180** > Caspofungin = **141** > **53** > **50**. The detailed view of the obtained results shows, that structures **71** and **72** occupy the protein pocket in the same way as

Caspofungin, structures **53**, **50** and **144d** bind in the Kanamycin binding pocket, and structures **141**, **143c** and **180** occupy another protein pocket (Figure 6.16).

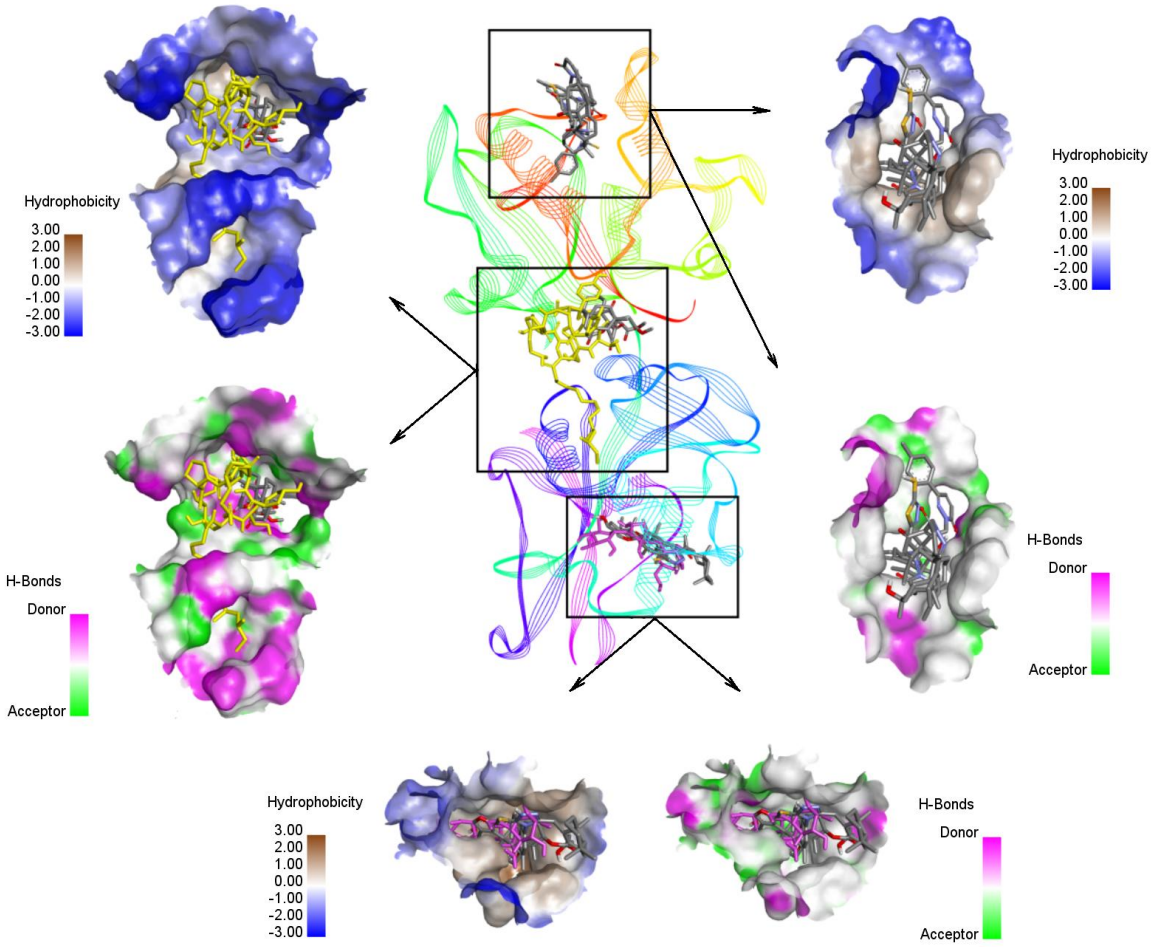


Fig. 6.16. The positions of the highest scoring conformations of all investigated compounds in the protein pocket of 3QLS (Caspofungin - yellow, Kanamycin - violet).

For *E. coli* MurB (2Q85), the activity sequence of the investigated compounds according to the binding energy with the structure of the bacterium is as follows: **141** > **53** = Kanamycin > **144d** > **143c** ≈ **180** > **71** > Caspofungin ≈ **50** > **72**. The detailed view of the obtained results shows, that all the structures of the synthesized compounds except **53** fit into the protein pocket in the same way as Caspofungin, and Kanamycin and compound **53** occupy two distinct pockets (Figure 6.17).

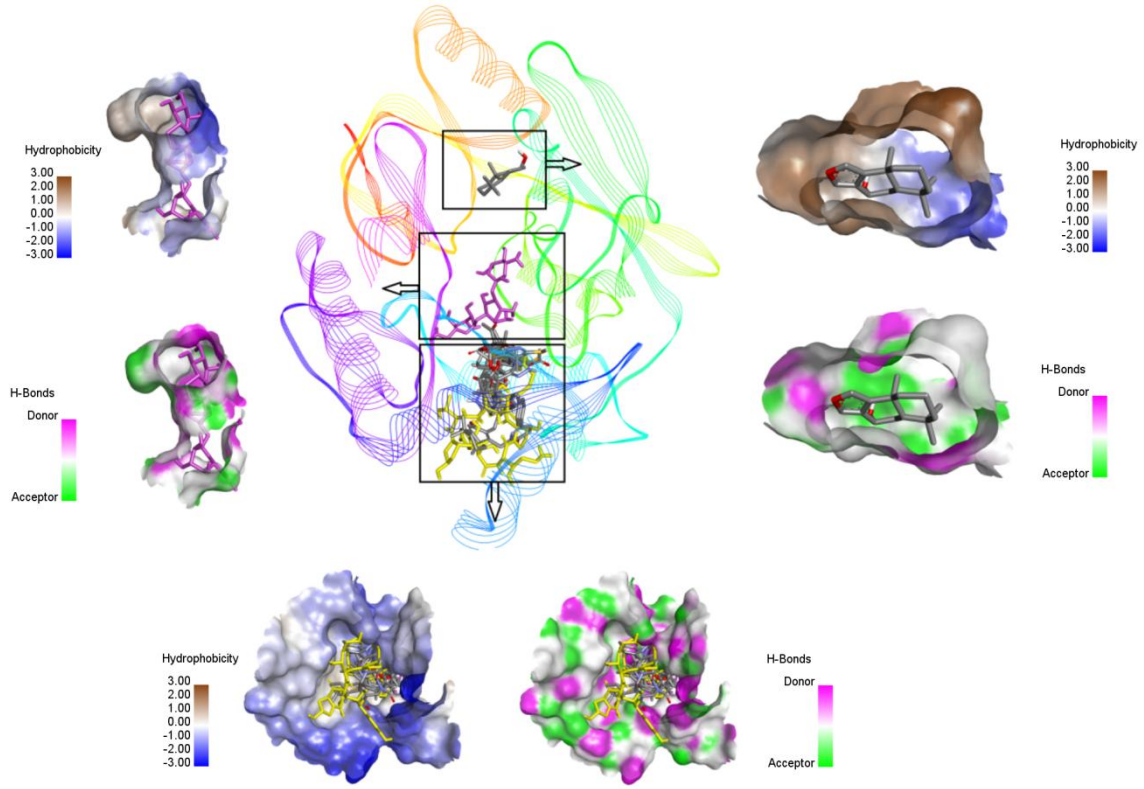


Fig. 6.17. The positions of the highest scoring conformations of all investigated compounds in the protein pocket of 2Q85 (Caspofungin - yellow, Kanamycin - violet, **53** - gray).

The interactions take place at the protein-ligand interface, and the obtained results demonstrate the formation of hydrogen bonds and hydrophobic interactions that stabilize the ligands in the substrate binding sites.

Thus, the results of the computational simulations correlate very well with the experimental ones, the three most active compounds being correctly identified, namely **141**, **144d** and **143c**. At the same time, such simulations provide a more comprehensive insight into how compounds bind to target proteins and act against pathogenic microorganisms, making it possible in the future to identify potential antimicrobial compounds prior to their synthesis by testing various molecular structures and optimizing compound properties in a faster and more efficient way. This highly valuable approach may lead to the development of more effective and safer compounds with antimicrobial activity.

GENERAL CONCLUSIONS AND RECOMMENDATION

The main concept of the present thesis refers to the development of new chemo-, regio- and stereoselective synthesis methods, based on (-)-sclareol and (+)-larixol, of norlabdane compounds with an advanced degree of functionalization, analogues of natural compounds and terpene-heterocyclic molecular hybrids, by combining classical synthesis methods with non-conventional electrochemical (anodic oxidation), photochemical (photolytic degradation, sensitized photooxidation, photooxidative dehydrogenation) and electromagnetic (microwave irradiation) methods, which proved to be an effective tool for transformation of terpene substrates according to modern green chemistry approaches. Thus, the results obtained can be summarized by the following general conclusions:

1. Making use of the *non-conventional methods* mentioned in the preamble, the conditions of the key Stoll-Hinder reactions in the chemistry of (-)-sclareol were *considerably streamlined*, many

short and efficient syntheses of the target compounds, of the molecular hybrids in terms of reaction times – total yields were achieved and *established the optimal conditions* of the reactions to obtain some important synthesis intermediates;

2. For the first time, *compounds with unusual structures* were obtained, such as tetranorlabdane dimers, a sesquiterpenoid with an aromatic B ring and a skeleton analogous to the natural bicyclic diterpenoids fregenedadiol and isofregenedadiol, tetra- and pentanorlabdane lactones and lactams with a regrouped skeleton;

3. *Effective syntheses of analogues of natural and biologically active compounds*, such as (-)-7-ketoeuryfuran, (-)-albrassitriol, (-)-6-*epi*-albrassitriol, (+)-6-ketoeuryfuran, pereniporin B , cinnamosmolide, (+)-fragrolide, (+)-crotonadiol, and *for the first time* (+)-6-ketowinterin, derivatives of (+)-larixol with an advanced degree of functionalization in the B ring and the (*Z*)-isomer of crotonadiol were perfomed;

4. Following various strategies, for the first time, based on (-)-sclareol, series of terpene-heterocyclic molecular hybrids were synthesized: 6 *compounds with azaheterocyclic units* obtained by the coupling reaction of some tetra- and pentanorlabdane bromides with 6-(*p*-tolyl)-3(2*H*)-pyridazinone; 4 *compounds with 1,2,4-triazole units* obtained by the heterocyclization reactions of the corresponding tetranorlabdane hydrazinecarbothioamides; 12 *compounds with 1,3-thiazole units* obtained by the heterocyclization reactions of the corresponding di-, tri, tetra-, pentanorlabdane thiosemicarbazones; 5 *compounds with 1,3,4-oxadiazole* units obtained by coupling reactions of the intermediate tetranorlabdane hydrazide with 1,1'-carbonyldiimidazole (CDI) or with cyanogen bromide (CNBr), or by heterocyclization of intermediate hydrazinecarbothioamides in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC); 5 *compounds with 1,3,4-oxadiazole or 1,3,4-thiadiazole units* by coupling reactions of tetranorlabdane hydrazide with substituted isothiocyanates (R-NCS), or with variable amounts of tetramethylthiuram disulfide (TMTD), with subsequent alkylation which led to 3 *new N- and S-substituted derivatives*;

5. The probable mechanisms of the reactions were also proposed: *anodic oxidation* of the mixture of bicyclohomofarnesenic esters; *formation of tetranorlabdane dimers* with unusual structure; *formation of lactones and lactams with a regrouped skeleton*, including pentanorlabdane thiolactam; and *conversion of 1,3,4-oxadiazole fragments into 1,3,4-thiadiazole* under the action of tetramethylthiuram disulfide (TMTD);

For the first time, a phytochemical study was carried out that allowed:

6. *Comparative quantification* of the terpene components in the samples of volatile oil of lavender *L. angustifolia* Mill., obtained from 7 producers from different geographical areas of the Republic of Moldova and the samples of volatile oil obtained from fresh and dried plants of corymflower tansy *T. corymbosum* (L .) Shi. Bip., which made it possible to highlight the relationship between the chemical composition and the production conditions of the lavender oil, and the processing conditions of the corymflower tansy plants. It was established that the volatile oil of corymflower tansy of Moldovan origin belongs to the *chemotype germacrene D*;

7. *Quantification of triterpene oleanolic and ursolic acids* from the composition of apple fruits from 12 cultivars grown in different geographical areas of Moldova and Romania, in lavender plants and its dried waste, in species of local medicinal plants and wild fruits, a fact that confirmed, the relationship between content - area of origin and variety, and that apples from this area represent an important source of bioactive compounds, as well as medicinal plants and wild fruits used in food and therapeutic treatments; The use of lavender plants and its waste, in which

the content of oleanolic and ursolic acids is respectively 5% and 1%, as raw materials to obtain them, was argued;

8. *Quantification of phenolic acids, flavonoids* and *(E)-resveratrol* in the mentioned apple varieties, finding significant variations in the content of bioactive components from variety to variety, and the fact that apple varieties from this area represent an important source of antioxidants;

9. *Determination of the mineral composition* of parts of lavender plants (roots, stalks, leaves and inflorescences) and of corymbflower tansy plants, establishing the "*chemical fingerprint*" for both species. The obtained data allowed the calculation of the coefficients of transfer and accumulation of chemical elements from the soil in the organs of the lavender plants and the fact that the mineral composition of the corymbflower tansy plants corresponds to the requirements set for medicinal plants, and the results obtained generally correspond, with some exceptions , the norms accepted by the World Health Organization (WHO) and confirms the specific mineral composition of the soils of the Republic of Moldova.

10. New synthetic compounds, samples of volatile lavender oil, floral waters, ethanolic extracts from lavender waste, from lavender and calendula plants were tested *in vitro* on 6 strains of fungi and 7 species of non-pathogenic and phytopathogenic bacteria, establishing that 29 *compounds and extracts possess good to moderate antifungal and antibacterial activity*, and 8 *compounds and floral waters excellent activity* compared to reference compounds. Additionally, 2 *hybrid compounds* were tested on the ovarian cancer cell lines A2780 and A2780cis and the non-cancerous human embryonic kidney cell line HEK293, showing *moderate cytotoxic activity* at millimolar concentrations. Based on the obtained data, hypotheses *were issued regarding the structure-activity relationship* for the most active compounds, which were *confirmed by molecular docking* on four protein targets. The antimicrobial properties of some synthetic compounds *have been protected by 3 patents*.

Based on the general conclusions, the following recommendations can be made:

1. *Carrying out preclinical and clinical testing* of norlabdane compounds, including molecular hybrids with patented high antimicrobial activity, in the perspective of their use in the pharmaceutical industry as antifungal and antibacterial agents;
2. *Valorization* of apples, wild fruits, volatile oil producing, aromatic, medicinal plants and their wastes in order to obtain products with increased biological potential such as terpenes, phenolic acids and flavonoids;
3. *Cultivation* on large areas of the varieties of lavender Moldoveneasca 4 and Chisiniovskaia 90, the volatile oil of which corresponds to the International Standard and of the local corymbflower tansy, which has a specific chemical composition and belongs to the Germacren D chemotype.

SELECTED BIBLIOGRAPHIE

1. FRIJA, L., FRADE, R., AFONSO, C. Isolation, chemical and biotransformation routes of labdane-type diterpenes. In: *Chemical Reviews*. 2011, nr. 111, 8, pp. 4418-4452.
2. KOLTSA, M., MIRONOV, G., MALINOVSKII, S., VLAD, P. Synthesis of drim-8(9)-en-7-one, drima-5,8(9)-dien-7-one, and their 11,12-dibromo derivatives from norambreinolide. In: *Russian Chemical Bulletin*. 1996, nr. 45, 1, pp. 208–214.
3. VLAD, P., ARYKU, A., CHOKYRLAN, A. Synthesis of (+)-drim-9(11)-en-8α-ol from sclareol. In: *Russian Chemical Bulletin*. 2004, nr. 53, 2, pp. 443-446.
4. VLAD P., COLTSA, M., ARICU, A., CIOCARLAN, A., GORINCIOI, E., EDU, C., DELEANU, C. Photooxidative dehydrogenation of Δ^8-drimen- and Δ^8-11-homodrimen-7-ones into α,α'-dienones. In: *Russian Chemical Bulletin*. 2006, nr. 55, 4, pp. 703-707.

5. VLAD, P., CIOCARLAN, A., MIRONOV, G., COLTSA, M., SIMONOV, YU., KRAVTSOV, V., LIPKOWSKI, J. Synthesis of 7*α*- and 17-bromonorambreinolides from norambreinolide. In: *Chemistry Journal of Moldova*. 2007, nr. 2, 1, pp. 114-118.
6. VLAD P., CIOCARLAN, A., COLTSA, M., BARANOVSKY, A., KHRIPACH, N. Synthesis of drim-7,9(11)-diene and its hydroxylated derivatives. In: *Synthetic Communications*. 2008, nr. 38, 22, pp. 3960-3972.
7. VLAD, P., EDU, K., KOLTSA, M., **CIOCARLAN**, **A**., NIKOLESCU, A., DELYANU, K. Enantioselective synthesis of 11-homodrim-7-en-9*α*,12,13-triol. In: *Chemistry of Natural Compounds*. 2011, nr. 47, 4, pp. 574-578.
8. VLAD, P., **CIOCARLAN**, **A**., EDU, C., ARICU, A., BIRIIAC, A., COLTSA, M., D'AMBROSIO, M., DELEANU, C., NICOLESCU, A., SHOVA, S., VORNICU, N., DE GROOT, A. Regio- and stereoselective synthesis of (+)-6-ketoeuryfuran, (+)-6-ketowinterin, and (-)-7-ketoeuryfuran from accessible labdane diterpenoids (+)-larixol and (-)-sclareol. In: *Tetrahedron*. 2013, nr. 69, 2, pp. 918-926.
9. **CIOCARLAN**, **A**., EDU, C., BIRIIAC, A., LUNGU, L., ARICU, A., D'AMBROSIO, M., SHOVA, S., NICOLESCU, A., DELEANU, C., VORNICU, N. Synthesis of polyfunctional drimanes from drim-7,9(11)-diene and drim-8-en-7-one. In: *Synthetic Communications*. 2013, nr. 43, 22, pp. 3020–3033.
10. **CIOCARLAN**, **A**., LUNGU, L., BLAJA, S., DRAGALIN, I., ARICU, A. The use of some non-conventional methods in chemistry of bicyclohomofarnesenic methyl esters. In: *Chemistry Journal of Moldova*. 2020, nr. 15, 2, pp. 69-77.
11. DUCA, Gh., ARICU, A., LUNGU, L., TENU, N., CIOCARLAN, A., GUTU, Y., DRAGALIN, I., BARBA, A. Synthesis of new homodrimane sesquiterpenoids containing diazine, 1,2,4-triazole and carbazole rings. In: *Chemistry Journal of Moldova*. 2018, nr. 13, 1, pp. 69–73.
12. ARÎCU, A., MANGALAGIU, I., **CIOCARLAN**, **A.**, LUNGU, L., ZBÂNCIOC, Gh., VORNICU, N. *11,12-Bis-p-tolil-piridazonil-drim-5(6),8(9)-dien-7-onă care manifestă proprietăţi antifungice şi antibacteriene.* MD Patent 2015, No 4370
13. **CIOCARLAN**, **A**., ARICU, A., LUNGU, L., EDU, C., BARBA, A., SHOVA, S., MANGALAGIU, I.I., D'AMBROSIO, M., NICOLESCU, A., DELEANU, C., VORNICU, N. Synthesis of novel tetranorlabdane derivatives with unprecedented carbon skeleton. In: *Synlett*. 2017, nr. 28, 5, pp. 565–571.
14. CLENNAN, E., PACE, A. Advances in singlet oxygen chemistry. In: *Tetrahedron.* 2005, nr. 61, pp. 6665−6691.
15. MIRONOV, G., COLȚA, M., VLAD, P., **CIOCÂRLAN**, **A**. *Electrochemical process for obtaining 7β,11-diacetoxy-drim-8-ene*. MD Patent 2009, No 3967.
16. **CIOCARLAN**, **A**., LUNGU, L., BLAJA, S., SHOVA, S., VORNICU, N., BOLOCAN, N. ARICU, A. Synthesis of norlabdane compounds with rearranged cycle B and molecular docking studies. In: *Acta Chimica Slovenica* (in press).
17. **CIOCARLAN**, **A**., ARICU, A., BIRIIAC, A., LUNGU, L., NICOLESCU, A., DELEANU, C., VORNICU, N. New antifungal and antibacterial nitrogen containing terpenes. In: Book of Abstracts of the International Conference dedicated to the 55th anniversary from the foundation of the Institute of Chemistry of the Academy of Sciences of Moldova, May 28-30, 2014, Chisinau (Moldova), p. 196.
18. CIOCARLAN, A., LUNGU, L., BLAJA, S., SHOVA, S., ARICU, A. Synthesis of cycle B functionalized derivatives of (+)-larixol. 2024, In: *Chemistry Journal of Moldova*. (in press).
19. VLAD, P., **CHOKYRLAN, A.**, D'AMBROSIO, M., KOLTSA, M., BARBA, A., EDU, K., BYRYYAK, A., NIKOLESCU, A., MARI, A., DELEANU, K. Synthesis of *ent*-crotonadiol and compounds related to it from (+)-larixol. In: *Chemistry of Natural Compounds*. 2011, nr. 47, 3, pp. 397-403.
20. LAGNEL, B., MORIN, C., DE GROOT, A. Synthesis of drimanes from (+)-larixol. In: *Synthesis*. 2000, nr. 13, pp. 1907-1916.

21. VLAD, P., **CIOCARLAN**, **A**., COLTSA, M., EDU, C., BIRIIAC, A., BARBA, A., DELEANU, C., NICOLESCU, A., D'AMBROSIO, M., DE GROOT, A. Synthesis of (-)-albrassitriol and (-)-6-*epi*-albrassitriol from (+)-larixol. In: *Natural Product Research*. 2013, nr. 27, 9, pp. 809-817.
22. VLAD, P., CIOCARLAN, A., COLTSA, M., DELEANU, C., COSTAN, O., SIMONOV, YU., KRAVTSOV, V., LIPKOWSKI, J., LIS, T., DE GROOT, A. Photodegradation of some 14,15-bisnorlabdene-13-ones, derived from larixol. Synthesis of drimane dienes with functional groups at C-6. In: *Tetrahedron*. 2006, nr. 62, 36, pp. 8489-8497.
23. GARCIA-GRANADOS, A., LOPEZ, P., MELGUIZO, E., PARRA, A., SIMEO, Y. Reactivity of chiral sesquiterpene synthons obtained by the degradation of maslinic acid from olive-pressing residues. In: *Synthetic Communications*. 2006, nr. 36, 20, pp. 3001-3018.
24. **CIOCARLAN**, **A**., ARICU, A., UNGUR, N., BIRIIAC, A., COLTSA, M., NICOLESCU, A., DELEANU, C., VORNICU, N. Formal synthesis of (-)-pereniporin B and (-)-cinnamosmolide. In: *Natural Product Research*. 2014, nr. 28, 19, pp. 1619-1625.
25. ZHOU, H., ZHU, T., CAI, S., GU, Q., LI, D. Drimane sesquiterpenoids from the mangrove-derived fungus *Aspergillus ustus*. In: *Chemical and Pharmaceutical Bulletin*. 2011, nr. 59, 6, pp. 762-766.
26. BÉRUBÉ, G. An overview of molecular hybrids in drug discovery. In: *Expert Opinion on Drug Discovery*. 2016, nr. 11, 3, pp. 281-305.
27. ARICU, A., **CIOCARLAN**, **A**., LUNGU, L., BARBA, A., SHOVA, S., ZBANCIOC, Gh., MANGALAGIU, I.I., D'AMBROSIO, M., VORNICU, N. Synthesis, antibacterial and antifungal activities of new drimane sesquiterpenoids with azaheterocyclic units. In: *Medicinal Chemistry Research*. 2016, nr. 25, 10, pp. 2316–2323.
28. KUCHKOVA, K., ARICU, A., BARBA, A., VLAD, P., SHOVA, S., SECARA, E., UNGUR, N., ZBANCIOC, Gh., MANGALAGIU, I.I. An efficient and straightforward method to new organic compounds: homodrimane sesquiterpenoids with diazine units. In: *Synlett*. 2013, nr. 24, 6, pp. 697–700.
29. KUCHKOVA, K., ARICU, A., SECARA, E., BARBA, A., VLAD, P., UNGUR, N., TUCHILUS, C., SHOVA, S., ZBANCIOC, Gh., MANGALAGIU, I.I. Design, synthesis, and antimicrobial activity of some novel homodrimane sesquiterpenoids with diazine skeleton. In: *Medicinal Chemistry Research*. 2014, nr. 23, 3, pp. 1559–1568.
30. KUCHKOVA, K., ARYCU, A., SEKARA, E., BARBA, A., VLAD, P., MAKAEV, F., MEL'NIK, E., KRAVTSOV, V. Synthesis and structure of homodrimane sesquiterpenoids containing 1,2,4-triazole and carbazole rings. In: *Chemistry of Natural Compounds*. 2015, nr. 51, 14, pp. 684–688.
31. CUCICOVA, C., RUDIC, V., ARICU, A., CEPOI, L., RUDI, L., SECARA, E., VALUTA, A., BARBA, A., MISCU, V., VLAD, P., CHIRIAC, T. *N-($\Delta^{8,13}$-Bicyclohomofarnesenoyl)-3-amino-1,2,4-triazole compound and process for cultivation of Nostoc linckia cyanobacterium with its use*. MD Patent 2013, No 4327.
32. LUNGU, L., **CIOCARLAN**, **A**., BARBA, A., SHOVA, S., POGREBNOI, S., MANGALAGIU, I., MOLDOVEANU, C., VORNICU, N., D'AMBROSIO, M., BABAK, M., ARION, V., ARICU, A. Synthesis and evaluation of biological activity of homodrimane sesquiterpenoids bearing hydrazinecarbothioamide or 1,2,4-triazole unit. In: *Chemistry of Heterocyclic Compounds*. 2019, nr. 55, 8 pp. 716–724.
33. STYNGACH, E., MALINOVSKII, S., BETS, L., VLAD, L., GDANETS, M., MAKAEV, F. Crystal and molecular structure of (1*S*,2*S*,4a*S*,8a*S*)-*N*-(*N*-allyldiaminomethanethione)-1-(2-hydroxy-2,5,5,8*a*-tetramethyldecahydro-naphtalenyl) acetamide. In: *Journal of Structural Chemistry*. 2005, nr. 46, 4, pp. 765–769.
34. NAMRATHA, B., GAONKAR, S. 1,2,4-Triazoles: synthetic strategies and pharmacological. In: *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014, nr. 6, 8, pp. 73-80.

35. JOGDAND, G.F. *RMN studies on five membered 1,2,3/1,2,4-triazoles and their hybrid* systems. PhD thesis in philosophy in chemistry. 2016. 324 p.
36. MACAEV, F., RUSU, G., POGREBNOI, S., GUDIMA, A., STINGACI, E., VLAD, L., SHVETS, N., KANDEMIRLI, F., DIMOGLO, A., REYNOLDS, R. Synthesis of novel 5-aryl-2-thio-1,3,4-oxadiazoles and the study of their structure–anti-mycobacterial activities. In: *Bioorganic & Medicinal Chemistry*. 2005, nr. 13, 16, pp. 4842-4850.
37. BLAJA, S., LUNGU, L., KUCHKOVA, K., **CIOCARLAN**, **A**., BARBA, A., VORNICU, N., ARICU, A. Norlabdane compounds containing thiosemicarbazone or 1,3-thiazole fragments: synthesis and antimicrobial activity. In. *Chemistry of Natural Compounds*. 2021, nr. 57, 1, pp. 101–110.
38. CUCICOVA, C., ARICU, A., SECARA, E., VLAD, P., UNGUR, N. *Process for producing 14,15-bisnorlabdane-8(9)-en-13-one*. MD Patent 2013, No 4248.
39. LUNGU, L., **CIOCARLAN**, **A**., SMIGON, C., OZER, I., SHOVA, S., GUTU, I., VORNICU, N., MANGALAGIU, I., D'AMBROSIO, M., ARICU, A. Synthesis and evaluation of biological activity of homodrimane sesquiterpenoids bearing 1,3,4-oxadiazole or 1,3,4-tiadiazole units. In: *Chemistry of Heterocyclic Compounds*. 2020, nr. 56, 5, pp. 578–585.
40. GRZESZCZUK, M., JADCZAK, D. Estimation of biological value of some species of mint (*Mentha* L.). In: *Herba Polonica*. 2009, nr. 55, pp. 193-199.
41. **CIOCARLAN, A.**, LUPASCU, L., ARICU, A., DRAGALIN, I., POPESCU, V., GEANA, E.-I., IONETE, R.-E., VORNICU, N., DULIU, O.G., HRISTOZOVA, G., ZINICOVSCAIA, I. Chemical composition and assessment of antimicrobial activity of lavender essential oil and some by-products. In: *Plants*, 2021, nr 10, 9, pp. 1829-1843.
42. PIRES, T., DIAS, M., BARROS, L., ALVES, M., OLIVEIRA, M., SANTOS-BUELGA, C., FERREIRA, I. Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica* Borkh. cv Bravo de Esmolfe). In: *Food Chemistry*. 2018, nr. 240, pp. 701–706.
43. ALARCÓN-FLORES, M.I., ROMERO-GONZÁLEZ, R., MARTÍNEZ VIDAL, J.L., GARRIDO FRENICH, A. Evaluation of the presence of phenolic compounds in different varieties of apple by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. In: *Food Analytical Methods*. 2015, nr. 8, pp. 696–709.
44. CARGNIN, S.T., GNOATTO, S.B. Ursolic acid from apple pomace and traditional plants: A valuable triterpenoid with functional properties. In: *Food Chemistry*. 2017, nr. 220, pp. 477–489.
45. GEANĂ, E., IONETE, R., **CIOCARLAN**, **A**., ARICU, A., FULGA, A., UNGUR, N., PODOGOVA, M., NIKOLAEVA, D. HPLC determination of oleanolic and ursolic acids in apples and by-products. In: *Progress of Cryogenics and Isotopes Separation*. 2014, nr. 17, pp. 53–62.
46. **CIOCARLAN**, **A**., ARICU, A., GEANA, E.-I. Investigating the therapeutic potential of some medicinal plant and wild fruits based on terpene ursolic and oleanolic acids. In: *Cient Periodique Nutrition*. 2019, nr. 3, pp. 1–12.
47. KONG, L., LI, S., LIAO, Q., ZHANG, Y., SU, R., ZHU, X., ZHANG, Q., WANG, J., WU, X., FANG, X., ZHU, Y. Oleanolic acid and ursolic acid: novel hepatitis C virus antivirals that inhibit NS5B activity. In: *Antiviral Research*. 2013, nr. 98, 1, pp. 44-53.
48. GEANĂ, E., CIUCURE, C., IONETE, R., **CIOCARLAN**, **A**., ARÎCU, A., FICAI, A., ANDRONESCU, E. Profiling of phenolic compounds and triterpene acids in different apple (*Malus domestica* Borkh.) cultivars. In: *Foods*, **2021**, nr. 10, 2, pp. 267-282. ISSN 2304-8158.
49. BONDONNO, C., BONDONNO, N., SHINDE, S., SHAFAEI, A., BOYCE, M., SWINNY, E., JACOB, S., LACEY, K., WOODMAN, R., CROFT, K., CONSIDINE, M., HODGSON, J. Phenolic composition of 91 Australian apple varieties: Towards understanding their health attributes. In: *Food & Function*. 2020, nr. 11, pp. 7115–7125.

50. **CIOCARLAN A.**, HRISTOZOVA G., ARICU A., DRAGALIN I., ZINICOVSCAIA I., YUSHIN N., GROZDOV D., POPESCU V. Determination of the elemental composition of aromatic plants cultivated industrially in the Republic of Moldova using neutron activation analysis. In: *Agronomy*, 2021, nr. 11, 5, pp. 1011-1030.
51. ZINICOVSCAIA I., **CIOCARLAN A.,** LUPASCU L., ARICU A., DRAGALIN I., CIOCARLAN N., YUSHIN N. Chemical analysis of *Tanacetum corymbosum* (L.) Sch. Bip. using neutron activation analysis. In: *Journal of Radioanalytical and Nuclear Chemistry*, 2019, nr. 321, 2, pp. 349-354.
52. MARKERT, B., FRÄNZLE, S., WÜNSCHMANN, S. *Chemical Evolution - The biological system of the elements*. 1st ed. Switzerland: Springer International Publishing, 2015. 490 p.
53. ZINICOVSCAIA, I., GUNDORINA, S., VERGEL, K., GROZDOV, D., **CIOCARLAN, A.**, ARICU, A., DRAGALIN, I., CIOCARLAN, N. Elemental analysis of *Lamiaceae* medicinal and aromatic plants growing in the Republic of Moldova using neutron activation analysis. In: *Phytochemistry Letters*. 2020, nr. 35, pp. 119–127.
54. ZINICOVSCAIA, I., DULIU, O., CULICOV, O., FRONTASYEVA, M., STURZA, R. Major and trace element distribution in soil of two Moldavian vineyards: Romanesti and Cricova. In: *Romanian Reports in Physics*. 2018, nr. 70, 2, p. 701.
55. BLAJA, S., LUNGU, L., ARÎCU, A., **CIOCÂRLAN, A.**, VORNICU, N. *(Z/E)-2-(1-((1R,2R,8aS)-2-hidroxi-2,5,5,8a-tetrametildecahidronaftalen-1-il)propan-2-iliden)hidrazincarbotioamidă pentru utilizare în calitate de remediu antifungic*. MD Patent 2021, No 4769
56. ARICU, A., **CIOCARLAN, A.**, LUNGU, L., VORNICU, N. *Compus (1R,2R,8aS)-1-((5-mercapto-1,3,4-tiadiazol-2-il)metil)-2,5,5,8a-tetrametildecahidro nafthalen-2-ol cu proprietăţi antifungice şi antibacteriene.* MD Patent 2019, MD 4580..
57. LUPASCU, G., **CIOCARLAN, A.**, DRAGALIN, I., LUPASCU, L. Antimicrobial activity of the Coriander oil (*Coriandrum sativum* L.). In: *Romanian Journal of Biology*. 2019, nr. 64, 1-2, pp. 31–42.

LIST OF PUBLICATIONS ON THE THESIS TOPIC

1. Monographs

1.1. chapter from monographs

1. ARICU, A., **CIOCARLAN**, **A**., LUNGU, L., CUCICOVA, C., BLAJA, S., SECARA, E., UNGUR, N. Synthesis of biologically active nitrogen and sulfur-containing terpenoids. In book: *Fundamental and Biomedical Aspects of Redox Processes. Chapter 17. IGI GLOBAL*, 2023, 31 p. DOI: 10.4018/978-1-6684-7198-2.ch017

2. Articles in scientific journals

2.1. in journals from the Web of Science and SCOPUS databases

1. **CIOCARLAN**, **A**., LUNGU, L., BLAJA, S., SHOVA, S., VORNICU, N., BOLOCAN, N. ARICU, A. Synthesis of norlabdane compounds with rearranged cycle B and molecular docking studies. In: *Acta Chimica Slovenica*, 2024, (in press). (**I.F. 1.51**)
2. **CIOCARLAN**, **A**., HRISTOZOVA, G., ARICU, A., DRAGALIN, I., ZINICOVSCAIA, I., YUSHIN, N., GROZDOV, D., POPESCU, V. Determination of the elemental composition of aromatic plants cultivated industrially in the Republic of Moldova using neutron activation analysis. In: *Agronomy*, 2021, vol. 11, nr. 5, pp.1011-1030. ISSN 2073-4395. DOI: 10.3390/agronomy11051011 (**I.F. 2.603**)
3. **CIOCARLAN**, **A**., LUPASCU, L., ARICU, A., DRAGALIN, I., POPESCU, V., GEANA, E.-I., IONETE, R.-E., VORNICU, N., DULIU, O.G., HRISTOZOVA, G., ZINICOVSCAIA, I. Chemical composition and assessment of antimicrobial activity of lavender essential oil and some by-products. In: *Plants*, 2021, vol. 10, nr. 9, pp. 1829-1843. ISSN 2223-7747. DOI: 10.3390/plants10091829 (**I.F. 3.935**)

4. BLAJA, S., LUNGU, L., KUCHKOVA, K., **CIOCARLAN**, **A**., BARBA, A., VORNICU, N., ARICU, A. Norlabdane compounds containing thiosemicarbazone or 1,3-thiazole fragments: Synthesis and antimicrobial activity. In: *Chemistry of Natural Compounds*, 2021, vol. 57, nr. 1, pp. 101-110. ISSN 0009-3130.
DOI: 10.1007/s10600-021-03292-3 (**I.F. 0.653**)
5. GEANĂ, E., CIUCURE, C., IONETE, R., **CIOCARLAN**, **A**., ARÎCU, A., FICAI, A., ANDRONESCU, E. Profiling of phenolic compounds and triterpene acids in different apple (*Malus domestica* Borkh.) cultivars. In: *Foods*, 2021, vol. 10, nr. 2, pp. 267-282. ISSN 2304-8158. DOI: 10.3390/foods10020267 (**I.F. 4.092)**
6. LUNGU, L., **CIOCARLAN**, **A**., SMIGON, C., OZER, I., SHOVA, S., GUTU, I., VORNICU, N., MANGALAGIU, I., D'AMBROSIO, M., ARICU, A. Synthesis and evaluation of biological activity of homodrimane sesquiterpenoids bearing 1,3,4-oxadiazole or 1,3,4-thiadiazole units. In: *Chemistry of Heterocyclic Compounds*, 2020, vol. 56, nr. 5, pp. 578-585. [*Khim. Geterotsikl. Soedin.* 2020, nr. 56, p. 578.]. ISSN 0009-3122. DOI: 10.1007/s10593-020-02703-7. (**I.F. 1.492**)
7. ZINICOVSCAIA, I., **CIOCARLAN**, **A**., LUPASCU, L., ARICU, A., DRAGALIN, I., CIOCARLAN, N., YUSHIN, N. Chemical analysis of *Tanacetum corymbosum* (L.) Sch. Bip. using neutron activation analysis. In: *Journal of Radioanalytical and Nuclear Chemistry*, 2019, vol. 321, nr. 2, pp. 349-354. ISSN 0236-5731.
DOI: 10.1007/s10967-019-06590-x (**I.F. 0.983**)
8. LUNGU, L., **CIOCARLAN**, **A**., BARBA, A., SHOVA, S., POGREBNOI, S., MANGALAGIU, I., MOLDOVEANU, C., VORNICU, N., D'AMBROSIO, M., BABAK, M., ARION V., ARICU A. Synthesis and evaluation of biological activity of homodrimane sesquiterpenoids bearing hydrazinecarbothioamide or 1,2,4-triazole unit. In: *Chemistry of Heterocyclic Compounds*, 2019, vol. 55, nr. 8, pp. 716-724. [*Khim. Geterotsikl. Soedin.* 2019, 55, 716.] ISSN 0009-3122. DOI: 10.1007/s10593-019-02526-1 (**I.F. 1.492**)
9. **CIOCARLAN**, **A**., ARICU, A., LUNGU, L., EDU, C., BARBA, A., SHOVA, S., MANGALAGIU I.I., D'AMBROSIO, M., NICOLESCU, A., DELEANU, C., VORNICU, N. Synthesis of novel tetranorlabdane derivatives with unprecedented carbon skeleton. In: *Synlett*, 2017, vol. 28, nr. 5, pp. 565-571. ISSN 0936-5214.
DOI: 10.1055/s-0036-1588651 (**I.F. 2.15**)
10. ARICU, A., **CIOCARLAN**, **A**., LUNGU, L., SHOVA, S., ZBANCIOC, Gh., MANGALAGIU I., D'AMBROSIO, M., VORNICU, N. Synthesis, antibacterial and antifungal activity of new drimane sesquiterpenoids with azaheterocyclic units. In: *Medicinal Chemistry Research*, 2016, vol. 25, nr. 10, pp. 2316–2323. ISSN 1054-2523. DOI: 10.1007/s00044-016-1665-0 (**I.F. 1.436**)
11. **CIOCARLAN**, **A**., ARICU, A., UNGUR, N., BIRIIAC, A., COLTSA, M., NICOLESCU, A., DELEANU C., VORNICU, N. Formal synthesis of (-)-pereniporine B and (-)-cinnamosmolide. In: *Natural Product Research*, 2014, vol. 28, nr. 19, pp. 1619-1625. ISSN 1478-6419. DOI: 10.1080/14786419.2014.930860 (**I. F. 1.02**)
12. VLAD P., **CIOCARLAN**, **A**. COLTSA, M., EDU, C., BIRIIAC, A., BARBA, A., DELEANU, C., NICOLESCU, A., D'AMBROSIO, M., DE GROOT, A. Synthesis of (-)-albrassitriol and (-)-*epi*-albrassitriol from (+)-larixol. In: *Natural Product Research*, 2013, vol. 27, pp. 809–817. ISSN 1478-6419.
DOI: 10.1080/14786419.2012.706297 (**I. F. 0.58**)

13. VLAD, P., **CIOCARLAN**, **A**., EDU, C., ARICU, A., BIRIIAC, A., COLTSA, M., D'AMBROSIO, M., DELEANU, C., NICOLESCU, A., SHOVA, S., VORNICU, N., DE GROOT A. Regio- and stereoselective synthesis of (+)-6-ketoeuryfuran, (+)-6-ketowinterin, and (-)-7-ketoeuryfuran from accessible labdane diterpenoids (+)-larixol and (-)-sclareol. In: *Tetrahedron*, 2013, vol. 69, nr. 2, pp. 918-926. ISSN 0040-4020.
DOI: 10.1016/j.tet.2012.10.096 (**I. F. 2.82**)
14. **CIOCARLAN**, **A**., EDU, C., BIRIIAC, A., LUNGU, L., ARICU, A., D'AMBROSIO, M., SHOVA, S., NICOLESCU, A., DELEANU, C., VORNICU, N. Synthesis of polyfunctional drimanes from drim-7,9(11)-diene and drim-8-en-7-one. In: *Synthetic communications*, 2013, vol. 43, nr. 22, p. 3020-3033. ISSN 0039-7911.
DOI: 10.1080/00397911.2012.762105 (**I. F. 1.02**)
15. VLAD, P., **CHOKYRLAN** (**CIOCARLAN**), **A**., D'AMBROSIO, M., KOLTSA, M., BARBA, A., EDU, K., BYRYYAK, A., NIKOLESCU, A., MARI, A., DELEANU, K. Synthesis of *ent*-crotonadiol and compounds related to it from (+)-larixol. In: *Chemistry of Natural Compounds*, 2011, vol. 47, nr. 3, pp. 397-403. ISSN 0009-3130. DOI: 10.1007/s10600-011-9943-z (**I. F. 0.53**)

2.2. in journals from other databases accepted by ANACEC

1. **CIOCARLAN, A**., LUNGU, L., BLAJA, S., SHOVA, S., ARICU, A. Synthesis of cycle B functionalized derivatives of (+)-larixol. In: *Chemistry Journal of Moldova*, **2024**, vol. 19, nr. 1. DOI: 10.19261/cjm.2024.1156
2. **CIOCARLAN**, **A**. From (-)-sclareol to norlabdane heterocyclic hybride compounds. In: *Chemistry Journal of Moldova*. 2022, vol. 17, nr. 2, pp. 19-34. ISSN 1857-1727. DOI: 10.19261/cjm.2022.958
3. **CIOCARLAN**, **A**., LUPASCU, L., ARICU, A., DRAGALIN, I., CIOCARLAN, N., ZINICOVSCAIA, I., SLANINA, V., YUSHIN, N. Chemical composition of the essential oil and antimicrobial properties of crude extract from *Tanacetum corymbosum* (L.). In: *Chemistry Journal of Moldova*, 2021, vol. 16, nr. 2, pp. 83-90. ISSN 1857-1727. DOI: 10.19261/cjm.2021.877
4. **CIOCARLAN**, **A**. (+)-Larixol: recent syntheses, phytochemical studies and biological activity assessments. In: *Chemistry Journal of Moldova*. 2021, vol. 16, nr. 1, pp. 30-45. ISSN 1857-1727.
DOI: 10.19261/cjm.2021.836
5. **CIOCARLAN**, **A**., LUNGU, L., BLAJA, S., DRAGALIN, I., ARICU, A. The use of some non-conventional methods in chemistry of bicyclohomofarnesenic methyl esters. In: *Chemistry Journal of Moldova*, 2020, vol. 15, nr. 2, pp. 69-77. ISSN 1857-1727. DOI: 10.19261/cjm.2020.791
6. **CIOCARLAN**, **A**., ARICU, A., GEANĂ E.-I. Investigating the therapeutic potential of some medicinal plant and wild fruits based on terpene ursolic and oleanolic acids. In: *Cient Periodique Nutrition*, 2019, vol. 3, nr. 5, pp. 1-12.
7. DUCA, GH., ARICU, A., LUNGU, L., TENU, N., **CIOCARLAN**, **A**., GUTU, YA., DRAGALIN, I., BARBA, A. Synthesis of new homodrimane sesquiterpenoids containing diazine, 1,2,4-triazole and carbazole rings. In: *Chemistry Journal of Moldova*, 2018, vol. 13, nr. 1, pp. 69-73. ISSN 1857-1727. DOI: 10.19261/cjm.2017.458
8. **CIOCARLAN**, **A**., VLAD, P., COLTSA, M., EDU, C., BIRIIAC, E., NICOLESCU, A., DELEANU, C. Synthesis of C_6 and C_7 functionaliazed drimanes from larixol and sclareol.

In: *Chemistry Journal of Moldova*, 2011, vol. 6, nr. 2, pp. 13-15. ISSN 1857-1727. DOI: 10.19261/cjm.2011.06(2).03

2.3. in journals from the National Register of professional field

- articles în journals of category B

1. GEANĂ, E.-I., IONETE, R.-E., **CIOCARLAN**, **A**., ARICU, A., FULGA, A., UNGUR, N., PODOGOVA, M., NIKOLAEVA, D. HPLC determination of oleanolic and ursolic acid in apples and apple pomance. Rezumat în extensor. In: *Progress of Cryogenics and Isotop Separation*. 2014, vol. 17, nr. 2, pp. 53-62. ISSN 1582-2575.

3. Articles in conference books and other scientific events

1. **CIOCARLAN**, **A**., COLTSA, M., VLAD, P.F., BIRIYAC, A., NICOLESCU, A., DELEANU, C., D'AMBROSIO, M. Photochemical transformation of drim-7,9(11)-diene. In: *Book of Abstracts of the International Conference dedicated to the 50th anniversary from the foundation of the Institute of Chemistry of the Moldavian Academy of Sciences 26 – 28 May,* 2009, Chisinau, Republic of Moldova, p. 131, ISBN 978-9975-62-258-5.
2. EDU, C., **CIOCARLAN**, **A**., COLTSA, M., D'AMBROSIO, M., NICOLESCU, A., DELEANU, C., VLAD, P.F. Syntheses of 11-bromo-drim-8-ene-7-one. In: *Book of Abstracts of the International Conference dedicated to the 50th anniversary from the foundation of the Institute of Chemistry of the Moldavian Academy of Sciences 26 – 28 May,* 2009, Chisinau, Republic of Moldova, p. 134. ISBN 978-9975-62-258-5.
3. **CIOCARLAN**, **A**., VLAD, P. F., COLŢA, M., BIRIIAC, A., NICOLESCU, A., DELEANU, C., D'AMBROSIO, M. Synthesis of C-6 and C-7 functionalized euryfurans from larixol and sclarol *via* photochemical transformations. In: *Culegerea de lucrări.* XXXI-*a Conferinta Nationala de Chimie,* 06-08 octombrie, 2010, Ramnicu Valcea, Romania, p. 20 (plus *comunicare orala*).
4. **CIOCARLAN**, **A**., EDU, C., BIRIIAC, A., COLTSA, M., VLAD, P., NICOLESCU, A., IVANOV, D., VORNICU, N. DELEANU, C. New drimane sesquiterpenoids with antibacterial and antifungal activity. In: *Book of abstracts. ZAI 2011, a XXIII-a sesiune de comunicari stiintifice "Progrese în știința compușilor organici and macromoleculeri, 28 septembrie-1 octombrie*, 2011, Iaşi, România, p. 58.
5. **CIOCARLAN**, **A**., EDU, C., BIRIIAC, A., SIRBU, T., BARBA, A., ARICU, A., SOVA, S., NICOLESCU, A., DELEANU, C. Synthesis of unusual tetranorlabdane dimer. In: *Book of abstracts. XXVII-a Conferinta Nationala de Chimie, 03-05 octombrie*, 2012, Calimanesti-Caciulata, Romania, p. 5.
6. EDU, C., **CIOCARLAN**, **A**., BIRIIAC, A., BARBA, A., ARICU, A., SHOVA, S., DELEANU, C., NICOLESCU, A., VORNICU, N. Synthesis of new and bioactive terpeno-lactones. In: *Book of Abstracts of the XXXII-nd Romanian Chemistry Conference, 02-05 octombrie*, 2012, p. 7.
7. BIRIIAC, A., **CIOCARLAN**, **A**., EDU, C., ARICU, A., COLTSA, M., NICOLESCU, A., DELEANU, C. VLAD, P. Regio- and stereoselective synthesis of (+)-6-ketowinterin from (+)- larixol. In: *Book of Abstracts of the XXXII-nd Romanian Chemistry Conference, 02-05 octombrie*, 2012, p. 27.
8. EDU, C., **CIOCARLAN**, **A**., SECARA, E., LUNGU, L., BIRIIAC, A., PAVALACHE, N., ARICU A., NICOLESCU, A., DELEANU, C., VORNICU, N. Biologically active brominated sesquiterpenes. In: *Book of Abstracts of the XXXII-nd Romanian Chemistry Conference, 02-05 octombrie*, 2012, p. 44.

9. **CIOCARLAN**, **A**., ARICU, A., BIRIIAC, A., LUNGU, L., EDU, C., GORINCIO,I E., BARBA, A., SHOVA, S. Synthesis of new homodrimanE dimers. In: *Book of Abstracts of the International Conference dedicated to the 55th anniversary of the Institute of Applied Physics of the A.S.M*., 2014, Chisinau, Republic of Moldova, p. 146.
10. **CIOCARLAN**, **A**., ARICU, A., LUNGU, L., BARBA, A., GORINCIOI, E., POPESCU, R., DINCA, O., ZBANCIOC, G., MOLDOVEANU, C., MANGALAGIU, I. Synthesis of hybrid terpeno-azaheterocyclic compounds. In: *Book of Abstracts of the International Conference dedicated to the 55th anniversary of the Institute of Chemistry of the A.S.M*. May 28-30, 2014, Chisinau, Republic of Moldova, p. 45. https://ibn.idsi.md/ro/vizualizare_articol/70797
11. **CIOCARLAN**, **A**., ARICU, A., BIRIIAC, A., LUNGU, L., NICOLESCU, A., DELEANU, C., VORNICU, N. New antifungal and antibacterial nitrogen containing terpenes. In: *Book of Abstracts of the International Conference dedicated to the 55th anniversary from the foundation of the Institute of Chemistry of the Academy of Sciences of Moldova*, May 28-30, 2014, Chisinau, Republic of Moldova, p. 196.
12. **CIOCARLAN**, **A**., ARICU, A., FULGA, A., IONETE, R., GEANA, I., UNGUR, N. HPLC analysis of ursolic and oleanolic acids in some by-products. In: *Book of Abstracts of the International Conference dedicated to the 55th anniversary from the foundation of the Institute of Chemistry of the Academy of Sciences of Moldova*, May 28-30, 2014, Chisinau, Republic of Moldova, p. 197. ISBN 978-9975-62-371-1.
13. **CIOCARLAN**, **A**., ARICU, A., FULGA, A., UNGUR, N., GEANA, I., IONETE, R., CIOCARLAN, N., VORNICU, N. Chemical composition and biological activity of lavender by-products. In: *Book of Abstracts of the XXXIII-nd Romanian Chemistry Conference*, 1-3 October, 2014, Calimanesti-Caciulata, Romania, p. 24.
14. GEANA, I., IONETE, R., **CIOCARLAN**, **A**., ARICU, A., FULGA, A., UNGUR, N., CIOCARLAN, N. RP-HPLC analysis of ursolic and oleanolic acids in some nedicinal plants sources. In: *Book of Abstracts of the XXXIII-nd Romanian Chemistry Conference*, 1-3 October 2014, Calimanesti-Caciulata, Romania, p. 22.
15. LUNGU, L., **CIOCARLAN**, **A**., ARICU, A., BARBA, A., GORINCIOI, E., ZBANCIOC, G., MANGALAGIU, I.I., VORNICU, N. Synthesis of new biological active pentanorlabdane-azaheterocyclic compounds. In: *Book of Abstracts of the XXXIII-nd Romanian Chemistry Conference,* 1-3 October, 2014, Calimanesti-Caciulata, Romania, p. 25.
16. BIRIIAC, A., **CIOCARLAN**, **A**., LUNGU, L., ARICU, A., BARBA, A., SHOVA, S. Synthesis of new drimane derivatives with unusual structures. In: *Book of Abstracts of the XXXIII-nd Romanian Chemistry Conference*, 1-3 October, 2014, Calimanesti-Caciulata, Romania, p. 26.
17. LUNGU, L., **CIOCARLAN**, **A**., ARICU, A., ZBANCIOC, GH., MANGALAGIU, I., VORNICU, N., SHOVA, S. Synthesis, biological activity and X-Rays analysis of 11,12-*p*-tolyl-pyridazonyl-drim-5(6),8(9)-en-7-one. In: *Book of Abstracts of the XVIII-th Conference "Physical Methods in Coordination and Supramolecular Chemistry"*, 8-9 October, 2015, Chișinău, Republica Moldova, p. 93.
18. LUNGU, L., ȘMIGON, C., ARICU, A., **CIOCARLAN**, **A**., BARBA, A., SHOVA, S. Synthesis and X-ray analysis of new tetranorlabdane compound with thiadiazole unit. In: *Book of Abstracts of the 8th International Conference on Materials Science and Condensed Matter Physics*, 12-16 September, 2016, Chișinău, Republica Moldova, p. 151.

19. LUNGU L., ARICU, A., **CIOCARLAN**, **A**., BARBA, A., VORNICU, N. Synthesis of new biological active tetranorlabdane compounds with triazole units. In: *Book of Abstracts of the XXXIV-th Romanian Chemistry Conference,* October 4-7, 2016, Calimanesti-Caciulata, Romania, p. 11.
20. ȘMIGON, C., LUNGU, L., ARICU, A., **CIOCARLAN, A**., BARBA, A., VORNICU, N. Synthesis of new biological active tetranorlabdane compounds with thiadiazole units. In: *Book of Abstracts of the XXXIV-th Romanian Chemistry Conference,* October 4-7, 2016, Calimanesti-Caciulata, Romania, p. 14.
21. LUNGU, L., SMIGON, C., ARICU, A., **CIOCARLAN, A.**, BARBA, A., MOLDOVEANU C. Synthesis of new tetranorlabdane compounds with oxadiazole units. In: *Book of Abstracts of „A.I. Cuza" University Days, Faculty of Chemistry Conference,* 27-29 October, 2016, p. 63.
22. ARICU, A., LUNGU, L., **CIOCARLAN**, **A**., BARBA A., DRAGANCEA, D. Synthesis of new biologically active polyfunctional terpenoids based on industrial wastes. In: *Book of Abstracts of the 6th International Conference Ecological & Environmental Chemistry*, 2-3 March, 2017, Chisinau, Republic of Moldova, p. 168.
23. LUNGU, L., ARICU, A., BLAJA, S., **CIOCARLAN**, **A**., BARBA, A. Synthesis of new potential biologically active tetra- and pentanorlabdane compounds with 1,3-thiazole units. In: *Book of Abstracts of the 20th Romanian International Conference on Chemistry and Chemical Engineering*, 6 – 9 September, 2017, Poiana Brasov, Romania, p. S2- 52.
24. LUNGU, L., BILAN, D., ARÎCU, A., **CIOCARLAN**, **A**., GORINCIOI, E., MACAEV, F. Synthesis and NMR characterization of some new compounds with combined skeleton containing heterocycle pharmacophores. In: *Book of Abstracts of 19th Central and Eastern European NMR Symposium & Bruker Users' Meeting*, 5-8 September, 2017, Timisoara, Romania, p. 22.
25. BLAJA, S., LUNGU, L., ARICU, A., **CIOCARLAN**, **A**. Synthesis of new potential active trinorlabdane compounds with 1,3-thiazole units. In: *Book of Abstracts of the XXXV-th Romanian Chemistry Conference,* 2–5 October, 2018, Calimanesti-Caciulata, Romania, p. 6.
26. ARICU, A., LUNGU, L., **CIOCARLAN**, **A**., BARBA, A., VORNICU, N., MANGALAGIU, I. Synthesis, structure and antimicrobial activity of new homodrimane sesquiterpenoids with oxa- and thiadiazole units. In: *Book of Abstracts of the XXXV-th Romanian Chemistry Conference,* 2 – 5 October, 2018, Calimanesti-Caciulata, Romania, p. 13.
27. LUNGU, L., TENU, N., ARICU, A., BARBA, A., **CIOCARLAN**, **A**., VORNICU, N. Synthesis of new biological active tetranorlabdane compounds with 1,3,4-thiadiazole units. In: *Book of Abstracts of the XXXV-th Romanian Chemistry Conference,* 2–5 October, 2018, Calimanesti-Caciulata, Romania, p. 16.
28. **CIOCARLAN**, **A**., LUNGU, L., ARICU, A., VORNICU, N. Antifungal and antibacterial activities of some new homodrimane sesquiterpenoids bearing 1,3,4-oxadiazole units. In: *Book of Abstracts of the XXXV-th Romanian Chemistry Conference,* 2–5 October, 2018, Calimanesti-Caciulata, Romania, p. 30.
29. LUNGU, L., **CIOCARLAN**, **A**., ARICU, A., FILIP, G., BIRCA, N. The use of some non-conventional methods in chemistry of bicyclohomofarnesenic methyl esters. In: *Book of Abstracts of the International Conference "Achievements and perspectives of modern chemistry" dedicated to the 60th anniversary of Institute of Chemistry*, 9-11 October, 2019,

Chisinau, Republic of Moldova, p. 53 (prezentare orală). ISBN 978-9975-62-428-2. https://ibn.idsi.md/sites/default/files/imag_file/53-53_12.pdf

30. LUNGU, L., **CIOCARLAN**, **A**., ARICU, A., OZER, I., BARBA A. Synthesis of new potential active homodrimane sesquiterpenoids with 1,3,4-thiadiazole units. In: *Book of Abstracts of the International Conference "Achievements and perspectives of modern chemistry" dedicated to the 60th anniversary of Institute of Chemistry.* 9-11 October 2019, Chisinau, Republic of Moldova, p. 225. ISBN 978-9975-62-428-2. https://ibn.idsi.md/sites/default/files/imag_file/225-225_2.pdf
31. LUNGU, L., BLAJA, S., **CIOCARLAN**, **A**., OZER, I., ARICU, A., VORNICU, N. Synthesis of new active nitrogen and sulfur containing norLABDANe COMPOUNDS. In: *Book of Abstracts of the International Conference "Achievements and perspectives of modern chemistry" dedicated to the 60th anniversary of Institute of Chemistry,* 9-11 October, 2019, Chisinau, Republic of Moldova, p. 226. ISBN 978-9975-62-428-2. https://ibn.idsi.md/sites/default/files/imag_file/226-226_3.pdf
32. LUNGU, L., BLAJA S., **CIOCARLAN**, **A**., DRAGALIN, I., ARICU, A. The electrochemical transformations of methyl bicyclohomofarnesoates. In: *Scientific seminar "New frontiers in natural product chemistry"6th Edition,* 4 iunie, 2021, Chișinău, Republica Moldova, p. 15 (poster). ISBN 978-9975-3336-7-2.
DOI: 10.19261/nfnpc.2021.ab08; https://ibn.idsi.md/ro/vizualizare_articol/131092
33. **CIOCARLAN**, A. Valorisation of some terpenes from vegetalwastes. In: *International Conference "Intelligent Valorisation of Agro-Industrial Wastes"*, 07-08 October, 2021, Chisinau, Republic of Moldova, p. 12. ISBN 978-9975-3464-2-9. https://ibn.idsi.md/sites/default/files/imag_file/12-12_38.pdf
34. ARICU, A., **CIOCARLAN**, **A**. Synthesis of biologically active terpenoids by valorization of some vegetalwastes. Conference "Ecological and environmental chemistry 2022" 7th Edition, Chisinau, Moldova. In: *Ecological and environmental chemistry*, 3-4 March, 2022, vol. 1, p. 146-147, 2022.
DOI: http://dx.doi.org/10.19261/eec.2022.v1
https://ibn.idsi.md/sites/default/files/imag_file/p-146-147_0.pdf
35. **CIOCARLAN**, **A**., ARICU, A, LUNGU, L., BLAJA, S., POPESCU, V., ZINICOVSCAIA, I., CARRUPT, V. Preliminary phytochemical analysis of crud extract from *Tanacetum corymbosum* (L.) Shi. Bip. National Conference with International Participation: *Life Sciences in the Dialogue of Generations: Connections Between Universities*, *Academia and Business Community*, In: *Book of abstracts*, 29-30 September, 2022, Chisinau, Republic of Moldova. p. 209. https://ibn.idsi.md/sites/default/files/imag_file/209_7.pdf
36. CIOCARLAN, A., LUNGU, L., BLAJA, S., CUCICOVA, C., ARICU, A. Photochemical transformation of some (+)-larixol derivatives. Seminar ştiinţific "*Noi frontiere în chimia compuşilor naturali*", 12-13 octombrie, 2023, Chișinău, R. Moldova. (cu prezentare orală).
37. LUNGU, L., CIOCARLAN, A., BLAJA, S., VORNICU, N., BOLOCAN, N., ARÎCU, A. Compuși norlabdanei biologic activi. Studiu comparativ: testările *in vitro* versus testul de andocare moleculară. Conferinţa ştiinţifică naţională cu participare internaţională „*Integrare prin Cercetare şi Inovare*". Noiembrie 09-10, 2023, Chisinau, Republica Moldova. (poster, rezumat în extenso)

4. Patents and other intellectual property objects (PIO)

4.2. granted by the State Agency for Intellectual Property

1. ARÎCU, A., **CIOCARLAN**, **A.**, LUNGU, L., BLAJA, S., VORNICU, N. *5-(Homodrim-6,8-dien-11-il)-1,3,4-tiadiazol-2(3H)-imină cu proprietăţi antifungice.* Brevet de invenție MD-4765 (13), B1 31.03.2012.
   http://www.db.agepi.md/Inventions/details/a%202019%200072/LinkTitluAcc
2. ARÎCU, A., LUNGU, L., **CIOCARLAN**, **A.**, VORNICU, N. *Compus (1R,2R,8aS)-1-((5-mercapto-1,3,4-thiadiazol-2-il)metil)-2,5,5,8atetrametildecahidro nafthalen-2-ol cu proprietăţi antifungice şi antibacteriene.* Brevet de invenție MD-4580 (13), B1 31.07.2018.
   http://www.db.agepi.md/Inventions/details/a%202017%200101/LinkTitluElib
3. ARÎCU, A., MANGALAGIU, I., **CIOCÂRLAN**, **A.**, LUNGU, L., ZBÂNCIOC, Gh., VORNICU, N. *11,12-Bis-p-tolil-piridazonil-drim-5(6),8(9)-dien-7-ona cu proprietăţi antifungale şi antibacteriene.* Brevet de invenție MD-4370 (13) C1 30.09.2015.
   http://www.db.agepi.md/Inventions/details/a%202015%200024/LinkTitluAcc
4. MIRONOV, G., COLȚA, M., VLAD, P., **CIOCÂRLAN**, **A**. *Electrochemical process for obtaining 7β,11-diacetoxy-drim-8-ene*. Brevet de invenție 3967 (13), C2 31.10.2009. http://www.db.agepi.md/Inventions/details/a%202009%200066/LinkTitluAcc

ADNOTARE

CIOCÂRLAN Alexandru: Sinteza derivaților norlabdanei biologic activi cu funcționalizare avansată and studiul fitochimic al unor surse vegetale locale, Teză de doctor habilitat în științe chimice, Chișinău, 2024

Structura tezei: Adnotare (în RO, EN and RU), Introducere, 6 capitole, Concluzii generale and recomandări, Bibliografie din 514 titluri, 235 pagini text de bază, 144 de figuri, 37 tabele. Rezultatele obținute au fost publicate în 66 lucrări științifice, inclusiv 15 articole cu factor de impact, un capitol în monografie and 4 brevete de invenție.

Domeniul de studii: 143.04 – Chimie bioorganică, chimia compușilor naturali and fiziologic activi.

Cuvinte-cheie: (-)-Sclareol, (+)-larixol, compuși norlabdanei, sinteza, transformări fotochimice, oxidare anodică, iradiere cu microunde, hibrizi moleculari terpeno-heterociclici, diazine, 1,2,4-triazoli, 2-amino-1,3-tiazoli, 1,3,4-oxadiazoli, 1,3,4-tiadiazoli, studiu fitochimic, activitate antifungică and antibacteriană, testări *in vitro*.

Scopul lucrării: Elaborarea unor scheme de sinteză a analogilor naturali, derivaților cu un grad avansat de funcționalizare and hibrizilor moleculari terpeno-heterociclici, biologic activi, prin metode clasice and neconvenționale în bază de (-)-sclareol and (+)-larixol, elucidarea mecanismelor de reacție, confirmarea structurilor, determinarea activității biologice a compușilor de sinteză and realizarea unui studiu fitochimic complementar al surselor vegetale locale de interes, testarea activității biologice.

Obiectivele lucrării: Constau în elaborarea metodelor noi de sinteză a analogilor naturali, compuși tetra- and pentanorlabdanei cu funcționalizare avansată and hibrizi moleculari diazinici, 1,2,4-triazolici, 2-amino-1,3-tiazolici, 1,3,4-oxadiazolici, 1,3,4-tiadiazolici din deșeuri vegetale, prin metode clasice and neconvenționale (foto- and electrochimice, iradiere cu microunde); elucidarea mecanismelor posibile de reacție; realizarea studiilor fitochimice ale surselor vegetale locare; testarea biologică a compușilor sintetizați and a extractelor vegetale pentru utilizarea lor ulterioară; stabilirea relației structură-activitate.

Noutatea and originalitatea științifică: În premieră, au fost realizate, sinteze eficiente, originale, stereo- and regioselective ale analogilor naturali, compușilor norlabdanei cu funcționalizare avansată and seriilor de hibrizi moleculari heterociclici; propuse mecanismele de reacție; realizate studii fitochimice ale surselor vegetale locale; identificată o serie de compuși de sinteză cu activitate antimicrobiană avansată and stabilită relația structură-activitate.

Rezultate principial noi pentru știință and practică obținute: Constă în elaborarea procedeelor noi de transformare a diterpenoidelor labdanice naturale (-)-sclareol and (+)-larixol, obținute din deșeurile de salvie tămâioasă and rășina de zadă, în compuși naturali or sintetici biologic activi cu posibile aplicații în industria farmaceutică, inclusiv prin metode de sinteză prietenoase mediului. A fost elaborată o direcție nouă de sinteză a unor hibrizi moleculari heterociclici ce posedă activitate biologică. A fost stabilită în premieră compoziția fitochimică a surselor locale de importanță alimentară and terapeutică, precum merele and fructele de pădure, plantele aromatice and etero-oleaginoase.

Semnificația teoretică: Rezultatele cercetărilor confirmă posibilitatea transformării deșeurilor locale în compuși de importanță practică; demonstrează utilitatea and eficiența aplicării metodelor de sinteză neconvenționale în chimia compușilor naturali; completează informațiile științifice privind mecanismele reacțiilor în șirul compușilor norlabdanei, inclusiv a hibrizilor heterociclici; and cele referitoare la relația structură-activitate ale compușilor de sinteză, compoziția calitativă and utilitatea materiilor vegetale locale and a produselor derivate din ele.

Valoarea aplicativă: Testarea activității antimicrobiene a compușilor de sinteză a confirmat, că 29 dintre aceștea manifestă activitate pronunțată. Studiul fitochimic al surselor vegetale locale a confimat corespunderea compoziției chimice a materiilor vegetale locale and a produselor derivate din ele standardelor în vigoare. Rezultatele obținute prezintă interes pentru firmele farmaceutice, producătorii locali de fructe, plante aromatice, etero-oleaginoase and medicinale, and cei ocupați de procesarea acestora. Utilizarea metodelor neconvenționale, precum degradările fotolitice, fotooxidările sensibilizate, oxidarea anodică and iradierea cu microunde, are impact pozitiv asupra mediului and justifică tehnologiile non-poluante, non-agresive şi ieftine. Au fost obținute 4 brevete de invenție în care sunt revendicate procedeele de sinteză ale compușilor bioactivi.

Implementarea rezultatelor științifice: Rezultatele cercetărilor au fost implementate în procesul de instruire la cursurile ”*Chimie bioorganică*” and ”*Obținerea sintetică and semisintetică a principiilor active*” la programele de studii de licență *Tehnologia produselor cosmetice and medicinale*. Au fost brevetate procedeele de sinteză and activitatea a 4 compuși norlabdanei noi, care pot fi implementați în practică în calitate de remedii antifungice.

SUMMARY

CIOCÂRLAN Alexandru: Synthesis of biologically active norlabdane derivatives with advanced functionalization and phytochemical study of some local plant sources, Thesis of doctor habilitate in chemistry, Chisinau, 2024

Structure of the thesis: Annotation (in RO, EN and RU), Introduction, 6 chapters, General conclusions and recommendations, Bibliography of 514 titles, 235 pages of basic text, 144 figures, 37 tables. The results obtained have been published in 66 scientific papers, including 15 articles with impact factor, one chapter in a book and 4 patents.

Field of studies: 143.04 – Bioorganic chemistry, chemistry of natural and physiologically active compounds.

Keywords: (-)-Sclareol, (+)-larixol, norlabdane compounds, synthesis, photochemical transformations, anodic oxidation, microwave irradiation, terpene-heterocyclic molecular hybrids, diazines, 1,2,4-triazoles, 2-amino-1,3-thiazoles, 1,3,4-oxadiazoles, 1,3,4-tiadiazoles, phytochemical study, antifungal and antibacterial activity, *in vitro* tests.

The aim of the work: Development of synthetic schemes of biologically active natural analogues, derivatives with an advanced degree of functionalization and terpene-heterocyclic molecular hybrids, by classical and non-conventional methods based on (-)-sclareol and (+)-larixol, elucidation of reaction mechanisms, the structures confirmation, determination of the biological activity of the synthetic compounds and carrying out a complementary phytochemical study of the local plant sources of interest, testing the biological activity.

The objectives of the work: Consist in the development of new methods for the synthesis of natural analogues, tetra- and pentanorlabdane compounds with advanced functionalization and diazine, 1,2,4-triazole, 2-amino-1,3-thiazole, 1,3,4-oxadiazole 1,3,4-tiadiazole molecular hybrids from vegetalwaste, by classical and non-conventional methods (photo- and electrochemical, microwave irradiation); elucidation of possible reaction mechanisms; carrying out phytochemical studies of local plant sources; biological testing of synthesized compounds and plant extracts for their further uses; establishing the structure-activity relationship.

Scientific novelty and originality: For the first time, efficient, original, stereo- and regioselective syntheses of natural analogues, norlabdane compounds with advanced functionalization and series of heterocyclic molecular hybrids were achieved; proposed the mechanisms of some reactions; carried out the phytochemical studies of local plant sources; identified a series of synthetic compounds with advanced antimicrobial activity and established the structure-activity relationship.

Fundamentally new results for science and practice obtained: Consist in the development of new procedures for the transformation of natural labdane diterpenoids (-)-sclareol and (+)-larixol, obtained from Clary sage waste and larch oleoresin, into biologically active natural or synthetic compounds with possible applications in the pharmaceutical industry, including through environmentally friendly methods of synthesis. A new direction of synthesis of heterocyclic molecular hybrids possessing biological activity

was developed. The phytochemical composition of local sources of food and therapeutic importance, such as apples and berries, aromatic and essential oil producing plants, was determined.

Theoretical significance: The research results confirm the possibility and practical utility of transforming local waste into compounds of practical importance; demonstrate the utility and efficiency of applying non-conventional synthetic methods in the chemistry of natural compounds; complete the scientific information regarding to mechanisms of reactions in the series of norlabdane compounds, including heterocyclic hybrids; and those related to the structure-activity relationship of synthetic compounds, the qualitative composition and usefulness of local plant materials and products derived from them.

Application value: Antimicrobial activity testing of synthetic compounds confirmed that 29 of them show pronounced activity. The phytochemical study of the local plant sources confirmed the conformity of the chemical composition of the local plant materials and the products derived from them to the standards in force. The obtained results are of interest to pharmaceutical companies, local producers of fruits, aromatic, essential oil producing and medicinal plants, and those involved in their processing. The use of non-conventional methods, such as photolytic degradations, sensitized photooxidations, anodic oxidation and microwave irradiation, have a positive impact on the environment and justify non-polluting, non-aggressive and cheap technologies. 4 invention patents were obtained in which the synthesis procedures of bioactive compounds are claimed.

Implementation of scientific results: The research results were implemented in the training process in the courses "*Bioorganic Chemistry*" and "*Synthetic and semi-synthetic preparation of active principles*" in the Bachelor's degree programs in the *Technology of cosmetic and medicinal products*. The synthesis procedures and the activity of 4 new norlabdane compounds that can be implemented in practice as antifungal remedies were clamed.

АННОТАЦИЯ

ЧОКЫРЛАН Александру: Синтез биологически активных высоко-функционализированых норлабдановых производных и фитохимическое исследование некоторых местных растительных источников, диссертация доктора химических наук, Кишинев, 2024

Структура диссертации: Аннотация (на RO, EN и RU), Введение, 6 глав, Общие выводы и рекомендации, Библиография из 514 источников, 235 страниц основного текста, 144 рисунков, 37 таблиц. Полученные результаты опубликованы в 66 научных статьях, в том числе в 15 статьях с импакт-фактором, в главе монографии и в 4 патентах на изобретение.

Ключевые слова: (-)-Склареол, (+)-лариксол, норлабдановые соединения, синтез, фотохимические превращения, анодное окисление, СВЧ-облучение, терпено-гетероциклические молекулярные гибриды, диазины, 1,2,4-триазолы, 2-амино-1,3-тиазолы, 1,3,4-оксадиазолы, 1,3,4-тиадиазолы, фитохимическое исследование, противогрибковая и антибактериальная активность, тесты *in vitro*.

Цель работы: Разработка схем синтеза биологически активных природных аналогов, производных с высокой степенью функционализации и терпен-гетероциклических молекулярных гибридов, классическими и неклассическими методами на основе (-)-склареола и (+)-лариксола, выяснение механизмов реакций, подтверждение структур, определение биологической активности синтезируемых соединений и проведение дополнительного фитохимического исследования местных растительных источников, представляющих интерес, тестирование биологической активности.

Задачи работы: Заключаются в разработке новых методов синтеза природных аналогов тетра- и пентанорлабдановых высоко-функционализированых соединений и диазиновых, 1,2,4-триазольных, 2-амино-1,3-тиазольных, 1,3,4-оксадиазольных, 1,3,4-тиадиазольных молекулярных гибридов, из отходов растительного происхождения, классическими и неклассическими методами (фото- и электрохимические, микроволновое облучение); выяснение возможных механизмов реакции; проведение фитохимических исследований местных расстительных источников; биологические тестирования синтезированных соединений и растительных экстрактов для дальнейшего их использования; установление связи структура-активность.

Научная новизна и оригинальность: Впервые осуществлены эффективные, оригинальные, стерео- и региоселективные синтезы природных аналогов, норлабдановых высоко-функционализированых соединений и ряда гетероциклических молекулярных гибридов; предложены механизмы некоторых реакций; проведены фитохимические исследования местных растительных источников; идентифицирован ряд синтетических соединений с повышенной противомикробной активностью и установлена взаимосвязь структура-активность.

Полученые принципиально новые результаты для науки и практики: Заключаются в разработке новых методов превращения природных лабдановых дитерпеноидов (-)-склареола и (+)-лариксола, полученных из отходов шалфея мускатного и смолы лиственницы, в биологически активные природные или синтетические соединения с возможным применением в фармацевтической промышленности, в том числе за счет экологически чистых методов синтеза. Разработано новое направление синтеза гетероциклических молекулярных гибридов, обладающих биологической активностью. Определен фитохимический состав местных источников пищевого и лечебного значения, таких как яблоки и лесные ягоды, ароматические и эфиромасличные растения.

Теоретическая значимость: Результаты исследований подтверждают важность и возможность преобразования некоторых отходов в соединения, имеющие практическое значение; демонстрируют полезность и эффективность применения неклассических методов синтеза в химии природных соединений; дополняет научные сведения о механизмах реакций ряда норлабдановых соединений, в том числе с гетероциклическими единицами; и о взаимосвязи структура-активность синтетических соединений, качестве и полезности местных растительных материалов и продуктов полученных из них.

Результаты исследований подтверждают возможность и практическую целесообразность преобразования местных отходов в соединения, имеющие практическое значение; демонстрируют полезность и эффективность применения неклассических методов синтеза в химии природных соединений; дополняют научную информацию о механизмах реакций в ряду норлабдановых соединений, в том числе гетероциклических гибридах; и о взаимосвязи структура-активность синтетических соединений, о качественном составе и практической пользе местного растительного сырья и продуктов, полученных из него.

Практическое значение: Тестирование антимикробной активности синтетических соединений подтвердило, что 29 из них проявляют выраженную активность. Фитохимическое исследование местного растительного сырья подтвердило соответствие химического состава местного растительного сырья и продуктов, полученных из него, действующим нормативам. Полученные результаты представляют интерес для фармацевтических компаний, местных производителей плодов, ароматических, эфиромасличных и лекарственных растений, а также лиц, занимающихся их переработкой. Использование неклассических методов, таких как фотолитическая деградация, сенсибилизированное фотоокисление, анодное окисление и микроволновое облучение, оказывают положительное влияние на окружающую среду и оправдывают экологически чистые, неагрессивные и дешевые технологии. Получено 4 патента на изобретения, в которых заявлены методы синтеза биоактивных соединений.

Внедрение научных результатов: Результаты исследований внедрены в учебный процесс по курсам «*Биоорганическая химия*» и «*Синтетическое и полусинтетическое получение активных веществ*» по учебной программе по специальности *Технология косметических и лекарственных средств*. Были запатентованы методы синтеза и активность 4 новых норлабдановых соединений, которые могут быть исспользованны на практике в качестве противогрибковых средств.

CIOCARLAN, ALEXANDRU

SYNTHESIS OF BIOLOGICALLY ACTIVE NORLABDANE DERIVATIVES WITH ADVANCED FUNCTIONALIZATION AND PHYTOCHEMICAL STUDY OF SOME LOCAL VEGETAL SOURCES

143.04 – BIOORGANIC CHEMISTRY, CHEMISTRY OF NATURAL AND PHYSIOLOGICALLY ACTIVE COMPOUNDS

Summary of doctor habilitat thesis in chimical sciences

Approved for print: *data* 21.03.2024 Paper format 60x84 1/16
Offset paper. Offset printing. Circulation 30 ex…
Pattern sheets.: 4.4 Order nr. 37/24

USM Editorial-Polygraphic Center
60 Al. Mateevici str., Chisinau, MD, 2009
e-mail: cep1usm@mail.ru