

**ISOLATION AND STRUCTURE ELUCIDATION  
OF SESQUITERPENOIDS FROM THE ESSENTIAL OILS  
OF SOME LIVERWORTS (HEPATICAE)**

DISSERTATION

In Fulfillment of the Requirements for the Degree of *Dr. rer. nat.*  
at the Institute of Organic Chemistry, University of Hamburg

by

**Adewale Martins Adio**  
from Ibadan (Nigeria)

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**1. Gutachter:** Prof. Dr. Dr. h. c. W. Francke  
Institut für Organische Chemie, Universität Hamburg

**2. Gutachter:** Prof. Dr. J. Voß  
Institut für Organische Chemie, Universität Hamburg

**1. Prüfer:** Prof. Dr. Dr. h. c. W. Francke  
Institut für Organische Chemie, Universität Hamburg

**2. Prüfer:** Prof. Dr. J. Voß  
Institut für Organische Chemie, Universität Hamburg

**3. Prüfer:** Prof. Dr. C. Meier  
Institut für Organische Chemie, Universität Hamburg

**4. Prüfer:** Dr. S. Franke  
Institut für Organische Chemie, Universität Hamburg

**5. Prüfer:** Dr. V. Sinnwell  
Institut für Organische Chemie, Universität Hamburg

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Dedicated to my Mother and Family

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## List of Abbreviations

base peak	peak representing the most abundant ion in the spectrum
C	carbon
C <sub>6</sub> D <sub>6</sub>	hexadeuteratedbenzene
CDCl <sub>3</sub>	deuteratedchloroform
<sup>13</sup> C-NMR	<sup>13</sup> carbon nuclear magnetic resonance
CD	cyclodextrin
CI	chemical ionization
COSY	correlation spectroscopy
2D	two dimensional
d	doublet
DEPT	distortionless enhancement by polarization transfer
eV	electron volt
EI	electron ionization
Fig.	figure
FPP	farnesyl pyrophosphate
GC	gas chromatography
H	proton
HPLC	high performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
Hz	hertz
J	coupling constant
<i>m/z</i>	mass-to-charge ratio
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
NOESY	nuclear overhauser spectroscopy
MS	mass spectrometry
m-CPBA	meta-chloroperbenzoic acid
prep.	preparative
ppm	parts per million
PDC	pyridinium dichromate
rel. int.	relative intensity
R <sub>f</sub>	ratio of fronts
s	singlet
t	triplet
TLC	thin layer chromatography

## **1. Introduction**

### **Development of Natural Products Chemistry**

Secondary metabolites are natural products resulting from metabolism that are not essential for normal growth, development or reproduction of the plant or organism. These plant constituents have diverse biological properties from an evolutionary point of view which go back to ancient times. The discovery of pure compounds as active principles in plants was first described at the beginning of the 19<sup>th</sup> century, and the art of exploiting natural products has become part of the molecular sciences. To date, more than 80% of the world's population use plants as their primary source of medicinal agents, and in many countries of the world there is a well-established system of traditional medicine, whose remedies are still being compiled.<sup>[1]</sup>

In the last two decades the approaches to the study of biologically active natural products have changed so dramatically that one is almost tempted to say that a new science has been born.<sup>[2]</sup> Of course, that is not really the case, but certainly the quiet, almost silent, revolution that has occurred has for ever altered the way that natural products research will be conducted. Eventhough, the alarming rate of disappearance of traditional healers and their apprentices has been expressed,<sup>[3]</sup> and the evolution of genomics, proteomics, combinatorial chemistry, and ultrahigh-throughput screening seem rapidly increasing, however, natural products discovery programmes are under increasing pressure.

Few areas in which strategies for the conduct of natural product chemistry have changed include plant selection and collection, isolation techniques, structure elucidations, biological evaluations, semisynthesis, dereplication, and biosynthesis.<sup>[1]</sup>

In addition, the natural product literature has also spawned several current awareness services, with more and more of the primary literature being available directly on-line, one can imagine that while strategies for publishing and accessing the chemical literature on natural products have changed substantially in the recent past, they will change even more dramatically in the very near future.<sup>[2]</sup>

It is apparent that the pace of natural products research and the level of global interest in this particular area of our environment has risen dramatically in the past few years. This trend is projected to continue for the future as the interface between chemistry and biology becomes even more interwoven, and the public demand rises for cost effective medications and biological agents from sustainable resources.

## 2. Objective of Research

Despite the suggestion that components of the oil bodies found in liverworts consisted of sesquiterpenes,<sup>[4]</sup> the chemical constituents have only been investigated during the last two decades because of the collection and separation of a large quantity of a single species and its identification are difficult and time-consuming.<sup>[5]</sup>

Previous reports on the chemical analysis of the liverworts revealed that often liverworts produce unique sesquiterpenoids with novel carbon skeletons which are not isolated from higher plants and most of the liverwort sesquiterpenoids correspond to the enantiomers of those of higher plants. In addition, several biologically active compounds<sup>[5]</sup> and a large variety of sesquiterpene hydrocarbons which are important intermediates in the biosynthesis of functionalized sesquiterpenes which may be useful reference compounds in studying the product specificity of sesquiterpene synthases, particularly after site-specific mutagenesis, have been isolated from liverworts.

Therefore, the isolation and structure elucidation of sesquiterpene hydrocarbons and oxygenated sesquiterpenes of some European liverworts from the families Gymnomitriaceae, Scapaniaceae, Plagiochilaceae and Jungermanniaceae, were carried out.

Isolation and Identification involve:

- Collection and proper botanical identification of the liverworts.
- Extraction of the ground liverwort materials; hydrodistillation, extraction with solvents of different polarity at room temperature e.g. hexane, dichloromethane, diethyl-ether.
- GC and GC-MS study of the essential oils and extracts by comparison of the mass spectra obtained with known compounds in a data bank established under identical experimental conditions.
- Isolation of compounds with unknown mass spectra by a combination of chromatographic techniques; column chromatography (CC), preparative gas chromatography (PGC) and thin layer chromatography (TLC), etc.
- Structure elucidation by mass spectrometry (MS), as well as one- and two dimensional NMR techniques (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, 2D- <sup>1</sup>H-<sup>1</sup>H-COSY, HMBC, HMQC, NOESY).
- Determination of absolute configuration of the isolated compounds by chemical correlation (hydrogenation, acid or thermal rearrangement reactions, etc.) with known compounds monitored by enantioselective gas chromatography (GC).

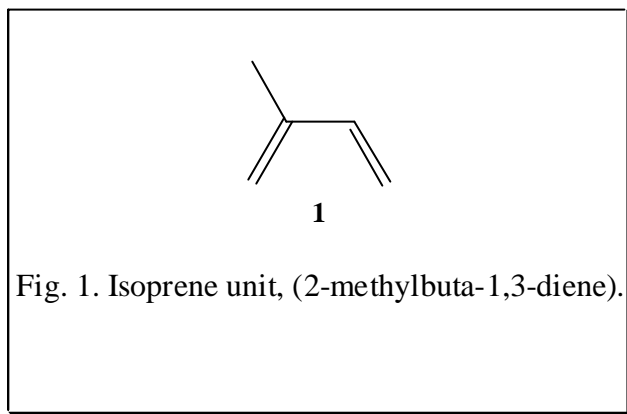


### 3. General Part

In this chapter, terpenoids and its biosynthesis, essential oils, methods of essential oil extraction, liverworts, germacrane, analytical methods, cyclodextrins, and determination of absolute configuration are discussed.

#### 3.1. Terpenoids

The terpenoids, also called isoprenoids, are one of the largest groups of natural products found in nature with more than 30,000 known examples, and their number is growing steadily.<sup>[6]</sup> Terpenoids are all based on the isoprene unit, 2-methylbuta-1,3-diene (**1**) (Fig. 1), and their carbon skeletons are built up from the union of two or more of these C<sub>5</sub> units - 'isoprene rule' proposed by Wallach, (1907 & 1909) and Ruzicka et al. (1953).<sup>[7-9]</sup>



Terpenoids range from the essential oil components, the volatile mono- and sesquiterpenes (C<sub>10</sub> and C<sub>15</sub>), through the less volatile diterpenes (C<sub>20</sub>) to the non-volatile triterpenoids and sterols (C<sub>30</sub>), carotenoid pigments (C<sub>40</sub>) and polyisoprene (C<sub>n</sub>). Each of these various classes of terpenoid are of significance in either plant growth, metabolism or ecology.<sup>[10]</sup> Most natural terpenoids have cyclic structures with one or more functional groups (hydroxyl, carbonyl, etc.), hence, the final steps in the synthesis involve cyclization and oxidation or other structural modification such as skeletal rearrangements.

#### 3.2. Terpenoid Biosynthesis

The formation of the common isoprene-derived subunit has been extensively studied over the last 50 years, leading to a generally accepted pathway from acetate activated as acetyl-coenzyme A (**2**), via acetoacetyl-coenzyme A (**3**), 3-hydroxy-3-methylglutaryl-coenzyme A

(5), and mevalonate (7) to isopentenyl diphosphate (IPP) (10), the first precursor possessing the branched C<sub>5</sub>-isoprenic skeleton<sup>[11-12]</sup> (Fig. 2).

A few years ago, however, incorporation of <sup>13</sup>C-labeled acetate and glucose into triterpenoids of the hopane series and the prenyl chain of ubiquinone from several bacteria proved unambiguously that the classical acetate / mevalonate pathway was not operating in all living organisms and that the isoprenic skeleton can be formed from triose phosphate derivatives via a non-mevalonate pathway.<sup>[13-15]</sup>

In addition, it has recently been established by incorporation of [1-<sup>13</sup>C]- and [U-<sup>13</sup>C<sub>6</sub>]glucose that monoterpene essential oils (geraniol, menthone, pulegone, thymol) are biosynthesised in plants by a pathway which is different from the established mevalonic acid route.<sup>[16]</sup>

### 3.2.1. The mevalonate pathway

The mevalonate pathway (Fig. 2) involves the enzymatic condensation of two molecules of acetyl-CoA (2) by acetoacetyl-CoA thiolase to form acetoacetyl-CoA (3). This reaction is followed by a nucleophilic attack of the acetyl-S-enzyme (4) derived from acetyl-CoA (2) to subsequently form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (5) by the enzyme HMG-CoA synthase. The enzyme HMG-CoA reductase (HMGR) catalyses the reductive deacylation of HMG-CoA (5) to mevalonate (MVA) (7) via mevaldate (6) and employs two equivalents of NADPH as reductant. This is followed by mevalonate kinase catalyses of the first ATP-dependent phosphorylation of mevalonate (7) to mevalonate 5-phosphate (8). Subsequently, mevalonate 5-diphosphate (9) is produced by the further action of phosphomevalonate kinase. These reactions lead to the formation of isopentenyl diphosphate (IPP) (10). The IPP isomerase catalyses the 1,3-allylic rearrangement reaction converting IPP (10) into dimethylallyl diphosphate (DMAPP) (11), IPP and DMAPP being the biogenetic isoprene units (Fig. 2).

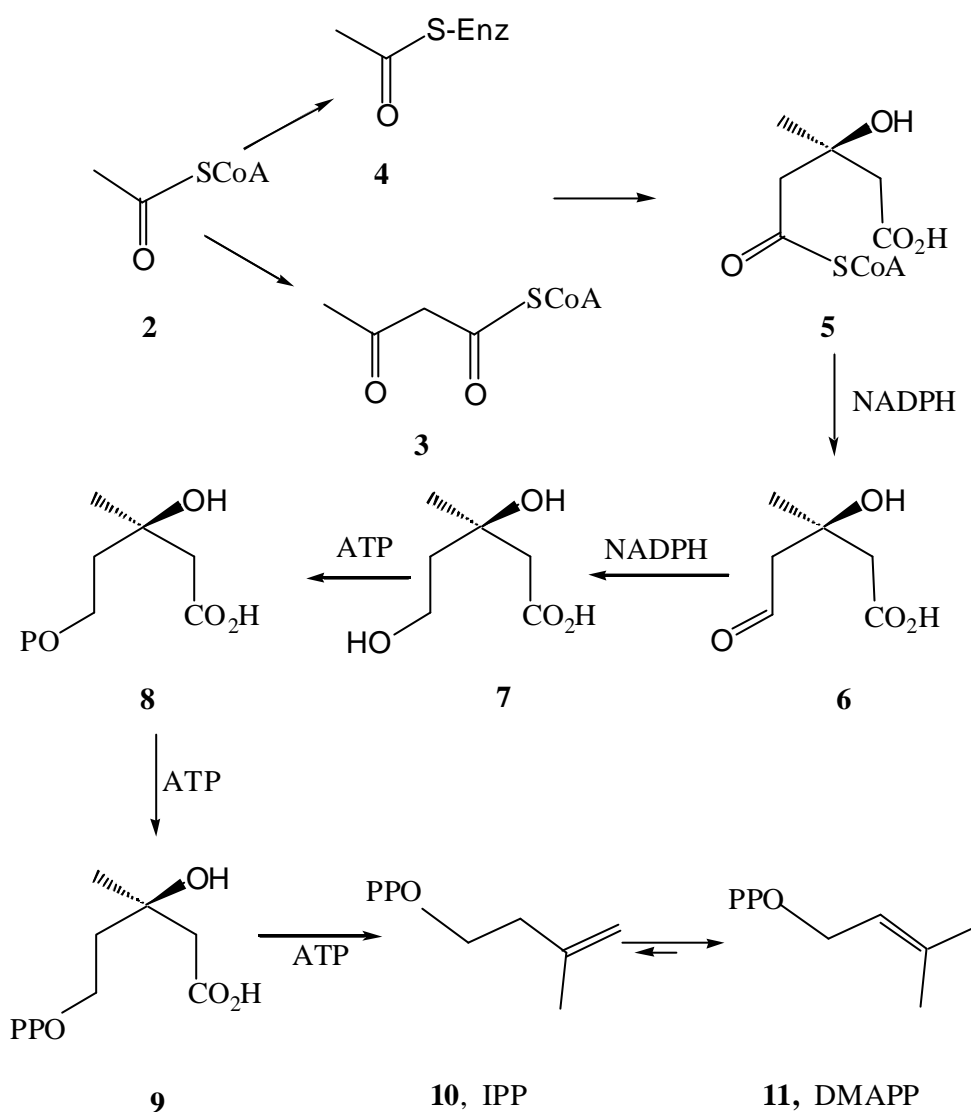


Fig. 2. The mevalonate pathway.

### 3.2.2. The mevalonate-independent (deoxyxylulose phosphate) pathway

The preliminary reactions in mevalonate-independent pathway (Fig. 3) involve the reaction of pyruvate (12) with thiamine diphosphate (13) to form pyruvate-thiamine diphosphate complex (14) which undergoes decarboxylation to generate (hydroxethyl)thiamine diphosphate (15). The first reaction of this pathway is a transketolase-like condensation between pyruvate (12) and D-glyceraldehyde 3-phosphate (16) to form 1-deoxy-D-xylulose 5-phosphate (DXP) (17). This involves condensation of (hydroxethyl)thiamine diphosphate (15), derived from pyruvate (12), with the aldehyde group of glyceraldehyde 3-phosphate (16). DXP (17) is then transformed into 2-C-methyl-D-erythritol-4-phosphate (MEP) (18). The anticipated intermediate aldehyde (2-C-methylerythrose-4-phosphate) (19) is not released

from the enzyme but is simultaneously reduced by NADPH. Subsequent reactions lead to the formation of isopentenyl diphosphate (IPP) (**10**) and DMAPP (**11**). In contrast to the mevalonate pathway, where IPP (**10**) isomerized to DMAPP (**11**), this last isomerization is yet to be confirmed as there is growing evidence that it may not occur in the non-mevalonate pathway.<sup>[12]</sup> Moreover, the non-mevalonate pathway for isoprenoid biosynthesis has been confirmed in several bacteria,<sup>[17-18]</sup> and recently for the biosynthesis of diterpenoids in two higher plants, *Ginkgo biloba* and *Salvia miltiorrhiza* <sup>[19]</sup> as well as for the formation of all isoprenoids (i.e. sterols, prenylquinones, phytol, carotenoids) of the unicellular green algae *Scenedesmus obliquus*.

Several terminologies commonly in use for this pathway, include mevalonate-independent pathway, glyceraldehyde 3-phosphate / pyruvate pathway, deoxyxylulose phosphate (DXP or DOXP) pathway, and methylerythritol phosphate (MEP) pathway.<sup>[12]</sup>

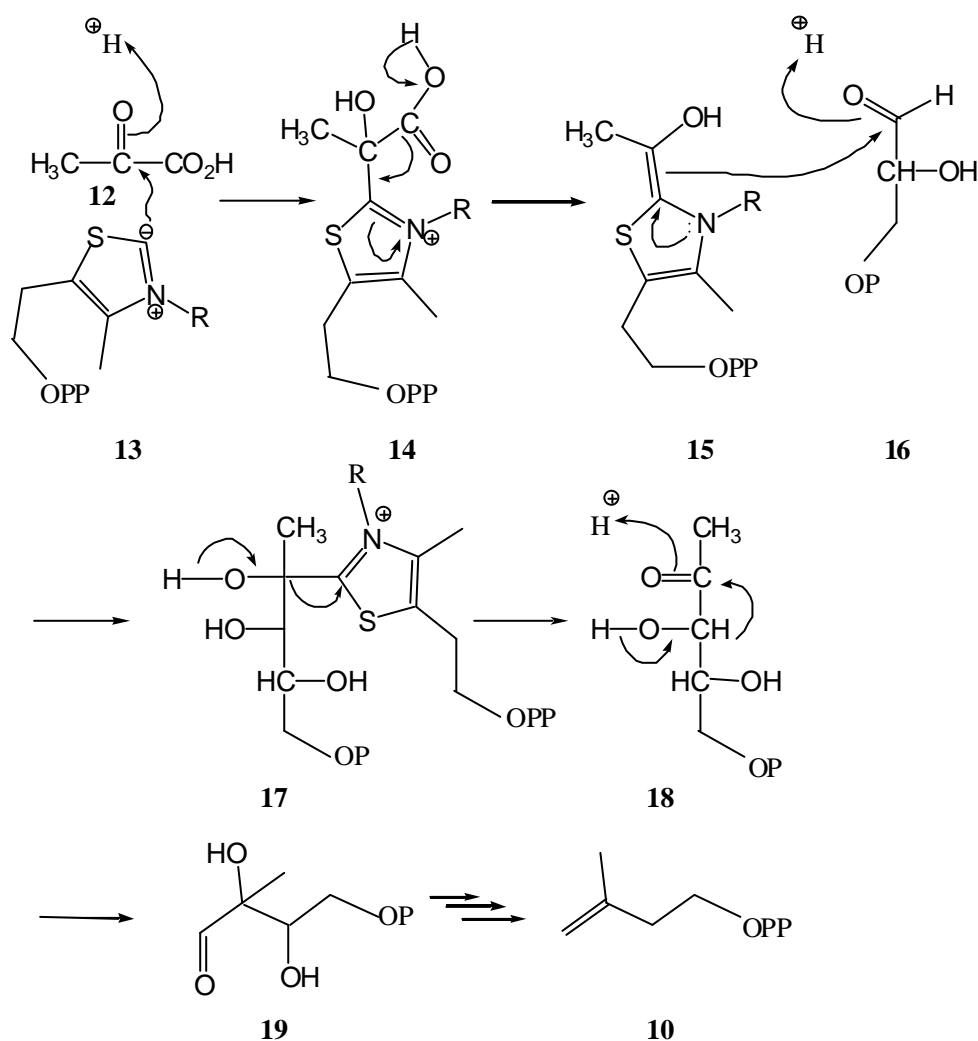


Fig. 3. Hypothetical biogenetic non-mevalonate pathway.

### 3.3. Prenyltransferases (Fig. 4)

Prenyltransferases are responsible for the alkylation steps involving DMAPP (**11**) and one or more IPP (**10**) residues. These reactions provide the polyprenyl diphosphate precursors for the various terpenoid families. Many polyprenyl diphosphate synthase enzymes are relatively non-specific and catalyse the condensation of DMAPP (**11**) with IPP (**10**) units to build up polyprenyl diphosphate chains of different lengths. Farnesyl diphosphate synthase catalyses the successive condensations of IPP (**10**) with both DMAPP (**11**) and geranyl diphosphate (GPP) (**20**), the precursor to a wide variety of monoterpeneoids, to give farnesyl diphosphate (FPP) (**21**), the intermediate in sesquiterpenoids formation. Geranylgeranyl diphosphate (GGPP) (**22**) synthase catalyses further the condensation step between IPP (**10**) giving GGPP (**22**) the normal progenitor of diterpenoids.<sup>[12]</sup>

Recently, the isolation of the cDNAs encoding (+)- and (-)-germacrene D-synthase,  $\alpha$ -gurjunene- and cascarilladiene synthase from *Solidago canadensis* L. and their functional expression in *E. coli* have been concluded.<sup>[21]</sup>

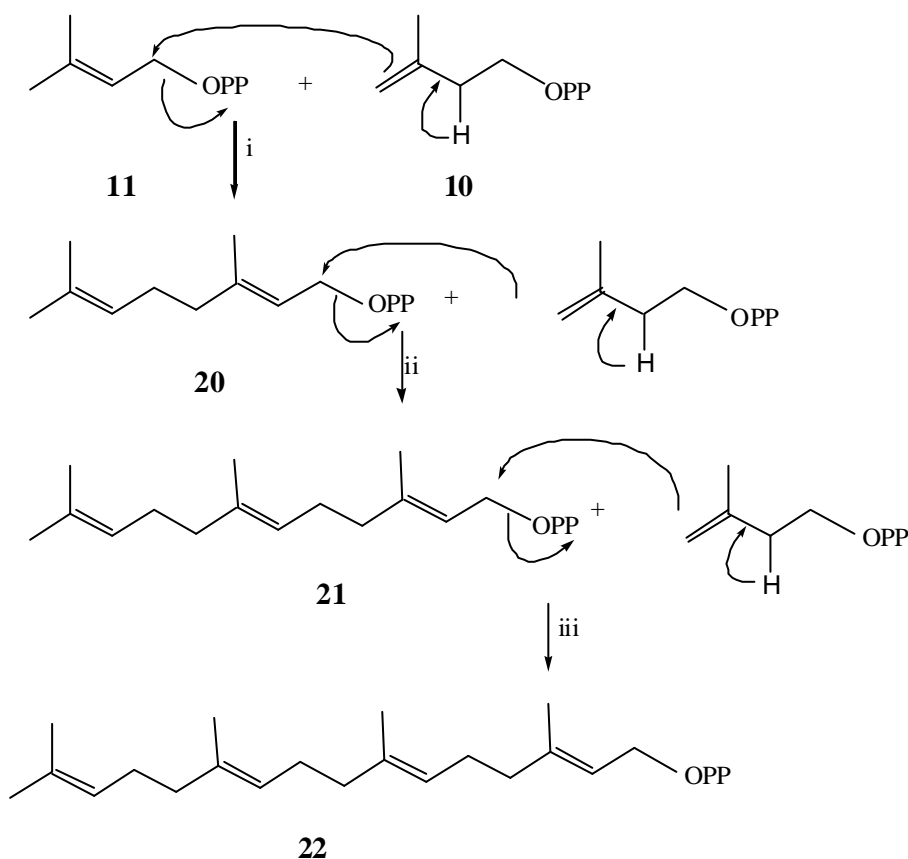


Fig. 4. Enzymes: i, geranyl diphosphate synthase; ii, farnesyl diphosphate synthase; iii, geranylgeranyl diphosphate synthase.

### 3.4. Biosynthesis of Sesquiterpenes

Sesquiterpenes are widespread in higher plants, liverworts and mosses, fungi, algae, arthropods, marine invertebrates, and microorganisms. In the last two decades studies of sesquiterpene biosynthesis have entered an entirely new phase, as several individual cyclases catalyzing the conversion of farnesylpyrophosphate (FPP) (**21**) to a variety of sesquiterpenes have been isolated and characterized. The availability of sesquiterpene synthases, in crude or purified form, has allowed detailed mechanistic analysis of the cyclization reactions which lies at the heart of terpenoid biosynthetic theory.<sup>[20]</sup> Hence, various carbon skeletons of sesquiterpenes were shown to be products of cyclisations of either farnesylpyrophosphate (FPP, **21**) or nerolidyl diphosphate (NPP) (Fig. 5).

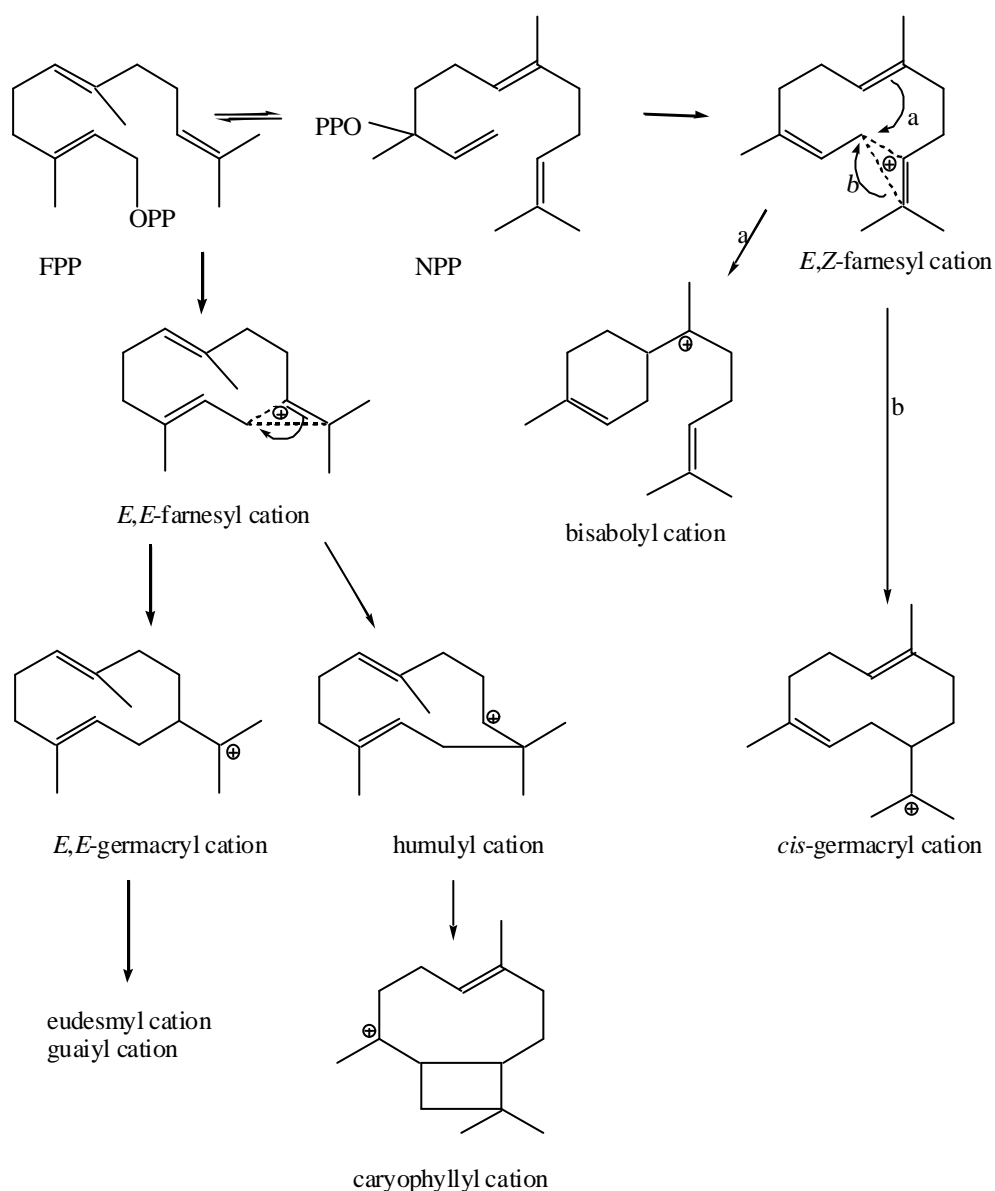


Fig. 5. Sesquiterpenes cyclization.

The initial step in these cyclisations involves a removal of the pyrophosphate anion accompanied by participation of either the central or terminal double bonds leading to the corresponding cations (e.g. germacryl, humulyl, bisaboly, etc.). Common naturally occurring cyclized sesquiterpene skeletons include eudesmane, longipinane etc. (Fig. 6).

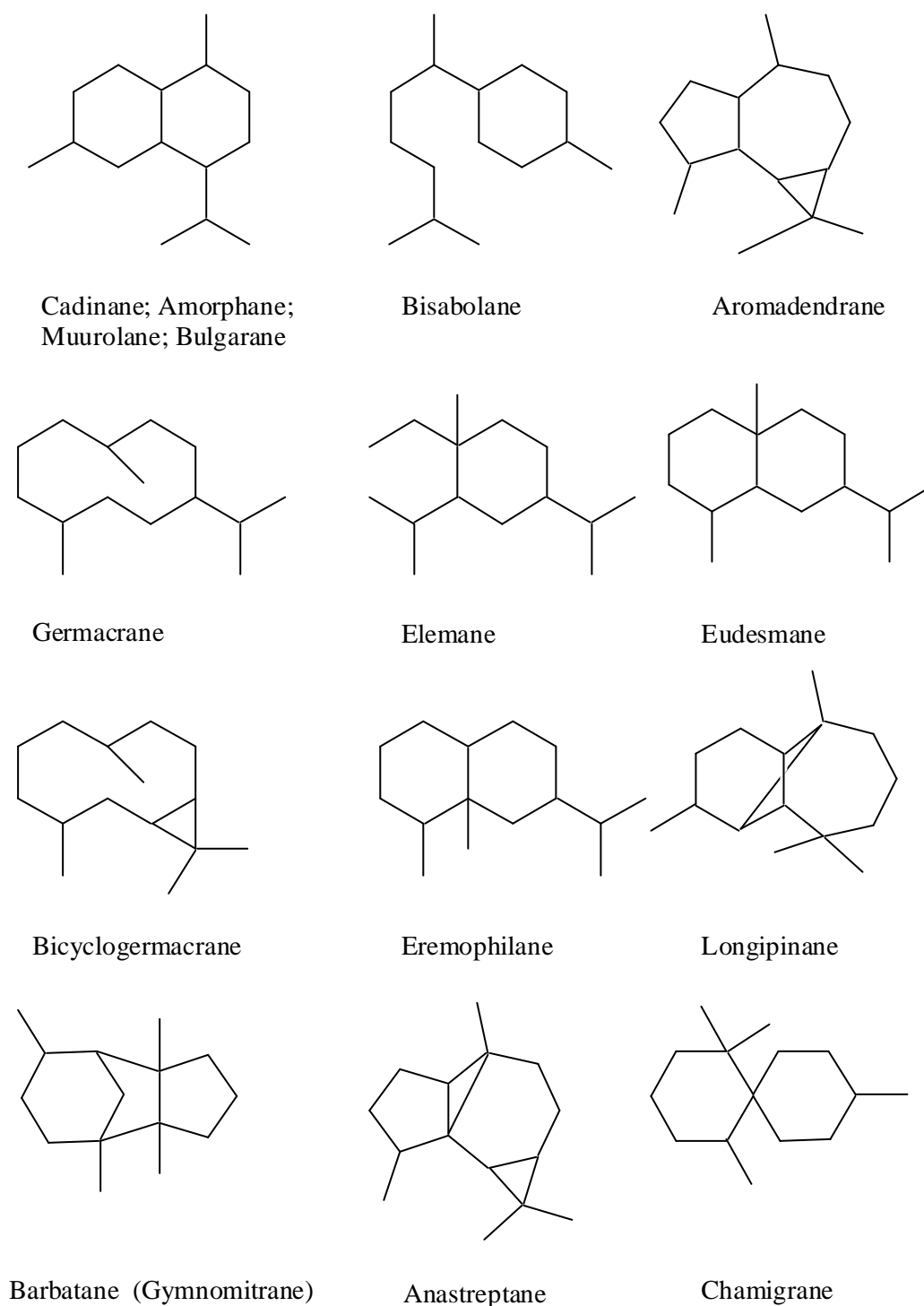


Fig. 6. Common cyclic sesquiterpene skeletons.

### **3.5. Essential Oil**

Essential oils, also known as volatile oils or essences, are volatile secondary metabolites that plants produce for their own needs other than nutrition (i.e. protectant or attractant).<sup>[22]</sup> The term 'essential oil' is also used to describe the oils captured via water or steam distillation or by mechanical processing of citrus rinds or dry distillation of natural materials. In general, they are complex mixtures of volatile organic compounds that give characteristic odour and flavour to the plants. Chemically, the terpenes essential oils can be divided into two classes, the mono-, and the sesquiterpenes, C<sub>10</sub> and C<sub>15</sub>-isoprenoids, which differ in their boiling point range (monoterpenes b.p. 140-180 °C, sesquiterpenes b.p. > 200 °C).<sup>[10]</sup>

These oils are found in special secretory glands or cells within plants,<sup>[4]</sup> and their accumulation is dependent on the developmental stage / phase as well as its concerned part / organ, tissue, and the cells. The essential oil production is highly integrated with the physiology of the whole plant and so depends on the metabolic state and preset developmental differentiation programme of the synthesising tissue and impact of abiotic stresses (moisture, soil salinity, temperature).<sup>[24]</sup> The same species of plant can produce an essential oil with different properties depending on whether it was grown in dry or damp earth, at high or low altitude or even in hot or cold climates.<sup>[22]</sup> Essential oils can be extracted from leaves, flowers, buds, twigs, rhizomes, heartwood, bark, resin, branches or whole plant, seeds and fruits.<sup>[23]</sup> Examples of higher plant families particularly rich in essential oils include the Compositae, Matricaria, Labiatae, e.g. the mints, Eucalyptus, Rutaceae, Citrus oils, dill etc. Essential oils are commercially important as the basis of natural perfumes and also of spices and flavourings in the food industry and are generally expensive. Thus, the high price of the natural oils coupled with their limited availability has encouraged a search for substitutes.

### **3.6. Methods of Extraction**

Several methods are used for the extraction of essential oils. The common methods are briefly discussed. The methods employed depend on the nature of the sample and other factors such as thermodynamic stability of the essential oil constituents.

#### **3.6.1. Hydrodistillation**

Hydrodistillation is one of the selective way of extracting essential oils. It involves boiling of the plant material in water and capturing the resultant steam, condensing it into water and essential oil, and then separating the two phases. This method requires a well regulated



heating system to avoid decomposition, rearrangements of terpenes, and hence, produces a lesser quality oil. The apparatus commonly used is called clevenger hydrodistillator.

### **3.6.2. Steam Distillation**

Steam distillation is the most common method used to capture essential oils. This involves placing the fresh plant material onto a grill situated inside a steel tank, followed by spraying pressurized steam through the material from below. The heat allows the essential oils to be released and travel with the steam up and into a condensation tube. Where, the steam is condensed to form an aqueous phase and essential oil, with the essential oil floating on top. Subsequently, the essential oils are skimmed off the top or since most are less dense than water, they are allowed to pour into a separate chamber. The water produced contains other important plant constituents, mostly water-soluble substances. Most plant materials that are steam distilled are fresh. This method yields higher quality essential oils than hydrodistillation since the amount of plant material exposed to the steam is minimal, thus reducing the deterioration of the essential oil components.

### **3.6.3. Solvent Extraction**

Solvent extraction involves soaking of the plant material into suitable solvent of various polarity, such as hexane or diethyl ether, to produce a crude extract. This extraction method yield, in addition to essential oils, substantial amount of other plant constituents such as resins, pigments, waxes, and other non volatile materials and avoids the danger of artifact formation via isomerization, polymerisation, dehydration etc., at raised temperature involved in methods such as hydrodistillation.

### **3.6.4. Cold Expression**

Cold expression involves mechanically pressing of the plant material, followed by spraying of water to ensure that all of the oil and pulp are captured. The mixture is then centrifuged. This form of extraction is commonly used for the citrus fruits.

### **3.6.5. Super Critical Carbon-Dioxide Extraction (CO<sub>2</sub>)**

Super critical carbon dioxide extraction process utilizes CO<sub>2</sub>, to extract most of the constituents of the plant material. The latter is placed in a stainless steel tank, the CO<sub>2</sub> is added, and pressure is increased inside the tank. With the high pressure and lower temperatures the CO<sub>2</sub> liquefies and acts as a solvent extracting the oils and other plant

constituents. Then the pressure is decreased to allow the CO<sub>2</sub> to evaporates leaving a pure extract completely free of solvents.

There are two types of CO<sub>2</sub> extracts, selective or total. Selective extracts are produced using lower pressures and contain the volatile oils of the plant, very much like those produced via steam distillation. Whereas in total extracts other plant constituents besides the volatile essential oil such as waxes, pigments, and fats are extracted using higher pressure.

### 3.7. Liverworts

The bryophytes are taxonomically placed between algae and pteridophytes, with approximately 18,000 species or more, world wide.<sup>[25-26]</sup> Bryophytes have been hypothesized to represent several quite separate evolutionary lines and classified into three coordinate phyla: Anthocerotophyta (hornworts), Bryophyta (mosses), and Hepaticae (liverworts).<sup>[25-26]</sup>

The Hepaticae contain oil bodies which are easily extracted with organic solvents whilst the other two classes do not contain oil bodies.<sup>[27]</sup> A typical liverwort *Marsupella emarginata* is shown in (Fig. 7).



Fig. 7. Liverwort: *Marsupella emarginata*.

Liverworts often produce unique sesquiterpenoids with carbon skeletons which are not isolated from higher plants, and the absolute configuration of most of the liverwort sesquiterpenoids correspond to the enantiomer of those of higher plants.<sup>[28-30]</sup> Exceptions are e.g. (-)-frullanolide (**23**) which occurs in both enantiomeric forms<sup>[31]</sup> or, (-)-caryophyllene (**24**) which is found in *Scapania undulata*.<sup>[32]</sup>

In higher plants mainly enantiomerically pure sesquiterpenoids<sup>[33]</sup> are formed and very few examples of the presence of their enantiomers have been reported.<sup>[34]</sup> An example is *Solidago canadensis* in which the presence of two enantiospecific synthases (cyclases) was found to be responsible for the generation of the two germacrene-D (**25**) enantiomers<sup>[21,35]</sup> (Fig. 8).

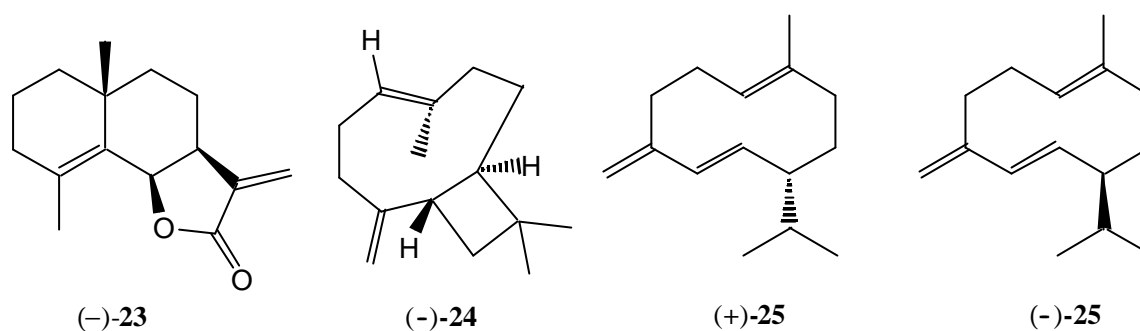


Fig. 8. Sesquiterpenes isolated from the liverworts *Scapania undulata*, *Frullania* species and *Solidago canadensis*.

Furthermore, in contrast to the liverworts, the optical antipodes of monoterpenoids generally occur in higher plants i.e they are often produced as mixture of enantiomers<sup>[36]</sup> (Fig. 9).

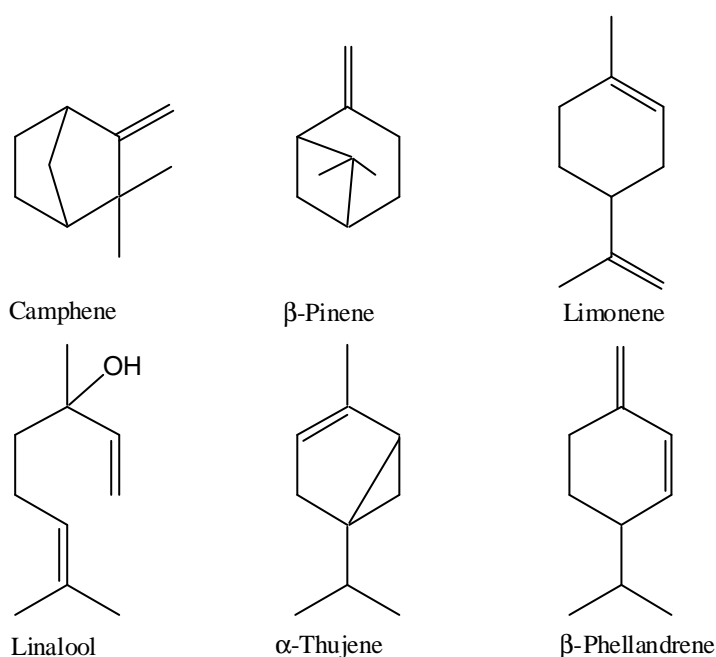


Fig. 9. Common monoterpenes in both liverworts and higher plants.

### 3.7.1. Biologically Active Compounds from Liverworts

Many natural products, other than alkaloids, show medicinal properties or other biological activities. Among these are sesqui- and diterpenoids as well as phenolic compounds isolated by solvent extraction from the oil bodies of bryophytes. Some of these biological activities include anti-microbial, anti-fungal, cytotoxic, insect antifeedant, muscle relaxing, some enzyme inhibitory, and apoptosis inducing activity, etc.<sup>[27]</sup>

Few of the biologically active compounds of liverwort origin include, Plagiochiline-A (**26**) from *Plagiochila fruticosa* and *P. ovalifolia* and Marsupellone (**27**) from *Marsupella emarginata*. Both **26** and **27** showed antitumor activity.<sup>[37]</sup> Lemnalone (**28**), the oxidative product of lemnalol (**29**) has been reported to exhibit remarkably strong cytotoxicity to DBA/MC fibrosarcoma cells *in vitro*.<sup>[38]</sup> Lemnalone has been isolated for the first time in this work from the liverwort *Marsupella emarginata* (Fig. 10).

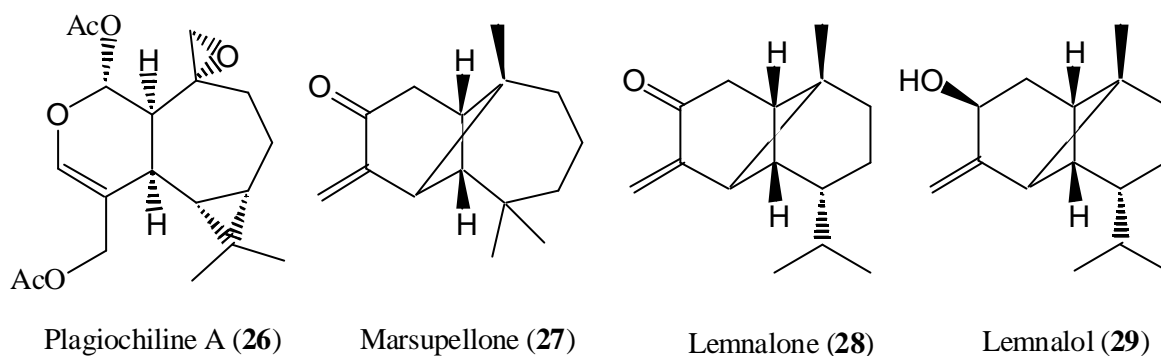


Fig. 10. Examples of biologically active compounds from liverworts.

Lemnalol (**29**) also inhibits tumor growth *in vivo*.<sup>[38]</sup> Another biologically active compound, Artemisinin (**30**), an antimalaria drug, was isolated from the Chinese medicinal plant *Artemisia annua*.<sup>[39]</sup> The (-)-enantiomer of (+)-amorpha-4,11-diene (**31**) isolated from *Marsupella aquatica*<sup>[40]</sup> has been described as the intermediate precursor of Artemisinin (**30**)<sup>[41]</sup> (Fig. 11).

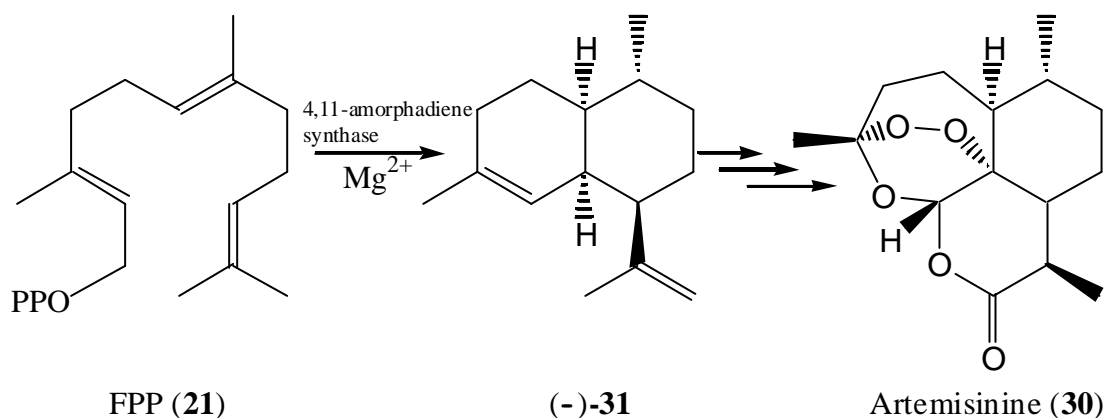


Fig. 11. Proposed pathway to Artemisinin (**30**) via Amorpha-4,11-dien (**31**).<sup>41</sup>

### 3.8. Germacranes

Germacranes are ten-membered monocyclic compounds which constitute important intermediates between farnesyl pyrophosphate (FPP) (**21**) and many other sesquiterpene structures (Fig. 5). Their unique structural and conformational features, general occurrence in nature, and their central role in the biosynthesis of other sesquiterpenes, have received much attention in modern organic chemistry.<sup>[42-44]</sup>

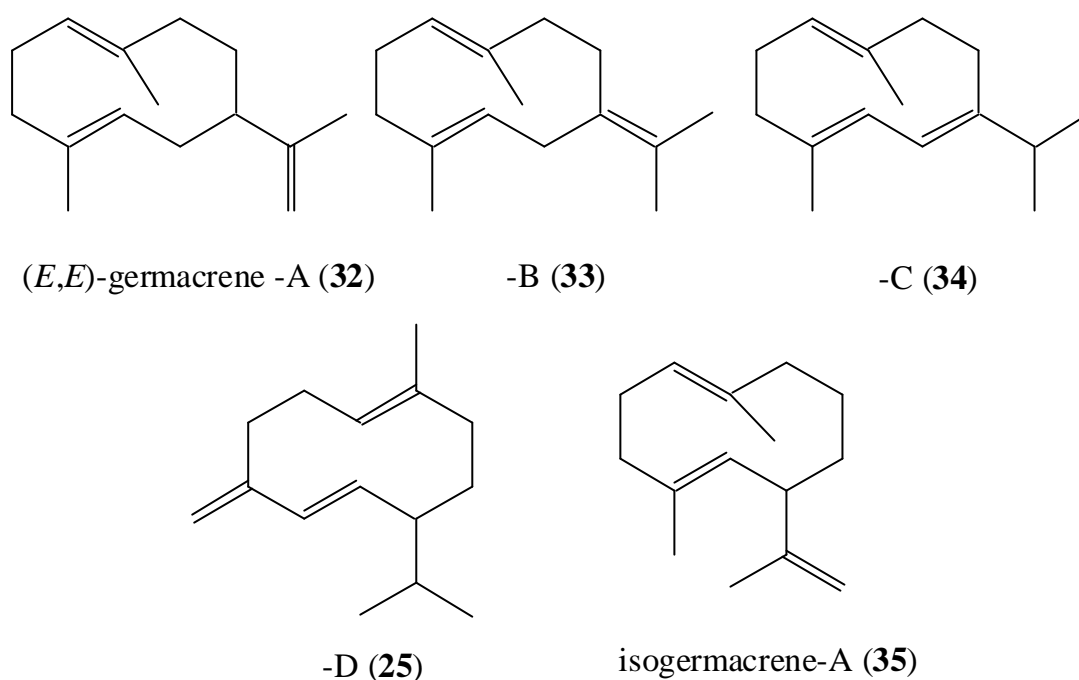


Fig. 12. Typical Germacrene structures.

The majority of known germacrenes (**32-34**) possesses the flexible (*E,E*)-cyclodeca-1(10),4-diene ring unit as the main structural characteristic (Fig. 12), but (*Z,E*)-germacrenes (melampolides), (*E,Z*)-germacrenes (heliangolides) and (*Z,Z*)-germacrenes are also found in nature (Fig. 13).<sup>[45]</sup> The new regioisomer of germacrene A (**32**), isogermacrene-A (**35**), recently isolated from the liverwort *Saccogyna viticulosa* (L.) Dum,<sup>[44]</sup> also belongs to the (*E,E*)-germacrene-group.

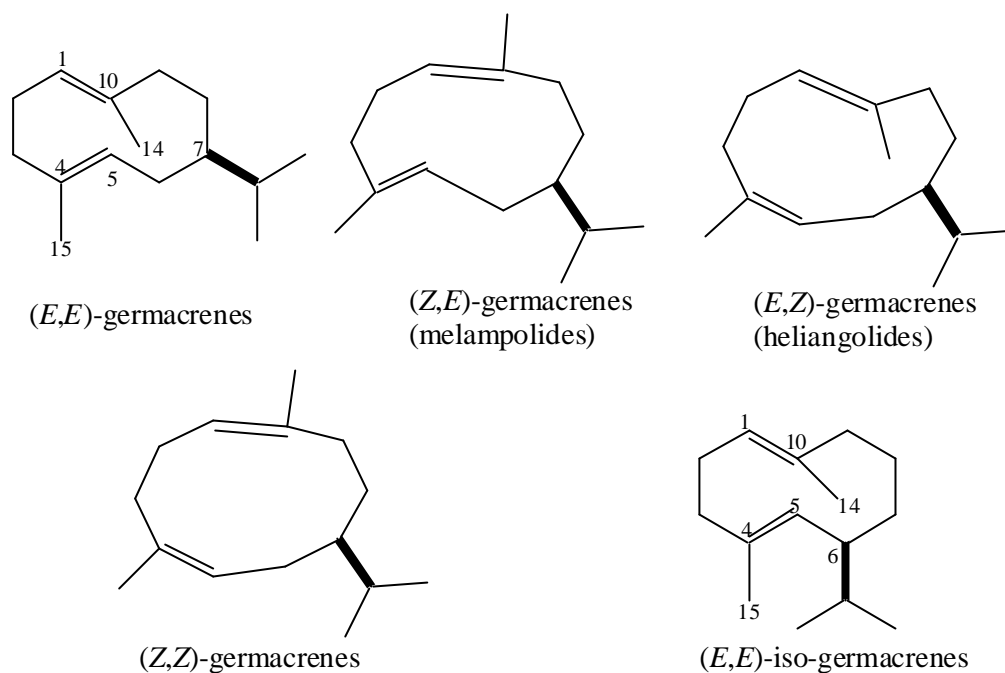


Fig. 13. Germacrene isomers.

The conformational behaviour of germacranes, in particular the (*E,E*)-germacranes, has been an area of study for decades.<sup>[46-48]</sup> These studies have shown that the 10-membered ring of germacranes can adopt four distinct conformations which can be denoted as UU, DU, DU, and DD. U (up) and D (down) refer to the orientation of the C(14) and C(15) methyl groups. These conformers have either a crossed (UU and DD) or parallel relation of the double bonds through the ring<sup>[49]</sup> and inversion of the C(7)-C(8) unit.<sup>[50]</sup> As indicated for hedycaryol (**36**), (Fig. 14) the conformation of the C(7)-C(8) segment in the 10-membered ring is largely determined by the C(7) substituent which tends to occupy a pseudoequatorial position.

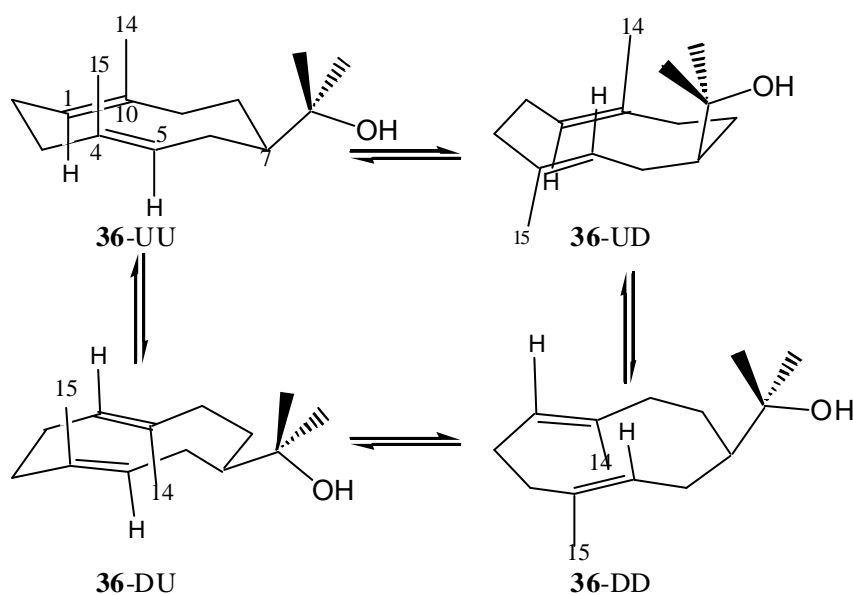


Fig. 14. Conformational behaviour of hedycaryol (**36**).

Many (*E,E*)-germacrenes show temperature-dependent NMR spectra indicative of conformational equilibria in solution. Hedycaryol (**36**), for example, exists in three different conformations at room temperature.<sup>[51]</sup> Since these conformations are interconverting at room temperature, transannular cyclization reactions of hedycaryol (**36**) only proceed via the most stable UU conformation.<sup>[52]</sup> This type of stereoselectivity in conformationally controlled reactions can often be predicted if the most stable conformation of the compound involved is known.<sup>[53]</sup> Correct predictions of most stable conformations can be produced by carrying out molecular mechanics calculations, often in combination with NMR and X-ray data.<sup>[54-55]</sup> The occurrence of these different germacrene conformer plays an important role in the biosynthesis of other sesquiterpenes.<sup>[56]</sup> The total synthesis of germacranes has been a long-standing problem mainly because of difficulties caused by their thermal instability and the ease with which these compounds undergo transannular cyclizations.<sup>[52,57-58]</sup>

### 3.8.1. The *Cope* Rearrangement

A very important reaction for the synthesis of germacrene sesquiterpenes is the [3,3] sigmatropic *Cope* rearrangement in which 1,2-divinylcyclohexanes are thermally converted into cyclodecadienes.<sup>[59]</sup> *Cope* rearrangement has been observed for members of the germacrene sesquiterpene skeleton family.<sup>[47]</sup> It is well known that germacrene C (**34**)<sup>[60]</sup> yields racemic  $\delta$ -elemene (**38**), germacrene B (**33**) forms racemic  $\gamma$ -elemene (**39**), isogermacrene A (**35**) yields iso- $\beta$ -elemene (**37**) and that (+)-germacrene A (**32**) is easily

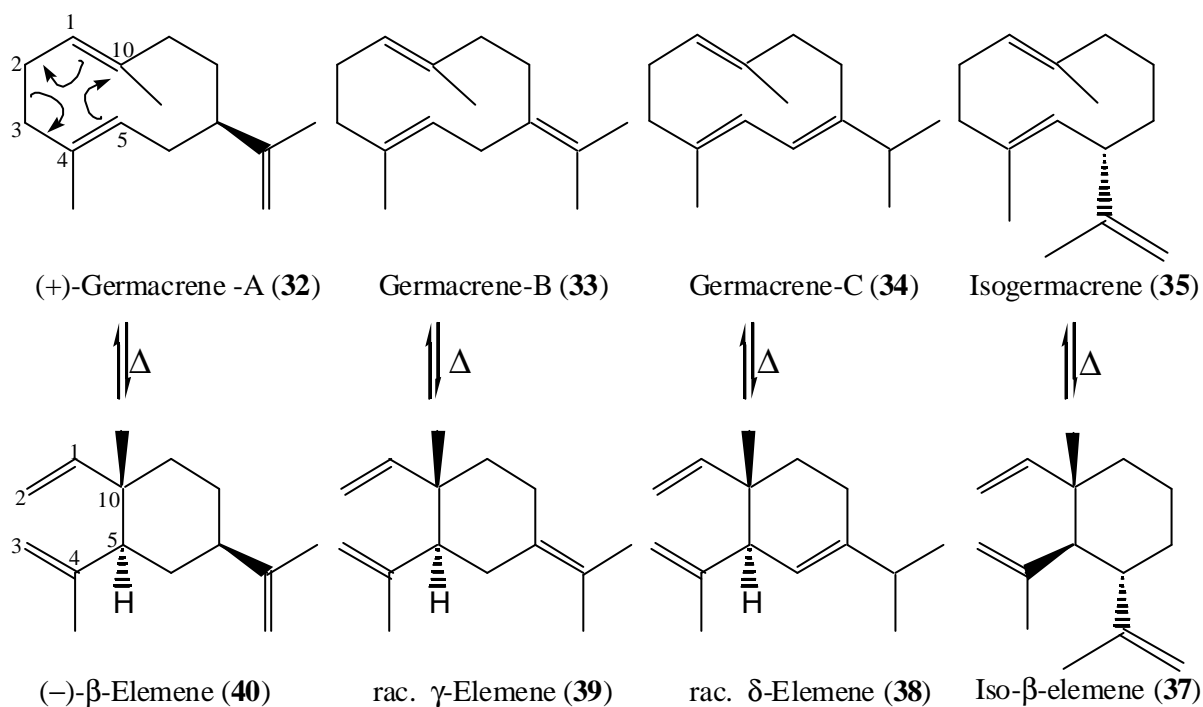


Fig. 15. Thermal or *Cope* rearrangements of germacrenes to elemenes.

converted into (-)- $\beta$ -elemene (**40**), (Fig. 15).<sup>[44, 46-47, 58]</sup>

Generally, the equilibrium is determined by the substitution pattern, conjugation effects, ring strain, conformation, and other factors.<sup>[61]</sup> Due to the reversible nature of the conventional *Cope* rearrangement, efforts to synthesize germacrene sesquiterpenes from 1,2-divinylcyclohexane precursors have met with only limited success.<sup>[62-63]</sup>

### 3.9. Analytical Methods

#### 3.9.1. Mass Spectroscopy

It is possible to determine the masses of individual ions in the gas phase. The most common method of ionisation involves electron impact (EI) and the alternative is chemical ionisation (CI). CI is a milder ionisation method than EI and leads to less fragmentation of the molecular ion. Other techniques developed to analyze complex and sensitive compounds include, fast atomic bombardment (FAB), matrix assisted laser desorption ionization time of flight (MALDI TOF), etc.

Three factors dominate the fragmentation processes, (i) weak bonds tend to be broken most easily, (ii) stable fragments (not only ions, but also the accompanying radicals and molecules) are produced most readily and (iii) some fragmentation processes depend on the ability of molecules to form cyclic transition states. Thus, fragmentation processes naturally occur more often, and ions thus formed give rise to strong peaks in the mass spectrum. Apart from giving the molecular weight of a substance, the molecular ion of a compound may provide additional information. For some elements where there is more than one isotope of high natural abundance (e.g. bromine has two abundant isotopes <sup>79</sup>Br 49% and <sup>81</sup>Br 51%; chlorine also has two abundant isotopes <sup>37</sup>Cl 25% and <sup>35</sup>Cl 75%). The relative intensities of the [M]<sup>+</sup>, [M+1]<sup>+</sup> and [M+2]<sup>+</sup> ions show a characteristic pattern depending on the elements which make up the ion. In addition, the 'nitrogen rule' states that a molecule with an even molecular weight must contain no nitrogen atoms or an even number of nitrogen atoms. Thus, a molecule with an odd molecular weight must contain an odd number of nitrogen atoms.

In High resolution mass spectra (HRMS) the use of double focussing allows the mass of an ion to be determined to an accuracy of approximately +/-0.0001 of a mass unit, hence, it is possible to obtain the exact atomic composition of each ion in a mass spectrum, unambiguously.

Another development which proved to be a very powerful technique is coupled gas chromatography-mass spectrometry (GC-MS), a technique that is widely used for the identification of volatile organic compounds in complex mixtures. Nevertheless, it has been



reported that even where GC-MS is used for analysis, assignments or identification of compounds cannot often be made on the basis of mass spectrometric data only,<sup>[67]</sup> since many terpenes have essentially identical mass spectra. This can be due to the initial similarity of structures, or due to various fragmentations and rearrangements after ionization. Hence, some knowledge of retention characteristics is often required to complement mass spectral data.

### **3.9.2. Kovats's Indices**

Gas chromatographic retention indices (Kovats indices) are a valuable aid in the identification of monoterpenes and sesquiterpenes in essential oils and related natural and synthetic products. Some 900 Kovat's indices of 400 individual compounds on methyl silicone (dimethyl polysiloxane) and/or Carbowax 20 M liquid phases are summarized from the general literature.<sup>[66]</sup> The dependence of retention index on temperature has been extensively described, with specific reference to terpenes. Temperature has a relatively small effect on Kovat's indices of terpenes on methyl silicone phases, but can have quite marked effects on the indices on CW 20M.<sup>[68-70]</sup>

### **3.9.3. Chromatography**

Chromatography is defined as the separation of a mixture of two or more different compounds (in some cases, ions) by distribution between stationary and moving phases. Various types of chromatography are possible, depending on the nature of the two phases involved; solid-liquid (column, thin-layer, and paper), liquid-liquid, and gas-liquid (vapour phase) chromatographic methods are commonly available. The choice of technique depends largely on the differential solubilities (or absorptivities) and volatilities of the compounds to be separated with respect to the two phases between which they are to be partitioned. The adsorption chromatography was discovered by the Russian botanist Michael Tswett in 1903,<sup>[71]</sup> and later developed in 1931 by Kuhn and Lederer.<sup>[72]</sup> The underlying mechanisms are the partitioning of the moving compounds between phases and also their being reversibly adsorbed on the surface of the stationary phase. There are, however, various qualitative factors to be considered in the choice of adsorbent and solvent(s), size of the column (both length and diameter) relative to the amount of material to be chromatographed and the rate of elution (or flow). These factors often enable the chromatographic behaviour of a substance to be predicted.

### 3.9.4. Thin Layer Chromatography (TLC)

Thin Layer Chromatography is one of the most important methods for analyzing essential oils apart from gas chromatography (GC).<sup>[73]</sup> The ratio of fronts ( $R_f$ ) value is dependent upon many variables which must be watched during the preparation and evaluation of the chromatogram if reproducible results are to be obtained : Quality of the layer material, activation grade of the layer, layer thickness, chamber saturation, quality of the solvent, development distance, and distance of the starting point from the surface of the solvent.<sup>[74]</sup> Sometimes a better separation is achieved by the stepwise elution technique, in which different solvents in succession are made to pass over the chromatogram in the same or rectangular direction.<sup>[75]</sup>

The effectiveness of TLC had also been improved by various modification techniques involving impregnation techniques using paraffin- and  $AgNO_3$ . The  $AgNO_3$ -impregnated-TLC allows the separation of terpenes according to the number of double bonds.<sup>[76-77]</sup> The biosynthesis of terpenes had been followed by TLC.<sup>[78]</sup>

### 3.9.5. Gas Chromatography and High Performance Liquid Chromatography

Gas chromatography (GC) finds its main application with volatile compounds, fatty acids, mono- and sesquiterpenes, and sulphur compounds. However, the volatility of higher boiling plant constituents (alcohols, acids) can be enhanced by conversion to esters and/or trimethylsilyl ethers so that there are only few classes which are completely unsuitable for GC separation. Alternatively, the less volatile constituents can be separated by HPLC, a method which combines column efficiency with speed of analysis. A major difference between HPLC and GLC is that the former procedure normally operates at ambient temperature, so that the compounds are not subjected to the possibility of thermal rearrangement during separation. HPLC is mainly used for those compounds which are non-volatile, e.g. higher terpenoids, phenolics of all types, alkaloids, lipids, and sugars. HPLC works best for compounds which can be detected in the ultraviolet or visible regions of the spectrum and provides a most important and versatile method of quantitative plant analysis.

### 3.9.6. Isolation Procedures

The method of isolation employed depends on the quantity and complexity of the essential oil as observed by GC or HPLC, in addition to other factors such as lability and thermal instability of the constituents. The flow chart below shows the interconnectivity of chromatographic techniques used in the isolation and purification of compounds (Fig. 16). Thus, there is

considerable overlap in the use of the chromatographic techniques and often a combination of conventional column chromatography (CC), TLC and HPLC or TLC and GLC may be the best approach for separating a particular class of plant compound.

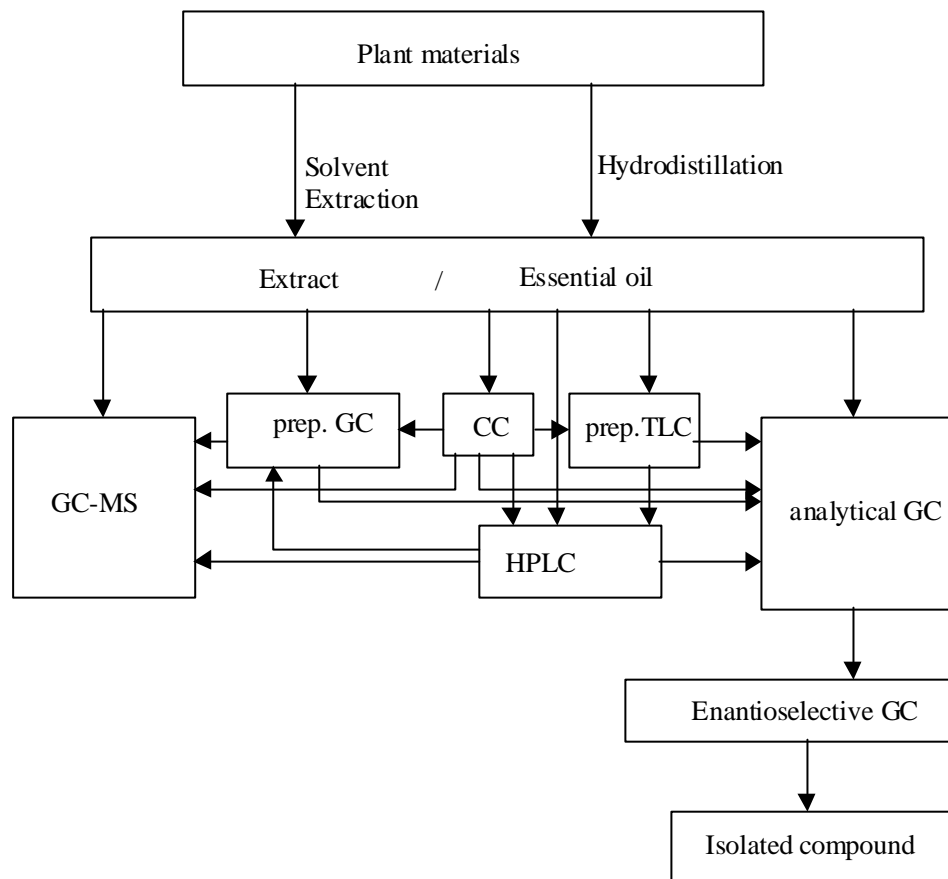


Fig. 16. Flow Chart of a simplified Isolation protocols

### 3.9.7. Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is a physical technique which enables investigation on the structure of a molecule at the atomic level. The nuclei of isotopes of most elements possess magnetic moments which arises from the spinning of a charged particle which generates a magnetic field. This involves the change of the spin state of a nuclear magnetic moment when the nucleus absorbs electromagnetic radiation in a strong magnetic field. Not all atomic nuclei are NMR-active.<sup>[64, 90]</sup> Both one- and two dimensional nuclear magnetic resonance (1D- and 2D-NMR) techniques were employed in the elucidation of structure of various compounds.

### 3.9.7.1. One Dimensional -NMR Experiments

One dimensional -NMR include,  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, spectra etc. The  $^1\text{H}$ -NMR spectrum, information relating to the number of different kinds of the environments of the hydrogen atoms, the ratio of the hydrogen causing each signal, the spin-spin splitting indicating the neighbouring nonequivalent protons, and the coupling constant ( $J$ ) which gives further information about stereochemical features in the molecule are observed.

### 3.9.7.2. $^{13}\text{C}$ -NMR Experiments

$^{13}\text{C}$  is a rare nucleus (1.1% natural abundance) and the most abundant isotope of carbon ( $^{12}\text{C}$ ) cannot be observed by NMR. Its low concentration coupled with the fact that  $^{13}\text{C}$  has a relatively low resonance frequency, leads to its relative insensitivity as an NMR-active nucleus (about 1/16000 as sensitive as  $^1\text{H}$ ). However, with increasing availability of routine pulsed FT-NMR spectrometers it is now common to acquire many spectra and sum them up, so  $^{13}\text{C}$ -NMR of good quality can be obtained readily. As a consequence,  $^{13}\text{C}$ - $^{13}\text{C}$  couple is not observed in  $^{13}\text{C}$ -NMR spectra. A  $^{13}\text{C}$  strongly couples to any proton which may be attached ( $^1J_{\text{CH}}$  is typically about 125 Hz for saturated carbon atoms in organic molecules). It is the usual practice to irradiate the  $^1\text{H}$  nuclei during  $^{13}\text{C}$  acquisition so that all  $^1\text{H}$  are fully decoupled from the  $^{13}\text{C}$  nuclei (usually termed broad band decoupling or noise decoupling).  $^{13}\text{C}$  spectra usually appear as a series of singlets when  $^1\text{H}$  is fully decoupled.

### 3.9.7.3. Distortionless Enhancement by Polarization Transfer (DEPT)

The DEPT experiment is commonly used to determine the multiplicity of  $^{13}\text{C}$  signals. The signals arising from carbons in  $\text{CH}_3$  and  $\text{CH}$  groups (i.e. those with an odd number of attached protons) appear oppositely phased from those in  $\text{CH}_2$  groups (i.e. those with an even number of attached protons) e.g. signals from  $\text{CH}_3$  and  $\text{CH}$  groups point upwards while signals from  $\text{CH}_2$  groups point downwards. Another  $^{13}\text{C}$ -experiment useful in structure elucidation is  $^{13}\text{C}$ -PENDANT. The observed spectrum from PENDANT is similar to that of DEPT with an additional less intensive signals of the quaternary carbon pointing in the same phase as the  $\text{CH}_2$  groups.

### 3.9.7.4. Two-dimensional NMR Experiments

The most important two-dimensional NMR experiments for solving structural problems are COSY (COrrelation SpectroscopY), NOESY (Nuclear Overhauser Enhancement SpectroscopY) and HSC (Heteronuclear Shift Correlation).

### 3.9.7.5. COrrelation SpectroscopY (COSY)

The COSY spectrum shows which pairs of nuclei in a molecule are coupled. The COSY spectrum is a symmetrical spectrum which has the  $^1\text{H}$ -NMR spectrum of the substance as both of the two chemical shift axes (F1 and F2). In a single COSY spectrum, all of the spin-spin coupling pathways in a molecule can be identified. Useful coupling correlations observed in COSY spectra include  $^2\text{J}$ ,  $^3\text{J}$ ,  $^4\text{J}$ , and  $^5\text{J}$  (Fig. 17)

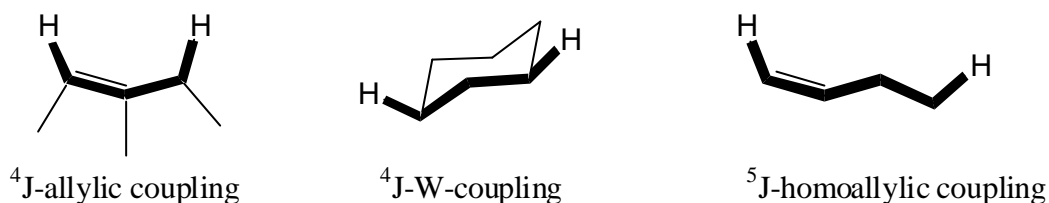


Fig. 17. Important coupling correlations

### 3.9.7.6. The Nuclear Overhauser Effect (NOE)

The irradiation of one nucleus while observing the resonance of another may result in a change in the amplitude of the observed resonance i.e. an enhancement of the signal intensity. This is known as the Nuclear Overhauser Effect (NOE). The NOE is a through space effect, and its magnitude is inversely proportional to the sixth power of the distance between the interacting nuclei. Therefore, because of the distance dependence of the NOE, it is an important method for establishing which groups are close together in space and can be measured quite accurately.

The **NOESY spectrum** relies on the Nuclear Overhauser Effect (NOE) and shows which pairs of nuclei in a molecule are close together in space. The NOESY is also a symmetrical spectrum which has the  $^1\text{H}$ -NMR spectrum of the substance as both of the two chemical shift axes (F1 and F2). The NOESY spectrum is very similar in appearance to a COSY spectrum. In a NOESY spectrum, all of the nuclei which are close in space ( $< 5 \text{ \AA}$ ) can be identified. From the analysis of the NOESY spectrum the relative configuration of a molecule can be determined when signals are not overlapped. In cases where there is an overlap, semi- or total synthesis or chemical interconversion of functional groups could be employed.

**3.9.7.7. The Heteronuclear Spectroscopy spectrum (HSC)** is the heteronuclear analogue of the COSY spectrum. The HSC spectrum has the  $^1\text{H}$ -NMR spectrum of the substance on one axis (F2) and the  $^{13}\text{C}$  spectrum (or the spectrum of some other nucleus) on the second axis (F1). In an HSC spectrum, the correlation between the protons in the  $^1\text{H}$ -NMR spectrum and the carbon nuclei in the  $^{13}\text{C}$  spectrum can be obtained. This include Heteronuclear Multiple-Bond Correlation (HMBC) and Heteronuclear Multiple Quantum-Coherence (HMQC). The HMQC is used to determine the one-bond proton-carbon shift correlation i.e.  $^1\text{J-C-H}$  correlation. While HMBC is used for long-range (two- and three-bonds)  $^1\text{H-}^{13}\text{C}$  interconnectivity i.e.  $^2\text{J}$  and  $^3\text{J}$  correlations.

### **3.9.7.8. Other Important Spectroscopic Methods**

Infrared (IR) and Ultraviolet and Visible (UV-VIS) spectroscopies are other useful analytical methods which furnish additional information in the elucidation of structures. Infrared (IR) is most widely used for the detection of functional groups in pure compounds, mixtures and for compound comparison. The main useful region of the infrared spectrum ranges from 4000-650  $\text{cm}^{-1}$ .

UV-VIS is primarily used to measure the multiple bond or aromatic conjugation within molecules. The UV region extends from 1000-4000 $\text{\AA}$  or 100-400 nanometers (nm).

### **3.9.8. Determination of Absolute Configuration**

Absolute configuration has become an important structural information, particularly because of the increasing number of cases where different biological properties can be related to stereoisomers. Several methods such as X-ray analysis, circular dichroism (CD), and NMR techniques have been used for the determination of the absolute configuration of numerous functionalised compounds. In addition, derivatisation, specifically Mosher's method has been widely used for the past decade because of its universality and accessibility.<sup>[91]</sup> Recently, a method has been developed for an acyclic secondary alcohol using the characteristic functions of 1,5-difluoro-2,4-dinitrobenzene (FFDNB).<sup>[92]</sup> Nevertheless, all these methods were not without limitations in their various applications. In hydrocarbons for example, the lack of functional groups for derivatisation, provided very limited applications of the aforementioned methods. Enantioselective GC is another method which became an essential tool for stereoselective analysis after the introduction of cyclodextrin derivatives as chiral stationary phases a decade ago.<sup>[80]</sup> The introduction of this analytical method enables a precise stereochemical analysis of the enantiomeric composition of a wide range of compounds.<sup>[88]</sup> In

addition, assignment of absolute configuration could be achieved in case a reference mixture of both enantiomers is available and the elution order of the enantiomers is known. This is determined by chemical correlations of unknown compounds with known structures by interconversion (hydrogenation, oxidation, acid- and thermal rearrangements, independent synthesis, etc.). Then, the universally used Cahn-Ingold-Prelog (CIP) rules<sup>[199,200]</sup> with the descriptors *R* and *S* are being applied for the specification of molecular configurations of enantiomers and diastereomers.

### 3.9.9. Cyclodextrins (CDs)

Cyclodextrins (CDs) are naturally occurring cyclic oligomers of  $\alpha$ -1,4-linked D-glucose units with a unique shape resembling a bottomless flowerpot.<sup>[79]</sup> CDs are derived by enzymatic hydrolysis of starch by cyclodextrin glycosyl transferases (CGTase) from *Klebsiella pneumonia*, *Bacillus macerans*, and other bacterial strains and are commercially available.<sup>[80]</sup> The common CDs contain six ( $\alpha$ ) (**41**), seven ( $\beta$ ) (**42**), and eight ( $\gamma$ ) D-glucose units (**43**), with each glucose being in a rigid chair conformation (Fig. 18).

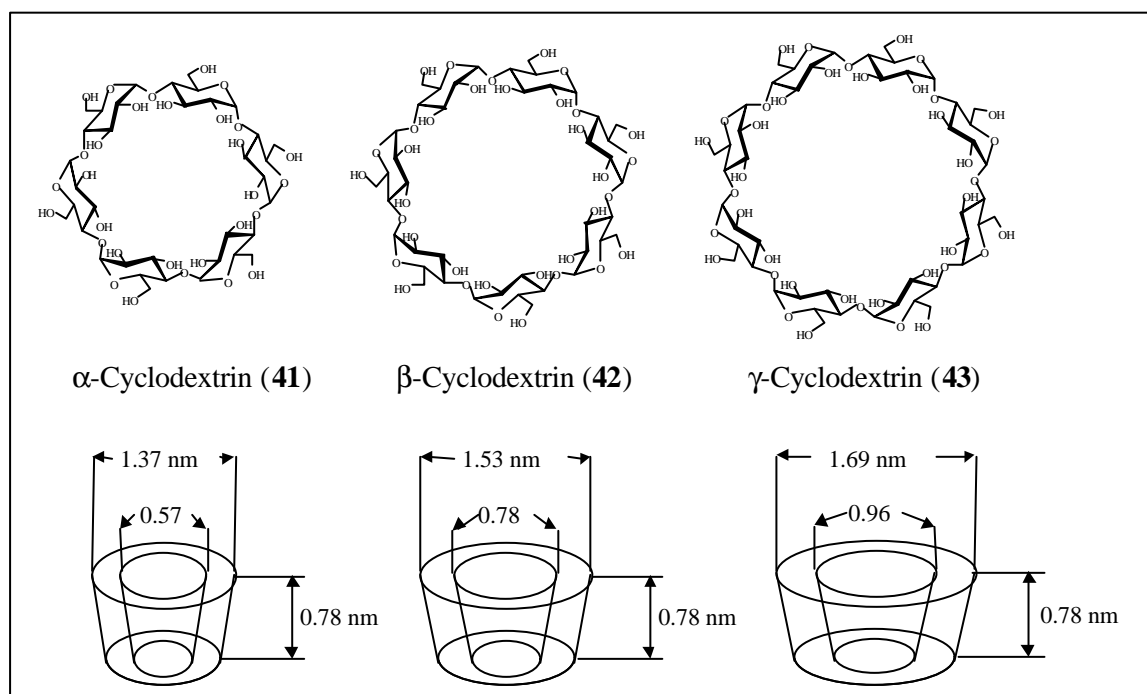


Fig. 18. Structures and dimensions of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins

Their overall conformation is mainly determined by  $\alpha$ -(1-4)-linked glucose units in the  ${}^4C_1$ -conformation and stabilized by intramolecular hydrogen bonding between the 2- and 3-hydroxy groups. Numerous CDs derivatives are obtained by modification of the 2-, 3-, and 6-hydroxy groups to O-alkylated and -acylated products. In contrast to the hydrophilic character of the outer surface, the cyclodextrin structure features a hydrophobic cavity that can encapsulate various guest molecules to produce supramolecular inclusion complexes.<sup>[81-82]</sup> They are widely used commercially as purification media. The extraordinary utility of CDs as chiral stationary phases for enantioselective gas chromatography was discovered by König's group when some of the characteristic properties (solubility, thermal stability, selectivity) of the CDs were improved by substitution of the sugar hydroxy groups with alkyl and acyl groups.<sup>[83-85]</sup> The successful application of CDs derivatives in packed columns for the preparative separation of enantiomers has furnished an efficient tool for the isolation of optical isomers.<sup>[86]</sup> In addition, capillary gas chromatography with modified CDs derivatives have been used extensively to resolve enantiomers composition of various monoterpene and sesquiterpene hydrocarbons. CD phases provide highly reliable tools for testing the authenticity of diverse essential oils.<sup>[87-89]</sup>



## 4. Special Part

This part involves the identification and characterization of the essential oils of the liverworts *Plagiochila asplenioides*, *Scapania undulata*, *Diplophyllum albicans*, *Marsupella emarginata*, *Marsupella aquatica*, *Marsupella alpina*, *Tritomaria polita* and *Barbilophozia floerkei*. In addition, isolation, structure elucidation and stereochemistry of new sesquiterpenes from these liverworts are discussed.

### 4.1. Chemical Analysis of the Essential Oil of the Liverwort *Plagiochila asplenioides*.

*Plagiochila asplenioides* is botanically classified as follows,<sup>[25-26]</sup>

Class : Hepaticae (Liverworts)

Subclass : Jungermanniidae

Order : Jungermanniales

Family : Plagiochilaceae

Species : *Plagiochila asplenioides*

The Plagiochilaceae form a large family amongst the Hepaticae, and many species occur world wide in reasonably wet, humus-rich habitats.<sup>[93]</sup> The number of species assigned to the genus *Plagiochila* is more than 1600,<sup>[102]</sup> and from these about 60 species have been chemically investigated.<sup>[94]</sup> These species are known to produce a broad and diverse spectrum of secondary metabolites comprised of mono-, sesqui- and diterpenoids as well as bibenzyls. Amongst the terpenes isolated from this family, sesquiterpenoids are most common. <sup>[5,93-101]</sup> Few examples of sesquiterpenoids isolated from *Plagiochila* species (*P. peculiaris*, *P. ovalifolia*, *P. porelloides*, *P. corrugata*, *P. asplenioides*, etc.) include peculiaroxide (**44**), 3 $\alpha$ -acetoxybicyclogermacrene (**45**), maaliol (**46**), maalian-5-ol (**47**), plagiochilide (**48**), cyclocolorone (**49**), etc. (Fig. 19).

Recently, *Plagiochila* species have been classified into ten chemotypes: the 2,3-seco-aromadendrane-type (I), the bibenzyl-type (II), the cuparane-herbertane sesquiterpene-type (III), the bibenzyl-cuparane-herbertane-type (IV), the gymnomitrane (= barbatane)-bicyclogermacrene sesquiterpene-type (V), the bicyclogermacrene-spathulenol-type (VI), pinguisane-type (VII), the 2,3-seco-aromadendrane-sesquiterpene lactone-type (VIII), the cyclic bis-bibenzyl-2,3-seco-aromadendrane-type (IX), and the sesquiterpene lactone-type.<sup>[94,103]</sup>

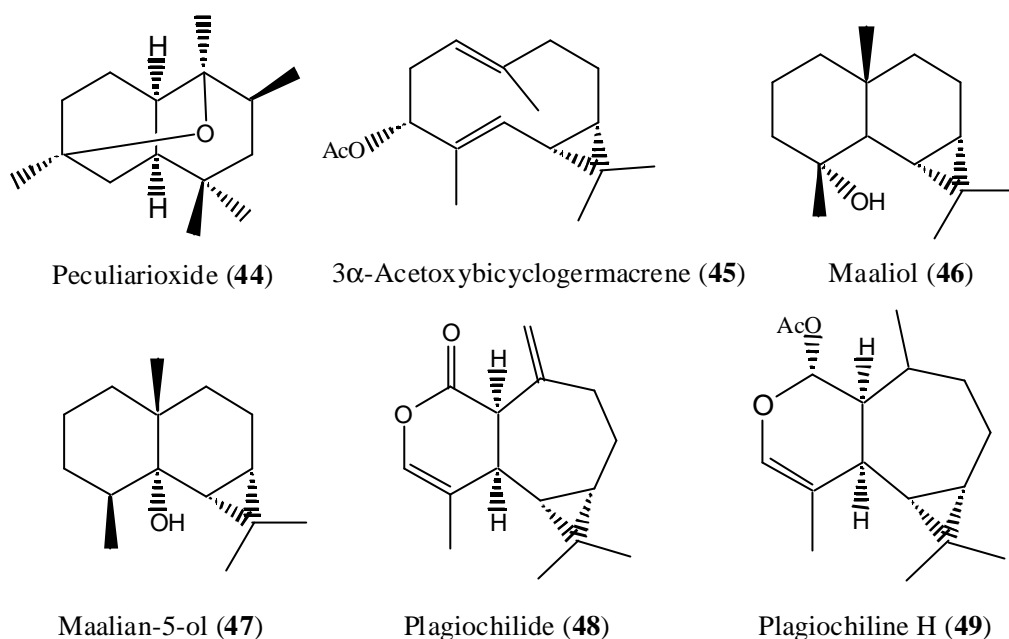


Fig.19. Isolated compounds from *Plagiochila* species.<sup>93-105</sup>

#### 4.1.1. Composition of the Essential Oils of *Plagiochila asplenioides*

The GC- and GC-MS-data of the essential oils of *Plagiochila asplenioides* collected from two different locations (Altenau and Kummerfeld) in Germany, in December 2001 and February 2004, respectively, were analysed (for GC traces see Figs. 20, 21 and for corresponding structures see Figs. 22 and 23). These essential oils revealed a complex mixture of sesquiterpene hydrocarbons including maali-1,3-diene (50),<sup>[104]</sup> anastreptene (51), italicene (52),  $\alpha$ -barbatene (53),  $\beta$ -funebrene (54),  $\gamma$ -maaliene (55),  $\alpha$ -maaliene (56),  $\beta$ -barbatene (57),  $\beta$ -acoradiene (58),  $\beta$ -chamigrene (59), (-)-bicyclogermacrene (60),  $\alpha$ -cuprenene (61),  $\alpha$ -chamigrene (62),  $\beta$ -bazzanene (63),  $\gamma$ -curcumene (64), leden (65) and fusicocca-3,5-diene (66). In the oxygenated fraction, 3 $\alpha$ -acetoxybicyclogermacrene (45), (+)-maalian-5-ol (47), plagiochilide (48), rosifoliol (67), gymnomitr-3(15)-en-4 $\beta$ -ol (68), were identified in the essential oils of *P. asplenioides* from both locations. All the above mentioned compounds were identified by comparison of their mass spectra and retention indices with a spectral library established under identical experimental conditions, (Joulain and König, 1998; MassFinder Software and Data Bank, Hochmuth et al. 2004).<sup>[107,108]</sup> The unknown constituents of the essential oils from both locations which could not be identified were selected for isolation, and combination of chromatographic techniques such as column chromatography, preparative gas chromatography and thin layer chromatography, etc., were used to isolate the new compounds.

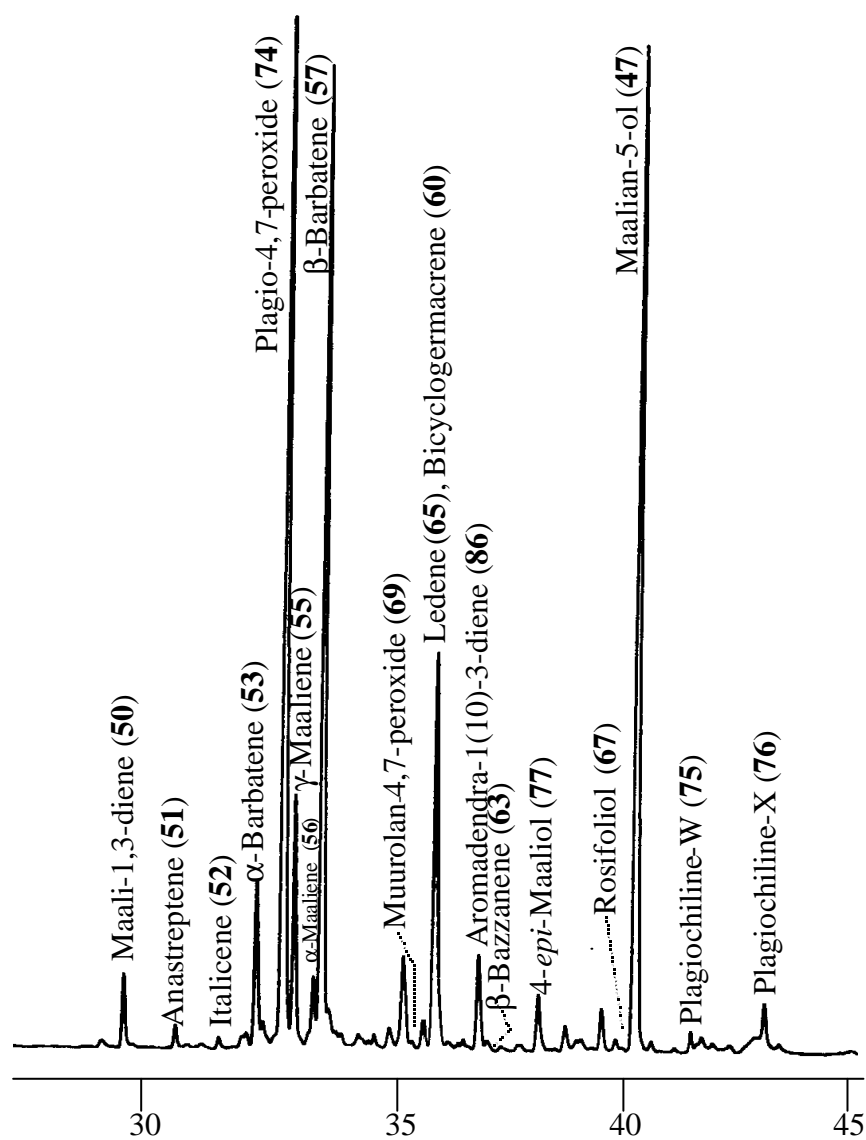


Fig. 20. Gas chromatogram of the essential oil of *Plagiochila asplenioides* from Kummerfeld, (Germany). (CPSIL 5, 50°C, 3°C/min., 230°C).

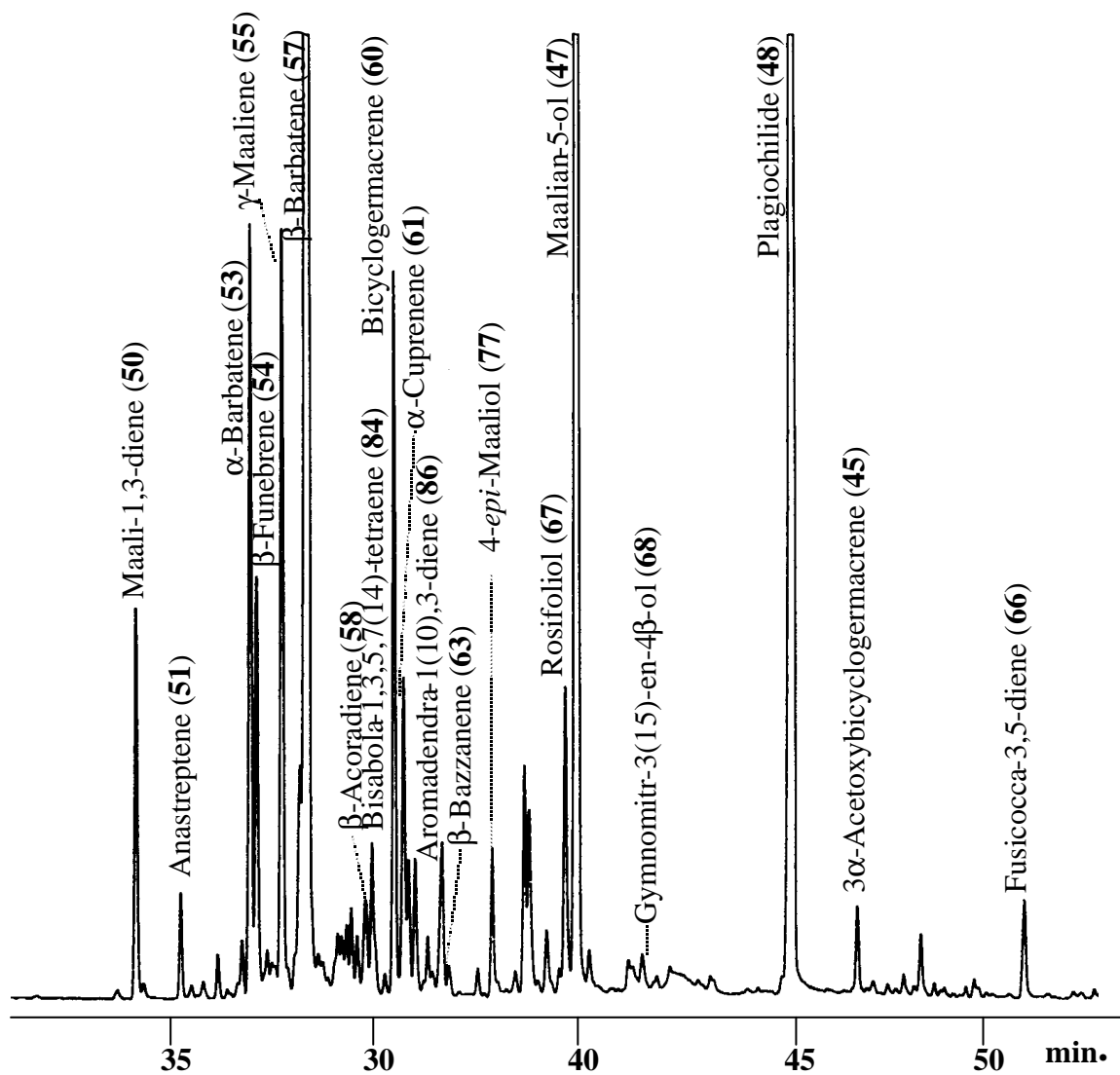


Fig. 21. Gas chromatogram of the essential oil of *Plagiochila asplenioides* from Altenau, Germany. (CPSIL 5, 50°C, 3°C/min., 230°C).



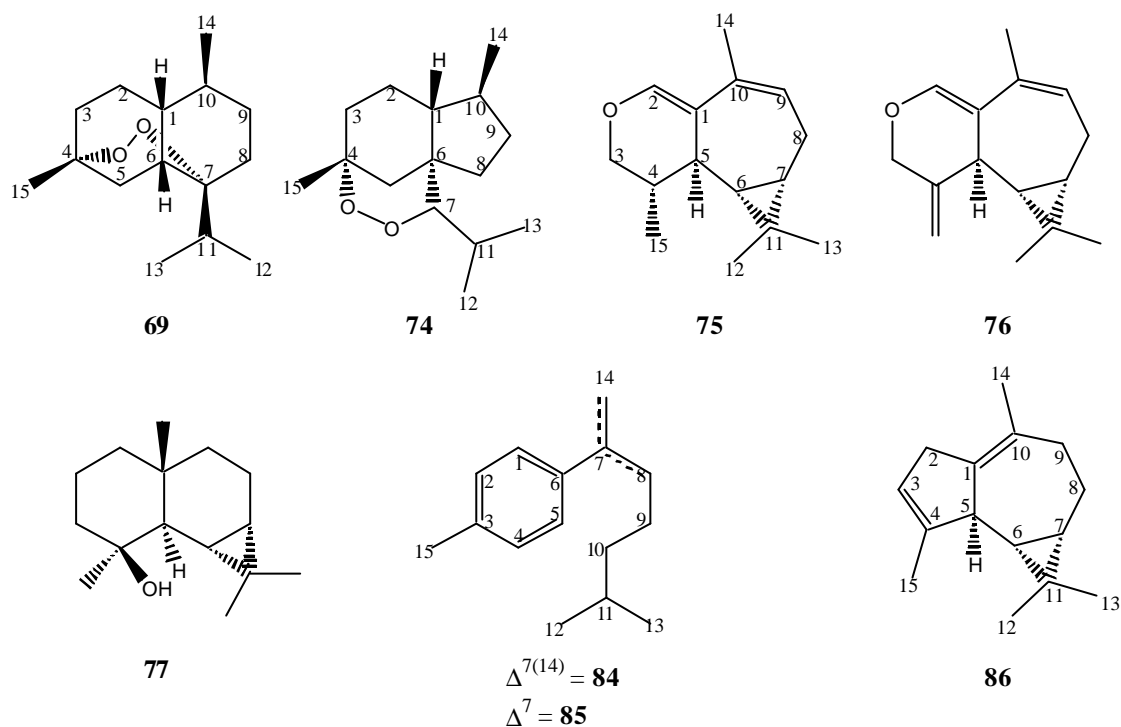


Fig. 23. New compounds from *Plagiochila asplenioides*.

#### 4.1.2. Structure of (+)-Muurolan-4,7-peroxide (**69**)

(+)-Muurolan-4,7-peroxide (**69**, 1.4%), a new tricyclic oxygenated muurolane derivative, gave a fragmented ion signal at  $m/z$  204 as the heaviest ion detected under EIMS. Since the EIMS gave no conclusive indication of the molecular ion, the chemical ionization was carried out. Compound **69** gave fragmented ion signals at  $m/z$  221 ( $M^+ + 1 - H_2O$ ) and  $m/z$  223 ( $M^+ + 1 - O$ ) on chemical ionization (CI) using *iso*-butane and ammonia as reactant gases, respectively. Hence the molecular formula could not be detected by CI-MS, however, the structure was concluded from the atmospheric pressure chemical ionization (APCI) technique which indicated a weak peak at  $m/z$  239  $[M+1]^+$ . The elemental analysis experiment of **69** confirmed the absence of nitrogen. Therefore, the molecular formula of **69** should be  $C_{15}H_{26}O_2$  to account for the observed chemical ionization fragments. The molecular formula revealed the presence of three degrees of unsaturation. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed four methyl

signals at  $\delta$  0.78 (3H, *d*, H-12,  $J = 6.9$  Hz), 0.87 (3H, *d*, H-14,  $J = 6.9$  Hz), 1.10 (3H, *d*, H-13,  $J = 6.6$  Hz), and 1.28 (3H, *s*, H-15) (Fig. 24).

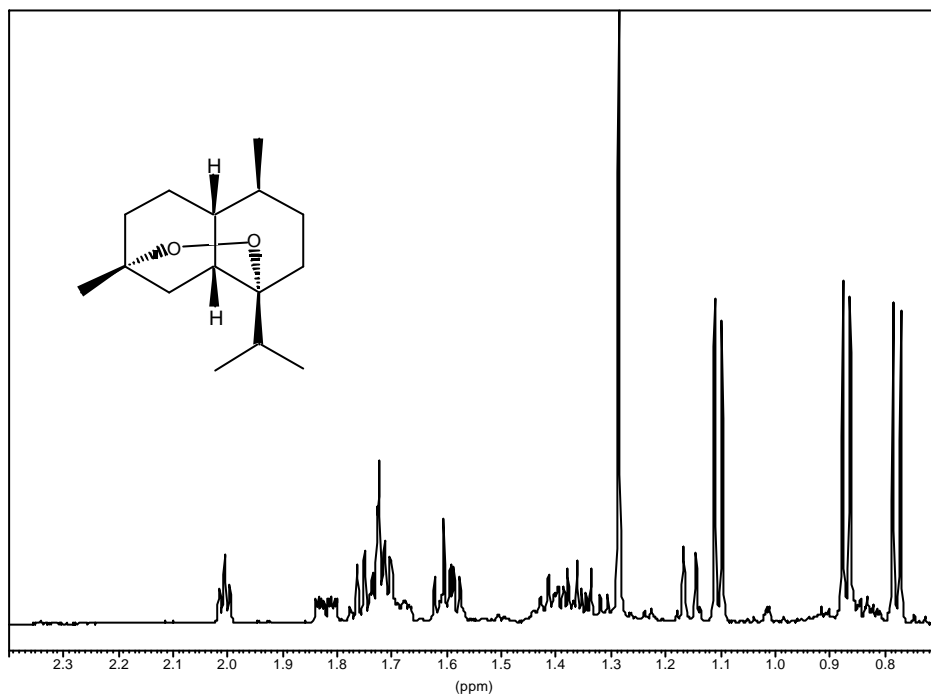


Fig. 24.  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ) of Muurolan-4,7-peroxide (**69**)

Additional structural information was obtained from the  $^{13}\text{C}$ -NMR spectrum of **69** which confirmed the presence of four methyl groups at  $\delta$  16.3, 19.3, 21.6 and 27.7, five methylene groups at  $\delta$  24.7, 28.1, 29.3, 39.4, and 41.5, four methine groups at  $\delta$  34.1, 37.0, 37.7, and 40.1, and two quaternary carbons at  $\delta$  79.7 and 86.1. The two quaternary carbon signals at  $\delta$  79.7(*s*) and 86.1(*s*) were assigned to the oxygen-linked carbons C-4 and C-7, respectively. The 2D- $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of **69** revealed the partial structure  $\text{CH}_2(3)\text{-CH}_2(2)\text{-CH}(1)\text{-CH}(10)\text{-CH}_2(9)\text{-CH}_2(8)$ -. The HMBC spectrum indicated that the tertiary methyl protons (H-14) correlated with the methine carbons (C-1, and C-10) and the secondary carbon (C-9). Other important HMBC correlations are shown in Fig. 25. All this information from the  $^{13}\text{C}$ -NMR in addition to 2D- $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC and HMBC (Fig. 25) led to structure **69**.

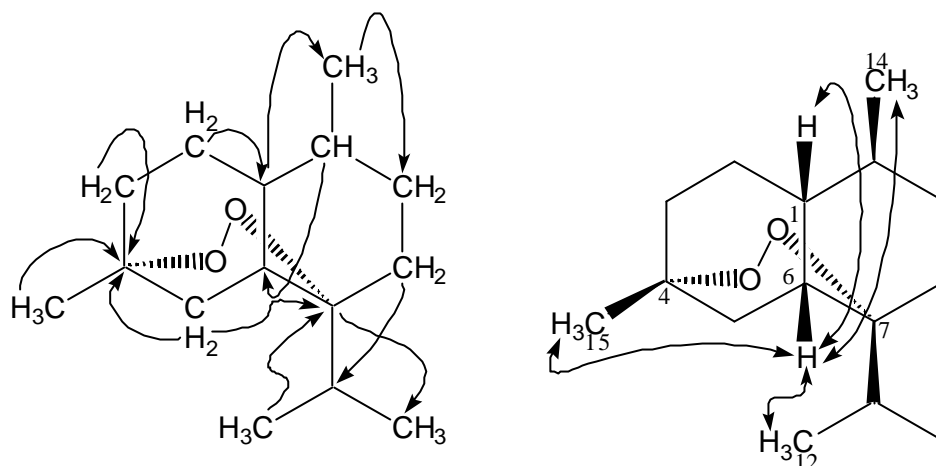


Fig. 25. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **69**. Fig. 26. NOE correlations of **69**.

Its relative configuration was inferred from the NOESY spectrum, in which NOEs were observed between the protons H-1 and H-6, as well as H-1 and H-14, (Fig. 26). The  $\alpha$ -orientation of the 1,2-dioxane ring was assigned from the spatial interactions of proton H-6 with methyl protons H-12 and H-15. The absolute configuration of **69** was deduced by both direct rigorous hydrogenation (Fig. 27) and acid rearrangement reactions (Fig. 28). The comparison of the four fully saturated diastereoisomeric amorphanes (molecular mass 208), with that of the fully hydrogenated products of an authentic (-)-amorpha-4,7(11)-diene (**70**, obtained from the liverwort *Marsupella aquatica*)<sup>[40]</sup> by enantioselective GC on a modified cyclodextrin stationary phase confirmed that the fully hydrogenated derivatives of **69** gave identical retention times with that of (-)-amorpha-4,7(11)-diene (**70**). Thus, the absolute configuration of **69** could be assigned as (1*R*, 4*R*, 6*R*, 7*R*, 10*S*). In addition, the treatment of (+)-muurolan-4,7-oxide (**69**) with an acidic ion exchange resin (Amberlyst) for two hours at room temperature afforded (-)-*epi*-zonarene (**71**, 85%)<sup>[108]</sup> as the major component with (+)-*trans*-calamenene (**72**) and (-)-*cis*-calamenene (**73**) as minor components. The gas chromatogram showing the comparison of (-)-*epi*-zonarene (**71**), the major product from the acid transformation of **69** with the authentic standards, is given below (Fig. 28). Compound **69** was resistant to treatment with lithium aluminium hydride ( $\text{LiAlH}_4$ ).



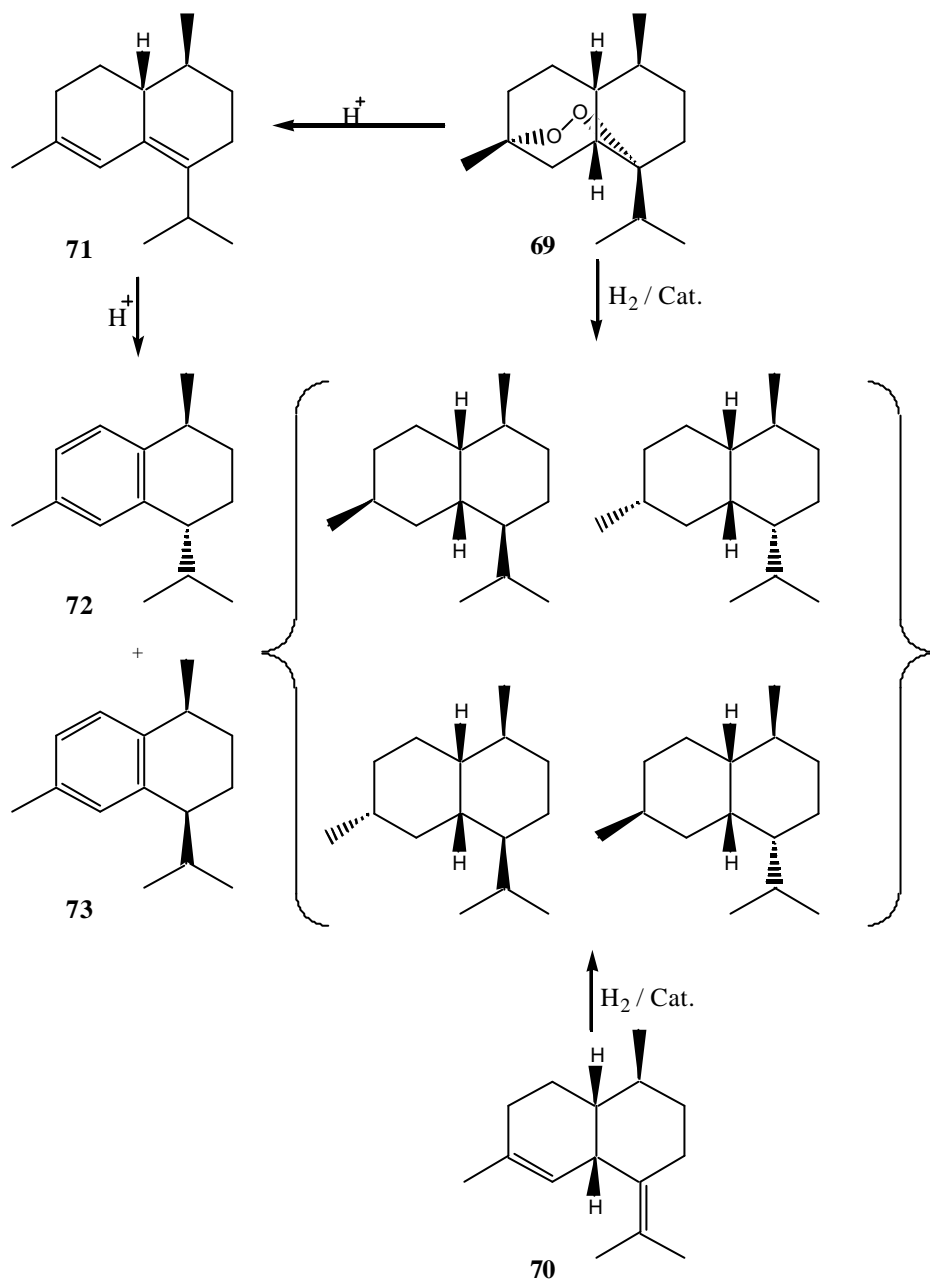


Fig. 27. Transformation and hydrogenation products of **69**.

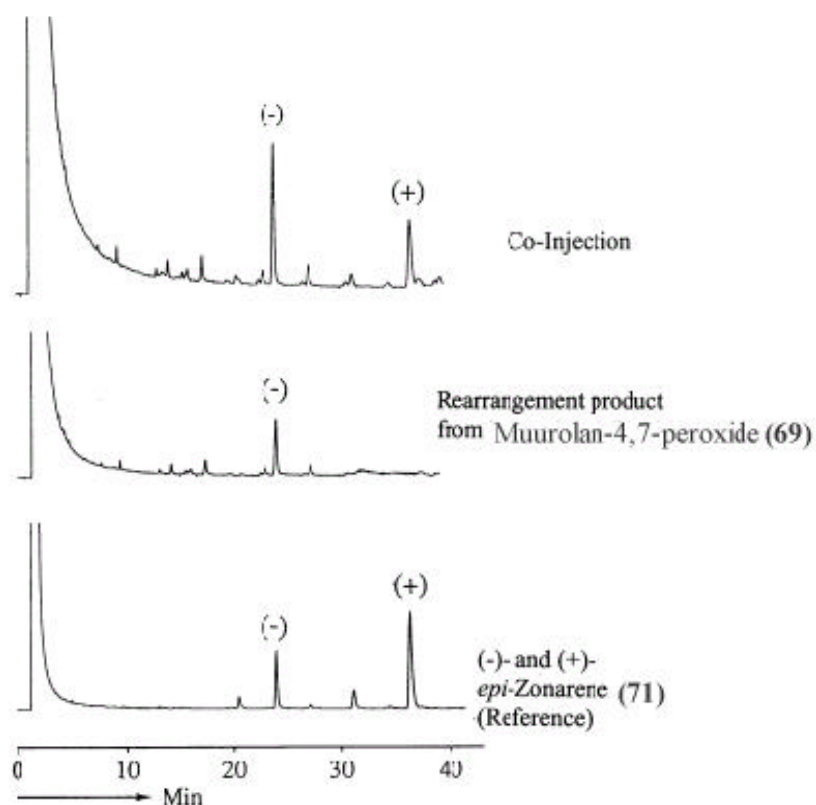
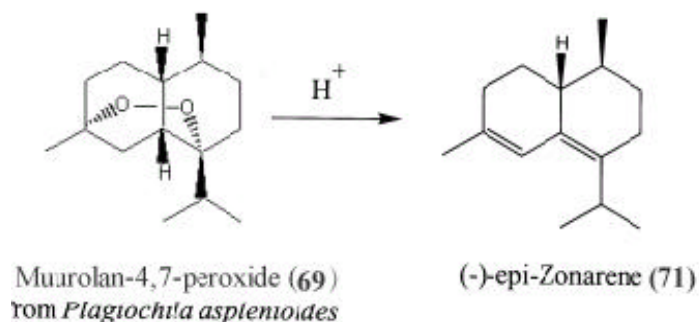


Fig. 28. Acid transformation of Muurolan-4,7-peroxide (**69**)

#### 4.1.3. Structure of (+)-Plagio-4,7-peroxide (**74**) (Fig. 23)

Compound **74**, named plagio-4,7-peroxide (19.5%) is an abeomurolane or abeoamorphane with a unique skeleton. **74** showed fragmented ion signals at  $m/z$  220 (1.7), 221 (1), 222 (<1), as the heaviest ions detected under direct inlet EIMS and a chemical ionization fragmented ion signals at  $m/z$  at 221 ( $M^+ + 1 - H_2O$ ) and  $m/z$  223 ( $M^+ + 1 - O$ ), similar to compound **69** using *iso*-butane and ammonia as reactant gases respectively. The molecular formula of  $C_{15}H_{26}O_2$  was inferred from the atmospheric pressure chemical ionization (APCI) technique which indicated a very weak peak at  $m/z$  239 [ $M+1$ ] $^+$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) of **74** indicated signals of four methyl groups at  $\delta$  0.81 (3H, *d*, H-12,  $J = 6.3$  Hz), 0.91 (3H, *d*, H-14,  $J = 6.3$

Hz), 1.23 (3H, *d*, H-13,  $J = 6.6$  Hz), and 1.30 (3H, *s*, H-15). A well resolved longrange  $^4J$ -coupling was observed for methylene proton H-5b at  $\delta$  1.94 (1H, *dd*,  $J = 1.9, 10.4$  Hz) and H-7 at  $\delta$  3.30 (1H, *d*,  $J = 10.4$  Hz), indicating that the 1,2-dioxane ring structure exhibits a fixed *W*-orientation of the four bonds. Additional useful information was obtained from the  $^{13}\text{C}$ -NMR data. All this information from  $^{13}\text{C}$ -NMR as well as from 2D  $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC and HMBC (Fig. 29) led to structure **74**. The relative configurations at C-1, C-4, C-6 and C-10 were established by the NOESY spectrum, which showed the spatial interactions of protons H-1 with H-14. Other important correlations are shown in Fig. 30. No visible transformation was observed when **74** was treated with an acidic ion exchange resin (Amberlyst) in hexane and few drops of hydrochloric acid (HCl) in methanol for 2 hours, respectively. Attempts to cleave the dioxane ring of **74** by rigorous hydrogenation for 2 hour failed to yield any transformation product. Compound **74** was also sufficiently stable to treatment with lithium aluminium hydride ( $\text{LiAlH}_4$ ). Thus, compound **74** was assumed to be a peroxide based on the interpretation of the atmospheric pressure chemical ionization (APCI) mass spectrometry only which does not correlate with epoxide function with a molecular mass of  $M = 222$ . Recently, the structure of **74** had been reported in the literature without any analytical data.<sup>[109]</sup>

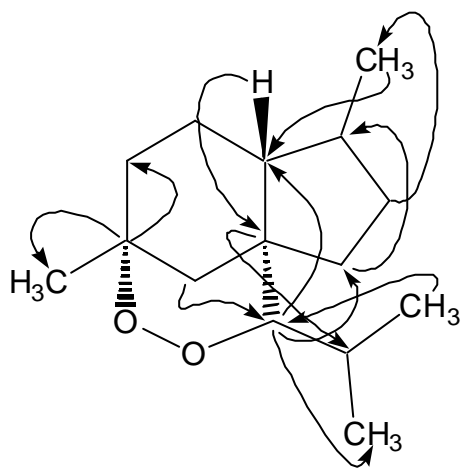


Fig. 29. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **74**.

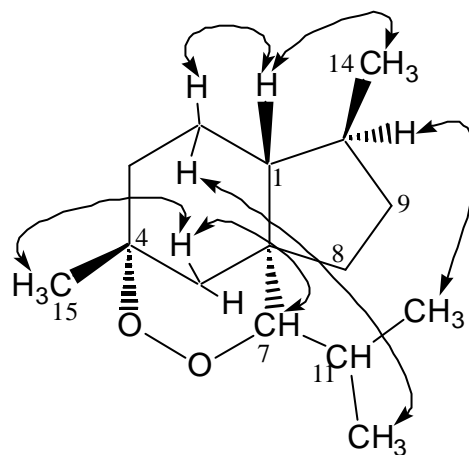


Fig. 30. NOE correlations of **74**.

A similar compound of the same skeletal backbone has recently been isolated from the seeds of *Artemisia annua* by Brown et al., (2003).<sup>[110]</sup> The unusual structure was proposed to be derived from amorphanes / muurolanes which have undergone rearrangement of the decalin ring. Thus, the novel skeleton might be derived by migration of the C-7-C8 bond to a new C6-C8 bond [i.e. plagio-4,7-peroxide (**74**) is an 8(7-6) abeo-amorphane / -muurolane] resulting in a contraction of ring B, (Fig. 31).

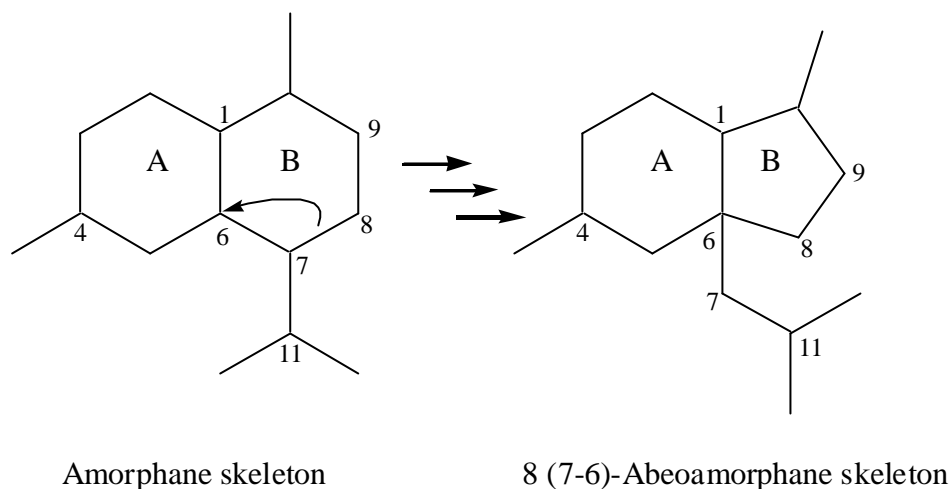


Fig. 31. Formation of 8 (7-6) abeoamorphane from an amorphane skeleton

Peroxides are important intermediates in organic chemistry and in biochemistry.<sup>[143]</sup> The peroxide bond (O-O) is a weak bond with dissociation energy of about 30-35 kcal/mol, consequently, the thermal, acid or base stability of the peroxide functional group makes the synthesis and characterization difficult. The biological assays of peroxides from natural products have revealed highly active antibacterial, fungicidal, cytostatic, and anticarcinogenic agents.<sup>[144]</sup>

#### 4.1.4. Structure of (+)-Plagiochiline-W (75) and (+)-Plagiochiline-X (76) (Fig. 23)

Plagiochiline-W (75) (0.8%) and Plagiochiline-X (76) (0.5%) are *seco*-aromadendranes isolated as minor components in addition to the known plagiochiline H (49, an acetylated hemiacetal isolated from the liverwort *Plagiochila yokogurensis*).<sup>[96]</sup> These colourless compounds were isolated from the oxygenated fraction using preparative GC with a octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\gamma$ -CD column. The assigned names, Plagiochiline-W (75) and Plagiochiline-X (76) are proposed in accordance with *seco*-aromadendranes of the structurally related plagiochiline A-V isolated from *Plagiochila* species.<sup>[95-100]</sup>

(+)-Plagiochiline-W (75), a tricyclic ether, showed a molecular ion signal at  $m/z$  218 (C<sub>15</sub>H<sub>22</sub>O). The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) of 75 indicated signals of four methyl groups at  $\delta$  0.89 (3H, *d*, H-15, J = 7.25 Hz), 0.92 (3H, *s*, H-12), 0.99 (3H, *s*, H-13) and 1.77 (3H, *s*, H-14). The highly deshielded signal at  $\delta$  6.78 (1H, *s*) was assigned to the olefinic protons H<sub>2</sub>-2 of the pyran ring. Plagiochiline-X (76), identical to 75 with an additional double bond at C<sub>4</sub>-C<sub>15</sub>, gave a molecular ion signal at  $m/z$  216 (C<sub>15</sub>H<sub>20</sub>O).

The  $^1\text{H}$ -NMR spectrum ( $\text{C}_6\text{D}_6$ ) of (+)-plagiochiline-X (**76**) indicated signals similar to plagiochiline-W (**75**). Compound **76** showed three methyl groups at  $\delta$  0.91 (3H, *s*, H-12), 1.02 (3H, *s*, H-13) and 1.73 (3H, *s*, H-14). The presence of a methyl signal at  $\delta$  0.89 (3H, *d*, H-15,  $J = 7.25$  Hz) of compound **75** was replaced by exo-methylene protons at  $\delta$  4.83 (1H, *s*, H-15a) and 4.95 (1H, *br.s.*, H-15b) in **76**. This was confirmed by the  $^{13}\text{C}$ -NMR signal at  $\delta$  111.57 (*t*) and 144.62 (*s*). The methylene protons  $\text{H}_2$ -3 of **75** at  $\delta$  3.49 (1H, *dd*,  $J = 5.1, 10.4$  Hz) and 3.78 (1H, *dd*,  $J = 2.5, 10.4$  Hz), were also slightly deshielded to  $\delta$  4.08 (1H, *d*,  $J = 11.3$  Hz) and 4.27 (1H, *d*,  $J = 11.3$  Hz) in **76**.

Additional structural information was obtained from the  $^{13}\text{C}$  NMR data of **75** and **76**. The information from  $^{13}\text{C}$ -NMR as well as from 2D  $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC and HMBC (Fig. 32) led to structures **75** and **76**. The proposed relative configurations at C-4, C-5, C-6 and C-7 was established by NOESY spectra of **75**, which revealed the spatial interactions of proton H-6 with H-7 and  $\text{H}_\beta$ -12. In addition, the interactions of proton H-5 with H-8a and  $\text{H}_\beta$ -13 were in agreement with a *cis*-fused cyclopropane ring, (Fig. 33). In addition the co-occurrence of (-)-bicyclogermacrene (**60**) and (+)-plagiochiline H (**49**) support the  $\alpha$ -orientation of the cyclopropane ring.

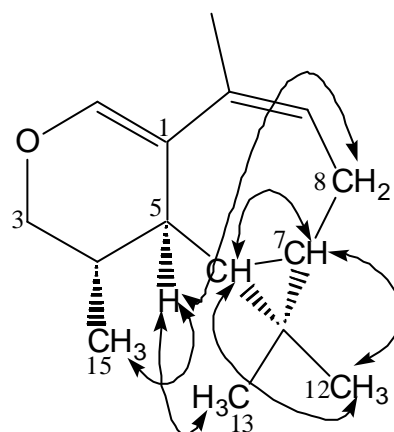
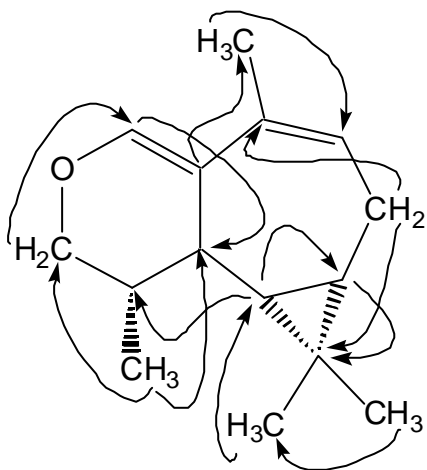


Fig. 32. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **75**. Fig. 33. NOE correlations of **75**.

#### 4.1.5. Structure of (-)-4-*epi*-Maaliol (**77**)

(-)-4-*epi*-Maaliol (**77**), a tricyclic sesquiterpene alcohol which exhibits a molecular ion signal at  $m/z$  222 corresponding to the molecular formula of  $\text{C}_{15}\text{H}_{26}\text{O}$  was isolated as a minor component (1.3%). This is the first occurrence of **77** in a liverwort (Hepaticae). The (+)-enantiomer of **77** with only the relative configuration determined from  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, and the NOED spectra has been isolated from the Brazilian Vassoura oil prepared from the

leaves of *Baccharis dracunculifolia* DC.<sup>[111]</sup> The (+)-enantiomer of **77** had been synthesized from 4-*nor*-maali-4-one<sup>[112]</sup> during the structure elucidation of maaliol (**46**), but it could only be characterized by its melting point and its infrared spectrum. The spectral data of **77** were totally consistent with those of the literature.<sup>[111]</sup> The structure and stereochemistry of (-)-4-*epi*-maaliol (**77**) was further confirmed by treatment with an acidic ion exchange resin (Amberlyst 15) at room temperature for two hours. The acid transformation of (-)-4-*epi*-maaliol (**77**) gave  $\beta$ -maaliene (**78**, 9.0%),  $\beta$ -gorgonene (**79**, 4.0%), selina-5,11-diene (**80**, 2.5%), (+)- $\delta$ -selinene (**81**, 75.0%, proved by comparison with an authentic reference compound by enantioselective GC), maaliolide (**82**, 1.0%), selina-5,7(11)-diene (**83**, 0.9%), and a trace of rosifoliol (**67**, 0.2%) (Fig. 34). According to the report literature, the dehydration of (-)-maaliol (**46**) using thionyl chloride in pyridine gave a mixture of  $\alpha$ -, (+)- $\beta$ - and (+)- $\gamma$ -maaliene, **55**, **56**, **78**.<sup>[104]</sup> Therefore, the formation of  $\beta$ -maaliene (**78**) through the acid transformation of **77** suggests that the hydroxy group at C-4 is  $\beta$ -oriented and *trans* to the  $\alpha$ -hydrogen at C-5.

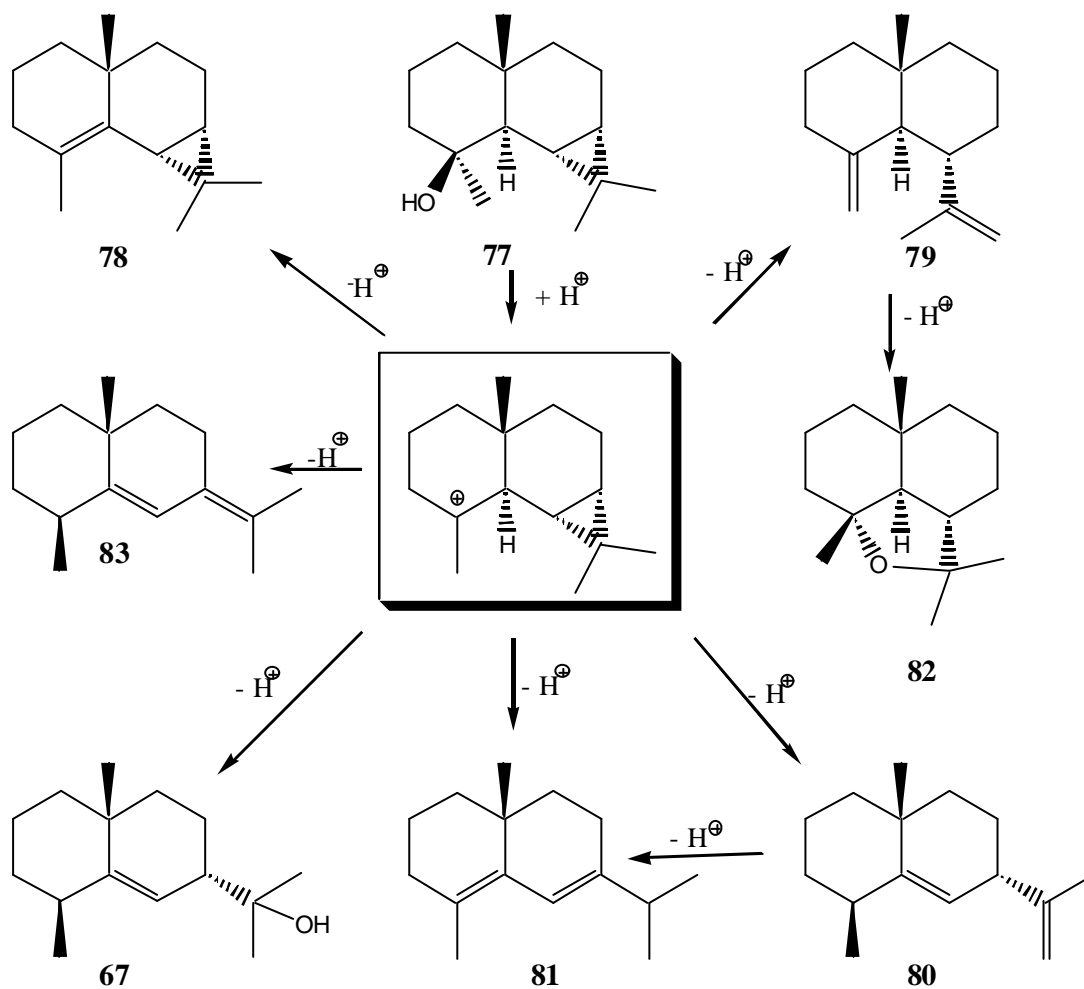


Fig. 34. Acid transformation products of (-)-4-*epi*-maaliol (**77**).

#### 4.1.6. Structure of Bisabola-1,3,5,7(14)-tetraene (**84**) (Fig. 23)

Bisabola-1,3,5,7(14)-tetraene (**84**, 3.2%), a *para*-substituted aromatic compound, showed a molecular ion signal at  $m/z$  202 consistent with the molecular formula of  $C_{15}H_{22}$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed one doublet and one singlet for methyl groups at  $\delta$  0.82 (6H, *d*, H-12, H-13,  $J = 6.6$  Hz) and 2.12 (3H, *s*, H-15), respectively. The olefinic carbon signals at  $\delta$  149.14 (*s*) and 111.77 (*t*), suggested an exomethylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  5.05 (1H, *d*, H-14a,  $J = 1.3$  Hz) and 5.34 (1H, *d*, H-14b,  $J = 1.6$  Hz). The downfield shifted signals at  $\delta$  7.01 (2H, *d*, H-2, H-4,  $J = 7.9$  Hz) and 7.33 (2H, *d*, H-1, H-5,  $J = 8.2$  Hz) were assigned to the *para*-substituted aromatic fragment since the symmetrical pattern of the  $^1H$ -NMR signal indicated a 1,4-disubstituted aromatic system. Additional structural information was obtained from the  $^{13}C$ -NMR data of **84**. All this information from  $^{13}C$ -NMR as well as from 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 35) led to structure **84**.

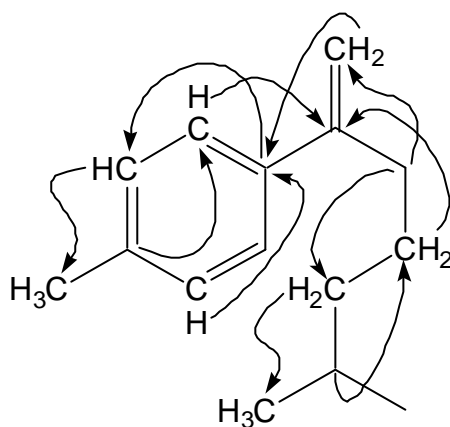


Fig. 35. Long-range  $^1H$ - $^{13}C$  correlations of **84**.

#### 4.1.7. Structure of Bisabola-1,3,5,7-tetraene (**85**) (Fig. 23)

Bisabola-1,3,5,7-tetraene (**85**, 2.3%), a colourless oil and double bond isomer of bisabola-1,3,5,7(14)-tetraene (**84**), gave a molecular ion signal at  $m/z$  202 ( $C_{15}H_{22}$ ). The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals similar to **84** except for the disappearance of the exomethylene protons, which were replaced by signals at  $\delta$  1.96 (3H, *s*, H-14) and a methine proton at  $\delta$  5.84 (1H, *br. t*, H-8,  $J = 6.94$  Hz). The  $^{13}C$ -NMR data of **85** as well as the information from 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 36) led to structure **85**.

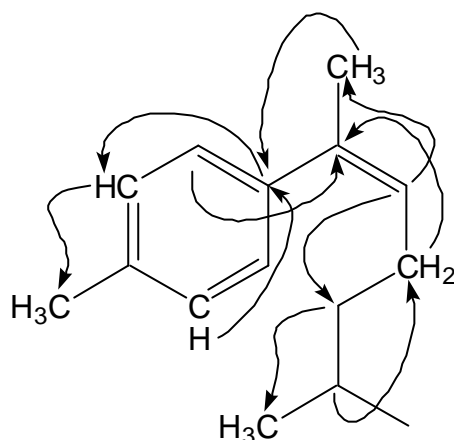


Fig. 36. Long-range  $^1H$ - $^{13}C$  correlations of **85**.

#### 4.1.8. Structure of (-)-Aromadendra-1(10),3-diene (**86**)

(-)-Aromadendra-1(10),3-diene (**86**) was isolated in trace amounts as a colourless oil from a non polar fraction. This tricyclic compound exhibited a molecular ion signal at  $m/z$  202 corresponding to a molecular formula of  $C_{15}H_{22}$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of four methyl groups at  $\delta$  0.98 (3H, *s*, H-12), 1.08 (3H, *s*, H-13), 1.55 (3H, *s*, H-14) and 1.68 (3H, *s*, H-15). Two highfield shifted protons at  $\delta$  0.55-0.60 (1H, *m*, H-7) and 0.68 (1H, *t*, H-6,  $J = 10.4$  Hz), with the methyl groups at  $\delta$  0.98 and 1.08, were assigned to the dimethyl substituted cyclopropane ring annulated to a hydroazulene skeleton. The  $^{13}C$ -NMR data of **86** as well as the information from 2D- $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 37) led to the proposed structure of **86**. Its relative configuration resulted from the NOESY spectrum (Fig. 38). Assignment of the  $\alpha$ -orientation of the cyclopropane ring was based on considerations concerning the biogenesis of **86** and its co-occurrence with (-)-bicyclogermacrene (**60**), isolated from the same essential oil (Fig. 39). The spatial interactions of proton H-6 with H-7 and one of the methyl group protons, H-12, in addition to the



interactions of proton H-5 with H-8a and the second methyl group, H13, were in agreement with the *cis*-fused cyclopropane ring. The co-occurrence of bicyclogermercene (**60**), aromadendra-1(10),3-diene (**86**) and 3 $\alpha$ -acetoxybicyclogermacrene (**45**) indicate a possible biogenetic relationship (Fig. 39).

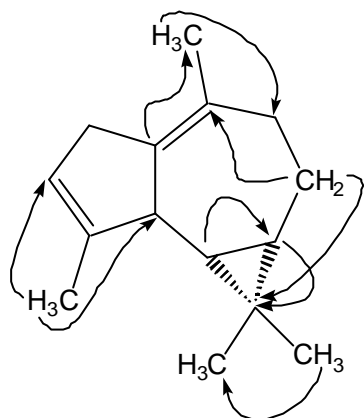


Fig. 37. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **86**.

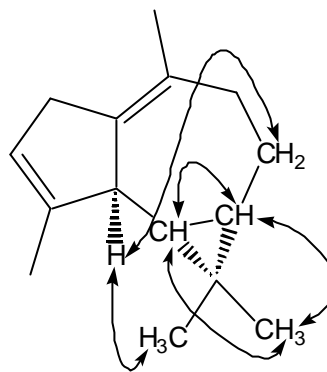


Fig. 38. NOE correlations of **86**.

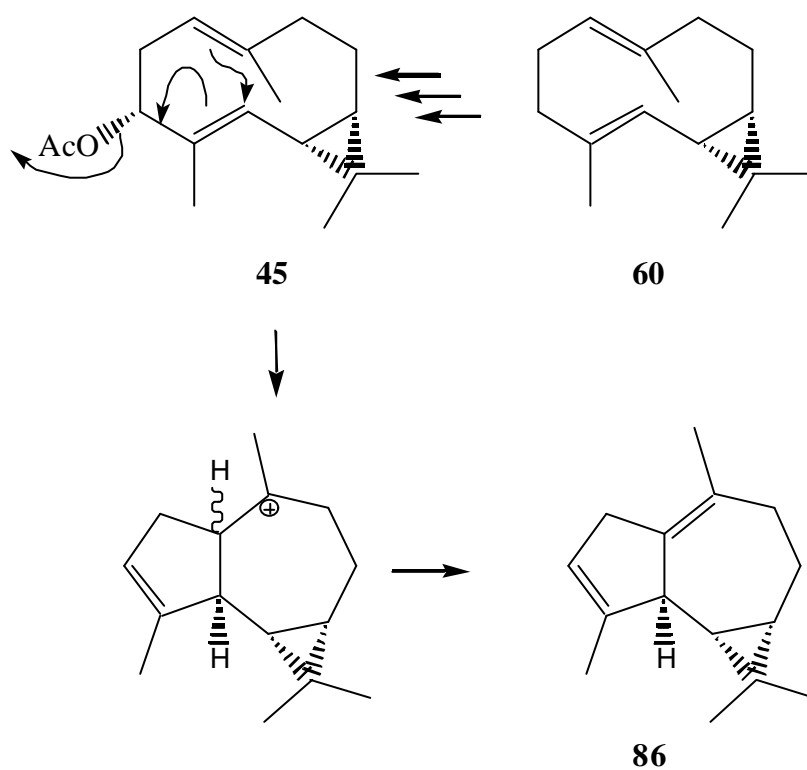


Fig. 39. Proposed biogenetic relationship of **45**, **60** and **86**.

#### 4.1.9. Comparative Study of the Essential Oils of *Plagiochila asplenioides*

Although the two *Plagiochila asplenioides* from Altenau and Kummerfeld showed quite similar sesquiterpene hydrocarbon composition, four new oxygenated compounds (+)-muurolan-4,7-peroxide (**69**), plagio-4,7-peroxide (**74**), plagiochiline-W (**75**), and plagiochiline-X (**76**) could be isolated and characterised from the essential oil of *P. asplenioides* collected in Kummerfeld which were not present in the oxygenated fraction collected in Altenau. In contrast, the aromatic sesquiterpene hydrocarbons bisabola-1,3,5,7(14)-tetraene (**84**) and bisabola-1,3,5,7-tetraene (**85**) which were not present in the hydrocarbon fractions of *P. asplenioides* from Kummerfeld were isolated from that of Altenau. (-)-Aromadendra-1(10),3-diene (**86**) and (-)-4-*epi*-maaliol (**77**) were present in the essential oils from both locations.

Therefore, considering the broad range of identified compounds from the two German *P. asplenioides* samples, they could be classified into both chemotypes-(I) and (V).<sup>[94]</sup>

#### 4.1.10. Thermal Transformation of Maalian-5-ol (**47**)

(+)-Maalian-5-ol (**47**, 18.7%) is one of the major oxygenated components of the essential oil of the liverwort *Plagiochila asplenioides* and a good source of (-)-selina-5,11-diene (**80**).<sup>[105]</sup> Thermal dehydration of maalian-5-ol (**47**) at 200 °C injector temperature by preparative GC gave (-)-selina-5,11-diene (**80**), (+)- $\delta$ -selinene (**81**), (-)-selina-5,7(11)-diene (**83**, 8.0%) and (-)-cascarilladiene (**87**) as reaction products. <sup>13</sup>C-NMR data of compounds **80** and **83** are reported for the first time (Fig. 40).

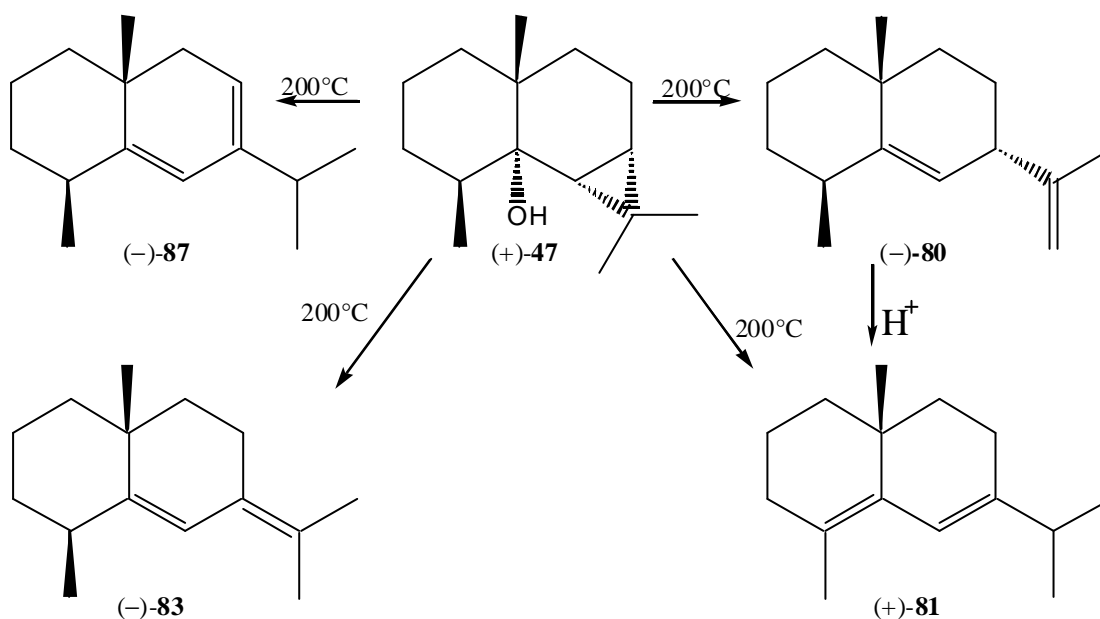


Fig. 40. Thermal transformation of maalian-5-ol (**47**).

## 4.2. Chemical Analysis of the Essential Oil of *Scapania undulata*

The botanical classification of *Scapania undulata*,<sup>[25-26]</sup>

Class : Hepaticae (Liverworts)

Subclass : Jungermanniidae

Order : Jungermanniales

Family : Scapaniaceae

Species : *Scapania undulata*,

Several chemotypes of *Scapania undulata* have been characterized and the European *S. undulata* have been classified into four chemical races: the longifolene-type, the longiborneol-type, the (+)-ent-*epi*-cubenol-type and the labdane-type.<sup>[113]</sup> The constituents of the liverwort *S. undulata* have been investigated, and its sesquiterpenoid features were reported to be very complex.<sup>[32,113,114]</sup> Since several interesting compounds have been reported from this family, a reinvestigation of the volatile constituents of *S. undulata* prepared by hydrodistillation or extraction with diethylether was carried out.

### 4.2.1. Composition of the Essential Oils of *Scapania undulata*

The essential oil of *Scapania undulata*, a liverwort which is very abundant in the Harz mountains near Altenau (Northern Germany), was prepared by hydrodistillation and analysed by GC and GC-MS. In order to exclude artefact formation by hydrodistillation also a diethyl ether extract was prepared and investigated by the same methods. The chemical constituents of the ether extract were consistent with the hydrodistilled product but different in yield. The following sesquiterpenes were identified in the order of their elution from a capillary column with polydimethylsiloxane (CPSIL-5) as constituents of the essential oil of *S. undulata*. For structures see Figs. 41a-b and for GC-traces see Figs. 41c-e. Relative concentrations of major compounds are given in parentheses, those below 1% are only listed:  $\alpha$ -longipinene (**88**, 1.6%),  $\alpha$ -ylangene (**89**), longicylene (**90**), sativene (**91**),  $\beta$ -longipene (**92**, 5%), (-)-*cis*-(5*R*, 7*R*, 10*S*)- $\beta$ -elemene (**93**), longifolene (**94**, 19%),  $\beta$ -maaliene (**78**),  $\alpha$ -barbatene (**53**), E- $\beta$ -caryophyllene (**24**),  $\beta$ -ylangene (**95**), (+)- $\beta$ -isolongibornene (**96**, 2.4%), isobazzanene (**97**),  $\beta$ -barbatene (**57**, 1.5%),  $\alpha$ -himachalene (**98**, 4.7%), ar-curcumene (**99**), isobicyclgermacrene (**100**), (+)- $\alpha$ -amorphene (**101**),  $\gamma$ -himachalene (**102**, 2.3%), isolepidozene (**103**)<sup>[115]</sup>  $\alpha$ -bisabolene (**104**, 1.6%), (+)- $\alpha$ -muurolene (**105**),  $\beta$ -himachalene (**106**, 1.1%), (+)-helminthogermacrene [(+)-1,5-dimethyl-8-(1-methylethenyl)-cyclodeca-1*E*,5*Z*-diene] (**107**, 1%), (+)- $\alpha$ -chamigrene (**62**, 2.2%),  $\delta$ -cadinene (**108**), E- $\alpha$ -bisabolene (**109**), (-)-perfora-1,7-diene (**110**, 1.1%), longipinanol (**111**, 7.2%, in the ether extract),

maaliol (**46**), longiborneol (**112**, 30.2%), 1-*epi*-cubenol (**113**), 2-himachalen-7-ol (**114**, 2.1%) and nerodilol (**115**). All known constituents were identified by computer-supported comparison of their mass spectra and gas chromatographic retention indices with a spectral library established under identical experimental conditions.<sup>[107-108]</sup>

An essential oil / extract of *Scapania undulata* obtained from Norway in June 2002 was also analysed by GC, GC-MS. The chemical composition of this *S. undulata* sample was consistent with the longiborneol-chemotype collected in Germany, except for the presence of mintsulphide (**116**) identified in Norway *Scapania undulata* sample. Thus, the possibility of a longiborneol-chemotype as one of the four chemical races in Norway is confirmed.

The identified constituents of *Scapania undulata* collected from the Harz area in Germany suggest that two of four-chemotypes predominate in this region: a chemotype with the major sesquiterpene constituents possessing a cadinane skeleton (1-*epi*-cubenol-type) and a chemotype with longiborneol and longipinane derivatives as major constituents (longiborneol type). Identified essential oil constituents of the 1-*epi*-cubenol-type consists of  $\alpha$ -longipinene (**88**),  $\beta$ -longipinene (**92**),  $\beta$ -ylangene (**95**), calarene (**117**), cadina-3,5-diene (**118**), germacrene-D (**25**), isolepidozene (**103**), cadina-1,4-diene (**119**), perfora-1,7-diene (**110**), maaliol (**46**), 4 $\alpha$ -hydroxygermacra-1(10),5-diene (**120**), scapanol (**121**), longiborneol (**112**), muurol-4-en-6 $\alpha$ -ol (**122**), 1-*epi*-cubenol (**113**), T-muurolol (**123**), and cubenol (**124**), (Fig. 41a-e).

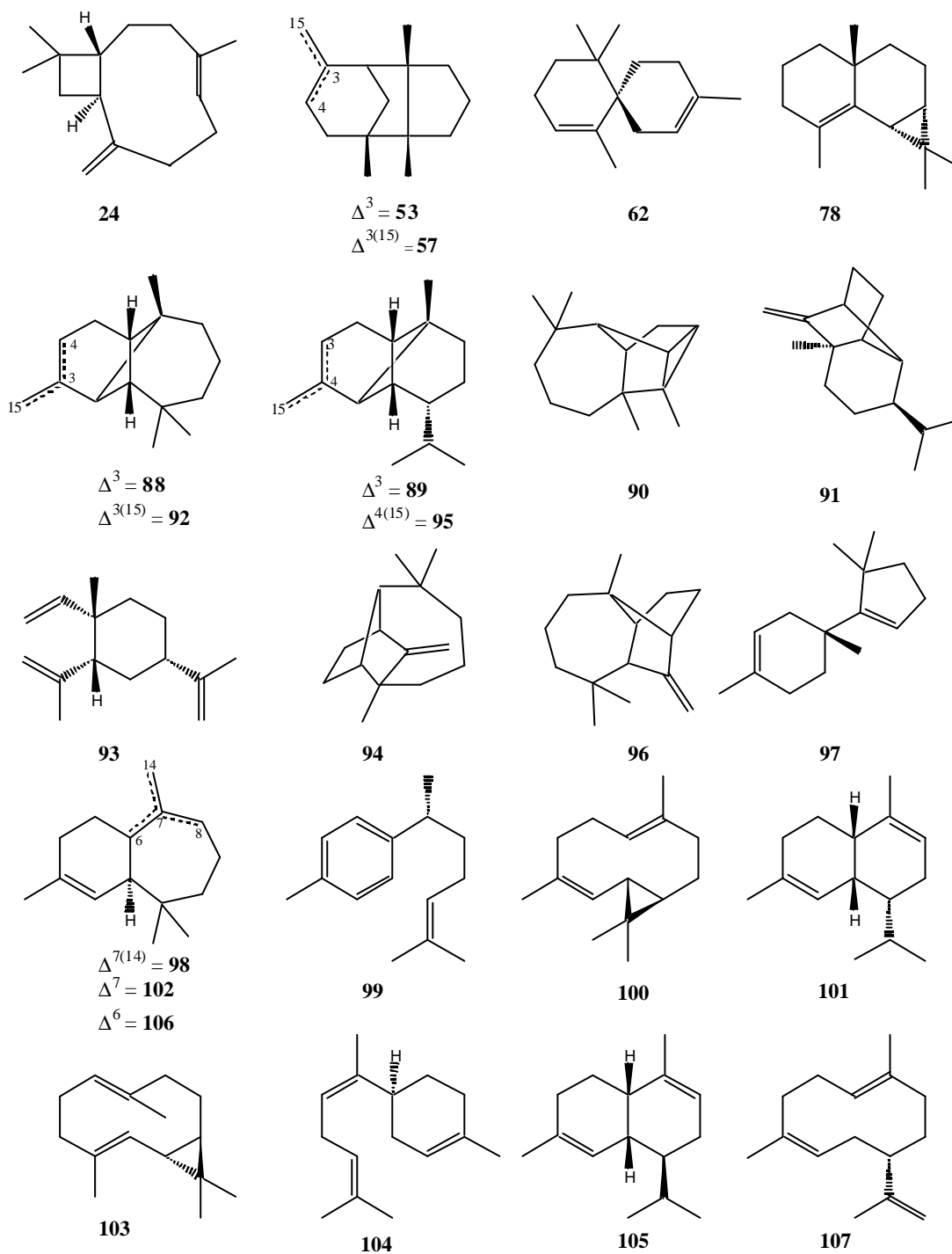


Fig. 41a . Sesquiterpenes of the essential oil of *Scapania undulata*.

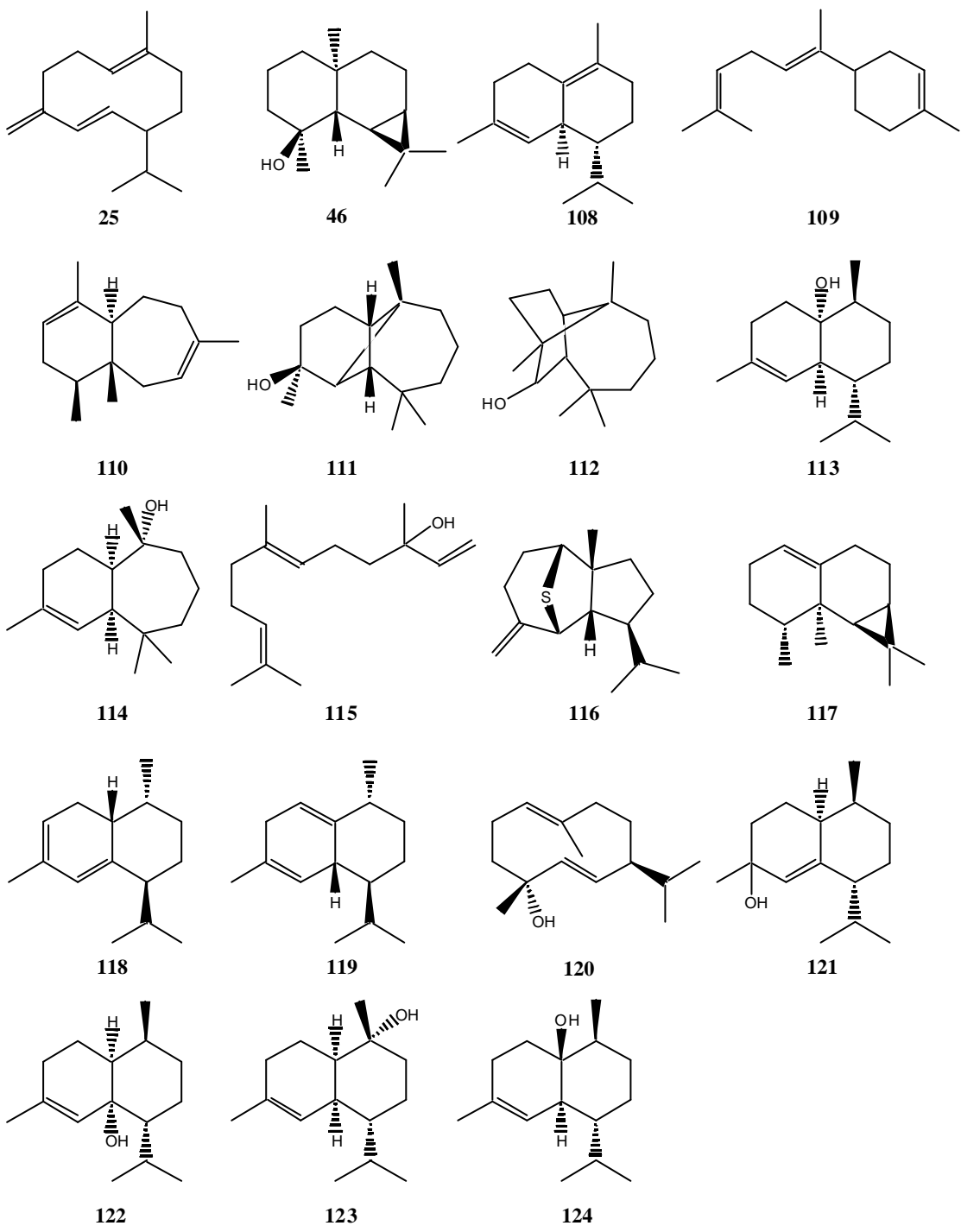


Fig. 41b. Sesquiterpenes of the essential oil of *Scapania undulata*.

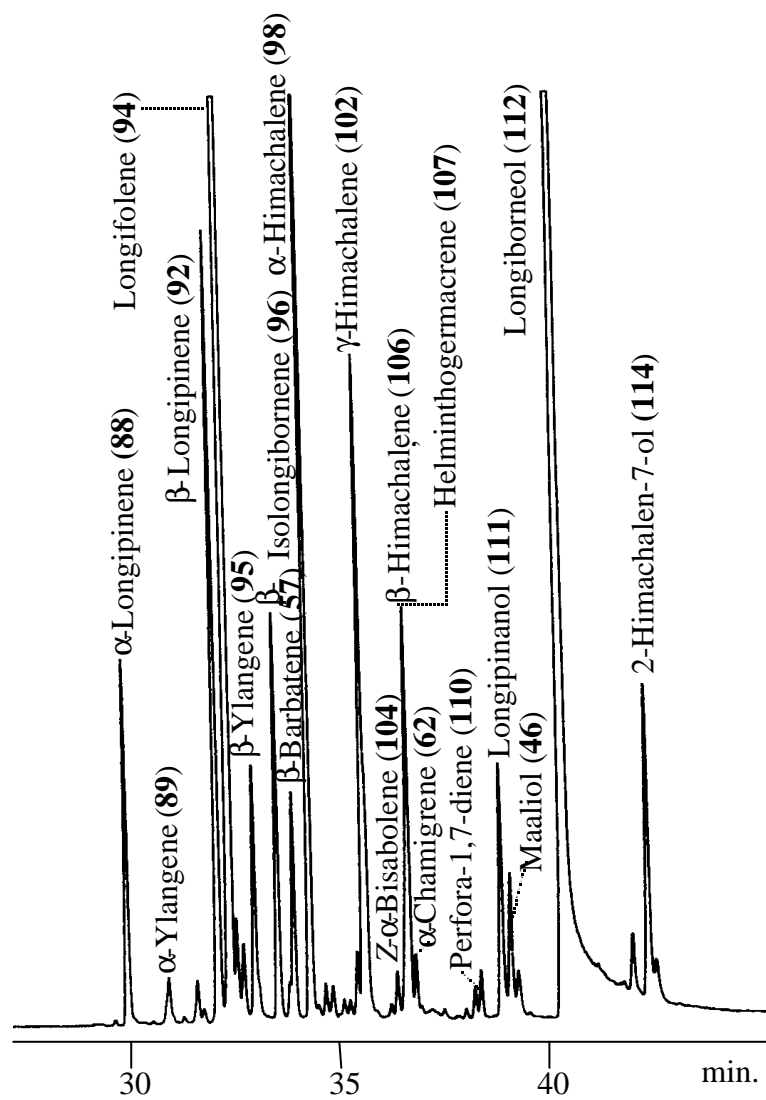


Fig. 41c. Gas chromatogram of the essential oil of *Scapania undulata* (Longiborneol-type) from Altenau (Germany). (CPSIL 5, 50°C, 3°C / min., 230°C).

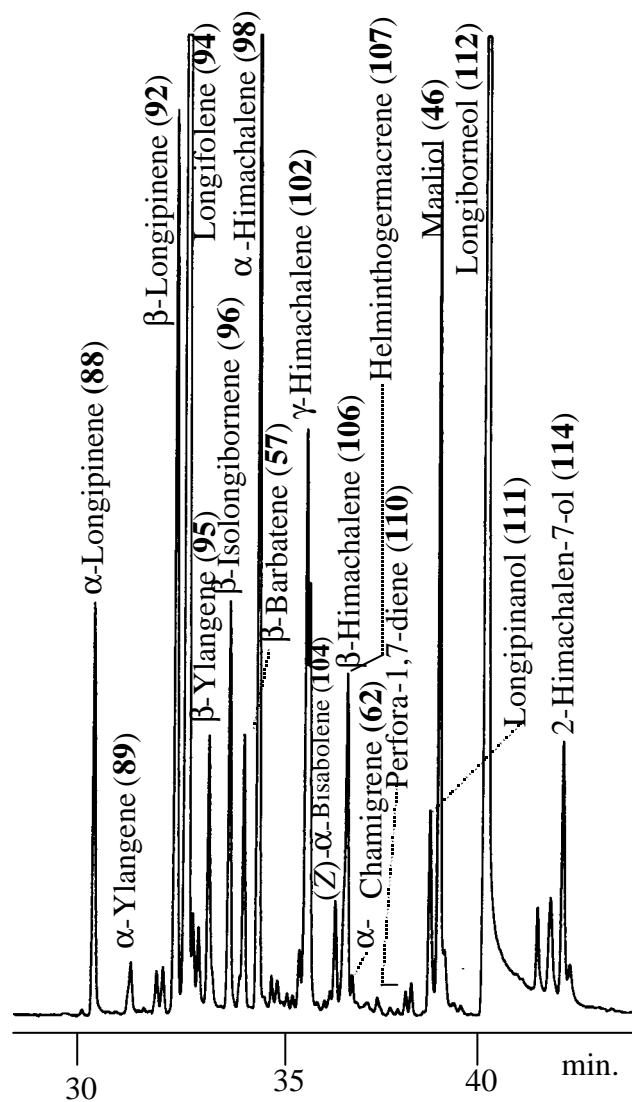


Fig. 41d. Gas chromatogram of the essential oil of *Scapania undulata* (Longiborneol-type) from Norway. (CPSIL 5, 50°C, 3°C / min., 230°C).



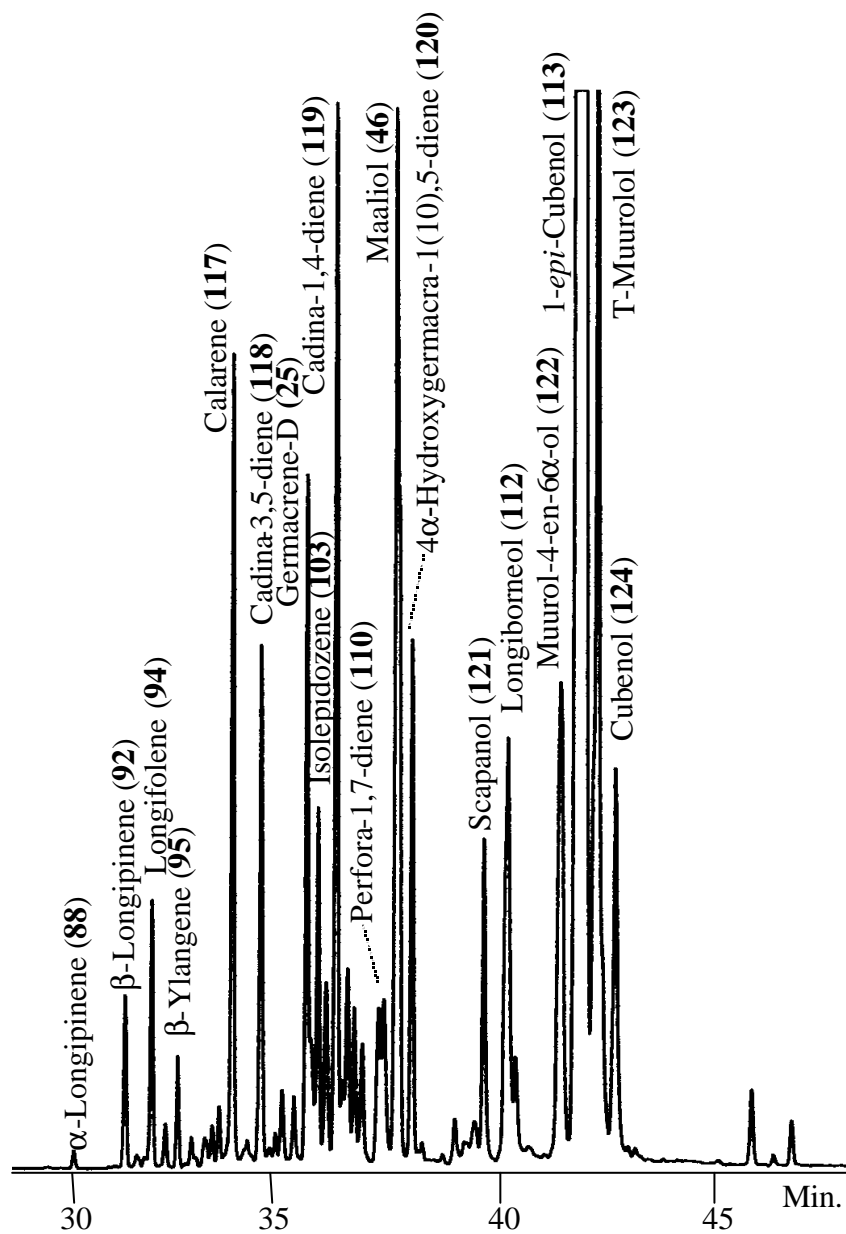


Fig. 41e. Gas chromatogram of the essential oil of *Scapania undulata* (Cadinane-type) from Okerstein (Harz, Germany). (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.2.2. Structure of (+)-Helminthogermacrene (**107**)

(+)-Helminthogermacrene (**107**) was isolated for the first time from a liverwort. The mass spectrum of the 10-membered monocyclic sesquiterpene hydrocarbon exhibits a molecular ion signal at  $m/z$  204 corresponding to the molecular formula of  $C_{15}H_{24}$ . The mass spectrum of **107** and the retention index on a non-polar stationary phase are identical to germacrene A (**32**). However, a separation from germacrene A is achieved on a more polar stationary phase as CPSil 19. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed broadened signals in the region between  $\delta$  1.84-2.24. The three downfield shifted singlets for the methyl groups at  $\delta$  1.57 (3H, *s*,  $CH_3$ -14), 1.66 (3H, *br.s*,  $CH_3$ -12) and 1.70 (3H, *s*,  $CH_3$ -15) are well resolved (Fig. 42.). In the  $^{13}C$ -NMR spectrum the olefinic carbon signals at  $\delta$  150.6 (*s*) and 109.3 (*t*) suggested an *exomethylene* double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at

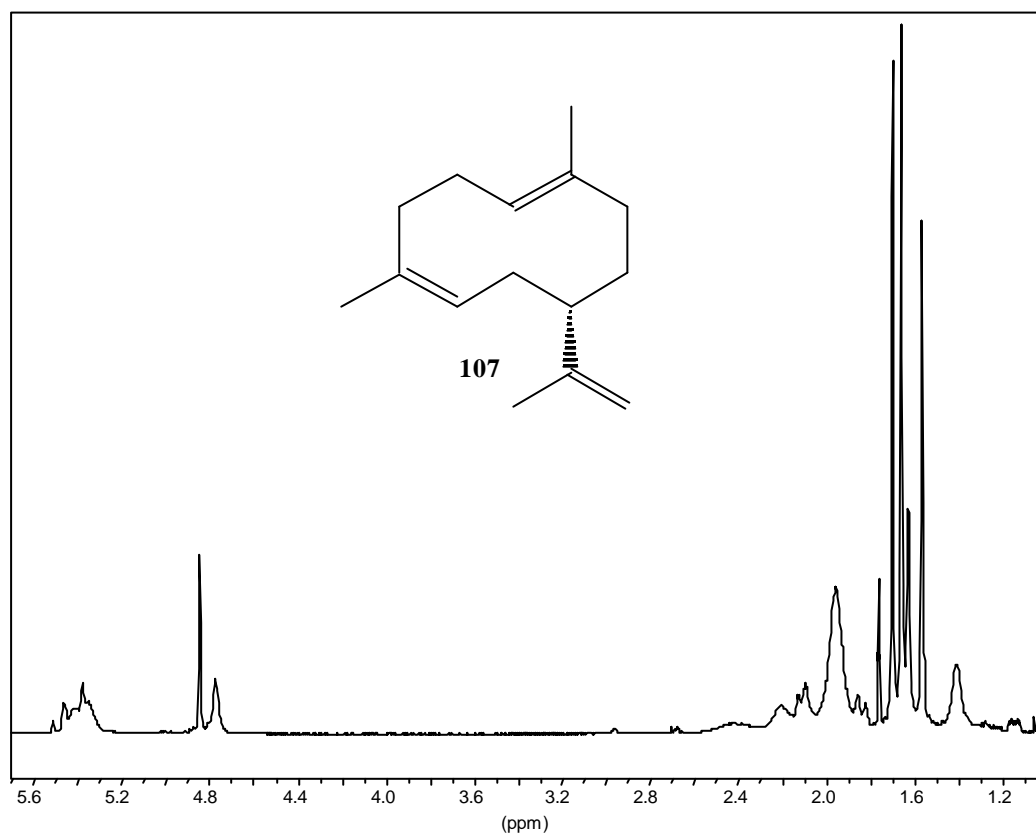


Fig. 42.  $^1H$ -NMR (500 MHz,  $C_6D_6$ ) of (+)-Helminthogermacrene (**107**) at room temp.

$\delta$  4.77 (1H, *br. s*) and 4.85 (1H, *s*). The multiplet signal at  $\delta$  5.30-5.44 (2H, *m*) was assigned to the two methine protons H-1 and H-5. In addition, the  $^1H$ -NMR of **107**, recorded in acetone- $d_6$  at  $-8$  °C and  $-16$  °C, showed less broadened, separated multiplet signals. Therefore, one conformation predominates, since only one set of signals was observed. Compared to many germacratrienes showing *E,E*-configuration of the double bonds in the cyclodecadiene system which show temperature dependent NMR spectra (multiple sets of

signals) indicative of conformational equilibria in solution,<sup>47,116</sup> **107** occurs essentially in one preferred conformation even at room temperature. All this information from as well as <sup>13</sup>C-NMR HMBC, HMQC and <sup>1</sup>H-<sup>1</sup>H-COSY led to structure **107**. Its relative configuration resulted from the NOESY spectrum which showed that the methyl group at  $\delta$  1.70 (3H, *s*, CH<sub>3</sub>-15) spatially interacts with the multiplet signal at  $\delta$  5.30-5.44. This interaction suggests that H-15 is in *cis*-configuration with the H-5 methine proton, which is only possible for a *Z*-4-5 double bond. Spatial interactions of H<sub>12</sub> at  $\delta$  1.66 (3H, *br.s*) with the *exo*-methylene protons H<sub>2</sub>-13 was also observed (Fig. 43).

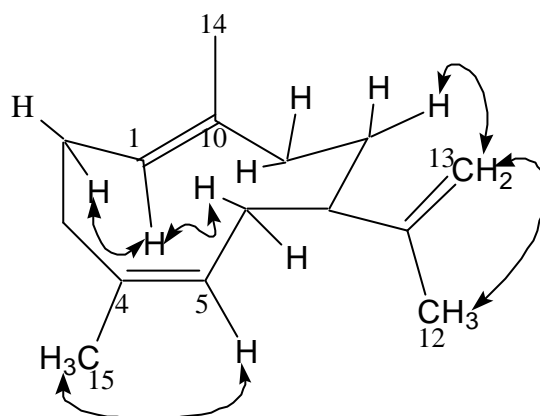


Fig. 43. NOE correlations of **107**

The <sup>1</sup>H-NMR spectra recorded in CDCl<sub>3</sub> and the <sup>13</sup>C-NMR recorded in acetone-d<sub>6</sub> were identical with those reported for the levorotatory enantiomer of **107** found in the defense secretion of the termite *Amitermes wheeleri*,<sup>[117]</sup> and the same enantiomer isolated from the mycelium of the fungus *Helminthosporium sativum* with its structure verified by total synthesis.<sup>[118-119]</sup> The unproven absolute configuration of the (-)-enantiomer of **107** was initially assigned H-7 $\alpha$  as concluded from the sesquiterpene congeners (-)-sativene (**91**) and (-)-longifolene (**94**) which were also isolated from *Helminthosporium sativum*.<sup>[118]</sup> To verify the absolute configuration, an indirect chemical correlation approach was employed. This involves a transannular cyclization reactions of **107**, to rearrangement products which could be easily identified by subjecting **107** to acid- and thermal (*Cope*) rearrangement reactions. The reason is that transannular cyclisations usually create chiral centers regio- and stereospecifically in reactions starting from trigonal carbons atoms.<sup>[43]</sup> Hence, the reacting conformations of **107** could be correlated with the configuration of the products.

#### 4.2.2.1. Acid Treatment of Helminthogermacrene (107)

(+)-(107), undergoes rearrangement when stored in  $\text{CDCl}_3$  at  $4^\circ\text{C}$  for at least one week to form a series of sesquiterpene hydrocarbons with mass 204 as observed on treatment of **107** with  $\text{BF}_3$  in diethyl ether or upon interaction with acidic ion exchange resin Amberlyst 15, respectively. The isolation of the unknown major compound common to all three treatments resulted in a product with  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ) and MS data totally consistent with (-)- $\alpha$ -helmiscapene (**125**), earlier isolated from *S. undulata*<sup>[32]</sup> and synthesized<sup>[121-123]</sup> with known absolute configuration. The sign of optical rotation of isolated **125** was negative as earlier reported.<sup>[32]</sup> The MS of **125** is very similar to that of its 10-epimer,  $\alpha$ -selinene (**126**). The formation of **125** from **107** can be rationalized as demonstrated (Fig. 44).

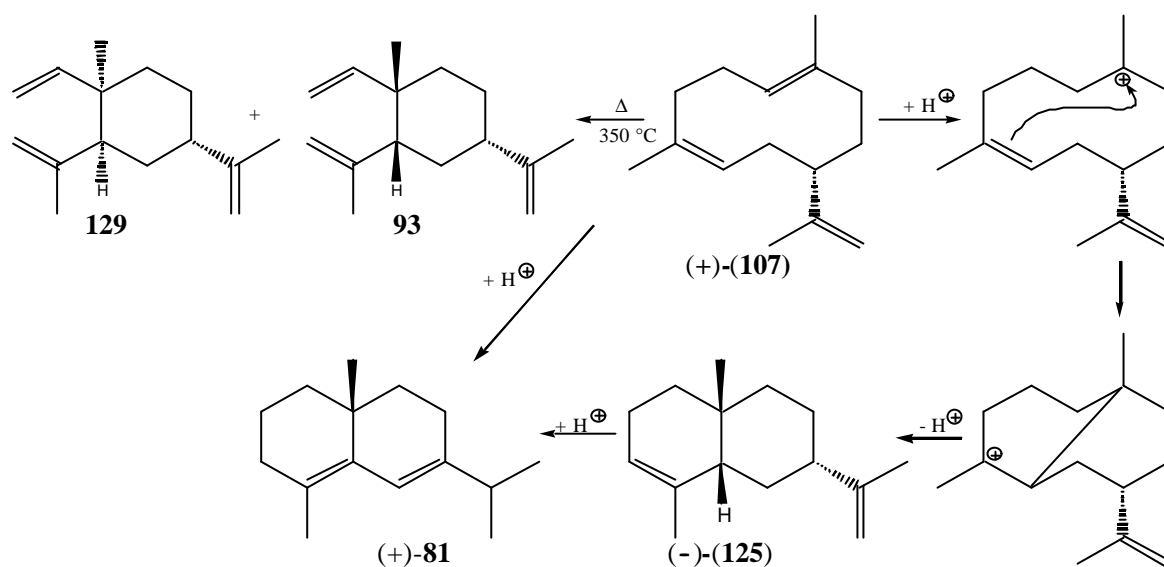


Fig. 44. Acid and thermal rearrangement of (+)-107.

In addition, the resolution of  $\delta$ -selinene (**81**), produced by treatment of **107** with  $\text{BF}_3$ , using heptakis(6-*O-tert*.butyldimethylsilyl-2,3-di-*O*-methyl)- $\beta$ -cyclodextrin as the stationary phase confirmed that a mixture of the (+)- and (-)-enantiomer in the ratio 5 : 1 was formed.

The formation of both enantiomers of **81** and the co-occurrence of a small amount of diastereomeric forms of *cis*- $\beta$ -elemene (**93**) suggest that more than one conformation might be present during the rearrangement process, although  $^1\text{H-NMR}$  recordings at ambient temperature,  $-8^\circ\text{C}$  and  $-16^\circ\text{C}$  did not reflect any significant doubling of signals. Thus, the conformational equilibrium in solution predominantly favoured the (+)-(*E,Z*)-107 configurational isomer.

No significant rearrangement products were observed when **107** was treated with silica gel for 3 hours. A series of *cis*-fused selinenes have been isolated from *S. undulata*,<sup>[32]</sup> *Helminthosporium sativum*<sup>[124]</sup> and the termite *Amitermes excellens*,<sup>[125]</sup> hence, **107** could be a possible biogenetic precursor rather than the *E,E*-germacrenes e.g. germacrene- A (**32**), Fig. 44.

#### 4.2.2.2. Hydrogenation of $\alpha$ -Helmiscapene (**125**) and Absolute Configuration of (+)-**107**

The comparison of hydrogenation products from (–)- $\alpha$ -helmiscapene (**125**) with the hydrogenated products of compounds of known absolute configuration like (+)- $\alpha$ -selinene (**126**), (+)- $\beta$ -selinene (**127**) and (–)-selina-5,11-diene (**80**), one of the dehydrated products of (–)-maalian-5-ol<sup>[105]</sup> (**47**) by enantioselective GC again confirmed the absolute configuration of (–)-(**125**) which is characterized by  $\beta$ -orientation of the angular methyl substituent at C-10 and the H-5 and H-7 protons and thus should be (5*S*, 7*S*, 10*S*). From this it can be concluded that the absolute configuration of the precursor (+)-helminthogermacrene (**107**) should also be 7*S* ( $\beta$ -orientation of the H-7 proton). Moreover the co-occurrence of (–)- $\alpha$ -ylangene (**89**)<sup>[32]</sup> and (+)- $\alpha$ -amorphene (**101**) in the essential oil of *S. undulata* is in agreement with the proposed absolute configuration (Fig. 45).

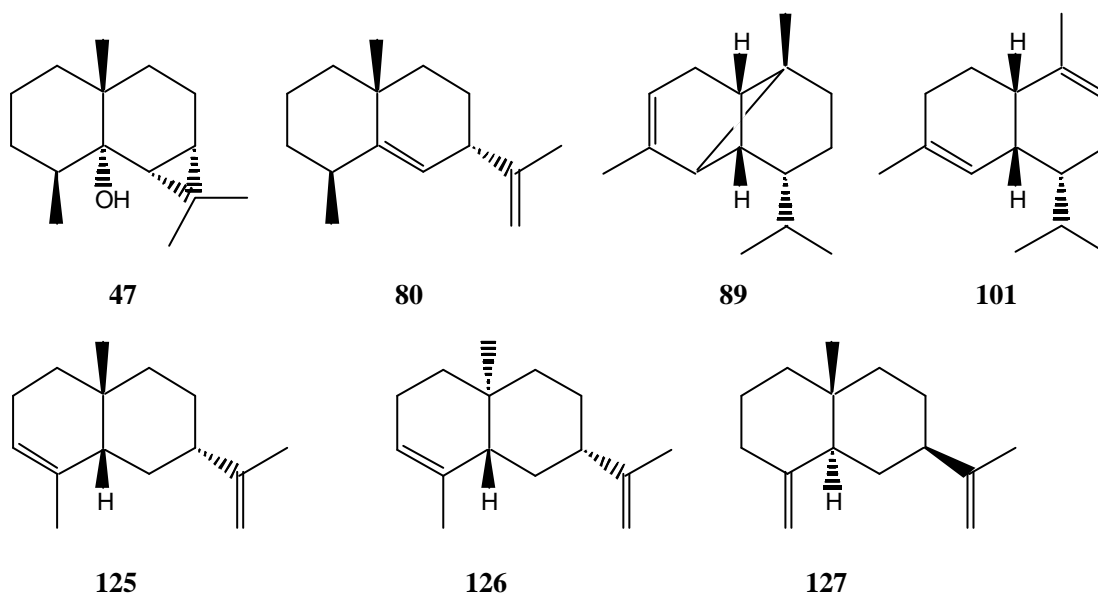


Fig. 45. Compounds mentioned in the text.

#### 4.2.2.3. Hydrogenation of Helminthogermacrene (107)

The hydrogenation of helminthogermacrene (**107**) for 2 hours at room temperature using palladium /charcoal in hexane resulted in complex mixtures of compounds with  $M = 206$  and  $M = 208$ . Attempts to compare the hydrogenation products of **107** with both (+)- and (-)-germacrene-D (**25**) isolated from *Solidago canadensis* and *Mentha piperita* was not conclusive since both enantiomers of **25** also gave similar complex mixture of compounds with  $M = 206$  and  $M = 208$ . Thus, the complexity of the product mixture showed that significant isomerization was occurring. Similar observations have been reported for (*E,E,E*)-1,7-dimethylcyclodeca-1,4,7-triene (pregeijerene B) (**128**) isolated from the foliage of *Juniperus erectopatens*<sup>[133]</sup> (Fig. 46).

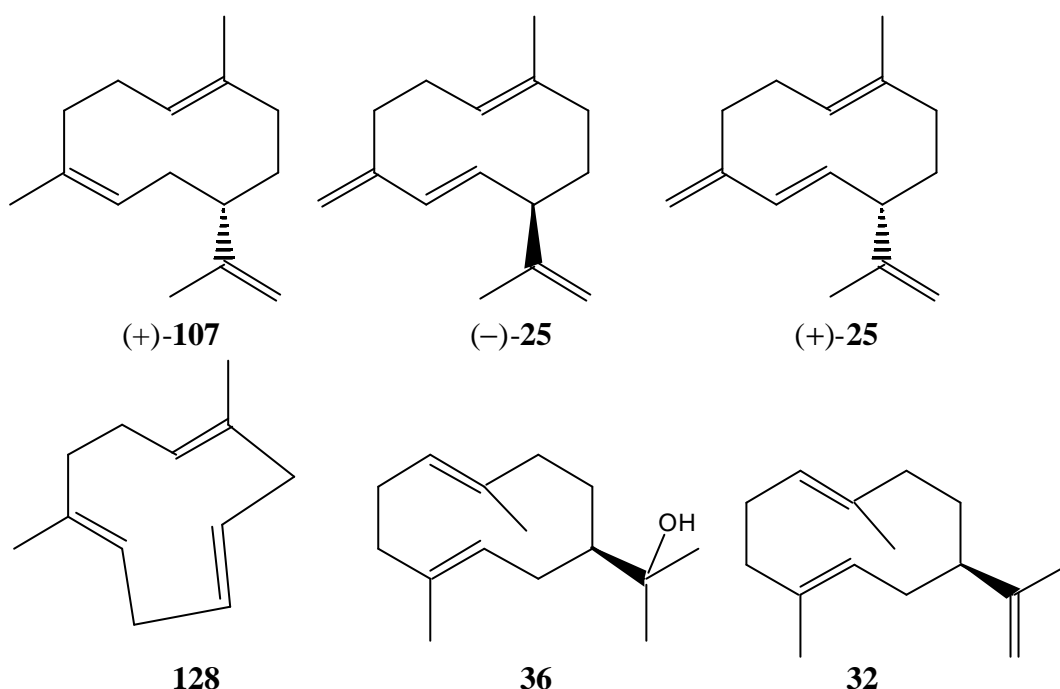


Fig. 46. Germacrenes mentioned in the text.

#### 4.2.2.4. Thermal Isomerization (Cope rearrangement) of (+)-**107** (see Fig. 44)

Thermal isomerization of **107** was achieved above 350 °C to afford *cis*- $\beta$ -elemene (**93**), another  $\beta$ -elemene diastereomer (**129**) and a trace of "normal" (-)- $\beta$ -elemene (**40**) in the ratio of (8 : 1 : ~0.08), respectively (elution order from CPSIL-5). **93** and **129** exhibit mass spectra undistinguishable from that of  $\beta$ -elemene (**40**) with a molecular ion signal at  $m/z$  204 and molecular formula  $C_{15}H_{24}$ . Further investigation of the products obtained from rearrangement of **107** by enantioselective GC on octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\gamma$ -cyclodextrin [Fig. 47] suggests that the third product formed is a racemic mixture of  $\beta$ -elemene (**40**) indicating

germacrene A (**32**) as precursor. The other conceivable possibility is that there are epimers formed that happened to coelute with the respective  $\beta$ -elemenes (**40**) which is highly unlikely as the separation is such that epimers should be resolved as well.

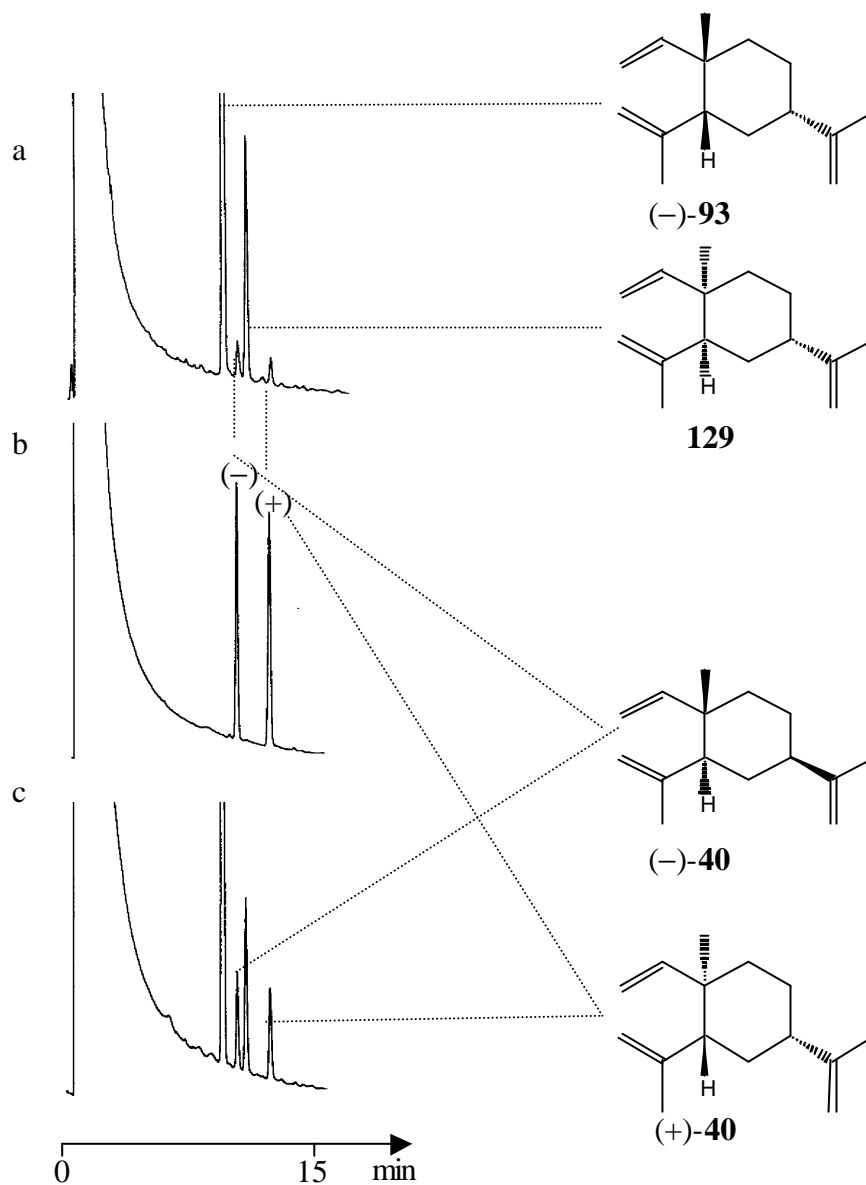


Fig. 47. Comparison of the elemenes from (+)-**107** (a); (+/-)-**40** (b) and co-injection (c) on the Cyclodextrin phase 2,6-Me-3-Pe- $\gamma$ -CD at 100 °C isothermal.

#### 4.2.3. Structure of (-)-*cis*-**β**-Elemene (**93**)

*cis*-**β**-Elemene (**93**) was detected only in trace amounts in the original essential oil of *S. undulata*. As shown above in Figs. 44 and 47, it can be generated from **107** by *Cope* rearrangement and isolated by preparative GC at an injector temperature of 390 °C. **93** exhibits a mass spectrum undistinguishable from **β**-elemene (**40**) with a molecular ion signal at  $m/z$  204 corresponding to the molecular formula  $C_{15}H_{24}$ .

The  $^1H$ -NMR of **93** was recorded in both  $C_6D_6$  and  $CDCl_3$  to achieve the best resolution over the whole chemical shift range. The  $^1H$ -NMR ( $C_6D_6$ ) showed signals of three methyl singlets at  $\delta$  1.05 (3H, *s*,  $CH_3$ -14), 1.66 (3H, *s*,  $CH_3$ -15) and 1.67 (3H, *s*,  $CH_3$ -12). Interestingly, the  $CH_3$ -15 signal in **93** is slightly up-field shifted by 0.04 ppm as compared to **β**-elemene (**40**). The two methylene protons at  $\delta$  5.01 (*ddd*, 2H, H-2,  $J = 1.26, 11.0, 17.3$  Hz) formed an ABX system with a methine proton at  $\delta$  6.29 (*dd*, 1H, H-1,  $J = 11.0, 17.3$  Hz). Additional structural information was obtained from the  $^{13}C$ -NMR spectrum of **93** which confirmed the presence of three methyl groups at  $\delta$  21.4, 22.9 and 27.8, six methylene groups at  $\delta$  28.0, 33.8, 42.2, 109.1, 112.9 and 113.4, three methine groups at  $\delta$  46.5, 56.2 and 143.3 and three quaternary carbons at  $\delta$  39.5, 147.2 and 150.3. The structure of compound **93** was elucidated based on information established by 2D- $^1H$ - $^1H$ -COSY, HSQC, HMBC (Fig. 48), and NOESY in comparison with the spectral data of authentic **β**-elemene (**40**)

For the relative configuration of **93** a *cis*-relationship at C-5 and C-10 was concluded from a strong NOE interaction of the H-5 and H-14 protons. Furthermore, interactions between H-9a with both H-5 and H-7 confirmed that the three hydrogens are on the same side of the molecule. Since **93** is formed from **107** with retention of its configuration at C-7,  $\beta$ -orientation for H-7, and consequently for  $CH_3$ -14, is suggested (Fig. 49).

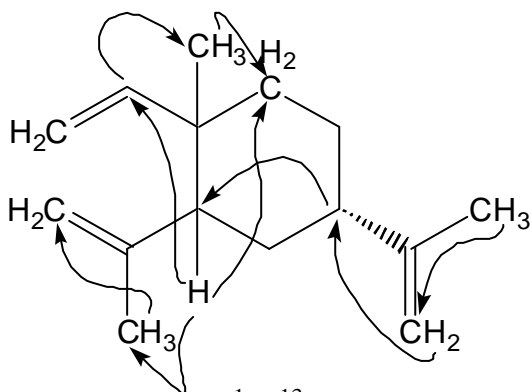


Fig. 48. Long-range  $^1H$ - $^{13}C$  correlations of **93**.

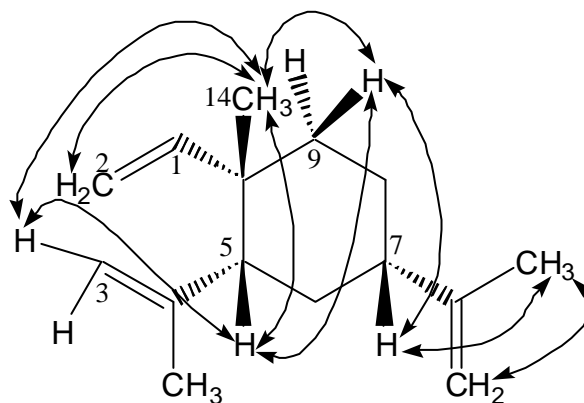


Fig. 49. NOE correlations of **93**.



The structure of compound **129**, a diastereomer of **93** (see Fig. 47), was assigned based on identical MS and  $^1\text{H-NMR}$  data with **93**. Although, the chemical shift of the signals of **129** in the  $^1\text{H-NMR}$  ( $\text{C}_6\text{D}_6$ ) were slightly shifted up-field with the methine proton at  $\delta$  6.22 (1H, *dd*,  $J = 11.0, 17.7$  Hz) compared to  $\delta$  6.29 in **93**. The slight up-field shift of the methine proton signal to  $\delta$  6.23 (*dd*, 1H,  $J = 11.4, 17.7$  Hz) was also observed in the  $^1\text{H-NMR}$ , recorded in  $\text{CDCl}_3$ , as compared to  $\delta$  6.31 (*dd*, 1H,  $J = 11.03, 17.7$  Hz) of **93**, while the corresponding proton of authentic  $\beta$ -elemene (**40**) absorbs at  $\delta$  5.80 (1H, *dd*,  $J = 10.7, 17.3$  Hz). The C-10 methyl singlet signal of **129** also appeared slightly shielded at  $\delta$  1.04 as compared to that of **93** at  $\delta$  1.05.

#### 4.2.4. Comparison of the *Cope* rearrangement of (+)-**107** and Germacrene A (**32**)

The *Cope* rearrangements products of (+)-helminthogermacrene (**107**) was compared with that of (+)-germacrene A (**32**), isolated from a diethyl ether extract of *Solidago canadensis* under identical conditions. *Cope* rearrangement of **32** gave two products in a ratio of 95:5 (Fig. 50a). The major compound was confirmed to be (-)- $\beta$ -elemene (**40**), while the new minor product **129a** was observed for the first time. The existence of an additional elemene-type compound has never been identified before even though it has been speculated.<sup>[116]</sup>

Compound **129a** elutes before (-)- $\beta$ -elemene (**40**) from the GC column during the *Cope* rearrangement of (+)-germacrene A (**32**), and it increases with increasing amount of rearranged **32**. The MS of **129a** is very similar to “normal”  $\beta$ -elemene (**40**).

Coinjection of the two *Cope* rearrangement products from helminthogermacrene (**107**) and germacrene A (**32**) on two columns in parallel with dimethylpolysiloxane CPSil-5 and the more polar CPSil-19 stationary phase, respectively, revealed that (**129a**) is not identical to

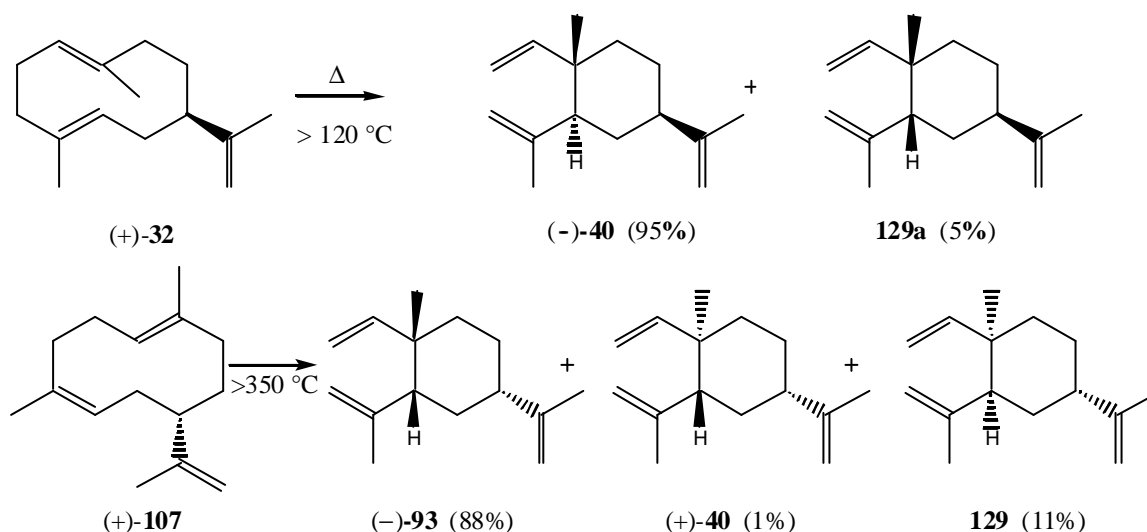


Fig. 50a. *Cope* rearrangement of germacrene-A (**32**) and helminthogermacrene (**107**).

*cis*- $\beta$ -elemene (**93**) but coelutes with **129**.

Coinjections using enantioselective GC shows separation of **129** and **129a**, hence both are of opposite absolute configuration. The mixture was hydrogenated to confirm that one more product was generated in addition to  $\beta$ -elemene (**40**).

The results of molecular mechanics calculations carried out to determine the relative stabilities of each conformation of germacrene-A (**32**) in their ground and transition states in the literature<sup>[127]</sup> were in agreement with the thermal isomerization products **40** and **129a**.

This observation is similar to the thermal isomerization of bicyclogermacrene (**60**)<sup>[126]</sup> (Fig. 50b) and hedycaryol (**36**).<sup>[120]</sup> Bicyclogermacrene (**60**) rearranged to bicycloelemene (**133**) and isobicyclogermacrene (**100**) as shown. The (*E,Z*)-isomer of **36** rearranged at 500 °C to the corresponding *cis*-isoelemol (**130**) and three different diastereoisomers (130a-c), (Fig. 50b).<sup>[120]</sup> In other cases, e.g. germacrene-A (**32**), -B (**33**) and -C (**34**), have been reported to produce only one type of diastereomer upon heating.<sup>[58,60]</sup> (Fig. 50b).

In addition, compound **129a**, a diastereoisomer of  $\beta$ -elemene (**40**) was also identified for the first time in fresh costus roots oils (*Saussurea lappa*) obtained from Japan in June 2002, (Adio, A. M. and König, W. A., Unpublished results).

Therefore, since helminthogermacrene (**107**) having 1(10),4-*E,Z* configuration undergoes thermal rearrangement at higher temperatures (> 300 °C) compared to germacrene A (**32**) with 1(10),4-*E,E*- configuration which undergoes *Cope* rearrangements at room temperature. This suggests that **107** is more stable than **32**.

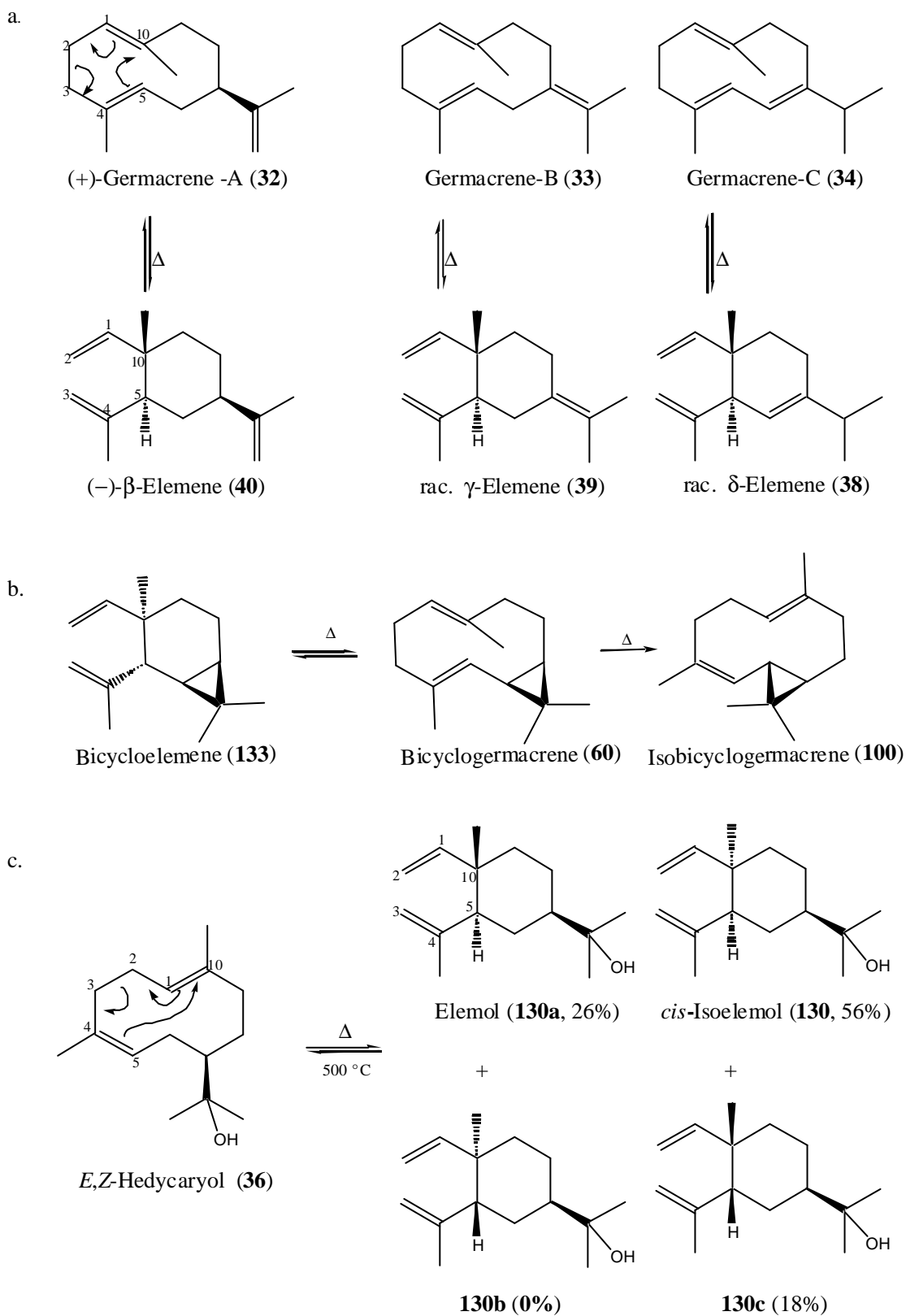


Fig. 50b. Thermal or *Cope* rearrangements of germacrene to elemene.

#### 4.2.5. Acid Transformations of Germacrene-A (32) and B (33)

When the diethyl ether extract of *S. canadensis* was exposed to silica gel at room temperature for 24 hours it gave (-)-selin-11-en-4-ol (**131**, 23%), (**131** had been reported as a major constituent of the essential oil of *Conocephalum conicum*<sup>[128]</sup>) in addition to reported *trans*-selinenes, **126**, **127** and **132**.<sup>[129]</sup> Thus, germacrene-A (**32**) rearranges to these bicyclic systems in an analogous fashion as in helminthogermacrene (**107**). The proposed pathway for the formation of **131** is depicted as shown (Fig. 51). This observation has never been reported before for compound **32**, even though it has been reported that germacrenes B (**33**) and -C (**34**) gave similar oxygenated products when subjected to acid rearrangement reactions.<sup>[130-132]</sup> Similar acid rearrangement reaction products of germacrene B (**33**) were obtained but it was noticed that this rearrangement occurred rather slowly with very low yield as compared to **32** under the same conditions. Therefore, considering the rate and the yield of *trans*-selinenes formed, it is suggested that **33** is more stable than **32** when treated with silica gel under the same conditions. **32** also proved to be much more stable than reported in literature with silica gel if the experiment was run within a very short period of time.

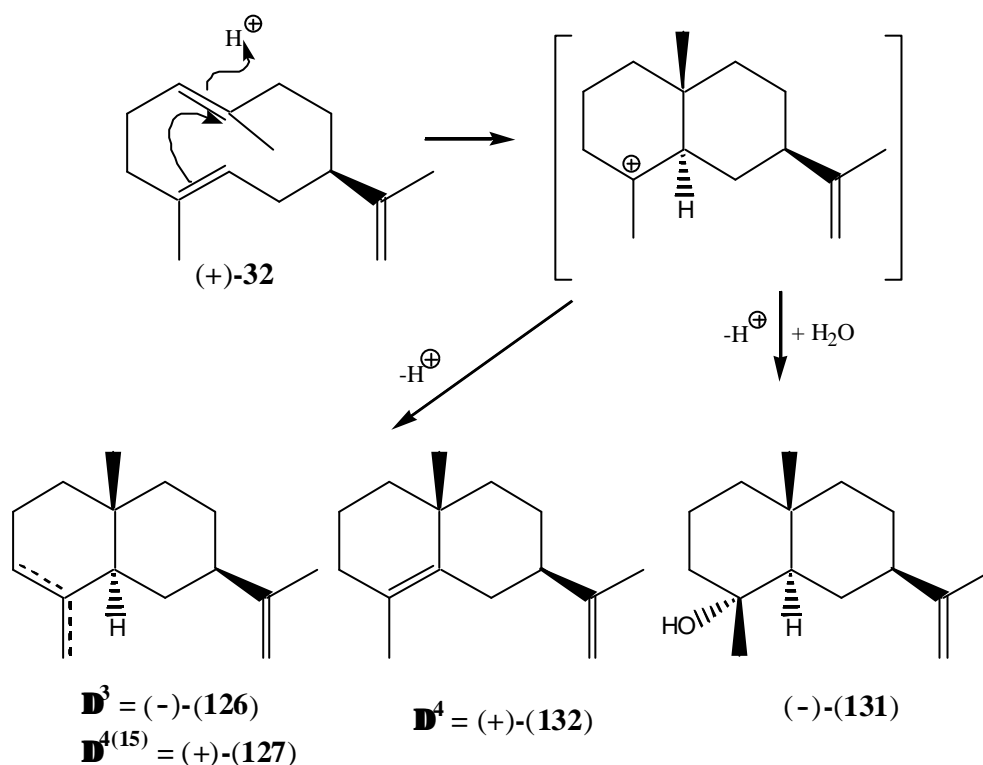


Fig. 51. Rearrangement of (+)-germacrene-A (**32**) to selinenes.

#### 4.2.6. Structure of (-)-Perfora-1,7-diene (**110**)

Perfora-1,7-diene (**110**), a new bicyclic sesquiterpene with perforane skeleton exhibited a molecular ion signal at  $m/z$  204 consistent with the molecular formula of  $C_{15}H_{24}$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of a doublet and three singlets for methyl groups at  $\delta$  0.82 (3 H, *d*, H-13,  $J = 6.6$  Hz), 0.74 (3 H, *s*, H-14), 1.62 (3 H, *br.s*, H-12) and 1.74 (3 H, *s*, H-15). The downfield shifted signals at  $\delta$  5.40 (*br.s*, 1 H) and 5.45 (*br.s* 1 H) were assigned to the olefinic methine protons H-2 and H-7, respectively. Additional structural information was obtained from the  $^{13}C$ -NMR spectrum. The connectivity of structure **110** by 2D- $^1H$ - $^{13}C$ -COSY and HMBC spectra (Fig. 52) confirmed a perforane skeleton. The relative configuration at C-4, C-5 and C-11 was established by the NOESY spectrum, which revealed the interaction of protons H-4 and H-11, H-13 and H-14, (Fig. 53).

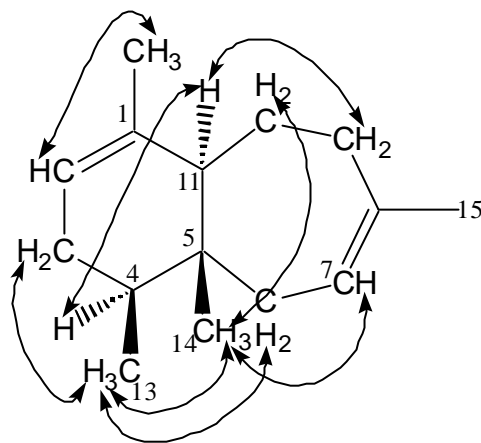
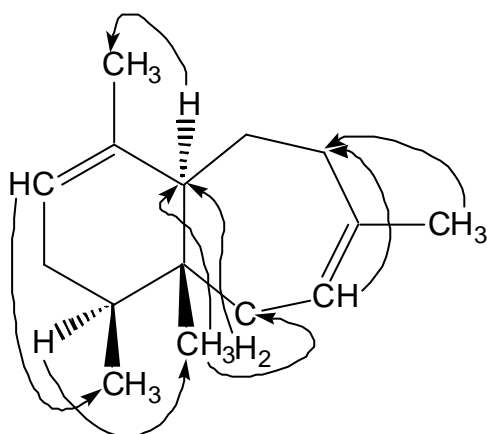


Fig. 52. Long-range  $^1H$ - $^{13}C$  correlations of **110**. Fig. 53. NOE correlations of **110**.

Compound **110** exhibits identical  $^1H$ -NMR and MS data as the unknown compound 'undulatene' whose incomplete  $^1H$ -NMR was earlier reported from the same species.<sup>[32]</sup> A possible biogenetic pathway for the perforane skeleton from *cis-trans*-farnesyl diphosphate is proposed (Fig. 54). Keto-halides and hydroxyl derivatives with perforane backbone have earlier been isolated from the marine algae *Laurencia perforata*, and their structures were confirmed by total synthesis.<sup>[134-135]</sup> Other hydroxy and acetoxy derivatives with perforane skeleton from a related *Laurencia* species have also been reported.<sup>[136]</sup>

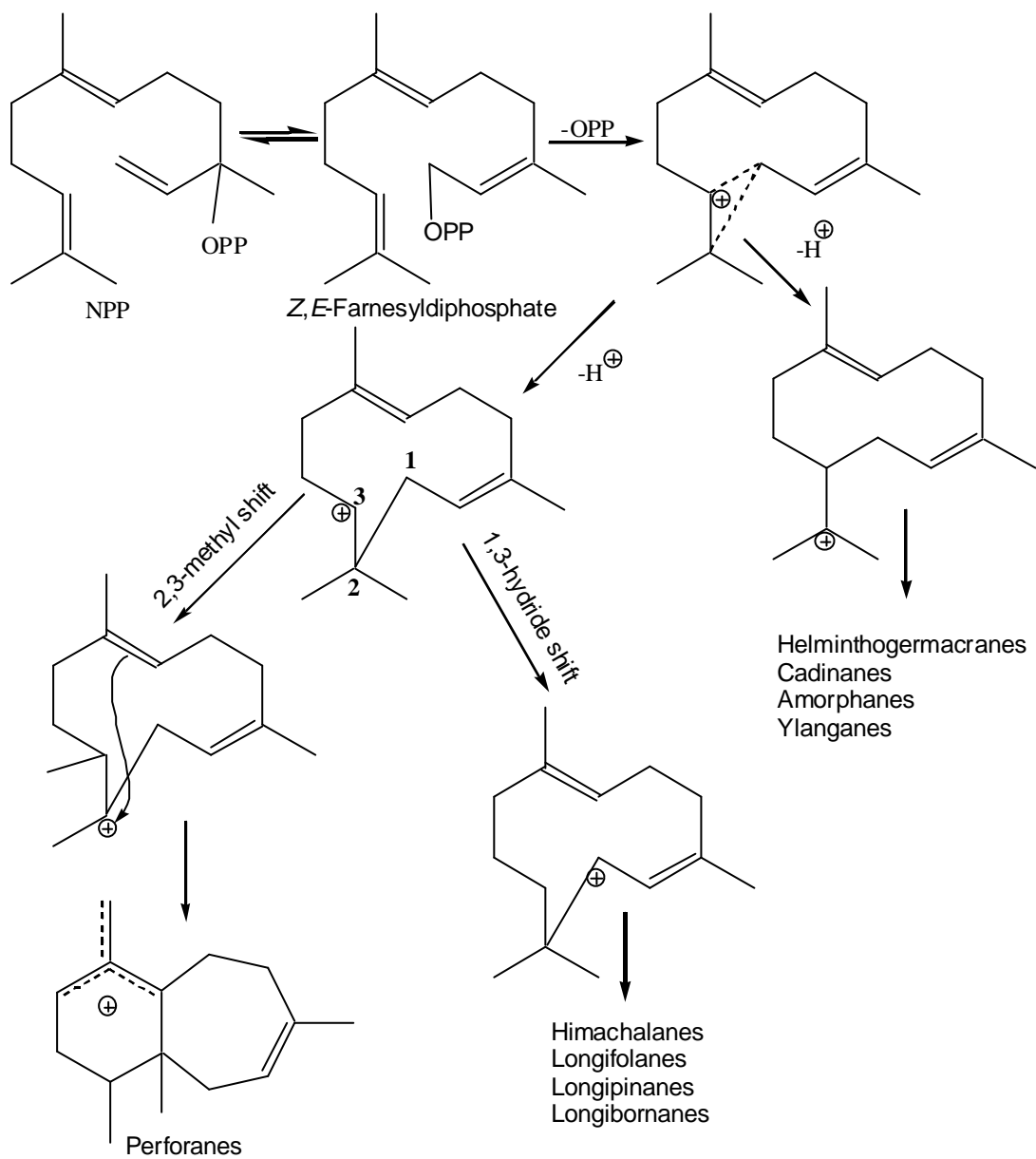


Fig. 54. Proposed biogenetic pathway for Perfora-1,7-diene (**110**).

### 4.3. Chemical Analysis of the Essential Oil of *Diplophyllum albicans*

*Diplophyllum* is another large genus of the Scapaniaceae. In this genus, five epiphytic species, *D. serrulatum*, *D. taxifolium*, *D. andrewsii*, *D. obtusifolium* and *D. albicans*, are known.<sup>[137]</sup> Previous investigations of both *D. taxifolium* and *D. albicans* species indicated the presence of *ent*-eudesmane-type sesquiterpenoids.<sup>[138,139]</sup> *D. taxifolium* and *D. albicans* species have been found to produce *ent*- $\alpha$ -selinene (**126**) and *ent*-selina-4,11-diene (**132**) together with anastreptene (**51**) and  $\beta$ -elemene (**40**).<sup>[137]</sup> In addition, from the polar constituents of these two species, an eudesmane-type sesquiterpene lactone, diplophyllin (**134**) was identified as a major component. Diplophyllin (**134**) and other sesquiterpene lactones were not reported in *D. serrulatum*. The constituents of *D. serrulatum* were found to contain drimane-type sesquiterpenoids e.g. albicanic acid (**135**), albicanal (**136**), and isoalbicanal (**137**) (Fig. 55).<sup>[137]</sup> Hence, *D. serrulatum* are chemically different from *D. taxifolium* and *D. albicans*.<sup>[137]</sup> *D. albicans* produced pungent substances which showed inhibitory activity towards the germination and root elongation of rice husks.<sup>[140]</sup> Diplophyllin (**134**) isolated from this liverwort showed significant activity against human epidermoid carcinoma.<sup>[139]</sup>

#### 4.3.1. Composition of the Essential Oil of *Diplophyllum albicans*

GC and GC-MS of the essential oil obtained by hydrodistillation from the liverwort *Diplophyllum albicans* collected in June 2002 from Altenau (Germany) was investigated. The essential oil revealed a complex mixture of sesquiterpene hydrocarbons including, bicycloelemene (**133**, 0.1%), maali-1,3-diene (**50**), anastreptene (**51**, 23.0%),  $\beta$ -elemene (**40**, 1.3%), tritomarene (**138**),  $\beta$ -barbatene (**57**, 0.3%),  $\beta$ -acoradiene (**58**, 0.3%),  $\delta$ -selinene (**81**, 0.7%), bicyclogermacrene (**60**, 14.0%),  $\beta$ -bazzanene (**63**, 0.1%), *ent*-(+)- $\alpha$ -selinene (**126**), *ent*-(-)-selina-4,11-diene (**132**), and the three tricyclic sesquiterpenes aromadendra-4,10(14)-diene (**139**, 0.8%), aromadendra-4,9-diene (**140**, 0.7%) and aromadendra-1(10),4-diene (**141**, 0.3%).<sup>[141]</sup> In the oxygenated fraction, 4-dehydroviridiflorol (**142**, 4.6%), 3 $\alpha$ -acetoxybicyclogermacrene (**45**, 0.8%),<sup>[142]</sup> (+)-8,9-epoxyselina-4,11-diene (**143**), (+)-*ent*-8 $\alpha$ -hydroxyeudesma-3,11-diene (**144**, 0.3%),<sup>[40,106]</sup> diplophyllin (**134**, 18.2%), *ent*-diplophyllolide (**145**, 8.1%), and *ent*-dihydrodiplophyllin (**146**, 2.5 %), could be identified in *D. albicans* by comparison of their mass spectra and retention indices with the spectral library established under identical experimental conditions.<sup>[107-108]</sup> (Fig. 55) Oxygenated sesquiterpenes **45**, **143**, **144** and sesquiterpene hydrocarbons **139**, **140** and **141** were identified for the first time in a *D. albicans*.

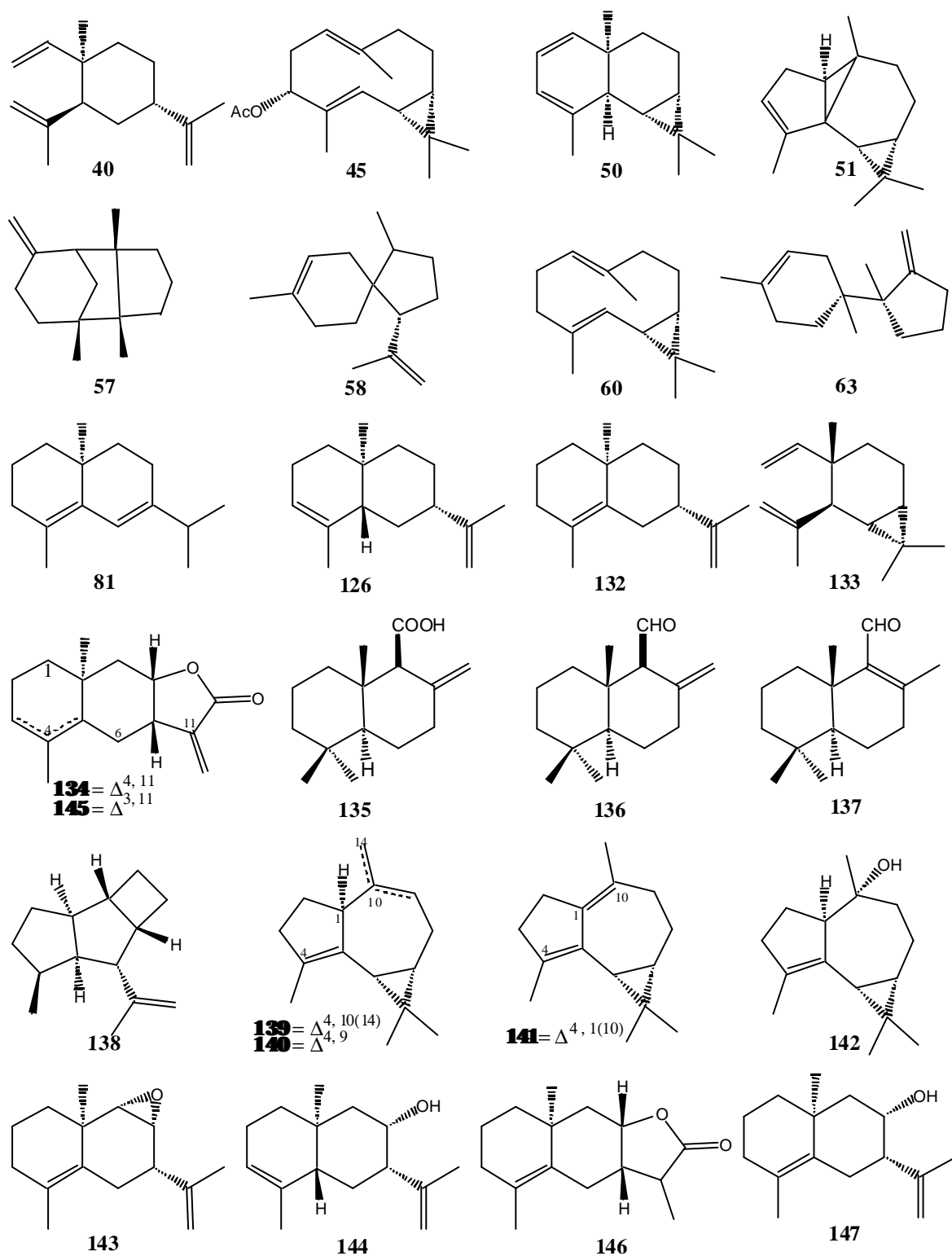


Fig. 55. Sesquiterpenoids identified in the essential oil of *Diplophyllum*.



### 4.3.2. Structure of (+)-Eudesma-4,11-dien-8 $\alpha$ -ol (**147**)

(+)-Eudesma-4,11-dien-8 $\alpha$ -ol (**147**) a double bond isomer of **144** was isolated for the first time in a liverwort *D. albicans*. **147** exhibited a molecular ion at  $m/z$  220 corresponding to the molecular formula of C<sub>15</sub>H<sub>24</sub>O. The <sup>13</sup>C-NMR spectrum revealed the presence of 15 carbon resonances. <sup>1</sup>H-NMR and HMQC spectroscopic analysis indicated a total of 23 protons directly attached to the carbon skeleton. The <sup>1</sup>H-NMR spectrum showed three singlets for methyl groups at  $\delta$  1.49 (3H, *s*, H-14), 1.53 (3H, *s*, H-12) and 1.58 (3H, *s*, H-15). The signal at  $\delta$  3.86 (1H, *d*,  $J = 2.2$ Hz) was assigned to the oxygenated methine H-8. Additional structural information was obtained from the <sup>13</sup>C-NMR spectrum which confirmed the presence of three methyl groups (*q*, at  $\delta$  19.6, 22.8 and 28.2), six methylene groups (*t*, at  $\delta$  19.3, 24.8, 33.6, 41.5, 48.1 and 111.6), two tertiary carbons (*d*, at  $\delta$  50.7 and 67.5) and four quaternary carbons (*s*, at  $\delta$  34.8, 124.8, 135.7 and 147.7). All the information from the 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC, and HMBC (Fig. 56a) analysis were consistent with the assigned structure of **147**. Its relative configuration was derived from the NOESY spectrum, which indicated NOE interactions of H-7 and H-8 (Fig. 56b).

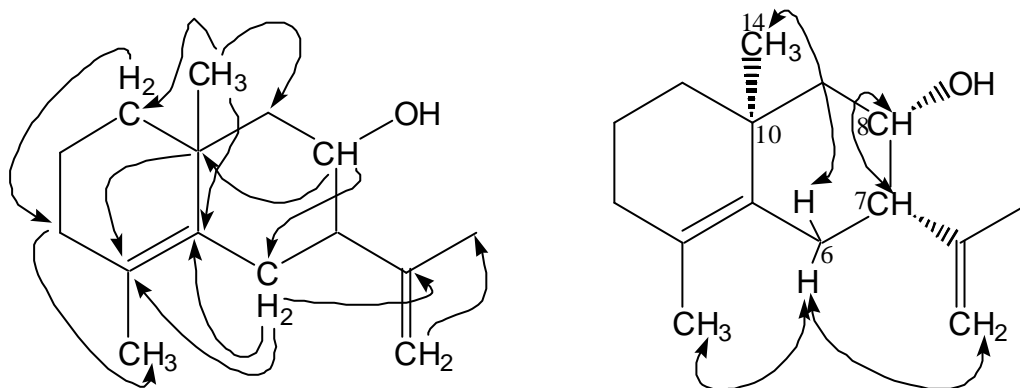


Fig. 56a. Long-range <sup>1</sup>H-<sup>13</sup>C correlations of **147**. Fig. 56b. NOE correlations of **147**.

The two saturated hydrogenation products of authentic (+)- $\alpha$ -selinene (**126**) have the same retention times with two of the four saturated major hydrogenation products of eudesma-4,11-dien-8 $\alpha$ -ol (**147**) using capillary GC with different cyclodextrin derived chiral stationary phases (Fig. 57). Therefore, the absolute configuration of **147** was to be (7*S*, 8*S*, 10*S*). The assigned absolute configuration is consistent with the co-occurrence of *ent*-(-)-selina-4,11-diene (**132**) and *ent*-(+)- $\alpha$ -selinene (**126**) in the hydrocarbon fraction of the liverwort sample.

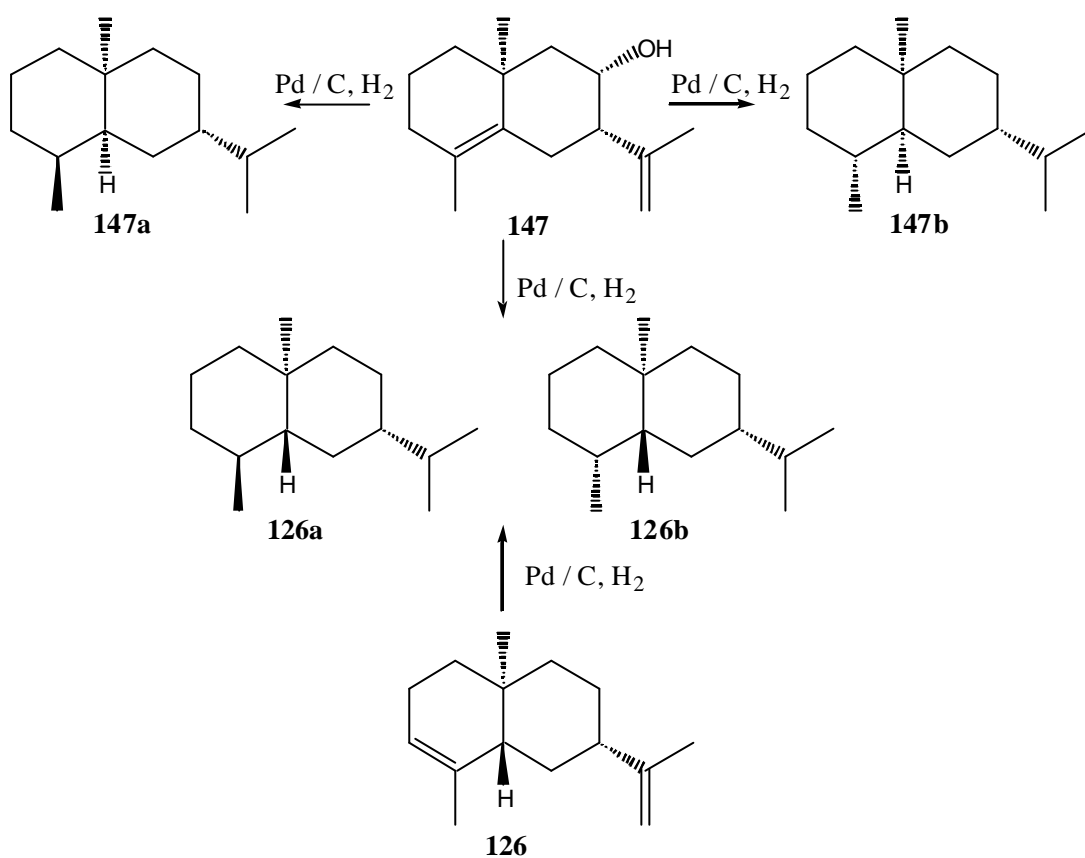


Fig. 57. Hydrogenation products of eudesma-4,11-dien-8 $\alpha$ -ol (**147**).

### 4.3.3. Dehydration of 4-Dehydroviridiflorol (142)

The sesquiterpene hydrocarbons **139**, **140** and **141**<sup>[141]</sup> were identified for the first time in the essential oil of *Diplophyllum albicans*. These compounds were also derived upon dehydration of 4-dehydroviridiflorol (**142**) (Fig. 58).

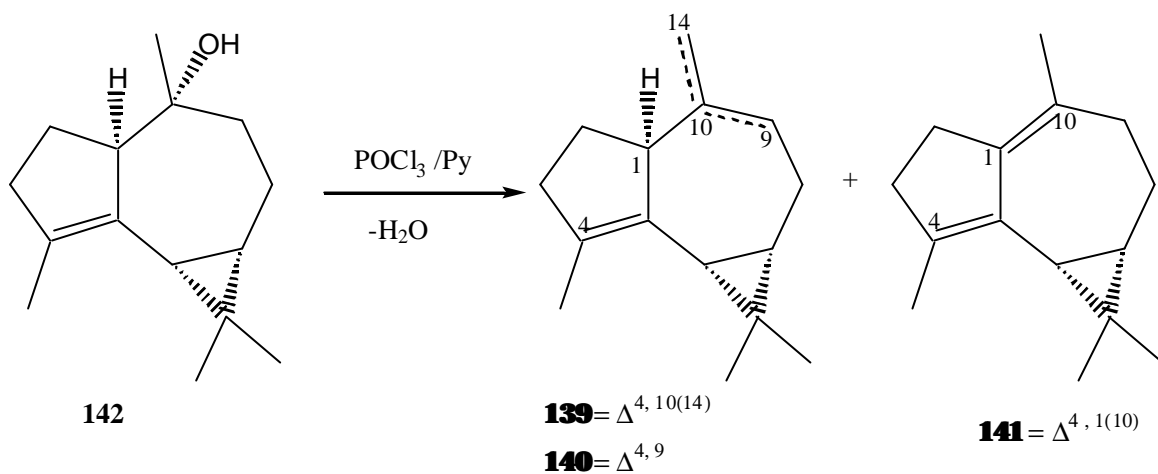


Fig. 58. Dehydration of 4-dehydroviridiflorol (**142**).

#### 4.4. Chemical Analysis of the Essential Oils of four *Marsupella* species

The botanical classification of *Marsupella* species<sup>[25,26]</sup>

Class : Hepaticae (Liverworts)

Subclass : Jungermanniidae

Order : Jungermanniales

Family : Gymnomitriaceae (Marsupellaceae)

Species : *Marsupella emarginata*, *M. aquatica*, *M. alpina*

The chemical composition of the liverwort *Marsupella emarginata*, common in areas of moderate elevation and mainly growing on water flooded rocks in small mountain rivers in Europe and in other countries has been investigated.<sup>[146-149]</sup>

Previously, four closely related species, namely *M. emarginata* ssp. *tubulosa*,<sup>[145]</sup> *M. emarginata* var. *patens*,<sup>[146]</sup> *M. emarginata*,<sup>[147-149]</sup> and *M. aquatica*,<sup>[150-151]</sup> have been chemically investigated. The main components of these species were *ent*-longipinane sesquiterpenoids which may be important chemical markers for *Marsupella* species. Only Japanese *M. emarginata* var. *patens*<sup>[146]</sup> did not contain longipinanes, *ent*-gymnomitrane sesquiterpenoids being isolated, instead. French *M. aquatica*<sup>[150]</sup> also afforded the novel (-)-gymnomitr-3(15)-en-9 $\beta$ -ol (**68**), in addition to longipinanes: 9-acetoxymarsupellol (**148**), 9,11 $\alpha$ ,14-triacetoxymarsupellone (**150**), 9-acetoxymarsupellone (**151**), marsupellol (**152**), and (+)-(4*S*,5*R*,7*S*,8*R*)-eremophila-9,11-dien-8 $\alpha$ -ol (**149**) which was found in French *M. emarginata* (Fig. 55a).<sup>[148]</sup>

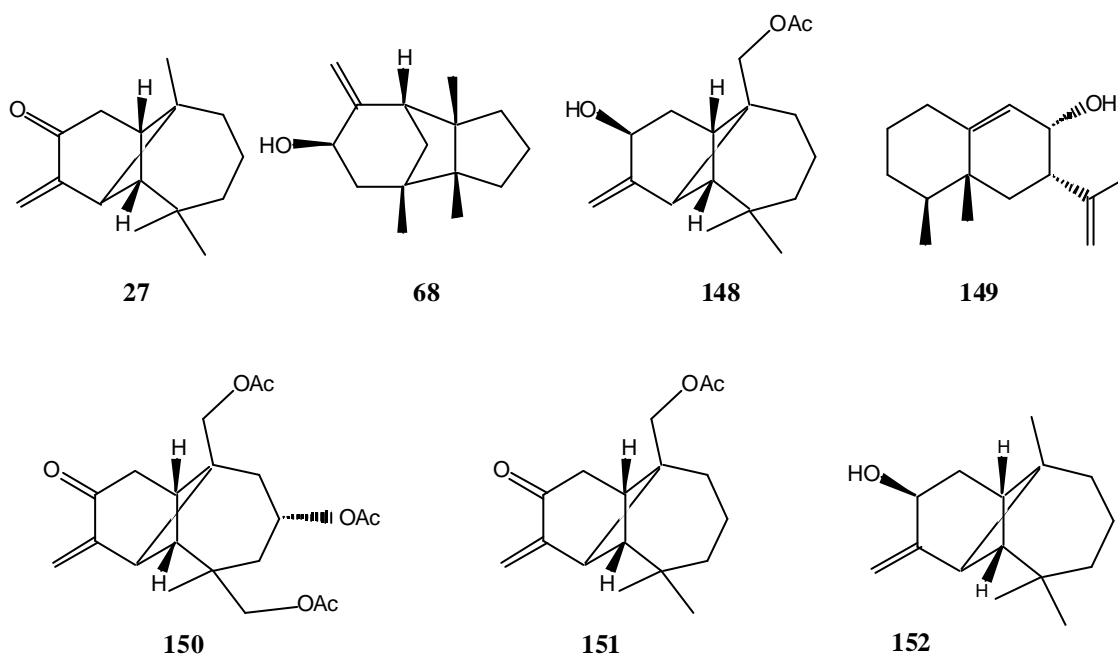


Fig. 59a. Sesquiterpene constituents of *Marsupella* species.<sup>[146-152]</sup>

A GC-MS analysis of a French *M. emarginata* indicated the absence of gymnomitrane sesquiterpenoids,<sup>[149]</sup> whilst highly acetylated longipinanes were found in German *M. emarginata* but not in the Russian species.<sup>[147]</sup>

Recent studies of the Scottish liverwort *M. emarginata* var. *aquatica* have failed to give longipinane, gymnomitrane, and eremophilane sesquiterpenoids but, instead, four novel amorphanes.<sup>[152]</sup> Thus, *M. aquatica* is closely related to *M. emarginata* as indicated by the presence of the same *ent*-longipinane type constituents. However, the two species clearly differ in their morphological properties and chemical composition.<sup>[153]</sup> Recently, a review on the chemistry of *M. emarginata* has been published.<sup>[94,113]</sup> Antitumor activity has been reported for (–)-marsupellone (**27**) and 9-acetoxymarsupellone (**151**).<sup>[154]</sup>

#### 4.4.1. Composition of the Essential Oil of the Liverwort *Marsupella emarginata*

The essential oil of the liverwort *M. emarginata* collected in early March 2001 near Altenau, Harz mountains (Germany) was obtained by hydrodistillation. The GC and GC-MS of the essential oil revealed a complex mixture of traces of monoterpenes [tricyclene (**153**),  $\alpha$ -pinene (**154**), camphene (**155**),  $\beta$ -pinene (**156**), limonene (**157**)], aliphatic and aromatic compounds [octan-3-one (**158**), oct-1-en-3-ol (**159**), oct-1-en-3-ylacetate (**160**), benzaldehyde (**161**), phenylacetaldehyde (**162**)] and a complex mixture of sesquiterpenes, including  $\alpha$ -longipinene (**88**, 0.3%), (–)- $\beta$ -longipinene **92**, 9.4%), longifolene **94**, 1.5%),  $\beta$ -ylangene **95**, 1.5%),  $\beta$ -barbatene (**57**, 1.7%),  $\alpha$ -himachalene (**98**, 0.4%), 2-*epi*- $\beta$ -caryophyllene (**163**, 0.7%),  $\beta$ -chamigrene (**59**, 0.75%), aristolochene (**164**, 0.9%), valencene (**165**, 0.9%) and traces of anastreptene (**51**),  $\alpha$ -ylangene (**89**), gymnomitra-3(15),4-diene (**166**), aristola-1(10),8-diene (**167**), thujopsene (**168**), isobazzanene (**97**), alloaromadendrene (**170**),  $\gamma$ -muurolene (**171**), *trans*- $\beta$ -bergamotene (**172**),  $\alpha$ -muurolene (**105**), cuparene (**169**),  $\alpha$ -cuprenene (**61**), and  $\delta$ -cadinene (**108**) could be identified from the hydrocarbon fraction. In the oxygenated fraction, marsupellol (**152**, 12.3%), marsupellone (**27**, 47.8%), (+)-lemnalol (**29**, 2.6%, enantiomeric to a compound previously identified as a constituent of the soft coral *Lemnalia tenuis*),<sup>[155]</sup> together with gymnomitr-8(12)-en-9 $\beta$ -ol (**68**, 1.2%)<sup>[150]</sup> constituted the major components identified by comparison of their mass spectra and retention indices with a spectral library established under identical experimental conditions<sup>[107-108]</sup> (Fig. 59b-c). Other unidentified constituents were selected for isolation.

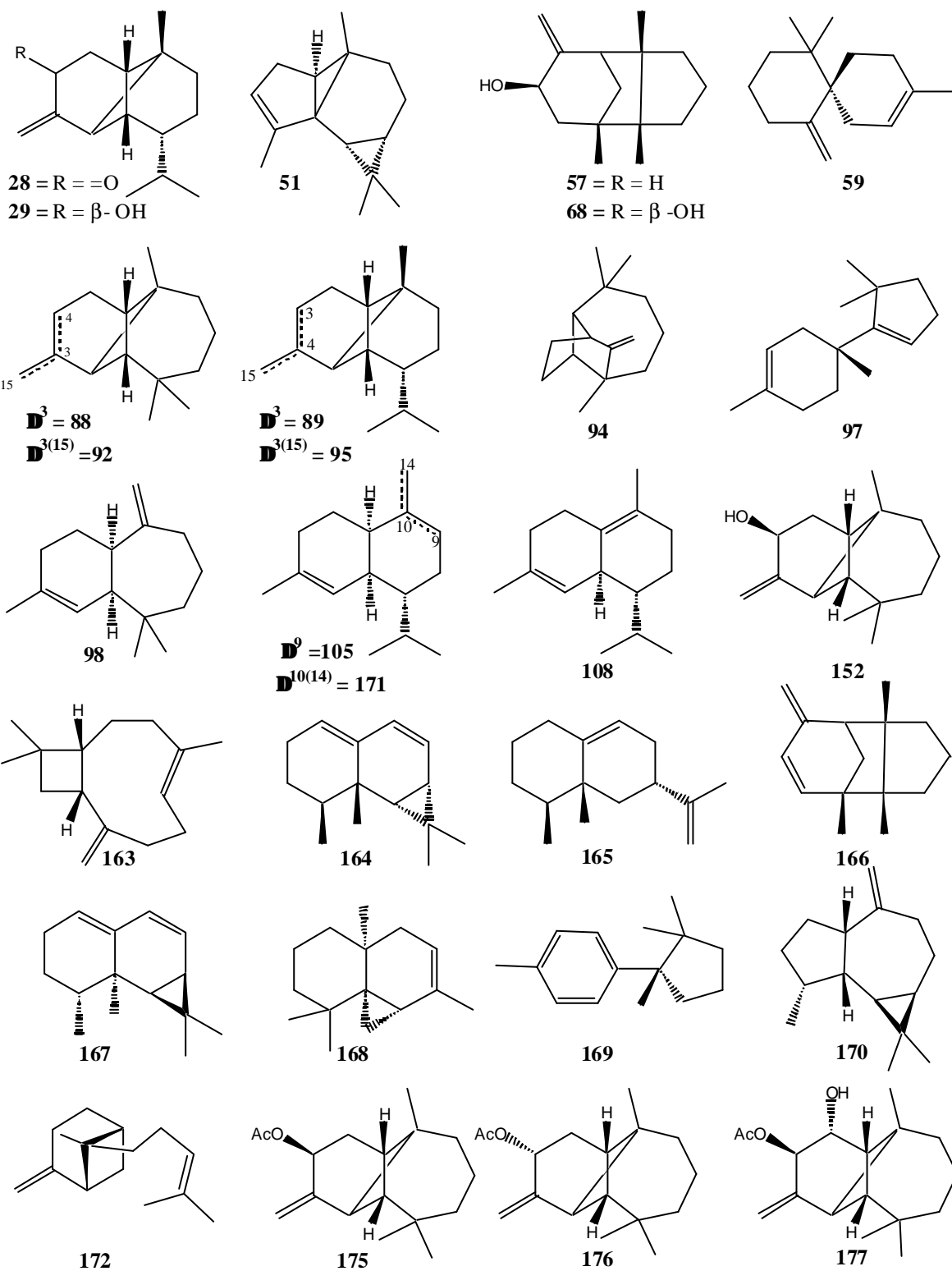


Fig. 59b. Sesquiterpene constituents of *Marsupella emarginata* (Altenau, Germany).

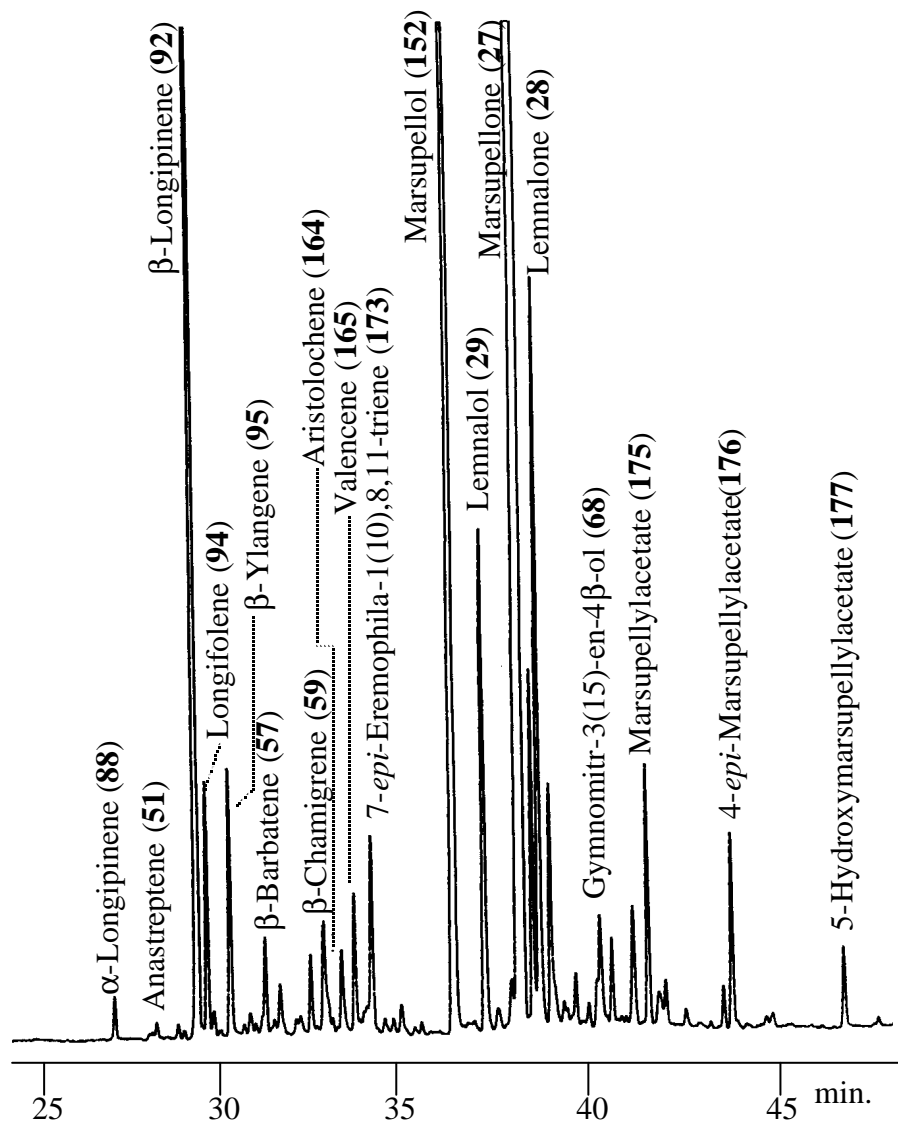


Fig. 59c. Gas chromatogram of the essential oil of *Marsupella emarginata* (Altenau, Germany). (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.4.2. Structure of (-)-7-*epi*-Eremophila-1(10),8,11-triene (**173**)

The new sesquiterpene hydrocarbon **173** was isolated from the hydrodistillation product of *M. emarginata* by preparative GC. The mass spectrum exhibits a molecular ion signal at  $m/z$  202 corresponding to the molecular formula of  $C_{15}H_{22}$ . The  $^{13}C$ -NMR and DEPT spectra of compound **173** indicated signals of three primary carbons ( $\delta$  15.7, 17.9, 20.4), four secondary carbons ( $\delta$  26.0, 27.1, 40.7, 110.7), five tertiary carbons ( $\delta$  38.9, 42.2, 124.6, 128.1, 130.0) and three quaternary carbons ( $\delta$  36.2, 141.4, 149.4). The  $^1H$ -NMR spectrum showed signals of a doublet and a singlet for methyl groups at  $\delta$  = 0.80 (3 H, *d*,  $J$  = 6.6 Hz) and 0.94 (3 H, *s*), respectively. The downfield methyl singlet ( $\delta$  1.68) indicated that this methyl group is attached to a double bond. The olefinic carbon signals at  $\delta$  149.4 (*s*) and 110.7 (*dd*) suggested a methylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  = 4.84 (1 H, *t*,  $J$  = 1.6 Hz) and 4.92 (1 H, *s*). The vinylic proton at  $\delta$  = 5.56 (1 H, *d*,  $J$  = 9.8 Hz) coupled with another vinylic proton at  $\delta$  = 6.08 (1 H, *dd*,  $J$  = 2.5, 9.8 Hz). The other vinylic proton at  $\delta$  = 5.41 (1 H, *t*,  $J$  = 3.5 Hz) coupled with the neighbouring methylene group at  $\delta$  = 2.01 (2 H, *br. d*,  $J$  = 4.0 Hz). All this information from  $^{13}C$ -NMR and DEPT as well as that from  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 60) spectra led to the proposed structure **173**. Its relative configuration resulted from the NOESY spectrum, which indicated a spatial interaction of protons H-14 with H-15 and H-7 (Fig. 61).

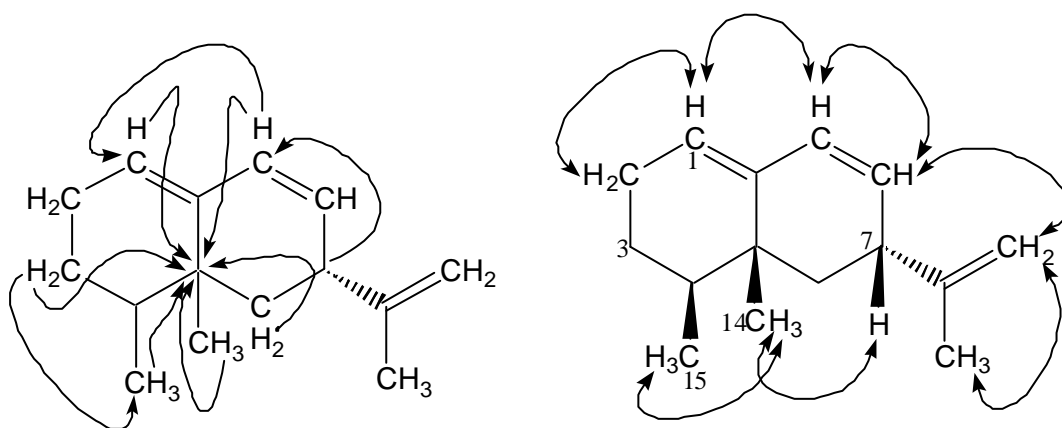


Fig. 60. Long-range  $^1H$ - $^{13}C$  correlations of **173**. Fig. 61. NOE correlations of **173**.



The absolute configuration was derived by comparison of the fully hydrogenated products of (+)- and (-)-valencene (**165**)<sup>[156]</sup> with those of **173** by enantioselective GC on a modified cyclodextrin stationary phase. The two tetrahydro derivatives of **173** and, those of (-)-valencene (**165**) are identical (Fig. 62).

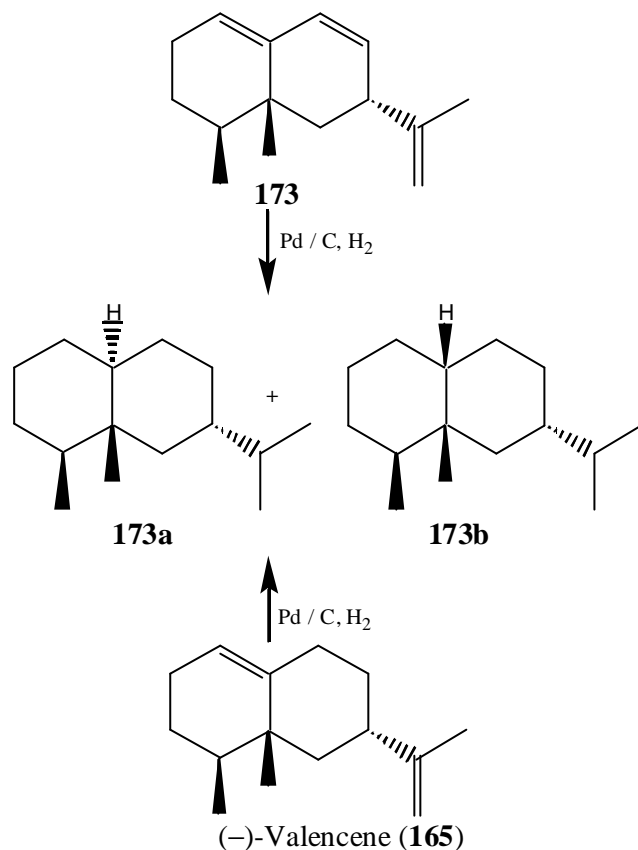


Fig. 62. Hydrogenation products of (-)-7-*epi*-eremophila-1(10),8,11-triene (**173**).

The formation of the sesquiterpene hydrocarbon **173** could be related to the hydroxy-derivative (**149**) (Fig. 63). (+)-Eremophila-9,11-dien-8 $\alpha$ -ol (**149**, RI<sub>CPSIL 5</sub> = 1666) was identified in the essential oil of this sample on the basis of its mass spectral fragmentation and retention index.<sup>[148]</sup>

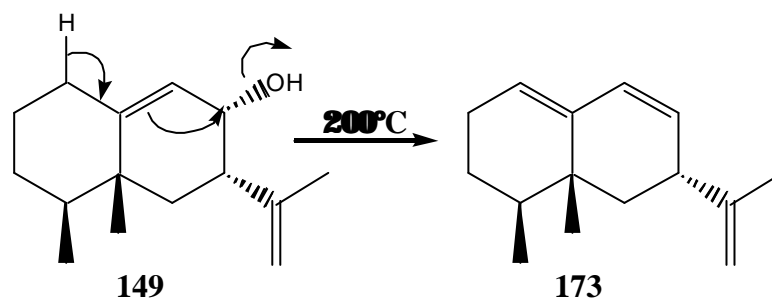


Fig. 63. Possible formation of **173** from **149**.

#### 4.4.3. Structure of (-)-Marsupellyl acetate (**175**)

**175**, the acetate of marsupellol (**152**), was isolated from the essential oil of *M. emarginata* by preparative GC. The mass spectrum exhibits a molecular ion signal at  $m/z$  262 corresponding to the molecular formula  $C_{17}H_{26}O_2$ . The  $^1H$ -NMR spectrum of **175** shows four singlets corresponding to methyl groups at  $\delta = 0.60$  (3 H, *s*), 0.89 (3 H, *s*), 0.92 (3 H, *s*) and 1.70 (3 H, *s*). The latter signal corresponds to an acetoxy methyl group. Two protons belonging to an exocyclic double bond absorbed at  $\delta = 4.86$  (1H, *s*) and 5.17 (1H, *s*). A signal at  $\delta$  5.90 (1 H, *dd*,  $J_1 = 1.3$ ,  $J_2 = 8.8$  Hz, H4) indicated a methine proton connected to an acetoxy group. The  $^{13}C$ -NMR and DEPT spectra showed signals for four primary carbon atoms ( $\delta$  21.2, 24.3, 27.9, 28.3), five secondary carbons ( $\delta$  22.0, 36.8, 39.7, 41.3, 112.3), four tertiary carbons ( $\delta$  38.9, 50.5, 54.1, 68.7) and four quaternary carbons ( $\delta$  32.9, 42.1, 153.2, 170.0). The signal at  $\delta$  68.7 was assigned to a tertiary carbon bound to an oxygen of the acetoxy group. The signals at  $\delta$  112.3 (*t*) and 153.2 (*s*) indicated the presence of an exocyclic double bond. The  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 64) spectra were in agreement with structure **175**. The relative configuration, particularly of C-4 carrying the acetoxy group was determined from a NOESY spectrum. In addition, NOEs were observed for protons H-6 with H-4 and H-1 (Fig. 65).

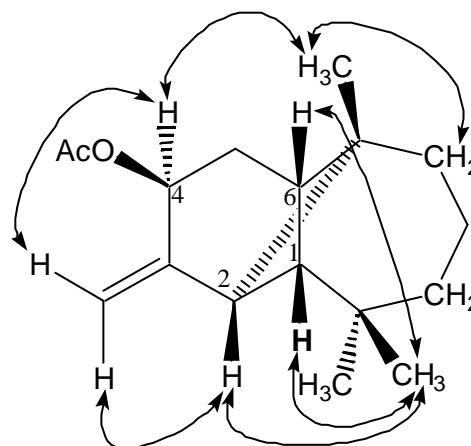
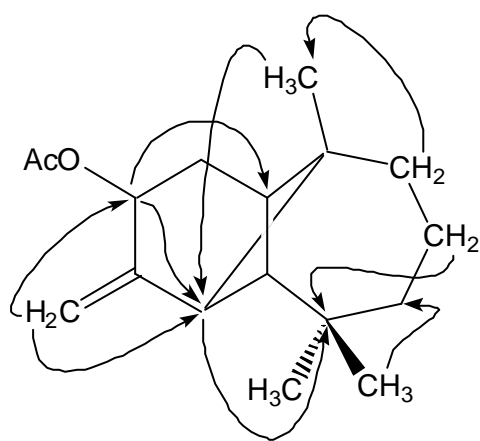


Fig. 64. Long-range  $^1H$ - $^{13}C$  correlations of **175**. Fig. 65. NOE correlations of **175**.

Treatment of **175** with potassium carbonate ( $K_2CO_3$ ) in methanol gave a product identical (same GC-MS characteristics and same retention times on polysiloxane and cyclodextrin derived GC phases) with marsupellol (**152**). Oxidation of **152** with pyridiniumdichromate (PDC) gave marsupellone (**27**). Both compounds **27** and **152** are present as major components of the essential oil of *M. emarginata*. (Fig. 66).

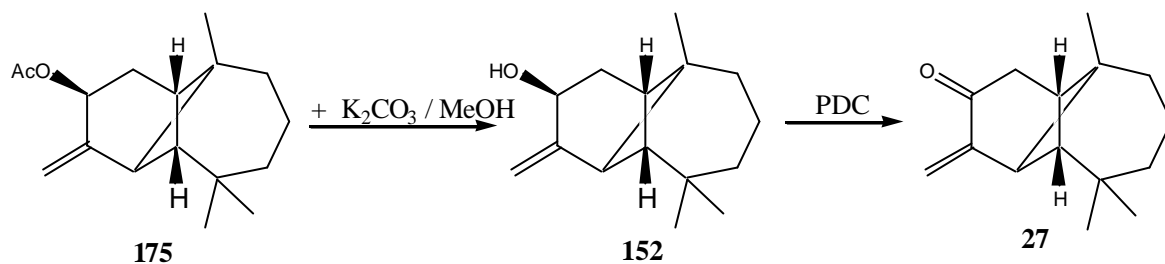


Fig. 66. Functional group conversion of **175** to **27**.

#### 4.4.4. Structure of (-)-4-*epi*-Marsupellyl acetate (**176**)

The mass spectrum of 4-*epi*-marsupellyl acetate (**176**) from *M. emarginata* exhibits a molecular ion peak at  $m/z$  262 consistent with the molecular formula of  $C_{17}H_{26}O_2$ . The  $^{13}C$ -NMR spectrum revealed the presence of 17 carbon resonances.  $^1H$ -NMR and HMBC demonstrated that a total of 26 protons were directly attached to the carbon skeleton. In addition, the molecular mass of 262 was confirmed by chemical ionization MS. The  $^1H$ - and  $^{13}C$ -NMR spectral patterns of **176** were almost identical to those of **175**, except for the NMR absorptions at  $\delta$  4.85 (1H, *d*,  $J = 1.3$  Hz) and 5.02 (1H, *dd*,  $J_1 = 0.6$  and  $J_2 = 1.9$  Hz) corresponding to the exomethylene protons. Also a chemical shift difference in the strongly lowfield shifted signal at  $\delta$  5.99-6.03 (1 H, *m*, H-4) for the oxygenated methine proton was observed. By examination of the 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 67) spectra the structure was identified as **176**. The relative configuration of **176** was derived from a NOESY spectrum, which indicated interaction of protons H-4 with H-1 and H-2 (Fig. 68a).

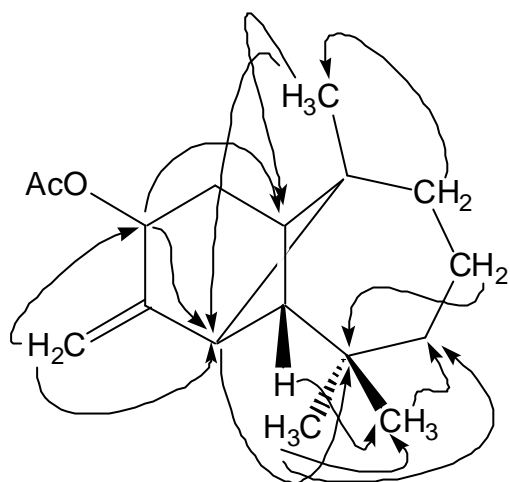


Fig. 67. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations **176**.

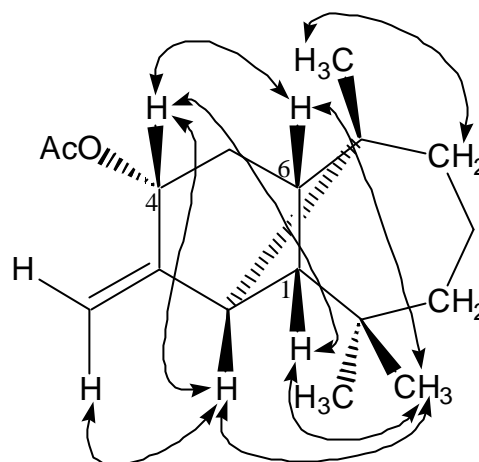


Fig. 68a. NOE correlations of **176**.

Compounds **175** and **176**, having the same skeleton and molecular formula  $\text{C}_{17}\text{H}_{26}\text{O}_2$ , showed almost identical mass spectrometric fragmentation patterns (EI and CI), however, their chromatographic retention times on diverse GC phases differed suggesting **175** and **176** to be stereoisomers. Compound **176**, an epimer of **175**, upon saponification yielded a corresponding 4-*epi*-marsupellol (**174**), which was also isolated as a trace component of *M. emarginata*. The relative configuration of **174** was deduced from **176** (Fig. 68b). Marsupellol (**152**) and 4-*epi*-marsupellol (**174**) showed similar  $^1\text{H}$ -NMR data and mass spectral fragmentation patterns but different retention times on both polysiloxane and cyclodextrin derived GC phases. In addition, oxidation of 4-*epi*-marsupellol (**174**) with pyridiniumdichromate (PDC) also gave marsupellone (**27**).

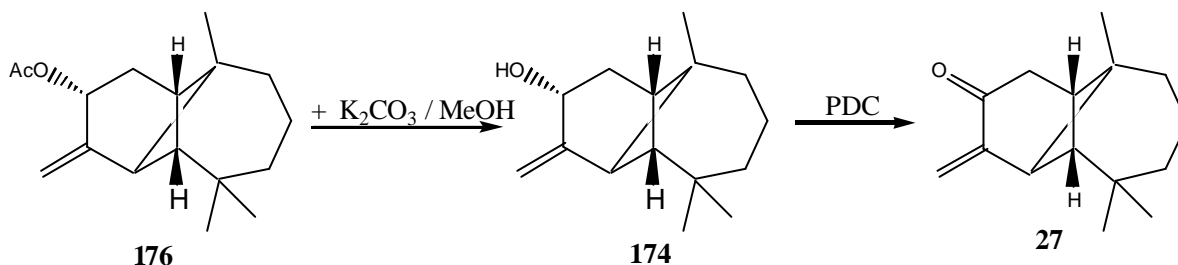


Fig. 68b. Functional group conversion of **176** to **27**.

#### 4.4.5. Structure of (+)-5-Hydroxymarsupellol acetate (**177**)

**177** has the same tricyclic sesquiterpene backbone as **175** and **176** but the mass spectrum exhibited a molecular ion peak at  $m/z$  278 corresponding to the molecular formula  $C_{17}H_{26}O_3$ . The  $^1H$ -NMR and  $^{13}C$ -NMR spectra of **177** were also similar to those of **175** and **176** except for the signals at  $\delta$  4.97 (1 H, *t*,  $J = 1.3$  Hz, H-15b), 5.44 (1 H, *d*,  $J = 1.9$  Hz, H-4) and 3.99 (1H, *s*, H-5). Also the  $^{13}C$ -NMR indicated a signal at  $\delta$  80.1 (*d*) which was assigned to the carbon attached to the hydroxy group. The relative configuration of **177** was derived from a NOESY spectrum, which indicated interactions of proton H-5 with H-1 and H-6 (Fig. 69). Sodium hydroxide hydrolysis of **177** gave a diol with a molecular ion peak at  $m/z$  236, suggesting a molecular formula  $C_{15}H_{24}O_2$ .

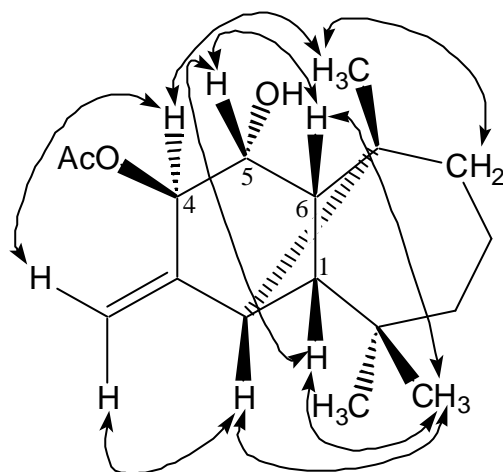


Fig. 69. NOE correlations of **177**.

#### 4.4.6. Structure of Lemnalone (**28**)

In addition, lemnalone (0.5%) (**28**), the ketone corresponding to lemnalol (**29**) was isolated as a trace component from the essential oil of *Marsupella emarginata*. This ketone, found for the first time in nature, is a colourless oil, exhibiting a molecular ion signal at  $m/z$  218 that correspond to a molecular formula of  $C_{15}H_{22}O$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ), showed signals of one singlet and two doublets for the methyl groups at  $\delta$  0.62 (3H, *s*), 0.76 (3H, *d*,  $J = 6.3$  Hz) and 0.78 (3H, *d*,  $J = 6.6$  Hz) respectively. **28** was further confirmed by comparison of its EI mass spectral data and retention times on both achiral and chiral GC with those of the oxidative product of (+)-lemnalol (**29**). Compound **28** has been reported to exhibit remarkably strong cytotoxicity to DBA/MC fibrosarcoma cells *in vitro*.<sup>[38]</sup> The relative configuration of **28** was deduced from lemnalol (**29**) (Fig. 70).

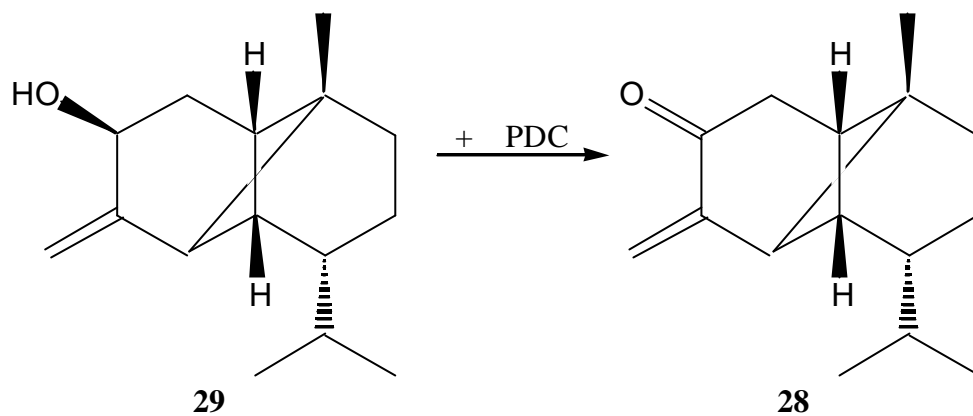


Fig. 70. Chemical conversion of lemnalol (**29**) to lemnalone (**28**).

Lemnalone (**28**) showed a very similar MS fragmentation pattern as marsupellone (**27**, 47.8%), the major component of the oxygenated fraction (Fig. 71).

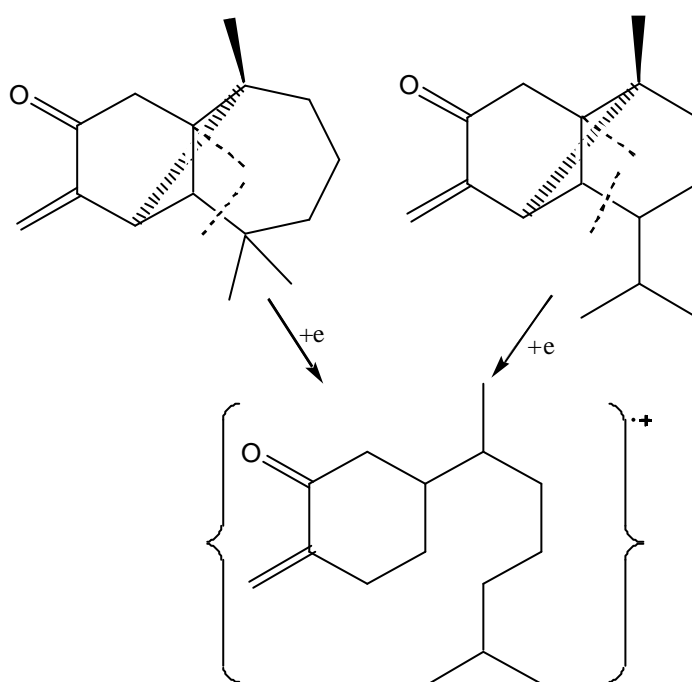


Fig. 71. Possible MS fragmentation of marsupellone (**27**) and lemnalone (**28**).

#### 4.5. Composition of the Essential Oil of another Sample of *Marsupella emarginata*

The essential oils of *M. emarginata* collected from a second location in Germany (Saarland) in March 2001, was obtained by hydrodistillation. This essential oil, in addition to the ether extract of *M. emarginata* (Ehrch.) Dum obtained from another location (Japan) in October 2000, was analysed by GC and GC-MS. All known constituents (Table 1, and Figs. 72a-b), were identified by mass spectrometry in connection with their gas chromatographic retention indices by comparison with a spectral library established under identical experimental conditions.<sup>[107-108]</sup> The relative concentrations of the identified compounds were illustrated with respect to the components present in trace amounts (Table 1). Gymnomitrane-4-one (**178**) and 15-*nor*-3-gymnomitrone (**179**, the first example of a naturally occurring *nor*-gymnomitrane derivative),<sup>[158]</sup> were also identified in the essential oil of *M. emarginata* for the first time. Structures of the newly isolated unknown constituents from the essential oil of this liverwort are discussed.

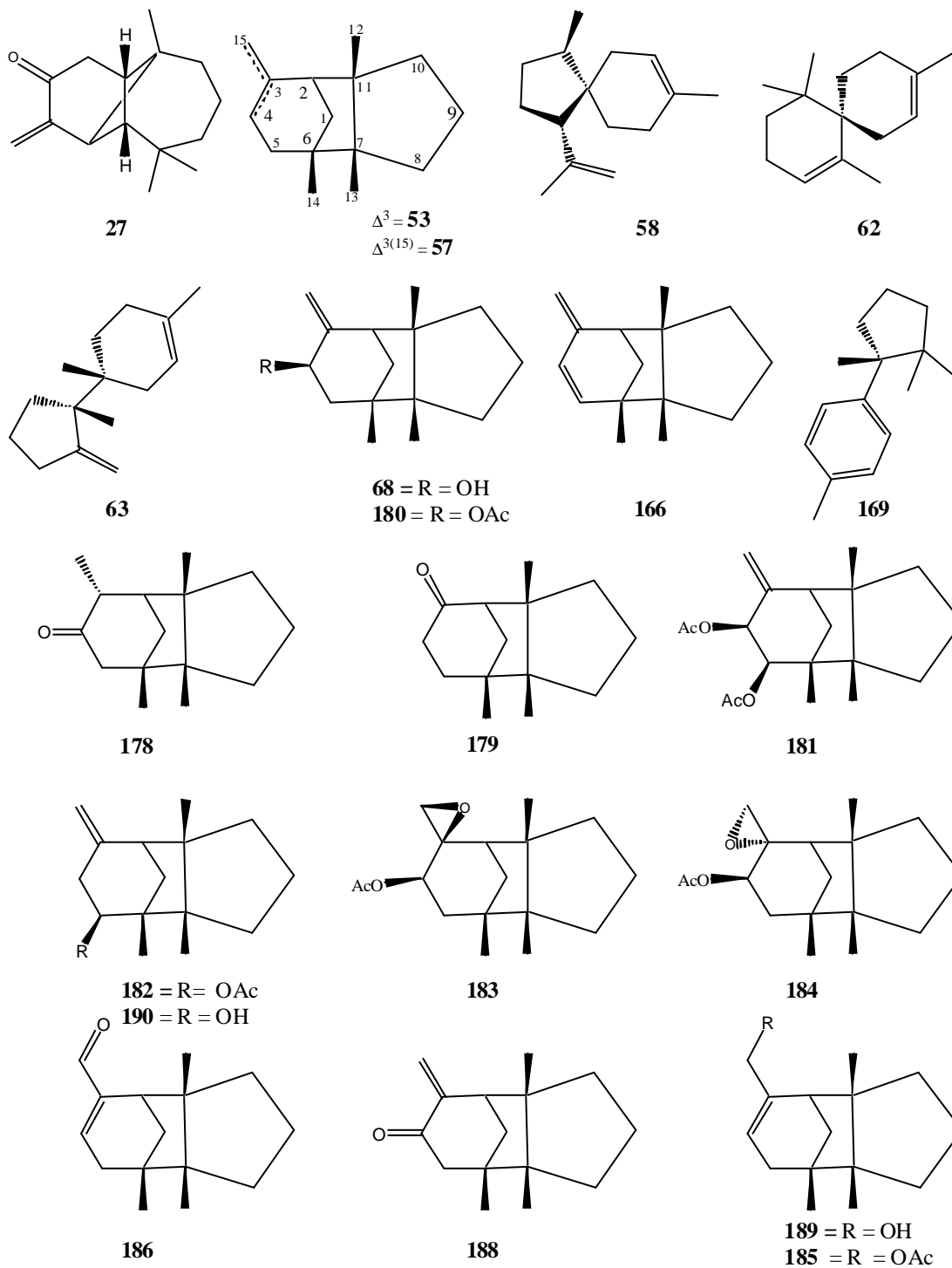


Fig. 72a. Gymnomitrane-type sesquiterpenoids from *Marsupella emarginata* (Saarland, Germany).



**Table 1.** Sesquiterpene constituents of the liverwort *Marsupella emarginata* from three different locations.

Sesquiterpene	Altenau	Saarland	Japan
Myltayl-4-ene (199)			x
Gymnomitra-3(15),4-diene (166)		x	x
$\alpha$ -Barbatene (53)		xx	x
$\alpha$ -Longipinene (88)	x		
Anastreptene (51)	x		
$\beta$ -Longipinene (92)	xxxxxx	x	
Longifolene (94)	xx		
$\beta$ -Ylangene (95)	xx		
Aristola-1(10),8-diene (167)	x		
Thujopsene (168)	x		x
Isobazzanene (97)	x	xx	xx
$\beta$ -Barbatene (57)	xx	xxxxxxxx	xxxxxxxx
trans $\alpha$ -Bergamotene		x	
Myltayl-4(12)-ene (198)			x
$\alpha$ -Himachalene (98)	x	x	
Allo-Aromadendrene (170)			x
$\beta$ -Acoradiene (58)		x	x
2- <i>epi</i> -(E)- $\beta$ -Caryophyllene (163)	x		
$\gamma$ -Muurolole (171)	x		x
Selina-4,11-diene (132)			xxxx
$\beta$ -Selinene (127)			x
$\beta$ -Bazzanene (63)		x	xx
$\beta$ -Chamigrene (59)	x	x	x
<i>trans</i> - $\beta$ -Bergamotene (172)		x	
$\delta$ -Cuperene (197)			x
Aristolochene (164)	x		
Valencene (165)	x		
$\alpha$ -Muurolole (105)	x		x
$\alpha$ -Chamigrene (62)		x	x
7- <i>epi</i> -Eremophila-1(10),8,11-triene (173)	xx		
Marsupellol (152)	xxxx	x	
Lemnalol (29)	xx		
Marsupellone (27)	xxxxxxxx	x	x
Lemnalone (28)	x		
4- <i>epi</i> -Marsupellol (174)	x		
Gymnomitr-3(15)-en-4 $\beta$ -ol (68)	x	xxxxxxxxxx	
Marsupellyl acetate (175)	x		
4- <i>epi</i> -Marsupellyl acetate (176)	x		
5 $\alpha$ -Hydroxymarsupellyl acetate (177)	x		
4 $\beta$ -Acetoxygymnomitr-3(15)-ene (180)	x	xxxxxxxxxx	
Gymnomitr-3(15)-en-12-ol (194)			x
Gymnomitr-3(15)-en-12-al (193)			xxx
Gymnomitr-3(15)-en-12-oic acid (192)			xxxxxxxx
12-Acetoxygymnomitr-3(15)-ene (187)			xx

Gymnomitr-3-en-15-ol ( <b>189</b> )		x	
$\alpha$ -Barbatenal ( <b>186</b> )		xx	
Gymnomitrane-4-one ( <b>178</b> )		x	
15- <i>nor</i> -3-Gymnomitrone ( <b>179</b> )		x	
Gymnomitr-3(15)-ene-4-one ( <b>188</b> )		x	
15-Acetoxygymnomitr-3-ene ( <b>185</b> )		x	
4 $\beta$ ,5 $\beta$ -Diacetoxygymnomitr-3(15)-ene ( <b>181</b> )		x	
3 $\alpha$ ,15 $\alpha$ -Epoxy-4 $\beta$ -acetoxygymnomitrane ( <b>184</b> )		x	
3 $\beta$ ,15 $\beta$ -Epoxy-4 $\beta$ -acetoxygymnomitrane ( <b>183</b> )		xx	
5 $\beta$ -Acetoxygymnomitr-3(15)-ene ( <b>182</b> )		x	
(+)-Eremophila-9,11-dien-8 $\alpha$ -ol ( <b>149</b> )	x		

X represents relative trace amount; number of x corresponds to the relatively increased amount from the gas chromatogram.

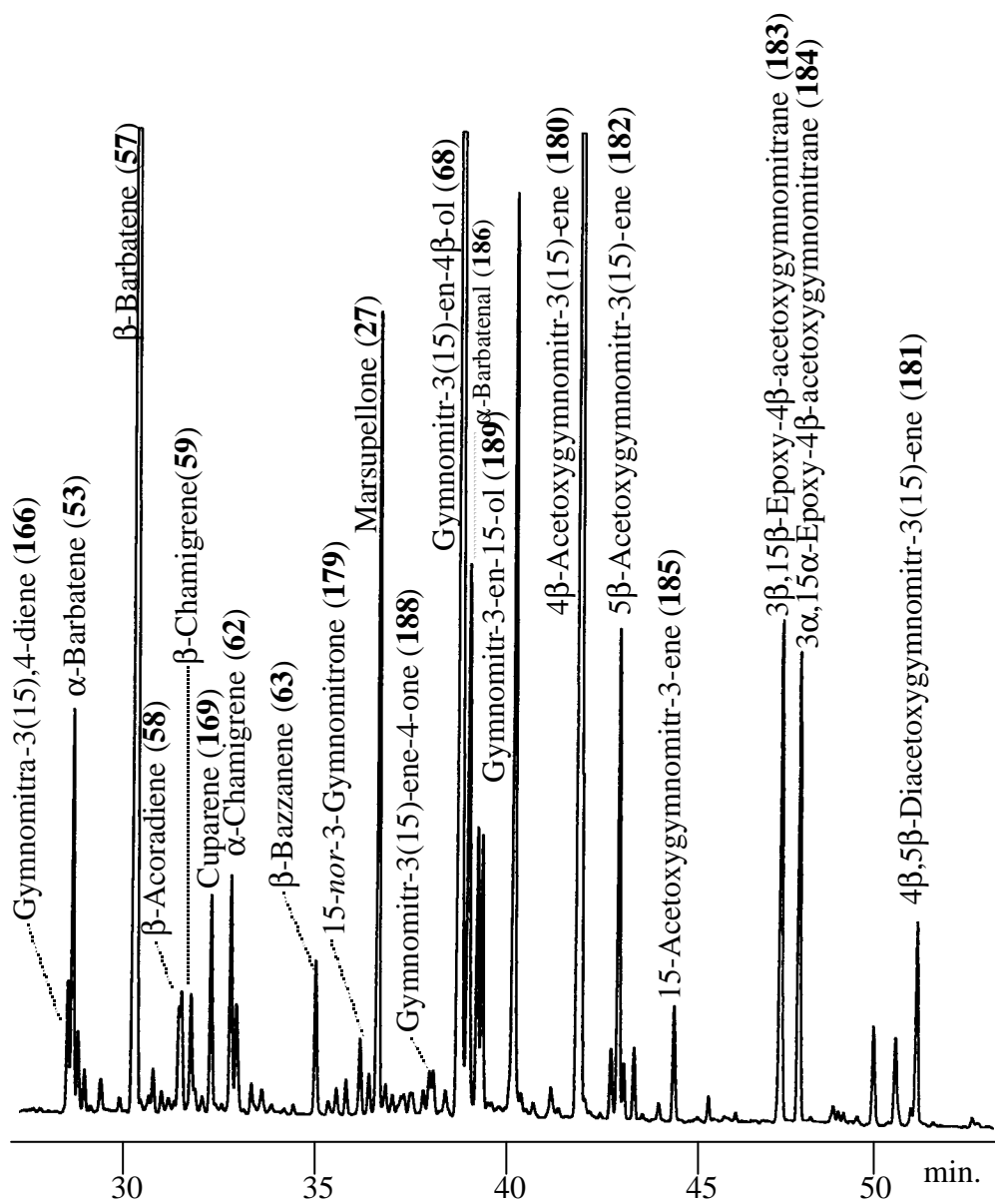


Fig. 72b. Gas chromatogram of the essential oil of *Marsupella emarginata* (Saarland, Germany). (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.5.1. Structure of (-)-4b-Acetoxygymnomitr-3(15)-ene (180)

The mass spectrum of **180** showed a molecular ion signal at  $m/z$  262 corresponding to  $C_{17}H_{26}O_2$ . In the  $^1H$ -NMR spectrum of **180** four singlet signals corresponding to methyl groups at  $\delta$  0.75 (6H, *s*), 0.90 (3H, *s*) and 1.75 (3H, *s*) were observed, the latter corresponding to a methyl group attached to a carbonyl of an acetoxy group. The olefinic carbon signals at  $\delta$  107.6 ( $\epsilon$ ) and 149.2 ( $\zeta$ ) suggested an exomethylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  4.84 (1H, *t*,  $J=2.2$  Hz) and 5.01 (1H, *t*,  $J=2.2$  Hz). The deshielded signal at  $\delta$  6.04 (1H, *ddt*,  $J_1=10.1$ ,  $J_2=4.7$ ,  $J_3=2.2$  Hz) was assigned to the oxygenated methine proton. The  $^{13}C$ -NMR and DEPT spectra of compound **180** indicated signals of four primary carbons ( $\delta$  20.6, 23.3, 24.1, 27.7), six secondary carbons ( $\delta$  27.7, 36.3, 37.7, 45.4, 47.0, 107.6), two tertiary carbons ( $\delta$  56.9, 70.4) and five quaternary carbons ( $\delta$  45.1, 54.8, 55.8, 149.2, 170.1). The 2D  $^1H$ - $^1H$ -COSY, HMQC, and HMBC (Fig. 73) spectra confirmed the structure of **180**. The spatial interactions of protons derived from the NOESY spectrum furnished the relative configuration of **180** (Fig. 74). A well-resolved longrange  $^4J$ -coupling was observed for methylene protons H10 and H8, indicating the five-membered ring structure to exhibit a fixed W-orientation of the four bonds. Spatial interactions were also observed between H4 and H10b, H8b, H5 and slightly with the H-15 protons, suggesting that they are on one side and between H-13/14, and H-12 and H-8a at the other side of the plane of the molecule, thus indicating that H-4 has  $\alpha$ -orientation. The absolute configuration of **180** was confirmed by chemical correlation.

The methanolic potassium carbonate hydrolysis of **180** gave a deacetylated product. The resulting alcohol gave the same GC-MS characteristics and retention times on polysiloxane

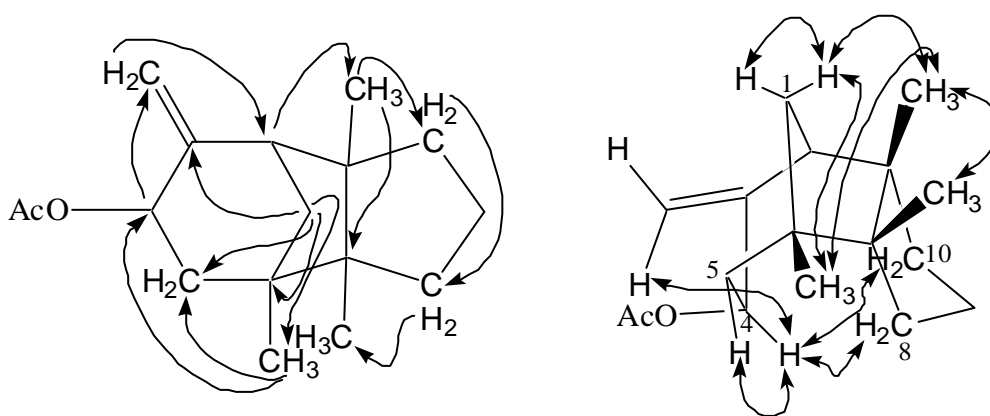


Fig.73. Long-range  $^1H$ - $^{13}C$  correlations of **180**. Fig. 74. NOE correlations of **180**.

and cyclodextrin derived GC phases with the known (-)-gymnomitr-8(12)-en-9 $\beta$ -ol (**68**).<sup>[150]</sup> Moreover, <sup>1</sup>H-NMR data of **68** measured in C<sub>6</sub>D<sub>6</sub> were totally identical with those reported.<sup>[150]</sup> Therefore, the new naturally occurring compound **180** should be an epimer of the product obtained by acetylation of (+)-gymnomitr-8(12)-en-9 $\alpha$ -ol.<sup>[159]</sup>

#### 4.5.2. Structure of (-)-4 $\beta$ ,5 $\beta$ -Diacetoxymygnomitr-3(15)-ene (**181**)

(-)-4 $\beta$ ,5 $\beta$ -Diacetoxymygnomitr-3(15)-ene (**181**), was obtained from the essential oil of *M. emaginata*. The chemical ionization mass spectrum of **181** exhibited a molecular ion at [M+NH<sub>4</sub><sup>+</sup>] *m/z* 338 and molecular formula C<sub>19</sub>H<sub>28</sub>O<sub>4</sub>. The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) showed signals of five singlets for methyl groups at  $\delta$  0.74 (3H, *s*, H-13), 0.78 (3H, *s*, H-14), 0.86 (3H, *s*, H-12), 1.76 (3H, *s*, H-19) and 1.80 (3H, *s*, H-17). The olefinic carbon signals at  $\delta$  146.82 ( $\psi$ ) and 107.85 ( $\phi$ ) suggested an exomethylene double bond, which was confirmed by two signals in the <sup>1</sup>H-NMR spectrum at  $\delta$  4.87 (1H, *t*, H-15a, *J* = 2.2 Hz) and 5.03 (1H, *t*, H-15b, *J* = 2.2 Hz). The signals at  $\delta$  5.48 (1H, *d*, *J* = 4.1 Hz) and 6.27-6.29 (1H, *m*), were assigned to the methine protons connected to the acetoxy groups at C-5 ( $\delta$  76.5) and C-4 ( $\delta$  69.6) respectively. Information from 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC, HMBC (Fig. 75) spectra in addition to the <sup>13</sup>C-NMR confirmed structure **181**. Its relative configuration resulted from the NOESY interactions of the two oxygenated methine protons H4 and H5, the interactions of both H4 and H5 with H8b and H10b, interactions of protons H17 and H15, in addition to the interactions between protons H19 and H14 (Fig. 76). The relative configuration of **181** at C-4 and C-5 is assumed to be 4 $\beta$ , 5 $\beta$  which is identical to the naturally occurring (-)-4 $\beta$ -acetoxymygnomitr-3(15)-ene (**180**) isolated from same essential oil.

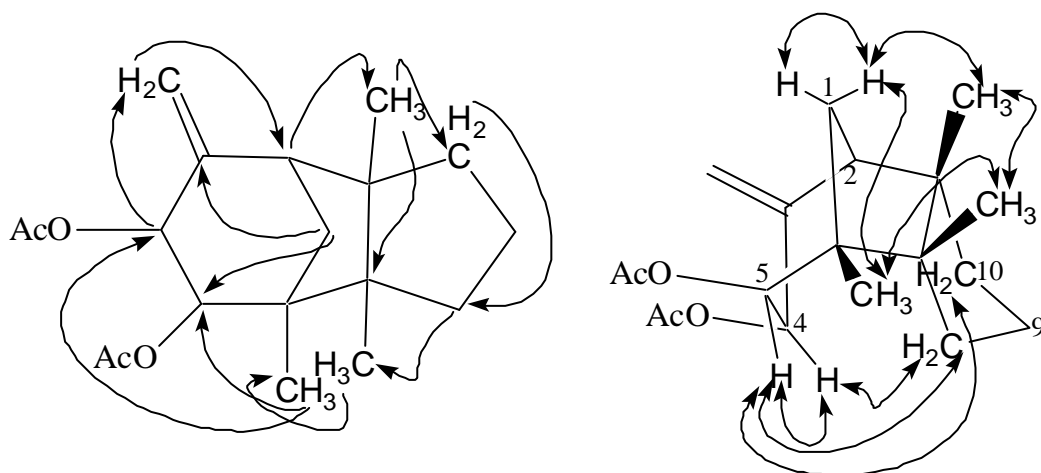


Fig.75. Long-range <sup>1</sup>H-<sup>13</sup>C correlations of **181**. Fig.76. NOE correlations of **181**.

#### 4.5.3. Structure of (+)-5 $\beta$ -Acetoxygymnomitr-3(15)-ene (**182**)

Compound **182**, (+)-5 $\beta$ -Acetoxygymnomitr-3(15)-ene, exhibits a molecular ion peak at  $m/z$  262 corresponding to  $C_{17}H_{26}O_2$ . The  $^{13}C$ -NMR spectrum revealed the presence of 17 carbon resonances.  $^1H$ -NMR and HMQC demonstrated that a total of 26 protons were directly attached to the carbon skeleton. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of four singlets for the methyl groups at  $\delta$  0.79 (3H, *s*, H-13), 0.83 (3H, *s*, H-14), 0.92 (3H, *s*, H-15) and 1.71 (3H, *s*, H-17). In addition, compound **182** shared several spectral similarities with **180** and **181**. The consideration of this data led to the conclusion that **182** is a structural isomer of **180** with the acetoxy group attached to C-5-position. The difference in substitution reflected principally in the  $^1H$ -NMR signals shift of methyls (H-13 and H-14), in addition to the  $^{13}C$ -NMR signal at C-5 ( $\delta$  76.4). Information from 2D  $^1H$ - $^1H$ -COSY, HMQC, HMBC (Fig. 77) spectra in addition to the  $^{13}C$ -NMR confirmed structure **182**. Its relative configuration from the NOESY spectrum could not be ascertained from the interactions of the oxygenated methine protons H5 with H-4b and the overlapped signal of protons H-8a, H-10a, H-9 and H-1a (Fig. 78).

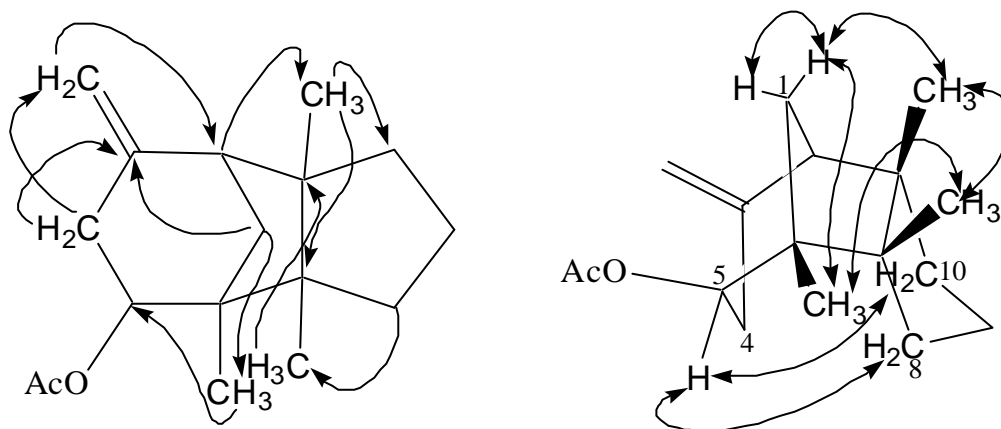


Fig.77. Long-range  $^1H$ - $^{13}C$  correlations of **182**. Fig. 78. NOE correlations of **182**.

Nevertheless, the configuration at C-5 of **182** was concluded to show a  $\beta$ -oriented acetoxy group on biogenetic grounds and the fact that its  $K_2CO_3$  /MeOH hydrolysis gave gymnomitr-3(15)-en-5 $\beta$ -ol (**190**), an epimer of (-)-gymnomitr-3(15)-en-5 $\alpha$ -ol (**191**) (Fig. 79a) isolated from the liverwort *Cylindrocolea recurvifolia*.<sup>[160]</sup>

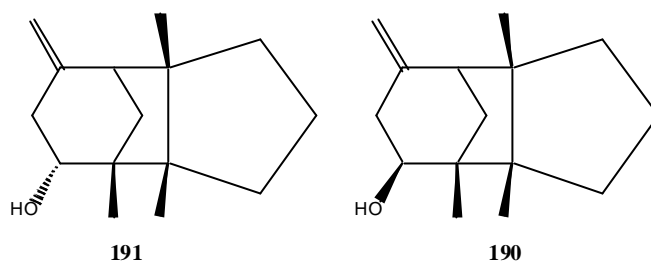


Fig. 79a. 5 $\alpha$ - and 5 $\beta$ - Hydroxygymnomitr-3(15)-en-ol (**191** and **190**).

Purification of compound **190** by preparative GC gave the sesquiterpene hydrocarbon (–)-gymnomitr-3(15),4-diene (**166**).<sup>161]</sup> The <sup>13</sup>C-NMR data of **166** are reported for the first time. Compound **166** occurs naturally in the essential oil of *M. emarginata*. **166**, also resulted from the thermal dehydration of (–)-gymnomitr-3(15)-en-4 $\beta$ -ol (**68**) (Fig. 79b).

Compound **68** is one of the major components obtained from hydrodistillation of *M. emarginata*. Oxidation of **68**, with pyridinium dichromate (PDC) in dry dichloromethane gave (–)-gymnomitr-3(15)-ene-4-one (**188**) which was also identified in the essential oil.

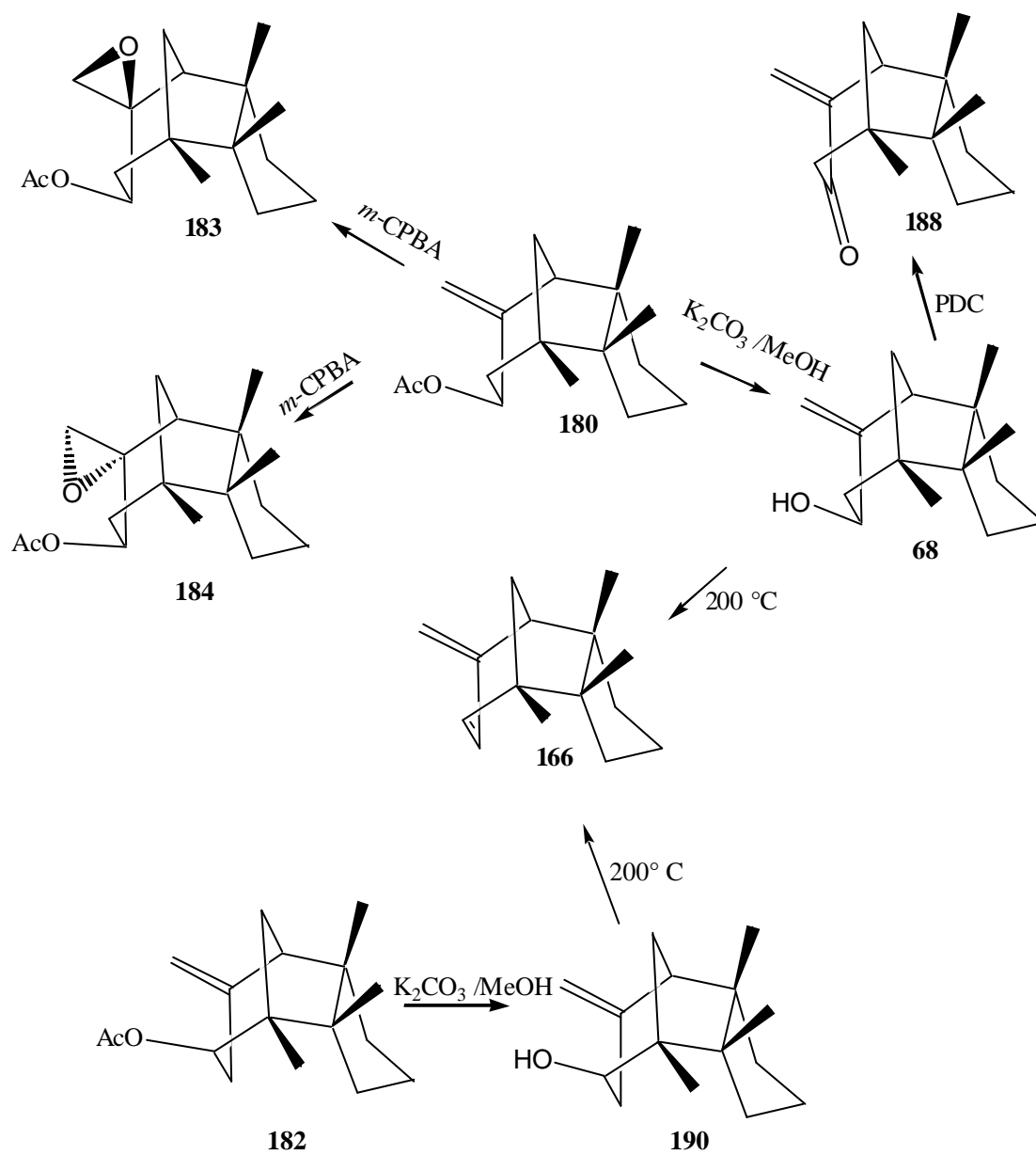


Fig. 79b. Functional group interconversions of related gymnomitrane sesquiterpenoids.

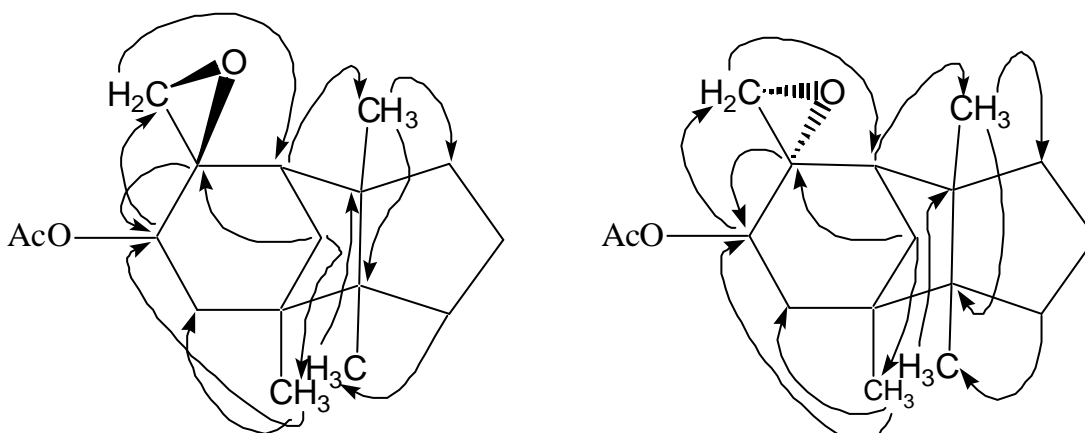


#### 4.5.4. Structure of (-)-**3b**,**15b**-Epoxy-**4b**-acetoxygymnomitrane (**183**)

Compound **183**, the  $3\beta,15\beta$ -epoxide of **180** and its structural isomer, (**184**,  $3\alpha,15\alpha$ -epoxide), were isolated. Both compounds, **183** and **184**, showed similar MS and NMR spectral data. Compounds **183** and **184** exhibited a molecular ion at  $m/z$  278 ( $M+NH_4^+ = 296$ ) corresponding to the molecular formula  $C_{17}H_{26}O_3$ .  $^1H$ -NMR and HMQC spectroscopic analyses indicated a total of 26 protons directly attached to the carbon skeleton. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) of **183** showed signals of four singlets for the methyl groups at  $\delta$  0.72 (3H, *s*, H-13), 0.73 (3H, *s*, H-14), 0.82 (3H, *s*, H-12) and 1.68 (3H, *s*, H-17). The downfield shifted signal at  $\delta$  2.27 (1H, *d*,  $J = 5.04$  Hz) and 2.60 (1H, *d*,  $J = 5.04$  Hz) were assigned to the epoxy-methylene at C-15. The proton signal at  $\delta$  5.85 (1H, *dd*,  $J = 7.6, 11.4$  Hz), attached to C-4 ( $\delta$  65.9) was assigned to the acetoxy methine proton, H-4.  $^{13}C$ -NMR data of **183** are consistent with the assigned structure.

#### 4.5.5. Structure of (-)-**3a**,**15a**-Epoxy-**4b**-acetoxygymnomitrane (**184**)

Compound **184**, the  $3\alpha,15\alpha$ -epoxide of compound **180**, also showed similar  $^1H$ -NMR ( $C_6D_6$ ). The  $^1H$ -NMR spectrum ( $C_6D_6$ ) of **184** showed signals of four singlets for the methyl groups at  $\delta$  0.73 (3H, *s*, H-13), 0.74 (3H, *s*, H-14), 0.87 (3H, *s*, H-12) and 1.60 (3H, *s*, H-17). The difference in stereochemistry of the epoxy-ring in compound **184** is reflected principally in the  $^1H$ -NMR signals shift of methyls (H-12 and H-17), the epoxy-methylene appeared at  $\delta$  2.09 (1H, *d*, H-15a,  $J = 5.7$  Hz), 2.90 (1H, *d*, H-15b,  $J = 5.7$  Hz) and the acetoxy methine proton signal appeared more deshielded at  $\delta$  5.95 (1H, *dd*, H-4,  $J = 7.6, 10.7$  Hz) as compared to that of compound **183**. In addition, the  $^{13}C$ -NMR signals at C-4 ( $\delta$  67.9) and C-3 ( $\delta$  61.9) were



Figs. 80 and 81. Long-range  $^1H$ - $^{13}C$  correlations of **183** and **184** respectively.

also slightly deshielded in **184**. All the information from the 2D  $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC, and HMBC (Figs. 80 and 81) analysis were consistent with the assigned structures **183** and **184**.

The relative configuration of compound **184** was derived from the NOESY spectrum (Fig. 82) which indicate spatial interactions of proton H-4 with both H-8a and H-10b. The NOEs observed for proton H-4 of compound **184**, were similar to those of the likely precursor, compound **180**. Thus, suggesting a  $\beta$ -orientation for the acetate group on C-4. Compound **183** demonstrated similar spatial interactions but in this case, H-4 interacted with the overlapped signal of protons H-8b, H-10b and H-5b (Fig. 83).

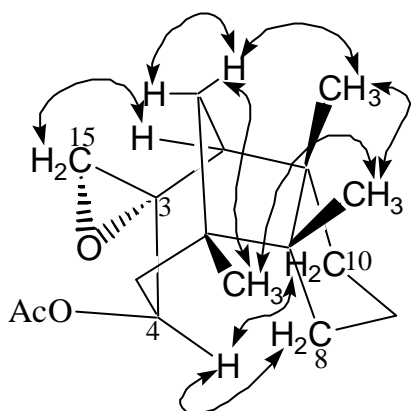


Fig. 82. NOE correlations of **184**.

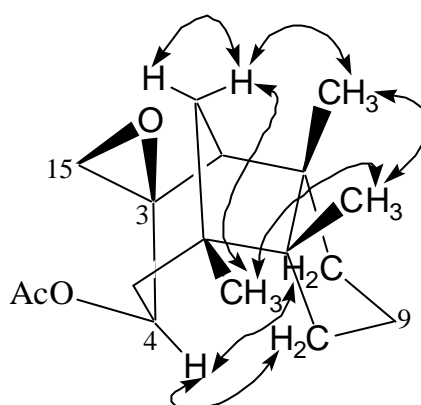


Fig. 83. NOE correlations of **183**.

Therefore, to determine the absolute configuration of compounds **183** and **184**, the supposed precursor, compound **180** of known absolute configuration was epoxidised with *m*-chloroperbenzoic acid, (*m*-CPBA) in chloroform. The epoxidation of **180** gave a mixture of two epoxides **183** and **184**, in the ratio of 10 : 1. Thus, confirming the  $\beta$ -orientation of the acetate group of compounds **183** and **184**. Although, *m*-CPBA epoxidation of **180** gave a ratio of 10 : 1 for compounds **183** and **184** respectively, the ratio of **183** and **184** in the essential oil of *M. emarginata* is 4 : 3. Therefore, **183** and **184** have the same configuration at C-4. The absolute configuration of the *epoxy*-ring in **183** and **184** were assigned by comparison of the shielding-deshielding effect of the oxirane ring on the chemical shifts of other neighbouring protons with respect to compound **180** by spectral analysis of the  $^1\text{H}$ -NMR ( $\text{C}_6\text{D}_6$ ). In the major epoxide **183**, deshielding of the chemical shifts of protons H-4, H-5a and the bridge protons, H<sub>2</sub>-1 (at  $\delta$  1.80 and 1.89) might be due to the proximity of the electronegative oxygen of the *epoxy*- ring. While in the minor epoxide **184**, although H-4, 8b, 10b were slightly deshielded, the bridge protons H<sub>2</sub>-1 (at  $\delta$  1.29 and 1.82) were shielded as compared to the bridge protons of **180** (at  $\delta$  1.40 and 1.92). Thus, the major epoxide was

assigned  $3\beta$ ,  $15\beta$  (**183**) and the minor  $3\alpha$ ,  $15\alpha$  (**184**). A similar effect has been reported for related gymnomitrane compounds isolated from the liverwort *Gymnomitrium obtusum* (Lindb) Pears.<sup>[162]</sup> The new compound **183** has recently been isolated by the Taiwanese group from *Marsupella emarginata* collected from Scotland.<sup>[163]</sup>

#### 4.5.6. Structure of (-)-15-Acetoxygymnomitr-3-ene (**185**)

(-)-15-Acetoxygymnomitr-3-ene (**185**), was isolated for the first time as a natural product. Compound **185** has been reported as a reaction product in the structural establishment of an allylic primary alcohol **189**, and only partial <sup>1</sup>H-NMR data of **185** are available in the literature.<sup>164</sup> The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) showed signals of four singlets for methyl groups at  $\delta$  0.78 (3H, *s*, H-13), 0.83 (3H, *s*, H-14), 0.94 (3H, *s*, H-12) and 1.7 (3H, *s*, H-17). The signals at  $\delta$  4.51 (2H, *d*,  $J = 8.8$  Hz) and 5.41 (1H, *br.s.*) were assigned to protons H-15 and H-4, respectively. All the information from the 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC (Fig. 84a) spectra analysis in addition to the <sup>13</sup>C-NMR (Table 3) were consistent with the assigned structure **185**. Treatment of **185** with K<sub>2</sub>CO<sub>3</sub> in methanol gave the de-acetate compound (+)-**189**, which was also isolated and identified in the essential oil of *M. emarginata* for the first time. The spectral data of **189** were totally consistent with literature data.<sup>[158]</sup>

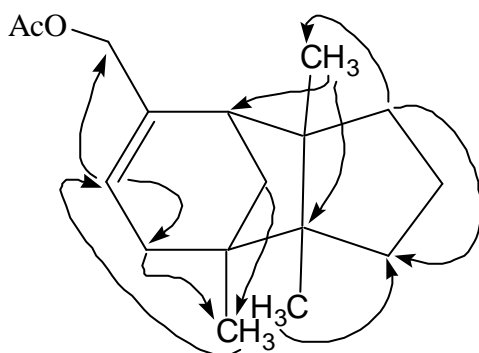


Fig. 84a. Long-range <sup>1</sup>H-<sup>13</sup>C correlations of **185**.

In addition, (+)- $\alpha$ -barbatenal (**186**), the ketone corresponding to **189**, was isolated for the first time from liverworts. The spectral data of **186** were consistent with those reported in the literature, and the polarimetric sign was opposite to the (-)-enantiomer isolated from the roots of the higher plant *Joannesia princeps*.<sup>[165]</sup> The <sup>13</sup>C-NMR (C<sub>6</sub>D<sub>6</sub>) of **186** are available in Table 3. The isolation procedures for (+)- $\alpha$ -barbatenal (**186**) isolated from the liverwort *Marsupella emarginata* is shown below (Fig. 84b).

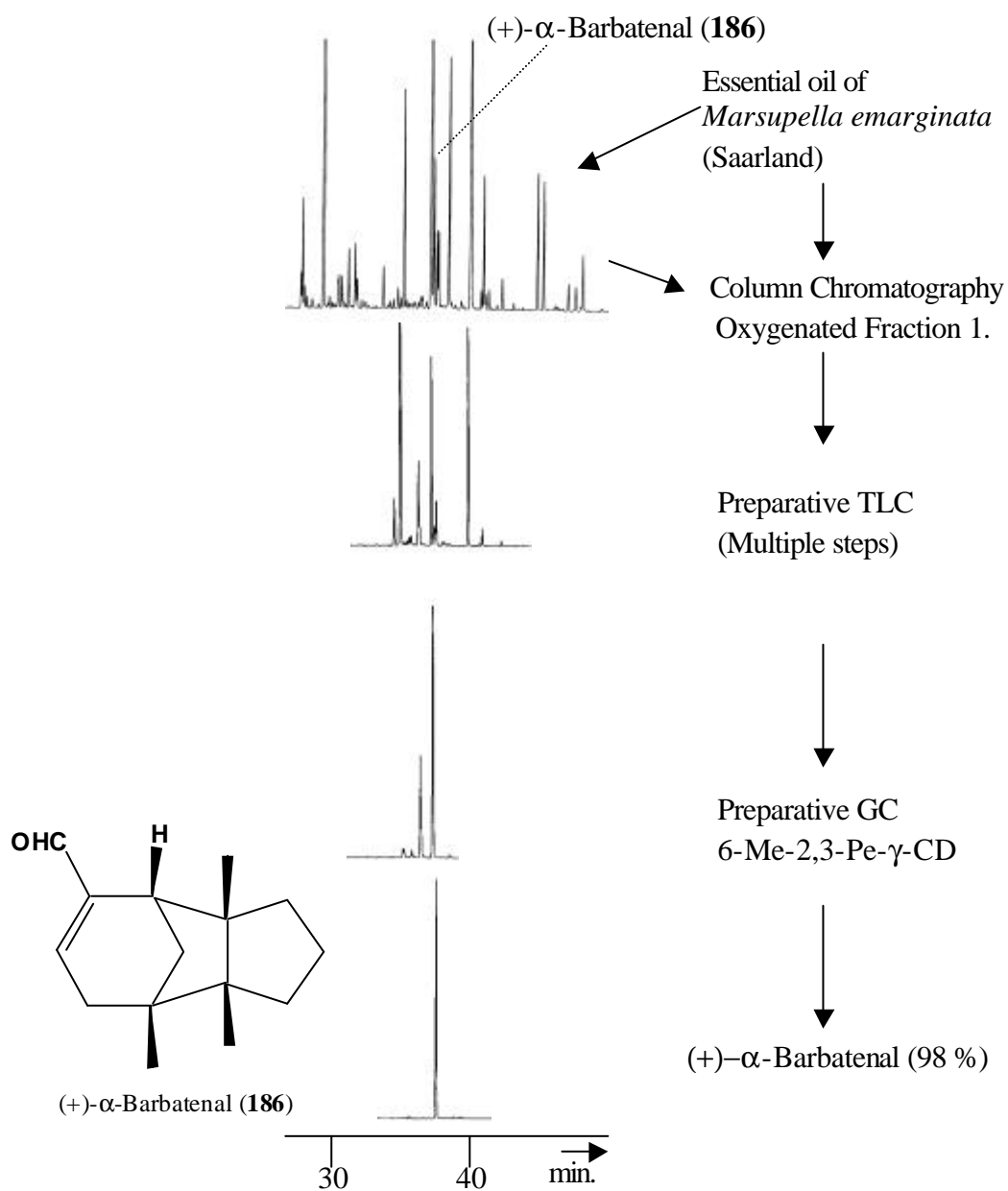


Fig. 84b. Isolation of (+)- $\alpha$ -Barbatenal (**186**) from *Marsupella emarginata* (Saarland, Germany)

#### 4.6. Composition of the Ether Extract of *M. emarginata* (Ehrch.) Dum from Japan

The GC and GC-MS of the ether extract of *M. emarginata* (Ehrch.) Dum collected from another region (Japan) was investigated. In the oxygenated fraction, the new compound, 12-acetoxygymnomitr-3(15)-ene (**187**, 2.1%), was isolated, in addition to the known (+)-gymnomitr-3(15)-en-12-oic acid (**192**, 35.9%), (-)-gymnomitr-3(15)-en-12-al (**193**, 10.7% ; <sup>13</sup>C-NMR data are reported for the first time, Table 4) and a trace of gymnomitr-3(15)-en-12-ol (**194**,<sup>[146]</sup> 0.3%, identified by MS fragmentation only, RI<sub>CPSIL 5</sub> = 1746). Identified sesquiterpene hydrocarbon constituents of the ether extract include  $\alpha$ -barbatene (**53**),  $\beta$ -barbatene (**57**), gymnomitr-3(15),4-diene (**166**), isobazzanene (**97**),  $\beta$ -bazzanene (**63**), selina-4,11-diene (**132**), thujopsene (**168**),  $\alpha$ -muurolene (**105**), as shown in Figs. 85a-b and Table 1.

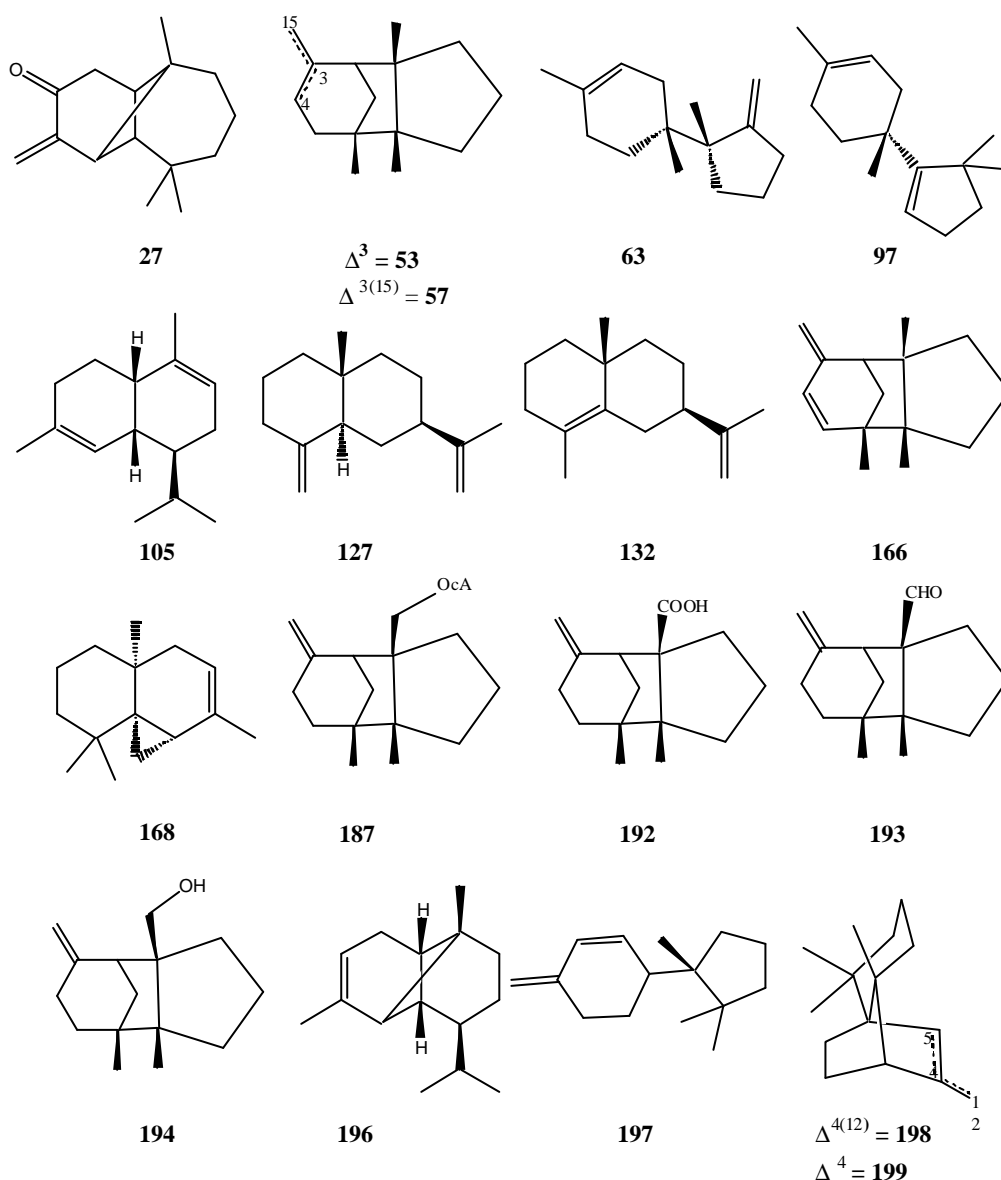


Fig. 85a Sesquiterpenoids constituents of *Marsupella emarginata* (Japan).

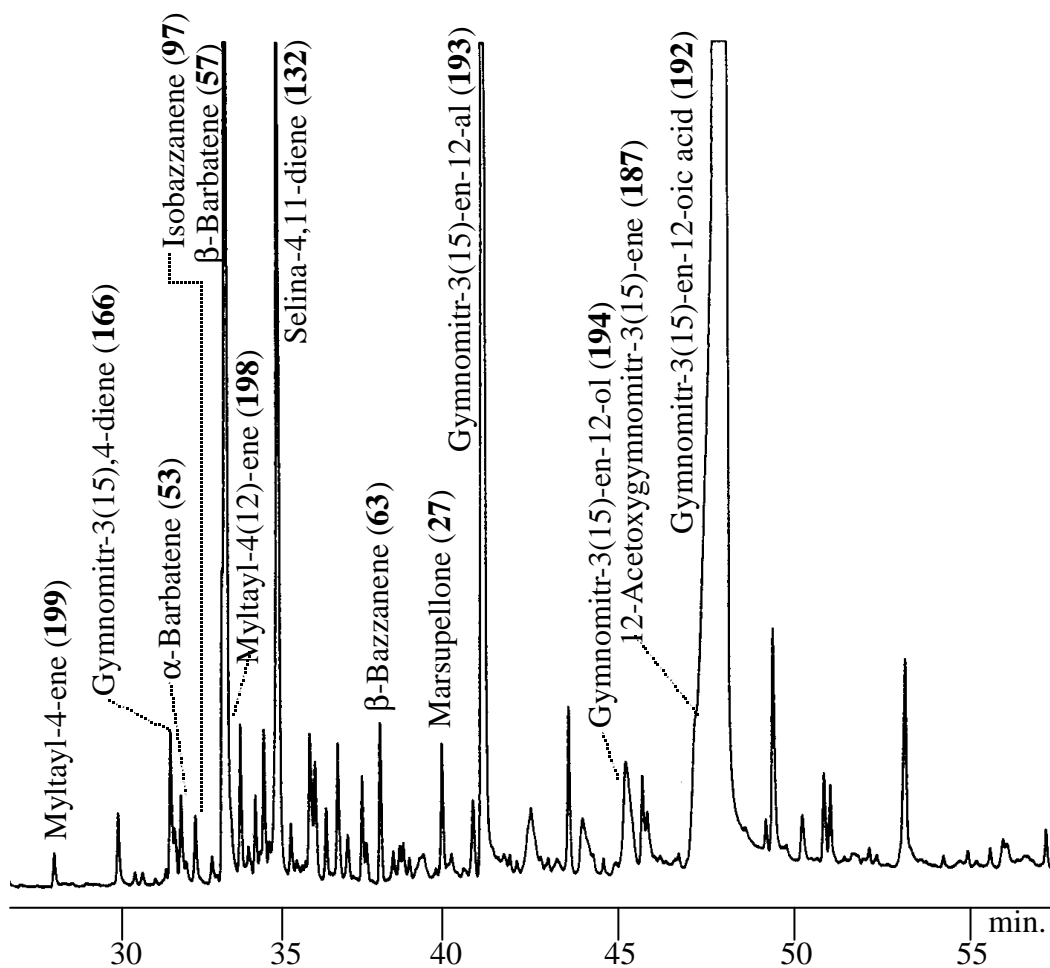


Fig. 85b. Gas chromatogram of the essential oil of *Marsupella emarginata* (Ehrch.) Dum. (Japan). (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.6.1. Structure of (+)-12-Acetoxygymnomitr-3(15)-ene (**187**)

Compound **187**, the acetate of **194**, exhibited a molecular ion at  $m/z$  262 corresponding to  $C_{17}H_{26}O_2$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of three singlets for the methyl groups at  $\delta$  0.72 (3H, *s*, H-14), 0.77 (3H, *s*, H-13) and 1.70 (3H, *s*, H-17). The olefinic carbon signals at  $\delta$  150.6 (*s*) and 108.9 (*t*) suggested an exomethylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  4.74 (1H, *t*, H-15a,  $J = 2.2$  Hz) and 4.77 (1H, *t*, H-15b,  $J = 2.2$  Hz). The signal at  $\delta$  4.00 (2H, *br.s*) was assigned to the methylene protons connected to the acetate group at C-12 ( $\delta$  68.9). Information from 2D  $^1H$ - $^1H$ -COSY, HMQC, HMBC (Fig. 85c) spectra in addition to the  $^{13}C$  NMR (extracted data from HMQC and HMBC) confirmed structure **187**. The shown relative configuration of **187** was assumed to be the same as the absolute configuration since the absolute configuration of the deacetylated form, **194** had been determined by X-ray.<sup>[146]</sup>

In conclusion, the essential oil obtained by hydrodistillation of *Marsupella emarginata* collected from Saarland (southern part of Germany) and the ether extract of the Japanese sample consists of a series of gymnomitrane structures in addition to a few known longipinane-based compounds (**27** and **92**), with no trace of lemnalol (**29**), lemnalone (**28**) and eremophila-9,11-dien-8 $\alpha$ -ol (**149**). While the extract of *Marsupella emarginata* from Altenau (Germany) consists of large numbers of longipinane-based compounds, few gymnomitrane (**180** and **166**) and in addition lemnalol (**29**), lemnalone (**28**), eremophila-9,11-dien-8 $\alpha$ -ol (**149**). This implies that the sesquiterpenoid compositional differences of the *M. emarginata* species are clear in the oxygenated fraction and in the relative amount of  $\beta$ -longipinene (**92**) and  $\beta$ -barbatene (**57**) in the sesquiterpene hydrocarbon fraction.

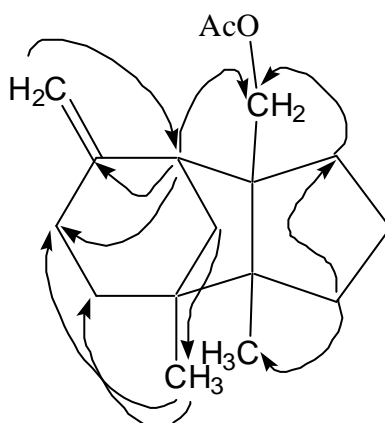


Fig. 85c. Long-range  $^1H$ - $^{13}C$  correlations of **187**.

#### 4.7. Composition of the Essential Oil of *Marsupella aquatica*

Fresh plant material of *Marsupella aquatica* collected near Gaschurn/Montafon, Austria, in July 2001 at an elevation of 1900 m was hydrodistilled. The essential oil yielded a complex mixture of volatiles from which the following constituents were identified by GC-MS comparison with a data bank.<sup>[107, 108]</sup> These include  $\beta$ -elemene (**40**, 0.4%),  $\alpha$ -barbatene (**53**, 0.4%), isobazzanene (**97**, 0.5%),  $\beta$ -barbatene (**57**, 3.2%),  $\beta$ -acoradiene (**58**, 1.3%), (+)-amorpha-4,11-diene (**31**, 9.6%), and (-)-amorpha-4,7(11)-diene (**70**, 25.2%). In addition, traces of  $\alpha$ -longipinene (**88**), cyclomylytayne (**195**),<sup>[167]</sup> anastreptene (**51**),  $\alpha$ -copaene (**196**),  $\beta$ -copaene, calarene (**117**),  $\alpha$ -chamigrene (**62**), cadina-1,4-diene (**119**), and  $\delta$ -cuprenene (**197**) could be identified (Fig. 86a-c). (+)-Amorpha-4,11-diene (**31**, Fig. 86d) and (-)-amorpha-4,7(11)-diene (**70**) were isolated for the first time from *M. aquatica*. The (-)-enantiomer of **31** has been described as an intermediate in artemisinin (**30**) biosynthesis,<sup>[41]</sup> while the (+)-enantiomer of **70** was a constituent of a higher plant, *Ageratina adenophora*.<sup>[166]</sup>







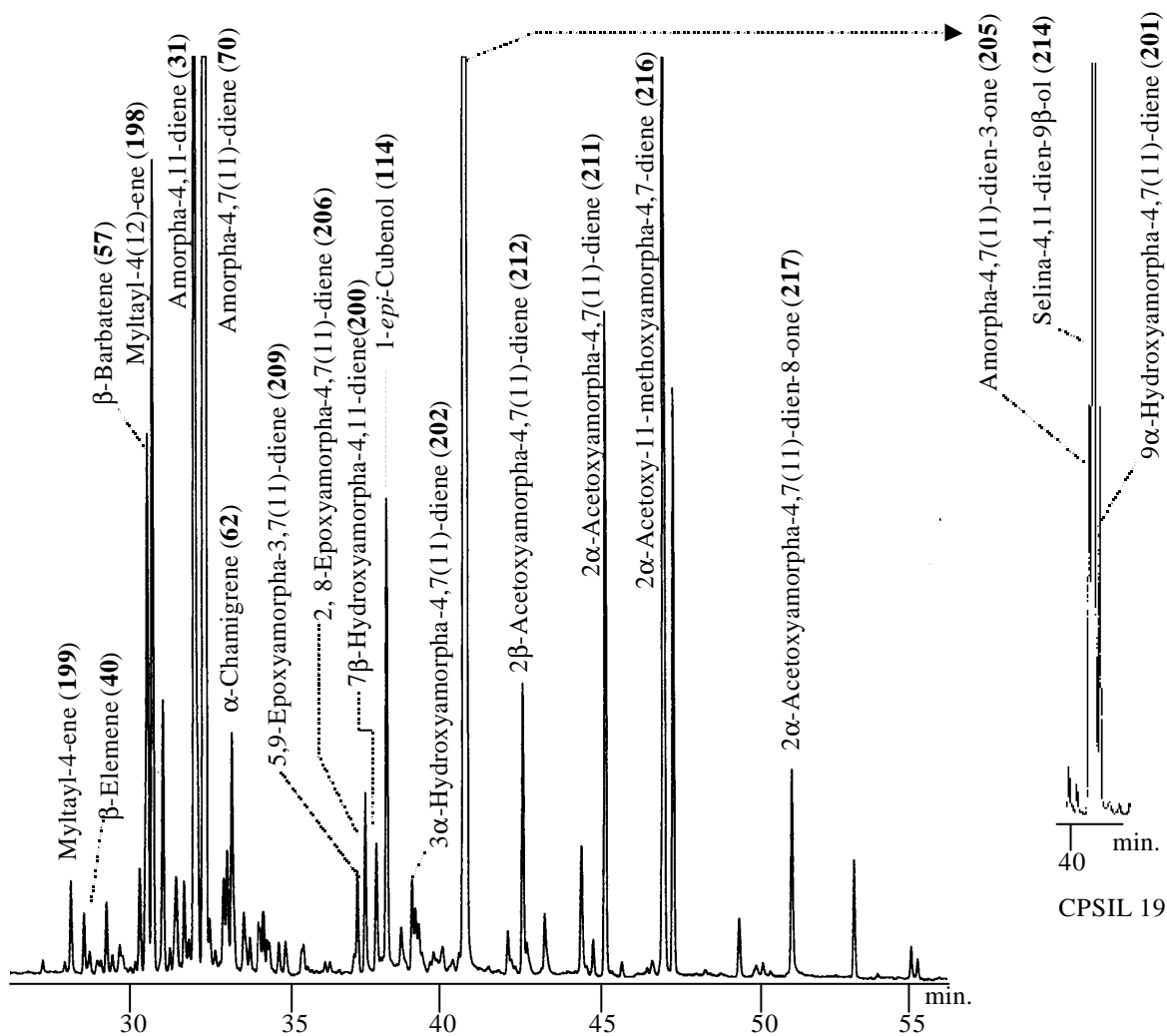


Fig. 86c. Gas chromatogram of the essential oil of *Marsupella aquatica* (Austria).  
(CPSIL 5, 50°C, 3°C/ min. 230°C).

Since (+)-Amorpha-4,11-diene (**31**) was isolated from the essential oil of *M. aquatica* for the first time, its absolute configuration was confirmed by coinjection with an authentic (–)-enantiomer of **31** using 6T-2,3-Me- $\beta$ -CD at 120 °C isothermal (Fig. 86d).

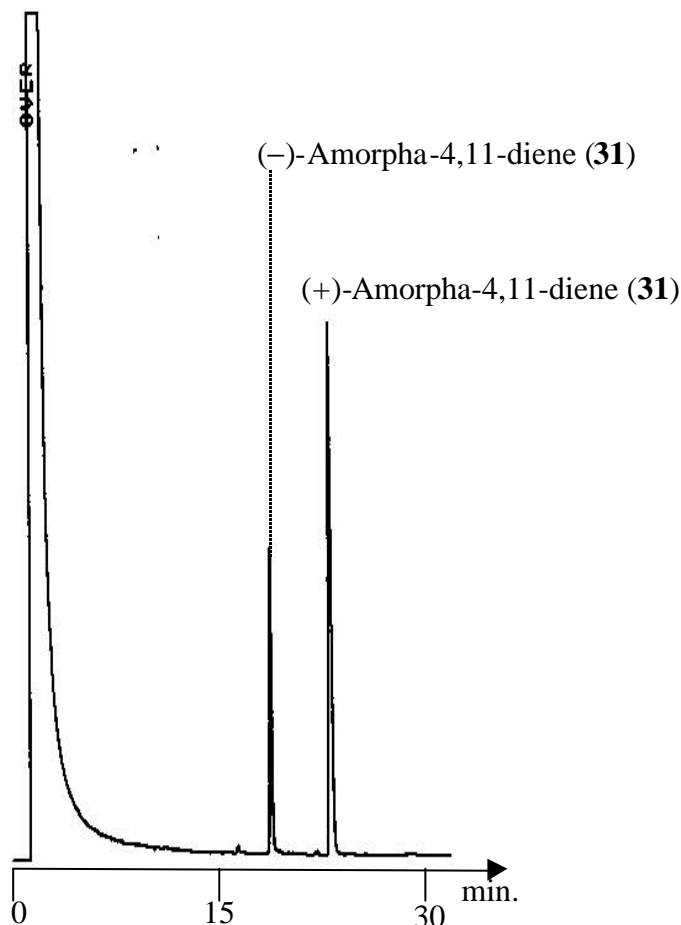


Fig. 86d. Separation of synthetic (–)-Amorpha-4,11-diene from (+)-Amorpha-4,11-diene isolated from *Marsupella aquatica* by Capillary gas chromatography, using 6T-2,3-Me- $\beta$ -CD at 120 °C isothermal.

#### 4.7.1. Structure of (–)-Myltayl-4(12)-ene (**198**)

A sesquiterpene hydrocarbon with an irregular carbon skeleton **198** eluted just after  $\beta$ -barbatene (**57**) from a non-polar dimethylpolysiloxane column and was isolated for the first time from a natural source. The mass spectrum exhibited a molecular ion signal at  $m/z$  204 corresponding to  $C_{15}H_{24}$ . The  $^1H$ -NMR spectrum of **198** was examined in both  $C_6D_6$  and  $CDCl_3$  solution for better resolution of the various multiplet signals. The  $^1H$ -NMR spectrum recorded in  $C_6D_6$  showed three methyl singlets at  $\delta$  0.77, 0.91 and 0.92. The olefinic carbon signals at  $\delta$  102.0 ( $t$ ) and 154.5 ( $s$ ) suggested an exocyclic double bond which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  4.69 (1 H,  $d$ ,  $J=1.6$  Hz) and 4.90 (1 H,  $d$ ,  $J=1.5$

Hz). A system of methylene protons coupling with one another at  $\delta$  1.77 (1 H, *br.d*,  $J=16.4$  Hz) and 2.47 (1 H, *d*,  $J=16.4$  Hz) was observed. The  $^{13}\text{C}$ -NMR and DEPT spectra of **198** indicated signals of three primary carbons ( $\delta$  19.4, 23.4, 28.9), seven secondary carbons ( $\delta$  19.5, 27.8, 28.1, 30.4, 36.6, 40.6, 102.0), one tertiary carbon ( $\delta$  58.0) and four quaternary carbons ( $\delta$  33.7, 47.2, 53.1, 154.5). The 2D  $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC and HMBC (Fig. 87a), spectra confirmed the structure of **198**. Important  $^1\text{H}$ - $^{13}\text{C}$  HMBC of (-)-myltayl-4(12)-ene (**198**) are shown (Fig. 87a). The relative configuration of **198** was deduced from the NOESY spectrum (Fig. 87b), which showed spatial interactions between protons H14 and H<sub>2</sub>-5, H-13 and H-3, H-8a/1a and also between H-15 and H-10a, 8a/1a.

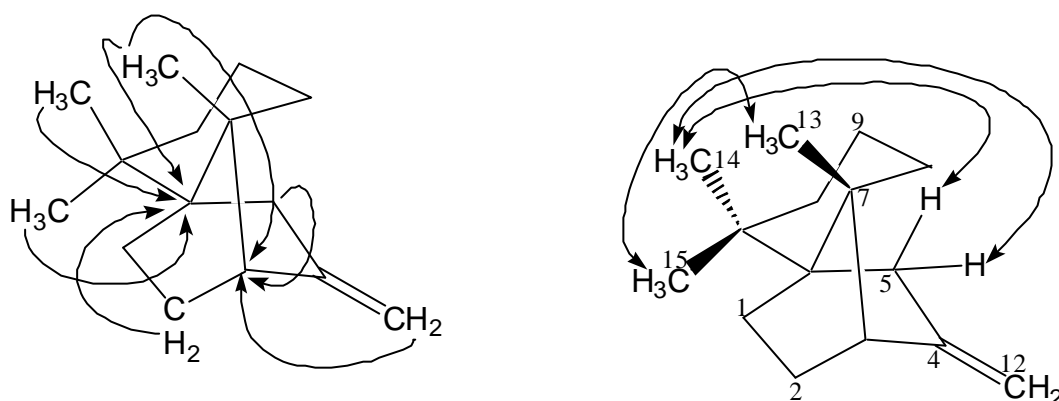


Fig. 87a. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **198**. Fig. 87b. NOE correlations of **198**.

The  $^1\text{H}$ -NMR recorded in  $\text{CDCl}_3$  was totally in agreement with that of synthetic ( $\pm$ )-myltayl-4(12)-ene.<sup>[168]</sup> The numbering system is consistent with that of (-)-myltaylenol, a sesquiterpene alcohol previously identified as a constituent of the liverwort *Mylia taylori*.<sup>[169]</sup> Takaoka et al., (1985)<sup>[169]</sup> proposed that the myltaylane framework may be derived from *cis*-farnesyl diphosphate through C-3 – C-7 cyclization of  $\beta$ -chamigrene followed by migration of the C-3 methyl group to the vicinal position via an anti-Markownikov route.

#### 4.7.2. Structure of (-)-Myltayl-4-ene (**199**)

Compound **199**, the double bond isomer of **198** eluted before  $\beta$ -elemene (**40**) from a non-polar dimethylpolysiloxane column. The mass spectrum of **199** exhibited a molecular ion signal at  $m/z$  204 consistent with the molecular formula  $\text{C}_{15}\text{H}_{24}$ , indicating four degrees of unsaturation. The  $^1\text{H}$ -NMR ( $\text{C}_6\text{D}_6$ ) showed three methyl singlets at  $\delta$  0.88 (3H, *s*), 0.94 (3H, *s*), 0.99 (3H, *s*) and one olefinic methyl at  $\delta$  1.62 (3H, *s*). The 2D  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra confirmed the structure of **199**. The relative configuration of **199** was deduced

from the NOESY spectrum which showed spatial interactions of the methyl group protons H 14 with methine H5, and H-10b / H-9a. Spatial interactions of H-13 protons with H-3, H-1b, H-2b, H-8a and H-15 protons were observed. In addition proton H5 also interacts with H-14 and H-9a /10b (Fig. 88).

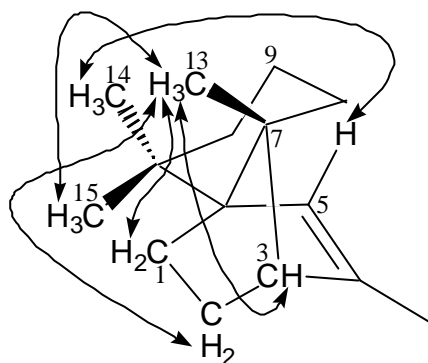


Fig. 88. NOE correlations of **199**.

The proposed mass spectral fragmentation of **199** is shown in (Fig. 89).

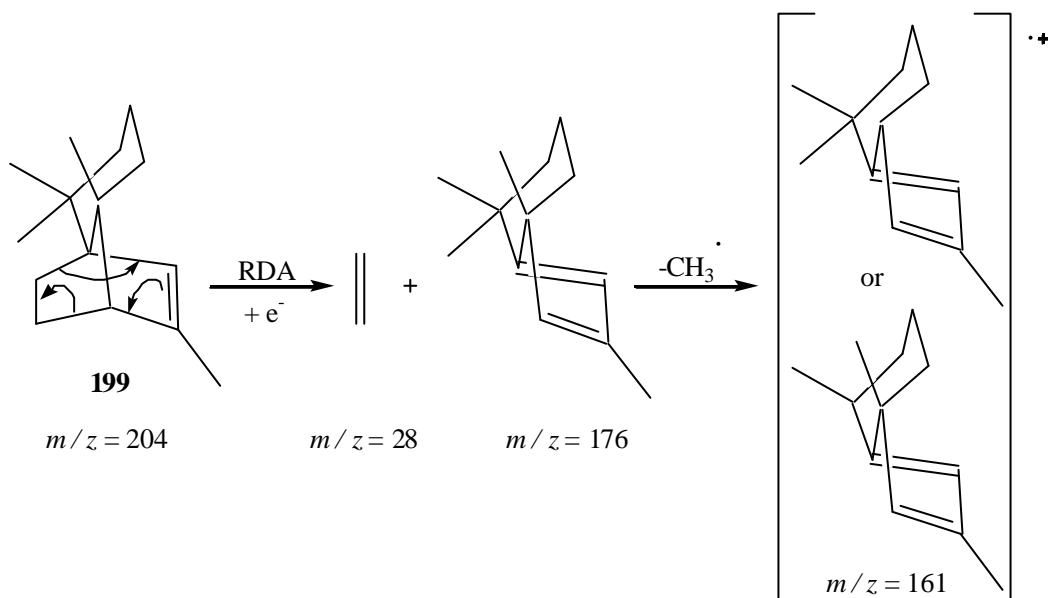


Fig. 89. Possible MS fragmentation pattern for **199**.

The biogenetic pathway via a Markownikov route is proposed in addition to the earlier suggested *anti*Markownikov route by Takaoka et al.<sup>169</sup> The co-occurrence of **198**, **199** and cyclomyltaylane (**195**) supports formation of the myltaylanyl cation in both routes (Fig. 90).

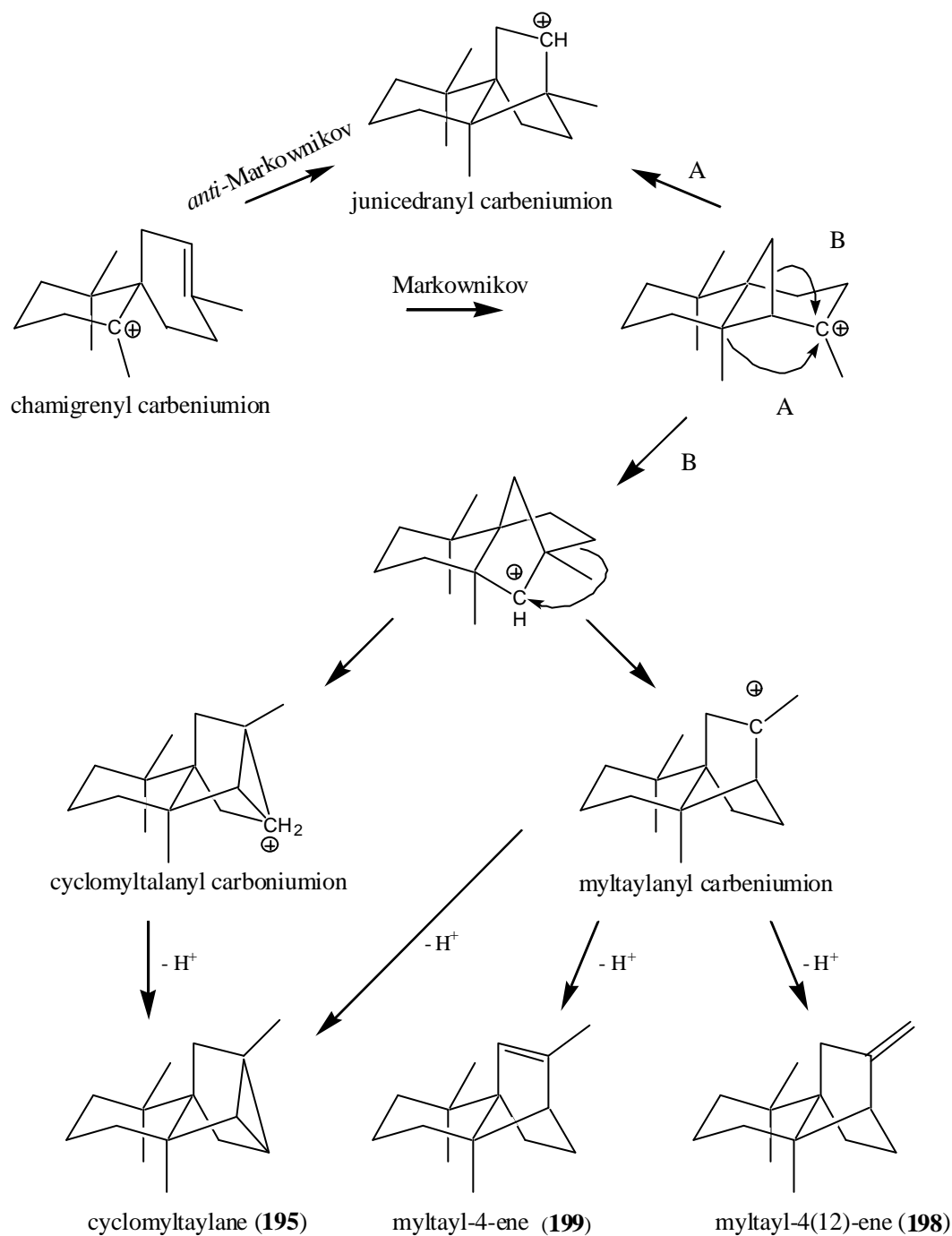


Fig. 90. Proposed biogenetic pathway for **195**, **198** and **199**.

#### 4.7.3. Structure of (+)-7**b**-Hydroxyamorpha-4,(11)-diene (**200**)

7 $\beta$ -Hydroxyamorpha-4,(11)-diene (**200**), showed a molecular ion signal at  $m/z$  220, and the molecular formula was determined as  $C_{15}H_{24}O$  ( $[M^+]$   $m/z$  220.1832) by HR-EIMS. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) of compound **200**, indicated signals of three methyl groups at  $\delta$  0.92 (3H, *d*, H-14,  $J = 6.3$ Hz), 1.57 (3H, *br.s*, H-15), and 1.81 (3H, *s*, H-12). Additional signals at  $\delta$  4.85 (1H, *s*, H-13a), 4.90 (1H, *s*, H-13b) and 1.81 (3H, *s*, H-12) revealed the existence of an isopropenyl group. The olefinic carbon signals at  $\delta$  150.92 (*s*) and 111.50 (*t*) confirmed the isopropenyl methylene double bond. The  $^{13}C$ -signal at  $\delta$  75.58 was assigned to the tertiary hydroxy group at C-7. The 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 91) spectra in addition to the  $^{13}C$ -NMR confirmed structure **200**. Its relative configuration resulted from the NOESY spectrum (Fig. 92a).

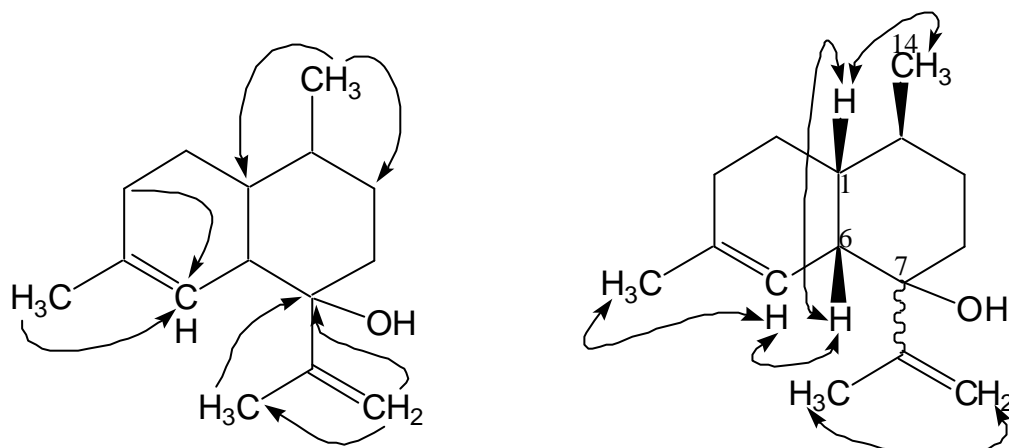


Fig. 91. Long-range  $^1H$ - $^{13}C$  correlations of **200**. Fig. 92a. NOE correlations of **200**.



The absolute configuration was determined by comparison of the fully hydrogenation products of (-)-amorpha-4,7(11)-diene (**70**) with (+)-**200**, by enantioselective GC on a cyclodextrin stationary phase. The four fully hydrogenated derivatives of **200** are identical (same GC-MS characteristics and same retention times on achiral polysiloxane and chiral cyclodextrin derived GC phases) with that of the hydrogenated products of compound **70** (Fig. 92b). Therefore the absolute configuration of **200** at C-1, C-6, and C-10 was concluded to be (1*R*, 6*S*, 10*S*). The 7*S*-configuration assigned to **200** was inferred from the four major hydrogenation products which suggested that proton H-6 must be *cis*-configuration to the hydroxy on C-7. In addition, the assigned 7*S*-configuration is consistent with (+)-amorpha-4,11-diene (**31**) and (+)-1-*epi*-cubenol (**113**) isolated and characterised in the *M. aquatica* oil.

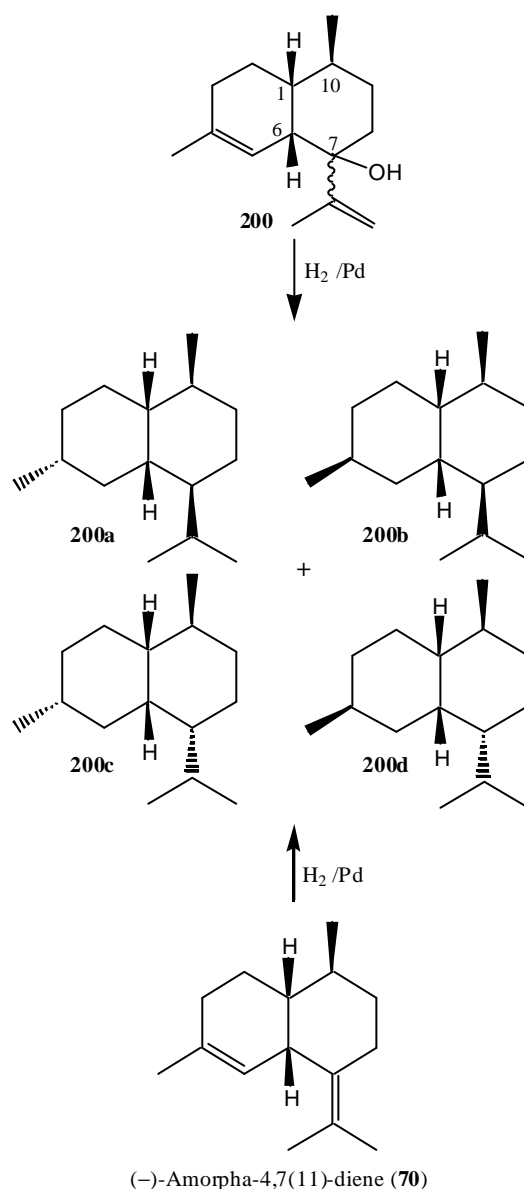


Fig. 92b. Complete hydrogenation products of **70** and **200**.

#### 4.7.4. Structure of (-)-9 $\alpha$ -Hydroxyamorpha-4,7(11)-diene (**201**)

(-)-9 $\alpha$ -Hydroxy-amorpha-4,7(11)-diene (**201**), has the same bicyclic sesquiterpene skeleton as **200**. Compound **201** showed a molecular ion signal at  $m/z$  220, and the molecular formula was determined as C<sub>15</sub>H<sub>24</sub>O ([M<sup>+</sup>]  $m/z$  220.1816) by HR-EIMS. The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) of **201** showed similar signals as **200**, except for the absence of the signal of the isopropenyl group. **201** indicated four methyl groups at  $\delta$  1.03 (3H, *d*, H-14,  $J$  = 6.6 Hz), 1.58 (3H, *s*, H-15) and 1.66 (6H, *br.s*, H-12 and H-13). A chemical shift signal at  $\delta$  2.98 (1H, *dt*, H-9,  $J$  = 4.1, 11.4 Hz) was assigned to the methine proton H-9, indicating the presence of a secondary hydroxy group at C-9. Additional useful structural information was obtained from the <sup>13</sup>C-NMR. The 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC (Fig. 93) spectra in addition to the <sup>13</sup>C-NMR data confirmed structure **201**. Its relative configuration resulted from the NOE spectrum, which indicated interactions of protons H-1 with H-6 and H-14 (Fig. 94). The absolute configuration of **201** was determined by using a similar method as for compound **200**. The  $\alpha$ -orientation of the secondary hydroxy group was deduced from the spatial interactions of H-9 and H-14.

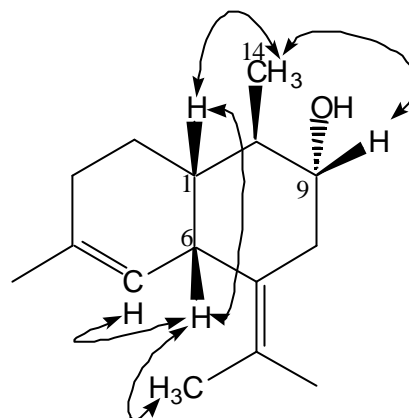
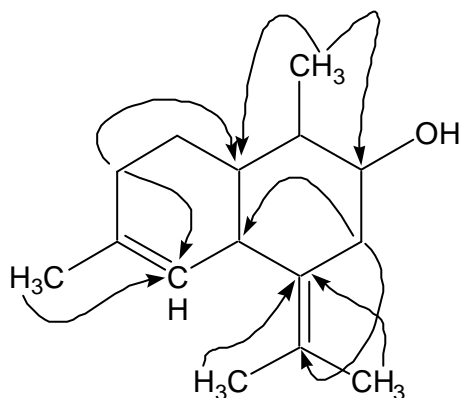


Fig. 93. Long-range <sup>1</sup>H <sup>13</sup>C correlations of **201**. Fig. 94. NOE correlations of **201**.

#### 4.7.5. Structure of (-)-3 $\alpha$ -Hydroxyamorpha-4,7(11)-diene (**202**)

(-)-3 $\alpha$ -Hydroxy-amorpha-4,7(11)-diene (**202**), C<sub>15</sub>H<sub>24</sub>O ([M<sup>+</sup>]  $m/z$  220.1826) exhibited a similar <sup>1</sup>H-NMR as **201** except that the  $\alpha$ -oriented secondary hydroxy group is now on C-3 at  $\delta$  67.4 (*d*). The 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC (Fig. 95) spectra in addition to the <sup>13</sup>C-NMR confirmed the structure. Its relative configuration was determined from the NOESY spectrum, which gave NOEs between protons H-1 and H-6, H-1 and H-14 (Fig. 96).

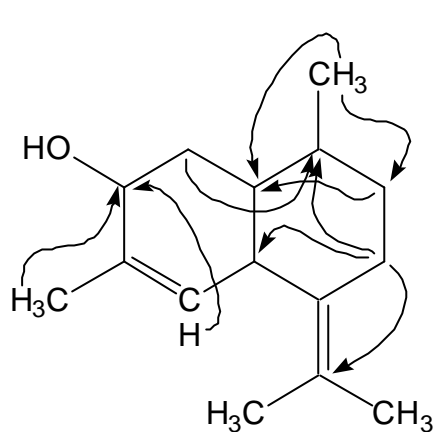


Fig. 95. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **202**.

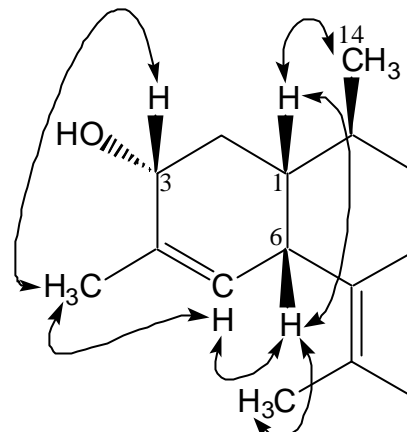


Fig. 96. NOE correlations of **202**.

Direct hydrogenation of compound **202** afforded identical products as in **200** and **201**. In addition, the alcohol **202** dehydrates at the injector port (200 °C) of the preparative GC during purification to afford (+)-amorpha-2,4,7(11)-triene (**203**). Compound **203** showed a molecular ion signal at  $m/z$  202 corresponding to the molecular formula of  $\text{C}_{15}\text{H}_{22}$ . The  $^1\text{H}$ -NMR spectrum ( $\text{C}_6\text{D}_6$ ) indicated the loss of a secondary hydroxy group at C-3 of compound **202** which is replaced by the formation of the endocyclic double bond at  $\delta$  5.85 (1H, *d*,  $J = 9.5$  Hz) and 6.08 (1H, *dd*,  $J = 6.0, 9.4$  Hz) (Fig. 97). The relative configuration of **203** was deduced from NOESY, which is similar to that of **202** (Fig. 96). The complete hydrogenation of **203** gave identical products as compounds **70**, **200**, **201** and **202**.

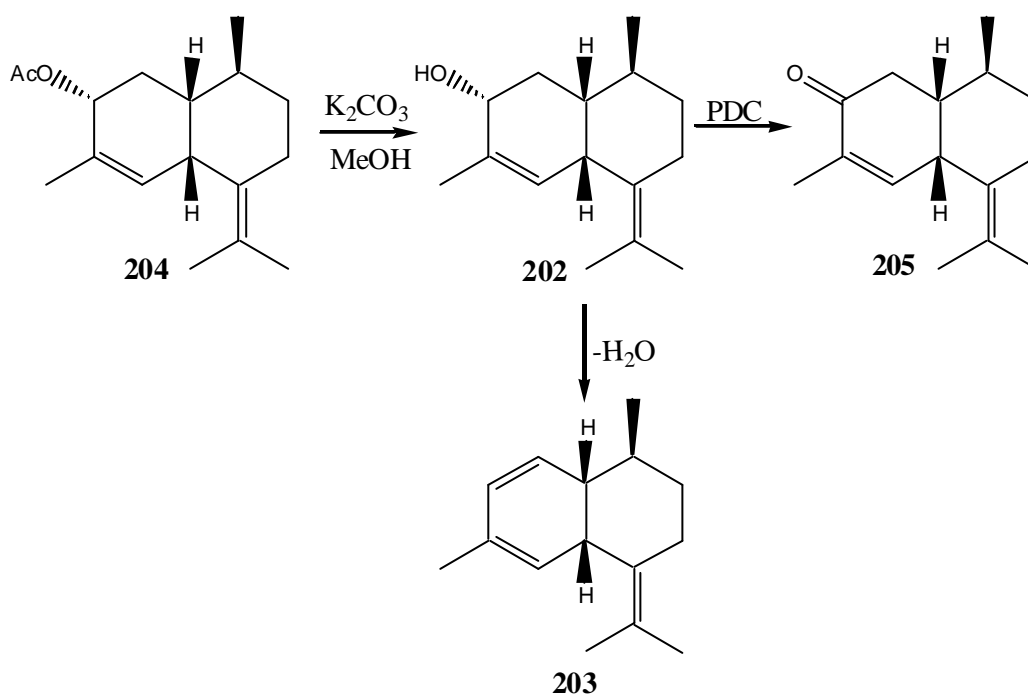


Fig. 97. Chemical interconversion of **202**.

#### 4.7.6. Structure of (-)-3 $\alpha$ -Acetoxyamorpha-4,7(11)-diene (**204**)

(-)-3 $\alpha$ -Acetoxyamorpha-4,7(11)-diene (**204**), the acetate of **202** was isolated from the same essential oil. It showed a molecular ion signal at 262 corresponding to the molecular formula of C<sub>17</sub>H<sub>26</sub>O<sub>2</sub>. The <sup>1</sup>H-NMR spectrum of **204** shows five singlets corresponding to methyl groups at  $\delta$  0.86 (3H, *d*, H-14, *J* = 6.3 Hz), 1.65 (9H, *br.s*, H-12, H-13 and H-15) and 1.74 (3H, *s*, H-17). The signal at  $\delta$  5.33 (1H, *br.d*, H-3, *J* = 5.0 Hz) was assigned to the methine proton connected to the acetoxy group. Additional structural information was obtained from <sup>13</sup>C-NMR. The relative configuration of **204** resulted from its NOESY spectrum, which is similar to that of **202** (Fig. 96). The hydrolysis of compound **204** using K<sub>2</sub>CO<sub>3</sub> / MeOH afforded compound **202**. Therefore **204** should have the same absolute configuration as **202** (Fig. 97).

#### 4.7.7. Structure of (-)-Amorpha-4,7(11)-dien-3-one (**205**)

(-)-Amorpha-4,7(11)-dien-3-one (**205**), an  $\alpha$ ,  $\beta$ -unsaturated ketone with amorphane skeleton, showed a molecular ion signal at *m/z* 218 and the molecular formula of C<sub>15</sub>H<sub>22</sub>O ([M<sup>+</sup>] *m/z* 218.1668) was determined by HR-EIMS. The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) indicated four methyl group signals at  $\delta$  0.72 (3H, *d*, H-14, *J* = 6.3 Hz), 1.59 (6H, *br.s*, H-12 and H-13) and 1.82 (3H, *s*, H-15). The proton signal at  $\delta$  5.91 was assigned to the methine proton H-5 with a downfield shifted carbon signal at  $\delta$  147.93 (*d*). The 2D <sup>1</sup>H-<sup>1</sup>H COSY, HMQC and HMBC (Fig. 98) in addition to the <sup>13</sup>C-NMR spectra confirmed the structure of **205**. Its relative configuration was determined from the NOESY spectrum, which was similar to that of compound **202** (Fig. 96).

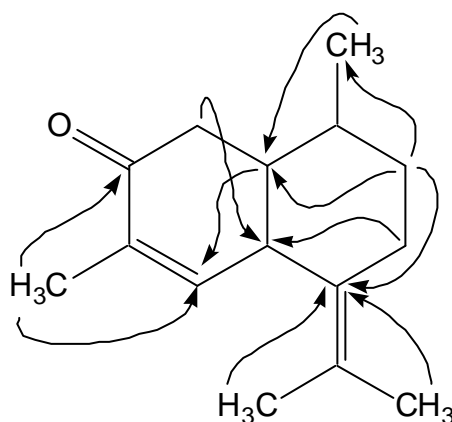


Fig. 98. Long-range <sup>1</sup>H-<sup>13</sup>C correlations of **205**.

Treatment of (–)-**202** with pyridinium dichromate (PDC) in dry dichloromethane gave a product identical (same GC-MS characteristics, same retention times on achiral polysiloxane and chiral cyclodextrin derived GC phases) with (–)-**205**. Hence (–)-**205** has the same absolute configurations at the stereogenic centres C-1, C-6 and C-10 as in **202** (Fig. 97).

#### 4.7.8. Structure of (+)-2,8-Epoxyamorpha-4,7(11)-diene (**206**)

(+)-2,8-Epoxyamorpha-4,7(11)-diene (**206**), showed a molecular ion signal at  $m/z$  at 218 and the molecular formula of  $C_{15}H_{22}O$  ( $[M^+]$   $m/z$  218.1680) was determined by HR-EIMS. The  $^1H$ -NMR spectrum ( $C_6D_6$ ) indicated signals of four methyl groups at  $\delta$  0.87 (3H, *d*, H-14,  $J = 7.25$  Hz), 1.39 (3H, *s*, H-13), 1.58 (3H, *s*, H-12) and 1.61 (3H, *br.s*, H-15). The signals at  $\delta$  4.04 (1H, *s*) and 4.49 (1H, *s*) are assigned to the two methine protons at the oxygenated C-2 and C-8 respectively. The 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC spectra (Fig. 99), in addition to the  $^{13}C$ -NMR extracted from the HMBC and HSQC data, confirmed structure **206**. Its relative configuration resulted from the NOESY spectrum, which indicated interactions of proton H-1 with H-6 and H-14 (Fig. 100). Direct rigorous hydrogenation of **206** gave similar products to that of the hydrogenation of (–)-amorpha-4,7(11)-diene (**70**), hence, the absolute configuration of **206** at C-1, C-6, and C-10 were confirmed. The  $\alpha$ -orientation of the oxane ring was deduced by the spatial interactions of protons H2 and H1. Therefore, **206** showed (1*R*,2*S*,6*R*,8*S*,10*S*)-configuration.

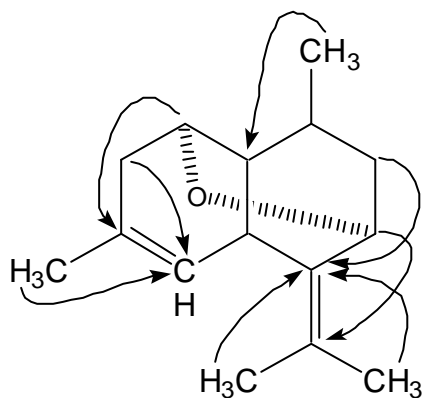


Fig. 99. Long-range  $^1H$ - $^{13}C$  correlations of **206**.

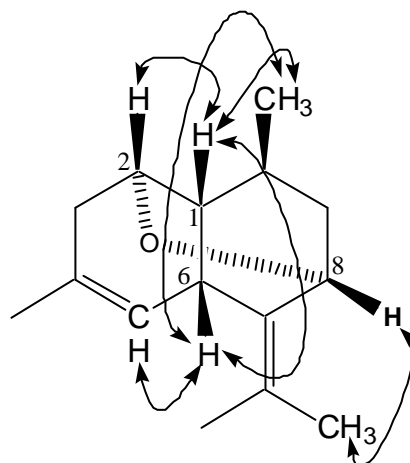


Fig.100. NOE correlations of **206**.

Treatment of **206** with acidic ion exchange resin (Amberlyst) for 2 hours at room temperature gave cadalene (**207**) and two additional compounds, the major one being identical to an authentic sample of  $\alpha$ -calacorene (**208**) (Fig. 101).

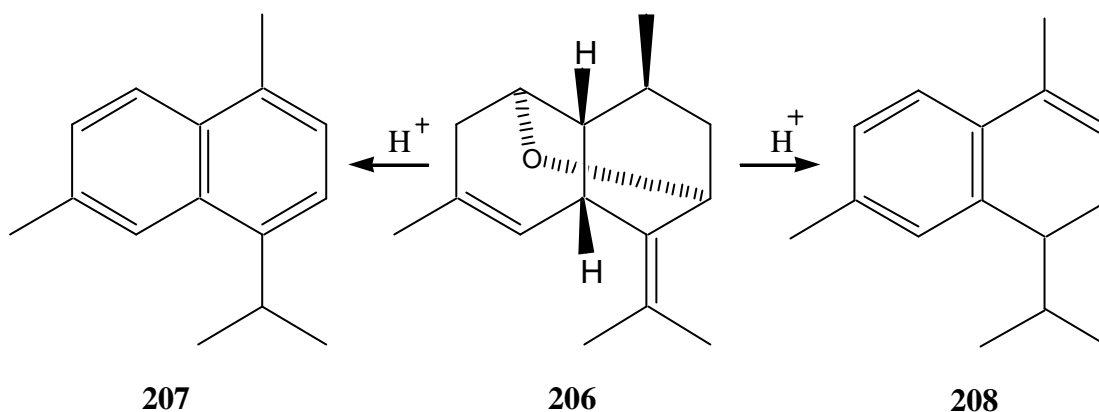


Fig. 101. Acid transformation of **206**.

#### 4.7.9. Structure of (+)-5,9-Epoxyamorpha-3,7(11)-diene (**209**)

(+)-5,9-Epoxyamorpha-3,7(11)-diene (**209**), a trace compound (0.05%),  $C_{15}H_{22}O$  ( $[M^+]$  218.1679) exhibited a similar  $^1H$ -NMR spectrum ( $C_6D_6$ ) as **206**, except that the position of the two methine protons at the oxygenated carbon have shifted to  $\delta$  3.70 (1H, *s*, H-9) and 3.93 (1H, *s*, H-5). In addition, the endocyclic double bond was situated at  $C^3$  compared to  $C^4$  in **206**. Additional structural information was obtained from HMBC and HMQC which confirmed the presence of two carbon-oxygen-linkages at  $\delta$  72.9 (*d*, C-9) and 73.9 (*d*, C-5). In addition, the methine carbon at  $\delta$  120.0 (*d*, C-3) and three quaternary carbons at  $\delta$  121.9 (*s*, C-11), 129.8 (*s*, C-7) and 137.8 (*s*, C-4) were confirmed from the HMBC spectrum. Its relative configuration was deduced from the NOESY spectrum, which indicated interactions of protons H-1 with H-14 and H-6. Proton H-5 also interacts with H-6 (Fig. 102).

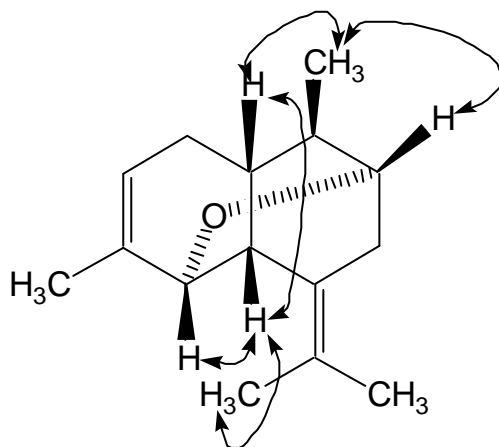


Fig.102. NOE correlations of **209**

Determination of the absolute configuration of **209** was achieved by hydrogenation as with **206**, and it was shown to be (1*S*,5*S*,6*R*,9*R*,10*R*). Subjected to acidic ion exchange resin (Amberlyst) treatment (Fig. 101), 10% of **209** formed similar compounds as **206**. Regarding, the yield of degradation products **209** was relatively stable compared to **206**.

#### 4.7.10. Structure of (–)-**2a**-Acetoxyamorpha-4,7(11)-diene (**210**)

The mass spectrum of this new sesquiterpene acetate exhibited a molecular ion signal at  $m/z$  262 corresponding to the molecular formula of  $C_{17}H_{26}O_2$ . The  $^1H$ -NMR and  $^{13}C$ -NMR spectral patterns of **210** were similar to those of amorpha-4,7(11)-diene (**70**) with additional signals due to the presence of an acetate group. The proton signal at  $\delta$  5.28 (1 H, *dt*,  $J_1=2.8$ ,  $J_2=8.5$  Hz) was assigned to an oxygenated methine carbon and  $\delta$  1.72 (3 H, *s*) to the methyl group of the acetyl moiety. By analysis of the  $^1H$ - $^1H$ -COSY, HMQC and HMBC spectra (Fig. 103) the structure was identified as **210**. The relative configuration of **210** was derived from the NOESY spectrum, which indicated NOEs between protons H1 and H6, H1 and H14, H-2 and H6 (Fig. 104). In addition, the orientation of the  $\alpha$ -acetoxy group was deduced from the spatial interaction of H-2 with H-1 and H-6 in the NOE spectrum.

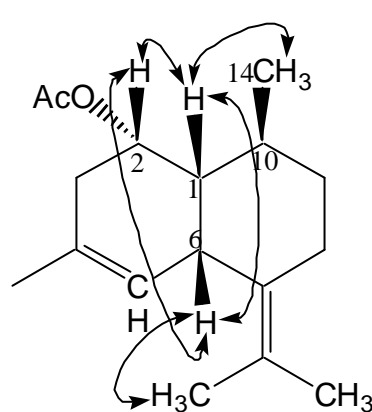
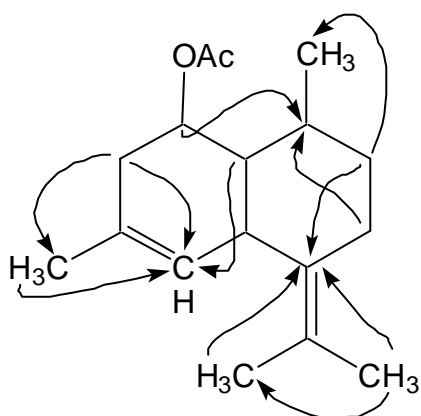


Fig. 103. Long-range  $^1H$ - $^{13}C$  correlations of **210**. Fig.104. NOE correlations of **210**.

To determine the absolute configuration of **210**, it was converted to the corresponding alcohol (**211**) by basic hydrolysis. Rigorous hydrogenation of the alcohol (**211**) resulted in eight fully saturated diastereoisomeric amorpha / cadinane / muurolanes (mass 208) which were compared to the corresponding fully hydrogenated products of (–)-amorpha-4,7(11)-diene (**70**) (see Fig. 92b).

GC investigations on a column with a cyclodextrin derived chiral stationary phase showed that the compounds **70** and **211** gave the same retention times for all the fully hydrogenated

products. Thus, the absolute configuration at the stereogenic centers C-1, C-6 and C-10 of **70** and **210** are identical.

(-)-2 $\alpha$ -Hydroxyamorpha-4,7(11)-diene (**211**), C<sub>15</sub>H<sub>24</sub>O ([M<sup>+</sup>] 220.1809), the de-acetylated form of (-)-2 $\alpha$ -acetoxyamorpha-4,7(11)-diene (**210**) was also isolated. The 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC spectra in addition to the <sup>13</sup>C-NMR (Table 5) confirmed the structure **210**. Its relative configuration was determined from the NOESY spectrum (Fig. 105). Its absolute configuration was deduced from direct hydrogenation of **211**.

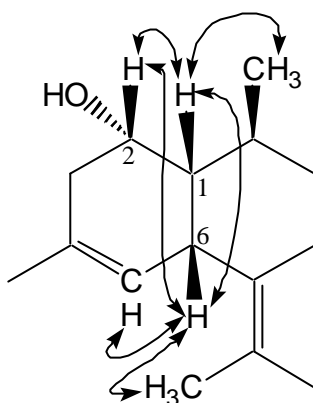


Fig.105. NOE correlations of **211**.

#### 4.7.11. Structure of (-)-**2b**-Acetoxyamorpha-4,7(11)-diene (**212**)

(-)-2 $\beta$ -Acetoxyamorpha-4,7(11)-diene (**212**) has the molecular formula C<sub>17</sub>H<sub>26</sub>O<sub>2</sub>. This could not be confirmed by mass spectrometry (the heaviest ion detected under EI conditions corresponds to C<sub>15</sub>H<sub>22</sub> due to immediate loss of acetic acid). The <sup>13</sup>C-NMR spectrum revealed the presence of 17 carbon resonances. <sup>1</sup>H-NMR and HMBC demonstrated that a total of 26 protons were directly attached to the carbon skeleton. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR were identical to those of (-)-2 $\alpha$ -acetoxyamorpha-4,7(11)-diene (**210**) except for slight chemical shift changes. The 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC spectra confirmed the structure of **212**. The spatial interactions of compound **212** protons were derived from the NOESY spectrum, which is similar to that of **210** (Fig. 104) except for the lack of spatial interactions between protons H-2 and H-6. The absolute configuration of **212** was confirmed by de-acetylation followed by pyridiniumdichromate (PDC) oxidation. The oxidation product (+)-amorpha-4,7(11)-dien-2-one (**213**) of **212**, gave identical analytical data (same <sup>1</sup>H-NMR, same GC-MS characteristics and same retention times on achiral polysiloxane and chiral cyclodextrin derived GC phases) with the PDC oxidation product of **210** (Fig. 106). Thus, the stereochemistry of **212** at the stereogenic centers C-1, C-6 and C-10 was confirmed.



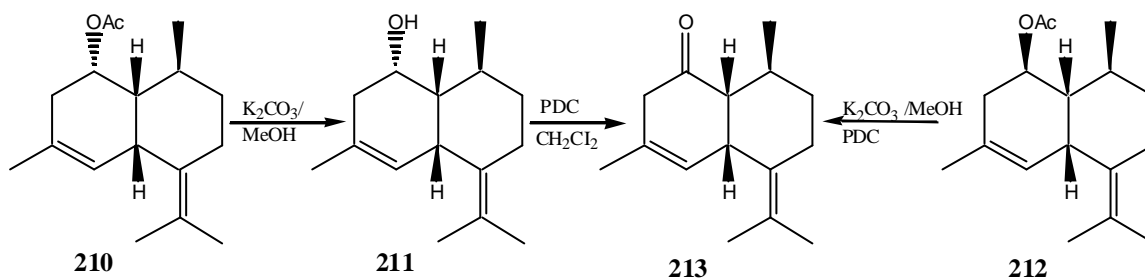


Fig. 106. Saponification and oxidation of **210** and **212**.

#### 4.7.12. Structure of (+)-Selina-4,11-dien-9**b**-ol (**214**)

The mass spectrum of this new sesquiterpene alcohol exhibits a molecular ion signal at  $m/z$  220 corresponding to the molecular formula of  $C_{15}H_{24}O$ . The  $^1H$ -NMR spectrum in  $C_6D_6$  was not well resolved in the range  $\delta$  1.65-1.90 but the overlapping signals integrated to 6 H. The  $^1H$ -NMR in  $CDCl_3$  showed moderately overlapping signals in this range. The  $^1H$ -NMR spectrum recorded in  $CDCl_3$  showed three methyl singlets at  $\delta$  1.10, 1.63 and 1.76. The deshielded signal at  $\delta$  3.37 (1 H, *dd*,  $J_1= 3.8$ ,  $J_2= 11.7$  Hz) was assigned to the proton adjacent to a hydroxy group. The exomethylene proton signals showed a multiplet at  $\delta$  4.72-4.75 in  $CDCl_3$ , and well resolved signals in  $C_6D_6$  at  $\delta$  4.80 (1 H, *s*) and 4.83 (1 H, *s*). The  $^{13}C$ -NMR and DEPT spectra showed singlets for three primary carbons ( $\delta$  17.7, 19.8, 20.8), six secondary carbons ( $\delta$  18.7, 30.0, 33.0, 35.6, 36.2, 108.8), two tertiary carbons ( $\delta$  43.2, 79.1) and four quaternary carbons ( $\delta$  40.1, 127.5, 133.1, 149.2). The 2D  $^1H$ - $^1H$ -COSY, HMBC (Fig. 107) and HMQC spectra confirmed the structure of **214**. The spatial interactions of protons H-7 and H-9 from the NOESY spectrum furnished the relative configuration (Fig. 108a).

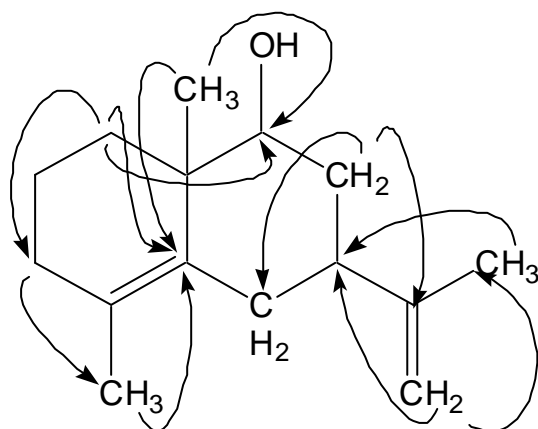


Fig. 107. Long-range  $^1H$ - $^{13}C$  correlations of **214**.

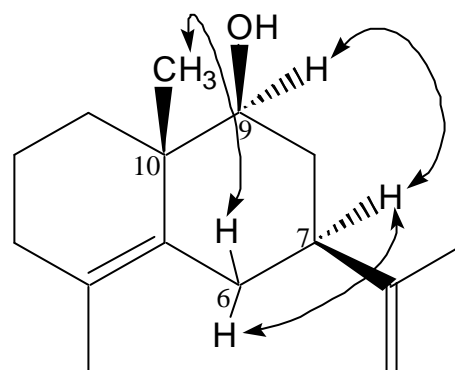


Fig. 108a. NOE correlations of **214**.

To determine the absolute configuration, a chemical correlation was carried out by comparing the fully hydrogenated products of **214** with the hydrogenation products of (+)- $\beta$ -selinene (**127**). The resulting GC with an achiral polysiloxane column showed that two out of the four hydrogenation products of **214** gave identical retention times with two hydrogenation products of (+)- $\beta$ -selinene (**127**). The same retention times were also observed when enantioselective GC was applied. This confirmed that the actual stereochemistry of **214** at the stereogenic centers C-7 and C-10 are the same with the authentic (+)- $\beta$ -selinene (**127**) (Fig. 108b).

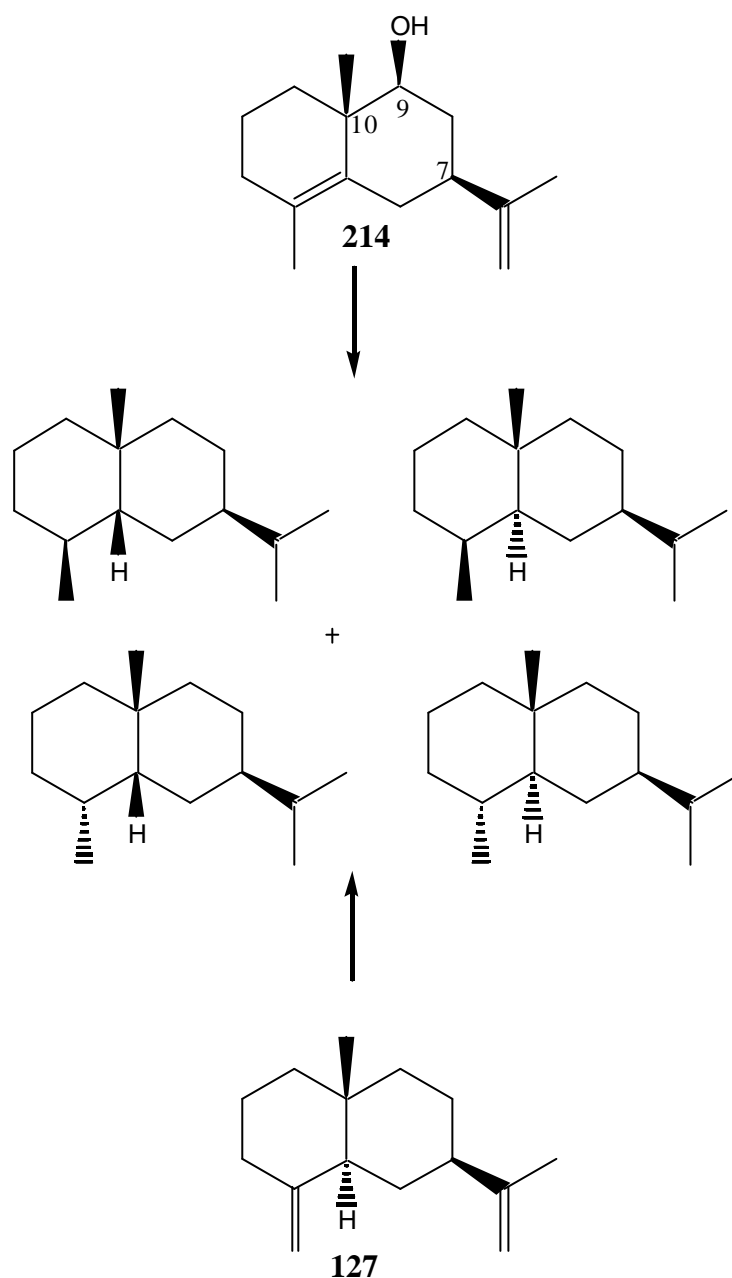


Fig. 108b. Hydrogenation of selina-4,11-dien-9 $\beta$ -ol (**214**)

The spatial interaction of H-7 and H9 confirmed that the hydroxy group on C-9 must be  $\beta$ -oriented. This was supported by the difficulty of dehydration, since the dehydration of **214** always resulted in the formation of the chlorinated derivative of **214**. Hence, the hydroxy group must be  $\beta$ -oriented to be sterically hindered.

Compound **214** was oxidized with PDC to (+)-eudesma-4,11-diene-9-one (**215**), (Fig. 108c).

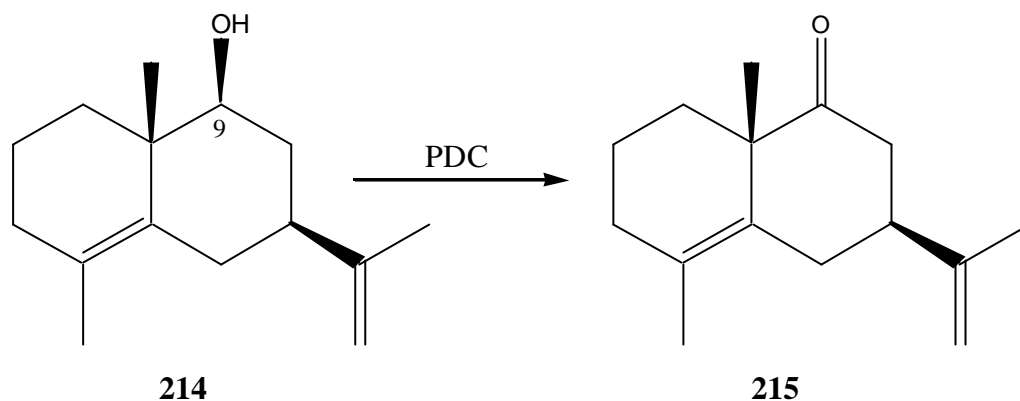


Fig. 108c. Oxidation of selina-4,11-dien-9 $\beta$ -ol (**214**).

In addition, (+)-1-*epi*-cubenol (**113**) was isolated and characterised. The spectral data of **113** were consistent with those reported in the literature.<sup>[114]</sup> Its absolute configuration was determined by treatment of **113** with acidic ion exchange resin (Amberlyst) for 2 hours at room temperature to afford (+)-cadina-1,4-diene (**119**, 85%) and (+)-*trans*-calamenene (**72**, 5%) (Fig. 109).

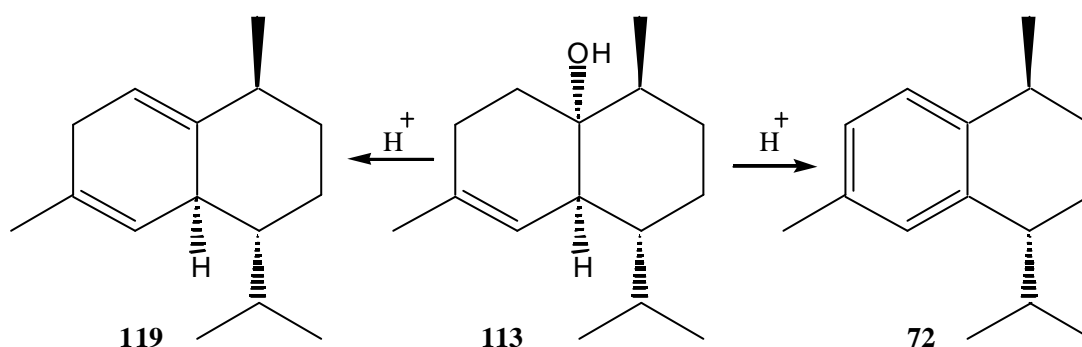


Fig. 109. Acid rearrangement of 1-*epi*-cubenol (**113**).

The co-existence of compounds (–)-**70**, (+)-**200**, (–)-**201**, (–)-**202**, (–)-**204**, (–)-**205**, (+)-**206**, (+)-**209**, (–)-**210**, (–)-**211**, (–)-**212** with (–)-(1*R*,2*S*,6*R*,10*S*)-2 $\alpha$ -acetoxy-11-methoxy-amorpha-4,7-diene (**216**) and (–)-(1*R*,2*S*,6*R*,10*S*)-2 $\alpha$ -acetoxyamorpha-4,7(11)-dien-8-one

(217)<sup>[152]</sup> identified on the basis of their mass spectral fragmentation and retention indices, suggested a common structural, stereochemical and biogenetical relationship.

Thus, the essential oil of the *Marsupella aquatica* sample collected in Austria consists of mainly amorphanes, traces of cadinane / muurolane (e.g. (+)-1-*epi*-cubenol (113), few gymnomitane based compounds e.g.  $\alpha$ -barbatene (53) and  $\beta$ -barbatene (57) with traces of longipinanes e.g.  $\alpha$ -longipinene (88). No trace of lemnalol (29) was detected. Hence, *Marsupella aquatica* is different from the two *Marsupella emarginata* samples in terms of their chemical composition.

#### 4.8. Composition of the Essential Oil of *Marsupella alpina*

*M. alpina* was collected in July 2001 near Gaschurn (Austria) at an elevation of 2000 m.  $\alpha$ -Pinene (**154**, 4.2%) was the only major monoterpene in the hydrodistillation product. In addition, limonene (**157**), 1-octen-3-ol (**159**, 1.1%) and its acetate (**160**, 1.2%) were detected. In the sesquiterpene region anastreptene (**51**, 0.8%), *cis*- $\alpha$ -bergamotene (**218**, 0.8%),  $\beta$ -santalene (**219**, 1.2%), (-)-selina-4,11-diene (**132**, 8.4%), eudesma-3,5,11-triene (**220**, 3%),<sup>[170,171]</sup> 2-pentylfuran (**221**), (+)- $\alpha$ -selinene (**126**, 3.7%) and the sesquiterpene lactones (-)-dihydrodiplophyllin (**146**, 2.5%),<sup>[140]</sup> and diplophyllolide, **145**, 24.3%),<sup>[138]</sup> were identified by comparison with a spectra library<sup>[107-108]</sup> (Fig. 110a-b). In this work the isolation of unknown compounds **222** (10.7%), **143** (7.8%) and **223** (3.1%) are discussed.

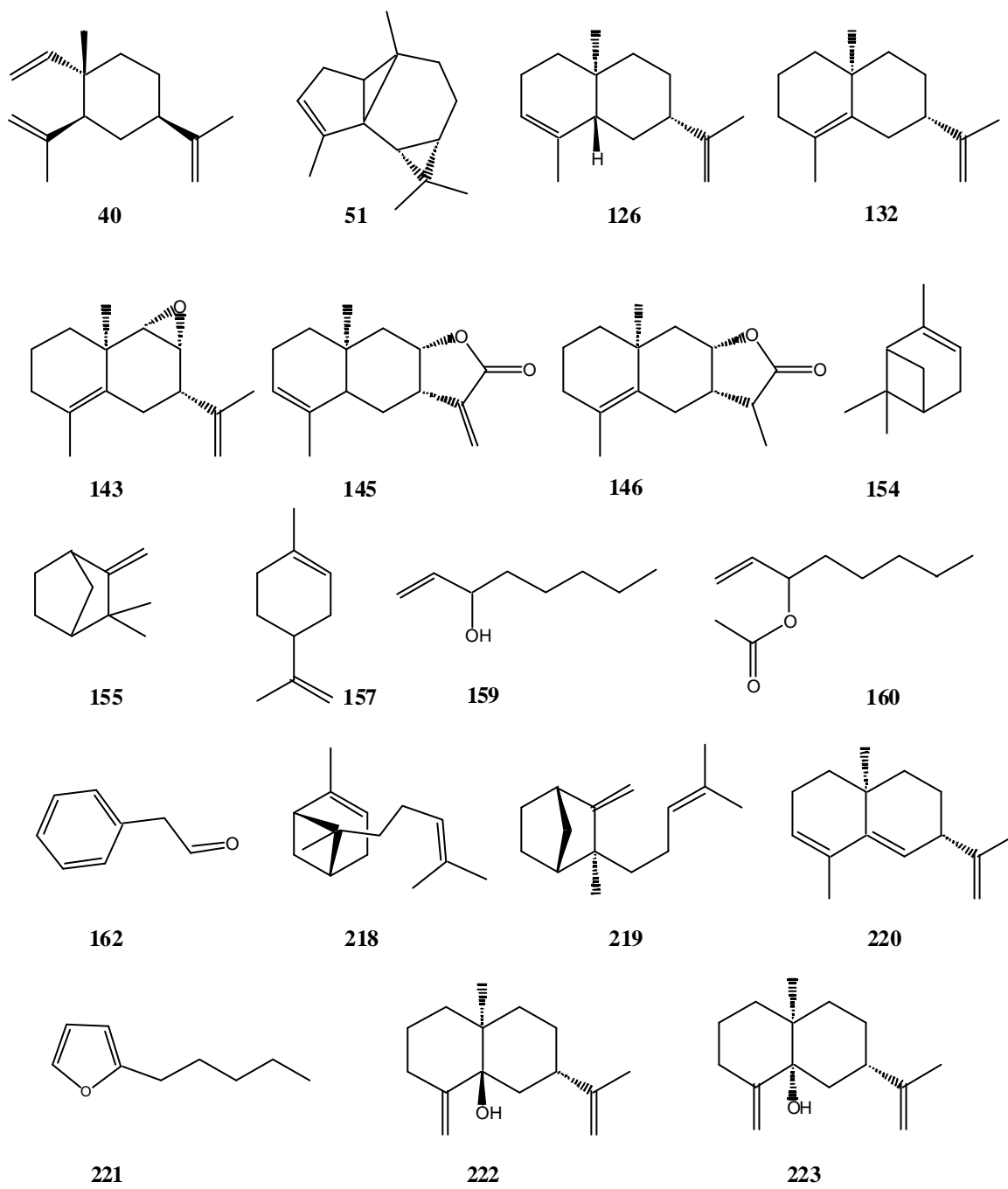


Fig. 110a. Constituents of *Marsupella alpina* (Austria).

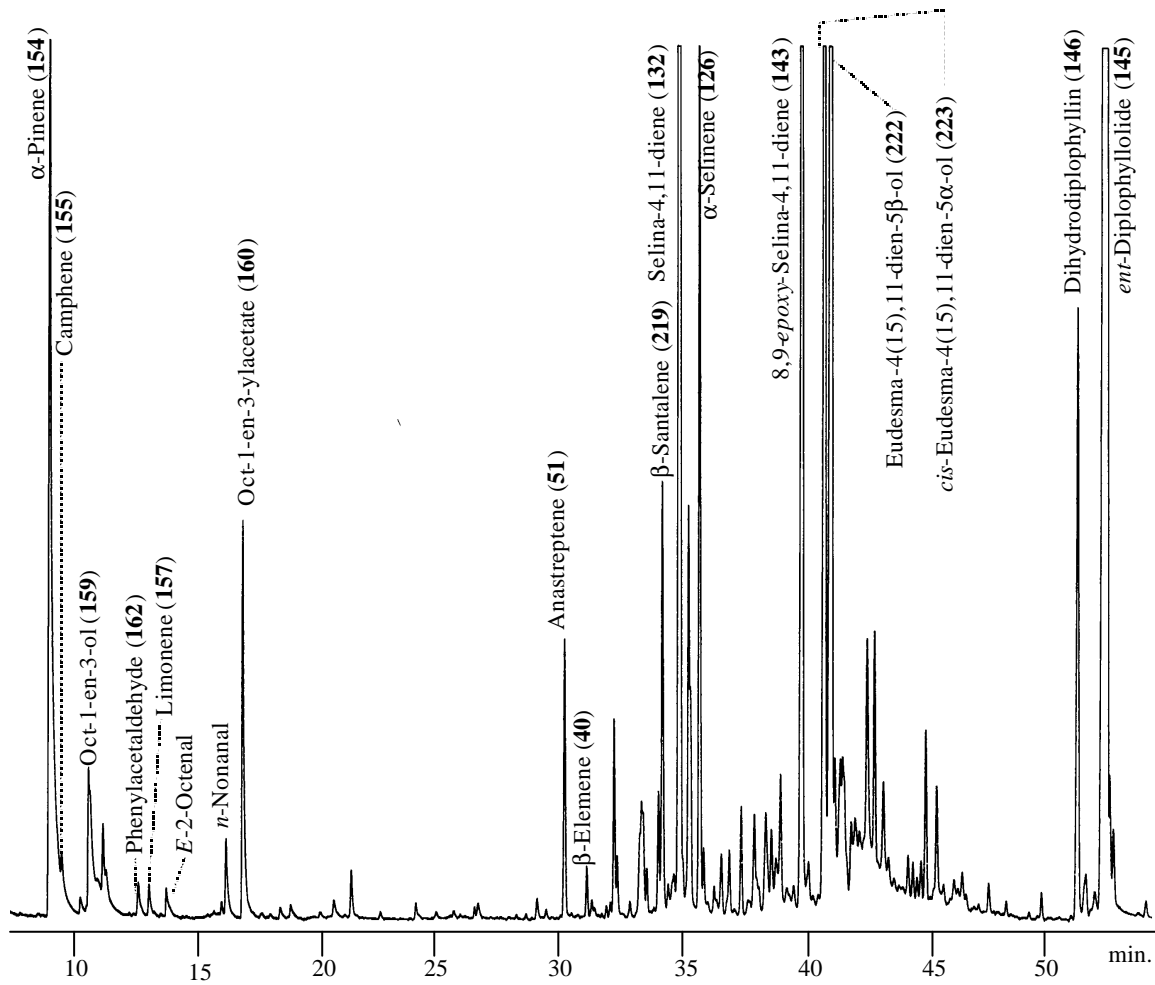


Fig. 110b. Gas chromatogram of the essential oil of *Marsupella alpina* (Austria). (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.8.1. Structure of (+)-8,9-Epoxyseolina-4-11-diene (**143**)

Eudesmane sesquiterpenoid **143** was isolated from *Marsupella alpina*. The mass spectrum exhibited a molecular ion signal at  $m/z$  218 corresponding to the molecular formula of  $C_{15}H_{22}O$ . The  $^{13}C$ -NMR and DEPT spectra of **143** showed signals of three primary carbons ( $\delta$  18.4, 21.2, 22.5), five secondary carbons ( $\delta$  19.0, 25.4, 32.1, 35.8, 111.4), three tertiary carbons ( $\delta$  46.4, 56.8, 60.6) and four quaternary carbons ( $\delta$  33.5, 124.5, 133.4, 148.0). The  $^1H$ -NMR spectrum showed signals of three singlets for the methyl groups at  $\delta = 1.27$  (3 H, *s*, H-14), 1.46 (3 H, *s*, H-15), 1.82 (3 H, *s*, H-13). The downfield methyl signals at  $\delta$  1.46 and 1.82 suggested that both are separately attached to double bonds. The olefinic carbon signals at  $\delta$  148.0 (*s*), and 111.4 (*t*), suggested a methylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  4.89 (1 H, *t*,  $J=1.6$  Hz) and 5.07 (1 H, *s*). The methine proton at  $\delta$  2.59 (1 H, *d*,  $J=3.8$  Hz) couples with the adjacent methine proton at another

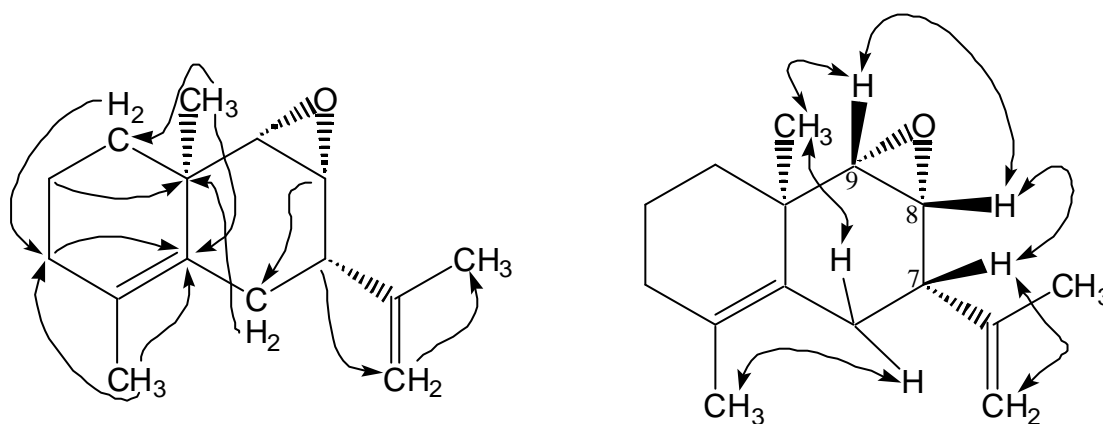


Fig. 111. Long-range  $^1H$ - $^{13}C$  correlations of **143**. Fig. 112. NOE correlations of **143**.

oxygenated carbon at  $\delta$  3.06 (1 H, *d*,  $J=3.8$  Hz). Information from 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC spectra (Fig. 111) in addition to the  $^{13}C$ -NMR and DEPT suggested structure **143**. Its relative configuration resulted from the NOESY spectrum, which indicated NOEs between protons H-7 and H-8, H-8 and H-9 (Fig. 112). The configuration of **143** at C-7 and C-10 was determined by chemical correlation with (+)- $\alpha$ -selinene (**126**), which was one of the major sesquiterpene hydrocarbons isolated from the same essential oil in conjunction with enantioselective gas chromatography. Rigorous catalytic hydrogenation of **143** resulted in two fully saturated diastereoisomeric eudesmanes (molecular mass 208), which were compared with the fully hydrogenated products of authentic (+)- $\alpha$ -selinene (**126**). The comparison by enantioselective GC showed that the two compounds gave the same retention times for all the fully hydrogenated products.



#### 4.8.2. Structure of (-)-Selina-4(15),11-dien-5b-ol (**222**)

The sesquiterpene alcohol **222** shows an eudesmane skeleton. The mass spectrum showed a molecular ion signal at  $m/z$  220 corresponding to the molecular formula of  $C_{15}H_{24}O$ . The  $^1H$ -NMR spectrum of **222** recorded in  $C_6D_6$  showed two singlet signals corresponding to methyl groups at  $\delta$  0.85 and 1.65. The presence of two exomethylene double bonds was confirmed by carbon signals at  $\delta$  107.3 ( $t$ ), 152.9 ( $s$ ) and 108.9 ( $t$ ), 150.8 ( $s$ ), respectively. This assignment was confirmed by three signals in the  $^1H$ -NMR spectrum at  $\delta$  4.59 (1 H,  $s$ ), 4.73 (1 H,  $s$ ) and 4.85 (2 H,  $d$ ,  $J=11.4$  Hz), respectively. The tertiary hydroxy group was assigned to C-5. The  $^{13}C$ -NMR and DEPT spectra of compound **222** indicated signals of two primary carbons ( $\delta$  20.0, 21.2), eight secondary carbons ( $\delta$  22.8, 26.5, 32.1, 34.6, 35.3, 35.8, 107.3, 108.9), one tertiary carbon ( $\delta$  40.3) and four quaternary carbons ( $\delta$  38.4, 75.5, 150.8, 152.9). The 2D  $^1H$ - $^1H$ -COSY, HMBC (Fig. 113) and HMQC spectra confirmed the structure of **222**. The spatial interactions of protons from the NOESY spectrum furnished the relative configuration

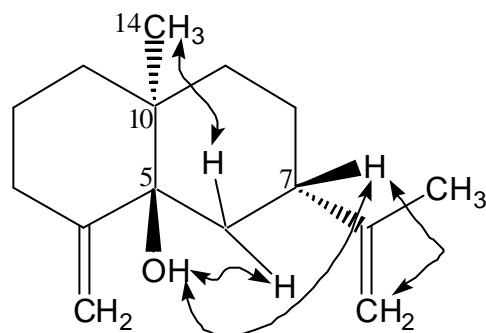
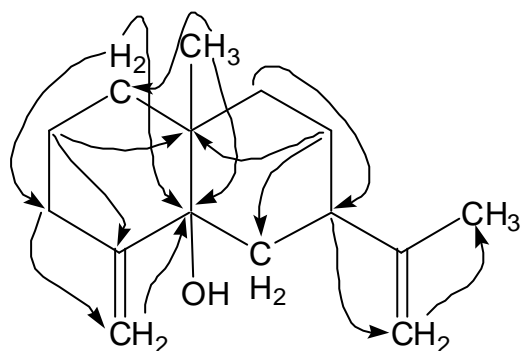


Fig. 113. Long-range  $^1H$ - $^{13}C$  correlations of **222**. Fig. 114. NOE correlations of **222**.

of **222** (Fig. 114). Rigorous catalytic hydrogenation of **222** resulted in simultaneous dehydration to two fully saturated diastereoisomeric eudesmanes (molecular mass 208), were compared with the fully hydrogenated products of authentic (+)- $\alpha$ -selinene (**126**). The comparison by enantioselective GC showed that the two compounds gave the same retention times for all the fully hydrogenated products. Thus, the absolute configuration of **222** at the stereogenic centers C-7 and C-10 were confirmed as (7*S*, 10*S*). The  $\beta$ -orientation of the hydroxy group was derived from the NOESY spectrum, which indicated the interaction of the hydroxy proton with H-7. Compound **222** exhibited opposite configuration to its (+)-enantiomer, (+)-5 $\alpha$ -hydroxy- $\beta$ -selinene which was isolated from the aerial parts of *Cassinia subtrapica* F. Mell.,<sup>[172]</sup> and later synthesized.<sup>[173-174]</sup> The  $^1H$ -NMR spectra recorded in  $CDCl_3$  were consistent with the three earlier reports.<sup>[172-174]</sup> The sign of optical rotation of **222** is negative. The assigned absolute configuration is consistent with the co-occurrence of *ent*-(-)-

selina-4,11-diene (**132**) and *ent*-(+)- $\alpha$ -selinene (**126**) in the hydrocarbon fraction of the liverwort sample.

#### 4.8.3. Structure of (+)-Selina-4(15),11-dien-5a-ol (**223**)

Only trace amounts of **223** could be isolated. The mass spectrum showed a molecular ion signal at  $m/z$  220 corresponding to the molecular formula of  $C_{15}H_{24}O$ . The  $^1H$ -NMR spectrum of **223** recorded in  $C_6D_6$  showed two singlets corresponding to methyl groups at  $\delta$  1.07 (3 H, *s*) and 1.66 (3 H, *s*). The presence of two methylene double bonds was confirmed by carbon signals at  $\delta$  109.0 (*t*), 110.2 (*t*) and 2x 149.6 (*s*). The carbon signal at  $\delta$  75.1 was assigned to a tertiary hydroxy group. The  $^1H$ -NMR,  $^{13}C$ -NMR, HMBC, HMQC and GC-MS data of **223** were almost identical to those of **222** except that the  $^1H$ -NMR spectrum of **223** was highly broadened at room temperature which is typical for *cis*-decalin derivatives.<sup>[198]</sup> Therefore, **223** should therefore be the C-5 epimer of **222**. The sense of optical rotation of **223** is positive. Compound **223** should therefore be the (+)-enantiomer of the reported (–)-5 $\beta$ -hydroxy- $\beta$ -selinene.<sup>[172-174]</sup> In conclusion, the chemical profile of this *Marsupella alpina* sample is different from that of *M. emarginata* and *M. aquatica* since it produces the eudesmane-type sesquiterpenoids chemically similar to *Diplophyllum albicans* which belongs to the Scapaniaceae.

#### 4.9. Chemical Analysis of the Essential Oil of *Tritomaria polita*

Class : Hepaticae (Liverworts)

Subclass : Jungermanniidae

Order : Jungermanniales

Family : Jungermanniaceae

Subfamily : Lophozioideae: *Tritomaria*

The chemical constituents of the Lophozioaceae are very complex, and substances structurally similar to those found in marine organisms have been found in a few species. Thus, based on terpenoid constituents, members of the Lophozioaceae are chemically divided into nine chemotypes.<sup>[94]</sup> *Tritomaria quinquedentata* and *T. polita* belong to chemotype-I which shows eudesmane-type sesquiterpenoids.

Previously, *T. polita* had been taxonomically separated and listed as *Saccobacis polita* (Nees) Buch.<sup>[153]</sup> *Tritomaria quinquedentata* has been investigated by several research groups,<sup>[175-176]</sup> while *Tritomaria polita* has not been chemically investigated. Recently, the eudesmanes 7-*epi*-isojunenol (**224**) and 7-*epi*-junenol (**225**) were isolated from *T. quinquedentata* (Fig. 115a).<sup>[176]</sup>

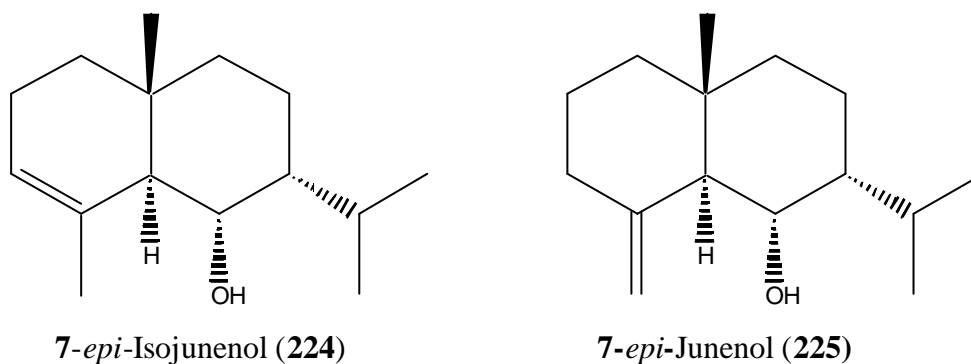


Fig. 115a. Compounds isolated from *Tritomaria quinquedentata*.

#### 4.9.1. Composition of the Essential Oil of *Tritomaria polita*

The essential oil of *T. polita* collected in Ötztal/Tyrol (Austria), was prepared by hydrodistillation and analysed by GC and GC-MS. As constituents of the essential oil of *Tritomaria polita*, the following known sesquiterpene hydrocarbons were identified in order of their elution from a capillary column with polydimethylsiloxane (CPSIL-5). Relative concentrations of major compounds are given in parentheses, those below 1% relative abundance are just listed:  $\beta$ -elemene (**40**), aromadendrene (**226**), *allo*-aromadendrene (**170**, 1.5%),  $\alpha$ -amorphene (**101**), 8 $\alpha$ -hydroxyeudesma-3,11-diene (**144**), eremophilene (**227**), (+)- $\alpha$ -selinene (**126**, 5%),<sup>[177]</sup>  $\delta$ -amorphene (**228**),<sup>[178]</sup> selina-3,7(11)-diene (**229**) (Fig. 115b-c). Thus, in the essential oil of *T. polita*, monoterpenes and sesquiterpene hydrocarbons are present only as minor constituents, but major peaks of oxygenated sesquiterpenes are unknown.

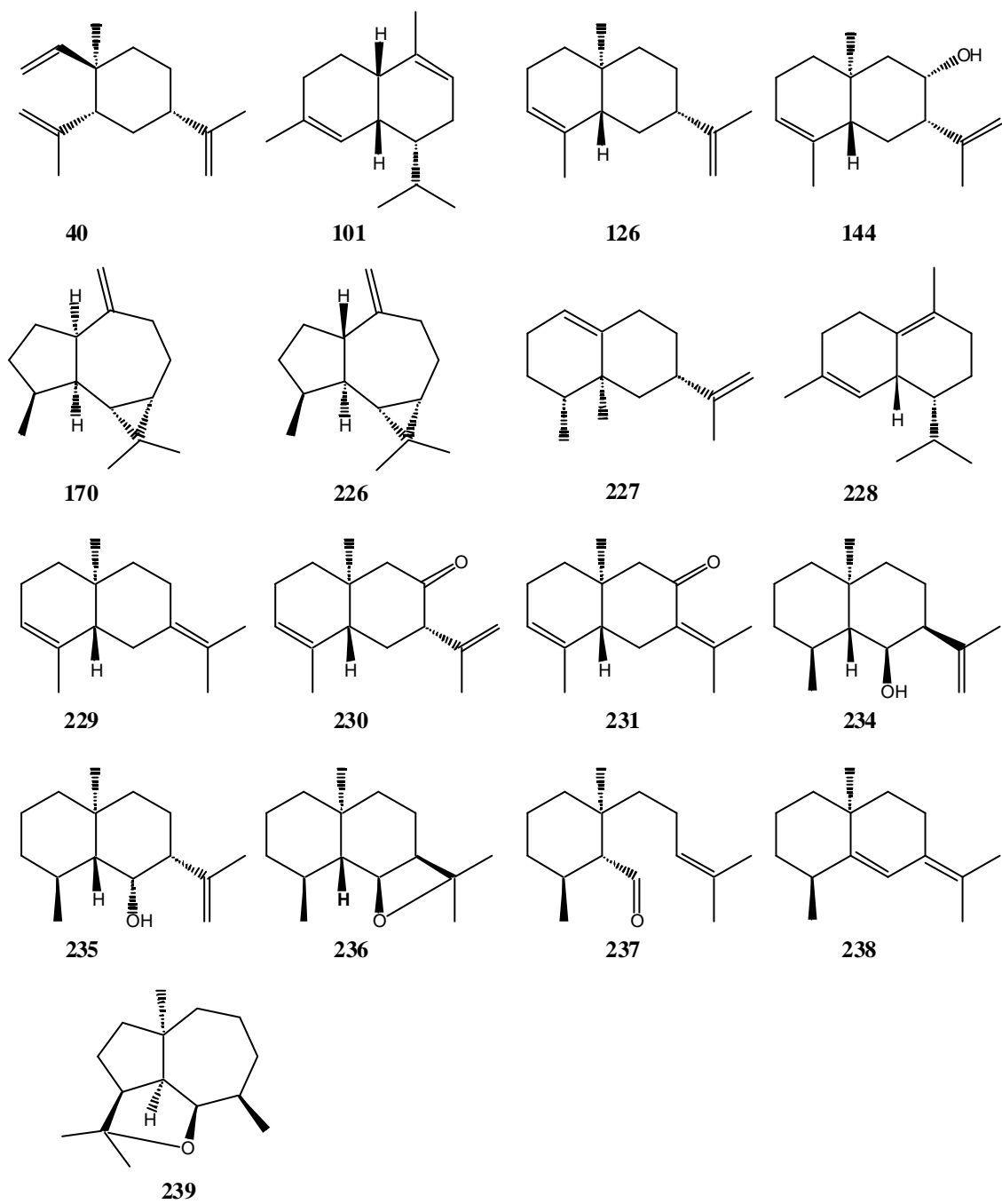


Fig.115b. Sesquiterpene constituents of the essential oil of *Tritomaria polita* from Austria.

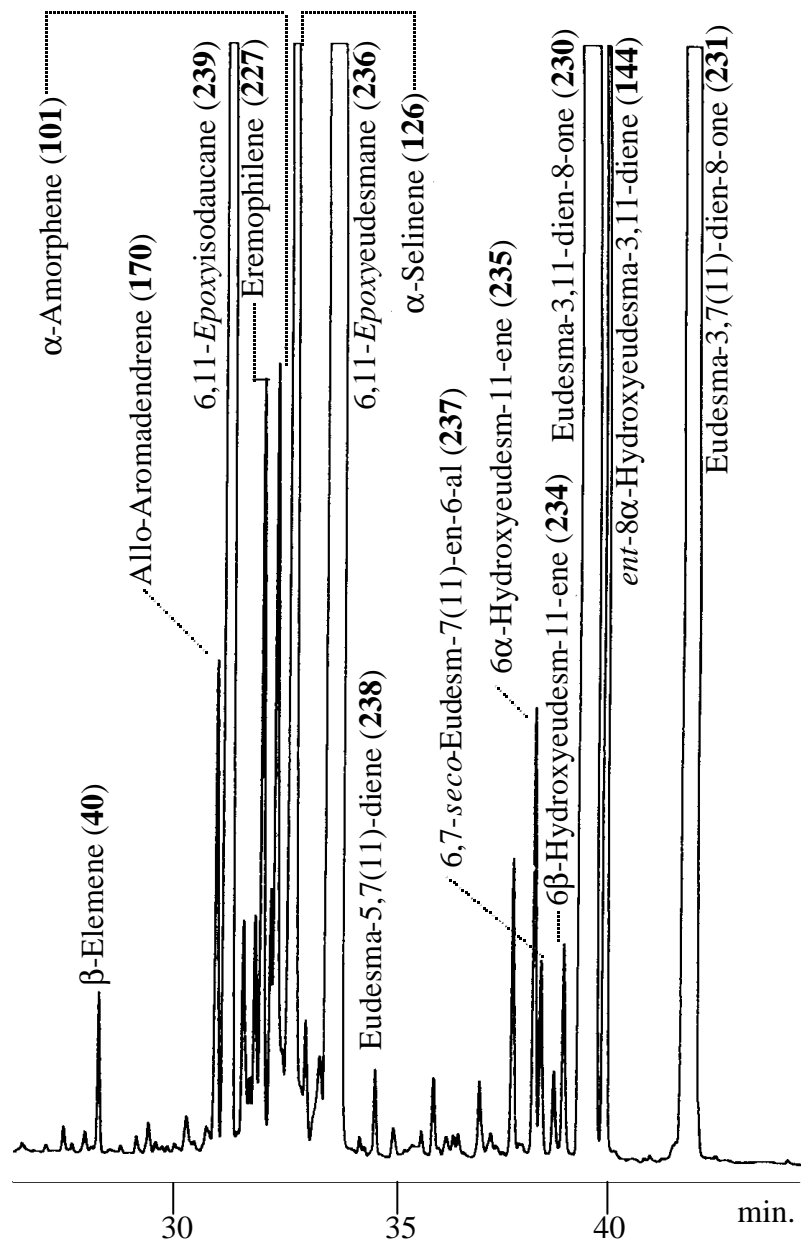


Fig. 115c. Gas chromatogram of the essential oil of *Tritomaria polita* from Austria. (CPSIL 5, 50°C, 3°C/min., 230°C).

#### 4.9.2. Structure of (+)-(5*S*,7*S*,10*S*)-Eudesma-3,11-dien-8-one (**230**)

(+)-Eudesma-3,11-dien-8-one (**230**), was isolated for the first time as a natural product. The  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) and the positive optical rotation of **230** were consistent with the reported synthesized oxidation product of *ent*-8 $\beta$ -hydroxy-eudesma-3,11-diene (**144**).<sup>[179]</sup> Its relative configuration was derived from the NOESY spectrum, indicated interaction of H-5 and H-7 (Fig. 116). Treatment of **230** with activated basic alumina for two days at room temperature afforded (+)-(5*S*,10*S*)-eudesma-3,7(11)-diene-8-one (**231**) and (+)-3,4,4*aR*,7,8,8*aR*-hexahydro-5,8*a*-dimethylnaphthalen-2(1*H*)-one (**233**) (Fig. 117).

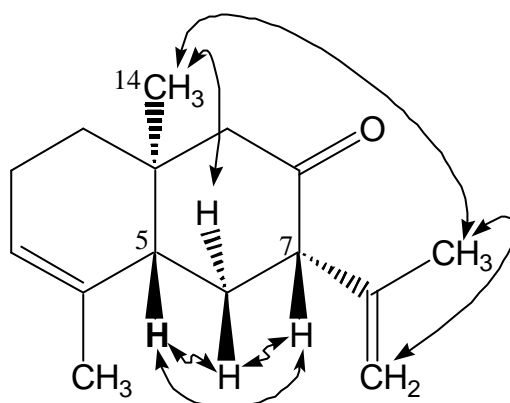


Fig. 116. NOE correlations of **230**.

#### 4.9.3. Treatment of (+)-Eudesma-3,11-dien-8-one (**230**) with alumina

(+)-3,4,4*aR*,7,8,8*aR*-hexahydro-5,8*a*-dimethylnaphthalen-2(1*H*)-one (**233**) is assumed to be a degradation product since it exhibited a molecular ion at  $m/z$  178 corresponding to  $\text{C}_{12}\text{H}_{18}\text{O}$ . A comparison of the  $^1\text{H-NMR}$  data ( $\text{C}_6\text{D}_6$ ) of **233** with **230** showed the disappearance of signals at  $\delta$  1.85 (s, 3H,  $\text{CH}_3$ -12), 4.77 (s, 1H, H-13*a*) and 4.97 (s, 1H, H-13*b*) and thus revealed the loss of an isopropenyl group. This was confirmed by the  $^1\text{H-NMR}$  spectrum in  $\text{CDCl}_3$ . It also showed the disappearance of the signals at  $\delta$  1.78 (s, 3H,  $\text{CH}_3$ -12), 4.77 (s, 1H) and 4.96 (s, 1H) corresponding to the isopropenyl residue. The GC-MS data and the  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) of **233** were consistent with the (–)-enantiomer obtained by degradation of podocarpic acid.<sup>[180]</sup> In addition, the mass spectrometric investigation of a sample of **233** kept in chloroform solution at room temperature for 24 hours showed a compound with a molecular ion at  $m/z$  288. This suggests further degradation of **233** in the presence of traces of HCl in the chloroform solution. **230** proved less stable in alumina and sensitive to heat. The attempt to isolate it by prep. GC always resulted in a mixture with **231** (8%). The thermal

instability of **230** could be due to formation of a resonance stabilized allylic carbanion ion by loss of hydrogen from the  $\alpha$ -position to the carbonyl group (Fig. 117a).

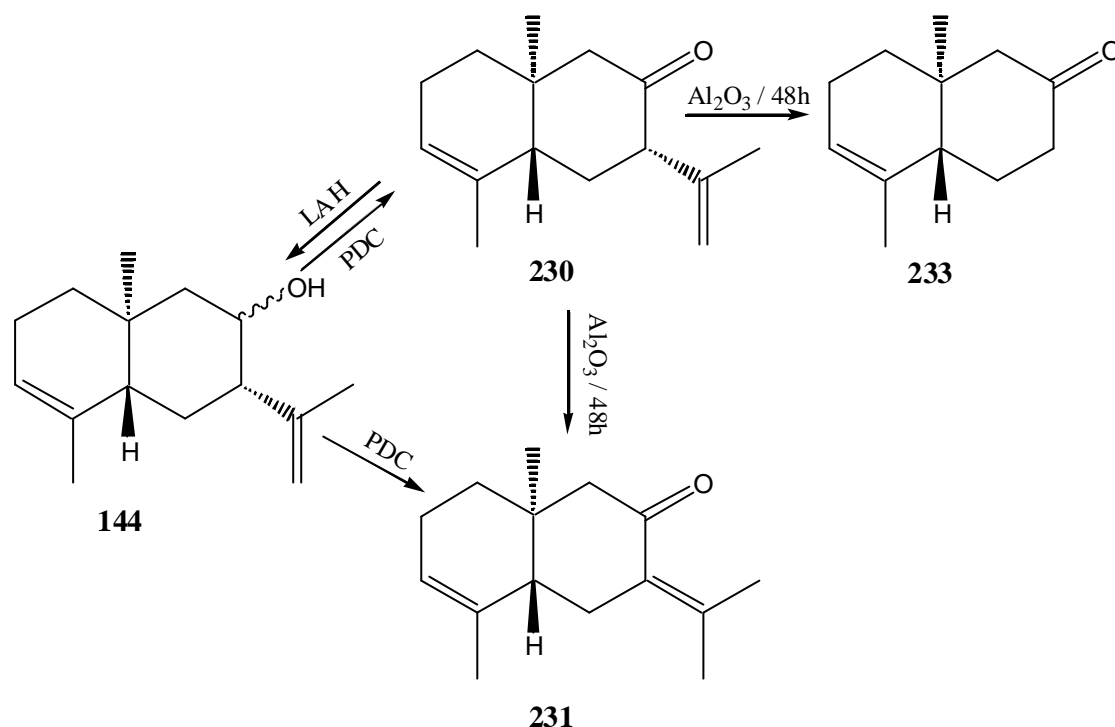


Fig. 117a. Rearrangement of Eudesma-3,11-dien-8-one (**230**) on treatment with basic alumina.

#### 4.9.4. Structure of (+)-(5*S*,10*S*)-Eudesma-3,7(11)-dien-8-one (**231**)

Compound **231**, an  $\alpha,\beta$ -unsaturated ketone with positive optical rotation, was isolated from the essential oil of *T. polita*. It showed identical mass spectrum and same retention times on both achiral and chiral capillary GC columns with the product formed from **230**. The (–)-enantiomer of **231** has been reported as a constituent of the liverwort *Bazzania fauriana*<sup>[181]</sup> and the racemic compound, resulting from the oxymercuration-demercuration reaction of germacrone (**232**) (Fig. 117b).<sup>[182]</sup> The <sup>1</sup>H-NMR data ( $\text{CDCl}_3$ ) of **231** are in agreement with the reported data. The <sup>13</sup>C-NMR data ( $\text{C}_6\text{D}_6$ ) of **230** and **231** are reported for the first time. The absolute configuration of **230** and **231** was deduced by chemical correlation with (+)- $\alpha$ -selinene (**126**). Reduction of **230** with lithium aluminium hydride gave a sesquiterpene alcohol whose GC-MS data were identical to the isolated *ent*-8 $\alpha$ -hydroxyeudesma-3,11-diene (**144**). Compound **231** (63%) was formed when **144** was treated with pyridinium dichromate (PDC) for 2-hours in addition to **230** (Fig. 117a). All spectral data and the sign of optical rotation were in agreement with a compound described as a constituent of the liverwort *Bazzania spiralis*.<sup>[179]</sup> The absolute configuration of **144** was determined by comparison of its



hydrogenated products with that of authentic (+)- $\alpha$ -selinene (**126**) by capillary GC with different cyclodextrin derived chiral stationary phases.

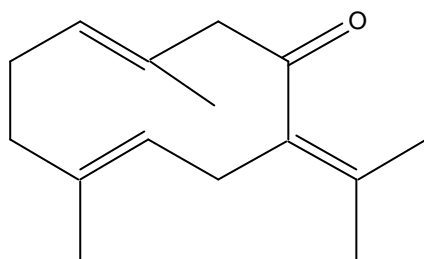


Fig. 117b. Germacrone (**232**)

#### 4.9.5. Structure of (+)-6b-Hydroxyeudesm-11-ene (**234**)

The sesquiterpene alcohol **234** showing an eudesmane skeleton exhibited a molecular ion at  $m/z$  222 corresponding to the molecular formula  $C_{15}H_{26}O$ . The  $^1H$ -NMR ( $C_6D_6$ ) spectrum showed signals of a doublet and two singlets for methyl groups at  $\delta$  1.31 ( $d$ , 3H,  $J = 6.3$  Hz), 0.82 ( $s$ , 3H) and 1.63 ( $s$ , 3H), respectively. The olefinic carbon signals at 113.2 ( $t$ ) and 146.5 ( $s$ ), suggested an *exo*-methylene double bond, which was confirmed by two signals in the  $^1H$ -NMR spectrum at  $\delta$  4.81 ( $s$ , 1H) and 4.88 ( $s$ , 1H). The signal at  $\delta$  70.5 was assigned to a secondary carbon with the hydroxyl group. All information from  $^{13}C$ -NMR and DEPT as well as from 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 118), were consistent with the assigned structure **234**. Its relative configuration was derived from the NOESY spectrum, which indicated spatial interactions of H6 and H7 with  $\alpha$ -H-14. Overlapped signals were observed for protons H-15, H-1b and protons H2a and H8b, hence, prevent a precise stereochemical assignment (Fig. 119).

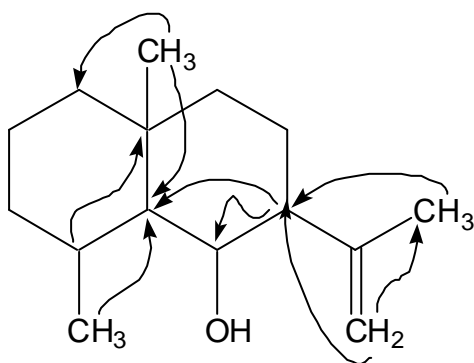


Fig. 118. Long-range  $^1H$ - $^{13}C$  correlations of **234**.

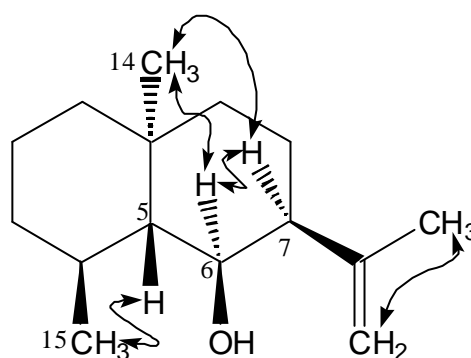


Fig. 119. NOE correlations of **234**.

The  $\beta$ -orientation of the hydroxyl group at C-6 and of the isopropenyl group at C-7 was derived from the spatial interactions of H-6 and H-7 with  $\alpha$ -H-14. Rigorous catalytic hydrogenation of **234** resulted in a simultaneous dehydration and hydrogenation to two fully saturated diastereoisomeric eudesmanes (molecular mass 208), which were compared with the fully hydrogenated products of authentic (+)- $\alpha$ -selinene (**126**). Enantioselective GC showed that one of the hydrogenation products of **234** gave identical retention times with a hydrogenation product of (+)-**126**. This is possible if an intermediate with a C-6–C-7 double bond is formed, which generates two diastereoisomeric products upon hydrogenation. Thus, the absolute configuration at the stereogenic centers C-5 and C-10 could be assigned. The  $\alpha$ -orientation of the C-10 methyl group (H-14) was further confirmed by the 95% conversion of **234** to (-)- $\delta$ -selinene (*ent*-**81**), when **234** was treated with acidic ion exchanger Amberlyst for 2-hours. As a result, **234** shows (4*S*,5*R*,6*R*,7*S*,10*S*)-configuration.

#### 4.9.6. Structure of (-)-6*a*-Hydroxyeudesm-11-ene (**235**)

compound **235**, a sesquiterpene alcohol with a molecular ion at  $m/z$  222 corresponding to the molecular formula of  $C_{15}H_{26}O$  showed similar mass spectrum as well as  $^1H$ -NMR,  $^{13}C$ -NMR, 2D  $^1H$ - $^1H$ -COSY, HMBC and HMQC data as **234**. Compound **235** gave similar results as **234** when subjected to catalytic hydrogenation, but the different relative configuration assigned to C-7 was shown by the NOESY spectrum, which indicated spatial interactions between protons H-5, H-6 and H-7. Therefore, H-7 is in  $\beta$ -orientation with respect to H-5 (Fig. 120).

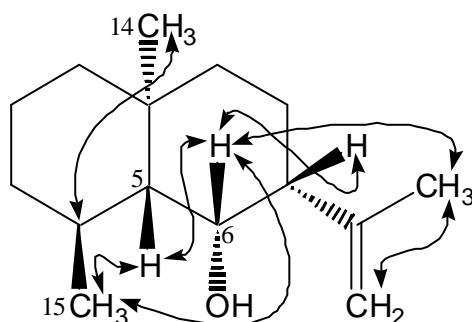


Fig. 120. NOE correlations of **235**.

To confirm the orientation of the methyl group at C-10, **235** was subjected to acid rearrangement reaction using Amberlyst for 2 hours at room temperature. From this reaction, only about 60% of compound **235** formed (-)- $\delta$ -selinene (**81**). Thus, the rate of loss water

molecule in both compounds **234** and **235** suggests that both have different hydroxy group orientation and consequently, **235** shows (4*S*,5*R*,6*S*,7*R*,10*S*)-configuration.

#### 4.9.7. Structure of (+)-6,11-Epoxyeudesmane (**236**)

The new tricyclic compound (+)-**236** exhibited a molecular ion at  $m/z$  222 corresponding to the molecular formula of  $C_{15}H_{26}O$ . The  $^{13}C$ -NMR spectrum revealed the presence of 15 carbon resonances.  $^1H$ -NMR and HMQC indicated a total of 26 protons directly attached to the carbon skeleton. The  $^1H$ -NMR spectrum showed signals of a doublet and three singlets for methyl groups at  $\delta$  1.09 (*d*, 3H,  $J = 5.9$  Hz), 0.58 (*s*, 3H), 1.30 (*s*, 3H) and 1.31 (*s*, 3H) respectively. The downfield shifted signal at  $\delta$  4.28 (*dd*, 1H,  $J = 7.4, 8.7$  Hz) was assigned to an oxygenated methine proton at H-6. Information from 2D  $^1H$ - $^1H$ -COSY, HMBC, HMQC spectra (Fig. 121) in addition to the  $^{13}C$ -NMR and DEPT confirmed structure **236**. Its relative configuration resulted from spatial interactions of H-14 with the protons of H-6, H-7 and H-15 as observed from the NOESY spectrum. The interactions of H-15 with H-6 may be due to constraints introduced by the orientation of the oxetane ring (Fig. 122).

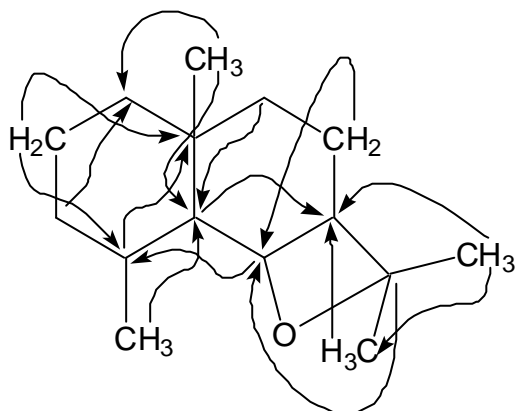


Fig. 121. Long-range  $^1H$ - $^{13}C$  correlations of **236**.

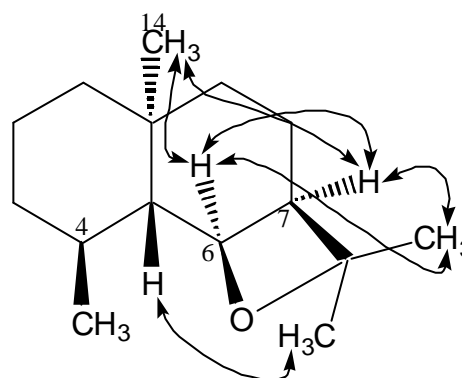


Fig. 122. NOE correlations of **236**.

#### 4.9.8. Structure of (-)-6,7-seco-Eudesma-7(11)-en-6-al (**237**)

Compound **237**, a colourless oil isolated as a trace component by preparative GC, shows a new *seco*-eudesmane skeleton. The mass spectrum exhibited a molecular ion at  $m/z$  222 and molecular formula  $C_{15}H_{26}O$ . The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of a doublet and three singlets for methyl groups at  $\delta$  0.68 (3H, *d*,  $J = 6.0$  Hz), 0.81 (3H, *s*), 1.60 (3H, *s*) and 1.68 (3H, *s*), respectively. The singlets of methyl groups at  $\delta$  1.60 and 1.68 indicate that they are attached to a double bond. The downfield shifted signal at  $\delta$  9.46 (1H, *d*, H-6,  $J = 4.4$  Hz) was assigned to the aldehyde proton. The  $^{13}C$ -NMR data are consistent with structure **237**.

The 2D  $^1\text{H}$ - $^1\text{H}$ -COSY, HMBC (Fig. 123) and HMQC spectra confirmed the structure of **237**. In the NOESY spectrum of **237**, spatial interactions between the aldehyde proton and the methyl protons H-14 and H-15 were observed (Fig. 124). This suggested that H-14 and H-15 might be on the same side of the molecule since the aldehyde group could be rotating. In addition, protons H-4 and H-5 protons overlapped and the exact stereostructure on C-4 and C-5 could not be deduced.

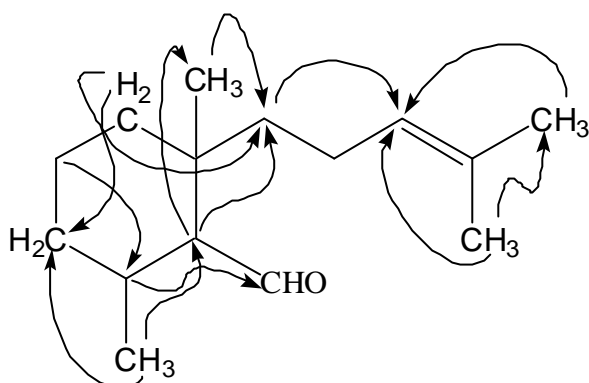


Fig. 123. Long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **237**.

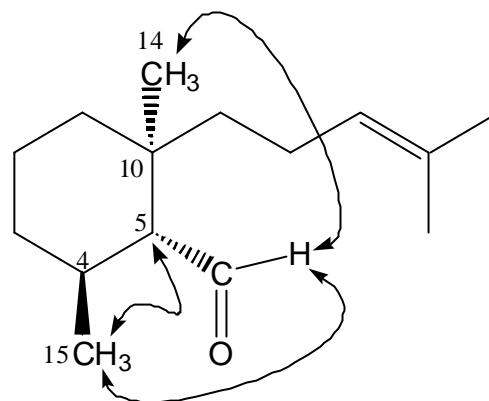


Fig. 124. NOE correlations of **237**.

Treatment of **237** with acidic ion exchange resin afforded (-)- $\delta$ -selinene (**81**, 13%) and compound **235** (75%). The correlation with (-)-**81** confirmed the  $\alpha$ -orientation assigned to the methyl group at C-10.

#### 4.9.9. 6,11-Epoxyudesmane (**236**) Transformations with Amberlyst

To determine the absolute configuration of **236**, it was treated with acidic ion exchange resin (Amberlyst) for 2 hours at room temp. This rearrangement gave an unknown hydrocarbon ( $m/z$  204, base peak 108) (4%) and **238** (7%), (-)- $\delta$ -selinene (**81**) (24%) (its stereochemical identity could be proved by comparison with an authentic reference compound by enantioselective GC with cyclodextrin columns) as well as compounds **234** (33%), **235** (19%), and **237** (2.1%), which were also originally present in the essential oil of *T. polita*. A possible biogenetic relationship between compounds **236**, **237**, **234** and **235** is shown (Fig. 125). Therefore, **237** could be a direct precursor of **235** as similar treatment of **237** with acidic ion exchange resin for 2 hours gave predominantly **235** (75%) and (-)- $\delta$ -selinene (**81**) (13%). **234** was not observed among the reaction products of **237**. This implied that **234** is formed directly by acid hydrolysis of **236** via a cationic intermediate. The formation of the isomeric alcohols **234** and **235** confirmed the C-O-linkage at C-6 in **236**. The proposed scheme also

suggests that **237** could be formed directly from **236** via a mechanism involving the opening of the oxetane ring in the absence of protons. From the co-occurrence of **236** and **237** it can not be excluded that the latter is an artefact formed during the isolation procedure. In analogy to reactions described,<sup>[183]</sup> it is also possible that one of the isomeric alcohols **234** or **235** is a precursor of **237**. The presence of (-)- $\delta$ -selinene (**81**) in the acid hydrolysis products confirmed the  $\alpha$ -orientation of the methyl group at C-10. As a result of these investigations, (4*S*,5*R*,6*S*,7*R*,10*S*)-configuration could be assigned to **236**.

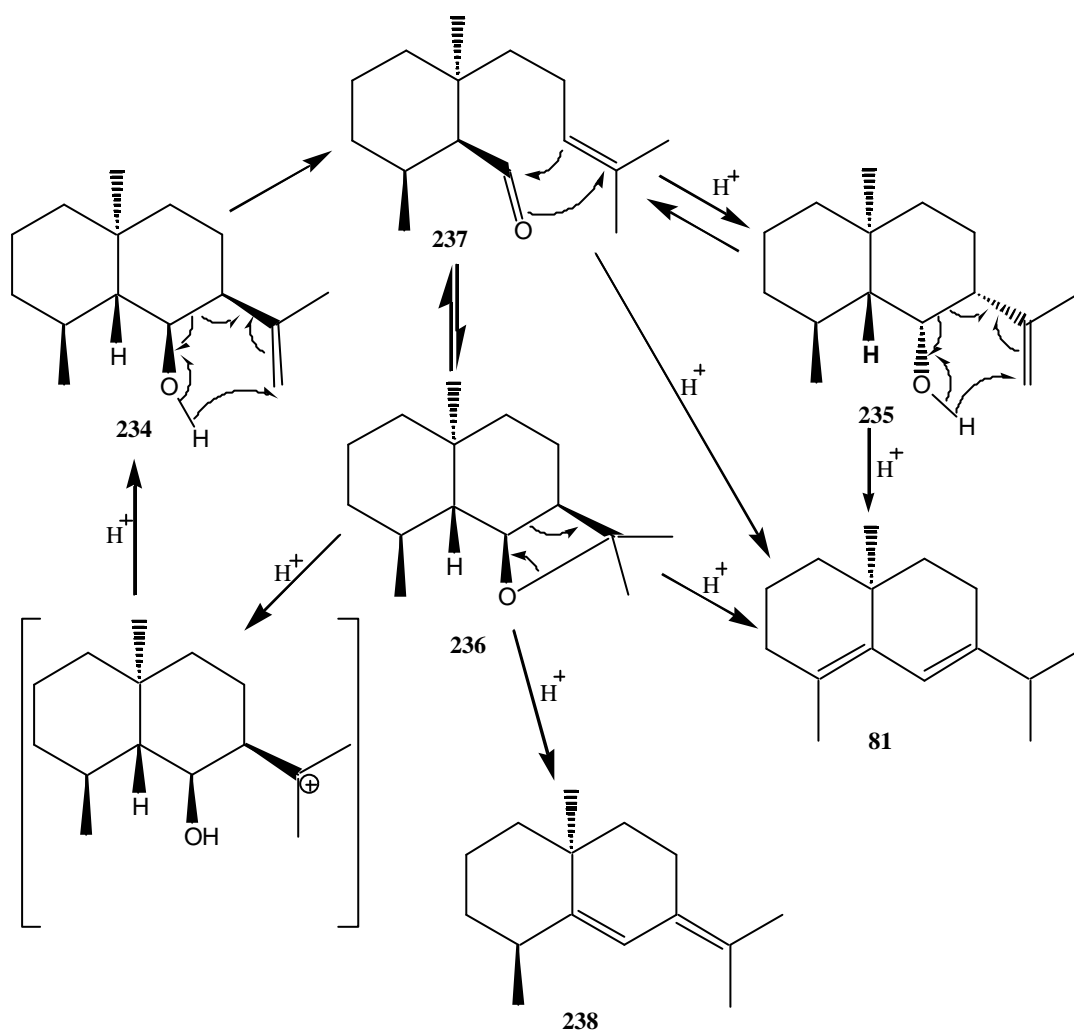


Fig. 125. Possible biogenetic scheme for the isolated compounds from *Tritomaria polita*.

#### 4.9.10. Structure of (+)-Eudesma-5,7(11)-diene (238)

The sesquiterpene hydrocarbon **238**, an acid hydrolysis product of **236**, exhibited a molecular ion at  $m/z$  204 corresponding to the molecular formula  $C_{15}H_{24}$  (Fig. 126a). The  $^1H$ -NMR spectrum ( $C_6D_6$ ) showed signals of a doublet and three singlets for methyl groups at  $\delta$  1.09 (*d*, 3H,  $J = 6.6$  Hz), 1.07 (*br.s*, 3H), 1.70 (*br.s*, 3H) and 1.80 (*br.s*, 3H), respectively. The signal at  $\delta$  6.32 (*br.s*, 1H) was assigned to the methine proton H-6. Compound **238** has a mass spectrum almost identical to (-)- $\delta$ -selinene (**81**). The structure of **238** was derived from the  $^1H$ -NMR data by comparison with the known data of **81**, the latter showing doublets for the geminal methyl groups. Its relative configuration was deduced from **236**. The absolute configuration of **238** was determined from its comparison with a closely related selina-5,7(11)-diene (**83**) of known absolute configuration. Compound **83** was generated from the thermal dehydration of (+)-maalian-5-ol (**47**) isolated from *Plagiochila asplenioides*, (Fig. 126b). The coinjection of **238** with **83** on CPSIL-5 and CPSIL-19 confirmed that the C-4-methyl group in compound **236** should be  $\beta$ -oriented. Hence, **238** should be the 10-epimer of (-)-selina-5,7(11)-diene (**83**) (Fig. 126) and, thus, should show (4*S*,10*S*)-configuration.

Compound **238** with unspecified configuration had previously been reported as a constituents of *Olibanum* oil.<sup>[184]</sup>

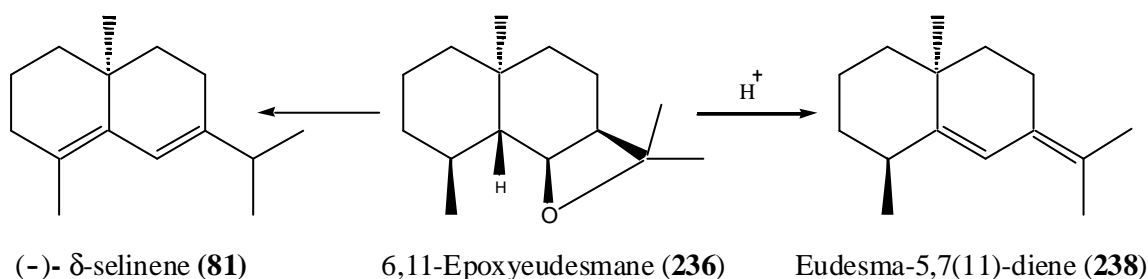


Fig. 126a. Acid transformation of 6,11-epoxyeudesmane (**236**).

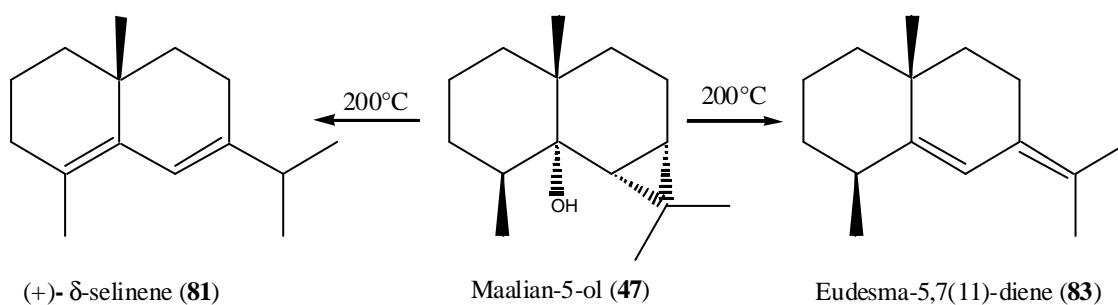


Fig. 126b. Thermal transformation of maalian-5-ol (**47**).

#### 4.9.11. Structure of (+)-6,11-Epoxyisodaucane (**239**)

From the hydrocarbon fraction of the liverwort **239**, a new tricyclic skeleton with isodaucane backbone was isolated. **239** exhibited a molecular ion at  $m/z$  222 corresponding to the molecular formula  $C_{15}H_{26}O$ . The  $^1H$ -NMR showed signals of a doublet and three singlets for methyl groups at  $\delta$  1.21 (*d*, 3H,  $J = 6.6$  Hz), 0.85 (*s*, 3H), 1.17 (*s*, 3H) and 1.30 (*s*, 3H), respectively. The downfield shifted signal at  $\delta$  3.22 (*t*, 1H,  $J = 9.8$  Hz) was assigned to the methine H6 which coupled with an adjacent proton H5 at  $\delta$  1.99 (*t*, 1H,  $J = 9.5$  Hz). The 2D  $^1H$ - $^1H$ -COSY, HMQC and HMBC (Fig. 127a) spectra in addition to the  $^{13}C$ -NMR confirmed the structure. Its relative configuration resulted from the NOESY spectrum, which showed an interaction of protons H-15 with H-4, and H-5 (Fig. 127b).

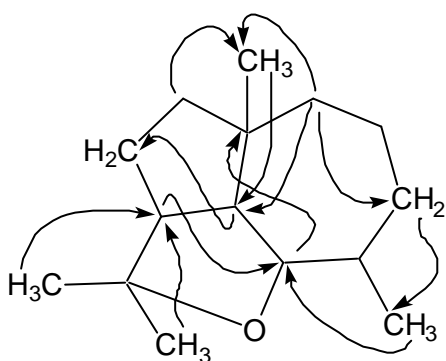
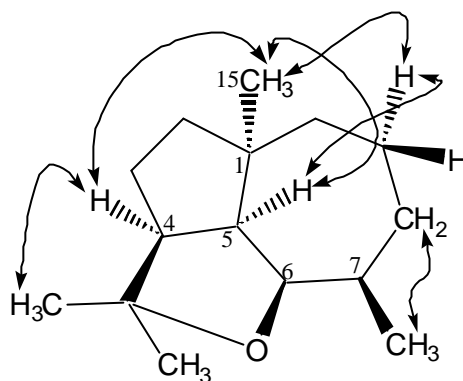


Fig. 127a. Long-range  $^1H$ - $^{13}C$  correlations of **239**.



#### 4.10. Chemical Analysis of the Liverwort *Barbilophozia floerkei*

*Barbilophozia* is another species of the Lophoziales family classified as chemotype-VI. This chemotype consists of daucane-sesquiterpenoid-dolabellane-type compounds.<sup>[94]</sup> *Barbilophozia* species are known to produce sesqui- and di-terpenoid substances with novel skeletons.<sup>[164,185-188]</sup> These species include *B. floerkeana*, *B. floerkei*, *B. hatcheri* and *B. lycopodioides*.

##### 4.10.1. Composition of the Essential Oil of the Liverwort *Barbilophozia floerkei*

GC and GC-MS of the essential oil obtained from the hydrodistillation of *Barbilophozia floerkei* collected in August 2002 from Altenau in Germany, revealed a complex mixture of mono-, sesqui-, and di-terpenes. The identified compounds include,  $\alpha$ -pinene (**154**), camphene (**155**), 3-octanone (**158**),  $\beta$ -pinene (**156**), limonene (**157**), trinoranastreptene<sup>[189,192]</sup> (**241**, 33.0%, also called inflatene and clavukerin B<sup>[190,190]</sup>), anastreptene (**51**), tritomarene (**138**), *trans*- $\alpha$ -bergamotene (**242**), alloaromadendra-4(15),10(14)diene (**243**), bicyclogermacrene (**60**),  $\beta$ -bisabolene (**240**), 1,4-dimethylazulene (**244**) and 3 $\alpha$ -acetoxybicyclogermacrene (**45**).<sup>[96]</sup> All these compounds were identified by comparison of their mass spectra and retention indices with a spectral library established under identical experimental conditions<sup>[107-108]</sup> (Fig. 128).



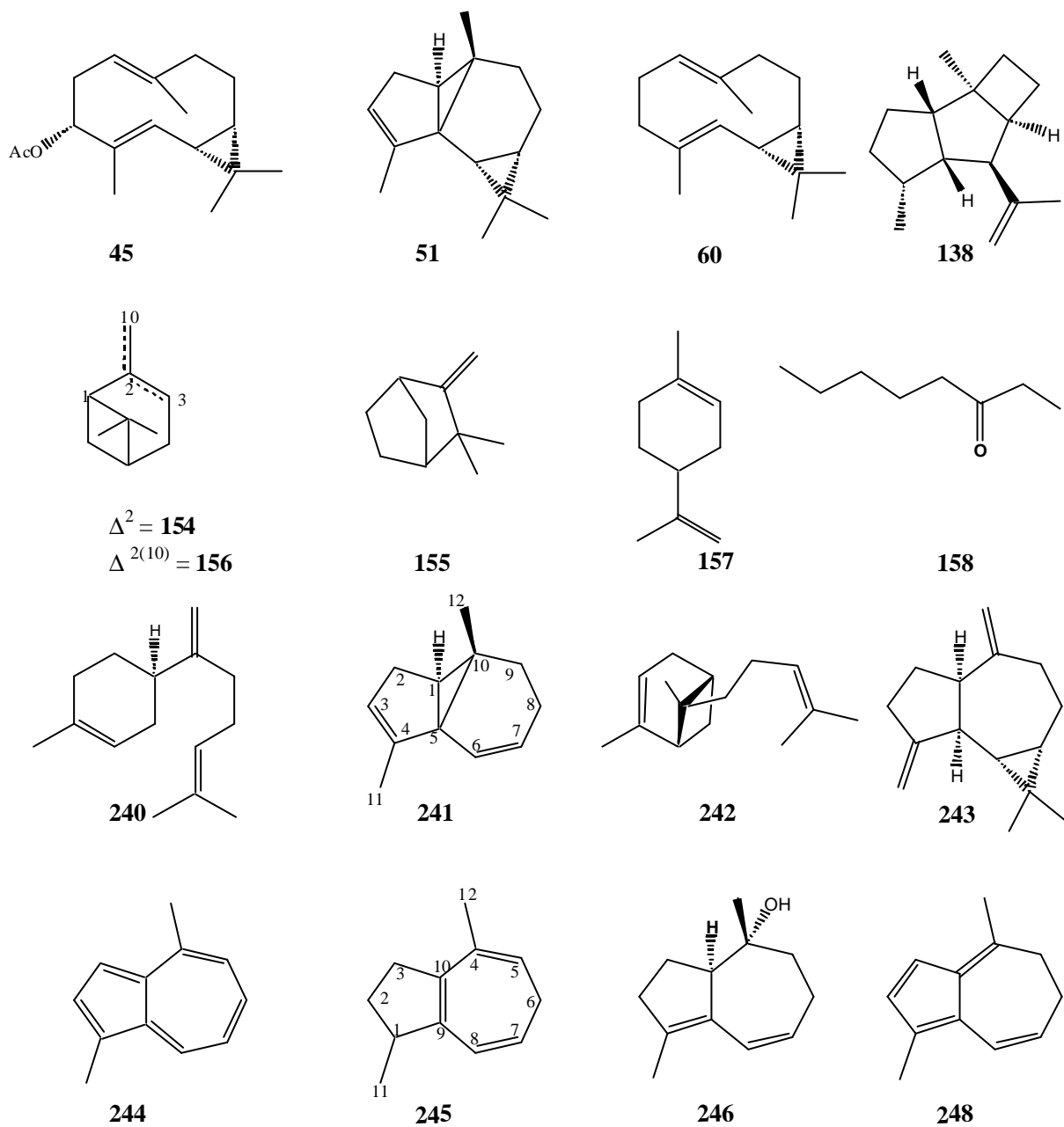


Fig. 128. Mono- and sesquiterpene constituents of the essential oil of *Barbilophozia floerkei*.

#### 4.10.2. Structure of (+)-1,2,3,6-Tetrahydro-1,4-dimethylazulene (**245**)

(+)-1,2,3,6-Tetrahydro-1,4-dimethylazulene (**245**), a bicyclic-C<sub>12</sub> compound was isolated by preparative GC. The mass spectrum exhibits a molecular ion at  $m/z$  160, consistent with the molecular formula of C<sub>12</sub>H<sub>16</sub> ( $[M^+]$   $m/z$  160.1240) was determined by HR-EIMS. The <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) showed signals of a doublet and a singlet for methyl groups at  $\delta$  1.12 (3H, *d*, H-11,  $J$  = 6.9 Hz) and 1.79 (3H, *s*, H-12), respectively. The methyl singlet signal at  $\delta$  1.79 indicated that this methyl group is attached to a double bond. The deshielded signals at  $\delta$  5.05 (1H, *t*,  $J$  = 6.6 Hz), 5.56 (1H, *dd*,  $J$  = 6.6, 16.1 Hz) and 6.10 (1H, *d*,  $J$  = 9.5 Hz) were assigned to the vinylic protons at H-5, H-7, and H-8, respectively. In addition, proton H-5 exhibits a long-range <sup>4</sup> $J$ -W-coupling with H-7. Additional structural information was obtained from the <sup>13</sup>C-NMR spectrum of **245** which confirmed the presence of two methyl groups (*q*, at  $\delta$  20.49 and 21.00), three methylene groups (*t*, at  $\delta$  28.18, 31.95 and 34.17), four tertiary carbons (*d*, at  $\delta$  42.55, 115.17, 119.19 and 125.28) and three quaternary carbons (*s*, at  $\delta$  133.82, 142.59 and 146.10). Since the <sup>13</sup>C-NMR, HMBC and HMQC spectra of **245** showed the presence of three double bonds and the molecular formula of **245** indicated the presence of five units of unsaturation, a bicyclic molecule was concluded. All the information from the 2D <sup>1</sup>H-<sup>1</sup>H-COSY, HMQC and HMBC (Fig. 129) analysis were consistent with structure **245**.

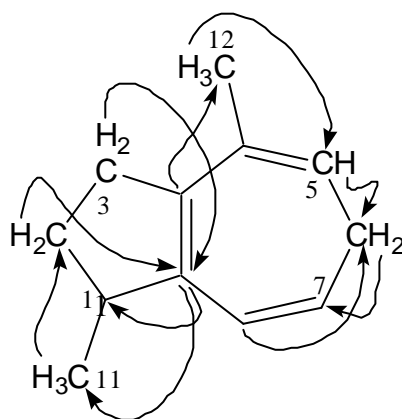


Fig. 129. Long-range <sup>1</sup>H-<sup>13</sup>C correlations of **245**.

#### 4.10.3. Structure of (-)-2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulen-4-ol (**246**)

(-)-2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulen-4-ol (**246**), a new trinor-guaiene alcohol, occurred as a trace component in the essential oil of *B. floerkei*. **246** was isolated from the oxygenated fraction by preparative GC. The bicyclic alcohol gave a molecular ion at  $m/z$  178, and molecular formula was determined as C<sub>12</sub>H<sub>18</sub>O ( $[M^+]$   $m/z$  178.1354 by HR-EIMS. <sup>1</sup>H-NMR spectrum (C<sub>6</sub>D<sub>6</sub>) of **246** showed signals of two singlets for methyl groups at  $\delta$  1.00 (3H,

*s*, H-12) and 1.57 (3H, *s*, H-11), respectively. The signals at  $\delta$  5.51 (1H, *m*) and 6.26 (1H, *d*,  $J = 11.7$  Hz) were assigned to the vinylic protons at H-7, and H-8, respectively. The Carbon signal at  $\delta$  74.7 was assigned to the tertiary hydroxy group. All this information from  $^{13}\text{C}$ -NMR as well as from  $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC and HMBC (Fig. 130a) led to structure **246**. Its relative configuration resulted from the NOESY spectrum (Fig. 130b) which showed a spatial interaction of H-10 with H-6a but no interaction with H-12. In contrast, H-12 interacted with H-6b, hence, H-10 and H-12 were not on the same side of the plane of the molecule. Moreover, the methyl protons H-11 interacted with the methine H-8. **246** could be the 4-hydroxy-derivative of the related structure, calvukerin A, (**247**),<sup>[193]</sup> (Fig.131).

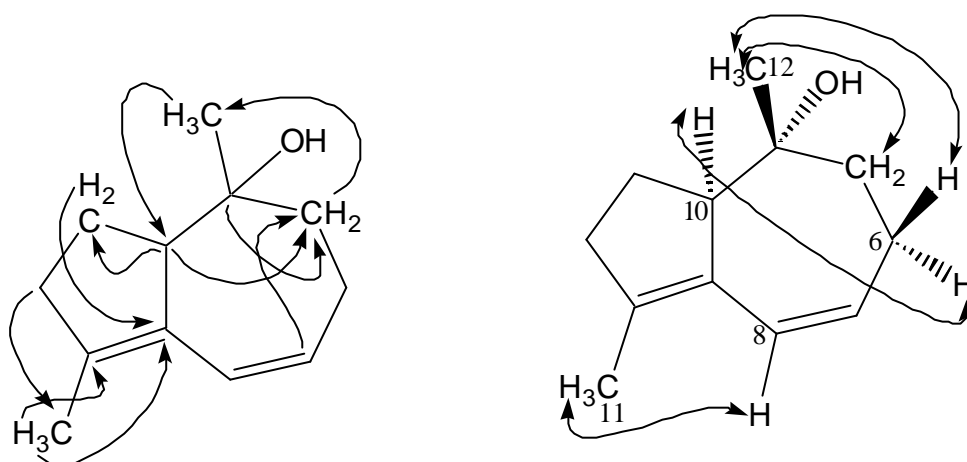


Fig. 130a. long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations of **246**. Fig. 130b. NOE correlations of **246**.

#### 4.10.4. Structure of 5,6-Dihydro-1,4-dimethylazulene (**248**)

5,6-Dihydro-1,4-dimethylazulene (**248**), a yellow oil, was isolated in trace amount by column chromatography. The mass spectrum exhibited a molecular ion signal at  $m/z$  158 and thus molecular formula of  $\text{C}_{12}\text{H}_{14}$ . The molecular formula of **248** indicated the presence of six units of unsaturation. The  $^1\text{H}$ -NMR spectrum ( $\text{C}_6\text{D}_6$ ) showed signals of two singlets for methyl groups at  $\delta$  1.81(3H, *s*) and 1.93 (3H, *s*) respectively. The strongly deshielded signals at  $\delta$  5.63-5.67 (1H, *m*), 6.29 (1H, *d*,  $J = 5.4$  Hz), 6.51 (1H, *d*,  $J = 5.4$  Hz) and 6.57 (1H, *d*,  $J = 11.0$  Hz) were assigned to the four vinylic protons. The presence of four protons multiplet signal in the allylic region of the  $^1\text{H}$ -NMR spectrum support the proposed structure **248**. Since the sample was not enough for the 2D-NMR experiments, additional structural information were obtained by spectral analysis and comparison of the  $^1\text{H}$ -NMR data with that of the 3,10-dihydro-1,4-dimethylazulene (**249**) and 1,6-dihydroazulen<sup>[194-196]</sup> (Fig. 131). Compound **248** decomposed during purification on preparative GC, hence, **248** was thermally unstable.

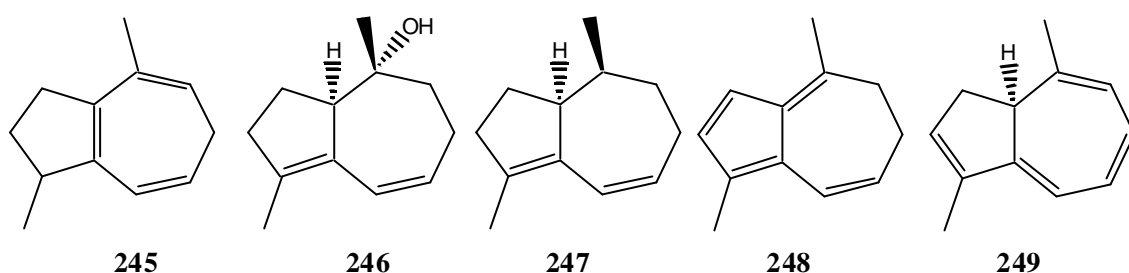


Fig.131. Structures of compounds **245**, **246**, **247**, **248** and **249**.

#### 4.10.5. Acid Rearrangement of (+)-Trisnoranastreptene (**241**)

Since traces of six unknown compounds with  $m/z$  160 were detected in the essential oil of *Barbilophozia floerkei*, **241** (33.0%, a major component) showing a mass spectra (MS) comparable to the trace components was subjected to acid transformation to determine if all the  $m/z$  160 compounds are related. Acid induced transformation of **241** afforded compounds **245**, **246** and three other labile compounds of  $m/z$  160 with two of them having a base peak of 131 and the third with a base peak of 117 (Fig. 132). These three compounds decomposed almost immediately after isolation by preparative GC, due to thermal instability. Attempts to isolate the three compounds with silica gel by preparative TLC also failed, and always resulted in numerous rearranged products. These three compounds are likely to be the other hydro-1,4-dimethylazulene-related compounds, since a member of this group of compounds, 3,10-dihydro-1,4-dimethylazulene (**249**) was labile.<sup>[194]</sup> From the co-occurrence of **245**, **246** and **241**, it cannot be excluded that the latter is the likely precursor of compounds **245** and **246**, and that they are artefacts formed during the isolation procedure. **241** has been reported to have ichthyotoxic properties.<sup>[191]</sup> The racemic mixture of **241** has been synthesized.<sup>[192]</sup>

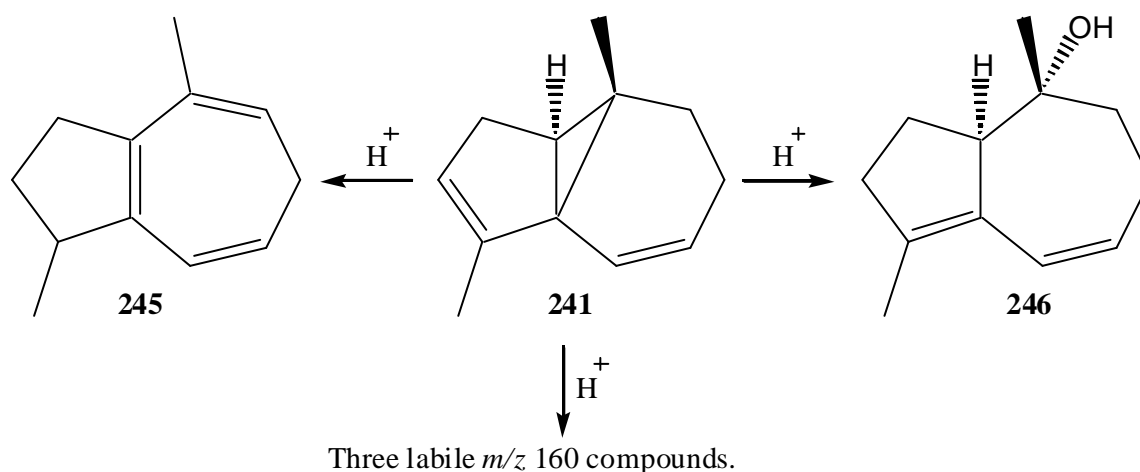


Fig. 132. Acid rearrangement of Trisnoranastreptene (**241**).

Compounds **241**, **245** and **246** are usually referred to as *nor*-compounds or considered as degraded sesquiterpenes formed by oxidative cleavage of carbon-carbon bonds. Although details of their biosynthesis are not known, it is possible that they are formed by enzymatic degradation of a parent sesquiterpene which is structurally related. Kobayashi et al, <sup>[190]</sup> proposed a scheme for the formation of the trisnoranastreptene (**241**) and other related compounds (Fig. 133). Known *nor*-sesquiterpenes include, cyprotene and cyperene from *Cyperus alopecuroides*<sup>[170]</sup> and albene and petasitene from *Petasites hybridus*.<sup>[197]</sup>

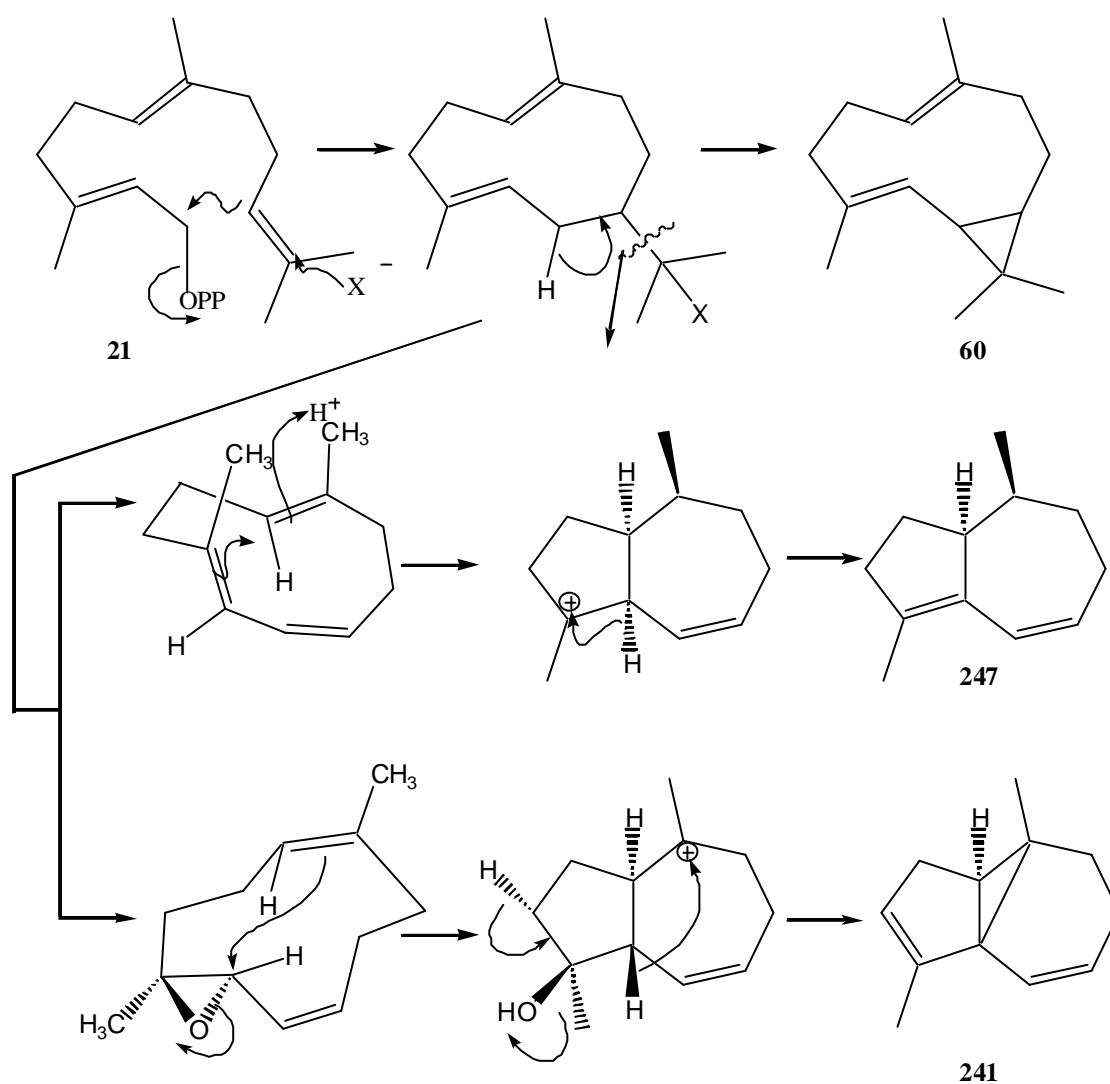


Fig. 133. Possible biogenetic pathways for trisnoranastreptene (**241**) and clavukerin (**247**) from farnesyl pyrophosphate (**21**).<sup>[190]</sup>

## 5. Summary

Sesquiterpene hydrocarbons and oxygenated sesquiterpenes from European liverworts of the families Gymnomitriaceae, Scapaniaceae, Plagiochilaceae, and Jungermanniaceae were investigated. The essential oils and extracts were analysed by GC and GC-MS. All known compounds were identified by comparison of their mass spectra and retention indices with a data bank established under identical experimental conditions.<sup>[107,108]</sup> Unknown compounds were isolated by combination of chromatographic techniques (prep. -GC, -TLC, CC, etc.), and their structures were elucidated by mass spectrometry (MS) and NMR-spectroscopic techniques (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, 2D-<sup>1</sup>H-<sup>1</sup>H-COSY, HMBC, HMQC, NOESY). Absolute configurations were determined by chemical correlation (hydrogenation, as well as acid or thermal transformation reactions, etc.) and monitored by enantioselective GC.

The essential oil of *Plagiochila asplenioides* collected from two different locations (Altenau and Kummerfeld) in Germany were analysed by GC and GC-MS. The analysis showed that the *P. asplenioides* species contained a broad range of compounds and hence could be classified into both chemotypes-(I) and (V). Four new oxygenated compounds, (+)-muurolan-4,7-peroxide (**69**), (+)-plagio-4,7-peroxide (**74**), (+)-plagiochiline-W (**75**), and (+)-plagiochiline-X (**76**) were isolated and characterised in the essential oil of *P. asplenioides* collected from Kummerfeld. In contrast, aromatic sesquiterpene hydrocarbons bisabola-1,3,5,7(14)-tetraene (**84**) and bisabola-1,3,5,7-tetraene (**85**) were isolated from *P. asplenioides* from Altenau. Whereas, compounds (–)-aromadendra-1(10),3-diene (**86**) and (–)-ent-4-epi-maaliol (**77**) were obtained from both locations (Fig. 134).

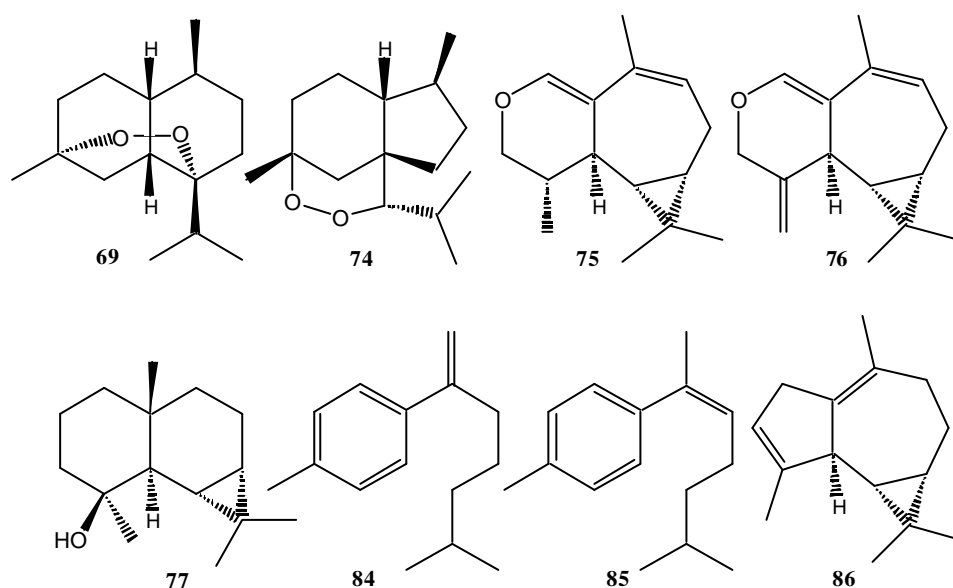


Fig. 134. New Sesquiterpenoids from *Plagiochila asplenioides*.

The European *Scapania undulata* have been characterised into four chemical races.<sup>[113]</sup> Two chemotypes predominate in the German Harz mountains: the cubenol-type and the longiborneol-type. (+)-Helminthogermacrene (**107**) ( Fig. 135) was isolated for the first time in a liverwort *S. undulata* collected in the Harz mountains. Its absolute configuration was determined by transannular cyclisation rearrangement reactions. Thermal isomerization of **107** at 350 °C afforded *cis*- $\beta$ -elemene (**93**), another  $\beta$ -elemene diastereomer (**129**) and a trace of "normal" *trans*-(+)- $\beta$ -elemene (**40**) in the ratio of (8 : 1 : ~0.08), respectively. In order to compare the characteristics of (+)-helminthogermacrene (**107**) (a *E,Z*-germacrene) with the common *E,E*-germacrene, (+)-germacrene-A (**32**) was isolated from *Solidago canadensis*. The thermal isomerization of **32** gave *trans*-(-)- $\beta$ -elemene (**40**) and the enantiomer of the  $\beta$ -elemene diastereomer **129a** in a ratio of 95:5, respectively at relatively low temperature (less than 120 °C). Thus, **107** is much less susceptible to *Cope* rearrangement than **32**. In addition, acid transformations of **107** gave (-)- $\alpha$ -helmiscapene (**125**) while **32** gave  $\alpha$ -selinene (**126**),  $\beta$ -selinene (**127**), selina-4,11-diene (**132**), and the alcohol 4 $\alpha$ -hydroxyselina-11-ene (**131**).

Perfora-1,7-diene (**110**) was the first perforane hydrocarbon isolated in nature. A possible biogenetic pathway for the perforane skeleton was proposed (Fig. 54).

Analysis by GC and GC-MS of the essential oil and diethyl ether extract of another *Scapania undulata* sample obtained from Norway in June 2002 confirmed the presence of the longiborneol- chemotype of *Scapania undulata* in Norway.

In the essential oil of *Diplophyllum albicans*, (+)-eudesma-4,11-dien-8 $\alpha$ -ol (**147**) was isolated and characterised.

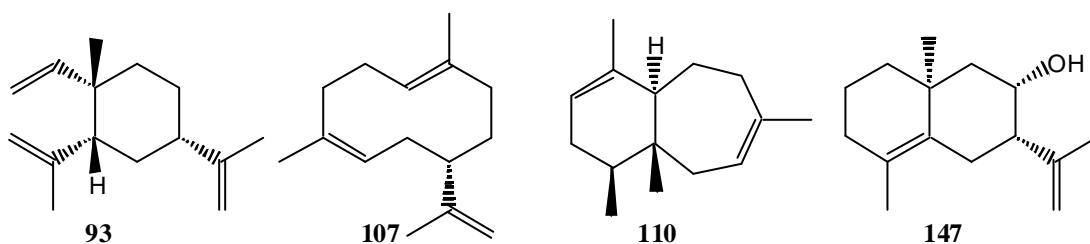


Fig. 135. New Sesquiterpenes from *Scapania undulata* and *Diplophyllum albicans*.

The essential oil of *Marsupella emarginata* obtained from Altenau (Germany), consists of a large number of longipinane-based compounds among which were isolated (-)-4-*epi*-marsupellol (**174**), (-)-marsupellyl acetate (**175**), (-)-4-*epi*-marsupellyl acetate (**176**), and (+)-5-hydroxymarsupellyl acetate (**177**). In addition, (-)-eremophila-1(10),8,11-triene (**173**) and lemnalone (**28**) were isolated. In the essential oil of *Marsupella emarginata* collected from Saarland (southwestern part of Germany) and the diethyl-ether extract of a Japanese sample, a series of gymnomitrane related compounds in addition to few known longipinane-based compounds were detected. The new gymnomitrane-type sesquiterpenoids include, (-)-4 $\beta$ -acetoxygymnomitr-3(15)-ene (**180**), (-)-4 $\beta$ ,5 $\beta$ -diacetoxygymnomitr-3(15)-ene (**181**), (+)-5 $\beta$ -acetoxygymnomitr-3(15)-ene (**182**), (-)-3 $\beta$ ,15 $\beta$ -epoxy-4 $\beta$ -acetoxygymnomitrane (**183**), (-)-3 $\alpha$ ,15 $\alpha$ -epoxy-4 $\beta$ -acetoxygymnomitrane (**184**), (-)-15-acetoxygymnomitr-3-ene (**185**), (+)- $\alpha$ -barbatenal (**186**) from the Saarland *M. emarginata*, while (+)-12-acetoxygymnomitr-3(15)-ene (**187**) was isolated from the Japanese *M. emarginata* (Fig. 136).

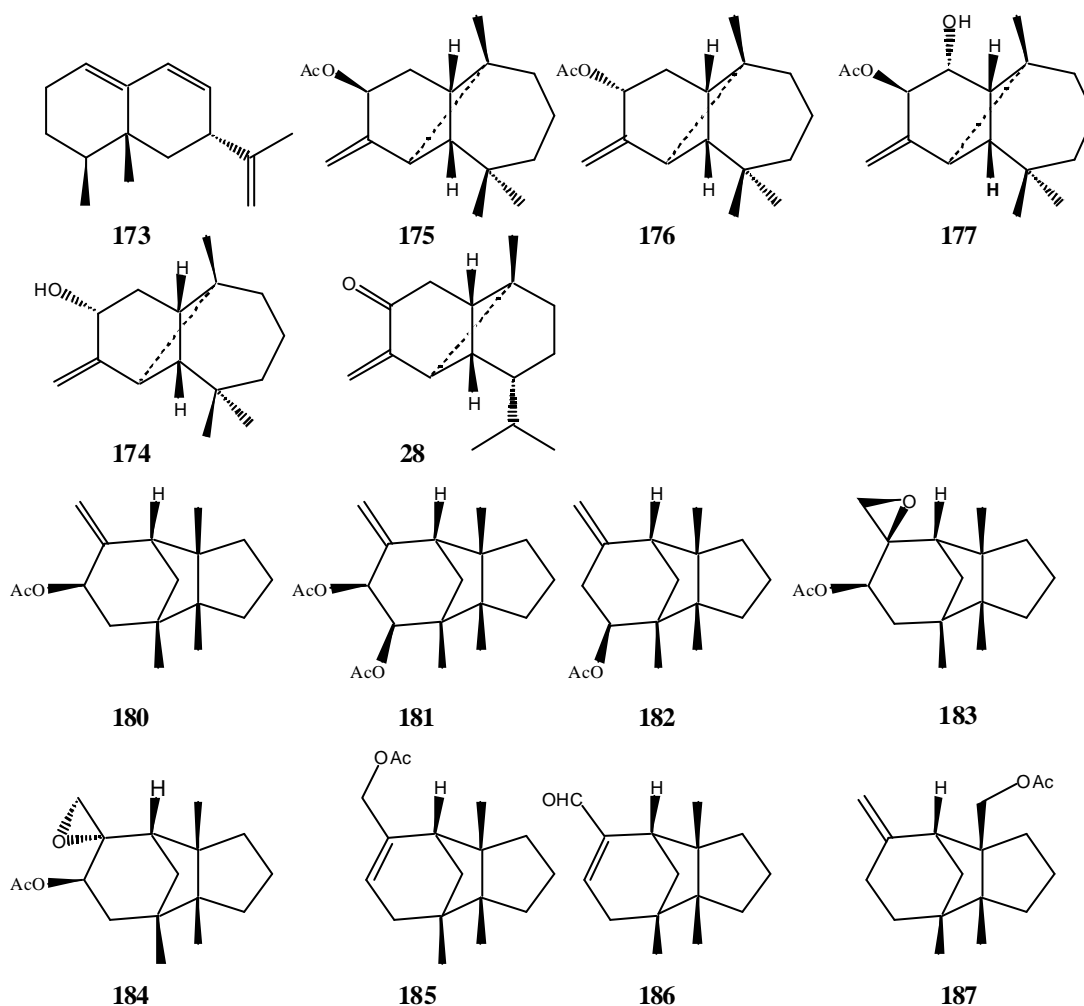


Fig. 136. New Sesquiterpenoids from *Marsupella emarginata* species.



In contrast to the *M. emarginata* species, the essential oil of *Marsupella aquatica* obtained from Austria mainly consists of amorphanes, traces of cadinane or muurolane, and few gymnomitrane based compounds with a trace of longipinane. The new isolated sesquiterpene hydrocarbons (Fig. 137) include (-)-myltayl-4(12)-ene (**198**) and (-)-myltayl-4-ene (**199**). These myltaylenes were the first myltaylane hydrocarbons to be isolated in nature thus, an additional biogenetic pathway in accordance with Markownikov rule was proposed.

The new amorphane-type sesquiterpenoids include, (+)-7 $\beta$ -hydroxyamorpha-4,11-diene (**200**), (-)-9 $\alpha$ -hydroxyamorpha-4,7(11)-diene (**201**), (-)-3 $\alpha$ -hydroxyamorpha-4,7(11)-diene (**202**), (-)-3 $\alpha$ -acetoxyamorpha-4,7(11)-diene (**204**), (-)-amorpha-4,7(11)-dien-3-one (**205**), (+)-2,8-epoxyamorpha-4,7(11)-diene (**206**), (+)-5,9-epoxyamorpha-3,7(11)-diene (**209**), (-)-2 $\alpha$ -hydroxyamorpha-4,7(11)-diene (**211**) and (-)-2 $\beta$ -acetoxyamorpha-4,7(11)-diene (**212**), (-)-2 $\alpha$ -acetoxyamorpha-4,7(11)-diene (**210**), (+)-amorpha-4,11-diene (**31**) and (-)-amorpha-4,7(11)-diene (**70**). The only new selinane isolated from *M. aquatica* was (+)-9 $\beta$ -hydroxyselina-4,11-diene (**214**).

In the essential oil of *Marsupella alpina*, the new compounds were (+)-8,9-epoxyselina-4,11-diene (**143**), (-)-selina-4(15),11-dien-5 $\beta$ -ol (**222**) and (+)-selina-4(15),11-dien-5 $\alpha$ -ol (**223**).

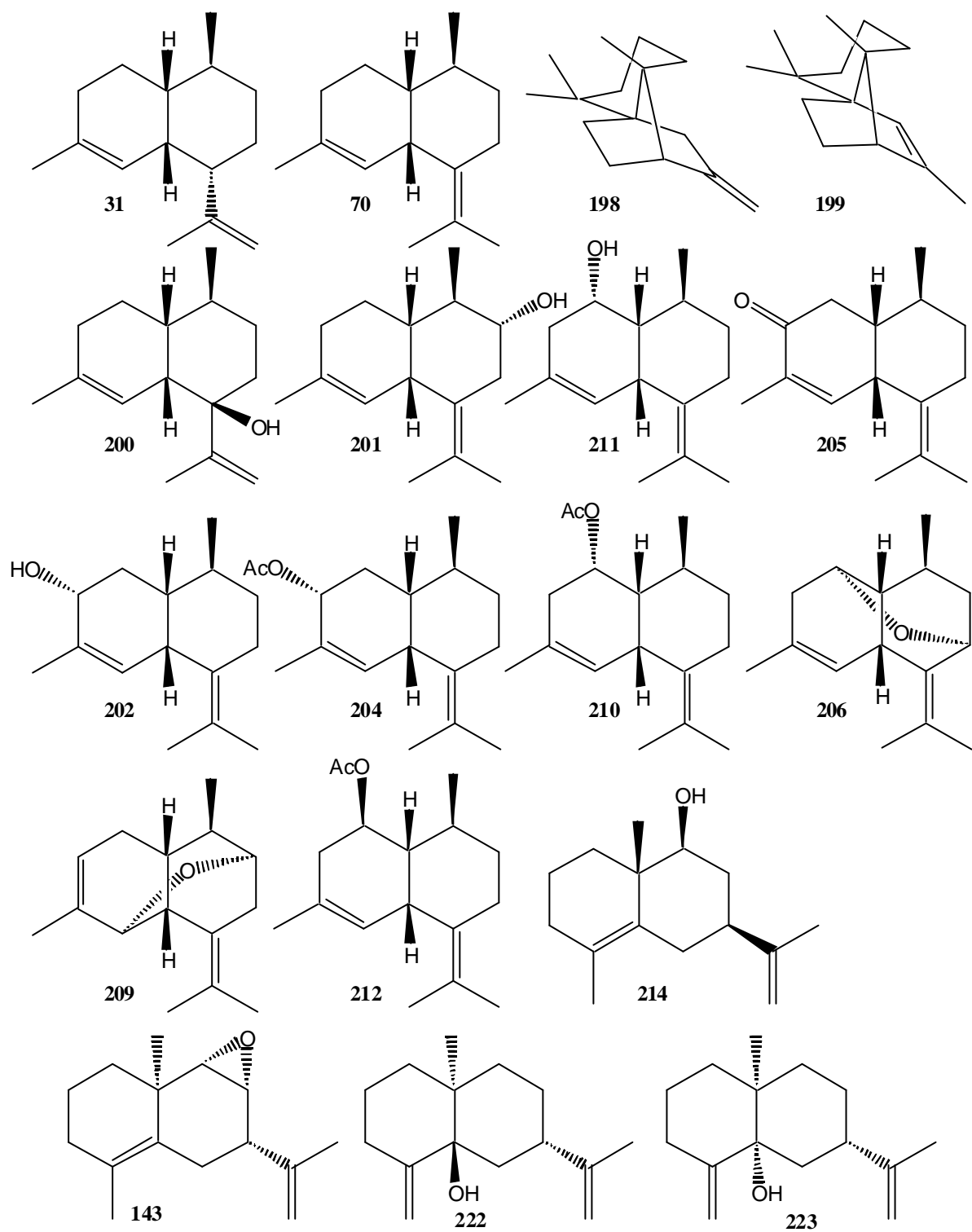


Fig. 137. New sesquiterpenoids from *M. aquatica* and *M. alpina*.

From the essential oil of *Tritomaria polita* the sesquiterpenoids (+)-eudesma-3,11-dien-8-one (**230**), (+)-eudesma-3,7(11)-dien-8-one (**231**), (+)-6,11-epoxyeudesmane (**236**), (-)-6,7-secoeudesm-7(11)-en-6-al (**237**), (+)-6 $\beta$ -hydroxyeudesm-11-ene (**234**), (-)-6 $\alpha$ -hydroxyeudesm-11-ene (**235**), (+)-6,11-epoxyisodaucane (**239**) were identified for the first time as natural compounds.

Bicyclic C<sub>12</sub>-norsesquiterpenes including, (+)-1,2,3,6-tetrahydro-1,4-dimethylazulene (**245**), (-)-2,3,3a,4,5,6-hexahydro-1,4-dimethylazulen-4-ol (**246**) and 5,6-dihydro-1,4-dimethylazulene (**248**) were isolated from the essential oil of *Barbilophozia floerkei* (Fig. 138).

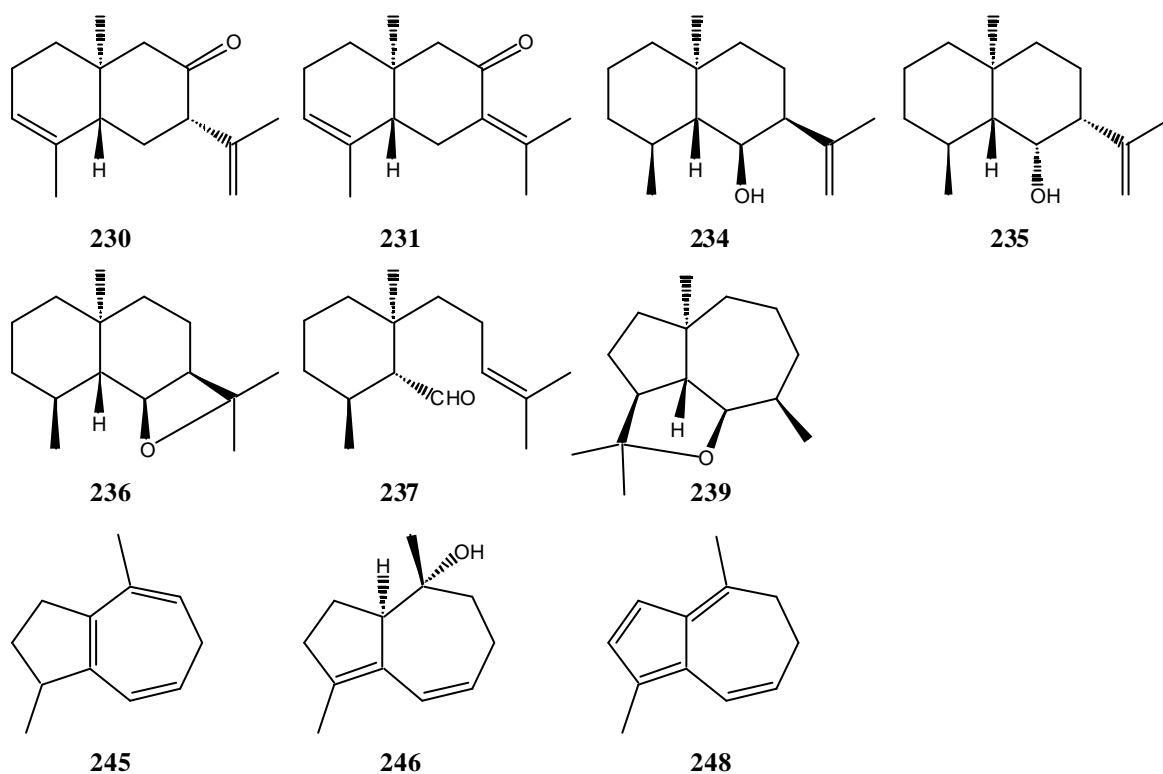


Fig. 138. New sesquiterpenoids from *T. polita* and *B. floerkei*.

The complete NMR data of some naturally occurring compounds which are not available in the literature were presented. These include (-)-gymnomitr-3(15),4-diene (**166**), (-)- $\beta$ -longipinene (**92**), (+)-germacrene-A (**32**) and germacrene-B (**33**) (Fig. 139). In addition, complete NMR data of (-)-selina-5,7(11)-diene (**83**) and (-)-selina-5,11-diene (**80**) obtained as thermal transformation products of (+)-maalian-5-ol<sup>[105]</sup> (**47**, 18.7%) at 200 °C injector temperature using preparative GC were also presented. In addition, partial NMR data of a  $\beta$ -elemene diastereomer (**129**), and five new reaction products including (+)-amorpha-2,4,7(11)-triene (**203**), a thermal transformation product of (-)-3 $\alpha$ -hydroxyamorpha-4,7(11)-diene (**202**) (Fig. 97), (+)-amorpha-4,7(11)-dien-2-one (**213**), an oxidation product of (-)-2 $\alpha$ -hydroxyamorpha-4,7(11)-diene (**211**) (Fig. 106), (+)-eudesma-4,11-dien-9-one (**215**) an oxidation product of (+)-9 $\beta$ -hydroxyselina-4,11-diene (**214**) (Fig. 108b), (+)-3,4,4a*R*,7,8,8a*R*-hexahydro-5,8a-dimethylnaphthalen-2(1H)-one (**233**), a degradation product of eudesma-3,11-dien-8-one (**230**) (Fig. 117a), and eudesma-5,7(11)-diene (**238**), an acid transformation product of (+)-6,11-epoxyeudesmane (**236**) (Fig. 125) were presented.

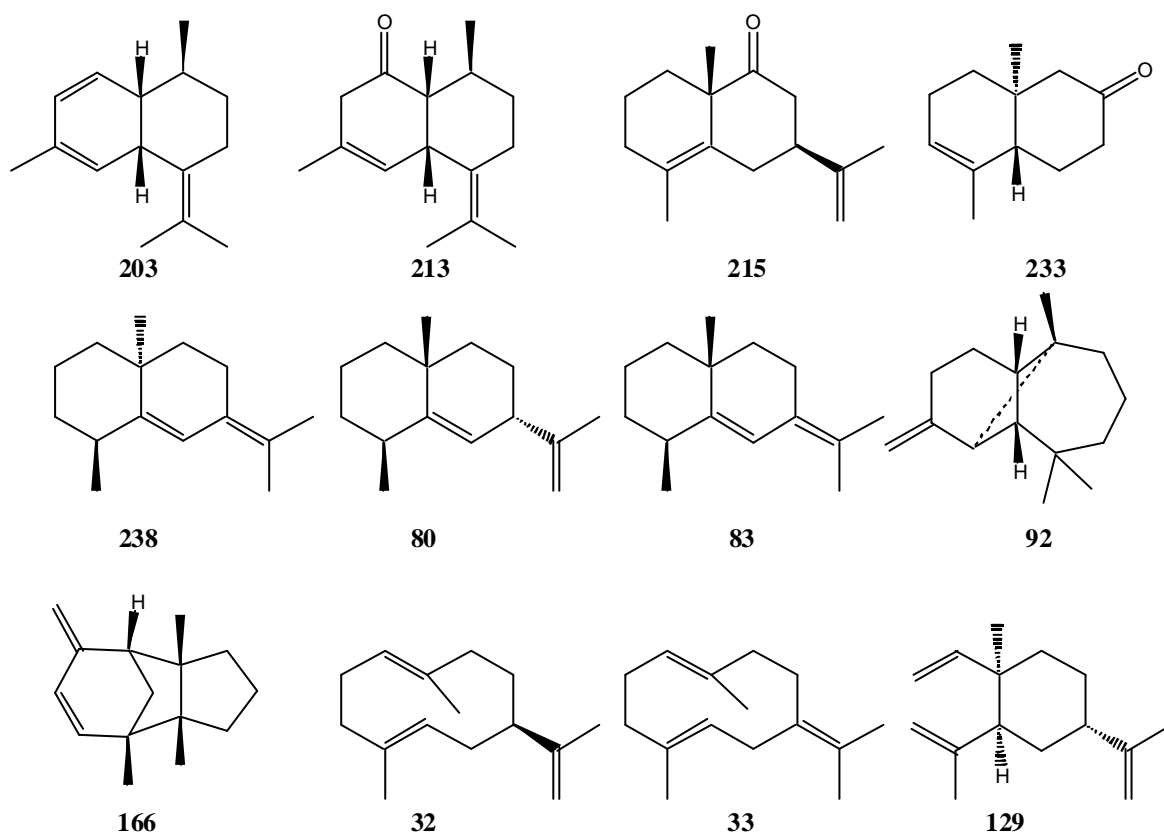


Fig.139. New reaction products and known compounds with NMR data.

## 6. Zusammenfassung

Es wurden die Sesquiterpenfraktionen europäischer Lebermoose der Familien Gymnomitriaceae, Scapaniaceae, Plagiochilaceae and Jungermanniaceae untersucht. Die ätherischen Öle und Extrakte wurden mittels GC und GC/MS analysiert. Alle bekannten Verbindungen wurden durch Vergleich ihrer Massenspektren und Retentionsindices mit einer Substanzbibliothek bei identischen experimentellen Bedingungen identifiziert. Unbekannte Verbindungen wurden durch Anwendung verschiedener chromatographischer Techniken (prep. -GC, -TLC, CC, etc.) isoliert und die Strukturen durch der Massenspektrometrie und NMR-spektroskopische Techniken ( $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ,  $2\text{D-}^1\text{H-}^1\text{H-COSY}$ ,  $\text{HMBC}$ ,  $\text{HMQC}$ ,  $\text{NOESY}$ ) aufgeklärt. Die Zuordnung von absoluten Konfigurationen erfolgte mittels chemischer Korrelation (Hydrierung, säurekatalysierte oder thermische Umwandlungen, etc.), die mittels enantioselektive GC verfolgt wurden.

Das ätherische Öl von *Plagiochila asplenoides*, gesammelt an zwei unterschiedlichen Fundorten in Deutschland (Altenau und Kummerfeld), wurde mittels GC und GC-MS untersucht. Die Analyse zeigte, dass diese *P. asplenoides* Arten eine Vielzahl von Verbindungen enthalten und somit den Chemovarietäten (I) und (V) zugeordnet werden könnten. Aus der Probe aus Kummerfeld wurden vier neue oxygenierte Verbindungen (+)-Murolan-4,7-peroxid (**69**), (+)-Plagio-4,7-peroxid (**74**), (+)-Plagiochiline-W (**75**), and (+)-Plagiochiline-X (**76**) isoliert und charakterisiert (Fig. 134). Dagegen wurden aus der Altenau-Probe die aromatischen Sesquiterpenkohlenwasserstoffe Bisabola-1,3,5,7(14)-tetraen (**84**) and Bisabola-1,3,5,7-tetraen (**85**) isoliert. (-)-Aromadendra-1(10),3-dien (**86**) and (-)-*ent*-4-*epi*-Maaliol (**77**) wurden aus den ätherischen Ölen beider Proben erhalten.

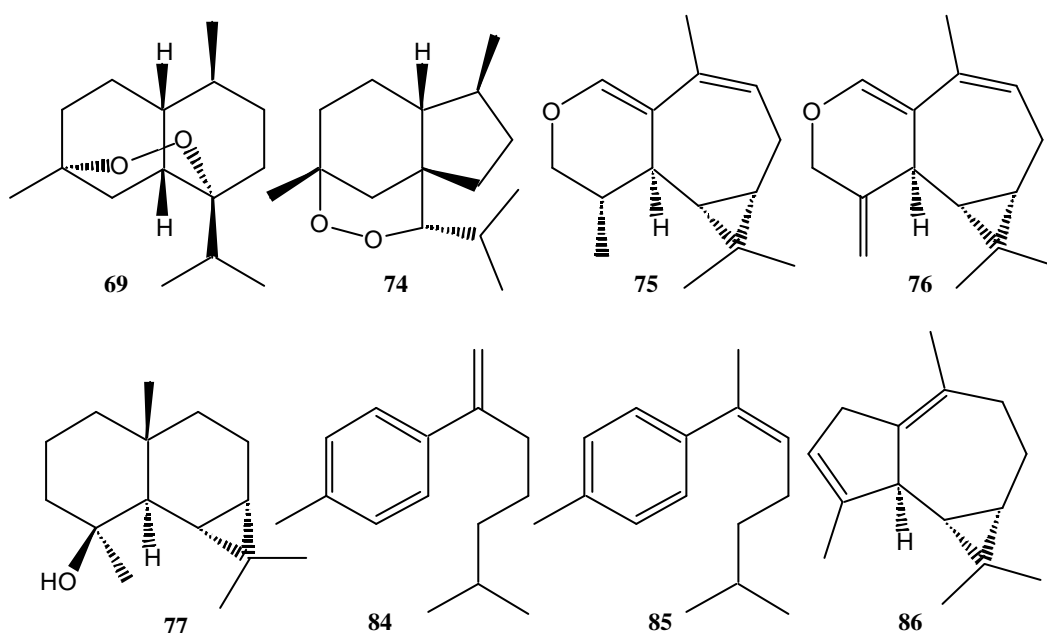


Fig. 134. Neue Sesquiterpenoide Verbindungen aus *Plagiochila asplenoides*.

Von dem europäischen Lebermoos *Scapania undulata* wurde vier Chemotypen charakterisiert. Zwei dieser Chemotypen dominieren im deutschen Harz-Gebirge: der 1-*epi*-Cubenol-Typ und der Longiborneol-Typ. (+)-Helminthogermacren (**107**) (Fig. 135) konnte erstmal aus einem Lebermoos isoliert werden. Die absolute Konfiguration konnte durch eine transannulare Cyclisierungsreaktion bestimmt werden. Die thermische Isomerisierung von **107** bei 350°C führte zu *cis*- $\beta$ -Elemen (**93**), einen weiteren  $\beta$ -Elemen Diastereomer (**129**) und zu Spuren von "normalem" *trans*-(+)- $\beta$ -Elemen (**40**) im Verhältnis 8 : 1 : ~0.08. Um die Eigenschaften von (+)-Helminthogermacren (**107**), einem *E,Z*-Germacren, mit denen des üblichen *E,E*-Germacren zu vergleichen, wurde Germacren A (**32**) aus *Solidago canadensis* isoliert. Die thermische Isomerisierung von **32** führte bei wesentlich geringeren Temperaturen (weniger als 120°C) zu *trans*-(-)- $\beta$ -Elemen (**40**) und dem Enantiomer des  $\beta$ -Elemen Diastereomers **129a** im Verhältnis 95:5. Folglich ist **107** gegenüber der *Cope*-Umlagerung deutlich weniger empfindlich als **32**. Zusätzlich führte die säurekatalysierte Umlagerung von **107** zu (-)- $\alpha$ -Helmiscapen (**125**), während **32**  $\alpha$ -Selininen (**126**),  $\beta$ -Selininen (**127**), Selina-4,11-dien (**132**) und den Alkohol 4 $\alpha$ -Hydroxy-selina-11-en (**131**) ergab.

(-)-Perfora-1,7-dien (**110**) ist der erste Perforan-Kohlenwasserstoff, der aus der Natur isoliert wurde. Eine mögliche Biogenese für das Perforan-Skelett wurde vorgeschlagen.

Die GC- und GC/MS-Analyse des ätherischen Öls und Diethylether-Extraktes einer weiteren *Scapania undulata* Probe aus Norwegen zeigte, dass der Longiborneol-Chemotyp auch in Norwegen gefunden wird.

Aus dem ätherischen Öl von *Diplophyllum albicans* wurde (+)-Eudesma-4,11-dien-8 $\alpha$ -ol (**147**) isoliert und charakterisiert.

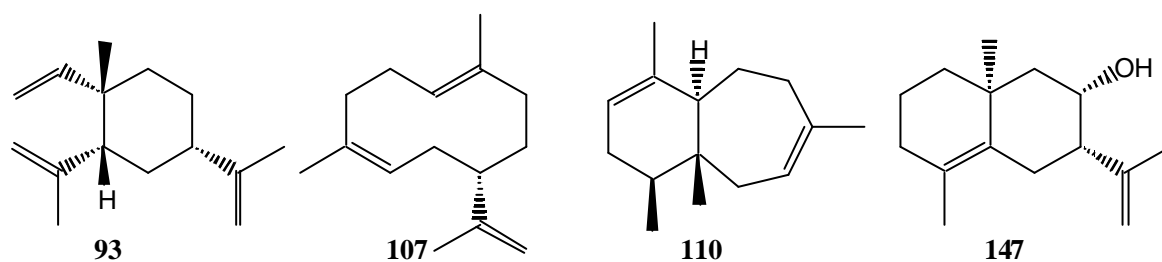


Fig. 135. Neue Sesquiterpenoide Verbindungen aus *Scapania undulata* und *Diplophyllum albicans*.

Aus *Marsupella emarginata*, gesammelt in Altenau (Deutschland), wurde eine Vielzahl von Longipinan basierten Verbindungen isoliert (Fig. 136), einschließlich (-)-4-*epi*-Marsupellol (**174**), (-)-Marsupellylacetat (**175**), (-)-4-*epi*-Marsupellylacetat (**176**) und (+)-5-Hydroxymarsupellylacetat (**177**). Zusätzlich konnten Eremophila-1(10),8,11-trien (**173**) und Lemnalon (**28**) isoliert werden. In dem ätherischen Öl von *M. emarginata*, gesammelt im Saarland (Süddeutschland), und dem Diethylether-Extrakt einer japanischen Probe wurde eine Reihe Gymnomitran verwandter Verbindungen neben einigen Longipinan-basierten Verbindungen detektiert. Die neuen Sesquiterpenoide vom Gymnomitran-Typ beinhalten (-)-4 $\beta$ -Acetoxygymnomitr-3(15)-en (**180**), (-)-4 $\beta$ ,5 $\beta$ -Diacetoxygymnomitr-3(15)-en (**181**), (+)-5 $\beta$ -Acetoxygymnomitr-3(15)-en (**182**), (-)-3 $\beta$ ,15 $\beta$ -Epoxy-4 $\beta$ -acetoxygymnomitran (**183**), (-)-3 $\alpha$ ,15 $\alpha$ -Epoxy-4 $\beta$ -acetoxygymnomitran (**184**), (-)-15-Acetoxygymnomitr-3-en (**185**) und (+)- $\alpha$ -Barbatenal (**186**) aus der Saarland-Probe, während (+)-12-Acetoxygymnomitr-3(15)-en (**187**) aus dem japanischem *M. emarginata* isoliert wurde.

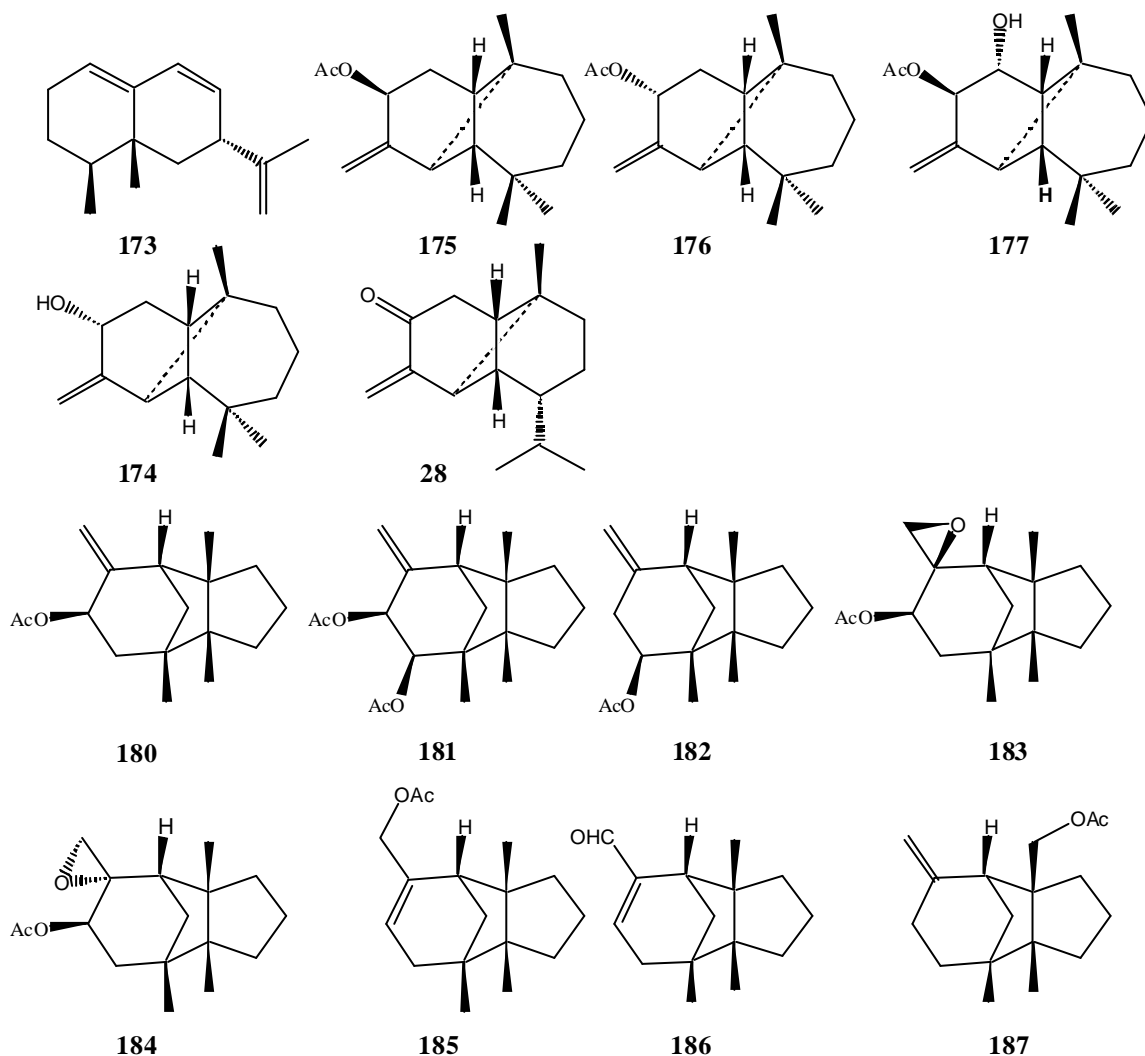


Fig. 136. Neue Sesquiterpenoide Verbindungen aus *Marsupella emarginata* species.

Neu isolierte Sesquiterpen-Kohlenwasserstoffe sind (-)-Myltayl-4(12)-en (**198**) and (-)-Myltayl-4-en (**199**) die ersten isolierten Myltaylan-Kohlenwasserstoffe darstellen (Fig. 137). Eine Biogenese wurde als im Einklang mit der Markownikov-Regel postuliert.

während die Sesquiterpenoiden vom Amorphan-Typ (+)-7 $\beta$ -Hydroxyamorpha-4,11-dien (**200**), (-)-9 $\alpha$ -Hydroxyamorpha-4,7(11)-dien (**201**), (-)-3 $\alpha$ -Hydroxyamorpha-4,7(11)-dien (**202**), (-)-3 $\alpha$ -Acetoxyamorpha-4,7(11)-dien (**204**), (-)-Amorpha-4,7(11)-dien-3-on (**205**), (+)-2,8-Epoxyamorpha-4,7(11)-dien (**206**), (+)-5,9-Epoxyamorpha-3,7(11)-dien (**209**), (-)-2 $\alpha$ -Hydroxyamorpha-4,7(11)-dien (**211**), (-)-2 $\beta$ -Acetoxyamorpha-4,7(11)-dien (**212**), (-)-2 $\alpha$ -Acetoxyamorpha-4,7(11)-dien (**210**), (+)-Amorpha-4,11-dien (**31**) und (-)-Amorpha-4,7(11)-dien (**70**) beinhalten. (+)-9 $\beta$ -Hydroxyselina-4,11-dien (**214**) ist das einzige neue Selinan, dass aus *M. aquatica* isoliert wurde.

Im Gegensatz zu den *M. emarginata* Arten, enthält das ätherische Öl von *M. aquatica* aus Österreich hauptsächlich Amorphane, Spuren von Cadinanen oder Muurolanen sowie einige Gymnomitran basierte Verbindungen mit Spuren von Longipinane.

Aus dem ätherischen Öl von *M. alpina* wurden die neuen Verbindungen (+)-8,9-Epoxyelina-4,11-dien (**143**), (-)-Selina-4(15),11-dien-5 $\beta$ -ol (**222**) und (+)-Selina-4(15),11-dien-5 $\alpha$ -ol (**223**) isoliert.



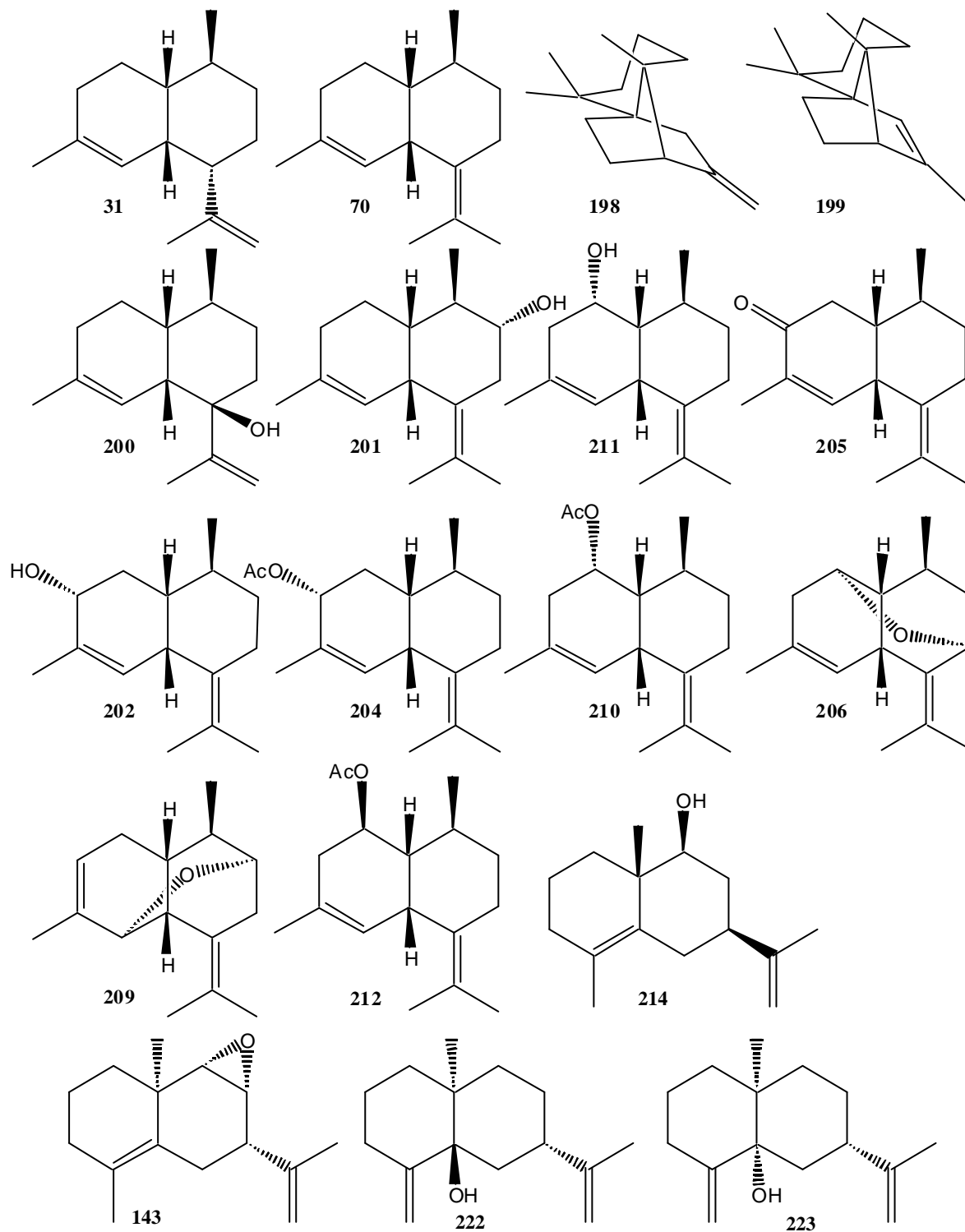


Fig. 137. Neue Sesquiterpenoide aus *M. aquatica* und *M. alpina*.

Die Sesquiterpeneoide (+)-Eudesma-3,11-dien-8-on (**230**), (+)-Eudesma-3,7(11)-dien-8-on (**231**), (+)-6,11-Epoxyeudesman (**236**), (-)-6,7-*seco*-Eudesm-7(11)-en-6-al (**237**), (+)-6 $\beta$ -Hydroxyeudesm-11-en (**234**), (-)-6 $\alpha$ -Hydroxyeudesm-11-en (**235**), (+)-6,11-Epoxyisodaucan (**239**) wurden erstmalig als Naturstoffe aus dem ätherischen Öl von *Tritomaria polita* identifiziert (Fig. 138). (+)-3,4,4a*R*,7,8,8a*R*-Hexahydro-5,8a-dimethylnaphthalen-2(1H)-on (**233**) wurde als Abbauprodukt von **230**. Die Norsesquiterpene mit bicyclischem C<sub>12</sub>-Gerüst, (+)-1,2,3,6-Tetrahydro-1,4-dimethylazulen (**245**), (-)-2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulen-4-ol (**246**) und 5,6-Dihydro-1,4-dimethylazulen (**248**), wurden aus dem ätherischen Öl von *Barbilophozia floerkei* isoliert.

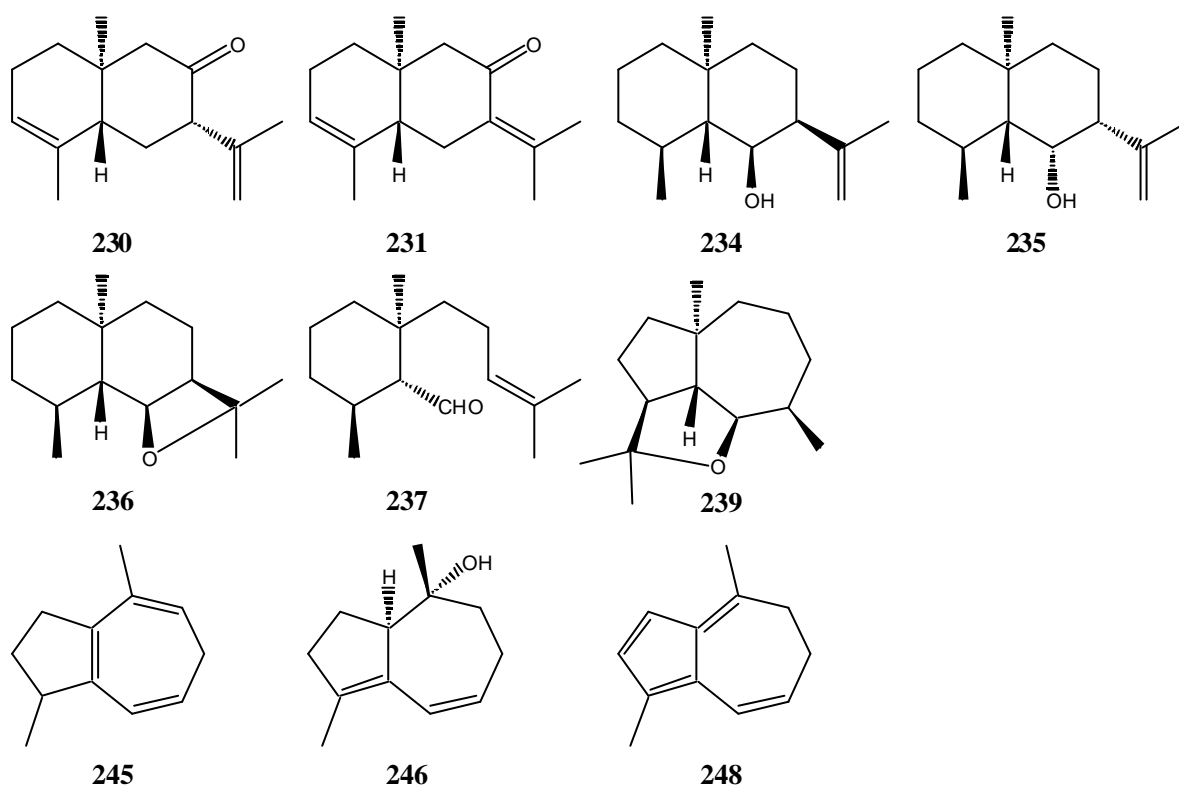


Fig. 138. Neue Sesquiterpeneoide aus *T. polita* und *B. floerkei*.

Es wurden die vollständigen NMR-Daten von bekannten natürlich vorkommenden Verbindungen, die nicht in der Literatur vorhanden sind, präsentiert (Fig. 139). Diese beinhalten (-)-Gymnomitr-3(15),4-dien (**166**), (-)- $\beta$ -Longipinen (**92**), (+)-Germacren-A (**32**) und Germacren-B (**33**). Zusätzlich wurden die vollständigen NMR-Daten von (-)-Selina-5,7(11)-dien (**83**) and (-)-Selina-5,11-dien (**80**) präsentiert, welche als thermische Umlagerungsprodukte von (+)-Maalian-5-ol bei einer Temperatur von 200°C im Injektorport des präparativen GC erhalten wurden. Zusätzlich teilweise NMR-Daten eines.  $\beta$ -Elemene Diastereomer (**129**) und fünf neuer Reaktionsprodukte einschließlich (+)-Amorpha-2,4,7(11)-trien (**203**), ein thermisches Umwandlungsprodukt von (-)-3 $\beta$ -Hydroxyamorpha-4,7(11)-dien (**202**) (Fig. 97), (+)-Amorpha-4,7(11)-dien-2-on (**213**), ein Oxidationsprodukt von (-)-2 $\alpha$ -Hydroxyamorpha-4,7(11)-dien (**211**) (Fig. 106), (+)-Eudesma-4,11-dien-9-on (**215**) ein Oxidationsprodukt von (+)-9 $\beta$ -Hydroxyselina-4,11-dien (**214**) (Fig. 108b), (+)-3,4,aR,7,8,8aR-Hexahydro-5,8a-dimethylnaphthalen-2(1H)-on (**233**), ein Verminderungsprodukt von Eudesma-3,11-dien-8-on (**230**) (Fig. 117a) und Eudesma-5,7(11)-dien (**238**), ein saures Umwandlungsprodukt von (+)-6,11-Epoxyeudesman (**236**) (Fig. 125) wurden dargestellt.

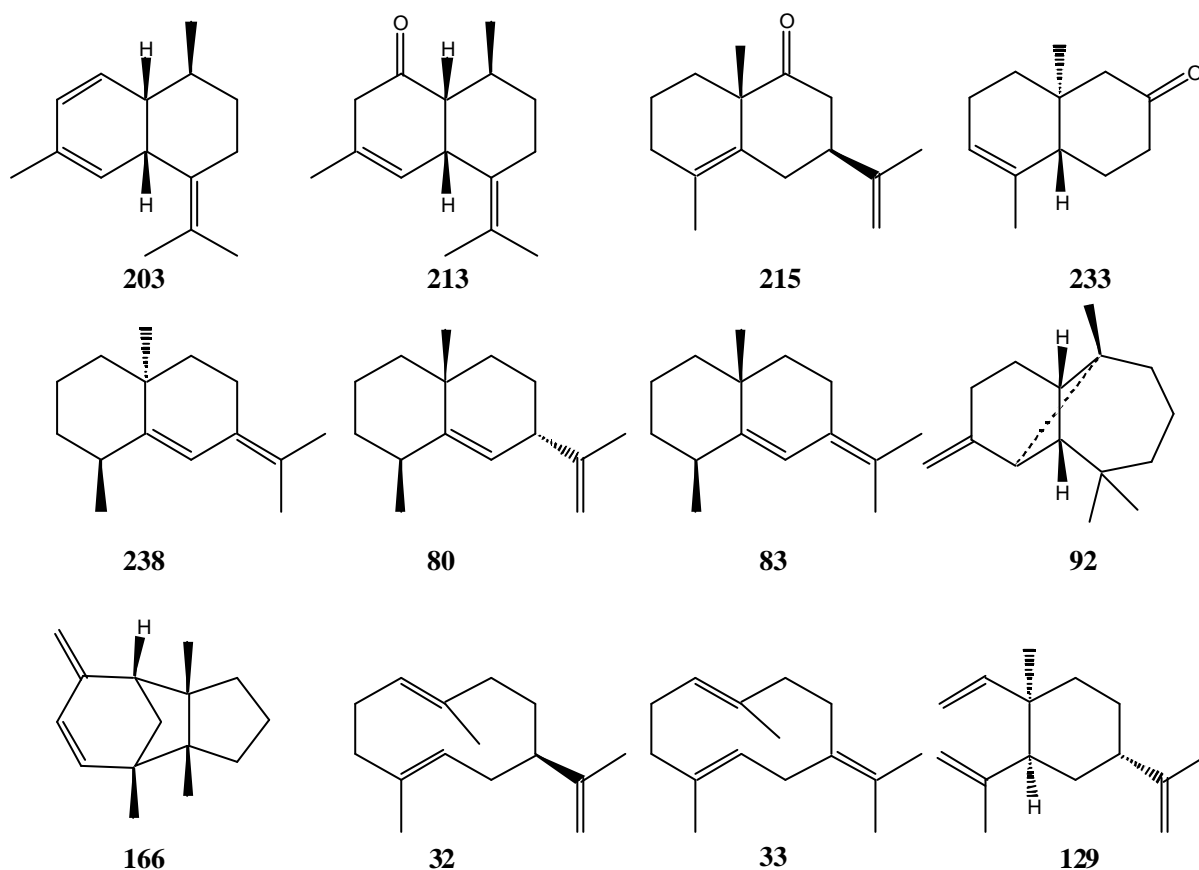


Fig.139. Neue reaktionsprodukte und die NMR-Daten von bekannten natürlich.

## 7. Experimental Part

### 7.1. General Experimental Procedures

#### 7.1.1. Gas chromatography

Orion Micromat 412 double column instrument with 25 m fused silica capillaries with polysiloxane CPSil-5 and polysiloxane CPSil-19 (Chrompack); Carlo Erba Fractovap 2150 or 4160 gas chromatographs with 25 m fused silica capillaries with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\gamma$ -cyclodextrin, heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\beta$ -cyclodextrin or heptakis(6-*O*-tert.-butyldimethylsilyl-2,3-di-*O*-methyl)- $\beta$ -cyclodextrin in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H<sub>2</sub>; injector and detector temperatures were 200 and 250 °C, respectively.

#### 7.1.2. Preparative Gas Chromatography

Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m x 4.3 mm) with 10% polydimethylsiloxane SE-30 on Chromosorb W-HP or with 15% SE-52 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\gamma$ -cyclodextrin in OV-170 (50%, w/w) on Chromosorb G-HP or with 6% heptakis(6-*O*-tert.-butyldimethylsilyl-2,3-di-*O*-methyl)- $\beta$ -cyclodextrin in SE-52 (50%, w/w) on Chromosorb W-HP; FID; helium as carrier gas at a flow rate of 120 ml/min.; injector and detector temperatures were 200 and 250 °C, respectively (Hardt and König, 1994).<sup>[86]</sup>

#### 7.1.3. Gas chromatography-Mass spectrometry

Electron impact (70 eV) and chemical ionization (using *iso*-butane and ammonia as reagent gases) GC-MS was carried out on a Hewlett Packard HP 5890 gas chromatograph equipped with a 25m CPSIL 5 CB capillary column and coupled with a VG Analytical 70-250S mass spectrometer. The atmospheric pressure chemical ionization (APCI) probe was carried out using MAT 95 XL Thermo Quest Finnigan, and the corona discharge was kept at 4.43 kV.

#### 7.1.4. Nuclear Magnetic Resonance spectroscopy

NMR measurements were carried out with a Bruker WM 400 or a Bruker WM 500 instrument in C<sub>6</sub>D<sub>6</sub> and/or CDCl<sub>3</sub> using tetramethylsilane (TMS) as internal standard.

### 7.1.5. Polarimetry

Measurements were performed with a polarimeter 341 (Perkin-elmer) using a wavelength of 589 nm at 20 °C. Due to the very small amounts of isolated compounds only the sense of optical rotation is given to avoid inaccuracies.

### 7.1.6. Thin Layer Chromatography

Thin layer chromatography was effected using glass and aluminum plates of silica 60 F<sub>254</sub> (Merck). An ethanolic solution of sulfuric acid (10%) and anisaldehyde was used as spray reagent. The solvent system used was n-hexane : ethyl acetate (3 : 1, v / v).

### 7.1.7. Reactions

**7.1.7.1.** *Hydrogenation* was performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 ml *n*-hexane and 0.5 mg Pd/C at room temperature for 1 h. The reaction mixture was filtered through a short column packed with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The filtered reaction products were analyzed by GC-MS and GC on several capillary columns with cyclodextrin derivatives.

**7.1.7.2.** *Saponification* of the acetates was achieved by treatment of ca. 1 mg of the sample with excess K<sub>2</sub>CO<sub>3</sub> in methanol for at least 12 h at room temperature. The reaction mixture was extracted with diethylether, and the organic phase was filtered through a short column packed with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic phase was analyzed by GC-MS and GC on several capillary columns with cyclodextrin derivatives.

**7.1.7.3.** *Dehydration* was carried out with ca. 1 mg of sample in 0.5 ml pyridine, follow by addition of 1 drop of phosphoryl chloride under ice cooling. After 1 h of stirring at room temperature the reaction was quenched by adding a few drops of water, and the mixture was extracted three times with hexane. The organic phase was washed several times with water and dried over Na<sub>2</sub>SO<sub>4</sub>. The organic phase was analyzed by GC-MS and GC on several capillary columns with cyclodextrin derivatives.

**7.1.7.4.** *Oxidation* reactions were carried out with ca. 1 mg of sample in dry CH<sub>2</sub>Cl<sub>2</sub> and ca. 2 mg of PDC. After 3 h of stirring at room temp., Et<sub>2</sub>O (5 ml) was added, and the resulting mixture was filtered through a short column packed with florisil. The extracted diethylether fraction was analyzed by GC-MS and GC on several capillary columns with cyclodextrin derivatives.

**7.1.7.5.** *Epoxidation* reactions were performed with ca. 2 mg of sample in 5 ml chloroform and ca. 2 mg *m*-chloroperbenzoic acid (*m*-CPBA). After 1 h of stirring at room temp., the

reaction mixture was filtered through a short column of florisil and the reaction products were analyzed by GC-MS and by GC on several capillary columns with cyclodextrin derivatives.

**7.1.7.6.** *Reduction* reactions were performed by adding a suspension of LiAlH<sub>4</sub> (2 mg) in dry Et<sub>2</sub>O to the sample in dry Et<sub>2</sub>O and stirred at room temp. for 1 h. The reaction mixture was quenched with water and partitioned with diethyl ether. The mixture was filtered, and the reaction products were analyzed by GC-MS and by GC on several capillary columns with cyclodextrin derivatives.

## 7.2. Chemical Analysis of *Plagiochila asplenioides*

*P. asplenioides* samples were collected from Altenau and Kummerfeld in Germany in December 2001 and February 2004 respectively. Essential oil was obtained by hydrodistillation (2 h) of aqueous homogenates of partially air-dried liverwort material using *n*-hexane as collection solvent. All isolations were carried out with SE-30- and/or SE-52-columns consecutively with at least one cyclodextrin phase column.

### 7.2.1. (+)-(1R,4R,6R,7R,10S)-Muurolan-4,7-peroxide (69)

Colourless oil; RI<sub>CPSIL 5</sub> = 1485; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.78 (3H, *d*, H-12, *J* = 6.9 Hz), 0.81-0.85 (1H, *m*, H-9a), 0.87 (3H, *d*, H-14, *J* = 6.9 Hz), 1.10 (3H, *d*, H-13, *J* = 6.6 Hz), 1.15 (1H, *d*, H-5a, *J* = 11.4 Hz), 1.28 (3H, *s*, H-15), 1.30-1.44 (3H, *m*, H-1, H-3a, H-10), 1.58-1.62 (2H, *m*, H-2), 1.66-1.78 (5H, *m*, H-3b, H-8, H-9b, H-11), 1.82 (1H, *ddd*, H-5b, *J* = 2.2, 4.7, 11.4 Hz), 2.00 (1H, *t*, H-6, *J* = 4.7 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): Table 2; MS (EI, 70eV), *m/z* (rel. int.): 204 (4), 180 (14), 179 (100), 162 (12), 161 (90), 135 (10), 119 (18), 105 (55), 95 (17), 81 (29), 67 (9), 55 (14), 43 (26). MS (CI, NH<sub>3</sub> gas), *m/z* (rel. int.): 223 (M<sup>+</sup>+1-O) (3), 221 (M<sup>+</sup>+1-H<sub>2</sub>O) (1), 205 (100), 179 (45), 161 (24), 149 (2), 135 (5). MS (CI, *iso*-butane gas), *m/z* (rel. int.): 223 (M<sup>+</sup>+1-O) (2), 221 (M<sup>+</sup>+1-H<sub>2</sub>O) (10), 205 (85), 179 (100), 161 (47), 149 (12), 135 (8).

### 7.2.2. (+)-(1R\*,4S\*,6S\*,7R\*,10S\*)-Plagio-4,7-peroxide (74)

Colourless oil; RI<sub>CPSIL 5</sub> = 1420; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.81 (3H, *d*, H-12, *J* = 6.3 Hz), 0.91 (3H, *d*, H-14, *J* = 6.3 Hz), 1.01 (1H, *dt*, H-1, *J* = 4.7, 12.6 Hz), 1.10-1.18 (1H, *m*, H-9a), 1.21-1.28 (2H, *m*, H-5a, H-3a), 1.23 (3H, *d*, H-13, *J* = 6.6 Hz), 1.30 (3H, *s*, H-15), 1.44-1.58 (3H, H-8, H-2a), 1.64 (1H, *dd*, H-3b, *J* = 5.7, 13.2 Hz), 1.68-1.76 (1H, *m*, H-10), 1.77-1.91 (2H, *m*, H-9b, H-2b), 1.94 (1H, *dd*, H-5b, *J* = 1.9, 10.4 Hz), 2.12-2.20 (1H, *m*, H-11), 3.30 (1H, *d*, H-7, *J* = 10.4 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>):

Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 222 (<1), 221 (1), 220 (1.7), 207 (3), 179 (8), 161(5), 150(47), 135(100), 121(13), 107 (21), 95(28), 94 (35), 93(21), 79 (17), 67 (10), 55 (16), 43 (27). MS (CI, NH<sub>3</sub> gas),  $m/z$  (rel. int.): 223 (M<sup>+</sup>+1-O) (3), 221 (M<sup>+</sup>+1-H<sub>2</sub>O) (1), 205 (100), 179 (3), 161 (2) 149 (2) 150 (22) 135 (34). MS (CI, *iso*-butane gas),  $m/z$  (rel. int.): 223 (M<sup>+</sup>+1-O) (2) 221 (M<sup>+</sup>+1-H<sub>2</sub>O) (18), 205 (100) 179 (12), 161 (8), 150 (76), 135 (67).

### 7.2.3. (+)-(4S\*,5S\*,6S\*,7S\*)-Plagiochiline-W (75)

Colourless oil; RI<sub>CPSIL 5</sub> = 1627; sense of optical rotation (benzene): (+) <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.49 (1H, *dd*, H-6,  $J$  = 9.5, 11.0 Hz), 0.89 (3H, *d*, H-15,  $J$  = 7.3 Hz), 0.92 (3H, *s*, H-12), 0.97-0.99 (1H, *m*, H-7), 0.99 (3H, *s*, H-13), 1.52-1.58 (1H, *m*, H-4), 1.77 (3H, *s*, H-14), 1.96 (1H, *dd*, H-5,  $J$  = 4.10, 11.0 Hz), 2.30 (2H, *t*, H-8,  $J$  = 7.6 Hz), 3.49 (1H, *dd*, H-3a,  $J$  = 5.1, 10.4 Hz), 3.78(1H, *dd*, H-3b,  $J$  = 2.5, 10.4 Hz), 5.44 (1H, *t*, H-9,  $J$  = 6.3 Hz), 6.78 (1H, *s*, H-2); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 218 [M<sup>+</sup>] (100), 203 (18), 175 (25), 161 (27), 147 (30), 133(28), 119 (42), 110 (22), 105 (57), 95 (52), 91(56), 77 (35), 69 (37), 55 (46), 41 (62). HREIMS calcd for C<sub>15</sub>H<sub>22</sub>O<sub>1</sub> [M<sup>+</sup>]  $m/z$  218.1671 found [M<sup>+</sup>]  $m/z$  218.1656.

### 7.2.4. (+)-(5S\*,6S\*,7S\*)-Plagiochiline-X (76)

Colourless oil; RI<sub>CPSIL 5</sub> = 1657; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.71 (1H, *dd*, H-6,  $J$  = 9.8, 11.0 Hz), 0.91 (3H, *s*, H-12), 1.01-1.03 (1H, *m*, H-7), 1.02 (3H, *s*, H-13), 1.73 (3H, *br.s*, H-14), 2.09 (2H, *br.t*, H-8,  $J$  = 7.9 Hz), 2.99 (1H, *d*, H-5,  $J$  = 11.0 Hz), 4.08 (1H, *d*, H-3a,  $J$  = 11.3 Hz), 4.27(1H, *d*, H-3b,  $J$  = 11.4 Hz), 4.83(1H, *s*, H-15a), 4.95 (1H, *br.s*, H-15b), 5.44 (1H, *t*, H-9,  $J$  = 6.0 Hz), 6.78 (1H, *s*, H-2); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 216 [M<sup>+</sup>] (91), 201 (26), 187 (16), 173 (43), 159 (45), 147 (72), 145 (68), 131 (36), 119 (48), 105 (58), 93 (45), 91(100), 77(52), 65 (26), 53 (28), 41 (79). HREIMS calcd for C<sub>15</sub>H<sub>20</sub>O<sub>1</sub> [M<sup>+</sup>]  $m/z$  216.1514 found [M<sup>+</sup>]  $m/z$  216.1488.

### 7.2.5. (-)-(4S,5R,6S,7R,10R)-4-*epi*-Maaliol (77)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1548$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.54-0.59 (2H, *m*, H-5, H-6), 0.64-0.71 (2H, *m*, H-9a, H-7), 0.86 (3H, *s*, H-12), 0.90 (1H, *dt*, H-1a,  $J = 3.5, 12.9$  Hz), 1.02 (6H, *s*, H-13, H-15), 1.10 (1H, *dd*, H-3a,  $J = 8.8, 13.2$  Hz), 1.17 (3H, *s*, H-14), 1.20 (1H, *dt*, H-3b,  $J = 4.4, 13.9$  Hz), 1.32-1.40 (2H, *m*, H-2a, H-1b), 1.52 (1H, *dd*, H-8a,  $J = 7.9, 15.1$  Hz), 1.56-1.61 (1H, *m*, H-9b), 1.79-1.88 (1H, *m*, H-8b), 1.92-2.01 (1H, *m*, H-2b);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  16.2 (*q*, C-12), 16.3 (*t*, C-8), 17.5 (*s*, C-11), 18.8 (*t*, C-2), 19.4 (*q*, C-14), 20.0 (*d*, C-6), 21.7 (*d*, C-7), 29.0 (*q*, C-13), 30.4 (*q*, C-15), 32.9 (*s*, C-10), 40.7 (*t*, C-1), 41.6 (*t*, C-9), 41.7 (*t*, C-3), 47.3 (*d*, C-5), 71.2 (*s*, C-4); MS (EI, 70eV),  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (6), 204 (40), 189 (72), 179 (8), 161(55), 147 (24), 133 (42), 123 (39), 121 (38), 109 (58), 107 (55), 105 (56), 91 (55), 81 (73), 67(44), 55 (46), 43 (100). HREIMS calcd for  $\text{C}_{15}\text{H}_{26}\text{O}_1$  [ $\text{M}^+$ ]  $m/z$  222.1984 found [ $\text{M}^+$ ]  $m/z$  222.1981.

### 7.2.6. (-)-(4S,7S,10R)-Selina-5,11-diene (80)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1447$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.14 (3H, *s*), 1.14-1.21 (2H, *m*), 1.17 (3H, *d*,  $J = 7.6$  Hz), 1.32-1.38 (1H, *m*), 1.45-1.55 (5H, *m*), 1.70 (3H, *s*), 1.71-1.80 (1H, *m*), 1.81-1.88 (1H, *m*), 2.45-2.51 (1H, *m*), 2.60 (1H, *t*,  $J = 5.4$  Hz), 4.88 (1H, *br.s*), 4.94 (1H, *br.s*), 5.34 (1H, *d*,  $J = 4.1$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  18.2 (*t*), 22.5 (*q*), 23.1(*q*), 23.2 (*t*), 27.2 (*q*), 34.2 (*t*), 35.1(*s*), 38.0 (*t*), 39.1(*d*), 42.4 (*d*), 42.6 (*t*), 112.0(*t*), 124.1(*d*), 148.1(*s*), 148.5(*s*); MS (EI, 70eV),  $m/z$  (rel. int.): 204 [ $\text{M}^+$ ] (40), 189 (42), 161(37), 147 (39), 133 (46), 121(38), 108 (100), 107(62), 105 (52), 93 (62), 81(48), 67 (22), 55(38), 41(49).

### 7.2.7. (-)-(4S,10R)-Selina-5,7(11)-diene (83)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1558$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.14 (3H, *s*, H-14), 1.17-1.25 (2H, *m*, H-1a, H-9a), 1.193H, *d*, H-15, 7.6 Hz), 1.35-1.60 (5H, *m*, H-3, H-1b, H-2a, H-9b), 1.67 (3H, *br.s*, H-13), 1.75-1.80 (1H, *m*, H-2b), 1.76 (3H, *br.s*, H-12), 2.24 (1H, *br.t*, H-8a,  $J = 14.2$ Hz), 2.44-2.55 (2H, *m*, H-4, H-8b), 6.32 (1H, *s*, H-6);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 204 [ $\text{M}^+$ ] (98), 189 (100), 161 (40), 148 (21), 147 (20), 133 (43), 119 (22), 105(30), 91(31), 77 (14), 55 (16), 41(32). HREIMS calcd for  $\text{C}_{15}\text{H}_{24}$  [ $\text{M}^+$ ]  $m/z$  204.1878 found [ $\text{M}^+$ ]  $m/z$  204.1903.



### 7.2.8. Bisabola-1,3,5,7(14)-tetraene (84)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1483$ ;  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.82 (6H, *d*, H-12, H-13,  $J = 6.6$  Hz), 1.16 (2H, *q*, H-10,  $J = 6.9$  Hz), 1.40-1.50 (3H, *m*, H-11, H-9), 2.12 (3H, *s*, H-15), 2.44 (2H, *t*, H-8,  $J = 7.6$  Hz), 5.05 (1H, *d*, H-14a,  $J = 1.3$  Hz), 5.34 (1H, *d*, H-14b,  $J = 1.6$  Hz), 7.01 (2H, *d*, H-2, H-4,  $J = 7.9$  Hz), 7.33 (2H, *d*, H-1, H-5,  $J = 8.2$  Hz);  $^{13}\text{C-NMR}$  (125.7 Hz,  $\text{C}_6\text{D}_6$ ): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 202 [ $\text{M}^+$ ] (18), 187 (5), 159 (8), 145 (32), 132 (100), 131 (35), 119 (17), 117 (40), 115 (26), 105 (17), 77 (8), 65 (10), 55 (9), 41 (21). HREIMS calcd for  $\text{C}_{15}\text{H}_{22}$  [ $\text{M}^+$ ]  $m/z$  202.1721 found [ $\text{M}^+$ ]  $m/z$  202.1723.

### 7.2.9. Bisabola-1,3,5,7-tetraene (85)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1557$ ;  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.88 (6H, *d*, H-12, H-13,  $J = 6.93$  Hz), 1.28 (2H, *q*, H-10,  $J = 6.9$  Hz), 1.48-1.60 (1H, *m*, H-11,  $J = 6.9$  Hz), 1.96 (3H, *s*, H-14), 2.14 (2H, *q*, H-9,  $J = 7.6$  Hz), 2.16 (3H, *s*, H-15), 5.84 (1H, *br. t*, H-8,  $J = 6.9$  Hz), 7.04 (2H, *d*, H-2, H-4,  $J = 8.2$  Hz), 7.34 (2H, *d*, H-1, H-5,  $J = 8.2$  Hz);  $^{13}\text{C-NMR}$  (125.7 Hz,  $\text{C}_6\text{D}_6$ ): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 202 [ $\text{M}^+$ ] (28), 145 (100), 132 (71), 131(40), 115 (20), 105(27), 91 (16), 77 (8), 65 (5), 53 (6), 41(18). HREIMS calcd for  $\text{C}_{15}\text{H}_{22}$  [ $\text{M}^+$ ]  $m/z$  202.1721 found [ $\text{M}^+$ ]  $m/z$  202.1726.

### 7.2.10. (-)-(5*R*\*,6*S*\*,7*S*\*)-Aromadendra-1(10),3-diene (86)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1512$ ; sense of optical rotation (benzene): (-)  $^1\text{HNMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.55-0.60 (1H, *m*, H-7), 0.68 (1H, *t*, H-6,  $J = 10.4$  Hz), 0.98 (3H, *s*, H-12), 1.08 (3H, *s*, H-13), 1.55 (3H, *s*, H-14), 1.56-1.62 (1H, *m*, H-8a), 1.66-1.73 (1H, *m*, H-8b), 1.68 (3H, *s*, H-15), 2.13-2.27 (2H, *m*, H-9), 3.00 (1H, *d*, H-2a,  $J = 20.5$  Hz), 3.06 (1H, *d*, H-5,  $J = 10.7$  Hz), 3.14 (1H, *d*, H-2b,  $J = 20.5$  Hz), 5.34-5.39 (1H, *m*, H-3);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 2; MS (EI, 70eV),  $m/z$  (rel. int.): 202 [ $\text{M}^+$ ] (22), 187 (10), 159 (44), 145 (26), 133 (79), 132 (100), 131 (30), 117 (18), 105 (58), 91 (21), 77 (16), 67 (8), 53 (8), 41 (26). HREIMS calcd for  $\text{C}_{15}\text{H}_{22}$  [ $\text{M}^+$ ]  $m/z$  202.1721 found [ $\text{M}^+$ ]  $m/z$  202.1729.

**Table 2.**  $^{13}\text{C}$ -NMR spectral data of compounds **84**, **85**, (-)-**86**, (-)-**83**, (+)-**69**, (+)-**74**, (+)-**75**, and (+)-**76** (125.7 MHz,  $\text{C}_6\text{D}_6$ ),  $\delta$  (ppm)<sup>a</sup>

C- no.	<b>84</b>	<b>85</b>	<b>86</b>	<b>83</b>	<b>69</b>	<b>74</b>	<b>75</b>	<b>76</b>
1	126.7	126.3	136.9	41.9	40.1	56.2	118.2	119.6
2	129.6	129.5	39.1	18.2	29.3	22.7	142.4	143.2
3	137.1	136.1	121.8	33.7	39.4	38.6	67.9	68.8
4	129.6	129.5	143.2	38.7	79.7	78.7	34.2	144.6
5	126.7	126.3	46.8	147.3	41.5	54.6	37.7	36.0
6	139.2	141.8	35.1	121.9	37.7	53.5	33.3	33.5
7	149.1	134.7	26.7	128.8	86.1	93.7	28.9	29.1
8	36.3	128.0	23.1	24.1	24.7	28.9	24.4	24.7
9	26.7	27.3	37.1	42.3	28.2	33.9	124.8	125.4
10	39.2	39.4	125.6	34.6	34.1	37.6	133.3	132.6
11	28.4	28.3	19.2	124.0	37.1	29.3	19.5	19.4
12	23.0	23.0	29.0	20.0	16.3	20.0	28.9	28.9
13	23.0	23.0	16.2	21.0	19.3	22.5	15.6	15.6
14	111.8	16.2	22.0	26.6	21.6	19.2	22.6	23.1
15	21.3	21.3	15.5	23.8	27.8	26.8	16.9	111.6

<sup>a</sup> All assignments were confirmed by HMBC and HMQC.

### 7.3. Chemical Analysis of *Scapania undulata*

#### 7.3.1. Isolation of Single Constituents of the Essential Oils

The isolation of **107** was carried out using preparative GC at an injector temp of 120-140 °C. The essential oil of *S. undulata* was fractionated using an SE-30 column from 90 °C to 150 °C with a heating rate of 2 °C/min. The fraction with high concentration in **107** was further purified using prep. GC columns with heptakis(6-*O*-tert-butyltrimethylsilyl-2,3-di-*O*-methyl)- $\beta$ -cyclodextrin (120 °C, isothermal) and octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- $\gamma$ -cyclodextrin (125 °C, isothermal) consecutively. The last stage of purification was achieved using SE-52 to remove a co-eluting impurity of  $\alpha$ -chamigrene of about 1%.

##### 7.3.1.1. Thermal isomerization of **107**

**107** undergoes thermal isomerization at an injector port temperature of at 390 °C of the preparative GC instrument. The isomerized products **40**, **93** and **129** were isolated using an SE 30 column.

### 7.3.1.2. Acid Rearrangement of **107**

To a solution of **107** in diethyl ether, (5 ml) BF<sub>3</sub> Et<sub>2</sub>O (3 ml) was added at room temp. The solution was stirred for 5 min. and allowed to stand for 3 hours. Then ice cooled aqueous 0.5 M KOH (20 ml) was added. The organic layer was washed several times with aqueous 0.5 M KOH, then with saturated aqueous NaCl solution and dried over MgSO<sub>4</sub>; the solvent was concentrated, and the residue analysed with GC and GC-MS using achiral polysiloxane and chiral cyclodextrin derived GC phases.

### 7.3.2. Isolation of (-)-**a**-Helmiscapene (**125**)

a) From the reaction mixture: A solution of ca. 1 mg of (+)-**107** in *n*-hexane (1.5 ml) was mixed with acidic ion exchange resin Amberlyst 15 and kept at room temperature for 2 hours until (+)-**107** had completely disappeared (GC control). The solution was filtered and the catalyst washed with *n*-hexane. The solution was shaken with a saturated aqueous solution of NaHCO<sub>3</sub> (3 ml). The organic layer was partitioned and dried over Na<sub>2</sub>SO<sub>4</sub>, the solvent was evaporated, and **125** was isolated using preparative GC.

b) From *Radula perrottettii* (liverwort): An independent isolation and structure elucidation of (-)-**125** recently isolated from the essential oil obtained by hydrodistillation of the liverwort *Radula perrottettii* collected in Tokushima, Japan, in October 2003,<sup>[201]</sup> and spectral comparisons confirmed complete identity.

### 7.3.3. (-)-(5*R*,7*S*,10*S*)-*cis*-**b**-Elemene (**93**)

Colourless oil; RI<sub>CPSIL5</sub> = 1382; sense of optical rotation (C<sub>6</sub>D<sub>6</sub>): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 1.05 (3H, *s*, CH<sub>3</sub>-14), 1.27 (*dt*, 1H, H-9a, *J* = 5.04, 12.9 Hz), 1.49-1.54 (*m*, 2H, 8-H<sub>2</sub>), 1.58-1.64 (*m*, 3H, 6-H<sub>2</sub>, H-9b), 1.66 (*s*, 3H, CH<sub>3</sub>-15), 1.67 (*s*, 3H, CH<sub>3</sub>-12), 1.87-1.92 (*m*, 1H, H-7), 1.94 (*dd*, 1H, H-5, *J* = 3.46, 12.0 Hz), 4.75 (*s*, 1H, H-3a), 4.82 (*d*, 2H, 13-H<sub>2</sub>, *J* = 13.2 Hz), 4.87 (*s*, 1H, H-3b), 5.01 (*ddd*, 2H, 2-H<sub>2</sub>, *J* = 1.3, 6.0, 11.0 Hz), 6.29 (*dd*, 1H, H-1, *J* = 11.0, 17.3 Hz); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): 1.03 (*s*, 3H), 1.42 (*dt*, 1H, *J* = 5.0, 12.9 Hz), 1.59-1.67 (*m*, 3H), 1.68 (*s*, 3H), 1.72 (*d*, 1H, *J* = 14.5 Hz), 1.75 (*s*, 3H), 1.97-2.01 (*m*, 1H), 2.03 (*dd*, 1H, *J* = 3.5, 12.3 Hz), 4.65 (*s*, 1H), 4.71 (*d*, 1H, *J* = 6.0), 4.78 (*s*, 1H), 5.01 (*ddd*, 2H, *J* = 1.6, 11.0, 18.9 Hz), 6.31 (*dd*, 1H, *J* = 11.0, 17.7 Hz); <sup>13</sup>C NMR [data taken from HSQC/HMBC (125.7 MHz, C<sub>6</sub>D<sub>6</sub> )]: δ = 21.4 (*q*, C-12), 22.9 (*q*, C-15), 27.8 (*q*, C-14), 28.0 (*t*, C-8), 33.8 (*t*, C-6), 39.5 (*s*, C-10), 42.2 (*t*, C-9), 46.5 (*d*, C-7), 56.2 (*d*, C-5), 109.1 (*t*, C-13), 112.9 (*t*, C-2), 113.4 (*t*, C-3), 143.3 (*d*, C-1), 147.2 (*s*, C-4), 150.3 (*s*, C-11); MS (EI, 70 eV), *m/z* (rel. int.): 204 [M<sup>+</sup>] (15), 189 (40), 175 (10), 162 (11), 161 (44), 147 (31), 133 (25), 121 (41),

119 (37), 107 (66), 105 (51), 95 (31), 93 (100), 91 (42), 81 (98), 79 (59), 77 (30), 68 (79), 67 (65), 55 (44), 53 (37), 41 (65).

#### 7.3.4. (+)-(7S)-Helminthogermacrene (107)

Colourless oil;  $RI_{CPSIL5} = 1503$ ; sense of optical rotation ( $CDCl_3$ ): (+);  $^1H$ -NMR (500 MHz,  $C_6D_6$ , 20 °C):  $\delta = 1.35$ -1.45 (*br.s.* 2H), 1.57 (*s*, 3H), 1.66 (*br.s.*, 3H), 1.70 (*s*, 3H), 1.85-2.01 (*m*, 6H), 2.05-2.15 (*m*, 2H), 2.16-2.25 (*m*, 1H), 4.77 (*br.s.*, 1H), 4.85 (*s*, 1H), 5.30-5.44 (*m*, 2H);  $^1H$ -NMR (400 MHz,  $CDCl_3$ , 20 °C): 1.40-1.53 (*m*, 2H), 1.67 (*br.s.*, 3H), 1.71(*s*, 6H), 1.80-2.05 (*m*, 6H), 2.18-2.45 (*m*, 3H), 4.66 (*br.s.*, 1H), 4.70 (*br.s.*, 1H), 5.19-5.24 (*m*, 1H), 5.28-5.34 (*m*, 1H);  $^1H$ -NMR (500 MHz, acetone- $d_6$ , -16°C): 1.25-1.40 (*m*, 2H), 1.45-1.58 (*m*, 2H), 1.68 (*br.s.*, 3H), 1.70 (*br.s.*, 3H), 1.72 (*br.s.*, 3H), 1.93-2.01(*m*, 3H), 2.13-2.19 (*m*, 2H), 2.29-2.36 (*br.s.*, 1H), 2.48-2.54 (*m*, 1H), 4.60 (*br.s.*, 1H), 4.67 (*br.s.*, 1H), 5.21 (*br.d.*, 1H,  $J = 10.7$  Hz), 5.33 (*br.s.*, 1H);  $^{13}C$  NMR [data taken from HSQC/HMBC (125.7 MHz,  $C_6D_6$ , 20 °C )]:  $\delta = 16.3$ , 24.3, 25.3, 29.5, 31.1, 33.2, 41.0, 49.5, 109.3, 124.7, 126.6, 132.0, 134.4, 150.6;  $^{13}C$  NMR [data taken from HSQC/HMBC (125.7 MHz, acetone- $d_6$ , -8 °C)]: 16.1, 19.2, 24.2, 25.5, 30.0, 30.9, 33.1, 41.3, 50.0, 109.3, 125.0, 126.6, 132.4, 134.8, 151.2; MS (EI, 70 eV),  $m/z$  (rel. int.): 204 [ $M^+$ ] (17), 189 (19), 175 (5), 161 (34), 147 (36), 133 (18), 121 (42), 107 (43), 93 (67), 81 (58), 68 (100), 67 (52), 53 (44), 41 (68).

#### 7.3.5. (-)-(4S\*,5R\*,11S\*)-Perfora-1,7-diene (110)

Colourless oil;  $RI_{CPSIL5} = 1543$  ; sense of optical rotation ( $C_6D_6$ ): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta = 0.74$  (*s*, 3H, H-14), 0.82 (*d*, 3H, H-13,  $J = 6.6$  Hz), 1.18 (*dq*, 1H, H-10a,  $J = 1.6$ , 12.3 Hz), 1.47-1.54 (*m*, 1H, H-4), 1.62 (*br.s.*, 3H, H-12), 1.74 (*s*, 3H, H-15), 1.71-1.80 (*m*, 3H, H-10b, H-3a, H-6a), 1.86-1.94 (*m*, 2H, H-3b, H-9a), 2.00 (*br.d.*, 1H, H-11,  $J = 12.3$  Hz), 2.16 (*br.t.*, 1H, H-9b,  $J = 13.2$  Hz), 2.25 (*dd*, 1H, H-6b,  $J = 9.2$ , 14.5 Hz), 5.40 (*br.s.*, 1H, H-2), 5.45 (*br.s.*, 1H, H-7).  $^{13}C$  NMR (125.7 MHz,  $C_6D_6$ ):  $\delta = 10.9$  (*q*,  $CH_3$ -14), 16.6 (*q*,  $CH_3$ -13), 23.1 (*q*,  $CH_3$ -12), 24.3 (*t*, C-10), 25.9 (*q*,  $CH_3$ -15), 33.7 (*t*, C-3), 35.0 (*t*, C-9), 37.2 (*s*, C-5), 37.8 (*t*, C-6), 38.9 (*d*, C-4), 56.2 (*d*, C-11), 122.9 (*d*, C-2), 123.9 (*d*, C-7), 136.3 (*s*, C-1), 140.4 (*s*, C-8); MS (EI, 70eV),  $m/z$  (rel. int.) : 204 [ $M^+$ ] (12), 189 (16), 176 (30), 161(13), 147 (7), 136 (55), 135 (17), 134 (10), 133 (18), 121 (100), 119 (21), 107 (37), 93 (23), 91 (25), 79 (21), 67 (18), 55 (24), 41 (49).

### 7.3.6. Characterisation of (-)-(5S,7S,10S)-**a**-Helmiscapene (125)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1451$ ; sense of optical rotation ( $\text{CDCl}_3$ ): (-);  $^1\text{H-NMR}$  (500MHz,  $\text{CDCl}_3$ ): 0.87 (3 H, *s*), 1.36-1.42 (2 H, *m*), 1.66 (3 H, *br.s*), 1.71 (3 H, *s*), 1.81-1.90 (4 H, *m*), 2.00-2.09 (2 H, *m*), 4.66 (1 H, *s*), 4.68 (1 H, *s*), 5.24 (1 H, *br.s*); MS (EI, 70eV), *m/z* (rel. int.): 204 [ $\text{M}^+$ ] (38), 189 (59), 175 (9), 161 (32), 147 (19), 133 (28), 121 (27), 119 (30), 107 (75), 105 (56), 93 (82), 91 (68), 81 (48), 79 (47), 77 (45), 67 (38), 55 (47), 53 (48), 41 (100).

### 7.3.7. (5S\*,7S\*,10R\*)-*cis*-**b**-Elemene diastereomer (129)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1387$ ;  $^1\text{H NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 1.04$  (*s*, 3H), 1.65 (*s*, 3H), 6.22 (*dd*, 1H,  $J = 11.0, 17.7$  Hz);  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ): 1.02 (*s*, 3H), 6.23 (*dd*, 1H,  $J = 11.4, 17.7$  Hz); MS (EI, 70 eV), *m/z* (rel. int.): 204 [ $\text{M}^+$ ] (5), 189 (32), 175 (9), 162 (8), 161 (33), 147 (28), 133 (31), 121 (40), 119 (38), 107 (67), 105 (46), 93 (100), 91 (47), 81 (76), 79 (59), 68 (55), 67 (56), 55 (37), 53 (32), 41 (49).

### 7.3.8. Isolation of (+)-Germacrene A (32) from *Solidago canadensis*

The macerated air-dried aerial parts of *S. canadensis*, collected in Hamburg, Germany in September 2003, were extracted for three days at room temperature with diethyl ether. The concentrated crude extract obtained below 40 °C by evaporation of the solvent was fractionated using flash silica gel column chromatography. The 100% *n*-pentane fraction (yellow solution) was then subjected to repeated TLC using aluminum coated silica gel at -25 °C, using *n*-pentane as the developing solvent to give (+)-germacrene A (**32**,  $R_f$  0.40), germacrene B (**33**,  $R_f$  0.45), both enantiomers of germacrene D (**25**,  $R_f$  0.61), and a single band [containing (-)- $\alpha$ -selinene (**126**, 41%), (+)- $\beta$ -selinene (**127**, 45%) and (+)-selina-4,11-diene (**132**, 12%)] ( $R_f$  0.74) and other known constituents in trace amounts adding up to 4%.

Germacrene A (**32**) analysed by GC at 120 °C injection port temperature gave a very small amount of 'normal' (-)- $\beta$ -elemene (**40**, ca. 5%), a broadened germacrene A (**32**) peak (ca. 81%) which was preceded by a "hump" in the baseline containing (**126**, 0.2%), (**127**, 0.3%) and (**132**, 0.08%).

### 7.3.9. Characterisation of (+)-Germacrene A (32)

Isolated from *S. canadensis* as colourless oil;  $RI_{\text{CPSIL } 5} = 1501$ ;  $R_{f(\text{pentane})} = 0.40$ ; sense of optical rotation (benzene): (+);  $^1\text{HNMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.31 (3H, *br. s*, H-14), 1.44 (3H, *br.s*, H-15), 1.48-1.55 (1H, *m*, H-8a), 1.67 (3H, *s*, H-12), 1.67-1.73 (1H, *m*, H-8b), 1.76-2.36 (9H, *br. m*, H-2ab, 3ab, 6ab, 7, 9ab), 4.68 (1H, *s*, H-13a), 4.51 (1H, *br. d*, 10.2 Hz, H-5), 4.79

(1H, *s*, H-13b), 4.71-4.75 (1H, *m*, H-1). <sup>13</sup>C NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ 16.6 (*q*, C-14), 17.0 (*q*, C-15), 20.6 (*q*, C-12), 26.8 (*t*, C-2 or C-3), 34.3 (*t*, C-8), 35.1 (*t*, C-6), 40.2 (*t*, C-3 or C-2), 42.3 (*t*, C-9), 52.1 (*d*, C-7), 108.1 (*t*, C-13), 126.9 (*d*, C-1), 128.8 (*s*, C-4), 131.9 (*d*, C-5), 137.7 (*s*, C-10), 153.1 (*s*, C-11); MS (EI, 70eV), *m/z* (rel. int.): 204 [M<sup>+</sup>] (10), 189 (25), 175 (8), 161 (28), 147 (32), 133 (25), 121 (42), 107 (59), 93 (84), 81 (82), 68 (100), 53 (57), 41 (82).

### 7.3.10. Isolation and Characterisation of Germacrene B (33)

**33** was isolated simultaneously with **32** by using same method since both are present in the same fraction. The NMR data of the **33** were recorded in C<sub>6</sub>D<sub>6</sub> in which all the methyls and the methine signals are well resolved.

Colourless oil; RI<sub>CPSIL 5</sub> = 1555; R<sub>f</sub> (penatne) = 0.45; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 1.46 (3H, *s*, H-14/15), 1.48 (3H, *s*, H-15/14), 1.63 (3H, *s*, H-12/13), 1.67 (3H, *s*, H-13/12), 1.93-2.27 (9H, *m*, H-2ab, 3ab, 9ab, 8ab, 6a), 2.53 (1H, *br.s*, H-6b), 4.67 (1H, *br.s*, H-1/5), 4.76 (1H, *br.d*, *J* = 12.0 Hz, H-5/1); <sup>13</sup>C NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): 16.7 (*q*, C-14/15), 16.7 (*q*, C-15/14), 20.6 (*q*, C-12/13), 20.7 (*q*, C-13/12), 26.5 (*t*, C-2), 32.8 (*t*, C-6), 39.3 (*t*, C-3 / C-8), 39.5 (*t*, C-3 / C-8), 41.3 (*t*, C-9), 126.3 (*s*, C-7/11), 126.9 (*d*, C-5/1), 128.3 (*d*, C-1/5), 131.6 (*s*, C-10/4), 133.9 (*s*, C-11/7), 136.8 (*s*, C-4/10); MS (EI, 70eV), *m/z* (rel. int.): 204 [M<sup>+</sup>] (28), 189 (20), 175 (5), 161 (31), 147 (19), 133 (27), 121 (100), 107 (53), 105 (51), 93 (69), 81 (47), 67 (55), 53 (48), 41 (85). The <sup>1</sup>H-NMR (CDCl<sub>3</sub>) of (**33**)<sup>[202]</sup> and the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of the (*E,Z*)-isomer of (**33**) have been reported before.<sup>[203]</sup>

## 7.4. Chemical Analysis of *Diplophyllum albicans*

The essential oil of macerated fresh plant materials of *Diplophyllum albicans*, collected in June 2002 from Altenau in Germany, was obtained by hydrodistillation. All isolations were carried out using prep. TLC, SE-30- and/or SE-52-columns consecutively with at least one cyclodextrin phase column.

### 7.4.1. (+)-(7*S*, 8*S*, 10*S*)-Eudesma-4,11-diene-8*a*-ol (147)

Colourless oil; RI<sub>CPSIL 5</sub> = 1648; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 1.25-1.31 (2H, *m*, H-1a, H-9a), 1.47-1.55 (2H, *m*, H-2a, H-1b), 1.49 (3H, *s*, H-14), 1.53 (3H, *s*, H-12), 1.58 (3H, *s*, H-15), 1.64-1.68 (1H, *m*, H-2b), 1.82-1.95 (3H, *m*, H-7, H-3a, H-3b), 2.01 (1H, *dd*, H-9b, *J* = 2.5, 14.2 Hz), 2.35-2.41 (1H, *m*, H-6a), 2.41-2.49 (1H, *m*, H-6b), 3.86 (1H, *d*, H-8, *J* = 2.2 Hz), 4.79 (1H, *s*, H-15a), 4.83 (1H, *s*, H-15b); <sup>13</sup>C NMR (125.7

MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  19.3 (*t*, C-2), 19.6 (*q*, C-15), 22.8(*q*, C-12), 24.8 (*t*, C-6), 28.2 (*q*, C-14), 33.6 (*t*, C-3), 34.8 (*s*, C-10), 41.4 (*t*, C-1), 48.1 (*t*, C-9), 50.7 (*d*, C-7), 67.5 (*d*, C-8), 111.6 (*t*, C-13), 124.8 ( $\delta$ , C-4), 135.7 ( $\delta$ , C-5), 147.7 ( $\delta$ , C-11); MS (EI, 70eV) *m/z* (rel.int.): 220 [M<sup>+</sup>] (28), 205 (10), 202 (21), 187 (60), 177 (22), 161(100), 147(28), 133 (29), 123 (36), 105 (68), 91(59), 79 (42), 67 (20), 55 (36).

### 7.5. Chemical Analysis of *Marsupella emarginata*

The essential oils of *Marsupella emarginata* collected in early March 2001 near Altenau, Harz mountains, (Germany), were prepared by hydrodistillation (2 h) of aqueous homogenates of fresh and green or air-dried plants using *n*-hexane as collection solvent.

#### 7.5.1. (1*S*\*,5*R*\*,6*S*\*,7*R*\*,10*R*\*)-Lemnalone (28)

Colourless oil; RI<sub>CPSIL 5</sub> = 1616; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  0.62 (3H, *s*), 0.76 (3H, *d*, *J* = 6.3 Hz), 0.78 (3H, *d*, *J* = 6.6 Hz), 1.33-1.45 (6H, *m*), 1.55 (1H, *s*), 1.65 (1H, *s*), 2.33(1H, *dd*, *J* = 2.8, 19.5 Hz), 2.53 (1H, *dd*, *J* = 2.8, 19.6 Hz), 2.56 (1H, *d*, *J* = 7.3), 4.80 (1H, *d*, *J* = 1.9 Hz), 6.21 (1H, *d*, *J* = 2.2 Hz); MS (EI 70eV), *m/z* (rel. int.): 218 [M<sup>+</sup>] (18), 203 (7), 185 (3), 175 (55), 161 (12), 147 (24), 134 (100), 119 (22), 105 (38), 91 (56), 79 (32), 77 (33), 69 (18), 55 (30), 41(43).

#### 7.5.2. (-)-**b**-Longipinene (92)

Colourless oil; RI<sub>CPSIL 5</sub> = 1405 ; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  (ppm) = 0.78 (*s*, 3H, CH<sub>3</sub>-14), 0.87 (*s*, 3H, CH<sub>3</sub>-12), 0.92 (*s*, 3H, CH<sub>3</sub>-13), 1.32-1.37 (*m*, 2H, H-10), 1.45-1.48 (*m*, 2H, H-8), 1.50-1.54 (*m*, 3H, H-1, H-9), 1.73 (*ddd*, 1H, H-5a, *J* = 2.3, 4.1, 10.9 Hz), 1.80(*ddd*, 1H, H-5b, *J* = 2.3, 6.6, 11.7 Hz), 1.88-1.94(*m*, 1H, H-6), 2.2(*ddt*, 1H, H-4a, *J* = 2.0, 10.6, 20.1 Hz), 2.47-2.55(*m*, 1H, H-4b), 2.57(*d*, 1H, H-2, *J* = 5.9 Hz), 4.70-4.73(*m*, 2H, H-15) ; <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 22.0 (*t*, C-9), 24.1 (*t*, C-4), 24.4 (*q*, C-14), 26.2 (*t*, C-5), 28.0 (*q*, C-12), 28.5 (*q*, C-13), 32.9 (*s*, C-11), 39.5 (*d*, C-6), 39.8 (*t*, C-10), 41.7 (*t*, C-8), 42.5 (*s*, C-7), 51.4 (*d*, C-2), 53.1(*d*, C-1), 105.6 (*t*, C-15), 153.7 (*s*, C-3).

#### 7.5.3. (-)-(4*S*,5*R*,7*S*)-7-*epi*-Eremophila-1(10),8,11-triene (173)

Colourless oil; RI<sub>CPSi15</sub> = 1507; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 0.80 (*d*, 3H, CH<sub>3</sub>-15, *J* = 6.6 Hz), 0.94 (*s*, 3H, CH<sub>3</sub>-14), 1.20-1.29 (*m*, 1H, H-6a),

1.31-1.40 (*m*, 2H, H-3a, H-4), 1.45-1.53 (*m*, 1H, H-3b), 1.68 (*s*, 3H, CH<sub>3</sub>-13), 1.90 (*dd*, 1H, H-6b, *J* = 4.4, 11.3 Hz), 2.01 (*br. d*, 2H, H-2, *J* = 4.0 Hz), 2.99 (*br. d*, 1H, H-7, *J* = 11.7 Hz), 4.84 (*t*, 1H, H-12a, *J* = 1.6 Hz), 4.92 (*s*, 1H, H-12b), 5.41 (*t*, 1H, H-1, *J* = 3.5 Hz), 5.56 (*d*, 1H, H-8, *J* = 9.8 Hz), 6.08 (*dd*, 1H, H-9, *J*<sub>1</sub> = 2.5, *J*<sub>2</sub> = 9.8 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 15.7 (*q*, C-15), 17.9 (*q*, C-14), 20.4 (*q*, C-13), 26.0 (*t*, C-2), 27.1 (*t*, C-3), 36.2 (*s*, C-5), 38.9 (*d*, C-4), 40.7 (*t*, C-6), 42.2 (*d*, C-7), 110.7 (*t*, C-12), 124.6 (*d*, C-1), 128.1 (*d*, C-8), 130.0 (*d*, C-9), 141.4 (*s*, C-10), 149.4 (*s*, C-11); MS (EI, 70 eV), *m/z* (rel. int.): 202 [M<sup>+</sup>] (100), 187 (59), 173 (15), 159 (38), 145 (97), 131 (91), 117 (55), 105 (71), 91 (90), 77 (47), 65 (24), 55 (29), 41 (78).

#### 7.5.4. (-)-4-*epi*-Marsupellol (174)

Colourless oil; RI<sub>CPSil5</sub> = 1614; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 0.78 (*s*, 3 H), 0.82 (*s*, 3 H), 0.85 (*s*, 3 H), 1.15 (*br.d*, 1 H, *J* = 6.0 Hz), 1.28- 1.33 (*m*, 2 H), 1.43-1.47 (*m*, 4 H), 1.71 (*ddd*, 1 H, *J*<sub>1</sub> = 1.9, *J*<sub>2</sub> = 6.3, *J*<sub>3</sub> = 11.7 Hz), 1.88-1.91 (*m*, 1 H), 2.24-2.29 (*m*, 1 H), 2.60 (*br.d*, 1 H, *J* = 6.0 Hz), 4.44-4.47 (*m*, 1 H), 4.82 (*br.s*, 1 H), 5.10 (*t*, 1 H, *J* = 1.9 Hz); MS (EI, 70 eV), *m/z* (rel. int.): 220 [M<sup>+</sup>] (2), 202 (14), 187 (17), 175 (8), 159 (24), 145 (20), 135 (26), 119 (25), 105 (39), 95 (41), 91 (63), 81 (54), 77 (46), 67 (48), 55 (62), 41 (100).

#### 7.5.5. (-)-Marsupellyl acetate (175)

Colourless oil; RI<sub>CPSil 5</sub> = 1673; R<sub>f</sub> = 0.93; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 0.60 (*s*, 3 H, CH<sub>3</sub>-14), 0.89 (*s*, 3 H, CH<sub>3</sub>-12), 0.92 (*s*, 3 H, CH<sub>3</sub>-13), 1.29-1.32 (*m*, 2 H, H-10), 1.40 (*br.t*, 2 H, H-8, *J* = 5.7 Hz), 1.44-1.48 (*m*, 2 H, H-9), 1.70 (*s*, 3 H, CH<sub>3</sub>CO-), 1.77-1.81 (*m*, 2 H, H-5a, H-1), 1.82-1.85 (*m*, 1 H, H-6), 2.41 (*ddd*, 1 H, H-5b, *J* = 2.2, 8.8, 14.8 Hz), 2.54 (*d*, 1 H, H-2, *J* = 5.7 Hz), 4.86 (*s*, 1 H, H-15a), 5.17 (*s*, 1 H, H-15b), 5.90 (*dd*, 1 H, H-4, *J* = 1.3, 8.8 Hz); <sup>13</sup>C-NMR (127.5 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  = 21.2 (*q*, CH<sub>3</sub>CO-), 22.0 (*t*, C-9), 24.3 (*q*, C-14), 27.9 (*q*, C-12), 28.3 (*q*, C-13), 32.9 (*s*, C-11), 36.8 (*t*, C-5), 38.9 (*d*, C-6), 39.7 (*t*, C-10), 41.3 (*t*, C-8), 42.1 (*s*, C-7), 50.5 (*d*, C-2), 54.1 (*d*, C-1), 68.7 (*d*, C-4), 112.3 (*t*, C-15), 153.2 (*s*, C-3), 170.0 (*s*, CH<sub>3</sub>CO-); MS (EI, 70 eV), *m/z* (rel. int.): 262 [M<sup>+</sup>] (1), 247 (2), 220 (6), 202 (10), 187 (13), 177 (10), 159 (21), 145 (19), 132 (27), 118 (35), 105 (34), 91 (36), 79 (19), 69 (27), 55 (34), 43 (100); MS (CI, NH<sub>3</sub>), *m/z* (rel. int.): 280 [M+NH<sub>4</sub>]<sup>+</sup> (1), 263 [M+1] (3), 249 (2), 233 (2), 229 (2), 220 (27), 203 (100), 187 (10), 177 (7), 159 (15), 143 (19), 133 (27), 119 (54), 105 (30), 95 (24), 91 (25), 78 (27), 69 (7), 65 (7), 60 (6).



### 7.5.6. (-)-4-*epi*-Marsupellyl acetate (176)

Colourless oil;  $RI_{CPSi15} = 1731$ ;  $R_f = 0.94$ ; sense of optical rotation (benzene): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta = 0.78$  (s, 3 H, CH<sub>3</sub>-13), 0.83 (s, 3 H, CH<sub>3</sub>-12), 0.87 (s, 3 H, CH<sub>3</sub>-14), 1.27-1.31 (m, 2 H, H-10), 1.42-1.45 (m, 4 H, H-9, H-8), 1.48 (br.s, 1 H, H-1), 1.75 (s, 3 H, CH<sub>3</sub>CO-), 1.78 (ddd, 1 H, H-5a,  $J_1 = 1.9$ ,  $J_2 = 6.0$ ,  $J_3 = 13.6$  Hz), 1.86 (br.t, 1 H, H-6,  $J = 5.0$  Hz), 2.56-2.59 (m, 1 H, H-5b), 2.61 (d, 1 H, H-2,  $J = 6.3$  Hz), 4.85 (d, 1 H, H-15a,  $J = 1.3$  Hz), 5.02 (dd, 1 H, H-15b,  $J_1 = 0.6$ ,  $J_2 = 1.9$  Hz), 5.99-6.03 (m, 1 H, H-4);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ ):  $\delta = 21.0$  (q, CH<sub>3</sub>CO-), 21.8 (t, C-9), 23.4 (q, C-14), 27.9 (q, C-13), 28.4 (q, C-12), 32.9 (s, C-11), 34.9 (t, C-5), 39.4 (d, C-6), 39.7 (t, C-10), 41.9 (t, C-8), 43.6 (s, C-7), 50.5 (d, C-2), 52.5 (d, C-1), 68.0 (d, C-4), 107.5 (t, C-15), 152.0 (s, C-3), 170.1 (s, CH<sub>3</sub>CO-); MS (EI, 70 eV),  $m/z$  (rel. int.): 262 [M<sup>+</sup>] (2), 247 (2), 220 (3), 202 (9), 187 (11), 177 (5), 159 (16), 145 (13), 131 (22), 118 (24), 105 (25), 91 (35), 77 (18), 69 (19), 55 (27), 43 (100); MS (CI, NH<sub>3</sub>),  $m/z$  (rel. int.): 280 [M+NH<sub>4</sub>]<sup>+</sup> (1), 263 [M+1] (5), 233 (1), 220 (14), 203 (100), 187 (7), 177 (4), 159 (10), 147 (13), 133 (19), 119 (37), 105 (19), 95 (17), 91 (16), 78 (11), 65 (4), 60 (6).

### 7.5.7. (+)-5a-Hydroxymarsupellyl acetate (177)

Colourless oil;  $RI_{CPSi15} = 1856$ ;  $R_f = 0.55$ ; sense of optical rotation (benzene): (+);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta = 0.79$  (s, 3 H, CH<sub>3</sub>-12), 0.83 (s, 3 H, CH<sub>3</sub>-13), 1.10 (s, 3 H, CH<sub>3</sub>-14), 1.22 (br.s, 1 H, H-1), 1.27 (br.t, 2 H, H-10,  $J = 5.0$  Hz), 1.40-1.49 (m, 3 H, H-9, H-8a), 1.56-1.61 (m, 1 H, H-8b), 1.62 (s, 3 H, CH<sub>3</sub>CO-), 2.45 (br.t, 1 H, H-6,  $J = 4.1$  Hz), 2.57 (d, 1 H, H-2,  $J = 5.7$  Hz), 3.99 (s, 1 H, H-5), 4.93 (s, 1 H, H-15a), 4.97 (t, 1 H, H-15b,  $J = 1.3$  Hz), 5.44 (d, 1 H, H-4,  $J = 1.9$  Hz);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ ):  $\delta = 19.3$  (q, CH<sub>3</sub>CO-), 20.5 (t, C-9), 24.6 (q, C-14), 26.3 (q, C-12), 26.6 (q, C-13), 31.1 (s, C-11), 38.1 (t, C-10), 39.9 (s, C-7), 40.6 (t, C-8), 44.3 (d, C-6), 49.9 (d, C-2), 52.2 (d, C-1), 79.6 (d, C-4), 80.1 (d, C-5), 109.5 (t, C-15), 149.0 (s, C-3), 173.1 (s, CH<sub>3</sub>CO-); MS (EI, 70 eV),  $m/z$  (rel. int.): 236 (7), 218 (11), 203 (7), 189 (15), 175 (8), 161 (7), 147 (10), 133 (12), 125 (20), 119 (14), 105 (21), 91 (27), 77 (22), 69 (17), 55 (27), 43 (100); MS (CI, *iso*-butane),  $m/z$  (rel. int.): 279 [MH<sup>+</sup>] (1), 261 (16), 236 (11), 219 (100), 201 (99), 189 (15), 175 (11), 161 (12), 145 (23), 135 (20), 119 (18), 109 (26), 95 (31), 81 (19), 69 (32), 61 (22).

## 7.6. Chemical Analysis of *Marsupella emarginata* (Saarland)

The essential oil was obtained by hydrodistillation.

### 7.6.1. (-)-Gymnomitr-3(15)-en-4b-ol (68)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1659$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.76 (6H, *s*, H-13, H-14), 0.92 (3H, *s*, H-12), 0.95-1.00 (1H, *m*, H-8a), 1.09 (1H, *dd*, H-5a,  $J = 10.4, 13.2$  Hz), 1.13-1.17 (1H, *m*, H-10a), 1.33 (1H, *d*, H-1a,  $J = 11.4$  Hz), 1.63-1.80 (4H, *m*, H-9a, H-9b, H-8b, H-10b), 1.91 (1H, *ddd*, H-1b,  $J = 3.2, 4.7, 11.7$  Hz), 2.07 (1H, *ddd*, H-5b,  $J = 2.8, 7.6, 13.2$  Hz), 2.27 (1H, *d*, H-2,  $J = 4.4$  Hz), 4.41 (1H, *br. t*, H-4), 4.83 (1H, *t*, H-15a,  $J = 2.2$  Hz), 5.19 (1H, *t*, H-15b,  $J = 2.2$  Hz);  $^{13}\text{C NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  23.3 (*q*, C-14), 24.2 (*q*, C-13), 27.7 (*t*, C-9), 27.8 (*q*, C-12), 36.0 (*t*, C-8), 37.5 (*t*, C-10), 45.3 (*s*, C-6), 47.2 (*t*, C-1), 50.0 (*t*, C-5), 54.6 (*s*, C-7), 55.4 (*s*, C-11), 56.7 (*d*, C-2), 68.0 (*d*, C-4), 107.5 (*t*, C-15), 154.4 (*s*, C-3); MS (EI, 70eV),  $m/z$  (rel. int.): 220 [ $\text{M}^+$ ] (3), 202 (7), 187 (5), 177 (2), 159 (4), 150 (4), 137 (8), 123 (100), 106 (87), 96 (90), 91(91), 81(84), 67 (22), 55 (39), 41 (42).

### 7.6.2. (-)-Gymnomitr-3(15)-4-diene (166)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1408$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.80 (3H, *s*, H-13), 0.87 (3H, *s*, H-14), 0.99 (3H, *s*, H-12), 1.02-1.08 (1H, *m*, H-8a), 1.17-1.23 (1H, *m*, H-10a), 1.50-1.56 (1H, *m*, H-9a), 1.58 (1H, *d*, H-1a,  $J = 10.7$  Hz), 1.68-1.74 (1H, *m*, H-9b), 1.80-1.85 (2H, *m*, H-1b, H-8b), 1.86-1.92 (1H, *m*, H-10b), 2.29 (1H, *d*, H-2,  $J = 4.4$  Hz), 4.68 (1H, *br.s*, H-15a), 4.80 (1H, *d*, H-15b,  $J = 2.5$  Hz), 5.55 (1H, *d*, H-5,  $J = 8.8$  Hz), 5.97 (1H, *d*, H-4,  $J = 9.5$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 202 [ $\text{M}^+$ ] (8), 106 (88), 91 (100), 81 (28), 65 (12), 53 (11), 41 (20).

### 7.6.3. 15-nor-3-Gymnomitrone (179)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1612$ ;  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.69 (3H, *s*), 0.71 (3H, *s*), 0.83 (3H, *s*), 1.13-1.18 (3H, *m*), 1.30-1.36 (1H, *m*), 1.33 (1H, *d*,  $J = 12.3$  Hz), 1.47-1.55 (3H, *m*), 1.67-1.75 (2H, *m*); 2.12 (1H, *dd*,  $J = 8.0, 9.1$  Hz), 2.16 (1H, *dd*,  $J = 3.8, 10.4$  Hz), 2.23 (1H, *d*,  $J = 4.4$  Hz);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.96 (3H, *s*), 1.00 (3H, *s*), 1.08 (3H, *s*), 1.17-1.24 (2H, *m*), 1.13-1.38 (2H, *m*), 1.59-1.67 (2H, *m*), 1.71 (1H, *d*,  $J = 12.6$  Hz), 1.79-1.87 (2H, *m*), 1.88-1.97 (1H, *m*), 2.09 (1H, *dt*,  $J = 4.1, 7.3$  Hz), 2.23 (1H, *d*,  $J = 4.4$  Hz), 2.33-2.40 (1H, *m*); MS (EI, 70eV),  $m/z$  (rel. int.): 206 [ $\text{M}^+$ ] (9), 191 (10), 188 (16), 177 (4), 163 (7), 149 (5), 137

(22), 121 (19), 111 (26), 110 (48), 109 (40), 96 (61), 95 (100), 94 (36), 81 (52), 67 (28), 55 (48), 41 (59).

#### **7.6.4. (-)-4b-Acetoxygymnomitr-3(15)-ene (180)**

Colourless oil;  $RI_{\text{CPSIL } 5} = 1723$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.75 (6H, *s*, H-13, H-14), 0.90 (3H, *s*, H-12), 1.08 (1H, *dd*, H-8a,  $J = 6.9, 12.9$  Hz), 1.20 (1H, *dd*, H-10a,  $J = 6.9, 13.2$  Hz), 1.35 (1H, *dd*, H-5a,  $J = 10.4, 12.9$  Hz), 1.40 (1H, *d*, H-1a,  $J = 11.7$  Hz), 1.64-1.73 (1H, *m*, H-9a), 1.75 (3H, *s*, H-17), 1.81-1.87 (1H, *m*, H-9b), 1.92 (1H, *ddd*, H-1b,  $J = 1.8, 4.7, 11.7$  Hz), 1.99 (1H, *dt*, H-10b,  $J = 6.9, 13.2$  Hz), 2.11 (1H, *dt*, H-8b,  $J = 6.9, 13.2$  Hz), 2.26-2.31 (1H, *m*, H-5b), 2.30 (1H, *d*, H-2,  $J = 4.7$  Hz), 4.84 (1H, *t*, H-15a,  $J = 2.2$  Hz), 5.01 (1H, *t*, H-15b,  $J = 2.2$  Hz), 6.04 (1H, *ddt*, H-4,  $J = 2.2, 4.7, 10.1$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  20.6 (*q*, C-17), 23.3 (*q*, C-13), 24.1 (*q*, C-14), 27.7 (*t*, C-9), 27.7 (*q*, C-12), 36.3 (*t*, C-8), 37.7 (*t*, C-10), 45.2 (*s*, C-6), 45.4 (*t*, C-5), 47.0 (*t*, C-1), 54.8 (*s*, C-7), 55.8 (*s*, C-11), 56.9 (*d*, C-2), 70.4 (*d*, C-4), 107.6 (*t*, C-15), 149.2 (*s*, C-3), 170.1 (*s*, C-16); MS (EI, 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ] (2), 247 (1), 220 (16), 202 (19), 187 (10), 159 (7), 145 (6), 123 (35), 106 (100), 96 (39), 95 (34), 91 (57), 81(26), 55 (11), 43 (28).

#### **7.6.5. (-)-4b,5b-Diacetoxygymnomitr-3(15)-ene (181)**

Colourless oil;  $RI_{\text{CPSIL } 5} = 1943$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.74 (3H, *s*, H-13), 0.78 (3H, *s*, H-14), 0.86 (3H, *s*, H-12), 1.01 (1H, *dd*, H-8a,  $J = 7.3, 13.6$  Hz), 1.21 (1H, *dd*, H-10a,  $J = 6.9, 12.3$  Hz), 1.67-1.72 (2H, *m*, H-1a, H-9a), 1.76 (3H, *s*, H-19), 1.80 (3H, *s*, H-17), 1.92-2.04 (2H, *m*, H-10b, H-9b), 2.10 (1H, *d*, H-1b,  $J = 12.0$  Hz), 2.25-2.32 (1H, *m*, H-8b,  $J = 6.9$  Hz), 2.28 (1H, *d*, H-2,  $J = 4.8$  Hz), 4.87 (1H, *t*, H-15a,  $J = 2.2$  Hz), 5.03 (1H, *t*, H-15b,  $J = 2.2$  Hz), 5.48 (1H, *d*, H-5,  $J = 4.1$  Hz), 6.27-6.29 (1H, *m*, H-4);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 200 (7), 185 (4), 164 (10), 153 (11), 122 (52), 105 (10), 96 (21), 95 (32), 91(17), 81(22), 67 (8), 55 (11), 43 (100); MS(CI,  $\text{NH}_3$  gas),  $m/z$  (rel. int.): 338 [ $\text{M}^+\text{NH}_4^+$ ] (75), 261(33), 236 (7), 219 (67), 201(25), 185 (5), 164 (10), 153 (12), 122 (100), 106 (84), 96 (64), 95 (76), 91(57), 81 (34).

#### **7.6.6. (+)-5b-Acetoxygymnomitr-3(15)-ene (182)**

Colourless oil;  $RI_{\text{CPSIL } 5} = 1755$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.79 (3H, *s*, H-13), 0.83 (3H, *s*, H-14), 0.89-0.94 (1H, *m*, H-8a), 0.92 (3H, *s*, H-15),

1.12-1.18 (1H, *m*, H-10a), 1.64-1.69 (3H, *m*, H-9, H-10b), 1.70-1.75 (2H, *m*, H-1a, H-8b), 1.71 (3H, *s*, H-17), 2.05 (1H, *d*, H-1b,  $J = 11.7$  Hz), 2.18 (1H, *d*, H-2,  $J = 4.7$  Hz), 2.38 (1H, *d*, H-4a,  $J = 18.0$  Hz), 2.73 (1H, *ddd*, H-4b,  $J = 2.5, 5.7, 18.0$  Hz), 4.68 (1H, *t*, H-15a,  $J = 2.2$  Hz), 4.74 (1H, *t*, H-15b,  $J = 2.5$  Hz), 5.10 (1H, *d*, H-5,  $J = 5.7$  Hz);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ] (2), 202 (10), 187 (6), 173 (2), 166 (2), 159 (4), 153 (6), 145 (4), 131 (6), 124 (6), 115 (4), 106 (100), 96 (36), 95 (54), 91 (94), 81(38), 67 (15), 55 (24), 43 (74).

#### **7.6.7. (-)-3b, 15b-Epoxy-4b-acetoxygymnomitrane (183)**

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1875$ ; sense of optical rotation (benzene): (-);  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.72 (3H, *s*, H-13), 0.73 (3H, *s*, H-14), 0.82 (3H, *s*, H-12), 1.09 (1H, *dd*, H-8a,  $J = 6.9, 13.2$  Hz), 1.18 (1H, *dd*, H-10a,  $J = 7.6, 14.8$  Hz), 1.19(1H, *d*, H-2,  $J = 4.7$  Hz), 1.50-1.58(1H, *m*, H-5a), 1.68 (3H, *s*, H-17), 1.68-1.80 (2H, *m*, H-1a, H-9a), 1.89 (1H, *d*, H-1b,  $J = 11.4$  Hz), 1.88-1.92 (1H, *m*, H-9b), 2.04-2.18 (3H, *m*, H-8b, H-10b, H-5b), 2.27 (1H, *d*, H-15a,  $J = 5.0$  Hz), 2.60 (1H, *d*, H-15b,  $J = 5.0$  Hz), 5.85 (1H, *dd*, H-4,  $J = 7.6, 11.4$  Hz);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 278 [ $\text{M}^+$ ] (2), 236 (2), 218 (8), 203 (4), 188 (4), 175 (4), 162 (4), 147 (6), 133 (5), 122 (22), 107 (29), 96 (91), 95 (100), 91(48), 81(79), 79 (31), 77 (30), 67 (18), 60 (10), 55 (35), 43 (56).

#### **7.6.8. (-)-3a, 15a-Epoxy-4b-acetoxygymnomitrane (184)**

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1887$ ; sense of optical rotation (benzene): (-);  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.73 (3H, *s*, H-13), 0.74 (3H, *s*, H-14), 0.87 (3H, *s*, H-12), 1.09 (1H, *dd*, H-8a,  $J = 6.9, 13.6$  Hz), 1.16-1.21 (2H, *m*, H-5a, H-2), 1.29 (1H, *d*, H-1a,  $J = 11.7$  Hz), 1.41 (1H, *dd*, H-10a,  $J = 7.6, 13.6$  Hz), 1.60 (3H, *s*, H-17), 1.67-1.75 (1H, *m*, H-9a), 1.82 (1H, *ddd*, H-1b,  $J = 3.2, 5.0, 11.7$  Hz), 1.91 (1H, *p*, H-9b,  $J = 6.9$  Hz), 2.09 (1H, *d*, H-15a,  $J = 5.7$  Hz), 2.17 (1H, *dt*, H-8b,  $J = 6.3, 12.9$  Hz), 2.30 (1H, *ddd*, H-5b,  $J = 3.2, 7.6, 13.2$  Hz), 2.65 (1H, *dt*, H-10b,  $J = 6.9, 13.9$  Hz), 2.90 (1H, H-15b, *d*,  $J = 5.7$  Hz), 5.95 (1H, *dd*, H-4,  $J = 7.6, 10.7$  Hz);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 236 (2), 218 (12), 203 (5), 188 (4), 181 (2), 175 (5), 161 (5), 147 (8), 133 (8), 122 (28), 107 (35), 96 (100), 95 (94), 91 (44), 81(79), 79 (42), 77 (31), 67 (18), 60 (13), 55 (34), 43 (53); MS (CI,  $\text{NH}_3$  gas),  $m/z$  (rel. int.): 279[ $\text{M}^+ + 1$ ](12), 236 (25), 219 (100), 205 (17), 122 (7), 106 (17), 96 (37), 95 (52), 81(22).

### 7.6.9. (-)-15-Acetoxygymnomitr-3-ene (185)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1784$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.78 (3H, *s*, H-13), 0.83 (3H, *s*, H-14), 0.94 (3H, *s*, H-12), 1.00 (1H, *dd*, H-8a,  $J = 6.9, 12.0$  Hz), 1.14-1.20 (1H, *m*, H-10a), 1.42 (1H, *d*, H-1a,  $J = 9.5$  Hz), 1.51-1.64 (3H, *m*, H-9, H-8b), 1.70 (3H, *s*, H-17), 1.78-1.87 (4H, *m*, H-2, H-1b, H-5a, H-10b), 2.10 (1H, *br d*, H-5b,  $J = 18.9$  Hz), 4.51(2H, *d*, H-15,  $J = 8.8$  Hz), 5.41 (1H, *br.s*, H-4);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ] (3), 220 (7), 202 (9), 187 (5), 159 (5), 136 (4), 131 (6), 121 (6), 106 (100), 95 (70), 91 (85), 81 (38), 67 (16), 55 (30), 43 (73).

### 7.6.10. (+)-**a**-Barbatenal (186)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1659$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.70 (3H, *s*, H-14), 0.72 (3H, *s*, H-13), 0.85-0.92 (1H, *m*, H-8a), 0.97 (3H, *s*, H-12), 1.05 (1H, *d*, H-1a,  $J = 11.5$  Hz), 1.12-1.21 (2H, *m*, H-9a, H-10a), 1.32-1.45 (2H, *m*, H-8b, H-9b), 1.50-1.55 (1H, *m*, H-10b), 1.65 (1H, *dd*, H-5a,  $J = 3.3, 21.1$ Hz), 1.71(1H, *dd*, H-1b,  $J = 4.6, 11.5$  Hz), 2.04 (1H, *dd*, H-5b,  $J = 2.8, 20.1$  Hz), 2.78 (1H, *d*, H-2,  $J = 4.3$  Hz), 5.87 (1H, H-4, *br.t*,  $J = 3.31$  Hz), 9.30 (1H, *s*, H-15);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 3; MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (8), 200 (4), 189 (3), 175(3), 161(3), 147 (6), 133 (5), 124 (16), 122(14), 107 (22), 96 (95), 95 (100), 81(75), 79 (33), 77 (36), 67 (17), 55 (32), 41 (48).

### 7.6.11. (+)-12-Acetoxygymnomitr-3(15)-ene (187)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1794$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.72 (3H, *s*, H-14), 0.77 (3H, *s*, H-13), 0.91-0.96 (1H, *m*, H-8a), 1.30 (1H, *d*, H-1a,  $J = 12.0$  Hz), 1.52-1.58 (3H, *m*, H-5, H-10a), 1.65-1.81 (4H, *m*, H-9, H-10b, H-8b), 1.70 (3H, *s*, H-17), 1.93 (1H, *ddd*, H-1b,  $J = 2.8, 4.7, 11.7$  Hz), 2.14 (1H, *dd*, H-4a,  $J = 8.2, 16.7$  Hz), 2.28-2.36 (1H, *m*, H-4b), 2.53 (1H, *d*, H-2,  $J = 4.7$  Hz), 4.00 (2H, *br.s*, H-12), 4.74 (1H, *t*, H-15a,  $J = 2.2$  Hz), 4.77 (1H, *t*, H-15b,  $J = 2.5$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 4; MS (EI, 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ], 202 (8), 187 (4), 173 (2), 159 (4), 153 (9), 145 (8), 131 (9), 119 (8), 109 (34), 108 (38), 107 (28), 94 (44), 93 (96), 91 (35), 79 (45), 67 (18), 55 (18), 43 (100).

### 7.6.12. (-)-Gymnomitr-3(15)-en-4-one (188)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1633$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.63 (3H, *s*), 0.68 (3H, *s*), 0.84 (3H, *s*), 1.02-1.08 (1H, *ddd*,  $J = 7.6, 9.8, 17.3$  Hz),

1.15-1.21 (1H, *m*), 1.23 (1H, *d*,  $J = 12.0$  Hz), 1.29-1.36 (2H, *m*), 1.57 (1H, *ddd*,  $J = 4.1, 6.6, 13.2$  Hz), 1.65-1.73 (2H, *m*), 1.88 (1H, *d*,  $J = 19.2$  Hz), 2.21 (1H, *d*,  $J = 4.7$  Hz), 2.63 (1H, *dd*,  $J = 3.5, 19.2$  Hz), 4.75 (1H, *d*,  $J = 2.2$  Hz), 6.16 (1H, *d*,  $J = 2.2$  Hz); MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $M^+$ ] (21), 203 (8), 190 (4), 175 (8), 161(5), 147(5), 133 (6), 122 (44), 107 (32), 96 (100), 81 (78), 79 (68), 77 (37), 67 (22), 53 (36), 41 (44).

#### 7.6.13. (+)-3-Gymnomitren-15-ol (189)

Colourless oil;  $RI_{CPSIL\ 5} = 1687$ ; sense of optical rotation (benzene): (+);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta$  0.65 (1H, *br.s*, OH), 0.81 (3H, *s*, H-13), 0.87 (3H, *s*, H-14), 0.94 (3H, *s*, H-12), 1.00-1.05 (1H, *m*, H-8a); 1.12-1.18 (1H, *m*, H-10a), 1.42 (1H, *d*, H-1a,  $J = 11.0$  Hz), 1.51-1.67 (3H, *m*, H-9, H-8b), 1.72-1.78 (2H, *m*, H-10b, H-2), 1.83-1.89 (2H, *m*, H-1b, H-5a), 2.13 (1H, *br.d*, H-5b,  $J = 18.3$  Hz), 3.78 (2H, *br.s*, H-15), 5.35 (1H, *s*, H-4);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ ):  $\delta$  23.8 (*q*, C-13), 24.9 (*q*, C-14), 27.6 (*t*, C-9), 27.6 (*q*, C-12), 37.5 (*t*, C-8), 38.7 (*t*, C-10), 40.6 (*t*, C-5), 43.0 (*t*, C-1), 44.1 (*s*, C-6), 47.4 (*d*, C-2), 55.7 (*s*, C-7), 58.6 (*s*, C-11), 67.1 (*t*, C-15), 120.6 (*d*, C-4), 144.1 (*s*, C-3); MS (EI, 70eV),  $m/z$  (rel. int.): 220 [ $M^+$ ] (8), 202 (8), 189 (9), 124 (22), 106 (83), 96 (52), 95 (78), 93 (51), 91(100), 81 (58), 67 (22), 55 (37), 41 (48).

#### 7.6.14. Gymnomitr-3(15)-en-5b-ol (190)

Colourless needles;  $RI_{CPSIL\ 5} = 1654$ ; sense of optical rotation (benzene): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta$  0.83 (3H, *s*), 0.89-0.94 (1H, *m*), 0.93 (3H, *s*), 0.94 (3H, *s*), 1.16-1.21 (1H, *m*), 1.64 (1H, *dd*,  $J = 4.7, 12.0$  Hz), 1.68-1.73 (4H, *m*), 1.96 (1H, *d*,  $J = 12.0$  Hz), 2.06 (1H, *d*,  $J = 17.0$  Hz), 2.16 (1H, *d*,  $J = 4.7$  Hz), 2.68 (1H, *ddd*,  $J = 2.5, 5.4, 17.0$  Hz), 3.38 (1H, *s*), 4.69(1H, *t*,  $J = 2.5$  Hz), 4.75 (1H, *t*,  $J = 2.2$  Hz);  $^1H$ -NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.94 (3H, *s*), 0.96 (3H, *s*), 1.05 (3H, *s*), 1.06-1.11 (1H, *m*), 1.19-1.22 (1H, *m*), 1.43 (1H, *d*,  $J = 6.0$  Hz), 1.77 (2H, *d*,  $J = 2.5$  Hz), 1.80-1.90 (3H, *m*), 2.18 (1H, *br.s*), 2.27 (1H, *d*,  $J = 17.0$  Hz), 2.90 (1H, *ddd*,  $J = 2.8, 5.4, 11.0$  Hz), 3.64 (1H, *t*,  $J = 5.4$  Hz), 4.66 (1H, *t*,  $J = 2.2$  Hz), 4.68 (1H, *t*,  $J = 2.5$  Hz); MS (EI, 70eV),  $m/z$  (rel. int.): 202 (5), 187 (2), 159 (2), 153 (4), 145 (2), 129 (4), 124 (10), 115 (8), 106 (88), 96 (30), 95 (39), 91 (100), 81(38), 67 (12), 55 (18), 41 (32).

#### 7.6.15. (+)-Gymnomitr-3(15)-en-12-oic acid (192)

Densed white oil;  $RI_{CPSIL\ 5} = 1796$ ; sense of optical rotation (benzene): (+);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta$  0.77 (3H, *s*, H-14), 1.05 (1H, *dd*, H-8a,  $J = 7.3, 12.6$  Hz), 1.14 (3H, *s*, H-13), 1.30 (1H, *ddd*, H-5a,  $J = 8.2, 12.3, 20.5$  Hz), 1.48 (1H, *d*, H-1a,  $J = 11.7$  Hz), 1.51-1.56 (1H, *m*, H-5b), 1.62-1.69 (1H, *m*, H-10a), 1.76 (1H, *dt*, H-8b,  $J = 6.9, 12.4$  Hz), 1.87 (1H, *m*, H-

9a), 2.08 (1H, *dd*, H-4a,  $J = 8.2, 16.7$  Hz), 2.17 (1H, *dd*, H-10b,  $J = 7.3, 13.6$  Hz), 2.21-2.34 (2H, *m*, H-4b, H-9b), 2.67 (1H, *dd*, H-1b,  $J = 3.2, 4.7, 11.7$  Hz), 3.15 (1H, *d*, H-2,  $J = 4.7$  Hz), 4.68 (2H, *br. s*, H-15);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 4; MS (EI, 70eV),  $m/z$  (rel. int.): 234 [ $\text{M}^+$ ] (2), 188 (3), 173 (3), 159 (2), 145 (4), 127 (32), 109 (100), 108 (64), 107 (44), 93 (80), 91 (46), 81(33), 79 (48), 77 (34), 67 (24), 53 (22), 41 (46).

**7.6.16. (-)-Gymnomitr-3(15)-en-12-al (193)**

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1632$ ; sense of optical rotation (benzene): (-);  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.67 (3H, *s*, H-14), 0.78 (3H, *s*, H-13), 0.90 (1H, *br.dd*, H-8a,  $J = 6.3, 12.9$  Hz), 1.25 (1H, *ddd*, H-5a,  $J = 8.2, 12.3, 20.2$  Hz), 1.36 (1H, *d*, H-1a,  $J = 11.7$  Hz), 1.46-1.53 (2H, *m*, H-10a, H-5b), 1.68 (1H, *dt*, H-8b,  $J = 6.9, 12.3$  Hz), 1.74-1.86 (3H, *m*, H-10b, H-9), 2.02-2.10 (2H, *m*, H-4a, H-1b), 2.15-2.24 (1H, *m*, H-4b), 2.75 (1H, *d*, H-2,  $J = 4.7$  Hz), 4.61 (1H, *t*, H-15a,  $J = 2.2$  Hz), 4.65 (1H, *t*, H-15b,  $J = 2.2$  Hz), 9.32 (1H, *s*, H-12);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ) Table 4; MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (4), 203 (5), 200 (4), 189 (9), 175 (5), 161 (7), 147 (9), 133 (11), 119 (13), 112 (23), 111 (70), 110 (37), 109 (74), 108 (84), 107 (56), 93 (100), 91 (62), 81 (34), 79 (52), 77 (34), 67 (28), 55 (23), 41 (28).

**Table 3.**  $^{13}\text{C}$  NMR spectral data of compounds **166**, **181**, **182**, **183**, **184**, **185**, and **186**; (125.7 MHz,  $\text{C}_6\text{D}_6$ );  $\delta$  (ppm)<sup>a</sup>

C-no.	<b>181</b>	<b>182</b>	<b>166</b>	<b>183</b>	<b>184</b>	<b>185</b>	<b>186</b>
1	41.7 ( <i>t</i> )	41.3 ( <i>t</i> )	44.0 ( <i>t</i> )	43.8 ( <i>t</i> )	45.4 ( <i>t</i> )	43.1 ( <i>t</i> )	41.7 ( <i>t</i> )
2	56.1 ( <i>d</i> )	56.2 ( <i>d</i> )	55.8 ( <i>d</i> )	54.3 ( <i>d</i> )	54.8 ( <i>d</i> )	48.0 ( <i>d</i> )	43.1 ( <i>d</i> )
3	146.8 ( <i>s</i> )	148.6 ( <i>s</i> )	149.0 ( <i>s</i> )	59.0 ( <i>s</i> )	61.9 ( <i>s</i> )	139.4 ( <i>s</i> )	147.5 ( <i>s</i> )
4	69.6 ( <i>d</i> )	36.2 ( <i>t</i> )	128.0 ( <i>d</i> )	65.9 ( <i>d</i> )	67.9 ( <i>d</i> )	124.7 ( <i>d</i> )	149.5 ( <i>d</i> )
5	76.5 ( <i>d</i> )	76.4 ( <i>d</i> )	144.0 ( <i>d</i> )	43.1 ( <i>t</i> )	44.4 ( <i>t</i> )	40.8 ( <i>t</i> )	41.9 ( <i>t</i> )
6	49.7 ( <i>s</i> )	47.4 ( <i>s</i> )	46.7 ( <i>s</i> )	44.9 ( <i>s</i> )	44.4 ( <i>s</i> )	44.2 ( <i>s</i> )	44.7 ( <i>s</i> )
7	55.3 ( <i>s</i> )	55.3 ( <i>s</i> )	56.7 ( <i>s</i> )	54.7 ( <i>s</i> )	55.1 ( <i>s</i> )	56.0 ( <i>s</i> )	55.7 ( <i>s</i> )
8	34.5 ( <i>t</i> )	35.1 ( <i>t</i> )	37.5 ( <i>t</i> )	36.3 ( <i>t</i> )	36.2 ( <i>t</i> )	37.7 ( <i>t</i> )	37.5 ( <i>t</i> )
9	28.2 ( <i>t</i> )	27.8 ( <i>t</i> )	27.1 ( <i>t</i> )	27.7 ( <i>t</i> )	28.0 ( <i>t</i> )	27.7 ( <i>t</i> )	27.3 ( <i>t</i> )
10	38.1 ( <i>t</i> )	38.1 ( <i>t</i> )	39.1 ( <i>t</i> )	36.8 ( <i>t</i> )	37.5 ( <i>t</i> )	38.9 ( <i>t</i> )	39.2 ( <i>t</i> )
11	55.9 ( <i>s</i> )	55.5 ( <i>s</i> )	57.9 ( <i>s</i> )	55.2 ( <i>s</i> )	55.5 ( <i>s</i> )	58.6 ( <i>s</i> )	58.1 ( <i>s</i> )
12	28.3 ( <i>q</i> )	27.8 ( <i>q</i> )	27.7 ( <i>q</i> )	27.9 ( <i>q</i> )	28.3 ( <i>q</i> )	27.9 ( <i>q</i> )	27.4 ( <i>q</i> )
13	24.2 ( <i>q</i> )	24.2 ( <i>q</i> )	23.1 ( <i>q</i> )	23.2 ( <i>q</i> )	23.0 ( <i>q</i> )	24.1 ( <i>q</i> )	24.5 ( <i>q</i> )
14	19.5 ( <i>q</i> )	19.8 ( <i>q</i> )	21.6 ( <i>q</i> )	24.0 ( <i>q</i> )	23.9 ( <i>q</i> )	25.0 ( <i>q</i> )	24.2 ( <i>q</i> )
15	107.9 ( <i>t</i> )	110.1 ( <i>t</i> )	110.1 ( <i>t</i> )	50.3 ( <i>t</i> )	47.9 ( <i>t</i> )	68.4 ( <i>t</i> )	191.7 ( <i>d</i> )
16	169.8 ( <i>s</i> )	169.7 ( <i>s</i> )		170.2 ( <i>s</i> )	170.0 ( <i>s</i> )	169.7 ( <i>s</i> )	
17	20.3 ( <i>q</i> )	21.2 ( <i>q</i> )		20.5 ( <i>q</i> )	20.4 ( <i>q</i> )	20.8 ( <i>q</i> )	
18	170.0 ( <i>s</i> )						
19	20.7 ( <i>q</i> )						

<sup>a</sup>All assignments were confirmed by HMBC and HMQC.



**Table 4.**  $^{13}\text{C}$  NMR spectral data of compounds **187**, **192**, and **193** (125.7 MHz,  $\text{C}_6\text{D}_6$ ); $\delta$  (ppm)<sup>a</sup>

C-no.	<b>187</b>	<b>192</b>	<b>193</b>
1	46.8 ( <i>t</i> )	49.8 ( <i>t</i> )	48.4 ( <i>t</i> )
2	50.2 ( <i>d</i> )	51.3 ( <i>d</i> )	48.3 ( <i>d</i> )
3	150.6 ( <i>s</i> )	150.0 ( <i>s</i> )	149.0 ( <i>s</i> )
4	28.8 ( <i>t</i> )	28.7 ( <i>t</i> )	29.0 ( <i>t</i> )
5	38.2 ( <i>t</i> )	38.3 ( <i>t</i> )	37.9 ( <i>t</i> )
6	43.3 ( <i>s</i> )	43.3 ( <i>s</i> )	42.8 ( <i>s</i> )
7	55.8 ( <i>s</i> )	60.2 ( <i>s</i> )	59.6 ( <i>s</i> )
8	36.0 ( <i>t</i> )	35.2 ( <i>t</i> )	35.1 ( <i>t</i> )
9	28.8 ( <i>t</i> )	31.1 ( <i>t</i> )	30.2 ( <i>t</i> )
10	32.2 ( <i>t</i> )	33.6 ( <i>t</i> )	30.1 ( <i>t</i> )
11	58.7 ( <i>s</i> )	68.4 ( <i>s</i> )	69.1 ( <i>s</i> )
12	68.9 ( <i>t</i> )	184.7 ( <i>s</i> )	203.3 ( <i>d</i> )
13	21.4 ( <i>q</i> )	25.0 ( <i>q</i> )	23.3 ( <i>q</i> )
14	24.5 ( <i>q</i> )	25.0 ( <i>q</i> )	24.3 ( <i>q</i> )
15	108.9 ( <i>t</i> )	110.4 ( <i>t</i> )	110.0 ( <i>t</i> )
16	170.5 ( <i>s</i> )		
17	20.8 ( <i>q</i> )		

<sup>a</sup>All assignments were confirmed by HMBC and HMQC.

## 7.7. Chemical Analysis of *Marsupella aquatica*

The essential oil of *Marsupella aquatica* collected near Gaschurn/Montafon, Austria, in July 2001 at an elevation of 1900m was obtained by hydrodistillation.

### 7.7.1. *ent*-(+)-(1*R*,6*R*,7*S*,10*S*)-Amorpha-4,11-diene (31)

Colourless oil; RI<sub>CPSil 5</sub> = 1480; sense of optical rotation (benzene): (+) ; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 0.88 (*d*, 3 H, CH<sub>3</sub>-14, *J* = 6.6 Hz), 0.88-0.98 (*m*, 1 H, H-9a), 1.61-1.21 (*m*, 1 H, H-1), 1.30-1.47 (*m*, 2 H, H-8a, H-10), 1.48-1.60 (*m*, 2 H, H-2a, H-3a), 1.61 (*s*, 3 H, CH<sub>3</sub>-15), 1.62-1.67 (*m*, 1 H, H-9b), 1.68-1.73 (*m*, 1 H, H-8b), 1.72 (*s*, 3 H, CH<sub>3</sub>-12), 1.78-1.97 (*m*, 3 H, H-3b, H-2b, H-7), 2.57 (*br. s*, 1 H, H-6), 4.81 (*s*, 1 H, H-13a), 5.00 (*dd*, 1 H, H-13b, *J*<sub>1</sub> = 1.3, *J*<sub>2</sub> = 3.3 Hz), 5.34 (*d*, 1 H, H-5, *J* = 1.3 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 20.1 (*q*, C-14), 22.7 (*q*, C-12), 23.8 (*q*, C-15), 26.2 (*t*, C-2), 26.5 (*t*, C-8), 26.7 (*t*, C-3), 28.2 (*d*, C-10), 35.8 (*t*, C-9), 38.0 (*d*, C-6), 42.1 (*d*, C-1), 48.0 (*d*, C-7), 110.4 (*t*, C-13), 121.4 (*d*, C-5), 134.8 (*s*, C-4), 148.2 (*s*, C-11); MS (EI, 70 eV), *m/z* (rel. int.) 204 [M<sup>+</sup>] (70), 189 (58), 175 (11), 162 (30), 147 (25), 133 (23), 121(100), 119 (97), 105 (41), 93 (68), 79 (53), 67 (26), 55 (33), 41 (45).

### 7.7.2. (-)-(1*R*,6*S*,10*S*)-Amorpha-4,7(11)-diene (70)

Colourless oil; RI<sub>CPSil 5</sub> = 1484; sense of optical rotation (chloroform): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 0.89 (*d*, 3 H, CH<sub>3</sub>-14, *J* = 6.3 Hz), 0.95-1.04 (*m*, 1 H, H-9a), 1.28-1.33 (*m*, 1 H, H-1), 1.50-1.60 (*m*, 2 H, H-2a, H-10), 1.61-1.70 (*m*, 2 H, H-9b, H-3a), 1.62 (*s*, 3 H, CH<sub>3</sub>-15), 1.69 (*s*, 3 H, CH<sub>3</sub>-12), 1.72 (*d*, 3 H, CH<sub>3</sub>-13, *J* = 2.2 Hz), 1.81-1.93 (*m*, 3 H, H-8a, H-2b, H-3b), 2.56 (*ddt*, 1H, H-8b, *J*<sub>1</sub> = 3.2, *J*<sub>2</sub> = 6.3, *J*<sub>3</sub> = 13.6 Hz), 3.51(*br.s*, 1 H, H-6), 5.12 (*s*, 1 H, H-5); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ = 0.88 (*d*, *J* = 6.6 Hz), 0.90-0.93 (1H, *m*), 1.24-1.27 (1H, *m*), 1.55-1.59 (2 H, *m*), 1.62 (3 H, *br.s*), 1.63-1.66 (1 H, *m*), 1.67 (3 H, *s*), 1.68 (3 H, *s*), 1.61-1.79 (2 H, *m*), 1.88-1.97 (2 H, *m*), 2.48 (1 H, *br.d*, *J* = 13.2 Hz), 3.36 (1 H, *br.s*), 4.97 (1 H, *s*); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 20.0 (2x *q*, C-12, C-14), 20.4 (*q*, C-13), 23.7 (*q*, C-15), 25.9 (*t*, C-3), 26.0 (*t*, C-2), 27.1 (*t*, C-8), 28.7 (*d*, C-10), 36.3 (*t*, C-9), 40.4 (*d*, C-6), 41.6 (*d*, C-1), 121.0 (*s*, C-11), 126.1 (*d*, C-5), 133.7 (*s*, C-4), 135.7 (*s*, C-7); MS (EI, 70 eV), *m/z* (rel. int.) 204 [M<sup>+</sup>] (67), 189 (41), 175 (5), 161 (100), 147 (18), 133 (26), 119 (45), 105 (56), 91 (45), 81 (67), 77 (31), 67 (17), 55 (33), 41 (59).

### 7.7.3. (-)-Myltayl-4(12)-ene (198)

Colourless oil;  $RI_{\text{CPSiL } 5} = 1455$ ; sense of optical rotation (chloroform): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 0.77$  (s, 3 H,  $\text{CH}_3$ -14), 0.91 (s, 3 H,  $\text{CH}_3$ -13), 0.92 (s, 3 H,  $\text{CH}_3$ -15), 1.06-1.22 (m, 3 H, H-1a, H-8a, H-10a), 1.33-1.52 (m, 4 H, H-2a, H-8b, H-9a, H-10b), 1.53-1.69 (m, 2 H, H-1b, H-9b), 1.77 (br.d, 1 H, H-5a,  $J = 16.4$  Hz), 1.83-1.90 (m, 1 H, H-2b), 2.05 (d, 1 H, H-3,  $J = 4.4$  Hz), 2.47 (d, 1 H, H-5b,  $J = 16.4$  Hz), 4.69 (d, 1 H, H-12a,  $J = 1.6$  Hz), 4.90 (d, 1 H, H-12b,  $J = 1.5$  Hz);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.80$  (3 H, s), 0.96 (3 H, s), 1.01 (3 H, s), 1.13-1.34 (3 H, m), 1.37-1.69 (4 H, m), 1.72-2.20 (5 H, m), 2.54 (1 H, d,  $J = 16.3$  Hz), 4.53 (1 H, s), 4.71 (1 H, s);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 19.4$  (q, C-13), 19.5 (t, C-9), 23.4 (q, C-14), 27.8 (t, C-2), 28.1 (t, C-1), 28.9 (q, C-15), 30.4 (t, C-8), 33.7 (s, C-11), 36.6 (t, C-10), 40.6 (t, C-5), 47.2 (s, C-7), 53.1 (s, C-6), 58.0 (d, C-3), 102.0 (t, C-12), 154.5 (s, C-4); MS (EI, 70 eV),  $m/z$  (rel. int.): 204 [ $\text{M}^+$ ] (25), 189 (20), 176 (4), 175 (3), 161 (19), 148 (12), 147 (10), 133 (22), 119 (42), 108 (100), 93 (60), 79 (37), 69 (32), 55 (31), 41 (56).

### 7.7.4. (-)-Myltayl-4-ene (199)

Colourless oil,  $RI_{\text{CPSiL } 5} = 1380$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 0.88$  (3H, br.s, H-13), 0.94 (3H, s, H-14), 0.99 (3H, s, H-15), 0.98-1.04 (2H, m, H-1a, H-2a), 1.21-1.26 (1H, m, H-10a), 1.39-1.48 (2H, m, H-9a, H-10b), 1.52-1.61 (2H, m, H-8a, H-9b), 1.62 (3H, s, H-12), 1.71-1.76 (1H, m, H-1b), 1.77-1.82 (1H, m, H-2b), 1.88-1.94 (2H, m, H-3, H-8b), 5.47 (1H, s, H-5);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 16.1$  (q, C-12), 20.0 (t, C-9), 21.5 (q, C-13), 23.0 (q, C-14), 25.2 (t, C-1), 25.4 (t, C-2), 29.4 (q, C-15), 30.3 (t, C-8), 33.2 (s, C-11), 39.3 (t, C-10), 56.1 (s, C-7), 58.5 (d, C-3), 60.8 (s, C-6), 131.0 (d, C-5), 142.4 (s, C-4); MS (EI, 70eV),  $m/z$  (rel. int.): 204 [ $\text{M}^+$ ] (16), 176 (44), 161 (100), 147 (8), 133 (12), 119 (21), 105 (18), 91 (17), 77 (8), 55 (8), 41(15). HREIMS calcd for  $\text{C}_{15}\text{H}_{24}$  [ $\text{M}^+$ ]  $m/z$  204.1878 found [ $\text{M}^+$ ]  $m/z$  204.1891.

### 7.7.5. (+)-(1R,6S,7S,10S)-7b-Hydroxyamorpha-4,11-diene (200)

Colourless oil,  $RI_{\text{CPSiL } 5} = 1614$ ;  $R_f = 0.75$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ): 0.92 (3H, d, H-14,  $J = 6.3$  Hz), 1.34-1.46 (4H, m, H-2a, H-8a, H-9a, H-10), 1.47-1.52 (1H, m, H-9b), 1.54-1.62 (1H, m, H-8b), 1.57 (3H, br.s, H-15), 1.62-1.68 (1H, m, H-3a), 1.75-1.82 (1H, m, H-3b), 1.81 (3H, s, H-12), 1.84-1.93 (2H, m, H-1, H-2b), 2.49 (1H, s, H-6), 4.85 (1H, s, H-13a), 4.90 (1H, s, H-13b), 5.15 (1H, s, H-5);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 5. MS (EI, 70eV),  $m/z$  (rel. int.): 220 [ $\text{M}^+$ ] (15), 202 (85), 187 (100), 173 (17),

159 (80), 146 (33), 145 (94), 134 (60), 132 (75), 133 (62), 121 (39), 119 (65), 105 (52), 91(53), 81 (38), 79 (47), 77 (46), 69 (30), 55 (40), 41 (75); HRMS calcd for C<sub>15</sub>H<sub>24</sub>O<sub>1</sub> [M<sup>+</sup>] *m/z* 220.1827 found [M<sup>+</sup>] *m/z* 220.1832.

#### 7.7.6. (-)-(1*S*,6*S*,9*R*,10*R*)-9*a*-Hydroxyamorpha-4,7(11)-diene (201)

White, viscous oil; RI<sub>CPSIL 5</sub> = 1680; R<sub>f</sub> = 0.56; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 1.03 (3H, *d*, H-14, *J* = 6.6 Hz), 1.24-1.29 (1H, *m*, H-1), 1.44-1.55 (3H, *m*, H-2, H-10), 1.58 (3H, *s*, H-15), 1.61-1.68 (1H, *m*, H-3a), 1.66 (6H, *br.s*, H-12, H-13), 1.78-1.86 (2H, *m*, H-3b, H-8a), 2.71 (1H, *dd*, H-8b, *J* = 4.1, 12.6 Hz), 2.98 (1H, *dt*, H-9, *J* = 4.1, 11.4 Hz), 3.36 (1H, *br.s*, H-6), 5.09 (1H, *s*, H-5); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): Table 5. MS (EI, 70eV), *m/z* (rel. int.) 220 [M<sup>+</sup>] (63), 202 (60), 187 (100), 173 (30), 159 (52), 147 (60), 145 (65), 131 (42), 119 (48), 105 (59), 91 (61), 77 (43), 55 (42), 41 (80); HRMS calcd for C<sub>15</sub>H<sub>24</sub>O<sub>1</sub> [M<sup>+</sup>] *m/z* 220.1827 found [M<sup>+</sup>] *m/z* 220.1816.

#### 7.7.7. (-)-(1*R*,3*R*,6*S*,10*S*)-3*a*-Hydroxyamorpha-4,7(11)-diene (202)

Colourless oil; RI<sub>CPSIL 5</sub> = 1666; R<sub>f</sub> = 0.68 ; sense of optical rotation (benzene): (-); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.88-0.97 (1H, *m*, H-9a), 1.01 (3H, *d*, H-14, *J* = 6.6 Hz), 1.24-1.28 (1H, *m*, H-1), 1.48-1.53 (1H, *m*, H-2a), 1.60-1.65 (1H, *m*, H-9b), 1.67 (3H, *d*, H-12, *J* = 0.9 Hz), 1.68 (3H, *d*, H-13, *J* = 2.2 Hz), 1.73 (3H, *br.s*, H-15), 1.80-1.88 (1H, *m*, H-8a), 1.99-2.05 (1H, *m*, H-10), 2.09-2.15 (1H, *m*, H-2b), 2.52-2.57 (1H, *m*, H-8b), 3.30 (1H, *br.s*, H-6), 3.68 (1H, *s*, H-3), 5.16 (1H, *s*, H-5); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): Table 5. MS (EI, 70eV), *m/z* (rel. int.): 220 [M<sup>+</sup>] (8), 218 (12), 202 (43), 187 (27), 177 (48), 159 (77), 145 (60), 131(39), 119 (47), 105 (61), 91 (75), 77 (49), 67 (31), 53 (41), 41(100). HRMS calcd for C<sub>15</sub>H<sub>24</sub>O<sub>1</sub> [M<sup>+</sup>] *m/z* 220.1827 found [M<sup>+</sup>] 220.1826.

#### 7.7.8. (+)-(1*S*,6*S*,10*S*)-Amorpha-2,4,7(11)-triene (203)

Colourless oil, RI<sub>CPSIL 5</sub> = 1449; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.76-0.85 (1H, *m*), 0.87 (3H, *d*, *J* = 6.3 Hz), 1.49-1.57 (2H, *m*), 1.62 (3H, *d*, *J* = 1.9 Hz), 1.66 (3H, *s*), 1.69 (3H, *br.s*), 1.70-1.76 (1H, *m*), 1.85-1.92 (1H, *m*), 2.59-2.64 (1H, *m*), 3.77 (1H, *s*), 5.20 (1H, *s*), 5.85 (1H, *d*, *J* = 9.5 Hz), 6.08 (1H, *dd*, *J* = 6.0, 9.4 Hz); MS (EI, 70eV), *m/z* (rel. int.): 202 [M<sup>+</sup>] (82), 187 (25), 173 (8), 160 (32), 159 (100), 145 (82), 131 (42), 119 (39), 105 (51), 91(38), 77 (22), 67 (20), 53 (18), 41(39).

### 7.7.9. (-)-(1R,3R,6S,10S)-3a-Acetoxyamorpha-4,7(11)-diene (204)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1780$ ;  $R_f = 0.94$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.86 (3H, *d*, H-14,  $J = 6.3$  Hz), 0.91 (1H, *dt*, H-9a,  $J = 3.5, 13.6$  Hz), 1.14-1.20 (1H, *m*, H-1), 1.52-1.61 (2H, *m*, H-2a, H-9b), 1.65 (9H, *br.s*, H-12, H-13, H-15), 1.74 (3H, *s*, H-17), 1.75-1.84 (1H, *m*, H-8a), 2.02-2.11 (1H, *m*, H-10), 2.29 (1H, *dd*, H-2b,  $J = 1.9, 15.5$  Hz), 2.54 (1H, *br.dd*, H-8b,  $J = 1.6, 13.9$  Hz), 3.25 (1H, *s*, H-6), 5.24 (1H, *br.s*, H-5), 5.33 (1H, *br.d*, H-3,  $J = 5.0$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 5; MS (EI, 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ] (20), 220 (4), 202 (98), 187 (50), 177 (21), 160 (55), 159 (100), 145 (70), 131 (33), 119 (42), 105 (44), 91 (38), 77 (23), 67 (18), 55 (23), 43 (63).

### 7.7.10. (-)-(1R,6R,10S)-Amorpha-4,7(11)-dien-3-one (205)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1676$ ;  $R_f = 0.86$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.72 (3H, *d*, H-14,  $J = 6.3$  Hz), 0.73-0.81 (1H, *m*, H-9a), 1.33-1.40 (1H, *m*, H-1), 1.41-1.58 (3H, *m*, H-8a, H-9b, H-10), 1.59 (6H, *s*, H-12, H-13), 1.82 (3H, *s*, H-15), 2.12 (1H, *dd*, H-2a,  $J = 4.7, 16.1$  Hz), 2.41 (1H, *br.d*, H-8b,  $J = 13.2$  Hz), 2.72 (1H, *dd*, H-2b,  $J = 2.5, 16.1$  Hz), 3.52 (1H, *br.s*, H-6), 5.91 (1H, *s*, H-5);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 5; MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (100), 203 (12), 189 (4), 176 (25), 175 (89), 161(49), 147 (28), 133 (30), 119 (41), 107 (35), 105 (47), 91(64), 77 (48), 67 (26), 55 (40), 41 (68); HRMS calcd for  $\text{C}_{15}\text{H}_{22}\text{O}_1$  [ $\text{M}^+$ ]  $m/z$  218.1671 found [ $\text{M}^+$ ]  $m/z$  218.1668

### 7.7.11. (+)-(1R,2S,6R,8S,10S)-2,8-Epoxyamorpha-4,7(11)-diene (206)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1597$ ;  $R_f = 0.75$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.87 (3H, *d*, H-14,  $J = 7.3$  Hz), 1.02-1.06 (1H, *m*, H-9a), 1.37-1.40 (1H, *m*, H-1), 1.39 (3H, *s*, H-13), 1.58 (3H, *s*, H-12), 1.61 (3H, *br.s*, H-15), 1.75-1.81 (1H, *m*, H-10), 1.95 (1H, *br.d*, H-3a,  $J = 18.0$  Hz), 2.18 (1H, *d*, H-3b,  $J = 18.6$  Hz), 2.21-2.26 (1H, *m*, H-9b), 3.07 (1H, *s*, H-6), 4.04 (1H, *s*, H-2), 4.49 (1H, *s*, H-8), 5.72 (1H, *d*, H-5,  $J = 6.0$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 5; MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (49), 200 (9), 185 (12), 175 (7), 157 (12), 143 (10), 138 (11), 133 (22), 121(100), 119 (66), 105 (28), 93 (36), 91(32), 77(26), 55 (21), 41(48); HRMS calcd for  $\text{C}_{15}\text{H}_{24}\text{O}_1$  [ $\text{M}^+$ ]  $m/z$  218.1671 found [ $\text{M}^+$ ]  $m/z$  218.1680

### 7.7.12. (+)-(1S,5S,6R,9R,10R)-5,9-Epoxyamorpha-3,7(11)-diene (209)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1595$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.71 (3H, *d*, H-14,  $J = 6.9$  Hz), 1.21-1.32 (1H, *m*, H-1), 1.57 (3H, *s*, H-12), 1.59 (3H,

*s*, H-13), 1.75 (1H, *d*, H-2a,  $J = 17.0$ ), 1.88 (3H, *s*, H-15), 1.87-1.95 (1H, *m*, H-10), 2.05-2.13 (1H, *d*, H-2b,  $J = 18.0$  Hz), 2.15-2.23 (1H, *d*, H-8a,  $J = 16.4$  Hz), 2.42 (1H, *s*, H-6), 2.45 (1H, *d*, H-8b,  $J = 13.6$  Hz), 3.70 (1H, *s*, H-9), 3.93 (1H, *s*, H-5), 5.32 (1H, *s*, H-3); MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $M^+$ ] (98), 203 (5), 185 (12), 175 (11), 157 (13), 145 (16), 135 (100), 119 (69), 105 (30), 93 (32), 91 (35), 77 (26), 55 (21), 41 (49); HRMS calcd for  $C_{15}H_{22}O_1$  [ $M^+$ ]  $m/z$  218.1671 found [ $M^+$ ]  $m/z$  218.1679.

#### 7.7.13. (-)-(1R,2S,6R,10S)-2a-Acetoxyamorpha-4,7(11)-diene (210)

Colourless oil;  $RI_{CPSIL\ 5} = 1800$ ; sense of optical rotation (benzene): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta = 0.96$ -1.01(*m*, 1 H, H-9a), 1.08 (*d*, 3 H,  $CH_3$ -14,  $J = 6.3$  Hz), 1.51 (*s*, 3 H,  $CH_3$ -15), 1.52-1.57 (*m*, 1 H, H-9b), 1.59 (*d*, 3 H,  $CH_3$ -12,  $J = 1.9$  Hz), 1.63 (*s*, 3 H,  $CH_3$ -13), 1.68-1.71 (*m*, 1 H, H-10), 1.72 (*s*, 3 H,  $CH_3CO$ ), 1.73-1.79 (*m*, 2 H, H-8a, H-1), 2.10 (*br.d*, 2 H, H-3,  $J = 8.5$  Hz), 2.46-2.50 (*m*, 1 H, H-8b), 3.57 (*br.s*, 1 H, H-6), 4.96 (*s*, 1 H, H-5) 5.28 (*dt*, 1H, H-2,  $J_1 = 2.8$ ,  $J_2 = 8.5$  Hz);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ ):  $\delta = 19.9$  (*q*, C-12), 20.3 (*q*, C-13), 21.1 (*q*,  $\underline{C}H_3CO$ ), 21.7 (*q*, C-14), 22.8 (*q*, C-15), 26.6 (*t*, C-8), 28.1 (*d*, C-10), 32.5 (*t*, C-3), 36.7 (*t*, C-9), 40.9 (*d*, C-6), 45.0 (*d*, C-1), 75.1 (*d*, C-2), 122.0 (*s*, C-11), 126.2 (*d*, C-5), 132.2 (*s*, C-4), 133.5 (*s*, C-7), 169.9 (*s*,  $CH_3\underline{C}O$ ); MS (EI, 70 eV),  $m/z$  (rel. int.): 262 [ $M^+$ ] (1), 219 (1), 202 (42), 187 (27), 173(4), 160 (88), 159 (93), 145 (100), 131 (22), 119 (21), 105 (30), 91 (26), 77 (17), 67 (13), 55 (22), 43 (73).

#### 7.7.14. (-)-(1R,2S,6R,10S)-2a-Hydroxyamorpha-4,7(11)-diene (211)

Colourless oil;  $RI_{CPSIL\ 5} = 1684$ ;  $R_f = 0.56$ ; sense of optical rotation (benzene): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta$  0.98-1.05 (1H, *m*, H-9a), 1.21 (3H, *d*, H-14,  $J = 6.6$  Hz), 1.31-1.35 (1H, *m*, H-1), 1.56 (3H, *br.s*, H-15), 1.56-1.60 (1H, *m*, H-9b), 1.67 (3H, *s*, H-12), 1.70 (3H, *d*, H-13,  $J = 1.9$  Hz), 1.70-1.82 (2H, *m*, H-8a, H-10), 1.94-2.09 (2H, *m*, H-3), 2.49 (1H, *br.d*, H-8b,  $J = 13.9$  Hz), 3.40 (1H, *s*, H-6), 3.78-3.82 (1H, *m*, H-2), 4.97 (1H, *s*, H-5);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ ):  $\delta$  Table 5; MS (EI, 70eV),  $m/z$  (rel. int.): 220 [ $M^+$ ] (100), 205 (22), 202 (24), 187 (78), 177 (33), 159 (85), 145 (53), 131 (29), 121 (30), 119 (31), 105 (38), 91(42), 55 (39), 41 (76); HRMS calcd for  $C_{15}H_{24}O_1$  [ $M^+$ ]  $m/z$  220.1827 found [ $M^+$ ]  $m/z$  220.1809.

#### 7.7.15. (-)-(1R,2R,6R,10S)-2b-Acetoxyamorpha-4,7(11)-diene (212)

Colourless oil;  $RI_{CPSIL\ 5} = 1721$ ;  $R_f = 0.94$ ; sense of optical rotation (benzene): (-);  $^1H$ -NMR (500 MHz,  $C_6D_6$ ):  $\delta$  0.86 (3H, *d*, H-14,  $J = 6.6$  Hz), 0.87-0.94 (1H, *m*, H-9a), 1.21-1.32 (1H, *m*, H-10), 1.46-1.51 (1H, *m*, H-9b), 1.54 (3H, *br.s*, H-15), 1.56-1.62 (1H, *m*, H-1), 1.67 (3H, *s*,

H-12), 1.71 (3H, *s*, H-17), 1.75 (3H, *s*, H-13), 1.75-1.83 (1H, *m*, H-8a), 1.95-2.08 (2H, *m*, H-3), 2.52 (1H, *br.d*, H-8b,  $J = 13.6$  Hz), 3.83 (1H, *s*, H-6), 5.18 (1H, *s*, H-5), 5.49 (1H, *s*, H-2);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 5; MS (EI 70eV),  $m/z$  (rel. int.): 262 [ $\text{M}^+$ ], 202 (98), 187 (89), 174 (11), 159 (98), 145 (97), 131 (41), 119 (32), 105 (55), 91 (50), 77 (30), 67 (21), 55 (29), 43 (100).

#### 7.7.16. (+)-(1R,6R,10S)-Amorpha-4,7(11)-dien-2-one (213)

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1645$ ; sense of optical rotation (benzene): (+);  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.79 (3H, *d*,  $J = 6.6$  Hz), 1.16 (3H, *dd*,  $J = 10.4, 17.7$  Hz), 1.41 (3H, *d*,  $J = 0.9$  Hz), 1.49 (3H, *d*,  $J = 1.9$  Hz), 1.58 (3H, *d*,  $J = 0.9$  Hz), 2.12 (1H, *dd*,  $J = 5.0, 11.4$  Hz), 2.41-2.62 (4H, *m*), 3.69 (1H, *br.s*), 5.02 (1H, *s*); MS (EI 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (100), 203 (21), 190 (8), 185 (8), 175 (82), 161(51), 147 (48), 133 (38), 119 (42), 105 (43), 91 (41), 77 (28), 55 (27), 41 (67).

#### 7.7.17. (+)-(7S,9S,10S)-9b-Hydroxyselina-4,11-diene (214)

White, viscous oil;  $\text{RI}_{\text{CPSIL } 5} = 1690$ ;  $R_f = 0.51$ ; sense of optical rotation (chloroform): (+);  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 1.10$  (*s*, 3 H,  $\text{CH}_3$ -14), 1.41 (*dt*, 1 H, H-1a,  $J_1 = 3.2, J_2 = 12.3$  Hz), 1.51-1.58 (*m*, 2 H, H-2a, H-8a), 1.59-1.65 (*m*, 1 H, H-2b), 1.63 (*s*, 3 H,  $\text{CH}_3$ -15), 1.71-1.85 (*m*, 3 H, H-1b, H-6a, H-8b), 1.76 (*s*, 3 H,  $\text{CH}_3$ -13), 1.89-2.1 (*m*, 3 H, H-3, H-7), 2.52 (*ddd*, 1 H, H-6,  $J_1 = 1.7, J_2 = 3.2, J_3 = 13.6$  Hz), 3.37 (*br.dd*, 1 H, H-9,  $J_1 = 3.8, J_2 = 11.7$  Hz), 4.72-4.75 (*m*, 2 H, H-12);  $^1\text{H}$ -NMR (500 MHz  $\text{C}_6\text{D}_6$ ):  $\delta = 1.05$  (*s*, 3 H), 1.37-1.43 (*m*, 1 H), 1.49-1.57 (*m*, 3 H), 1.54 (*s*, 3 H), 1.65-1.72 (*m*, 1 H), 1.66 (*s*, 3 H), 1.77-1.90 (*m*, 5 H), 2.56 (*d*, 1 H,  $J = 12.0$  Hz), 3.21 (*dd*, 1 H,  $J_1 = 4.1, J_2 = 11.7$  Hz), 4.80 (*s*, 1 H), 4.83 (*s*, 1H);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta = 17.7$  (*q*, C-14), 18.7 (*t*, C-2), 19.8 (*q*, C-15), 20.8 (*q*, C-13), 30.0 (*t*, C-6), 33.0 (*t*, C-3), 35.6 (*t*, C-8), 36.2 (*t*, C-1), 40.1 (*s*, C-10), 43.2 (*d*, C-7), 79.1 (*d*, C-9), 108.8 (*t*, C-12), 127.5 (*s*, C-4), 133.1 (*s*, C-5), 149.2 (*s*, C-11); MS (EI, 70 eV),  $m/z$  (rel. int.): 220 [ $\text{M}^+$ ] (35), 202 (17), 187 (17), 176 (25), 161 (23), 159 (21), 145 (17), 133 (16), 123 (100), 105 (44), 97 (73), 91 (44), 81 (57), 77 (30), 67 (27), 55 (33), 41 (69).

#### 7.7.18. (+)-(7S,10S)-Eudesma-4,11-dien-9-one (215)

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1649$ ; sense of optical rotation (benzene): (+);  $^1\text{H}$ -NMR (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.07 (3H, *s*), 1.46 (3H, *s*), 1.51 (3H, *s*), 1.63-1.78 (5H, *m*), 1.98-2.00 (3H, *m*), 2.14-2.20 (1H, *m*), 2.41-2.44 (1H, *m*), 2.60-2.64 (1H, *m*), 4.68 (1H, *s*), 4.71 (1H, *br.s*); MS (EI 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (41), 203 (29), 198 (5), 190 (5), 183 (12), 176 (100), 175 (58),

161 (27), 147 (52), 133 (75), 119 (38), 107 (69), 105 (50), 93 (64), 91 (62), 79 (50), 67 (29), 55 (32), 41 (74).

**Table 5.**  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{C}_6\text{D}_6$ )  $\delta$  (ppm) spectra data of compounds (+)-**200**, (-)-**201**, (-)-**202**, (-)-**204**, (-)-**205**, (+)-**206**, (-)-**211** and (-)-**212**

C	<b>200</b>	<b>201</b>	<b>202</b>	<b>204</b>	<b>205</b>	<b>206</b>	<b>211</b>	<b>212</b>
1	36.4	39.5	41.1	40.6	46.0	37.3	48.6	45.3
2	25.5	26.0	34.8	31.9	43.0	72.0	73.3	70.7
3	26.5	25.5	67.4	69.2	197.8	39.0	36.0	31.8
4	134.4	134.4	135.6	134.9	136.0	130.1	132.7	130.5
5	121.8	125.7	129.3	132.2	147.9	122.7	125.9	126.0
6	43.5	40.4	41.2	40.8	41.7	32.9	41.4	34.9
7	75.6	132.7	135.5	134.9	133.8	134.5	134.5	134.1
8	32.1	37.0	27.5	27.6	27.6	66.8	26.6	27.1
9	30.5	76.0	37.1	36.8	36.1	36.8	37.1	36.4
10	27.8	37.0	30.4	30.0	29.8	29.6	28.1	30.2
11	150.9	123.2	121.5	121.8	122.3	120.8	121.0	122.2
12	18.4	20.3*	20.6*	20.6*	20.2*	20.1	19.9	20.0
13	111.5	20.7*	20.7*	20.8*	20.4*	19.1	20.3	20.6
14	19.8	15.5	20.2	20.3	19.8	20.9	22.3	20.0
15	23.9	23.9	21.1	22.0	16.2	23.9	23.0	23.5
16				170.1				170.0
17				21.0				21.3

\* Interchangeable.

All assignments were confirmed by HMBC and HMQC.



## 7.8. Chemical Analysis of *Marsupella alpina*

The essential oil of *Marsupella alpina* collected in July near Gaschurn (Austria) at an elevation of 2000 m was obtained by hydrodistillation.

### 7.8.1. (+)-(7S,8R,9S,10R)-8,9-Epoxy Selina-4,11-diene (143)

Colourless oil; RI<sub>CPSil 5</sub> = 1596; R<sub>f</sub> = 0.94; sense of optical rotation (benzene): (+) ; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 1.27 (s, 3 H, CH<sub>3</sub>-14), 1.31-1.36 (m, 2 H, H-1), 1.46 (s, 3 H, CH<sub>3</sub>-15), 1.47-1.53 (m, 1 H, H-2a), 1.62-1.71 (m, 1 H, H-2b), 1.72-1.81 (m, 2 H, H-3), 1.82 (s, 3 H, CH<sub>3</sub>-13), 2.10 (t, 1 H, H-6a, *J* = 11.7 Hz), 2.15-2.19 (m, 1 H, H-7), 2.23 (dd, 1 H, H-6b, *J*<sub>1</sub> = 2.8, *J*<sub>2</sub> = 11.4 Hz), 2.59 (d, 1 H, H-9, *J* = 3.8 Hz), 3.06 (d, 1 H, H-8, *J* = 3.8 Hz), 4.89 (t, 1 H, H-12a, *J* = 1.6 Hz), 5.07 (s, 1H, H-12b); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 18.4 (q, C-15), 19.0 (t, C-2), 21.2 (q, C-13), 22.5 (q, C-14), 25.4 (t, C-6), 32.1 (t, C-3), 33.5 (s, C-10), 35.8 (t, C-1), 46.4 (d, C-7), 56.8 (d, C-8), 60.6 (d, C-9), 111.4 (t, C-12), 124.5 (s, C-4), 133.4 (s, C-5), 148.0 (s, C-11); MS (EI, 70 eV), *m/z* (rel. int.): 218 [M<sup>+</sup>] (26), 203 (27), 189 (20), 175 (22), 159 (17), 147 (32), 133 (31), 119 (39), 107 (67), 91 (64), 79 (46), 55 (45), 41 (100).

### 7.8.2. (-)-(5S,7S,10S)-trans-Selina-4(15),11-dien-5-ol (222)

Colourless oil; RI<sub>CPSil 5</sub> = 1629 ; R<sub>f</sub> = 0.87; sense of optical rotation (chloroform): (-) ; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 0.85 (s, 3 H, CH<sub>3</sub>-14), 0.95-1.00 (m, 1 H, H-1a), 1.09-1.14 (m, 1 H, H-9a), 1.39-1.44 (m, 1 H, H-6a), 1.47-1.65 (m, 4 H, H-2a, H-8, H-2b), 1.65 (s, 3 H, CH<sub>3</sub>-13), 1.73-1.76 (m, 1 H, H-6b), 1.92-2.05 (m, 3 H, H-9b, H-1b, H-3a), 2.52-2.65 (m, 2 H, H-7, H-3b), 4.59 (s, 1 H, H-15a), 4.73 (s, 1 H, H-15b), 4.85 (d, 2 H, H-12, *J* = 11.4 Hz); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ = 0.88 (s, 3 H), 1.06 (br.d, 1 H, *J* = 13.2 Hz), 1.20 (dt, 1 H, *J*<sub>1</sub> = 3.8, *J*<sub>2</sub> = 12.9 Hz), 1.51-1.62 (m, 5 H), 1.67 (dt, 1 H, *J*<sub>1</sub> = 4.7, *J*<sub>2</sub> = 13.2 Hz), 1.76 (s, 3 H), 1.79-1.88 (m, 2 H), 2.13 (dd, 1 H, *J*<sub>1</sub> = 4.7, *J*<sub>2</sub> = 3.2 Hz), 2.52 (m, 1 H, *J* = 4.1 Hz), 2.62 (dt, 1 H, *J*<sub>1</sub> = 6.3, *J*<sub>2</sub> = 13.2 Hz), 4.96 (br.s, 1 H), 4.73 (br.s, 1 H), 4.75 (br.s, 1 H), 4.82 (1H, t, *J* = 1.6 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ = 20.0 (q, C-14), 21.2 (q, C-13), 22.8 (t, C-2), 26.5 (t, C-8), 32.1 (t, C-3), 34.6 (t, C-9), 35.3 (t, C-1), 35.8 (t, C-6), 38.4 (s, C-10), 40.3 (d, C-7), 75.5 (s, C-5), 107.3 (t, C-15), 108.9 (t, C-12), 150.8 (s, C-11), 152.9 (s, C-4); MS (EI, 70 eV): *m/z* (rel. int.) 220 [M<sup>+</sup>] (7), 205 (24), 202 (25), 187 (57), 177 (15), 159 (13), 149 (13), 137 (32), 121 (22), 109 (33), 95 (65), 81 (52), 67 (50), 55 (54), 41 (100).

### 7.8.3. (+)-(5R,7S,10S)-cis-Selina-4(15),11-dien-5-ol (223)

Colourless oil;  $RI_{CPSil\ 5} = 1623$ ;  $R_f = 0.77$ ; sense of optical rotation (chloroform): (+);  $^1H$ -NMR (500 MHz,  $C_6D_6$ );  $\delta = 0.90$ -1.00 (*br.s*, 1 H), 1.07 (3 H, *s*), 1.05-1.17 (*br.s*, 1 H), 1.40-1.55 (*m*, 5 H), 1.66 (*s*, 3 H), 1.69-1.74 (*m*, 1 H), 1.80 (*br.d*, 1 H,  $J = 12.9$  Hz), 1.85-1.99 (*br.s*, 1 H), 2.03 (*br.d*, 1 H,  $J = 13.2$  Hz), 2.25-2.35 (*br.s*, 1 H), 2.45-2.55 (*br.s*, 1 H), 4.75-4.80 (*br.s*, 1 H), 4.81-4.90 (*br.s*, 3 H);  $^{13}C$ -NMR (125.7 MHz,  $C_6D_6$ );  $\delta = 21.2, 22.5, 22.6, 27.0, 33.3, 33.6, 36.9, 38.9, 43.3, 75.1$  ( $\zeta$ ), 109.0 ( $\epsilon$ ), 110.3 ( $\epsilon$ ), 2x 149.6 ( $\zeta$ ), one carbon signal not observed; MS (EI, 70 eV),  $m/z$  (rel. int.) 220 [ $M^+$ ] (13), 205 (25), 202 (32), 187(65), 177(15), 162 (24), 147 (23), 135 (40), 124 (62), 109 (52), 95 (72), 91 (54), 81 (63), 67 (62), 55 (61), 41 (100).

## 7.9. Chemical Analysis of *Tritomaria polita*

*Tritomaria polita* was collected in July 2001 in the Ötztal, Tyrol/Austria at an elevation of approx. 2300 m. A voucher specimen is kept at the Abteilung für Systematische Botanik und Ökologie, University of Ulm.

### 7.9.1. (+)-(5S,7S,10S)-Eudesma-3,11-dien-8-one (230)

Colourless oil;  $RI_{CPSIL\ 5} = 1668$ ;  $R_f = 0.81$ ; sense of optical rotation (chloroform): (+);  $^1H$ -NMR (500MHz,  $C_6D_6$ ):  $\delta$  0.67 (*s*, 3H,  $CH_3$ -14), 1.06 (*dd*, 1H, H-1a,  $J = 6.0, 12.6$  Hz), 1.23 (*dt*, 1H, H-1b,  $J = 6.6, 12.6$  Hz), 1.50 (*s*, 3H,  $CH_3$ -15), 1.59 (*dd*, 1H, H-6a,  $J = 12.6, 25.5$  Hz), 1.78-1.83 (*m*, 2H, H-9a, H-2a), 1.85 (*s*, 3H,  $CH_3$ -12), 1.87-1.93 (*m*, 2H, H-6b, H-2b), 2.02 (*br.d*, 1H, H-5,  $J = 12.9$  Hz), 2.08 (*d*, 1H, H-9b,  $J = 13.2$  Hz), 2.68 (*dd*, 1H, H-7,  $J = 6.3, 12.6$  Hz), 4.77 (*s*, 1H, H-13a), 4.97 (*s*, 1H, H-13b), 5.26 (*s*, 1H, H-3).  $^1H$ -NMR (500MHz,  $CDCl_3$ ):  $\delta$  0.79 (3H, *s*), 1.25 (1H, *br. s*), 1.42 (1H, *dd*,  $J = 6.0, 12.9$  Hz), 1.57 (1H, *dt*,  $J = 6.9, 12.6$  Hz), 1.68 (3H, *s*), 1.72 (1H, *dd*, 12.9, 25.9 Hz), 1.78 (3H, *s*), 1.99-2.11 (2H, *m*), 2.16 (1H, *ddd*, 3.8, 6.6, 13.7 Hz), 2.22 (1H, *dd*,  $J = 13.9, 29.6$  Hz), 2.49 (1H, *br. d*,  $J = 13.9$  Hz), 3.03 (1H, *dd*,  $J = 6.3, 12.3$  Hz), 4.77 (1H, *s*), 4.96 (1H, *s*), 5.42 (1H, *br. s*).  $^{13}C$ -NMR (125.7MHz,  $C_6D_6$ ) data Table 6; MS(EI, 70eV),  $m/z$  (rel. int.) 218 [ $M^+$ ] (45), 203 (12), 200 (12), 190 (3), 175 (9), 159 (14), 147 (22), 121 (33), 108 (100), 93 (74), 83 (30), 77 (38), 67 (26), 53 (37), 41 (74).

### 7.9.2. (+)-(5S,7S,8S,10S)-8a-Hydroxyeudesma-3,11-diene (144)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1669$ ;  $R_f = 0.77$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500MHz,  $\text{C}_6\text{D}_6$ ):  $\delta = 1.09$  (*dd*, 1H, H-9a,  $J = 3.4, 13.9$  Hz), 1.20-1.28 (*m*, 1H, H-1a), 1.31 (*s*, 3H,  $\text{CH}_3$ -14), 1.38 (*dd*, 1H, H-1b,  $J = 6.6, 12.6$  Hz), 1.55 (*s*, 3H,  $\text{CH}_3$ -12), 1.57-1.59 (*m*, 1H, H-6a), 1.62-1.70 (*m*, 1H, H-6b), 1.64 (*s*, 3H,  $\text{CH}_3$ -15), 1.81-1.88 (*br.d*, 2H, H-5, H-7,  $J = 11.4$  Hz), 1.92 (*dd*, 1H, H-9b,  $J = 2.2, 13.9$  Hz), 1.95-1.99 (*m*, 1H, H-2a), 2.09-2.16 (*m*, 1H, H-2b), 3.90 (*br.d*, 1H, H-8,  $J = 2.5$  Hz), 4.79 (*s*, 1H, H-13a), 4.85 (*s*, 1H, H-13b), 5.35 (*br.s*, 1H, H-3).  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.04 (*s*, 3H), 1.23-1.28 (*m*, 1H), 1.32 (*dd*, 2H,  $J = 4.1, 13.6$  Hz), 1.41 (*dd*, 1H,  $J = 6.3, 12.3$  Hz), 1.61 (*m*, 1H), 1.66 (3H, *br.s*), 1.81 (*s*, 3H), 1.90 (*dd*, 1H,  $J = 2.5, 14.5$  Hz), 1.96-2.00 (*m*, 2H), 2.06-2.12 (*m*, 1H), 2.12-2.17 (*m*, 1H), 4.05 (*br.d*, 1H,  $J = 2.52$  Hz), 4.87 (*s*, 1H), 5.02 (*s*, 1H), 5.32 (*br.s*, 1H).  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  18.1 (*q*, C-14), 21.4 (*q*, C-15), 22.6 (*q*, C-12), 23.1 (*t*, C-2), 23.3 (*t*, C-6), 32.3 (*s*, C-10), 38.7 (*t*, C-1), 45.9 (*t*, C-9), 47.6 (*d*, C-5), 51.3 (*d*, C-7), 67.1 (*d*, C-8), 111.9 (*t*, C-13), 121.5 (*d*, C-3), 134.7 (*s*, C-4), 147.2 (*s*, C-11). MS (EI, 70eV)  $m/z$  (rel. int.): 220 [ $\text{M}^+$ ] (33), 205 (12), 202 (31), 187 (41), 177 (17), 161 (44), 147 (34), 131 (22), 123 (23), 121 (54), 119 (49), 108 (47), 107 (78), 105 (67), 95 (31), 93 (84), 91 (77), 81 (54), 79 (51), 77 (49), 67 (35), 55 (54), 41 (100).

### 7.9.3. (+)-(5S,10S)-Eudesma-3,7(11)-dien-8-one (231)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1745$ ;  $R_f = 0.82$ ; sense of optical rotation (chloroform): (+);  $^1\text{H-NMR}$  (500MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.76 (*s*, 3H,  $\text{CH}_3$ -14), 1.12-1.17 (*m*, 2H, H-1), 1.48 (*s*, 3H,  $\text{CH}_3$ -12), 1.56 (*br.s*, 3H,  $\text{CH}_3$ -15), 1.74-1.83 (*m*, 1H, H-2a), 1.87-1.96 (*m*, 3H, H-6a, H-2b, H-5), 2.01 (*d*, 1H, H-9a,  $J = 16.3$  Hz), 2.20 (*s*, 3H,  $\text{CH}_3$ -13), 2.30 (*d*, 1H, H-9b,  $J = 16.2$  Hz), 2.57 (*dd*, 1H, H-6b,  $J = 3.8, 14.5$  Hz), 5.29-5.34 (*m*, 1H, H-3).  $^1\text{H-NMR}$  (500MHz,  $\text{CDCl}_3$ )  $\delta$  0.85 (*s*, 3H), 1.39-1.45 (*m*, 2H), 1.70 (*br.s*, 3H), 1.84 (*s*, 3H), 1.94-2.07 (*m*, 3H), 2.09 (*s*, 3H), 2.12-2.29 (*m*, 3H), 2.80 (*dd*, 1H,  $J = 3.8, 14.2$  Hz), 5.42 (*br.s*, 1H).  $^{13}\text{C-NMR}$  (125.7MHz,  $\text{C}_6\text{D}_6$ ) Table 6. MS (EI, 70eV),  $m/z$  (rel. int.): 218 [ $\text{M}^+$ ] (100), 203 (22), 189 (4), 175 (17), 161 (19), 147 (30), 135 (17), 121 (55), 108 (47), 98 (20), 93 (46), 91 (44), 83 (40), 77 (33), 67 (32), 53 (35), 41 (73).

### 7.9.4.(+)-(5S,10S)-3,4,4aR,7,8,8aR-Hexahydro-5,8a-dimethylnaphthalen-2(1H)-one (233)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1461$ ; sense of optical rotation (chloroform): (+);  $^1\text{H-NMR}$  (500MHz,  $\text{C}_6\text{D}_6$ ),  $\delta$  0.63 (*s*, 3H), 1.04 (*dd*, 1H,  $J = 6.0, 12.6$  Hz), 1.15-1.22 (*m*, 3H), 1.46 (*br.s*, 3H), 1.76 (*d*, 1H,  $J = 13.5$  Hz), 1.77-1.85 (*m*, 4H), 2.07 (*dd*, 1H,  $J = 2.0, 13.9$  Hz), 2.29

(*dt*, 1H,  $J = 2.5, 14.8$  Hz), 5.25 (*br.s*, 1H).  $^1\text{H-NMR}$  (500MHz,  $\text{CDCl}_3$ ),  $\delta$  0.78 (*s*, 3H), 1.41 (*dd*, 1H,  $J = 5.67, 12.6$  Hz), 1.67 (*s*, 3H), 2.02-2.10 (*m*, 3H), 2.13-2.22 (*m*, 3H), 2.28-2.39 (*m*, 3H), 2.44-2.50 (*m*, 1H), 5.41 (*br.s*, 1H). MS (EI, 70eV)  $m/z$  (rel. int.): 178 [ $\text{M}^+$ ] (45), 163 (100), 150 (47), 145 (10), 135 (18), 121 (40), 107 (41), 105 (37), 95 (40), 93 (61), 91 (42), 81 (82), 79 (63), 77 (39), 67 (37), 53 (38), 41 (63).

#### 7.9.5. (+)-(4*S*,5*R*,6*R*,7*S*,10*S*)-6*b*-Hydroxyeudesm-11-ene (234)

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1643$ ;  $R_f = 0.91$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.82 (*s*, 3H,  $\text{CH}_3$ -14), 0.97-1.03 (*m*, 1H, H-3a), 1.04-1.09 (*m*, 2H, H-1a, H-5), 1.15-1.21 (*m*, 1H, H-9a), 1.29-1.34 (*m*, 1H, H-1b), 1.31 (*d*, 3H,  $\text{CH}_3$ -15,  $J = 6.3$  Hz), 1.35-1.42 (*m*, 2H, H-9b, H-8a), 1.44-1.53 (*m*, 3H, H-8b, H-2a, H-4), 1.57-1.62 (*m*, 1H, H-2b), 1.63 (*s*, 3H,  $\text{CH}_3$ -12), 1.64-1.67 (*m*, 1H, H-3b), 2.42-2.47 (*m*, 1H, H-7), 3.61-3.66 (*m*, 1H, H-6), 4.81 (*s*, 1H, H-13a), 4.88 (*s*, 1H, H-13b).  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 7; MS (EI, 70eV)  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (3), 207 (7), 189 (6), 180 (3), 161 (2), 149 (2), 137 (47), 123 (57), 109 (39), 95 (27), 81 (48), 69 (44), 55 (56), 41 (100).

#### 7.9.6. (-)-(4*S*,5*R*,6*S*,7*R*,10*S*)-6*a*-Hydroxyeudesm-11-ene (235)

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1598$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.57 (*dd*, 1H, H-5,  $J = 2.2, 10.7$  Hz), 0.88-0.93 (*m*, 1H, H-3a), 0.98 (*d*, 3H,  $\text{CH}_3 = 15$ ,  $J = 6.3$ Hz), 1.02-1.08 (*m*, 2H, H-1), 1.23 (*s*, 3H,  $\text{CH}_3$ -14), 1.26-1.31 (*m*, 4H, H-2, H-9), 1.46-1.49 (*m*, 2H, H-8), 1.59 (*s*, 3H,  $\text{CH}_3$ -12), 1.71-1.78 (*m*, 2H, H-7, H-3b), 1.89-1.95 (*m*, 1H, H-4), 3.92 (*br.s*, 1H, H-6), 4.76 (*s*, 1H, 13a), 4.83 (*s*, 1H, 13b);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 7; MS (EI, 70eV),  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (6), 207 (8), 204 (16), 189 (14), 180 (5), 161 (6), 139 (35), 137 (56), 123 (100), 109 (40), 95 (32), 81 (52), 69 (41), 55 (53), 41 (79).

#### 7.9.7. (+)-(4*S*,5*R*,6*S*,7*R*,10*S*)-6,11-Epoxyeudesmane (236)

Colourless oil;  $\text{RI}_{\text{CPSIL } 5} = 1534$ ;  $R_f = 0.86$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.58 (*s*, 3H,  $\text{CH}_3$ -14), 0.90-1.00 (*m*, 1H, H-3a) 1.05-1.12 (*m*, 1H, H-1a), 1.09 (*d*, 3H,  $\text{CH}_3$ -15,  $J = 5.9$  Hz), 1.14-1.21 (*m*, 2H, H-4, H-9a), 1.22-1.28 (*m*, 2H, H-1b, H-5), 1.30 (*s*, 3H,  $\text{CH}_3$ -13), 1.31 (*s*, 3H,  $\text{CH}_3$ -12), 1.36-1.48 (*m*, 4H, H-9b, H-8a, H-2), 1.49-1.53 (*m*, 1H, H-8b), 1.55-1.62 (*m*, 1H, H-3b), 2.55-2.60 (*m*, 1H, H-7), 4.28 (*dd*, 1H, H-6,  $J = 7.4, 8.7$  Hz).  $^{13}\text{C-NMR}$ (125.7MHz,  $\text{C}_6\text{D}_6$ ): Table 7; MS (EI, 70eV),  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (4),

207 (5), 189 (3), 180 (3), 164 (34), 149 (77), 137 (40), 123 (50), 109 (90), 95 (35), 81 (50), 69 (46), 55 (52), 41 (100).

**7.9.8. (-)-(4S,5R,10R)-6,7-seco-Eudesm-7(11)-en-6-al (237)**

Colourless oil,  $RI_{\text{CPSIL } 5} = 1614$ ;  $R_f = 0.95$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.55-0.65 (*m*, 1H, H-3a), 0.68 (*d*, 3H,  $\text{CH}_3$ -15,  $J = 6.0$  Hz), 0.81 (*s*, 3H,  $\text{CH}_3$ -14), 0.93-1.03 (*m*, 1H, H-1a), 1.12-1.17 (*m*, 1H, H-1b), 1.20-1.32 (*m*, 4H, H-2, H-9), 1.42-1.47 (*m*, 1H, H-3b), 1.60 (*s*, 3H,  $\text{CH}_3$ -13), 1.61-1.65 (*m*, 2H, H-4, H-5), 1.68 (*s*, 3H,  $\text{CH}_3$ -12), 1.85-1.94 (*m*, 1H, H-8a), 2.06-2.13 (*m*, 1H, H-8b), 5.16 (*dt*, 1H, H-7,  $J = 1.6, 7.3$  Hz), 9.46 (*d*, 1H, H-6,  $J = 4.4$  Hz).  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 7; MS (EI, 70eV),  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (4), 207(4), 180 (3), 161 (2), 147 (2), 137 (38), 123 (7), 109 (35), 95 (21), 81 (22), 69 (53), 55 (39), 43 (23), 41 (100).

**7.9.9. (+)-(4S,10S)-Eudesma-5,7(11)-diene (238)**

Colourless oil;  $RI_{\text{CPSIL } 5} = 1543$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.07 (*br.s*, 3H), 1.09 (*d*, 3H,  $J = 6.6$  Hz), 1.70 (*br.s*, 3H), 1.80 (*br.s*, 3H), 6.32 (*br.s*, 1H). MS (EI, 70eV),  $m/z$  (rel. int.): 204 [ $\text{M}^+$ ] (90), 189 (100), 175 (5), 161 (38), 149 (23), 148 (25), 147 (21), 133 (57), 119 (30), 107 (26), 105 (52), 91 (56), 77 (25), 65 (18), 55 (31), 41 (69).

**7.9.10. (+)-(1R\*,4S\*,5S\*,6S\*,7R\*)-6,11-Epoxyisodaucane (239)**

Colourless oil;  $RI_{\text{CPSIL } 5} = 1468$ ;  $R_f = 0.97$ ; sense of optical rotation (benzene): (+);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  0.85 (*s*, 3H,  $\text{CH}_3$ -15), 0.94 (*dq*, 1H, H-8a,  $J = 2.5, 12.0$  Hz), 1.17 (*s*, 3H,  $\text{CH}_3$ -13), 1.15-1.23 (*m*, 1H, H-10a), 1.21 (*d*, 3H,  $\text{CH}_3$ -14,  $J = 6.6$  Hz), 1.22-1.27 (*m*, 1H, H-9a), 1.30 (*s*, 3H,  $\text{CH}_3$ -12), 1.40-1.49 (*m*, 3H, H-2, H-9b), 1.57-1.60 (*m*, 4H, H-7, H-3, H-10b), 1.66-1.72 (*m*, 1H, H-8b), 1.99 (*t*, 1H, H-5,  $J = 9.5$  Hz), 2.35-2.40 (*m*, 1H, H-4), 3.22 (*t*, 1H, H-6,  $J = 9.8$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ): Table 7; MS (EI, 70eV),  $m/z$  (rel. int.): 222 [ $\text{M}^+$ ] (4), 208 (16), 207 (100), 189 (16), 164 (10), 151 (17), 149 (30), 135 (9), 123 (33), 109 (22), 107 (24), 93 (26), 81 (64), 79 (28), 67 (26), 55 (42), 43 (83), 41 (77).

**Table 6.**  $^{13}\text{C}$ -NMR spectral data of compounds (+)-**230** and (+)-**231** (125.7 MHz,  $\text{C}_6\text{D}_6$ );  $\delta$  (ppm)

C	<b>230</b>	<b>231</b>
1	37.3 ( <i>t</i> )	36.8 ( <i>t</i> )
2	23.0 ( <i>t</i> )	22.8 ( <i>t</i> )
3	122.1 ( <i>d</i> )	122.5 ( <i>d</i> )
4	133.5 ( <i>s</i> )	133.9 ( <i>s</i> )
5	45.6 ( <i>d</i> )	43.8 ( <i>d</i> )
6	30.5 ( <i>t</i> )	29.4 ( <i>t</i> )
7	59.1 ( <i>d</i> )	145.1 ( <i>s</i> )
8	207.3 ( <i>s</i> )	200.6 ( <i>s</i> )
9	55.7 ( <i>t</i> )	56.4 ( <i>t</i> )
10	36.9 ( <i>s</i> )	33.4 ( <i>s</i> )
11	144.3 ( <i>s</i> )	131.2 ( <i>s</i> )
12	20.9 ( <i>q</i> )	22.7 ( <i>q</i> )
13	113.2 ( <i>t</i> )	23.8 ( <i>q</i> )
14	16.9 ( <i>q</i> )	17.2 ( <i>q</i> )
15	21.3 ( <i>q</i> )	21.0 ( <i>q</i> )

All assignments were confirmed by HMBC and HMQC.

**Table 7.**  $^{13}\text{C}$ -NMR spectra data for compounds (+)-**234**, (-)-**235**, (+)-**236**, (-)-**237** and (+)-**239** (125.7MHz,  $\text{C}_6\text{D}_6$ ),  $\delta$  (ppm)

C	<b>237</b>	<b>236</b>	<b>234</b>	<b>235</b>	<b>239</b>
1	37.3 ( <i>t</i> )	41.8 ( <i>t</i> )	44.0 ( <i>t</i> )	42.7 ( <i>t</i> )	42.6 ( <i>s</i> )
2	21.7 ( <i>t</i> )	22.2 ( <i>t</i> )	22.2 ( <i>t</i> )	21.4 ( <i>t</i> )	41.9 ( <i>t</i> )
3	34.5 ( <i>t</i> )	36.6 ( <i>t</i> )	38.0 ( <i>t</i> )	37.6 ( <i>t</i> )	26.4 ( <i>t</i> )
4	27.6 ( <i>d</i> )	33.8 ( <i>d</i> )	33.4 ( <i>d</i> )	30.0 ( <i>d</i> )	54.2 ( <i>d</i> )
5	64.2 ( <i>d</i> )	55.5 ( <i>d</i> )	53.7 ( <i>d</i> )	55.3 ( <i>d</i> )	62.8 ( <i>d</i> )
6	205.3 ( <i>d</i> )	78.0 ( <i>d</i> )	70.5 ( <i>d</i> )	66.6 ( <i>d</i> )	82.7 ( <i>d</i> )
7	125.2 ( <i>d</i> )	40.1 ( <i>d</i> )	46.3 ( <i>d</i> )	51.2 ( <i>d</i> )	41.7 ( <i>d</i> )
8	22.1 ( <i>t</i> )	18.9 ( <i>t</i> )	22.1 ( <i>t</i> )	22.5 ( <i>t</i> )	38.6 ( <i>t</i> )
9	43.9 ( <i>t</i> )	39.9 ( <i>t</i> )	38.8 ( <i>t</i> )	44.2 ( <i>t</i> )	23.5 ( <i>t</i> )
10	36.4 ( <i>s</i> )	33.1 ( <i>s</i> )	34.0 ( <i>s</i> )	34.0 ( <i>s</i> )	41.2 ( <i>t</i> )
11	131.2 ( <i>s</i> )	83.5 ( <i>s</i> )	146.5 ( <i>s</i> )	147.8 ( <i>s</i> )	81.0 ( <i>s</i> )
12	25.9 ( <i>q</i> )	31.9 ( <i>q</i> )	23.9 ( <i>q</i> )	23.0 ( <i>q</i> )	32.2 ( <i>q</i> )
13	17.6 ( <i>q</i> )	25.1 ( <i>q</i> )	113.2 ( <i>t</i> )	111.9 ( <i>t</i> )	23.8 ( <i>q</i> )
14	20.0 ( <i>q</i> )	18.2 ( <i>q</i> )	21.8 ( <i>q</i> )	20.6 ( <i>q</i> )	20.3 ( <i>q</i> )
15	20.9 ( <i>q</i> )	20.7 ( <i>q</i> )	22.8 ( <i>q</i> )	20.0 ( <i>q</i> )	28.1 ( <i>q</i> )

All assignments were confirmed by HMBC and HMQC.

## 8. Chemical Analysis of *Barbilophozia floerkei*

The essential oil of *Barbilophozia floerkei* was obtained by hydrodistillation of the plant material collected in August 2002 from Altenau in Germany.

### 8.1.1. (+)-Trisnoranastreptene (241)

Colourless oil; RI<sub>CPSIL 5</sub> = 1189; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 0.97 (3H, *s*, H-12), 1.55 (1H, *dt*, H-9a, *J* = 6.6, 12.3 Hz), 1.69 (3H, *br.s*, H-11), 1.70-1.84 (2H, *m*, H-8a, H-9b), 1.86-1.89 (1H, *m*, H-8b), 1.90-1.95 (2H, *m*, H-1, H-2a), 2.43 (1H, *dd*, H-2b, *J* = 7.9, 17.7 Hz), 5.12 (1H, *br.s*, H-3), 5.50-5.54 (1H, *dq*, H-7, *J* = 2.2, 9.5 Hz), 6.21 (1H, *dd*, H-6, *J* = 2.8, 10.1 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ 13.5 (*q*, C-12), 15.1 (*q*, C-11), 22.5 (*t*, C-8), 28.1 (*s*, C-10), 29.3 (*t*, C-9), 31.6 (*t*, C-2), 32.0 (*d*, C-1), 42.7 (*s*, C-5), 122.4 (*d*, C-7), 124.4 (*d*, C-3), 128.4 (*d*, C-6), 140.6 (*s*, C-4); MS (EI 70eV), *m/z* (rel. int.): 160 [M<sup>+</sup>] (34), 145 (100), 130 (33), 117 (42), 115 (43), 105 (35), 91 (59), 77 (38), 65 (27), 51 (37); HRMS calcd for C<sub>12</sub>H<sub>16</sub> [M<sup>+</sup>] *m/z* 160.1252 found [M<sup>+</sup>] *m/z* 160.1245.

### 8.1.2. 1,4-Dimethylazulene (244)

Blue oil; RI<sub>CPSIL 5</sub> = 1528; <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 2.53 (3H, *s*), 2.58 (3H, *s*), 6.75 (1H, *d*, *J* = 10.4 Hz), 6.79 (1H, *t*, *J* = 9.8 Hz), 7.20 (1H, *d*, *J* = 9.8 Hz), 7.33 (1H, *d*, *J* = 3.8 Hz), 7.66 (1H, *d*, *J* = 3.8 Hz), 8.05 (1H, *d*, *J* = 9.1 Hz).

### 8.1.3. (+)-1,2,3,6-Tetrahydro-1,4-dimethylazulene (245)

Colourless oil; RI<sub>CPSIL 5</sub> = 1244; sense of optical rotation (benzene): (+); <sup>1</sup>H-NMR (500 MHz, C<sub>6</sub>D<sub>6</sub>): δ 1.12 (3H, *d*, H-11, *J* = 6.9 Hz), 1.28-1.35 (1H, *m*, H-2a), 1.79 (3H, *s*, H-12), 1.97-2.03 (1H, *m*, H-2b), 2.04-2.10 (1H, *m*, H-6a), 2.35-2.40 (1H, *m*, H-6b), 2.45-2.51 (1H, *m*, H-3a), 2.52-2.58 (1H, *m*, H-3b), 2.84-2.90 (1H, *m*, H-1), 5.05 (1H, *t*, H-5, *J* = 6.6 Hz), 5.36 (1H, *dd*, H-7, *J* = 6.6, 16.1 Hz), 6.10 (1H, *d*, H-8, *J* = 9.5 Hz); <sup>13</sup>C-NMR (125.7 MHz, C<sub>6</sub>D<sub>6</sub>): δ 20.5 (*q*, C-12), 21.0 (*q*, C-11), 28.2 (*t*, C-6), 32.0 (*t*, C-2), 34.2 (*t*, C-3), 42.6 (*d*, C-1), 115.2 (*d*, C-5), 119.2 (*d*, C-7), 125.3 (*d*, C-8), 133.8 (*s*, C-4), 142.6 (*s*, C-10), 146.1 (*s*, C-9); MS (EI 70eV), *m/z* (rel. int.): 160 [M<sup>+</sup>] (22), 145 (100), 130 (23), 128 (29), 118 (39), 115 (36), 105 (12), 91 (28), 77 (22), 63 (22), 51(31); HRMS calcd for C<sub>12</sub>H<sub>16</sub> [M<sup>+</sup>] *m/z* 160.1252 found [M<sup>+</sup>] *m/z* 160.1240.



#### 8.1.4. (-)-2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulen-4-ol (246)

Colourless oil;  $RI_{\text{CPSIL } 5} = 1448$ ; sense of optical rotation (benzene): (-);  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.00 (3H, *s*, H-12), 1.57 (3H, *br.s*, H-11), 1.71-1.82 (3H, *m*, H-3a, H-5), 1.84-1.91 (1H, *m*, H-3b), 1.95-2.03 (1H, *m*, H-6a), 2.04-2.13 (1H, *m*, H-2a), 2.14-2.25 (2H, *m*, H-2b, H-6b), 2.93 (1H, *br.t*, H-10,  $J = 6.3$  Hz), 5.51-5.55 (1H, *m*, H-7), 6.26 (1H, *d*, H-8,  $J = 11.7$  Hz);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  14.6 (*q*, C-11), 20.7 (*q*, C-12), 24.8 (*t*, C-3), 25.9 (*t*, C-6), 37.3 (*t*, C-2), 44.4 (*t*, C-5), 60.3 (*d*, C-10), 74.7 (*s*, C-4), 124.8 (*d*, C-8), 128.7 (*d*, C-7), 133.7 (*s*, C-9), 139.1 (*s*, C-1); MS (EI 70eV):  $m/z$  (rel. int.): 178 [ $\text{M}^+$ ] (12), 160 (43), 131 (73), 117 (48), 115 (50), 105 (57), 91(100), 79 (59), 77 (70), 65 (35), 51 (53); HRMS calcd for  $\text{C}_{12}\text{H}_{16}\text{O}$  [ $\text{M}^+$ ]  $m/z$  178.1357 found [ $\text{M}^+$ ]  $m/z$  178.1354

#### 8.1.5. 5,6-Dihydro-1,4-dimethylazulene (248)

Yellow oil;  $RI_{\text{CPSIL } 5} = 1425$ ;  $^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  1.81 (3H, *s*), 1.93 (3H, *s*), 2.11-2.18 (4H, *m*), 5.63-5.67 (1H, *m*), 6.29 (1H, *d*,  $J = 5.4$  Hz), 6.51 (1H, *d*,  $J = 5.4$  Hz), 6.57 (1H, *d*,  $J = 11.0$  Hz); MS (EI, 70eV),  $m/z$  (rel. int.): 158 [ $\text{M}^+$ ] (30), 156 (84), 155 (83), 143 (55), 142 (22), 141 (100), 128 (78), 115 (59), 102 (9), 89 (10), 77 (24), 63 (29), 51 (32), 39 (33).

## 9. Hazardous chemicals

Compound	Hazard symbol	R(risk) phrases	S (safety) phrases
Acetone	F	11	9 - 16 - 23, 2 - 33
Benzene-d <sub>6</sub>	F, T	42, 2-11- 23 / 24 / 25- 48	53 - 16 - 29 - 44
Boron trifluoride-Diethyl ether Complex	C, F	15 - 34	16 - 23, 2 - 26 - 28, 1 - 36
Chloroform	Xn	22 - 38 - 40- 48 / 20 / 22	36 / 37
Chloroform-d <sub>1</sub>	Xn	22 - 38- 40 -48 / 20 / 22	36 / 37
m-Chloroperbenzoic acid	O, Xi	7- 36 / 37 / 38	3/7-14, 10-36 /37/ 39
Dichloromethane	Xn	20 - 40	24
Diethyl ether	F <sup>+</sup>	12 - 19	9 - 16 - 29 - 33
Ethanol	F	11	7-16
Ethyl acetate	F	11	16 - 23, 2 - 29 - 33
n-Hexane	Xn, F	11- 48 / 20	9- 16- 24 / 25-29-51
Hydrogen	F	12	9 - 16 - 33
Lithium aluminium hydride	F	15	7 / 8 - 24 / 25- 43
Methanol	F, T	11- 23 / 25	7 - 16 - 24 - 45
Palladium	A	-	-
Pentane	F	11	9 - 16 - 29 - 33
Pyridinium dichromate	T, N	49 - 43 - 50 / 53	53 - 45 - 60 - 61
Pyridine	Xn, F	11 - 20 / 21 / 22	26 - 28, 1
Phosphoryl chloride	C	34 - 37	7 / 8- 26 - 45
Sodium sulphate	-	-	-
Sodium carbonate	Xi	36	22 - 26
Sodium hydrogen carbonate	-	-	-
Sodium hydroxide	C	35	26 - 37 / 39 - 45
Silver nitrate	C, N	34 - 50 / 53	26 - 45- 60 - 61

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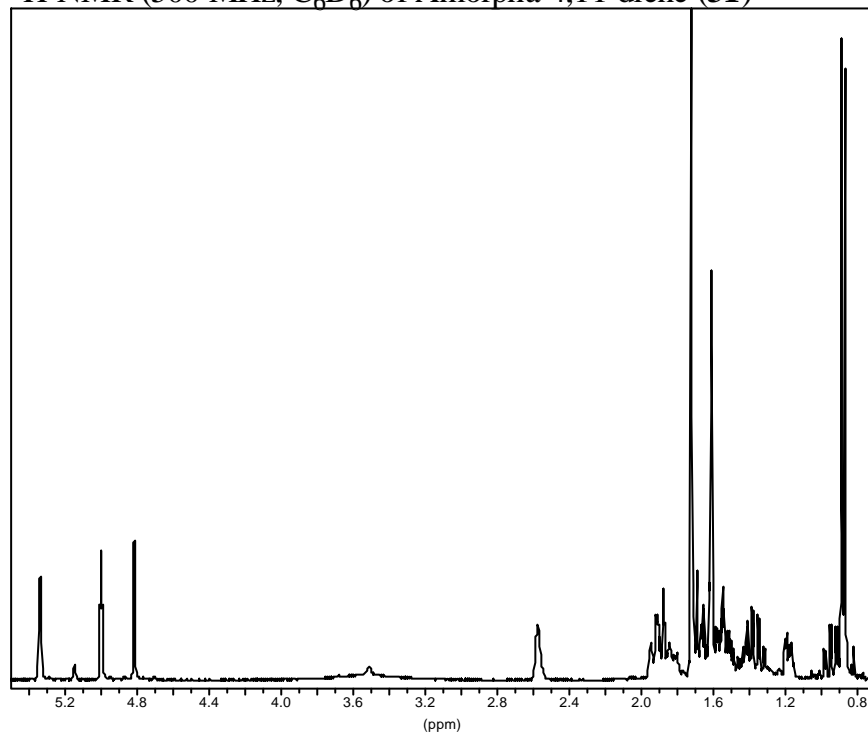
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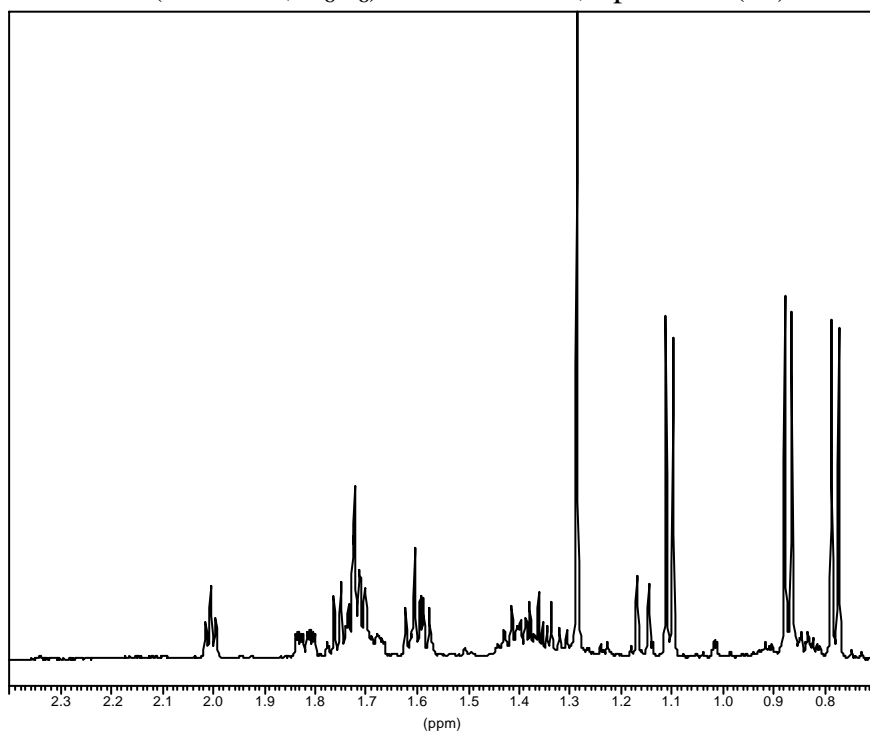
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## 11. $^1\text{H-NMR}$ Spectra of Compound.

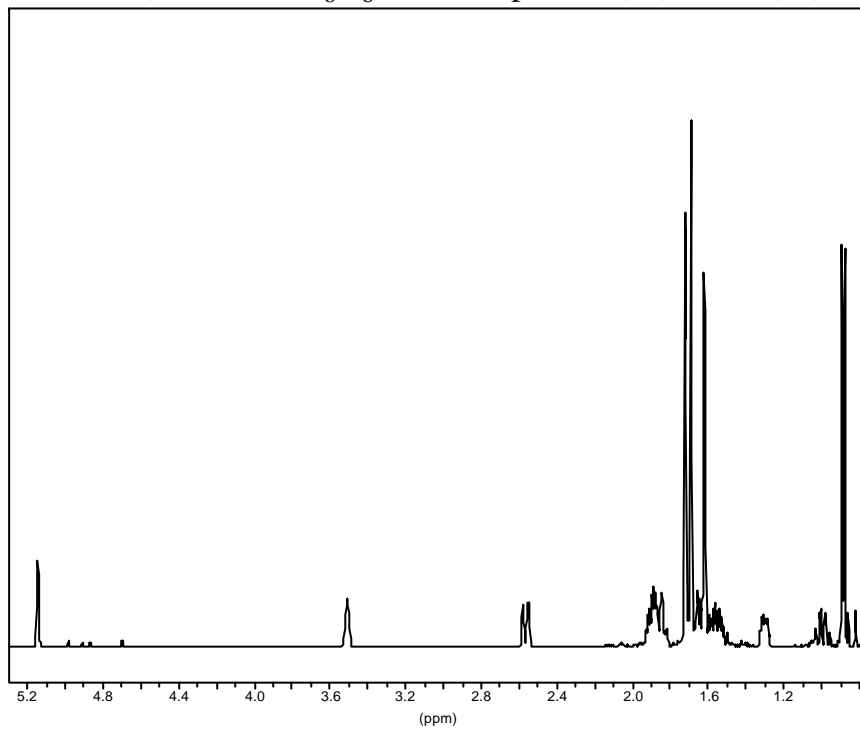
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Amorpha-4,11-diene (**31**)



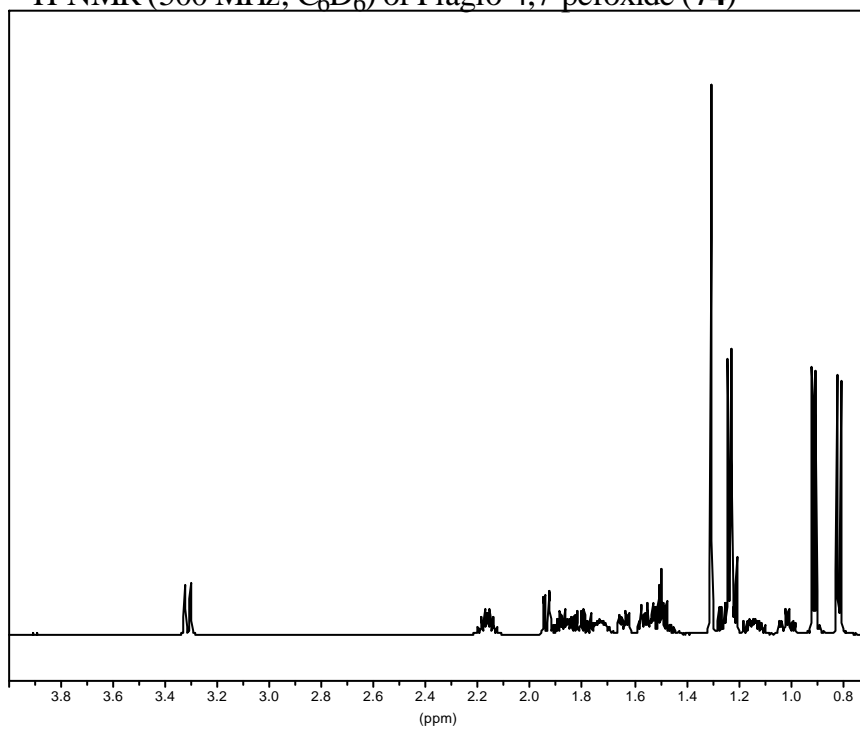
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Muurolan-4,7-peroxide (**69**)



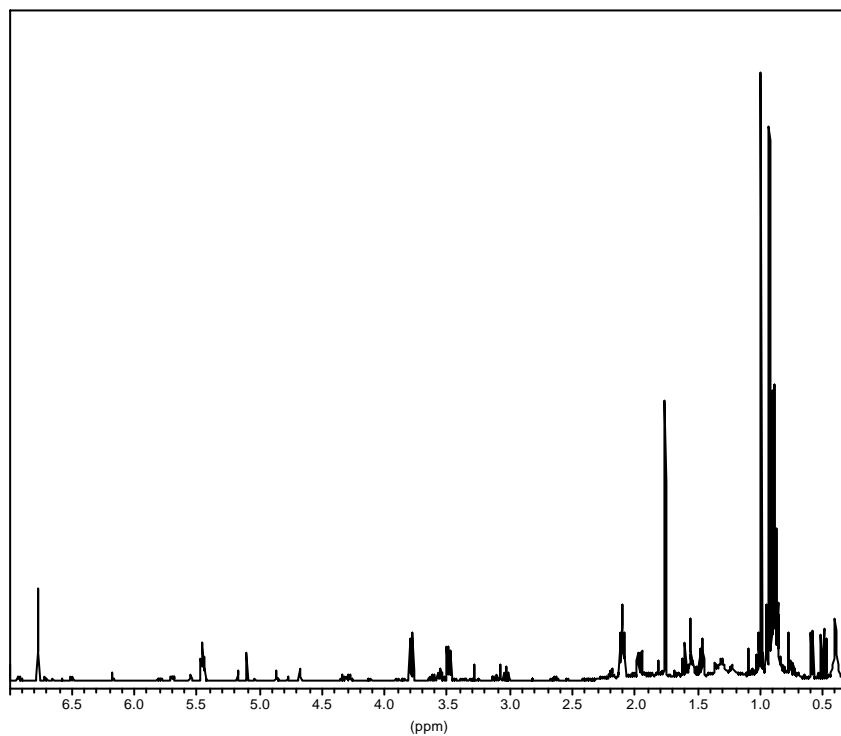
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Amorpha-4,7(11)-diene (**70**)



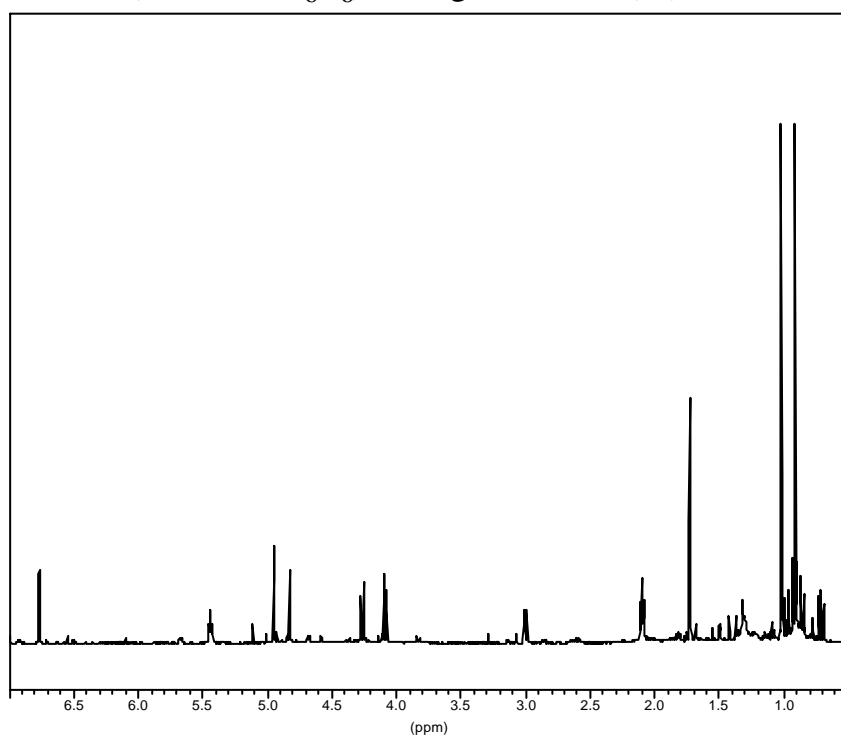
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Plagio-4,7-peroxide (**74**)



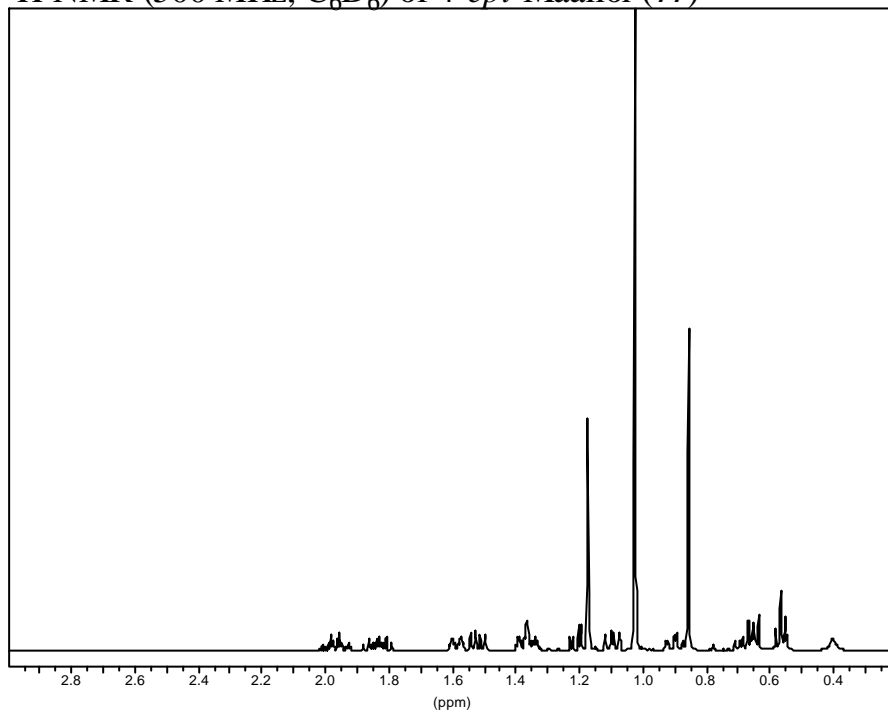
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Plagiochiline-W (**75**)



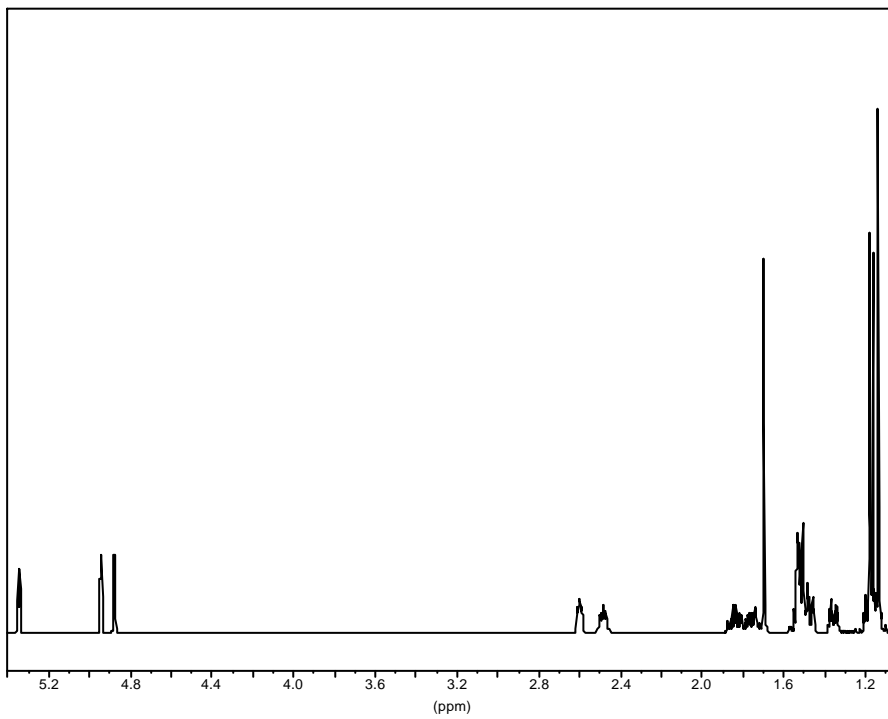
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Plagiochiline-X (**76**)



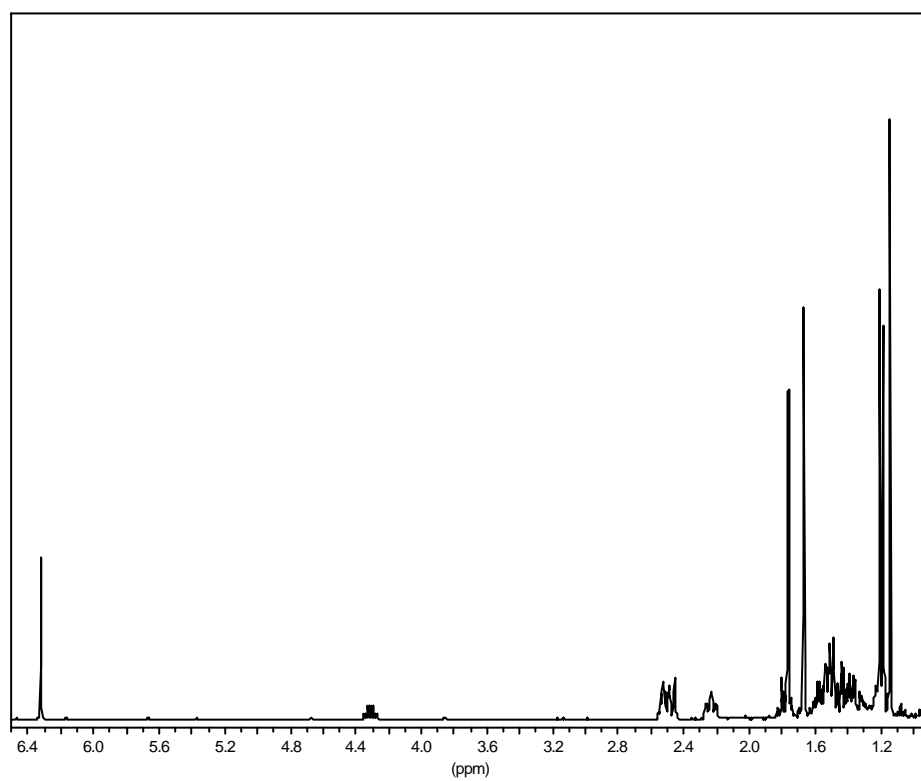
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 4-*epi*-Maaliol (**77**)



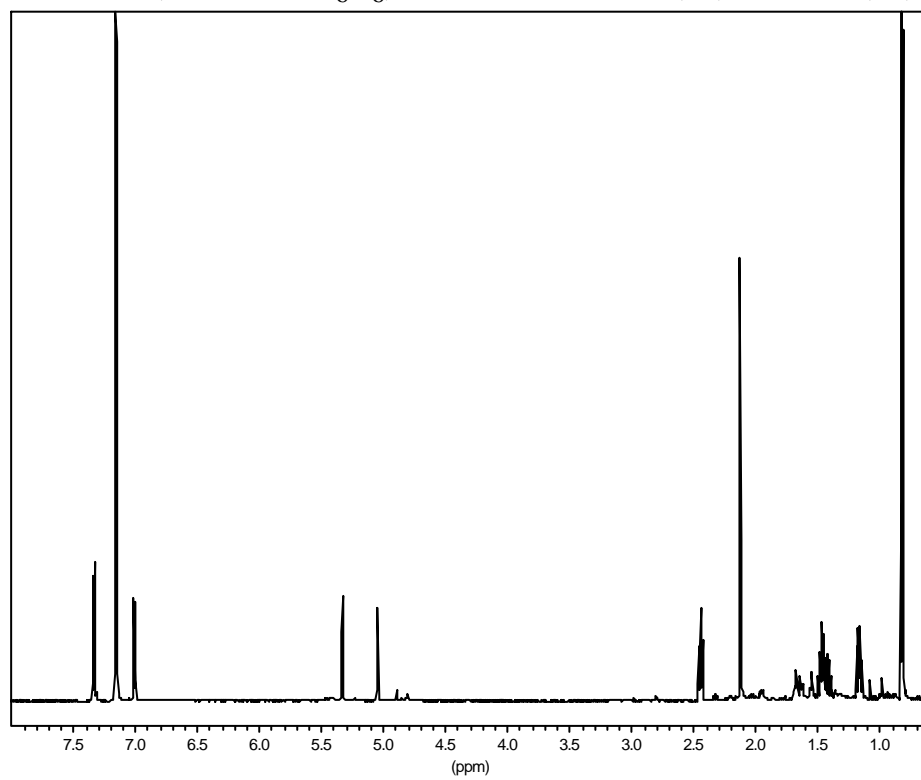
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Selina-5,11-diene (**80**)



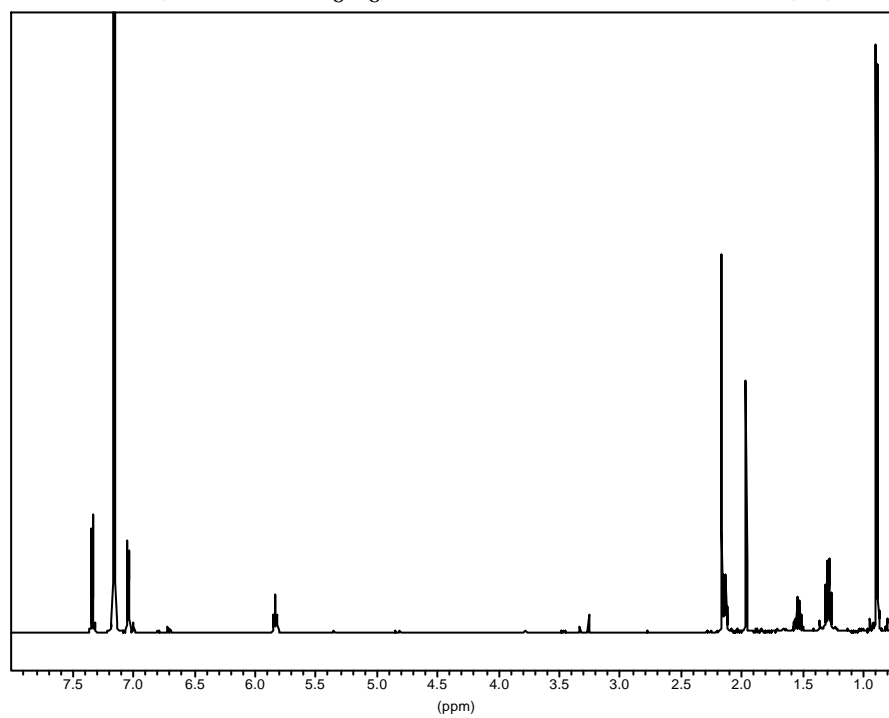
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Selina-5,7(11)-diene (**83**)



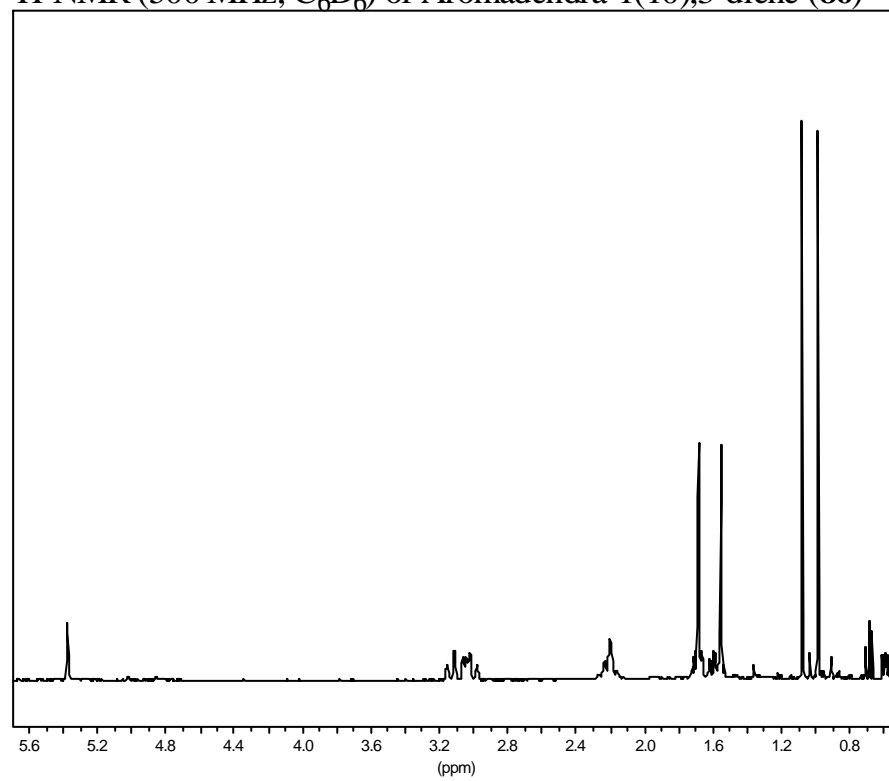
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Bisabola-1,3,5,7(14)-tetraene (**84**)



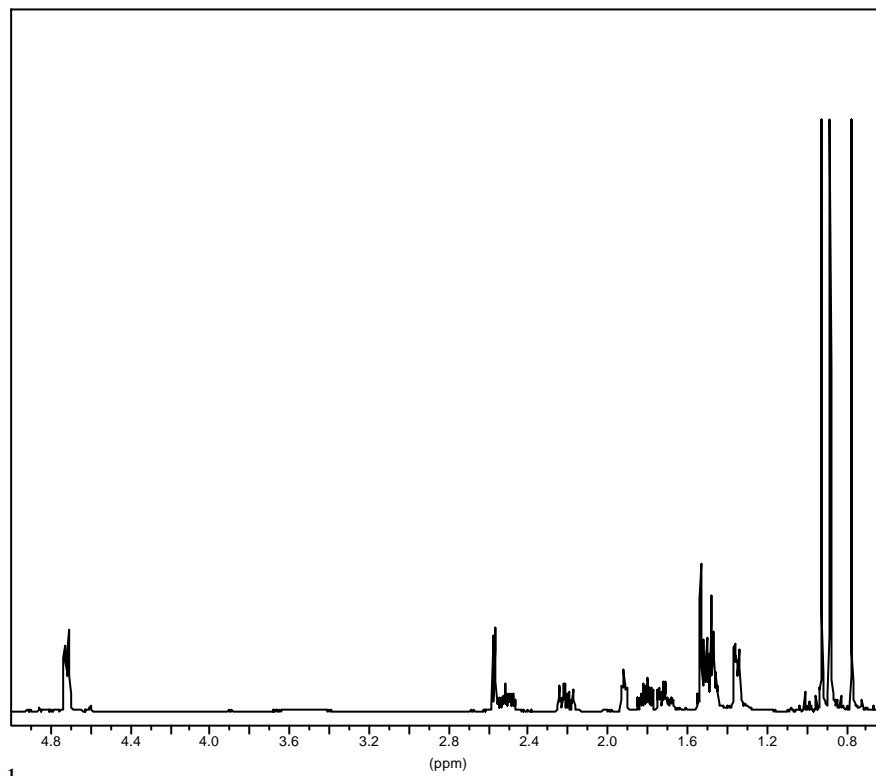
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Bisabola-1,3,5,7-tetraene (**85**)



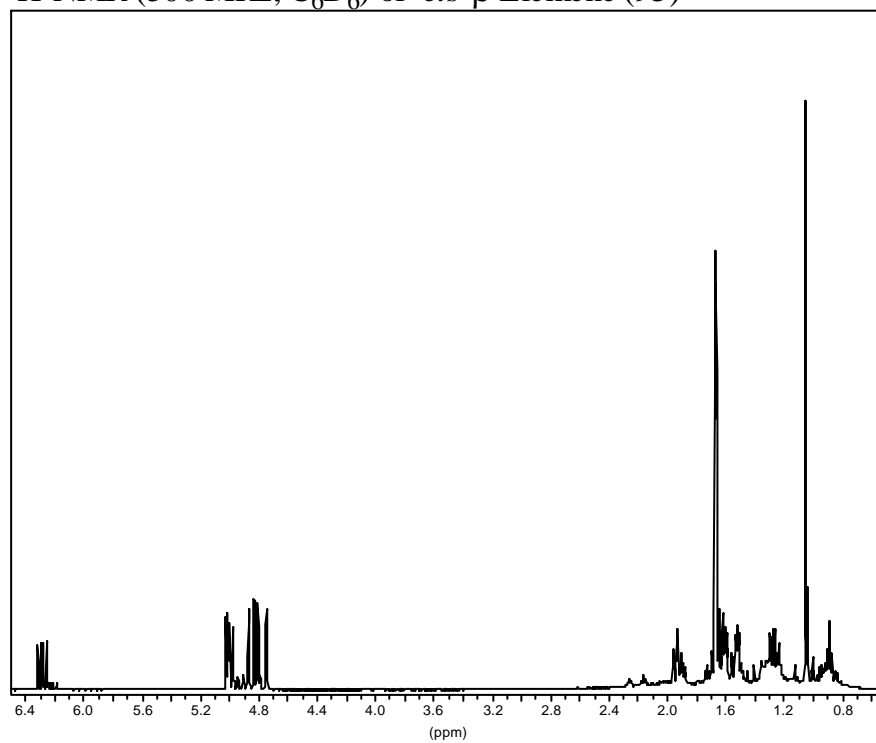
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Aromadendra-1(10),3-diene (**86**)



$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $\beta$ -Longipinene (**92**)

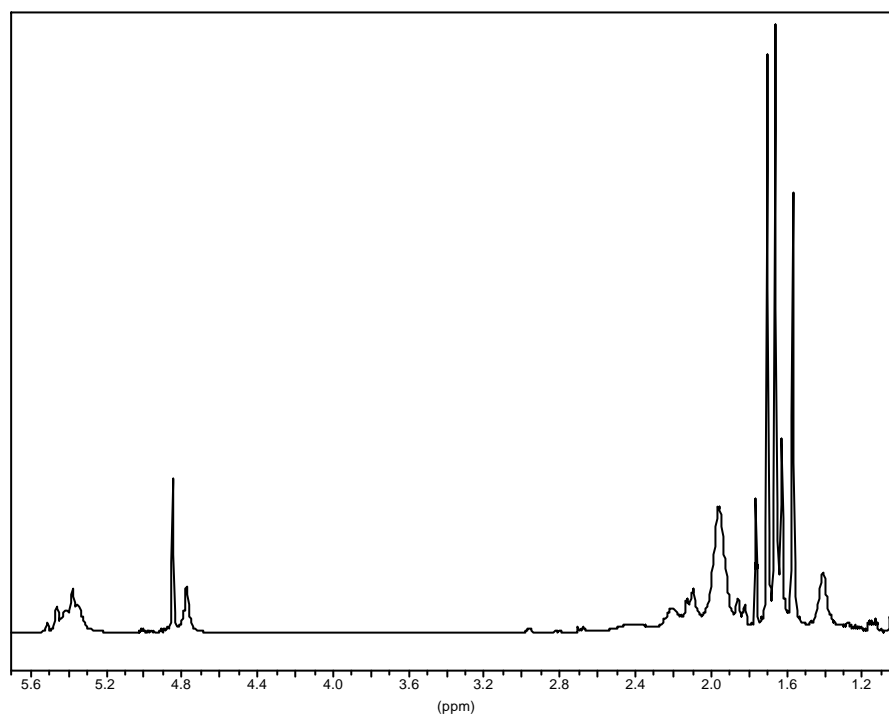


$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of *cis*- $\beta$ -Elemene (**93**)

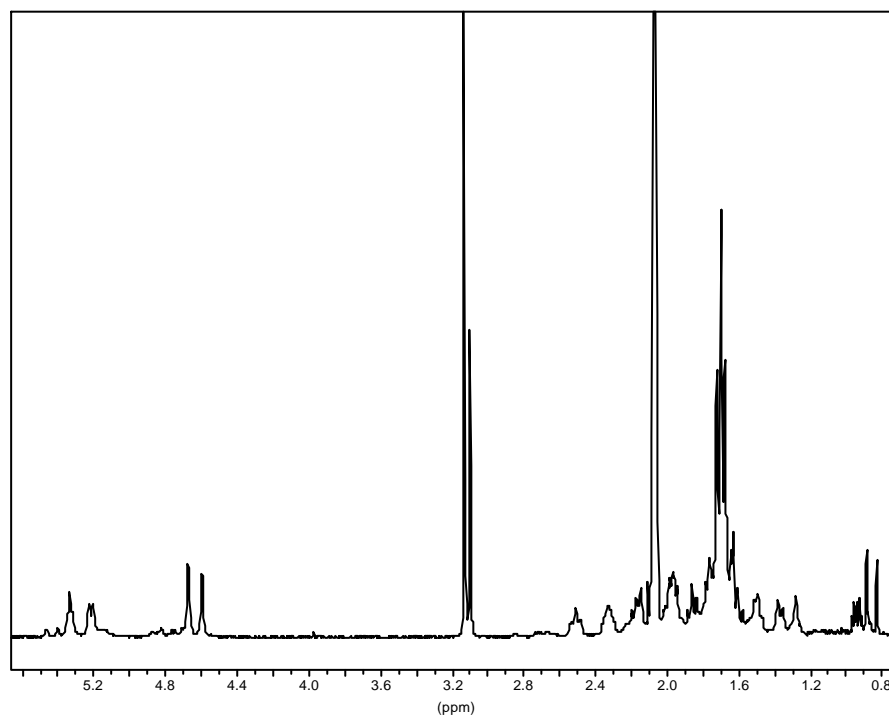




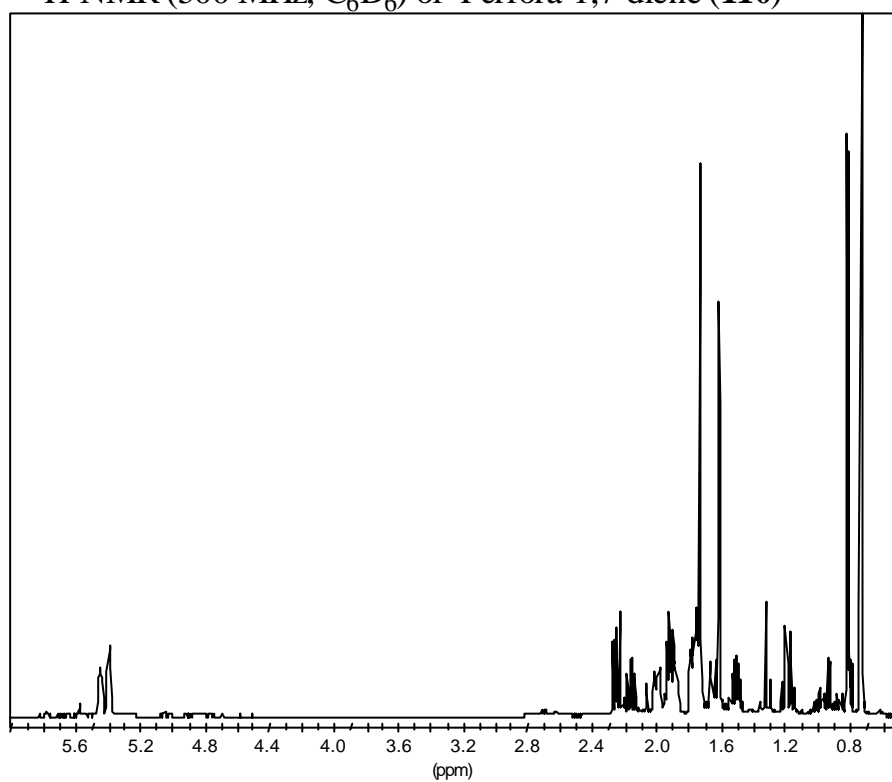
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Helminthogermacrene (**107**)



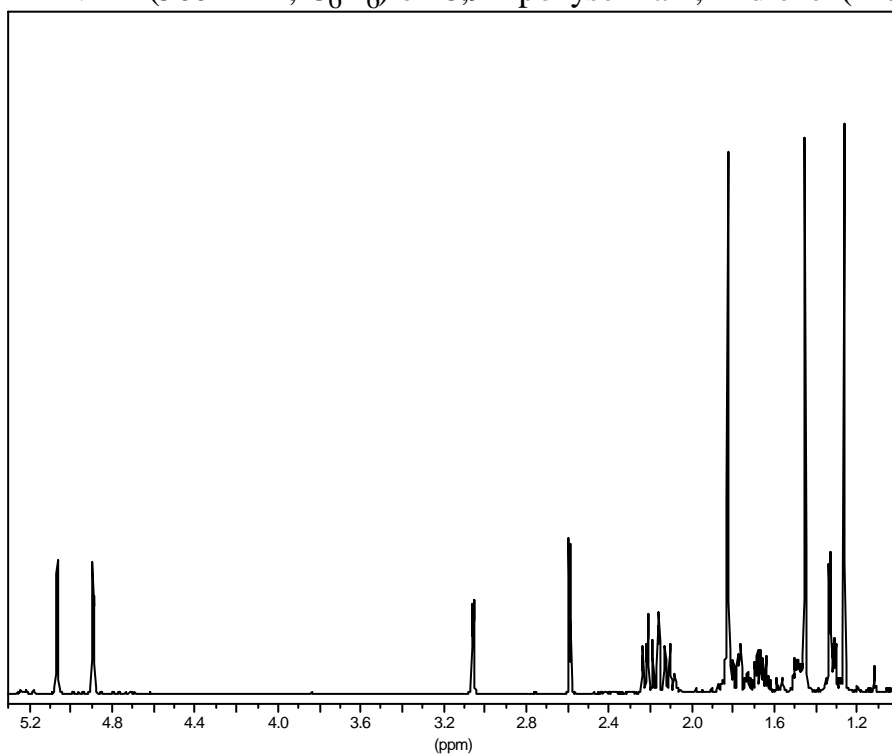
$^1\text{H-NMR}$  (500 MHz, acetone- $\text{d}_6$ ) of Helminthogermacrene (**107**)



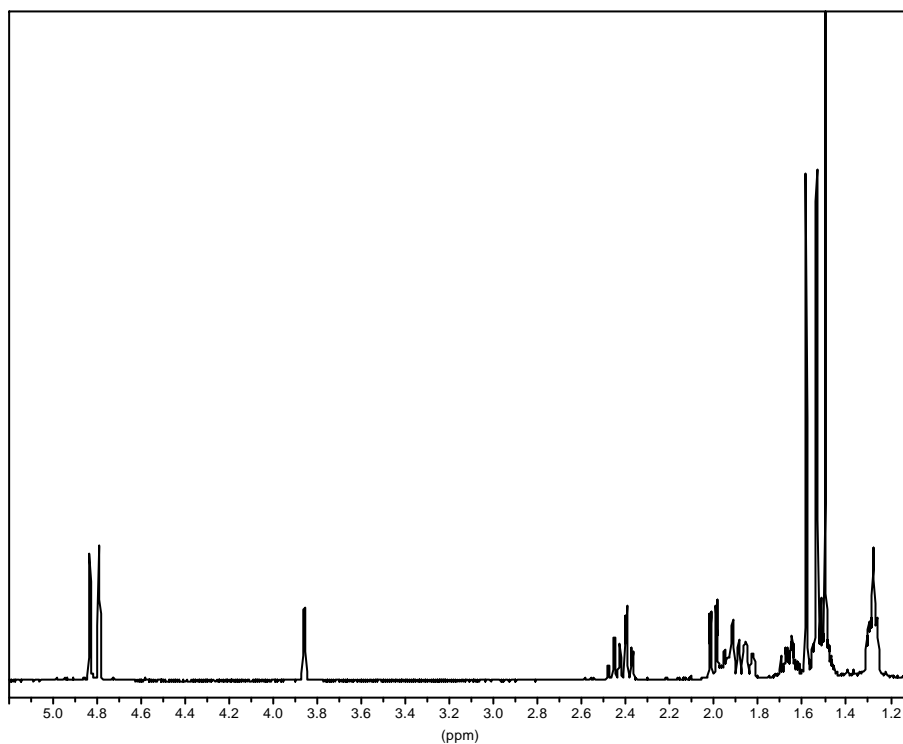
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Perfora-1,7-diene (**110**)



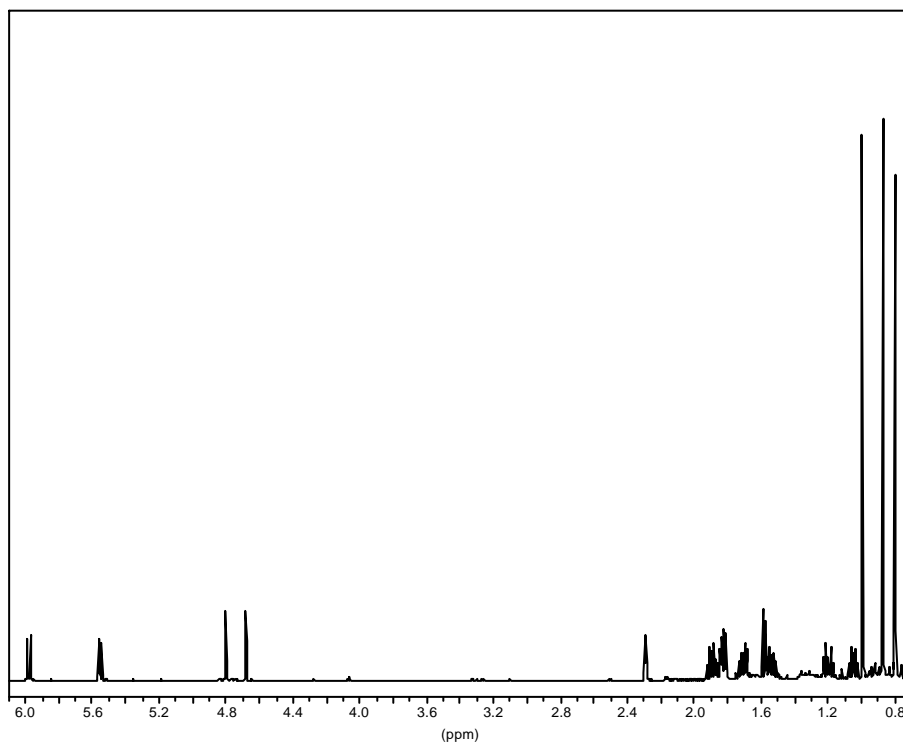
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 8,9-Epoxyelina-4,11-diene (**143**)



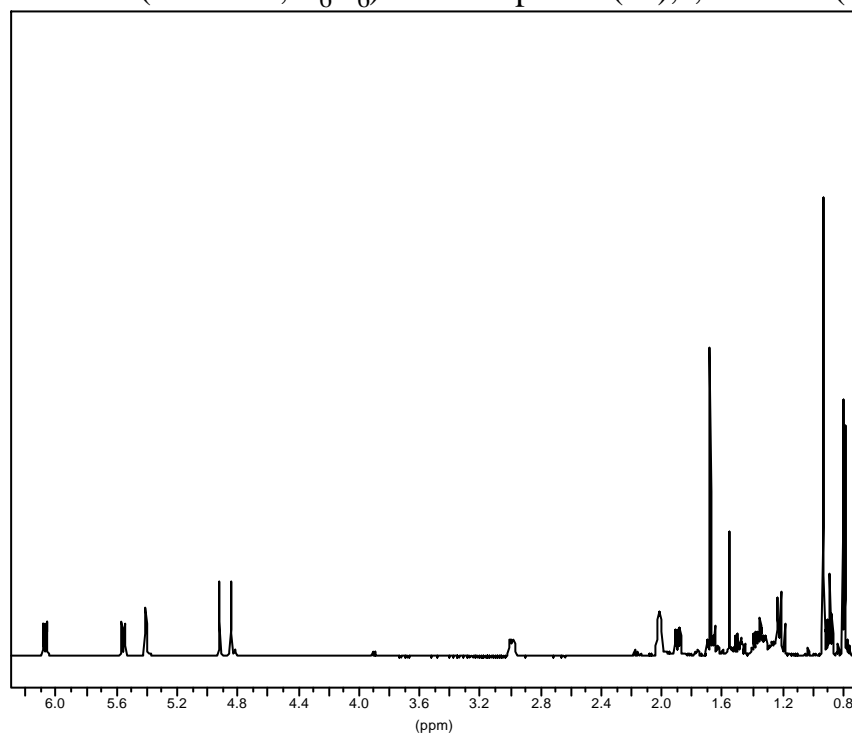
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Eudesma-4,11-dien-8 $\alpha$ -ol (**147**)



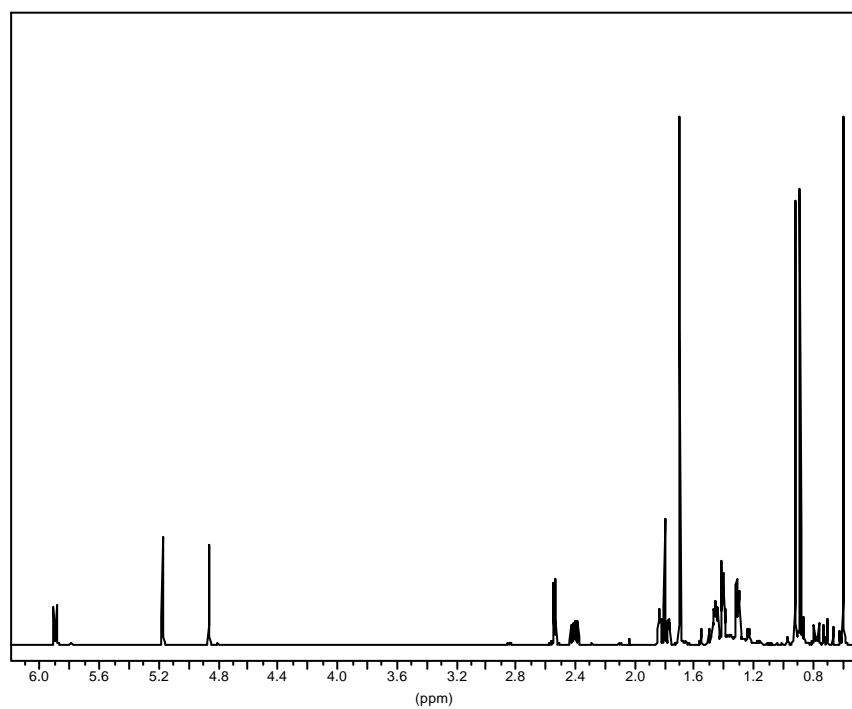
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Gymnomitr-3(15),4-diene (**166**)



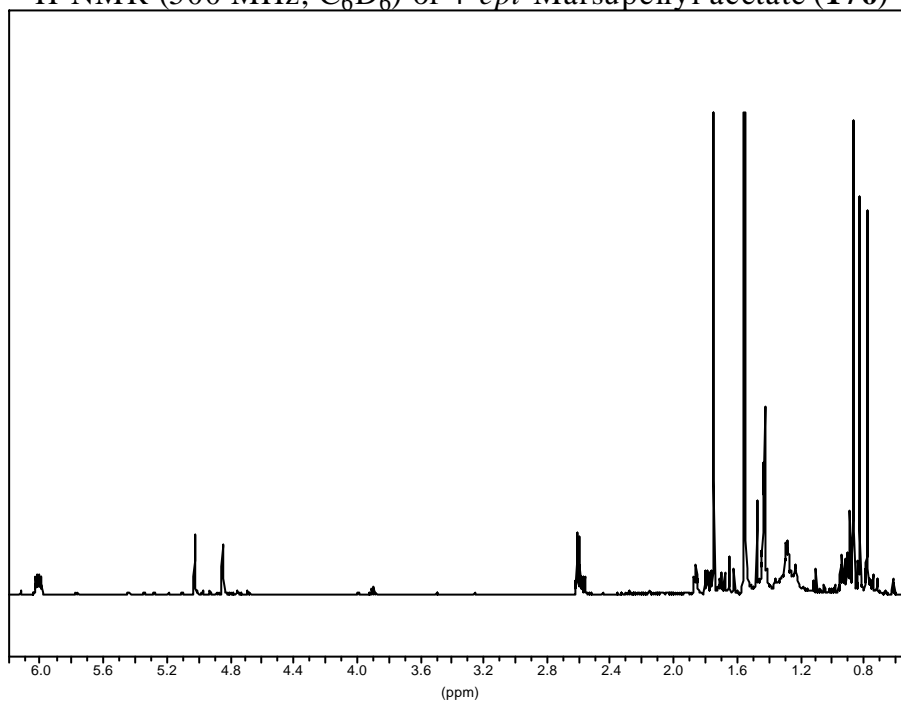
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Eremophila-1(10),8,11-triene (**173**)



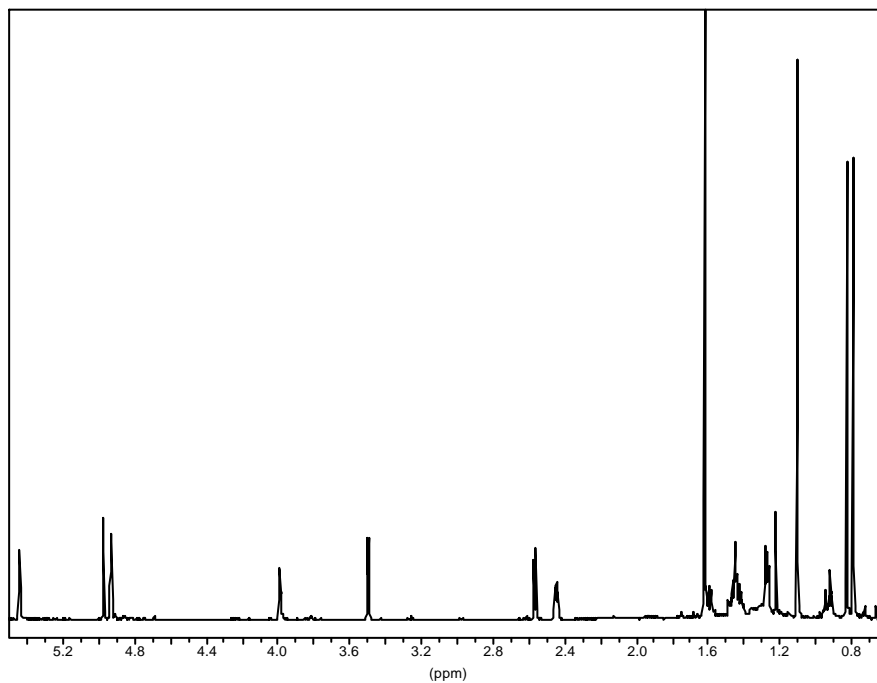
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Marsupellyl acetate (**175**)



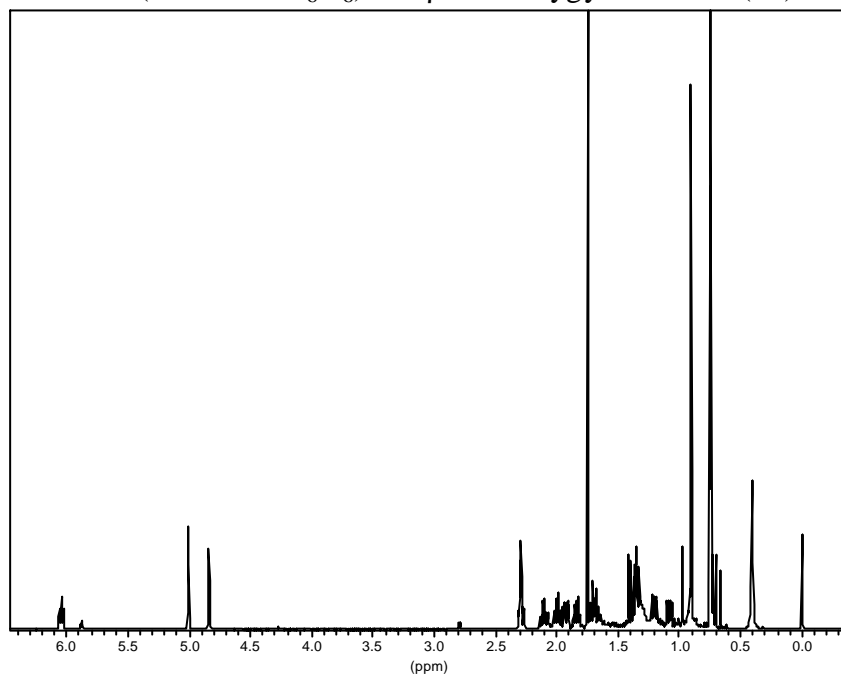
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 4-*epi*-Marsupellyl acetate (**176**)



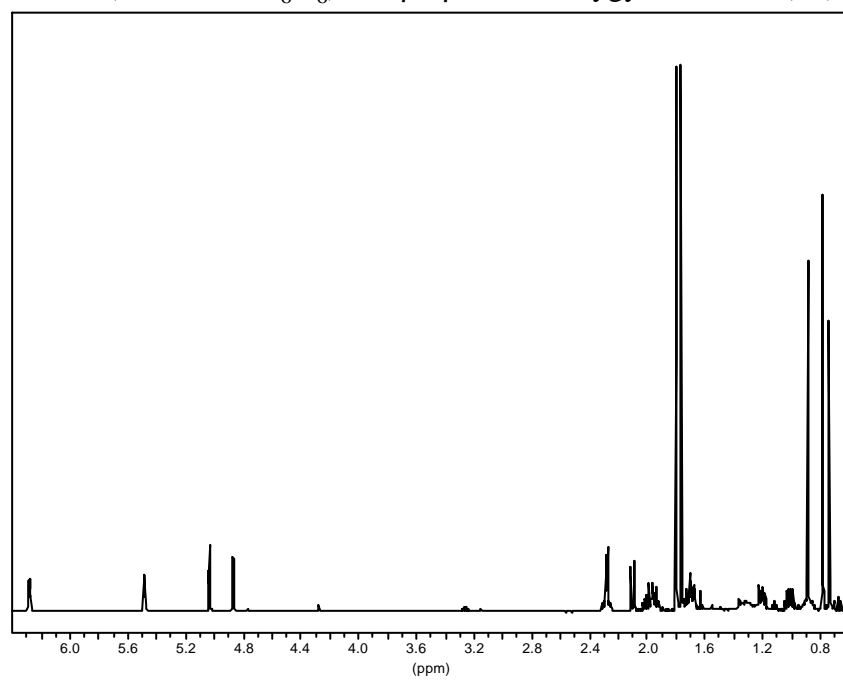
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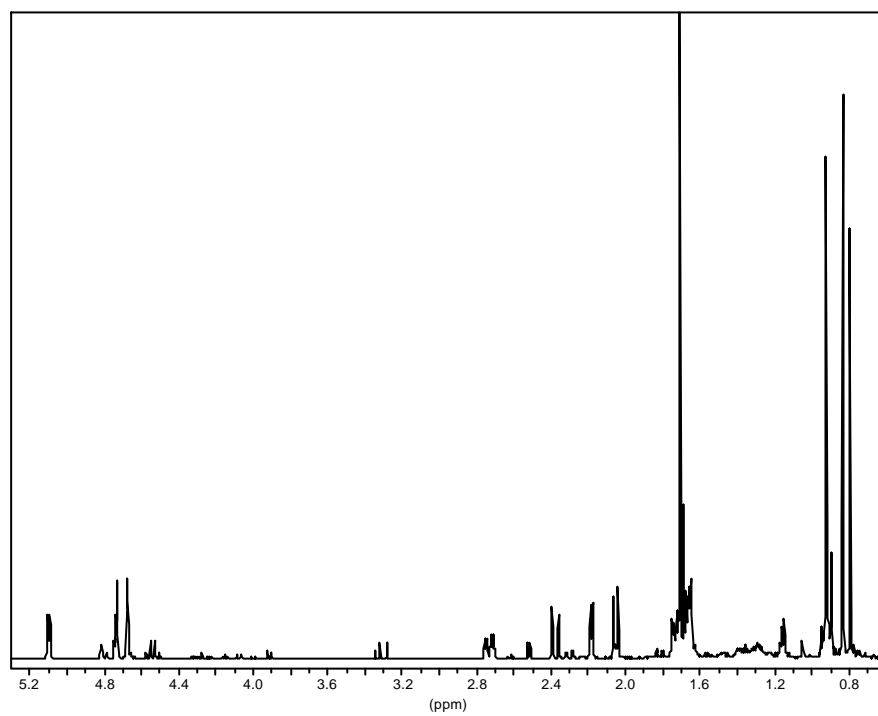
$^1\text{H-NMR}$  (500 MHz  $\text{C}_6\text{D}_6$ ) of  $4\beta$ -Acetoxygymnomitr-3(15)-ene (**180**)



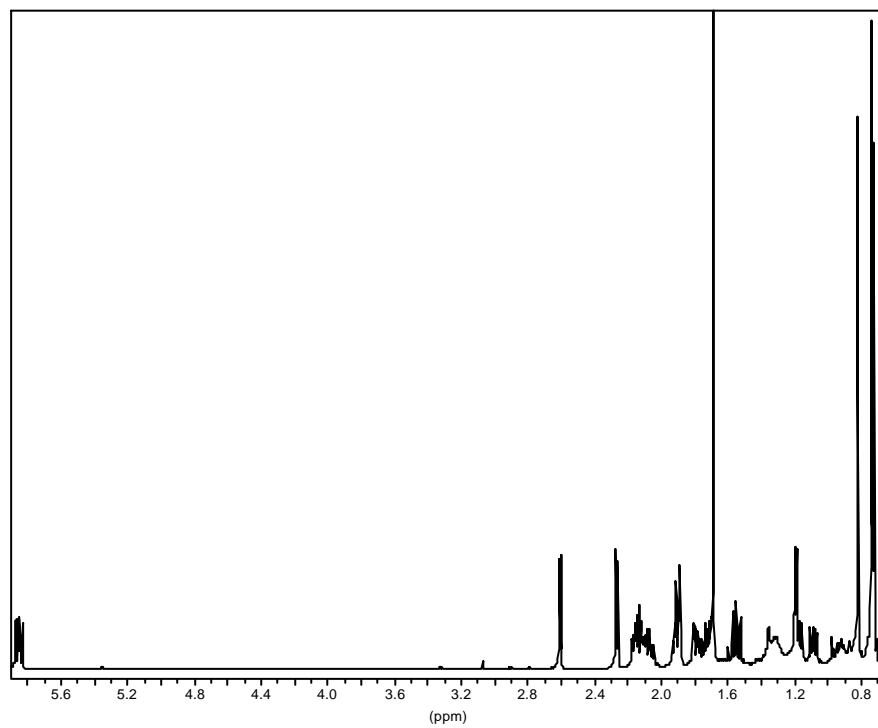
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $4\beta,5\beta$ -Diacetoxygymnomitr-3(15)-ene (**181**)



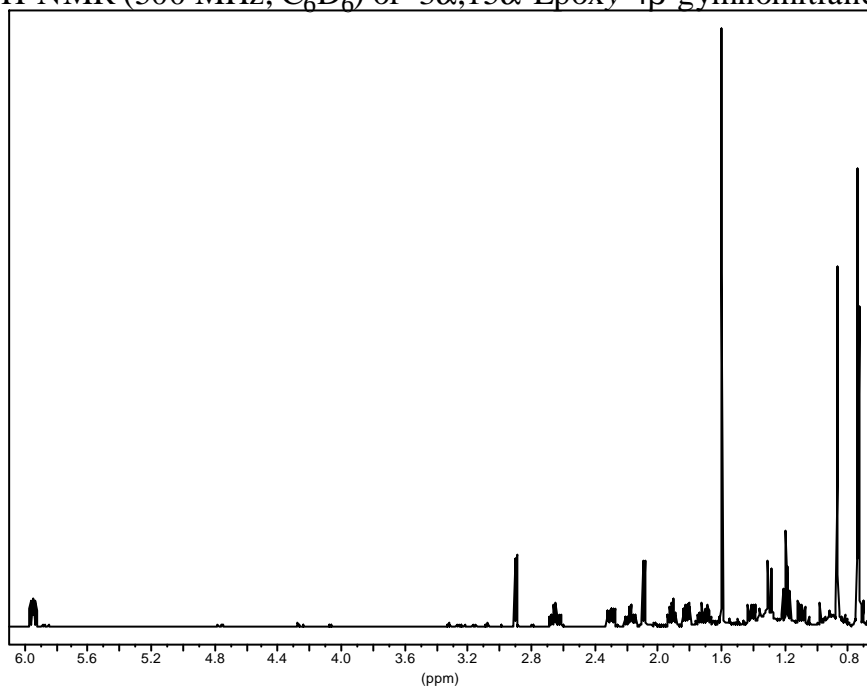
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $5\beta$ -Acetoxymygnomitr-3(15)-ene (**182**)



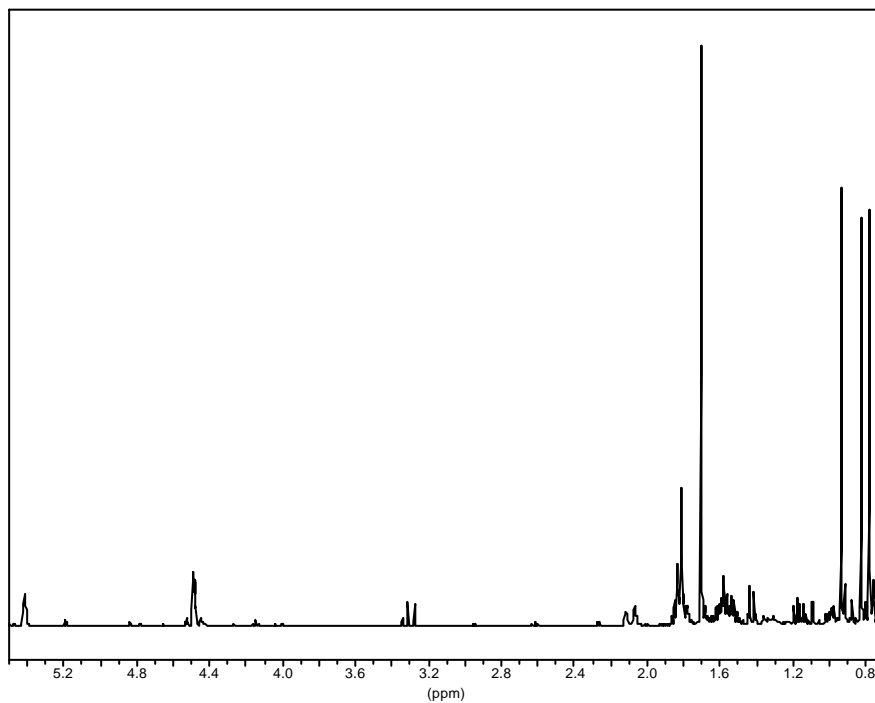
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $3\beta,15\beta$ -Epoxy-4 $\beta$ -mygnomitrane (**183**)



$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $3\alpha,15\alpha$ -Epoxy- $4\beta$ -gymnomitrane (**184**)

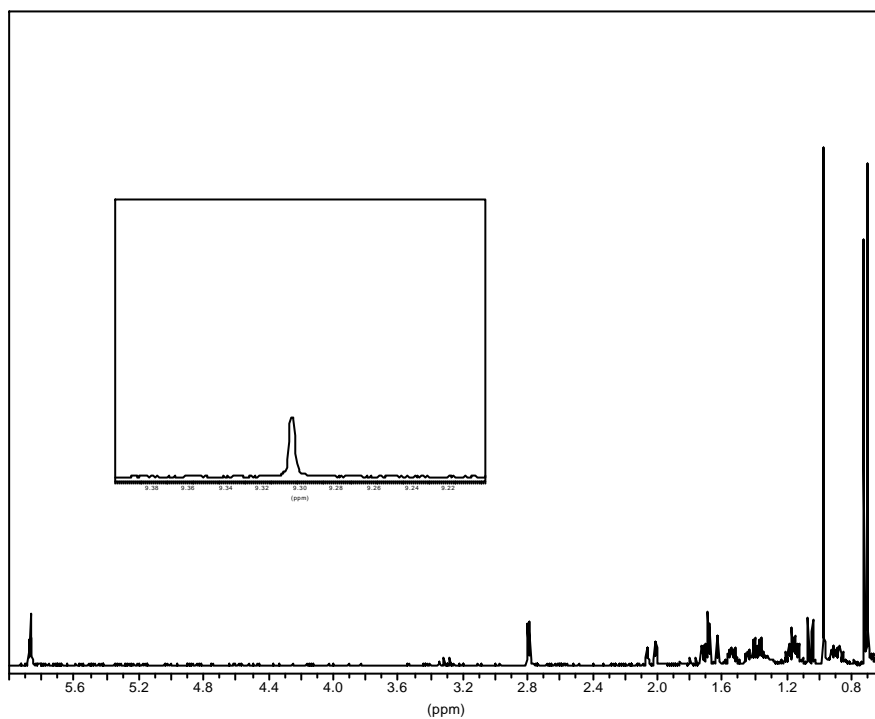


$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 15-Acetoxygymnomitr-3-ene (**185**)

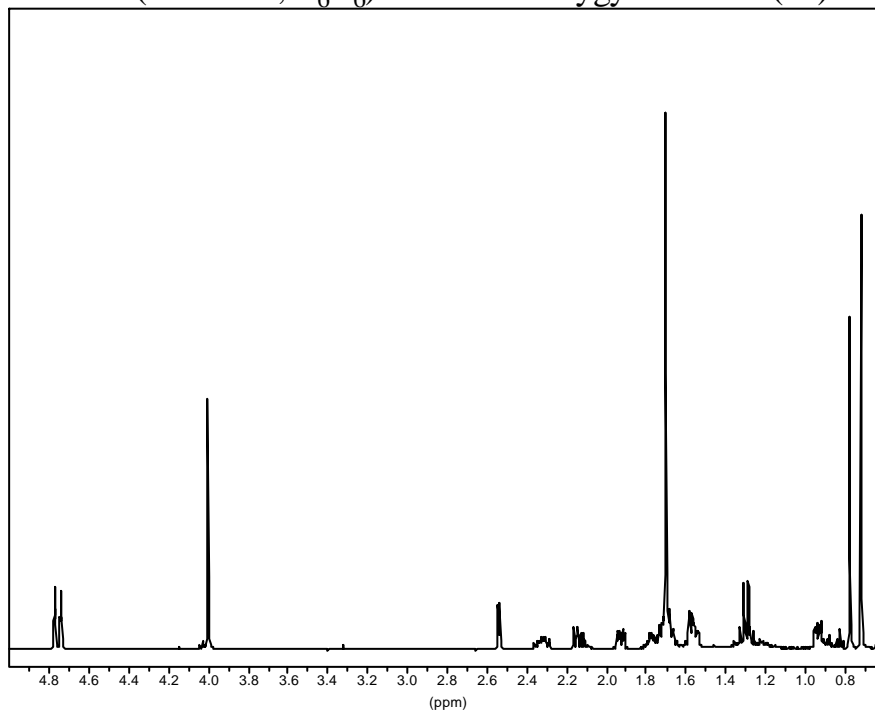




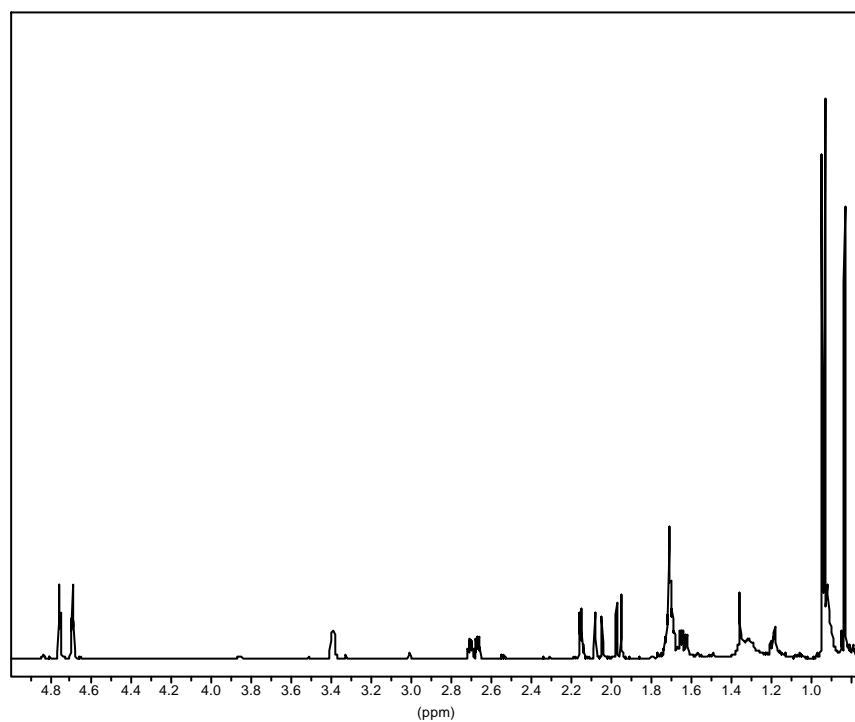
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $\alpha$ -Barbatenal (**186**)



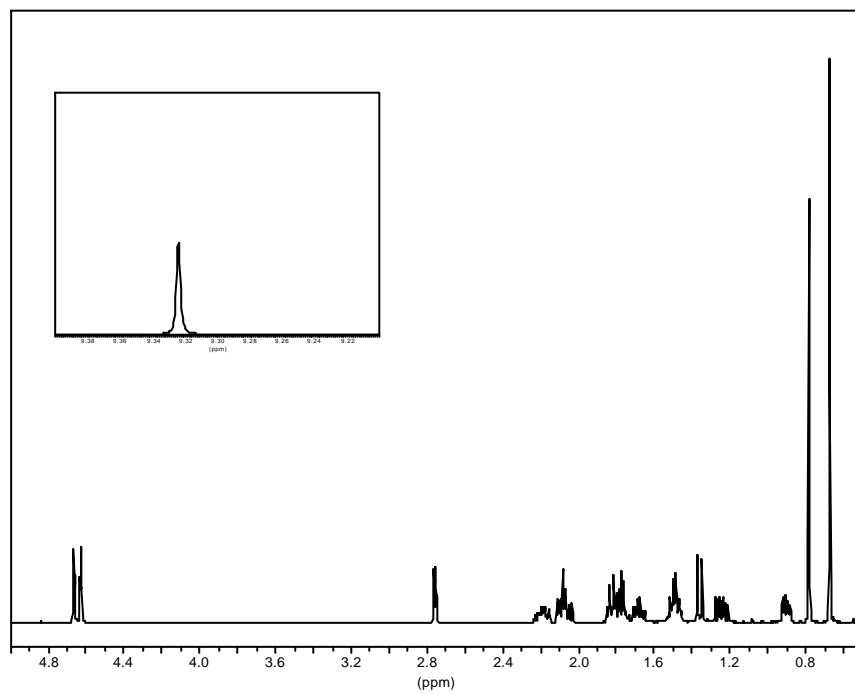
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 12-Acetoxygymnomitr-3(15)-ene (**187**)



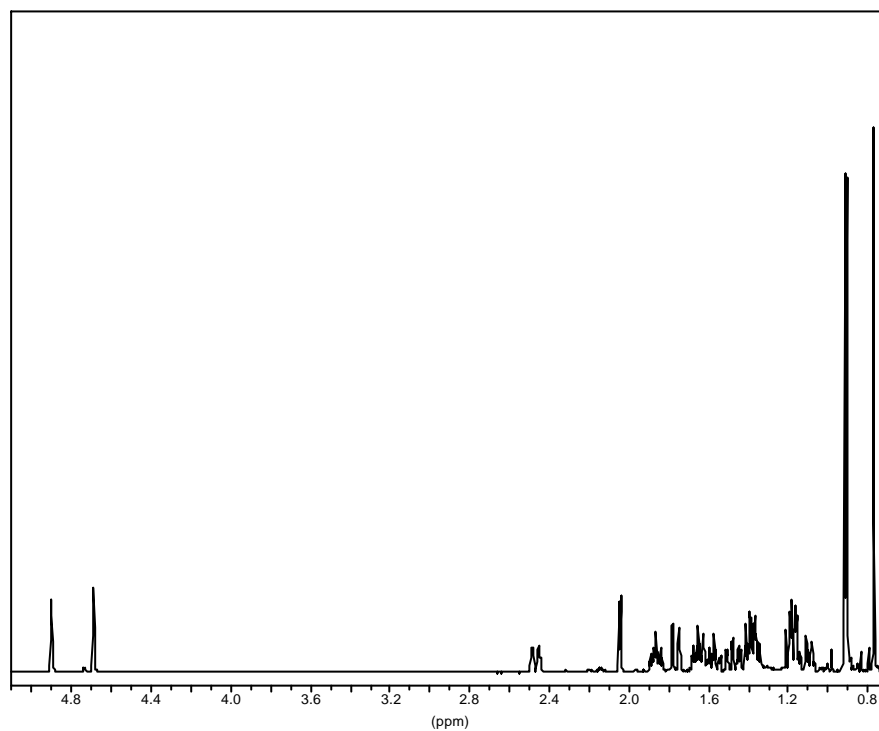
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 5 $\beta$ -Hydroxygymnomitr-3(15)-ene (**190**)



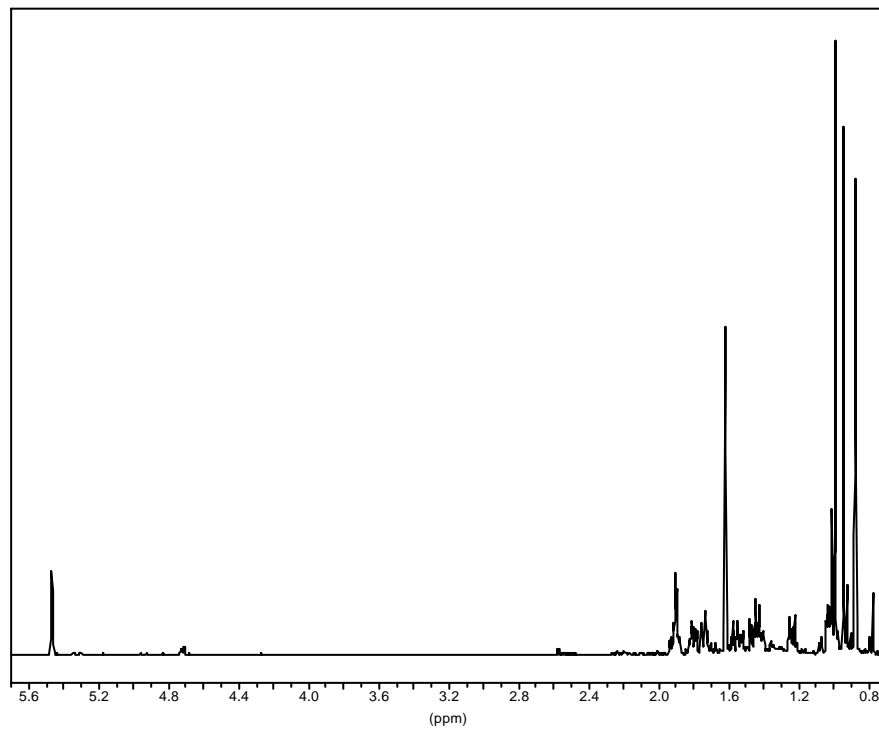
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Gymnomitr-3(15)-en-12-al (**193**)



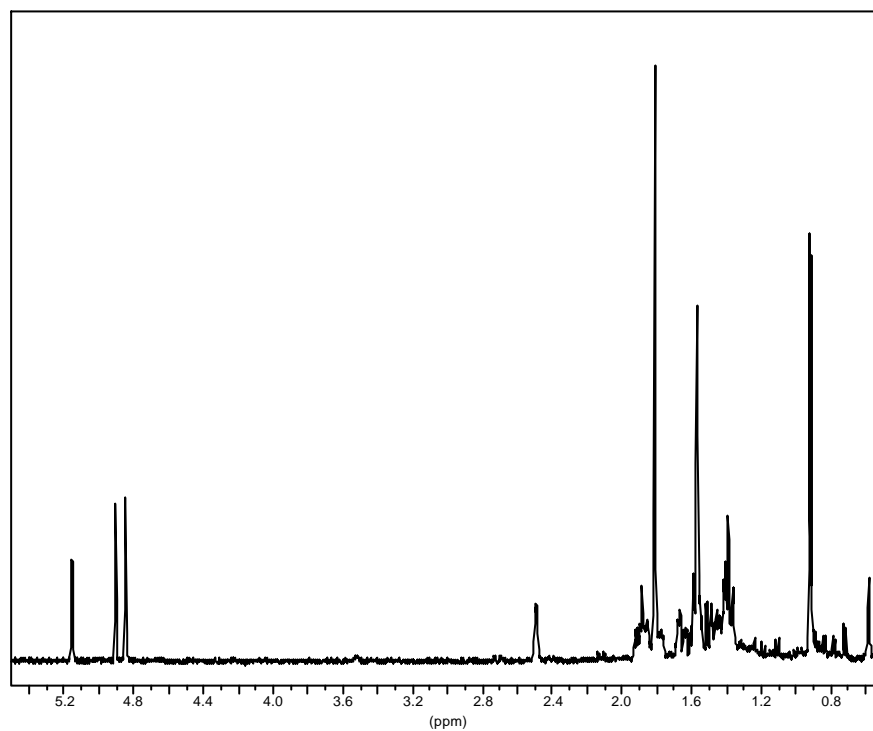
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Myltayl-4(12)-ene (**198**)



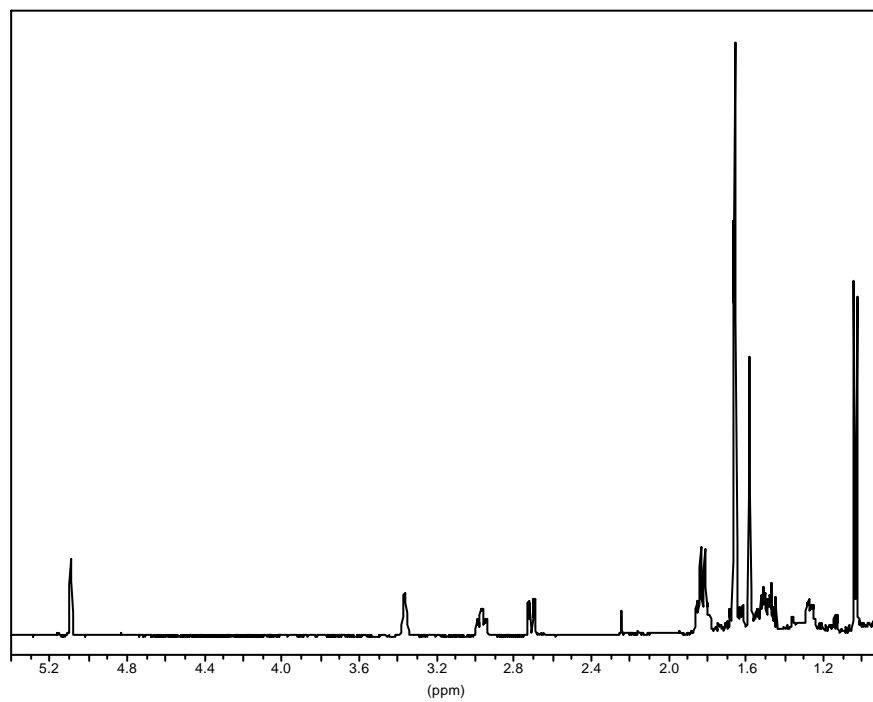
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Myltayl-4-ene (**199**)



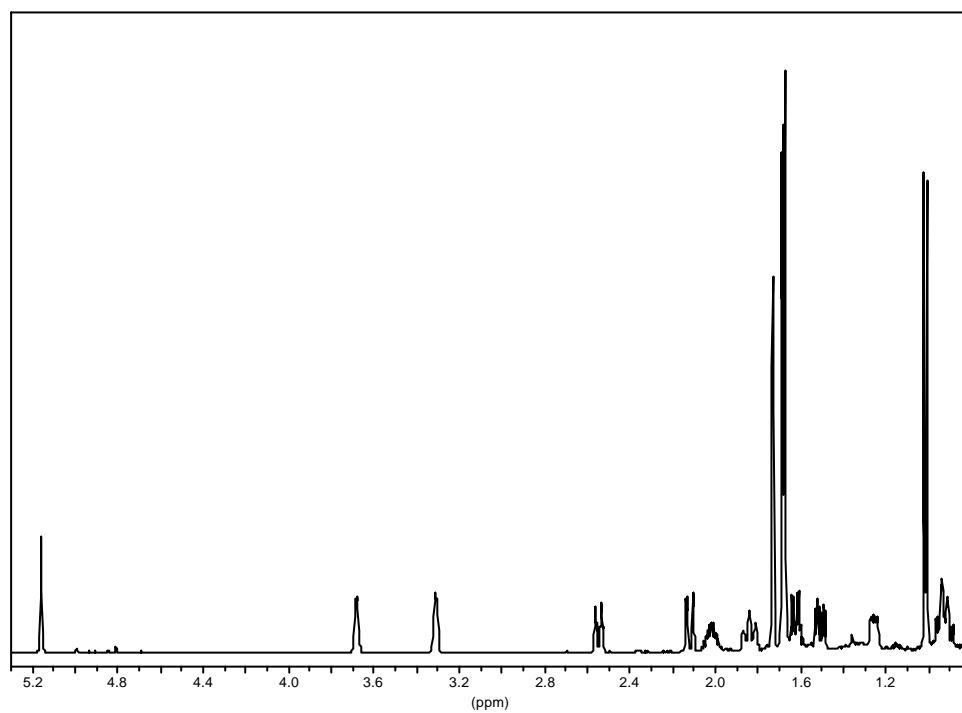
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 7 $\beta$ -Hydroxyamorpha-4,11-diene (**200**)



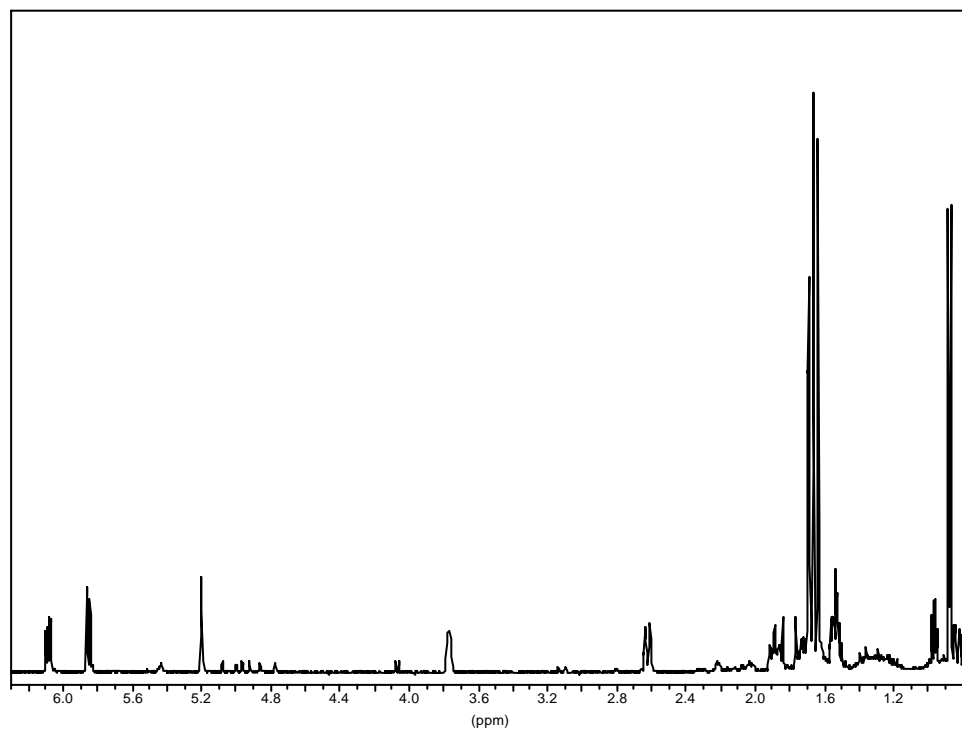
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 9 $\alpha$ -Hydroxyamorpha-4,7(11)-diene (**201**)



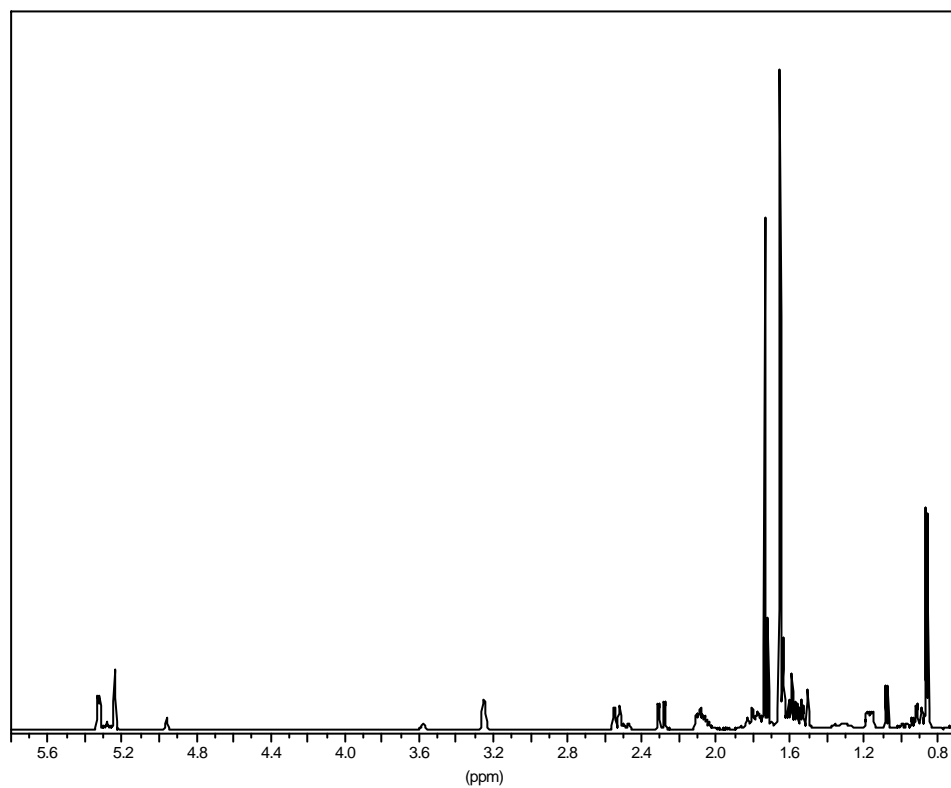
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $3\alpha$ -Hydroxyamorpha-4,7(11)-diene (**202**)



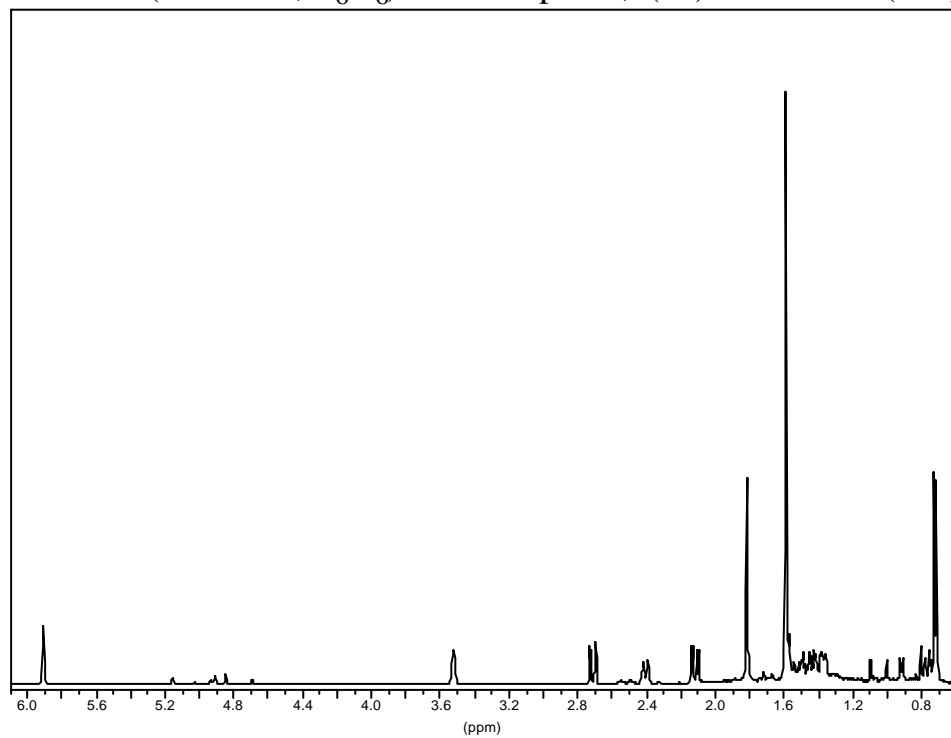
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Amorpha-2,4,7(11)-triene (**203**)



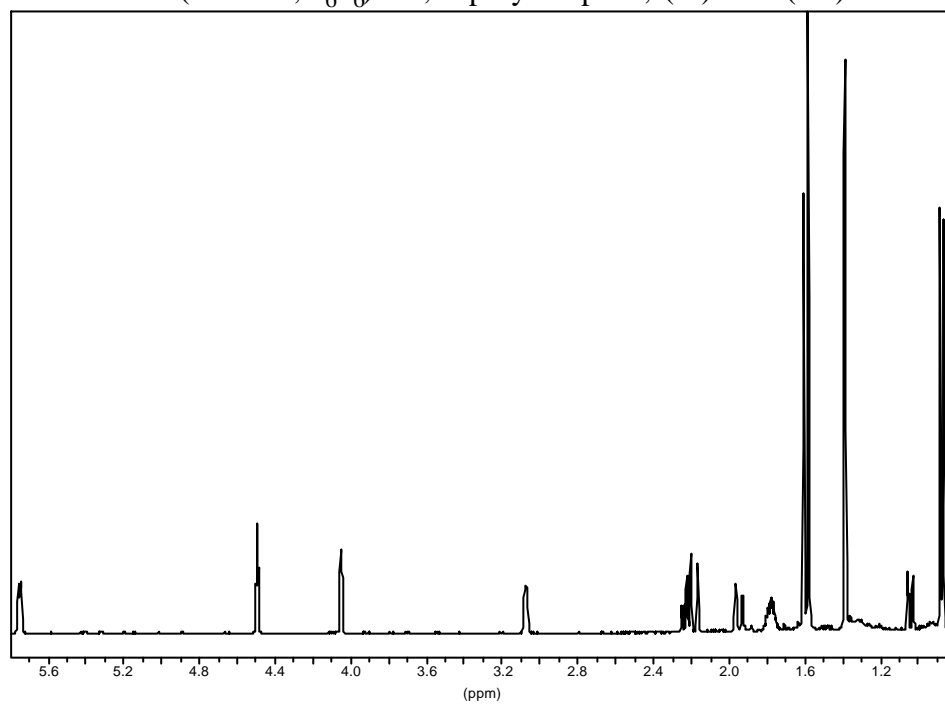
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $3\alpha$ -Acetoxymorpha-4,7(11)-diene (**204**)



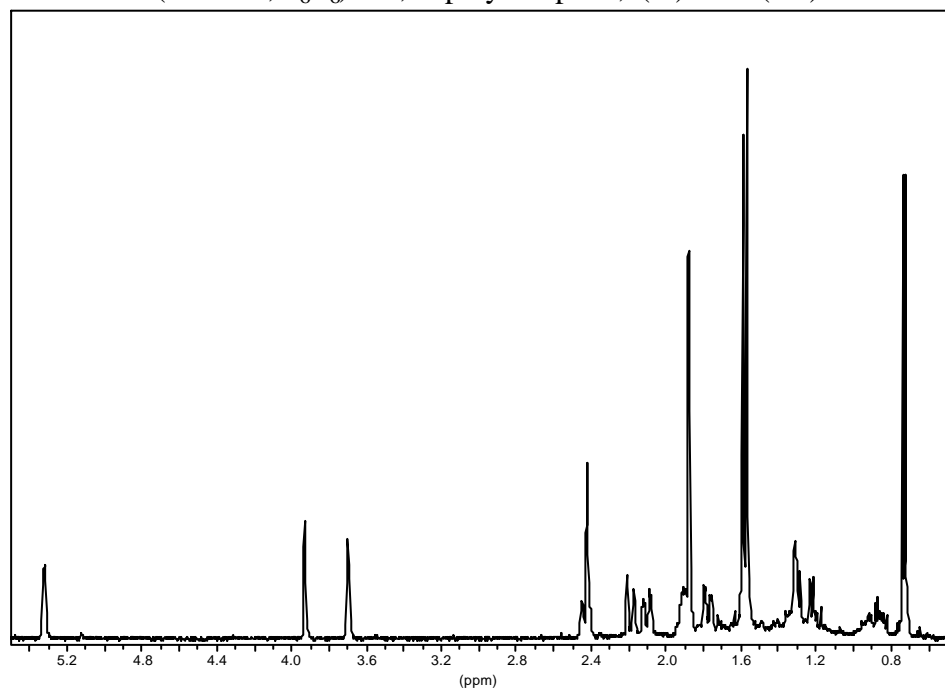
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Amorpha-4,7(11)-dien-3-one (**205**)



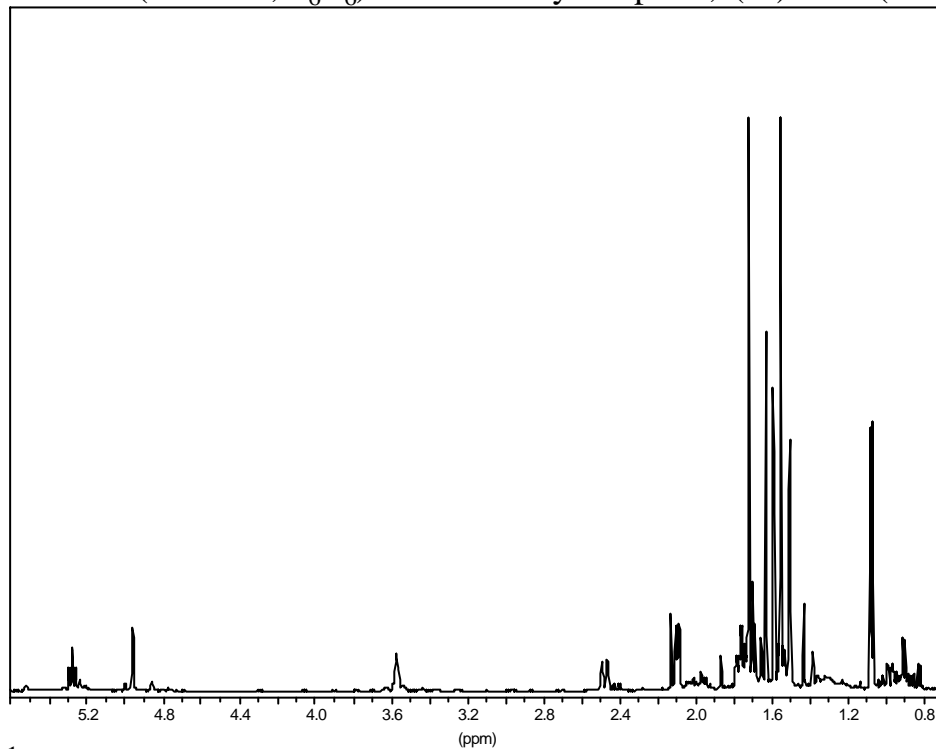
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 2,8-Epoxyamorpha-4,7(11)-diene (**206**)



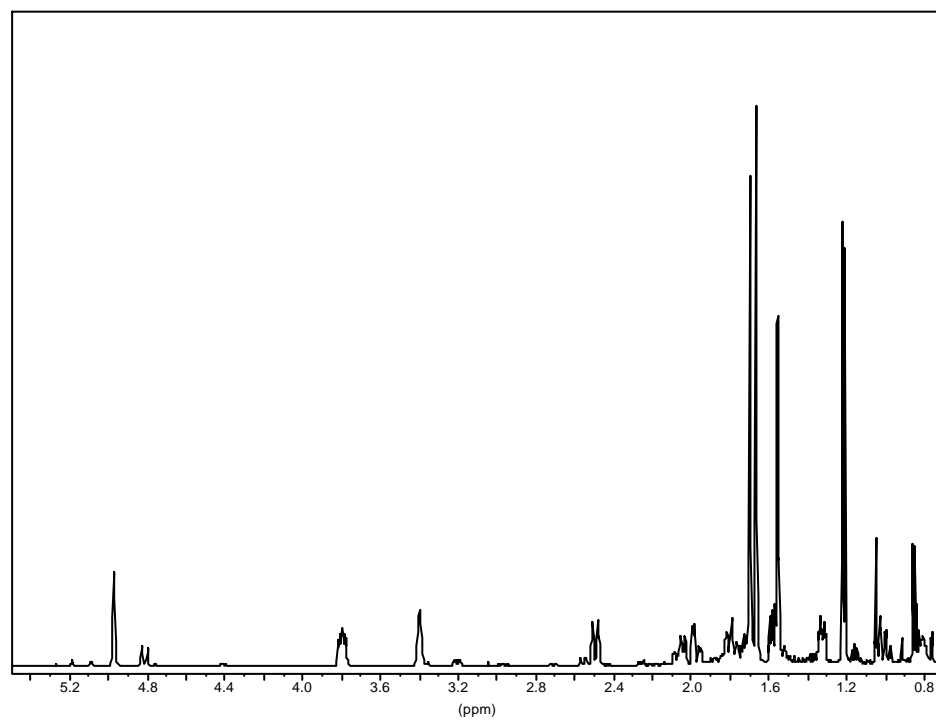
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 5,9-Epoxyamorpha-4,7(11)-diene (**209**)



$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $2\alpha$ -Acetoxyamorpha-4,7(11)-diene (**210**)

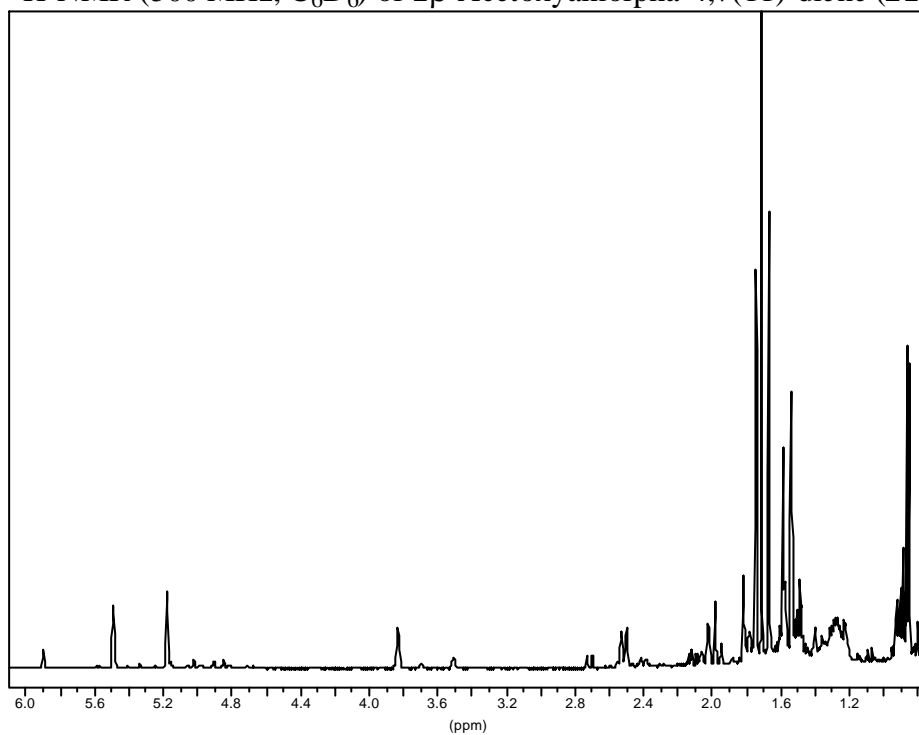


$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $2\alpha$ -Hydroxyamorpha-4,7(11)-diene (**211**)

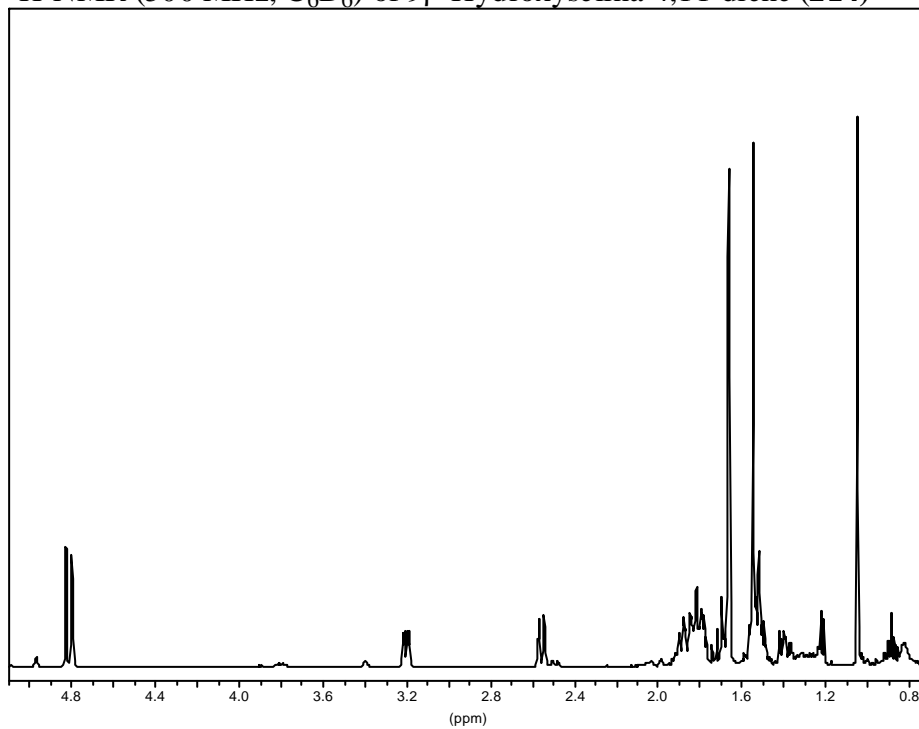




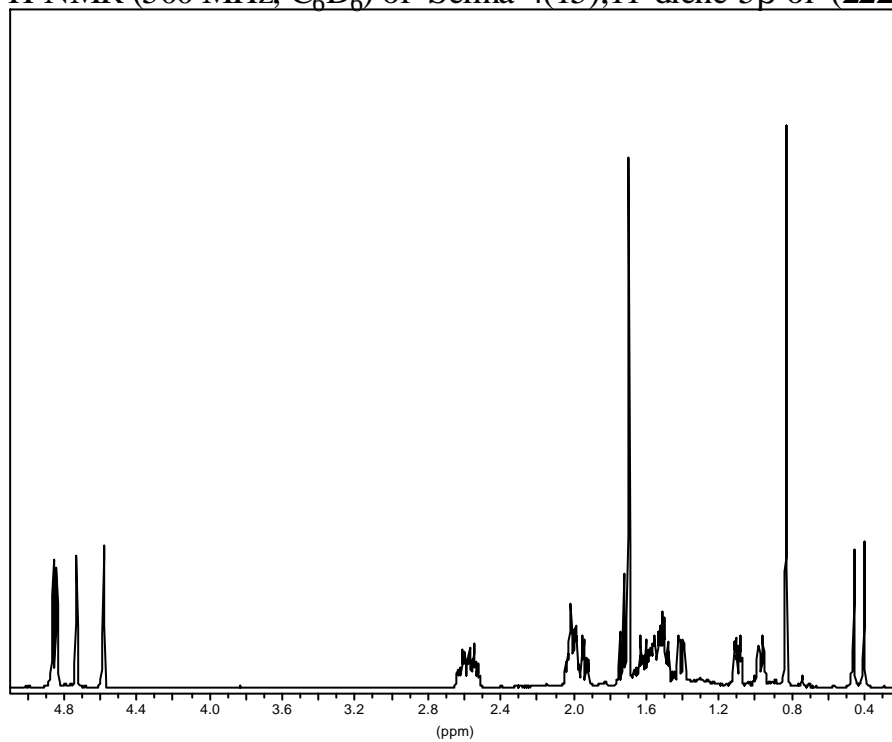
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $2\beta$ -Acetoxymorpha-4,7(11)-diene (**212**)



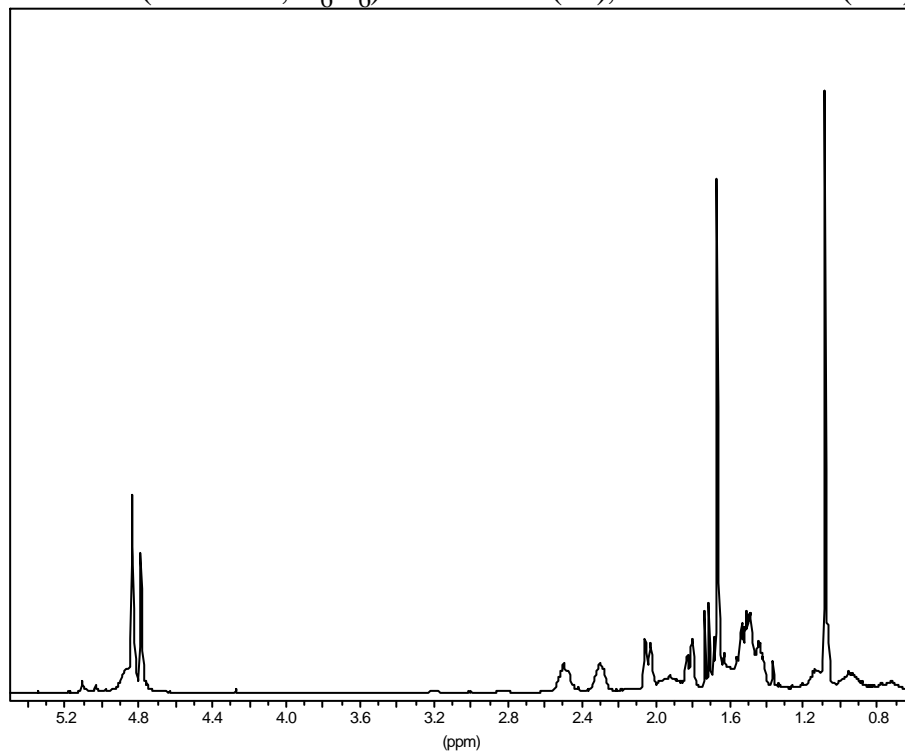
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $9\beta$ -Hydroxyselina-4,11-diene (**214**)



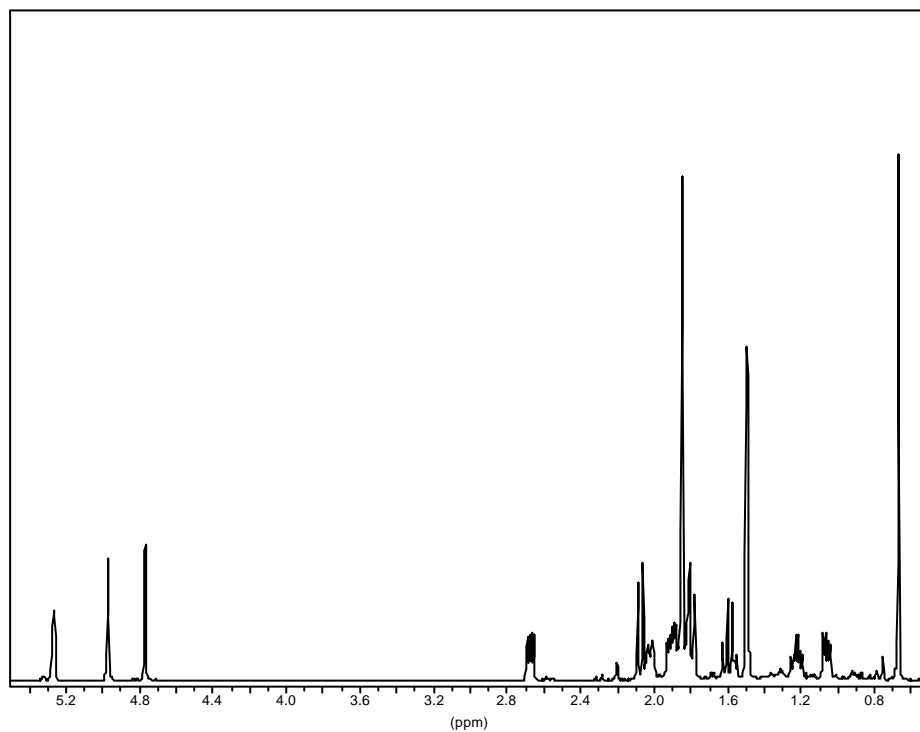
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Selina-4(15),11-diene-5 $\beta$ -ol (**222**)



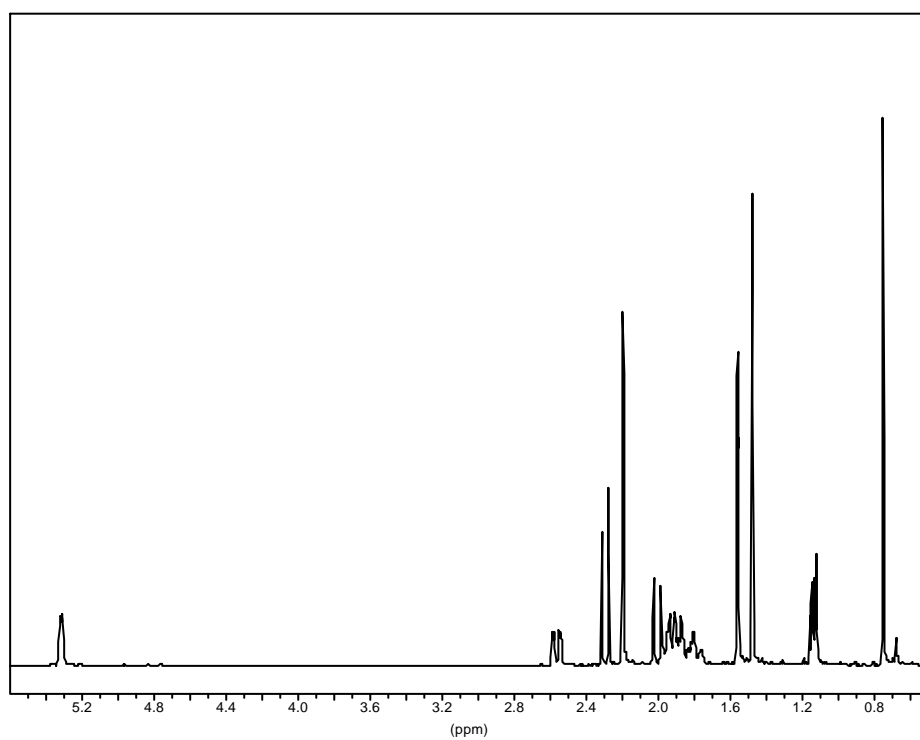
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Selina-4(15),11-diene-5 $\alpha$ -ol (**223**)



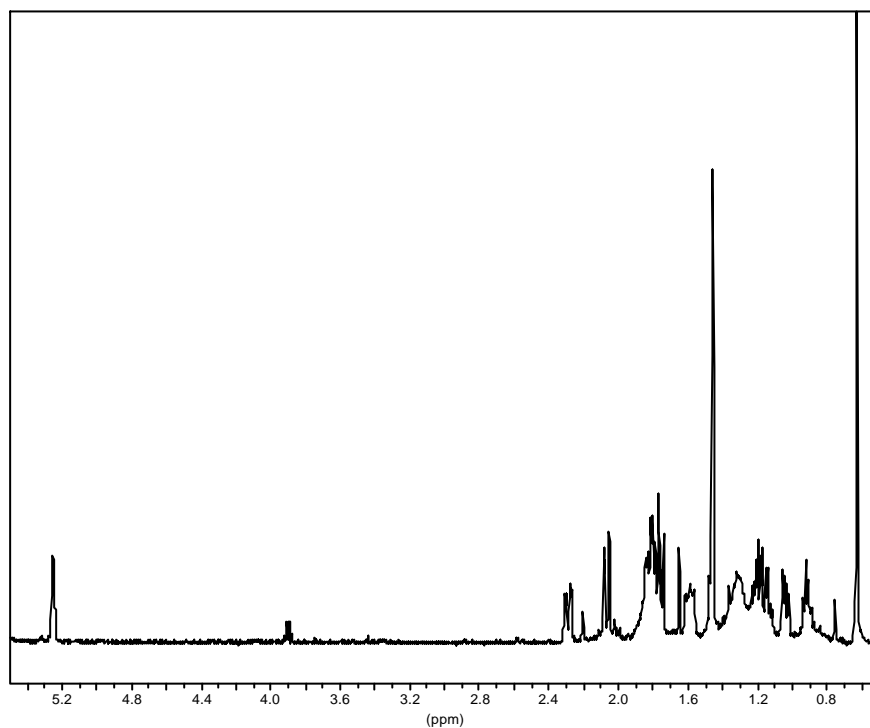
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Eudesma-3,11-dien-8-one (**230**)



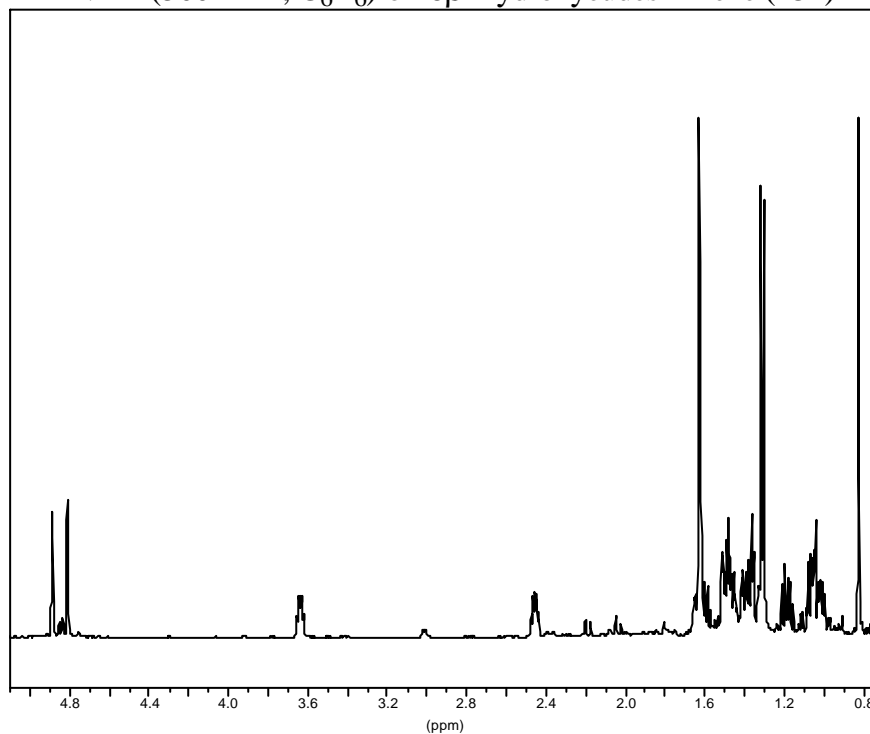
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of Eudesma-3,7(11)-dien-8-one (**231**)



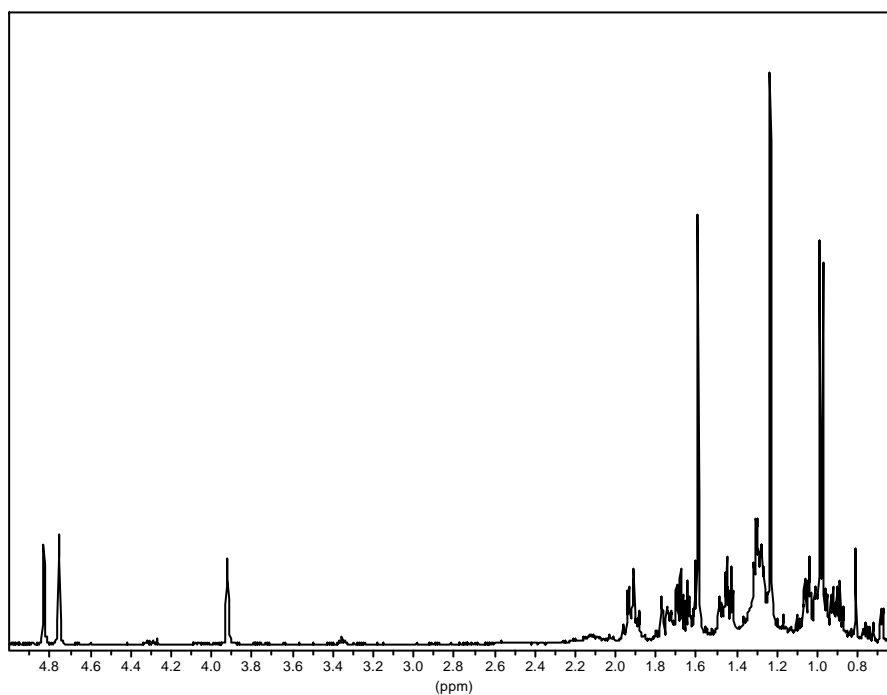
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of de-isopropyl eudesma-3,(11)-dien-8-one (**233**)



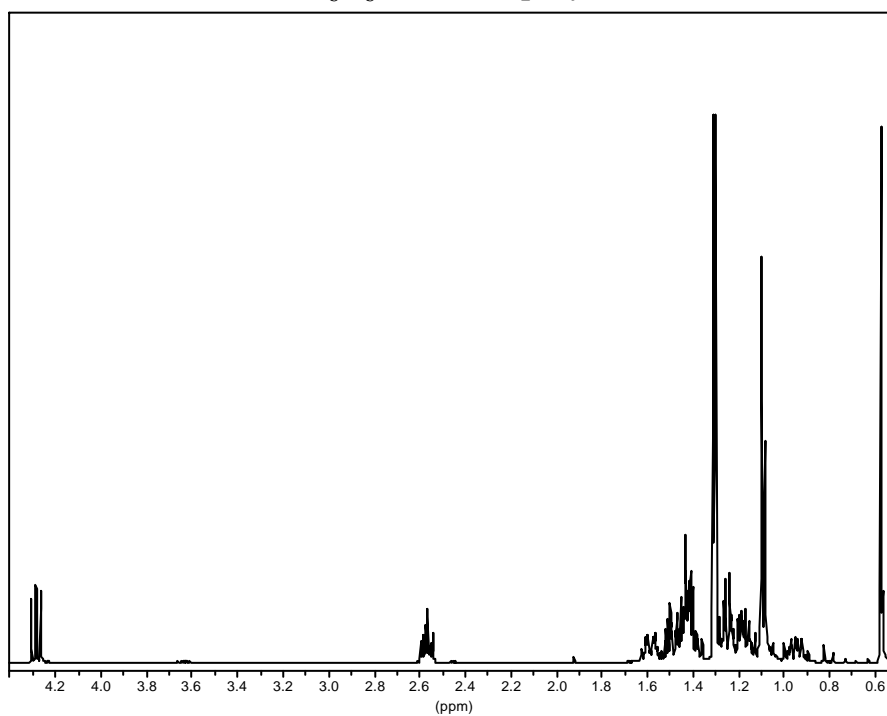
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of  $6\beta$ -Hydroxyeudes-11-ene (**234**)



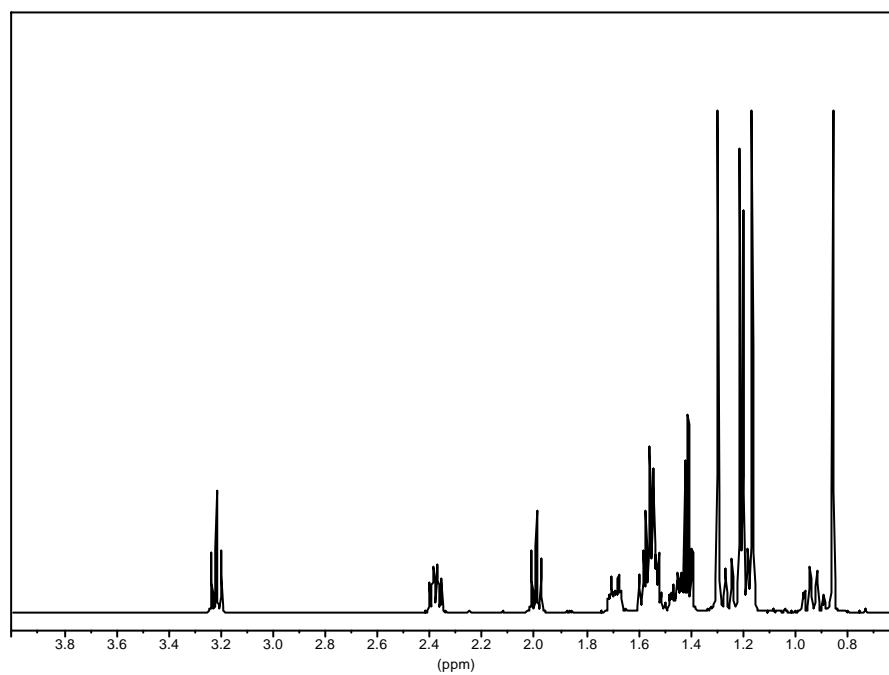
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 6 $\alpha$ -Hydroxyeudes-11-ene (**235**)



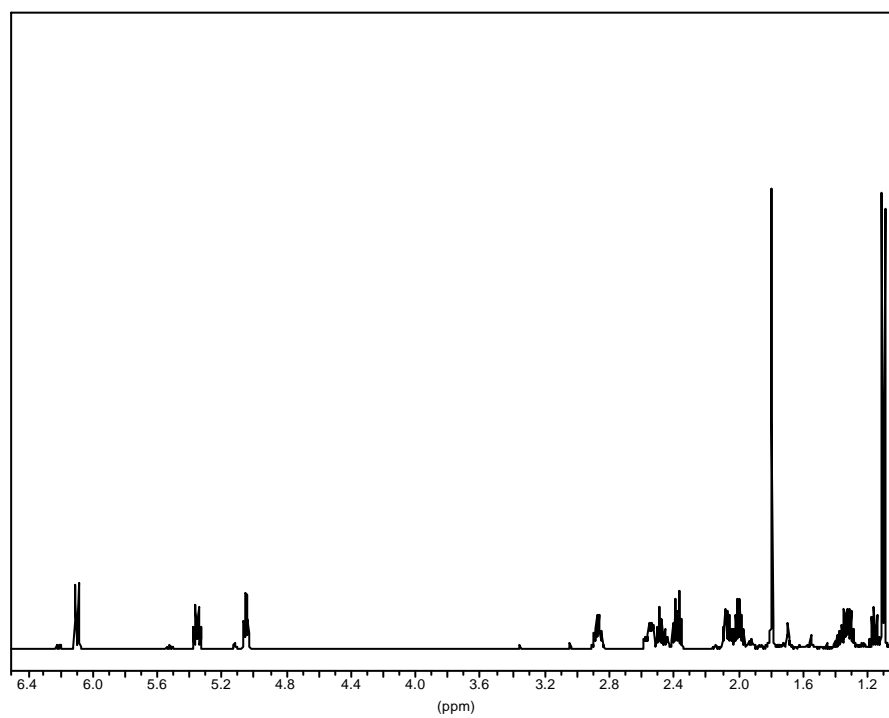
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 6,11-Epoxyeudesmane (**236**)



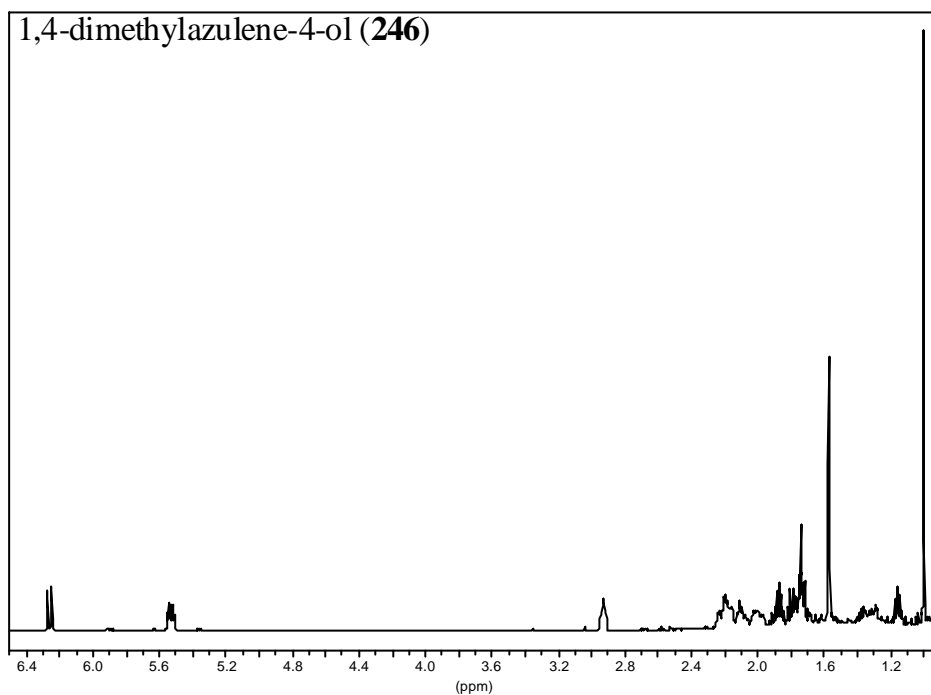
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 6,11-Epoxyisodaucane (**239**)



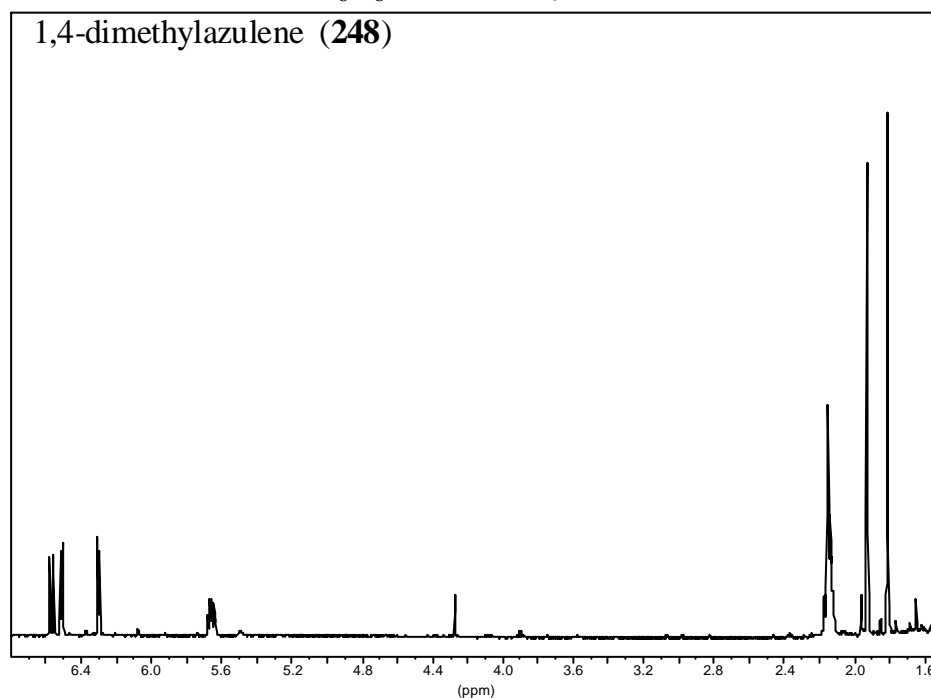
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 1,2,3,6-Tetrahydro-1,4-dimethylazulene (**245**)



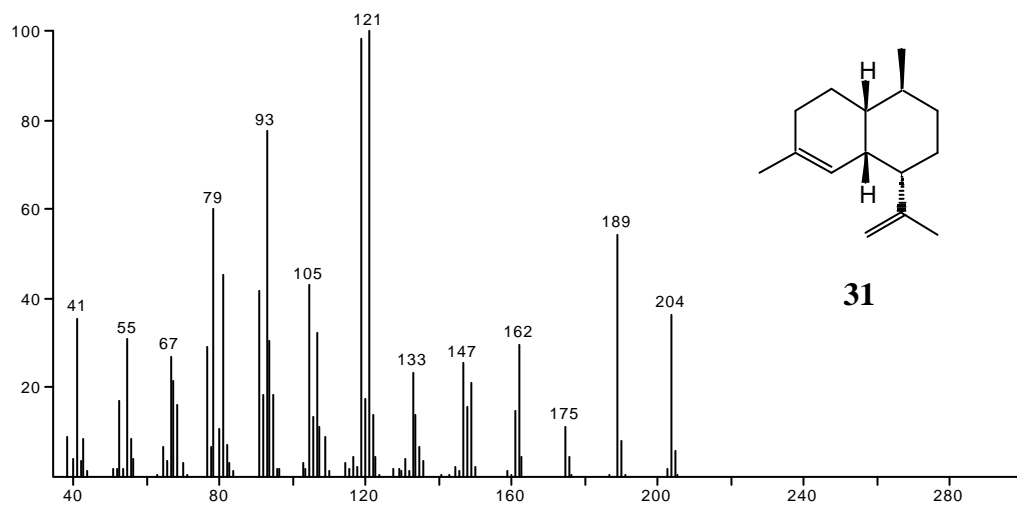
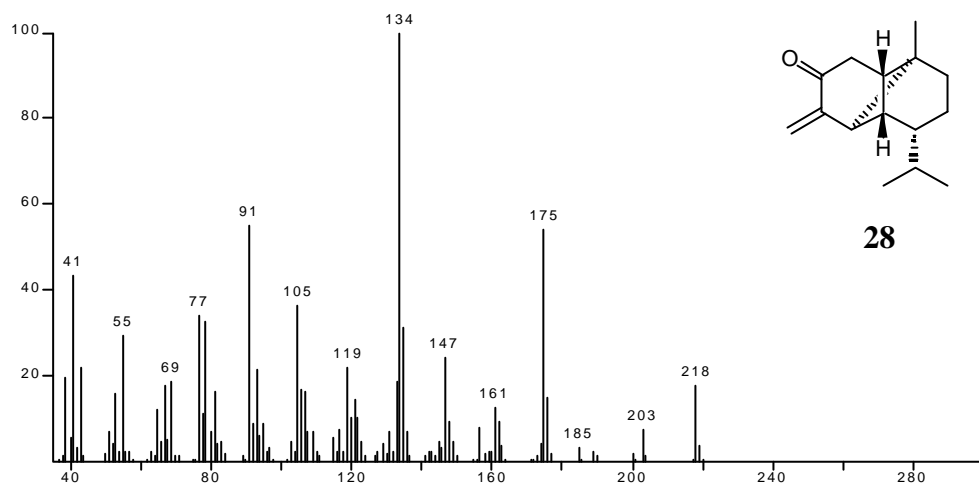
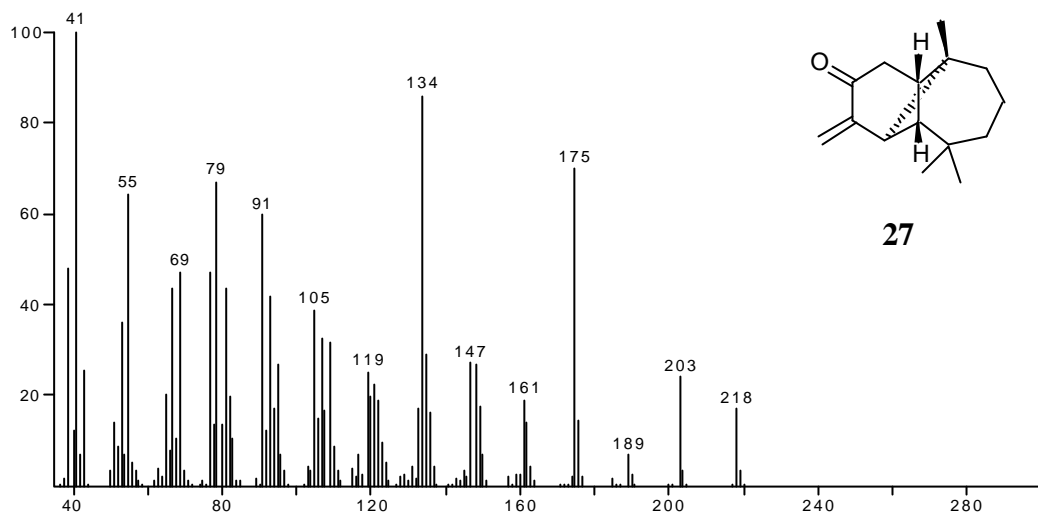
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulene-4-ol (**246**)



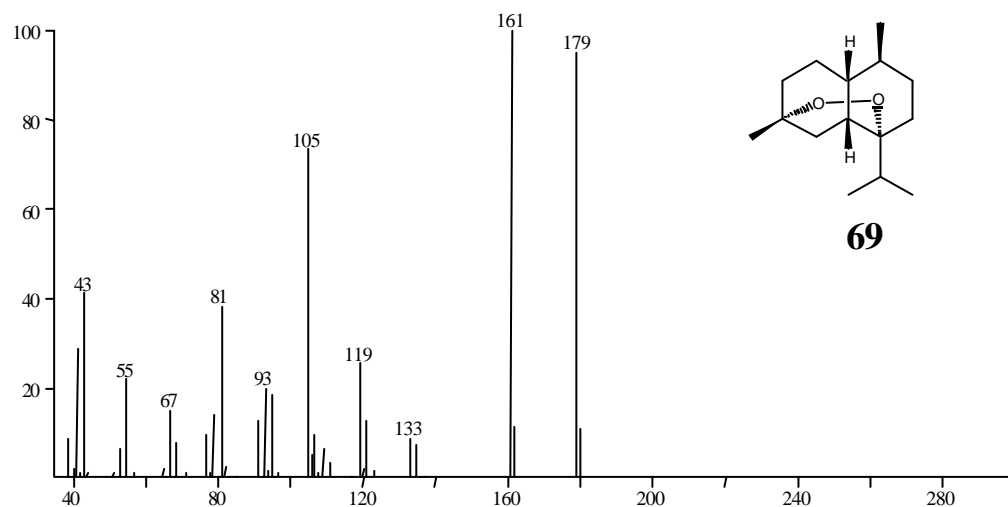
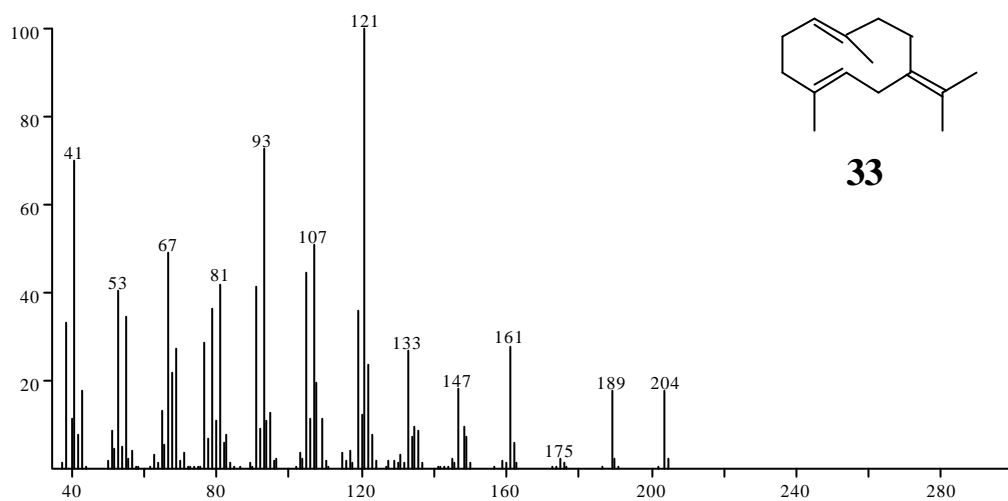
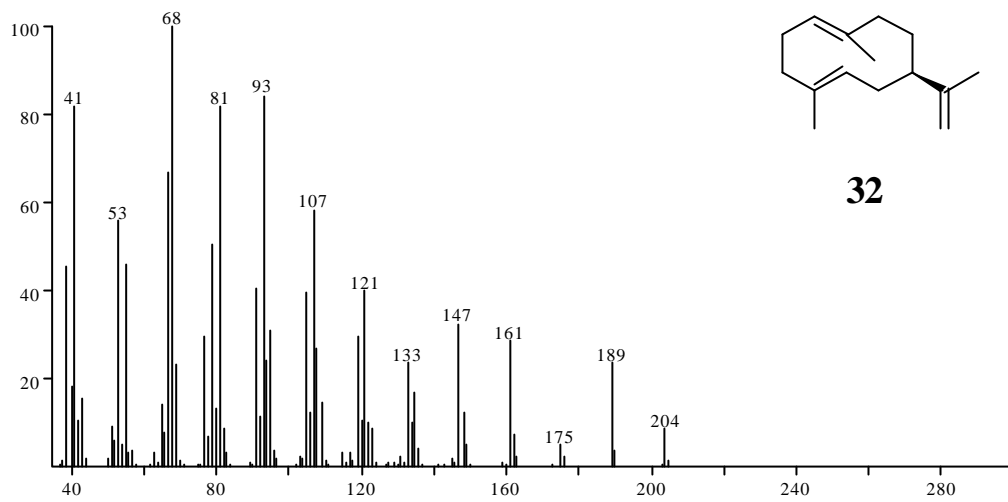
$^1\text{H-NMR}$  (500 MHz,  $\text{C}_6\text{D}_6$ ) of 5,6-Dihydro-1,4-dimethylazulene (**248**)

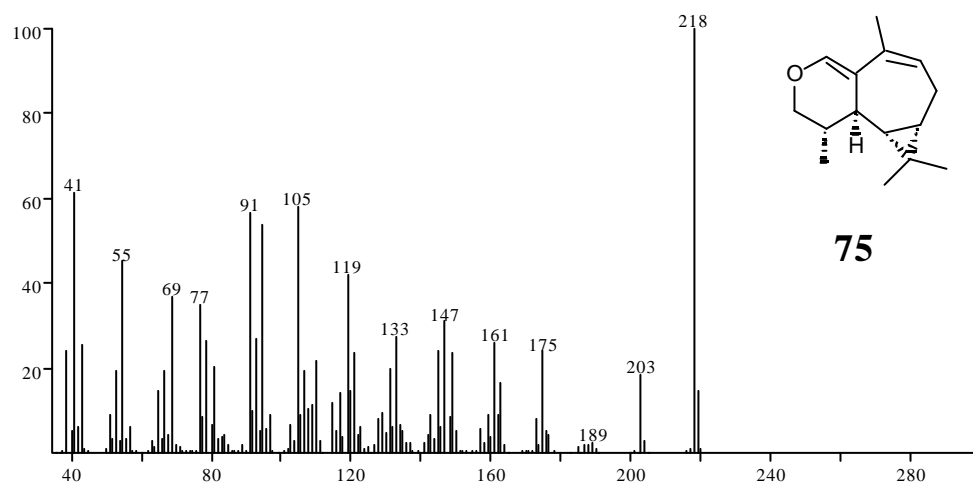
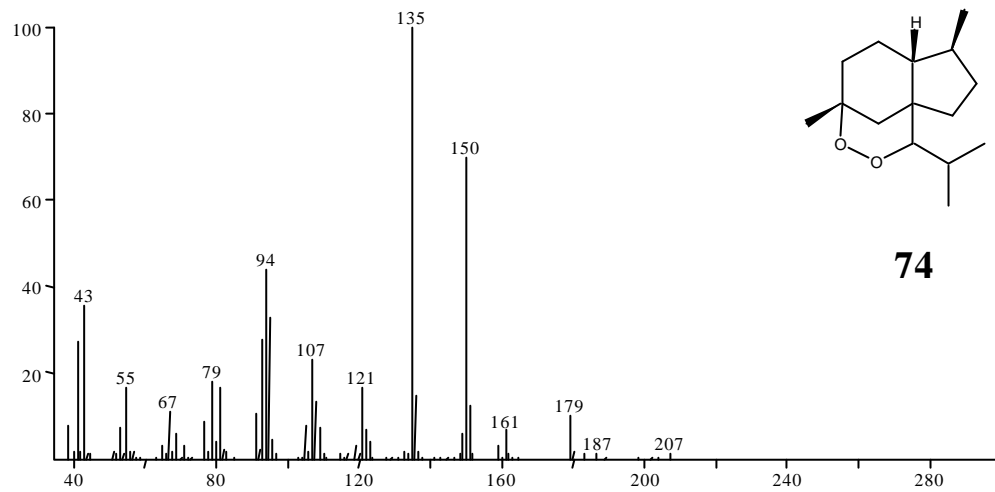
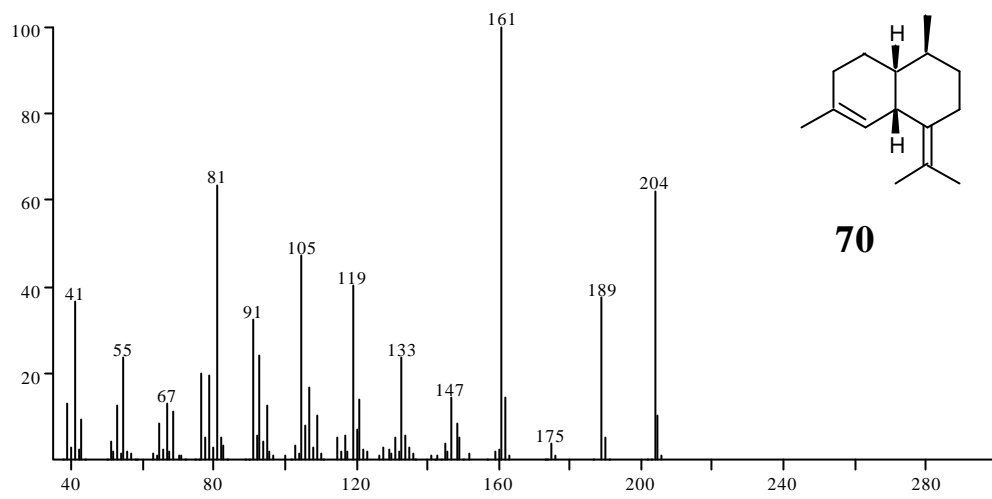


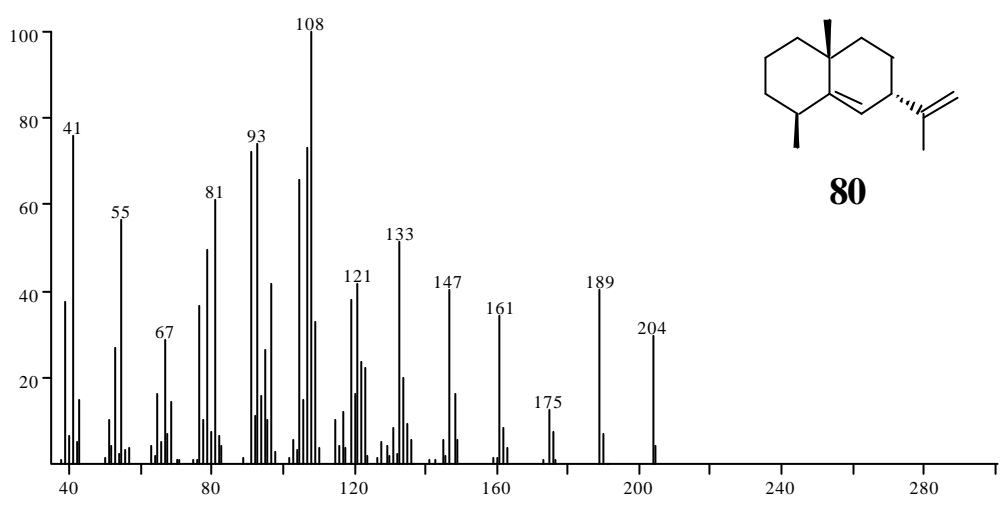
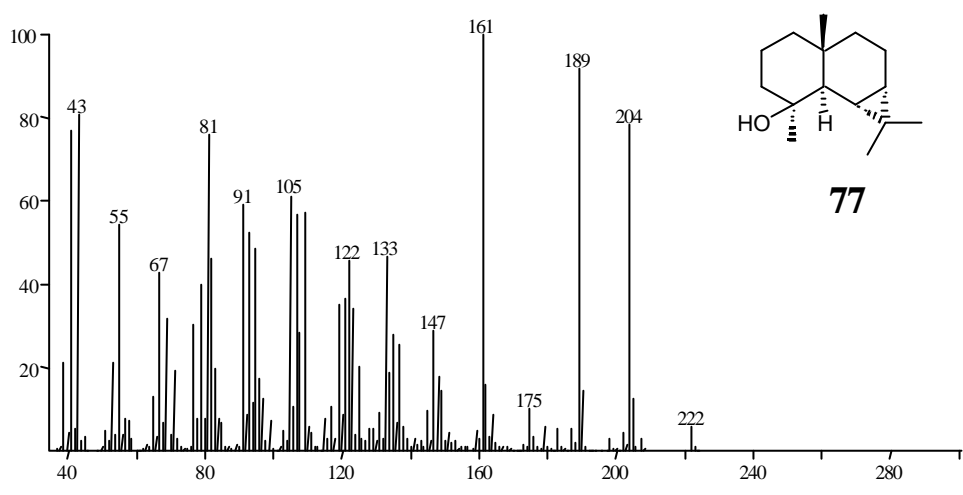
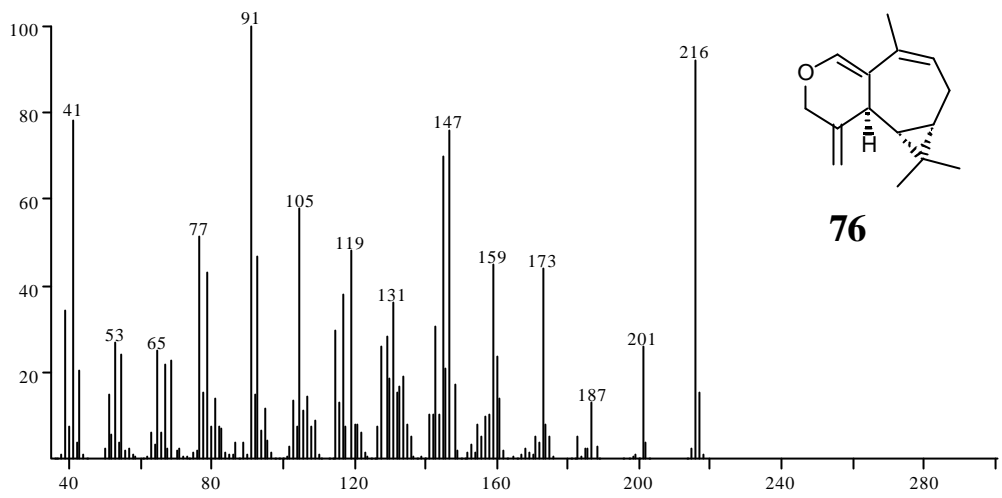
## 12. Mass spectra of Compounds

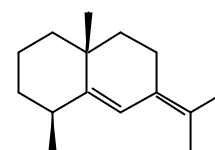
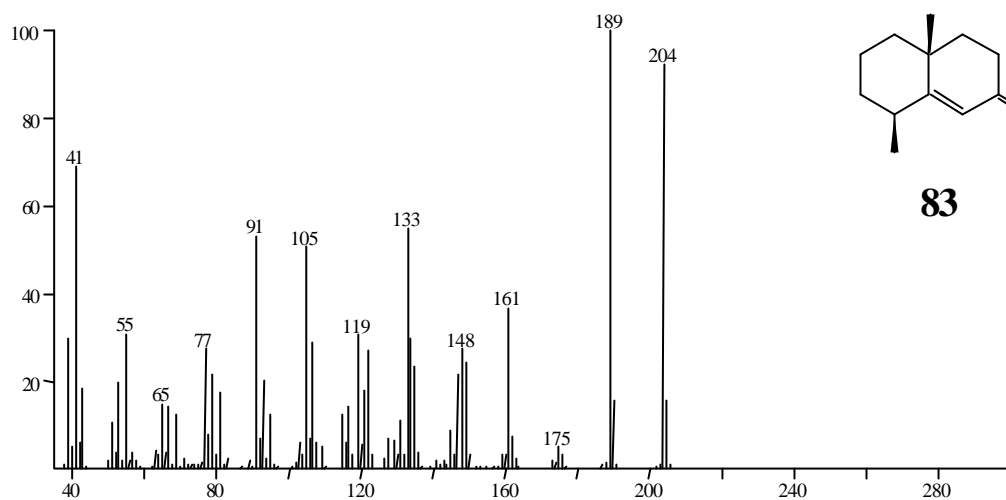




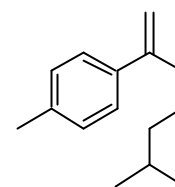
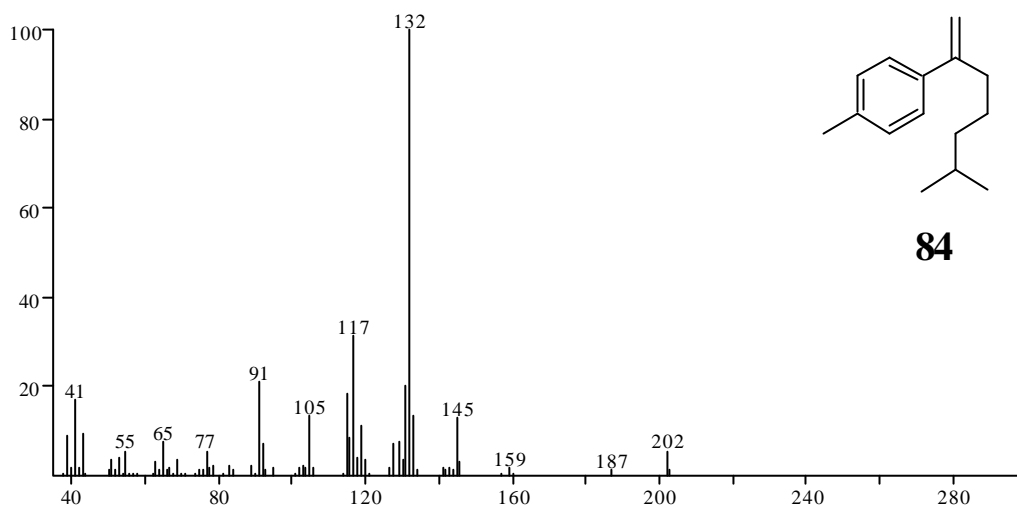




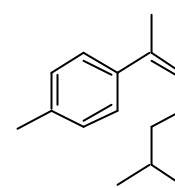
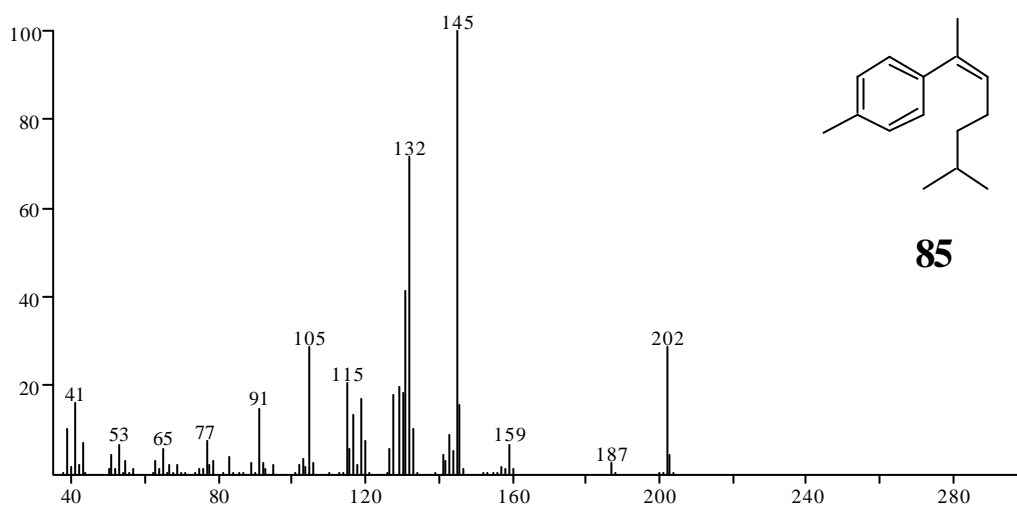




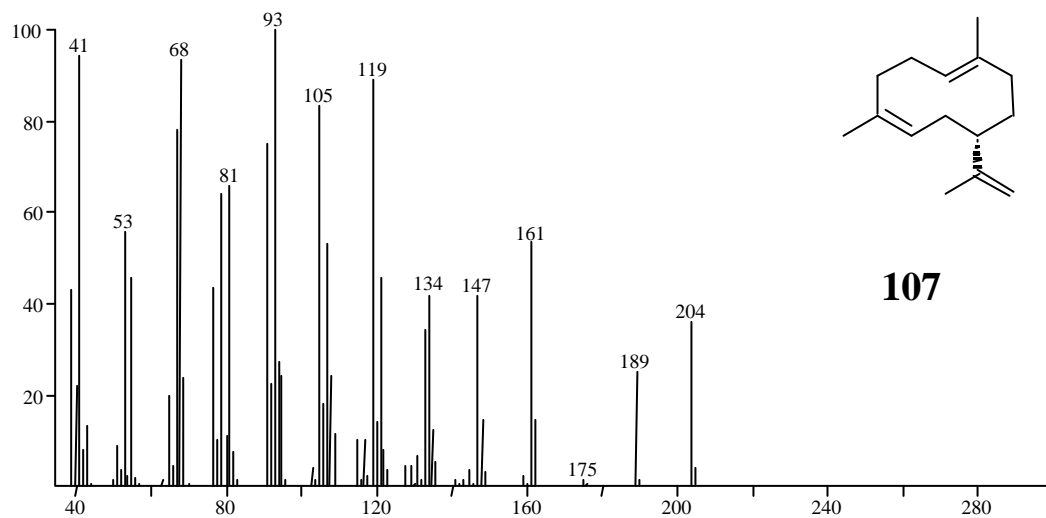
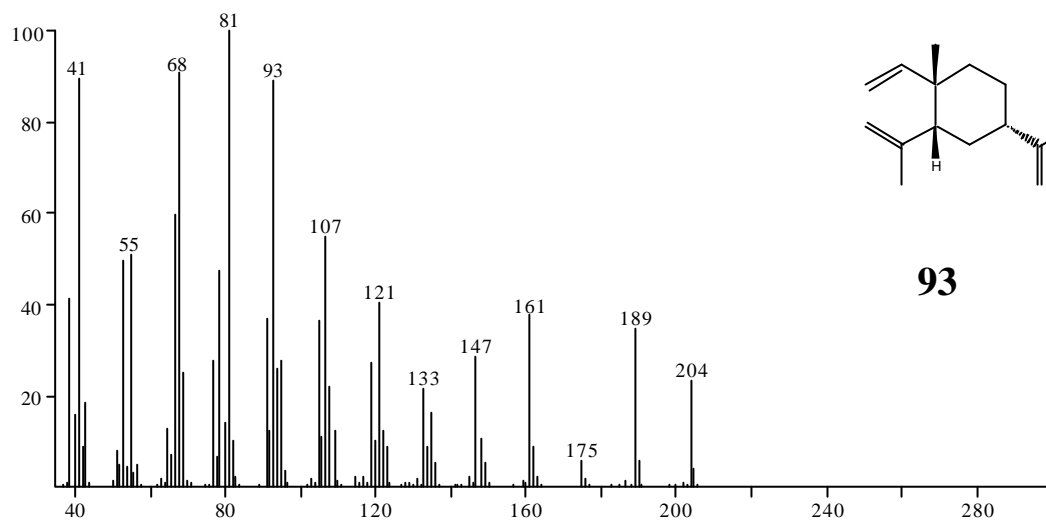
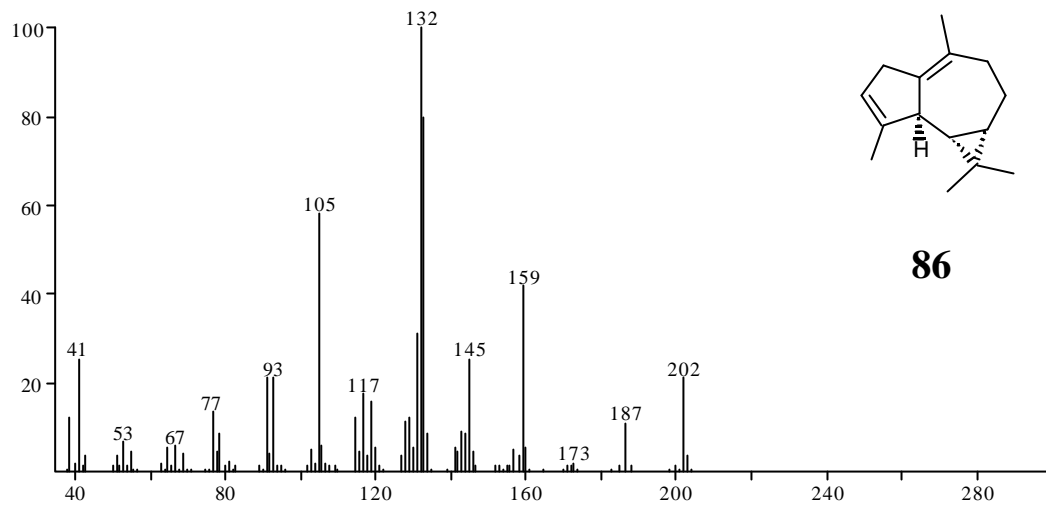
**83**

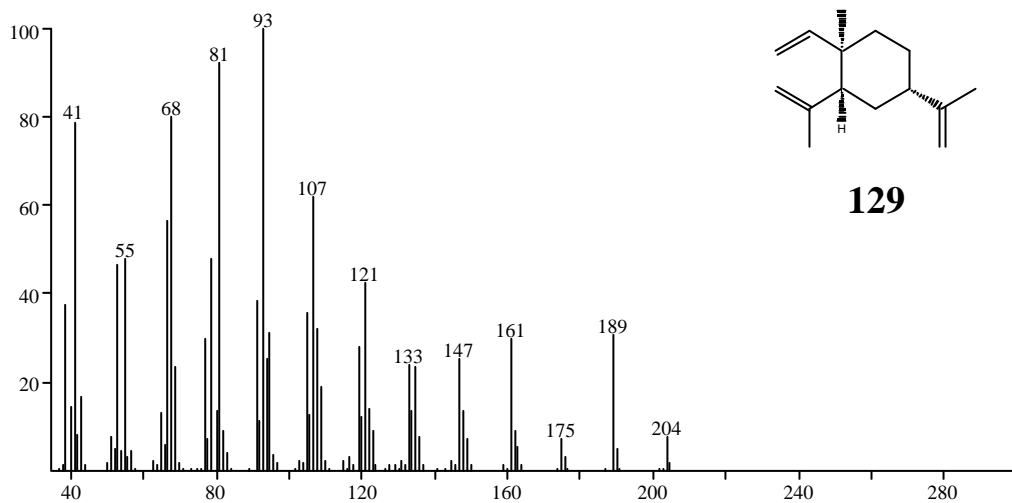
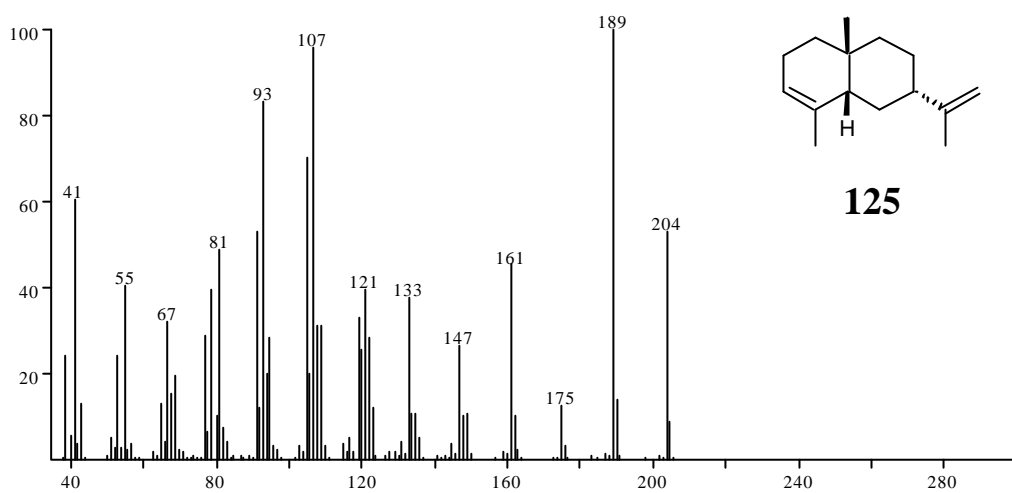
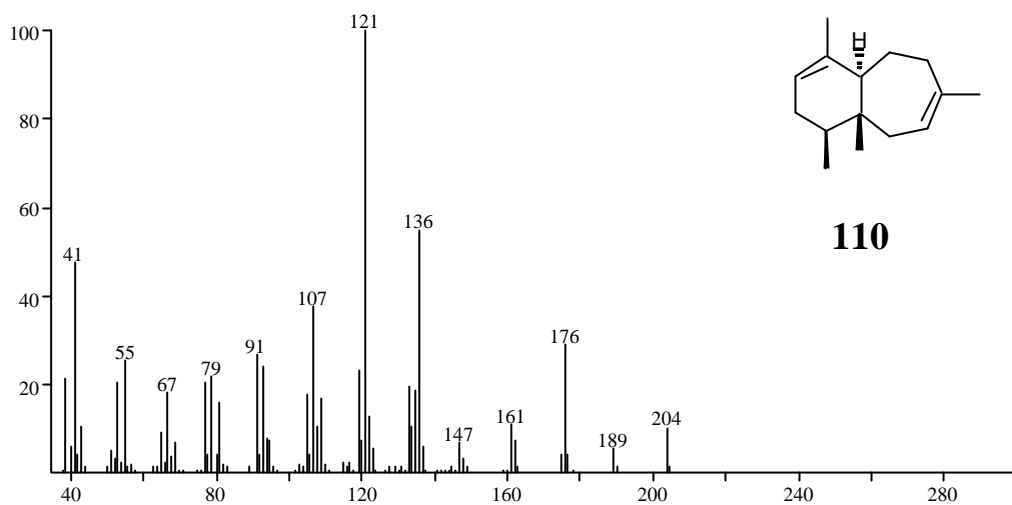


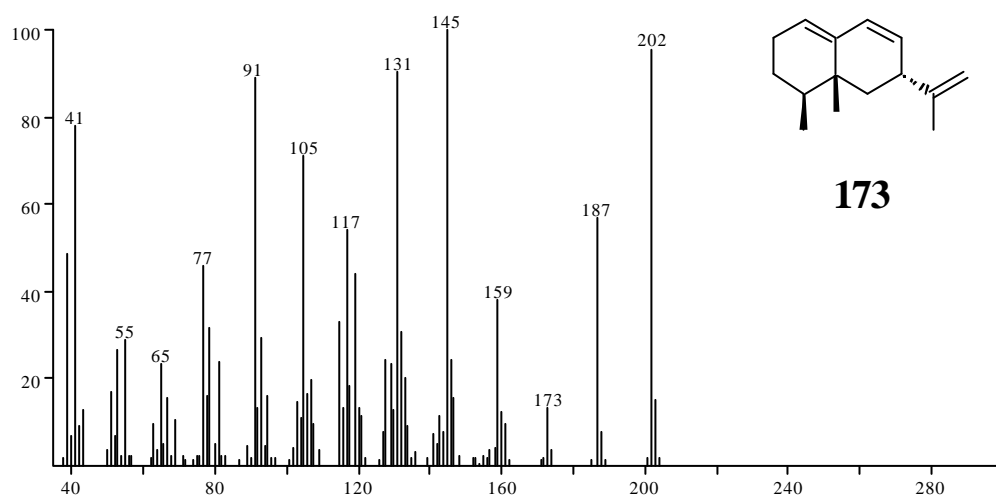
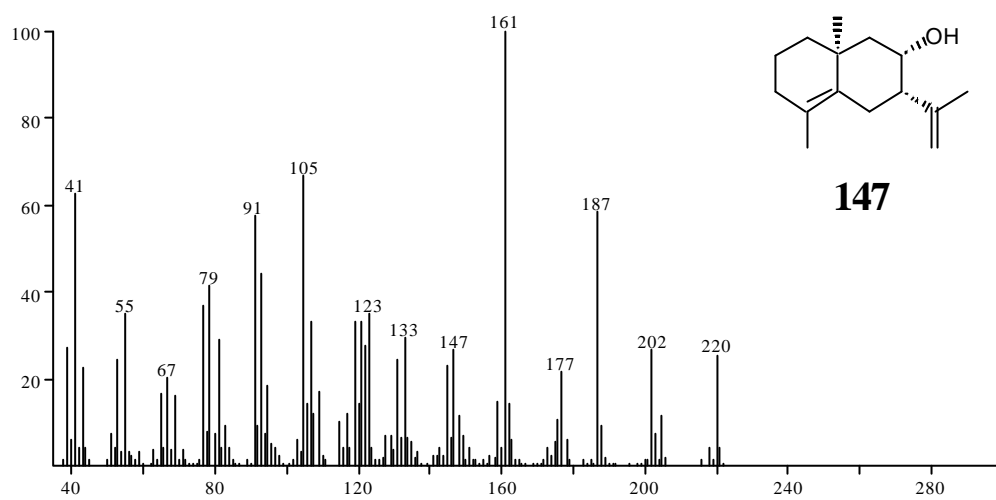
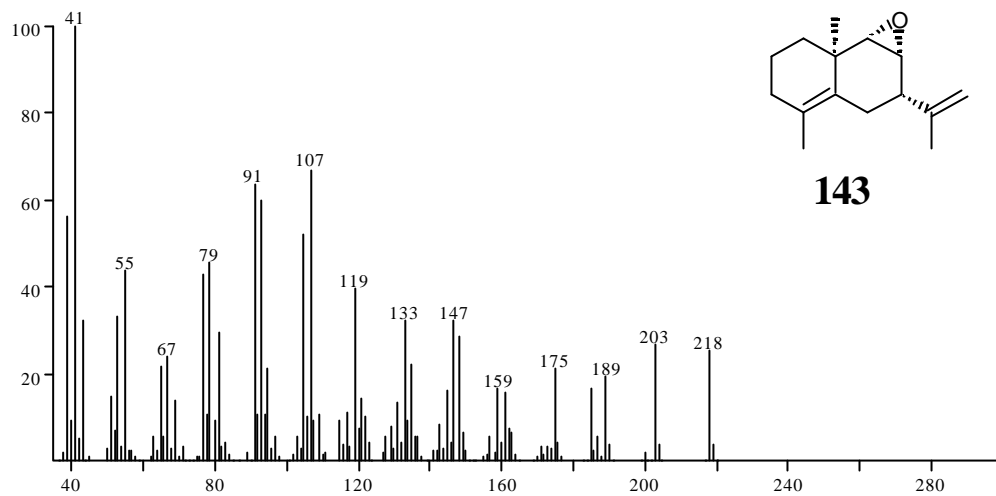
**84**

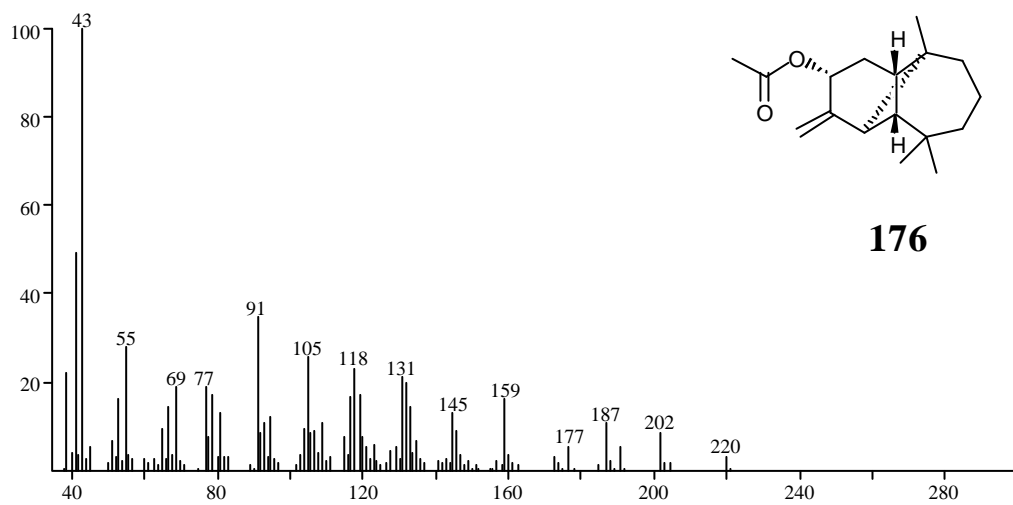
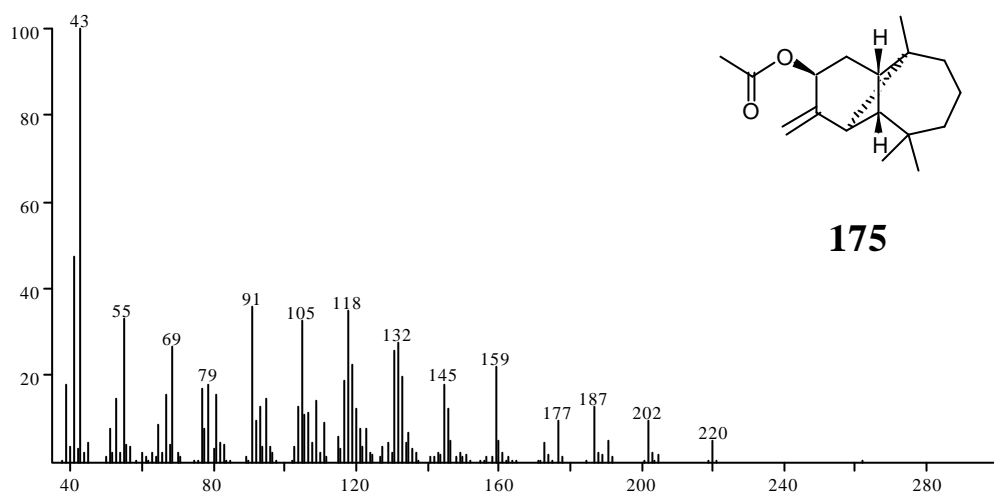
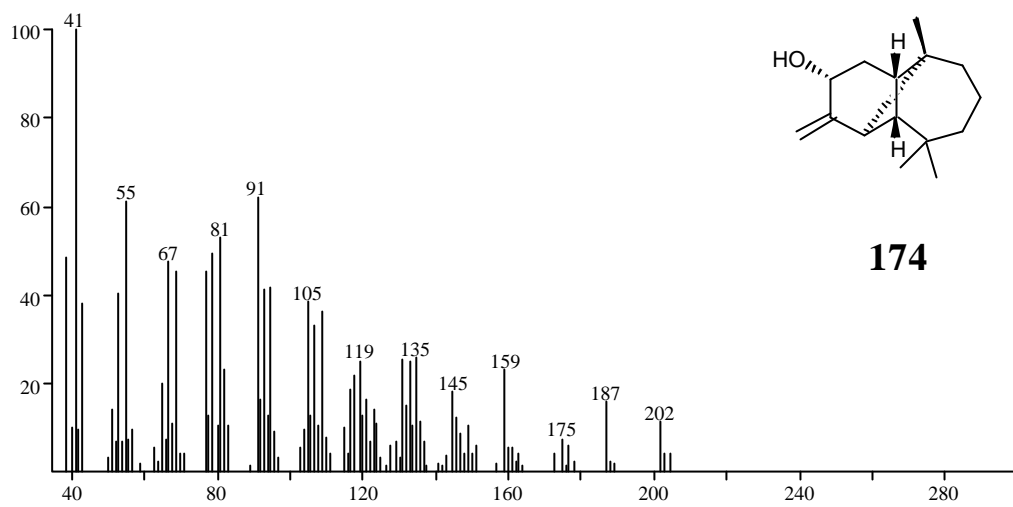


**85**

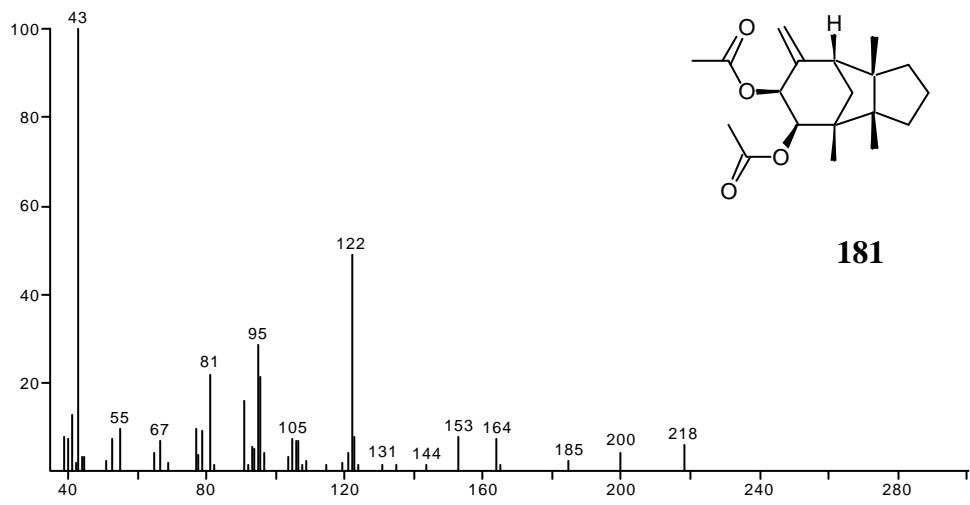
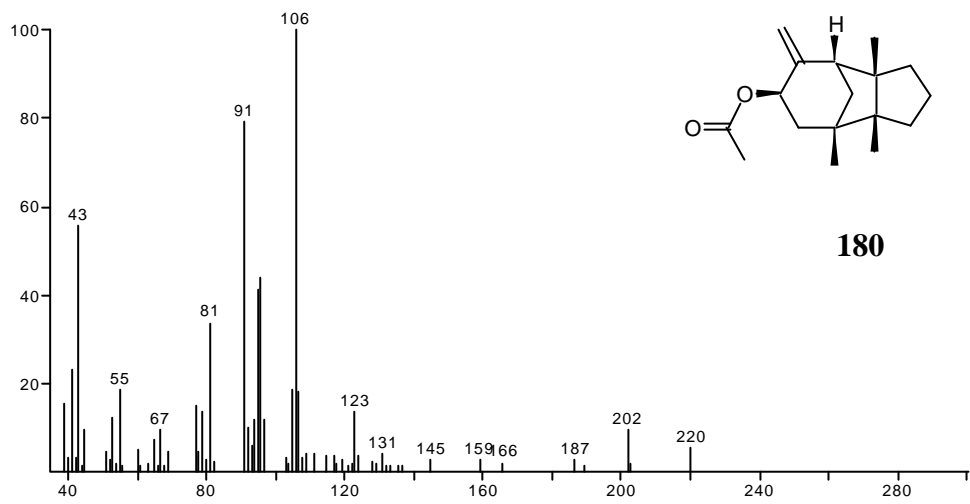
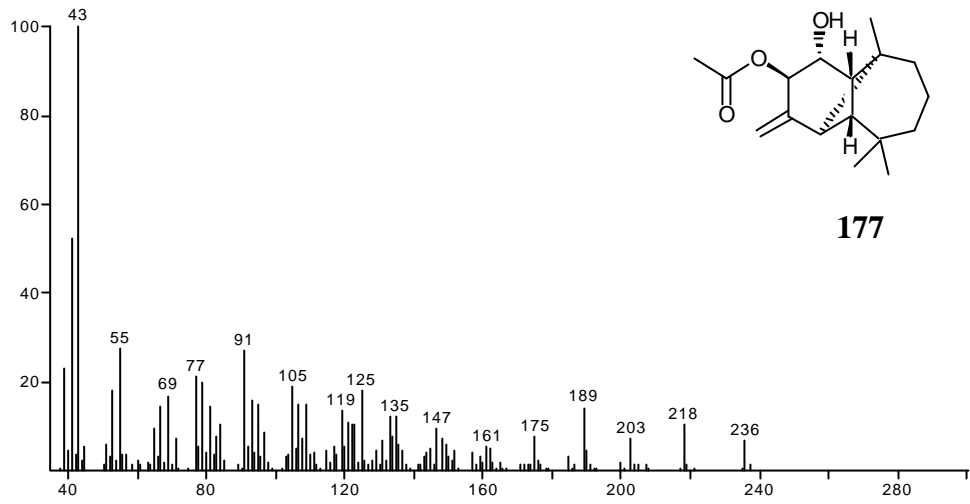


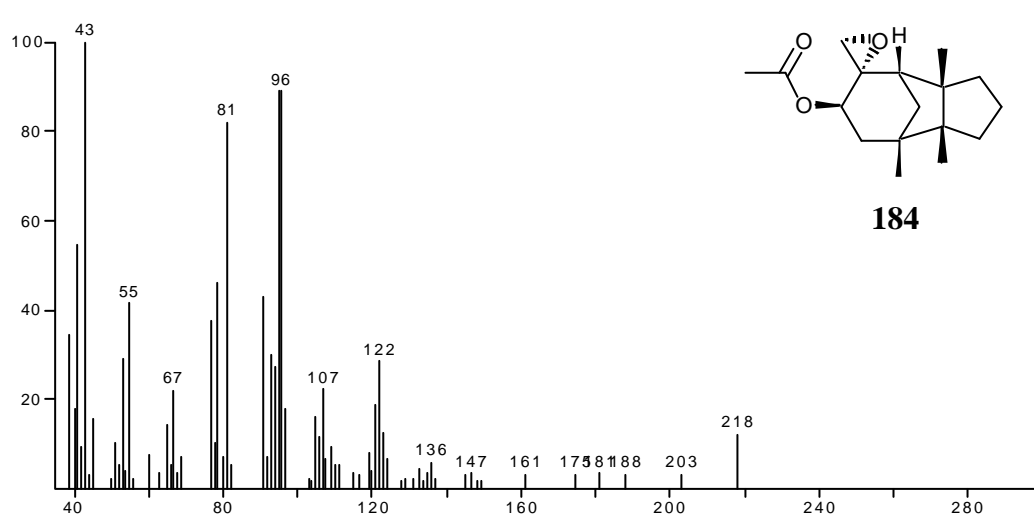
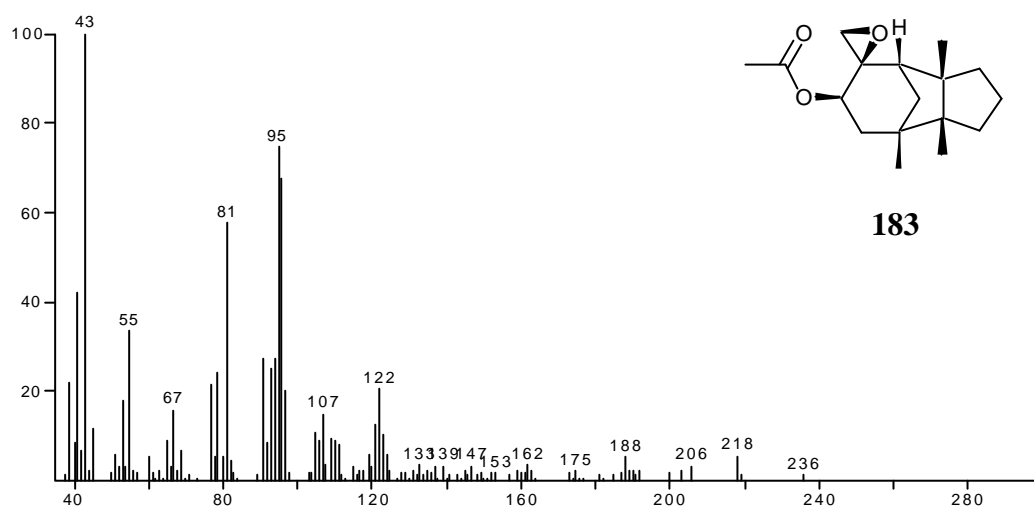
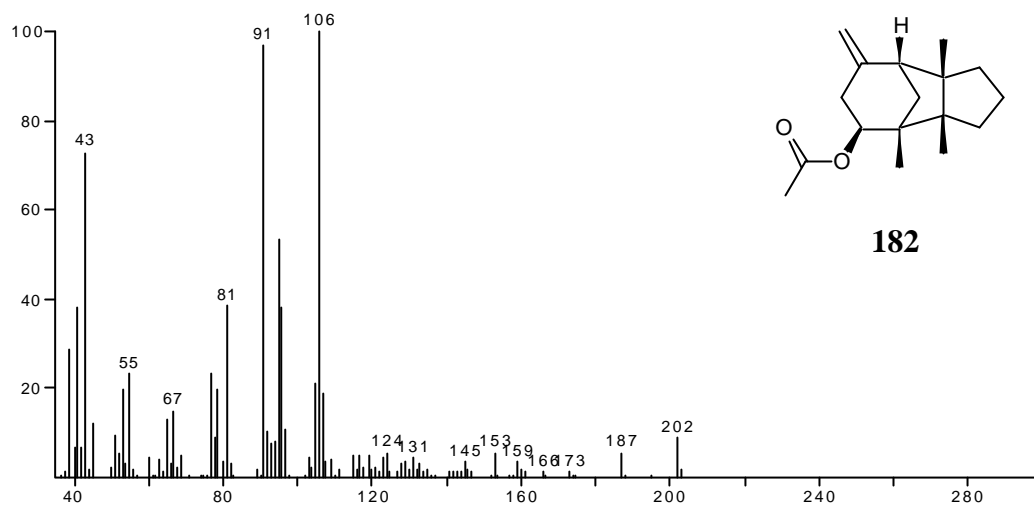


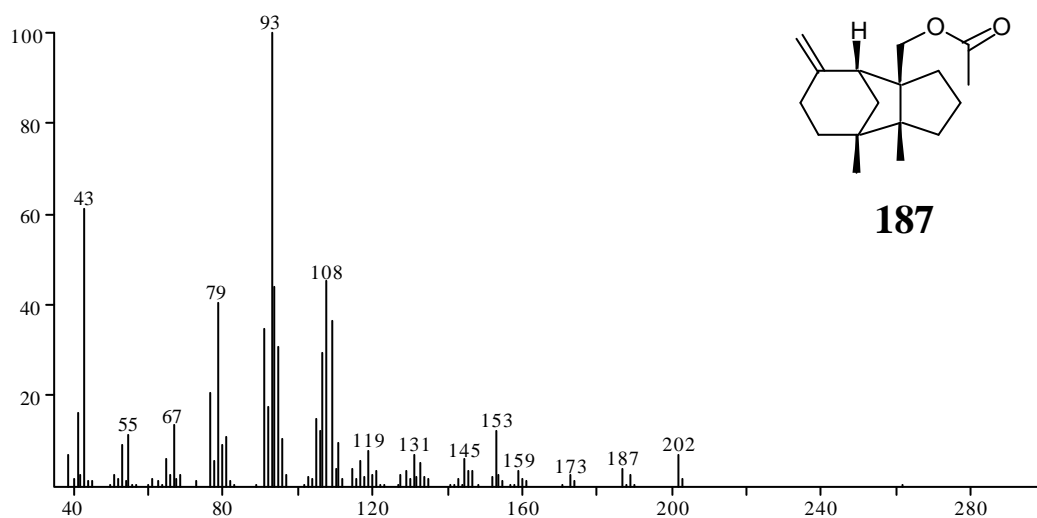
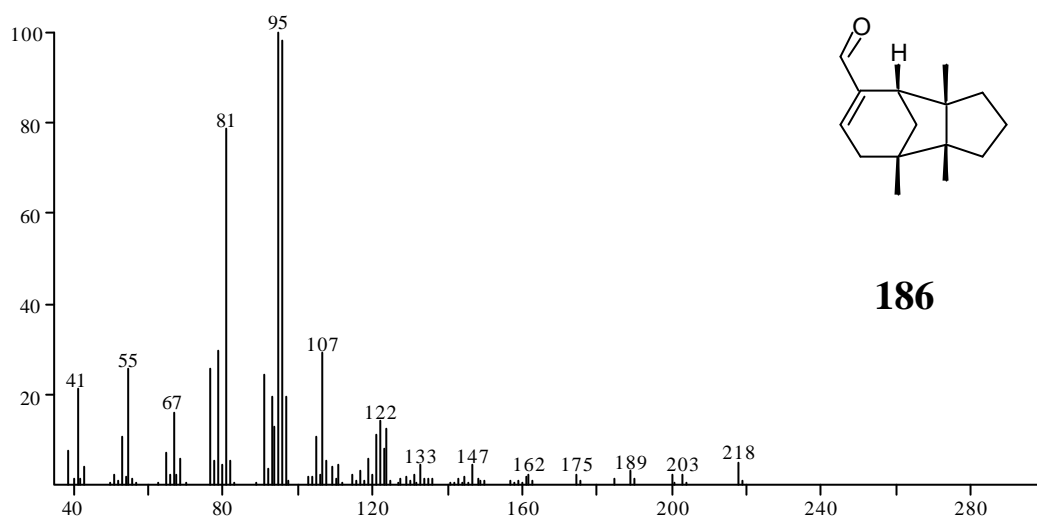
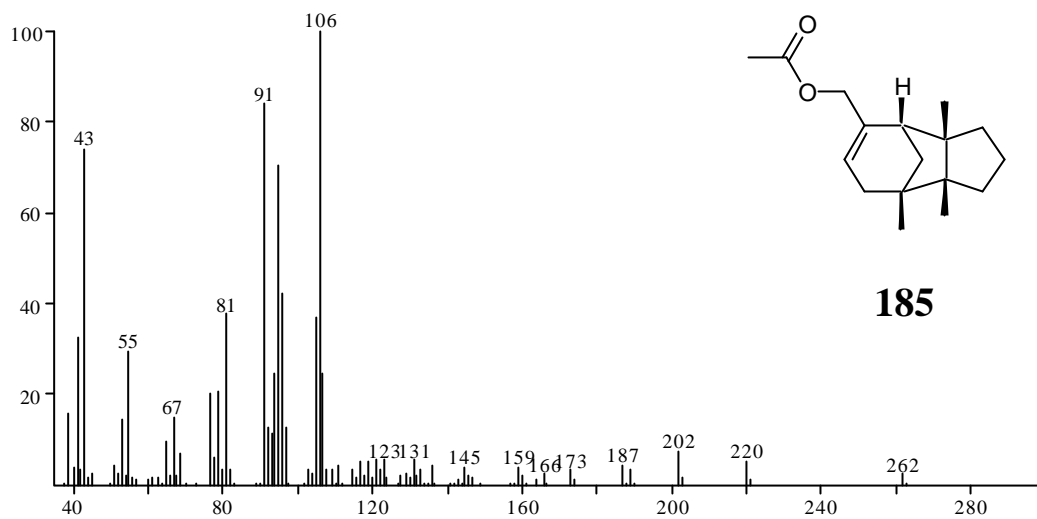


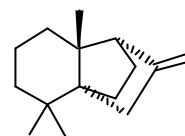
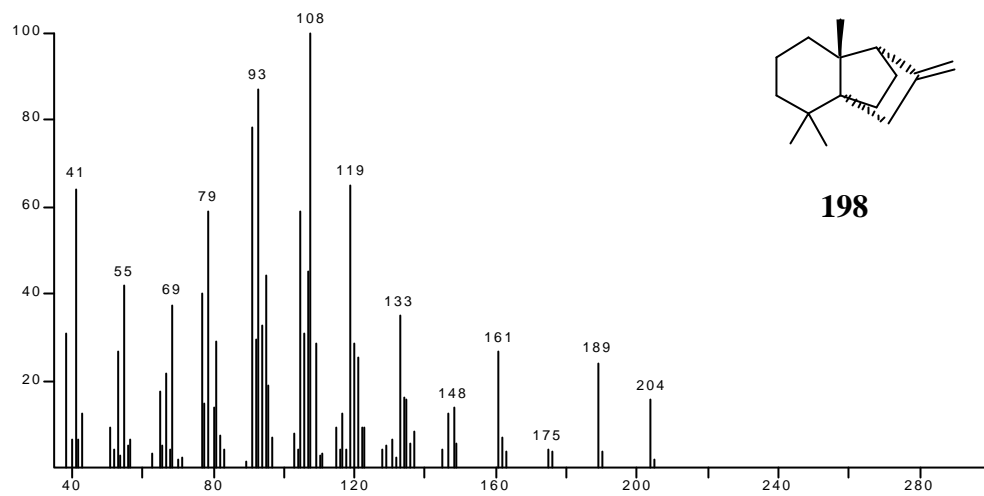




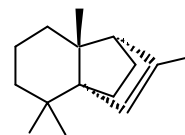
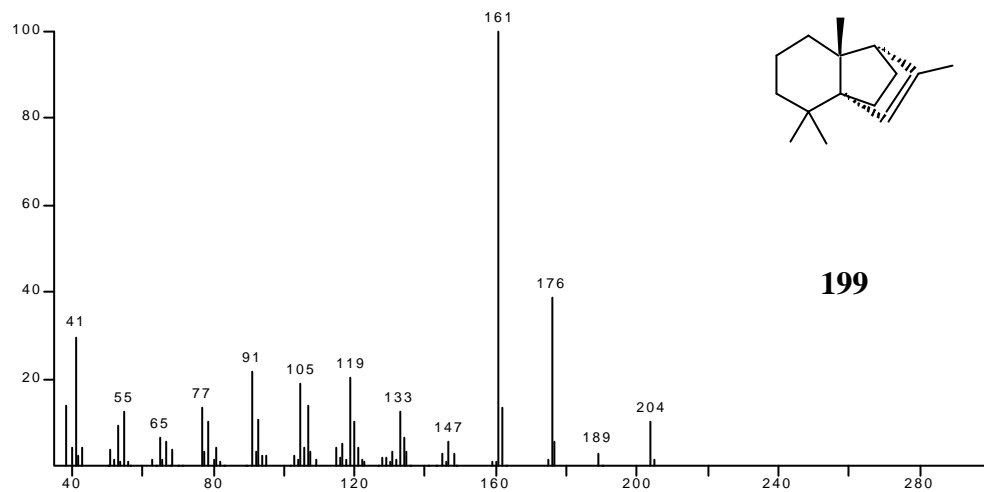




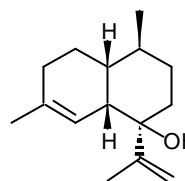
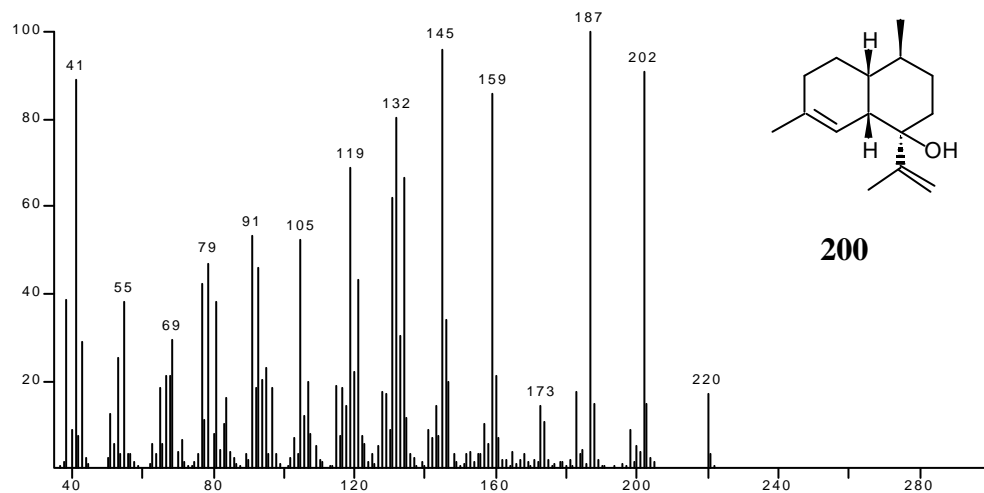




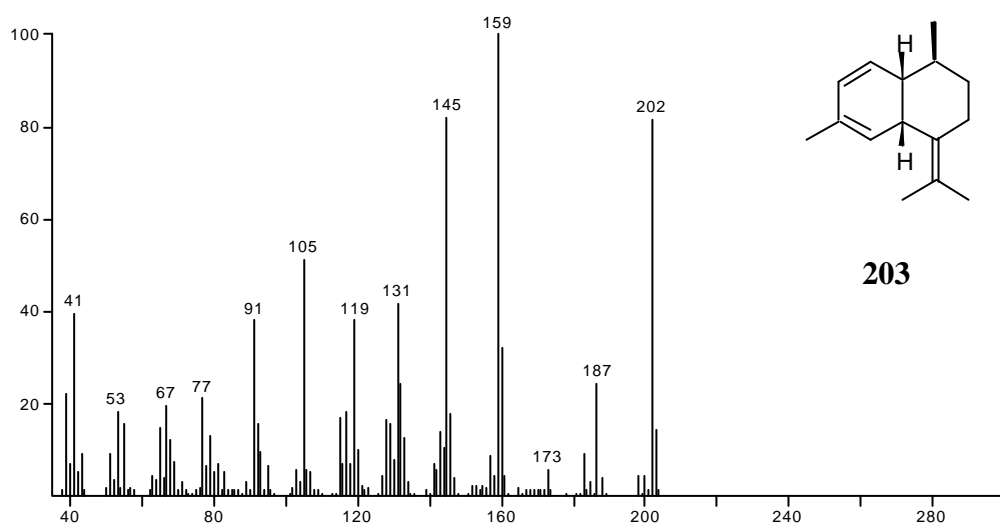
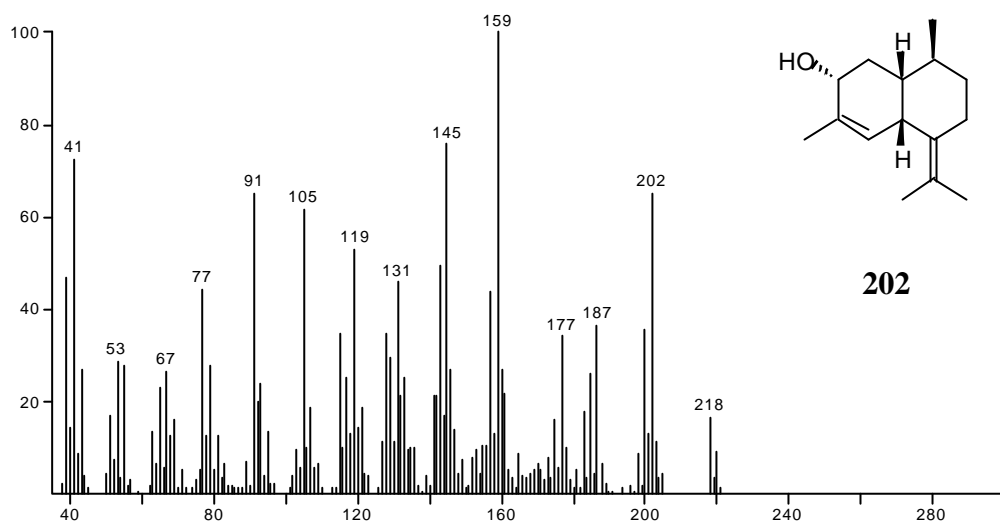
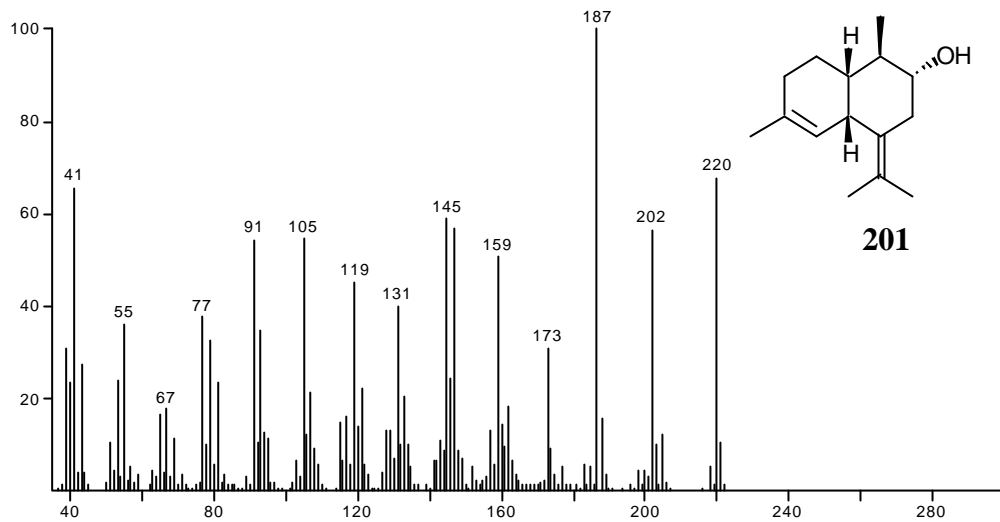
**198**

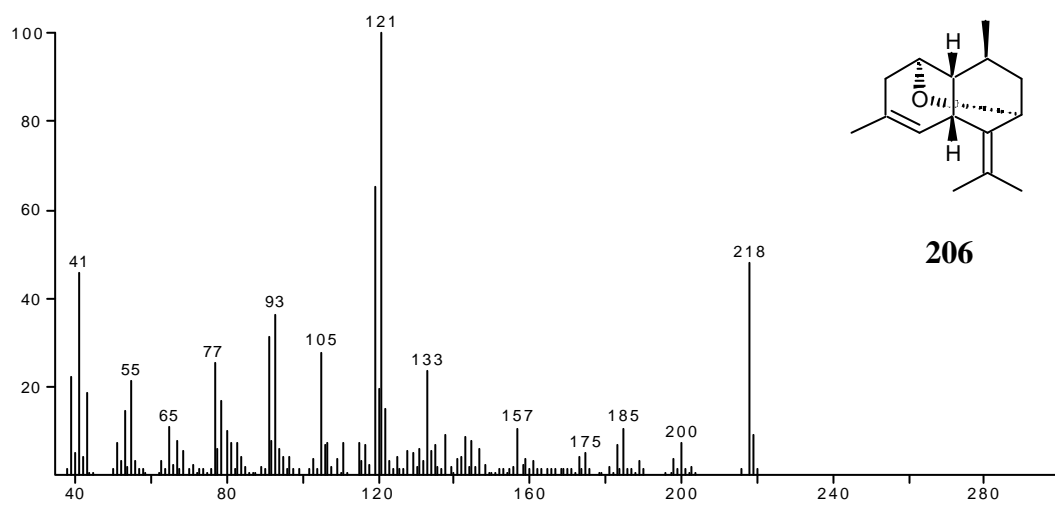
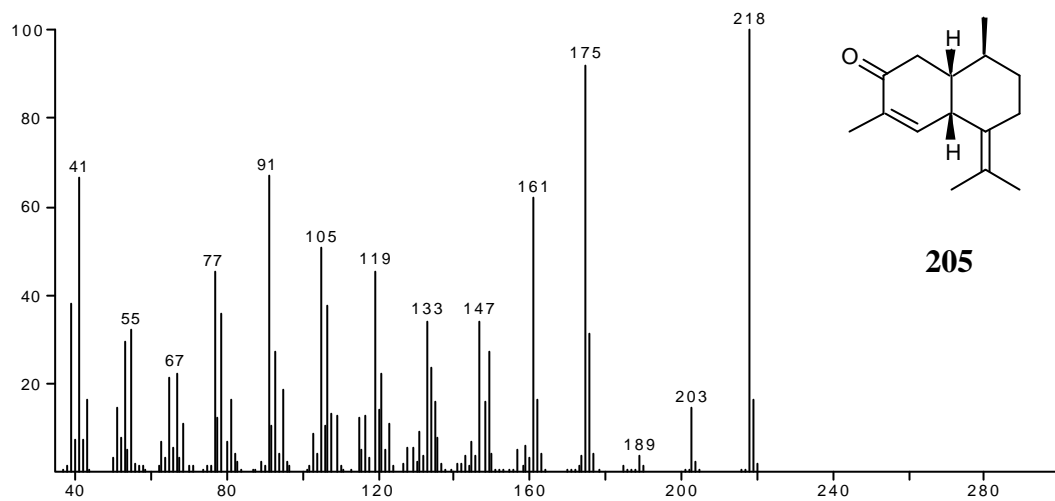
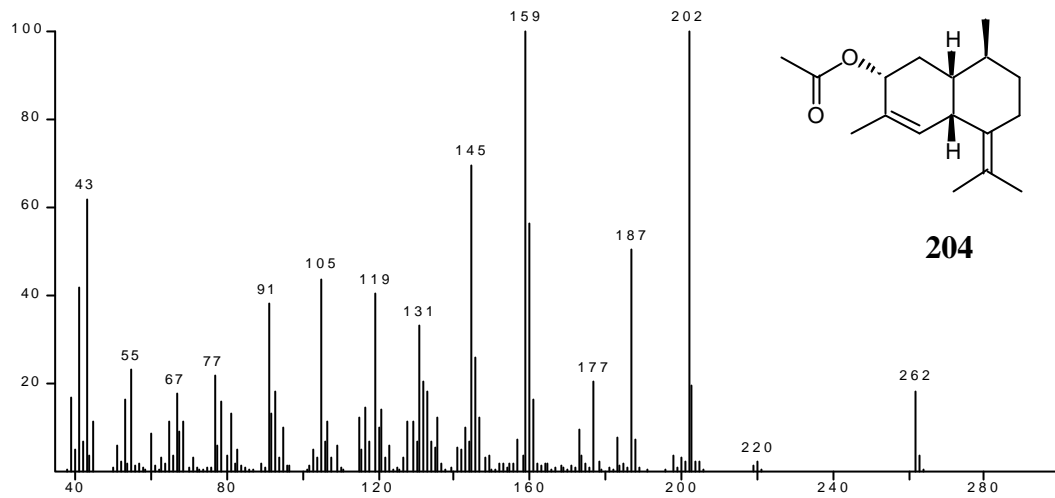


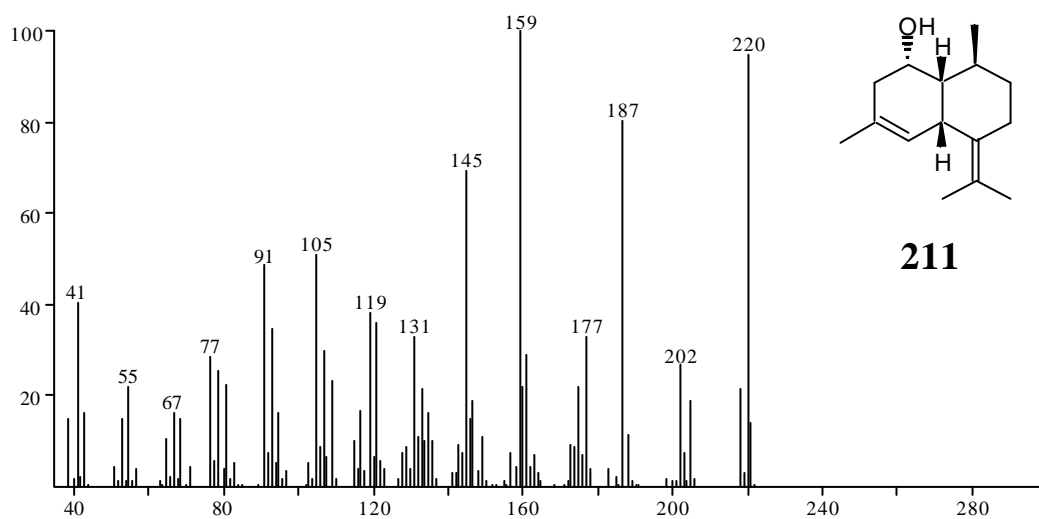
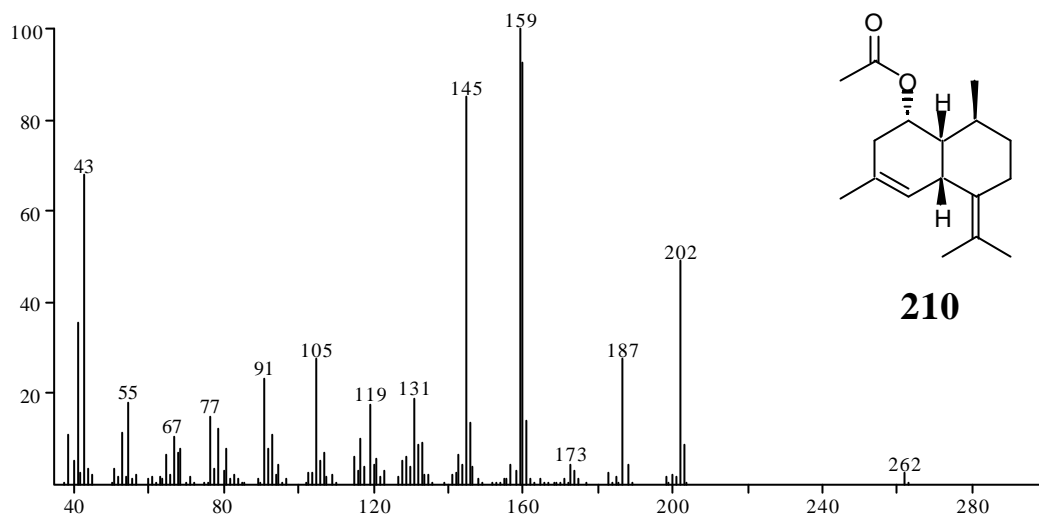
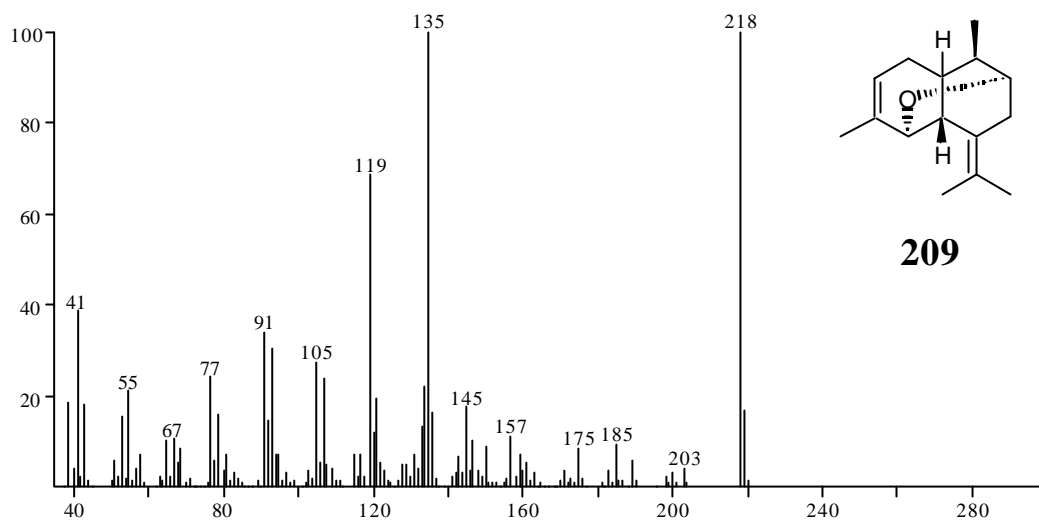
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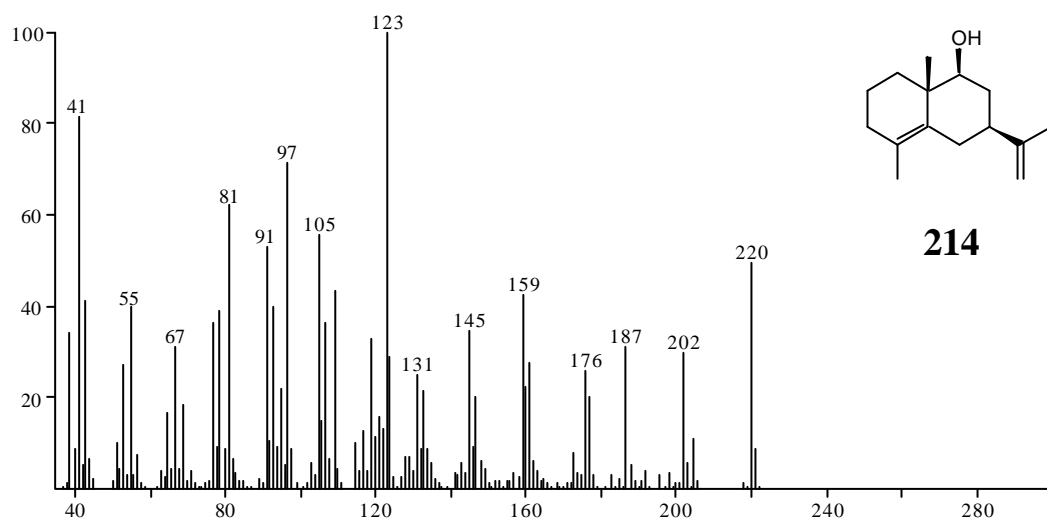
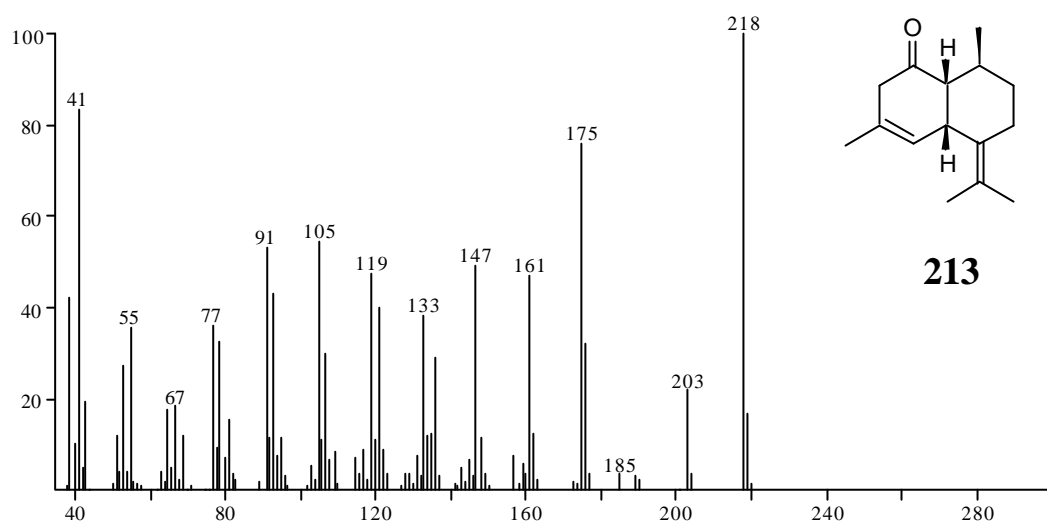
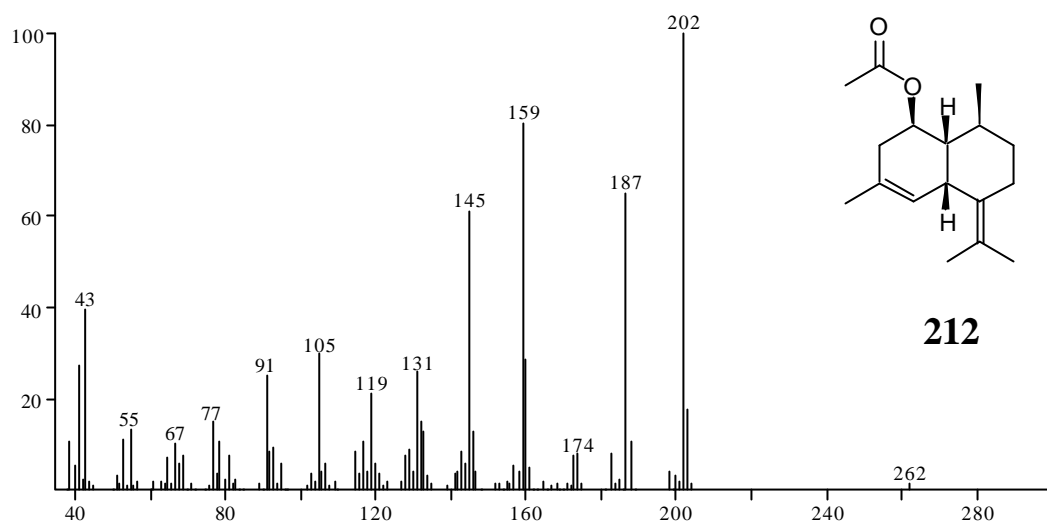


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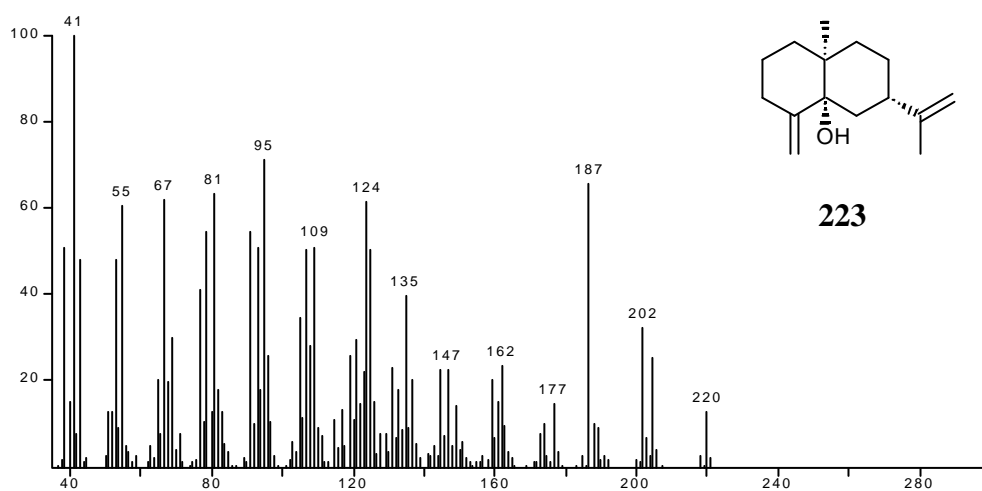
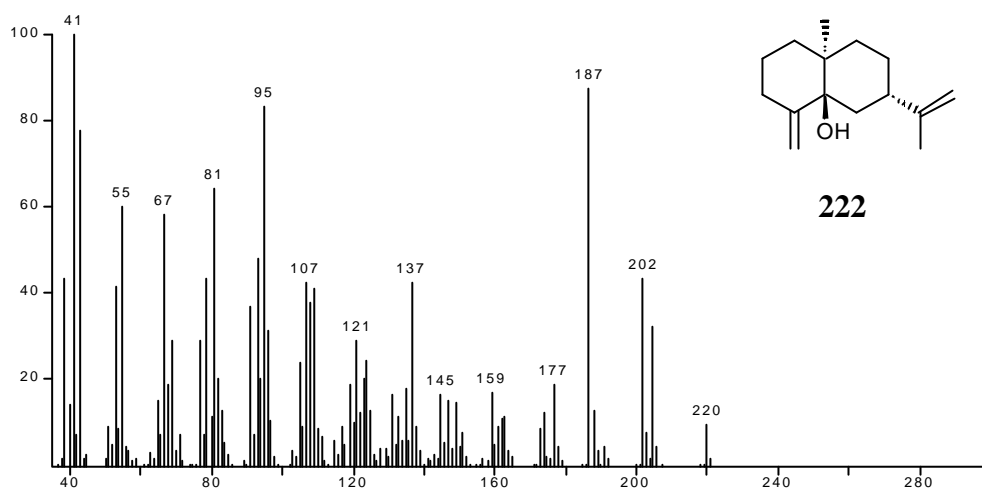
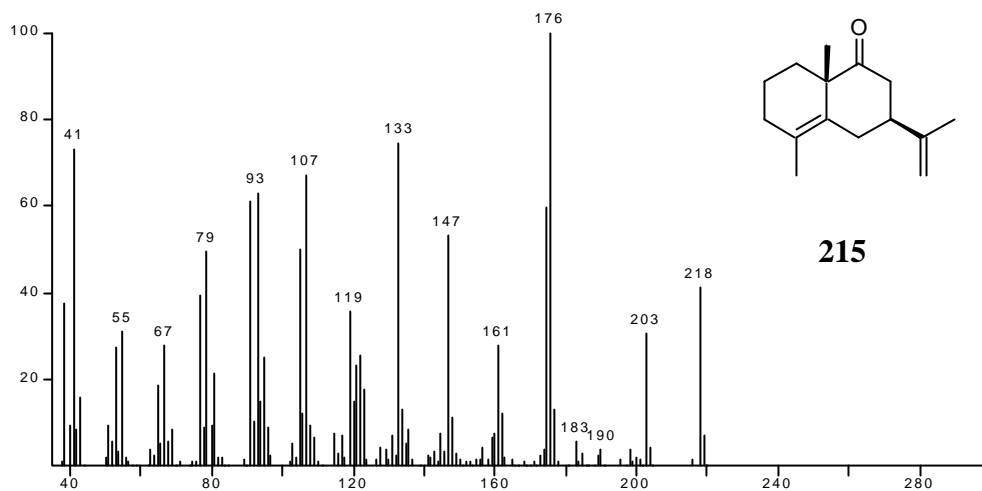


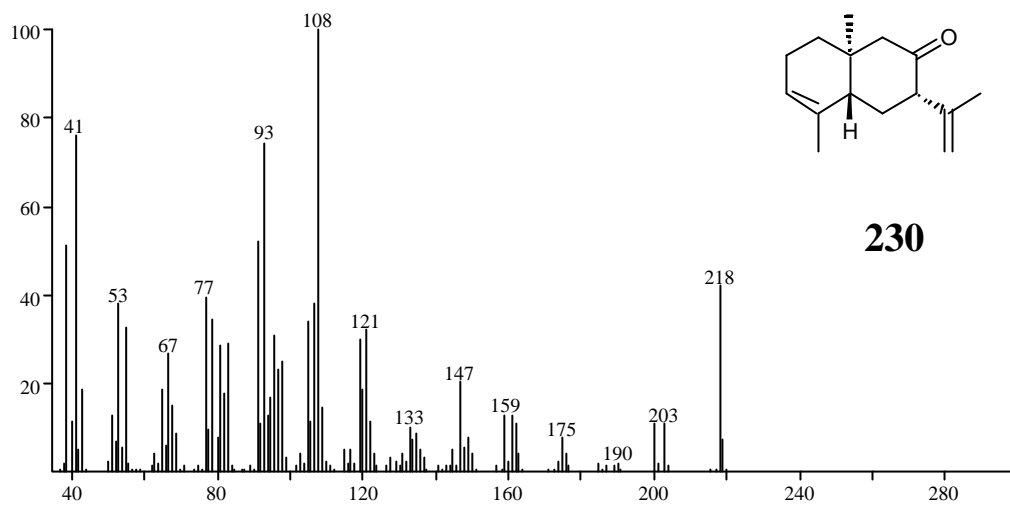




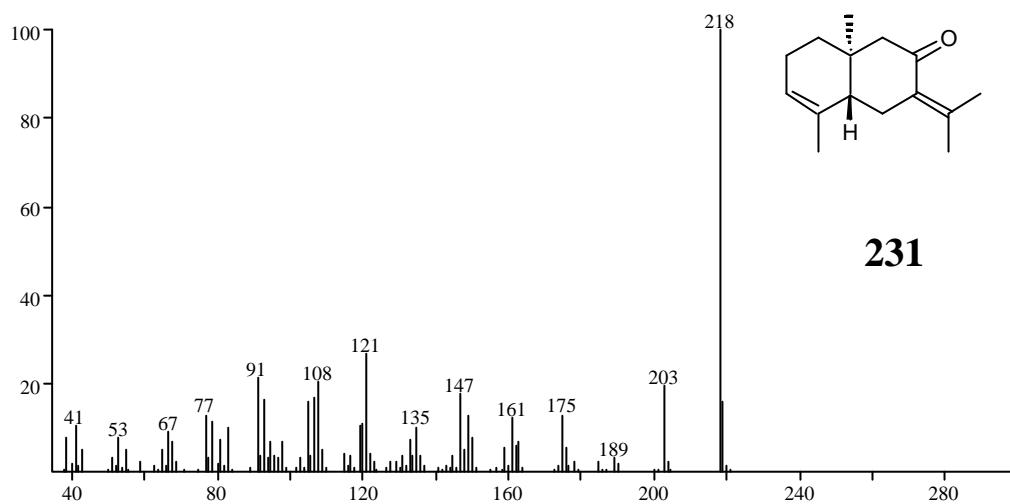




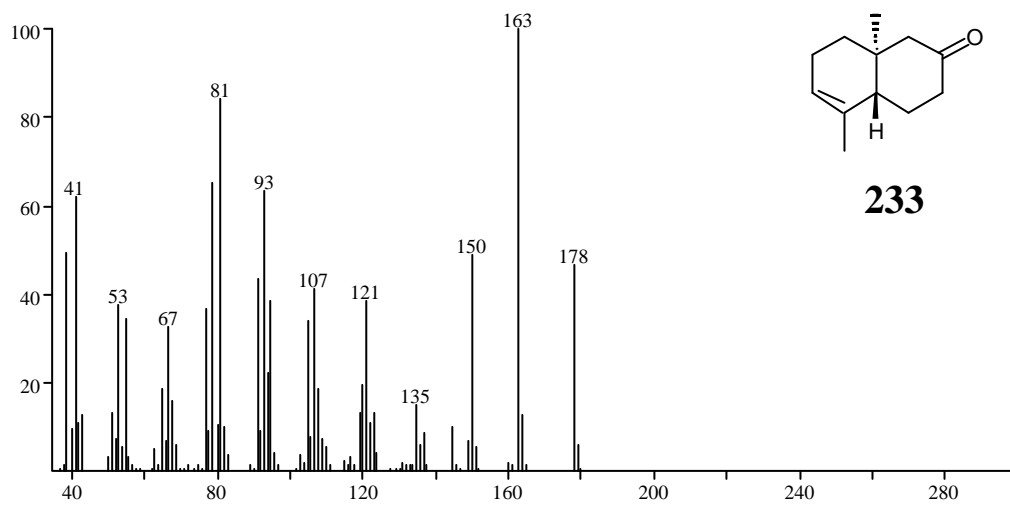




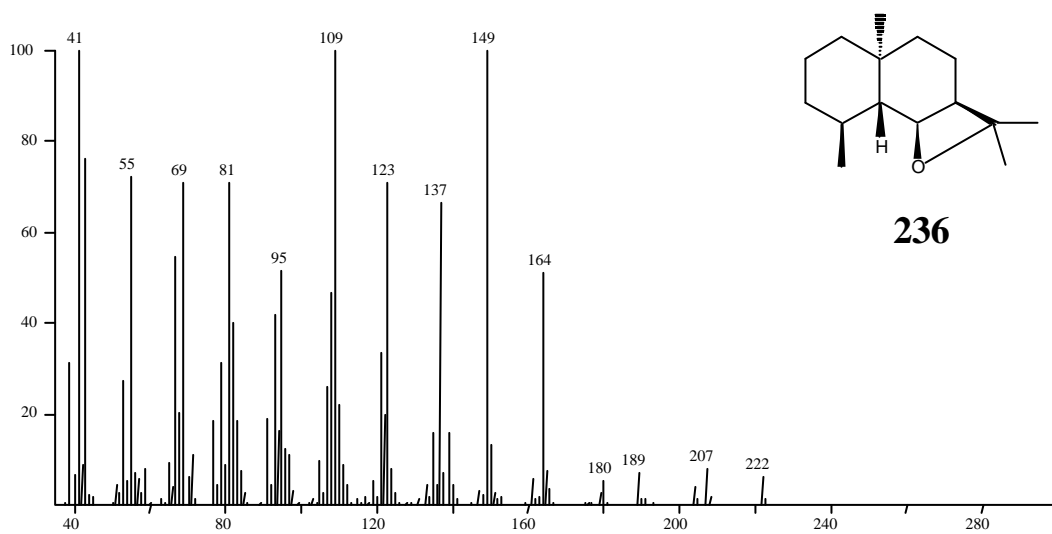
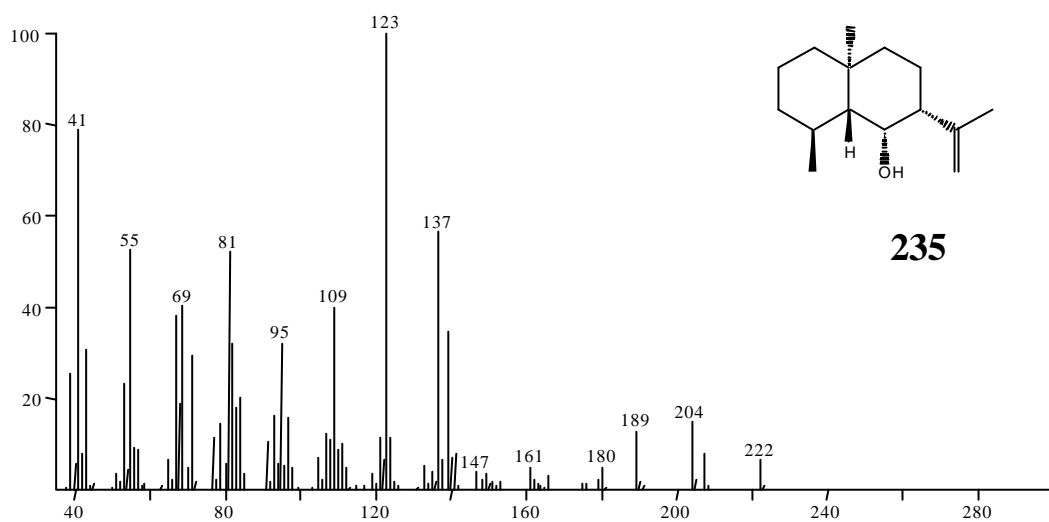
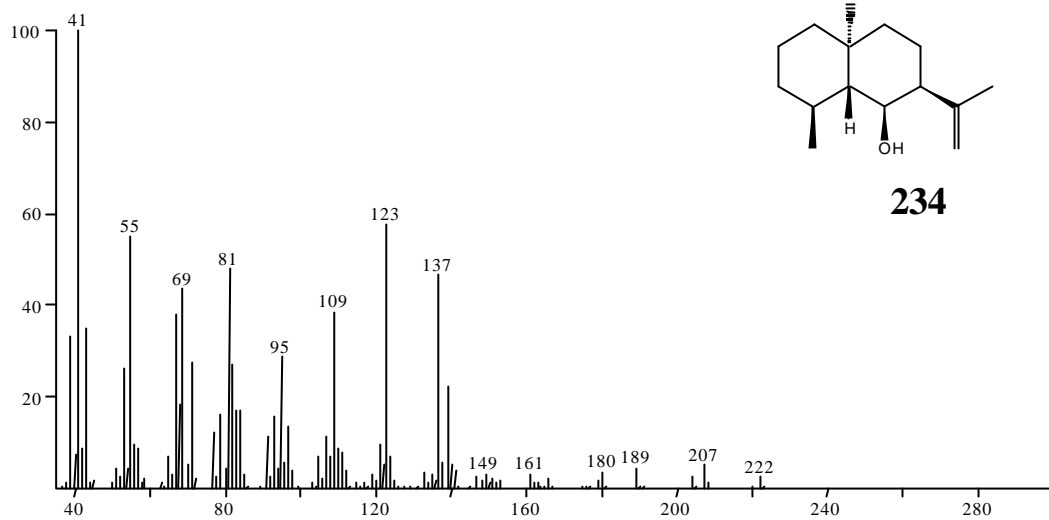
**230**

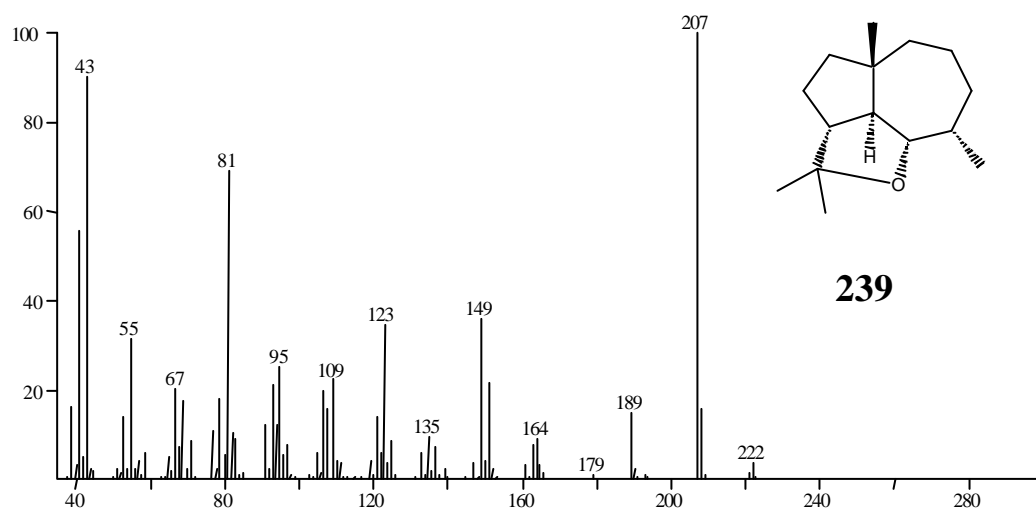
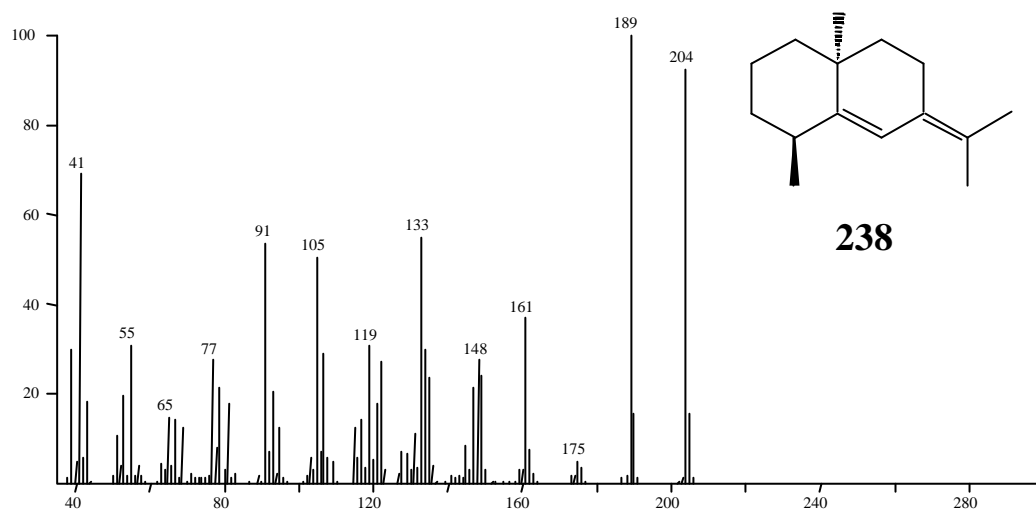
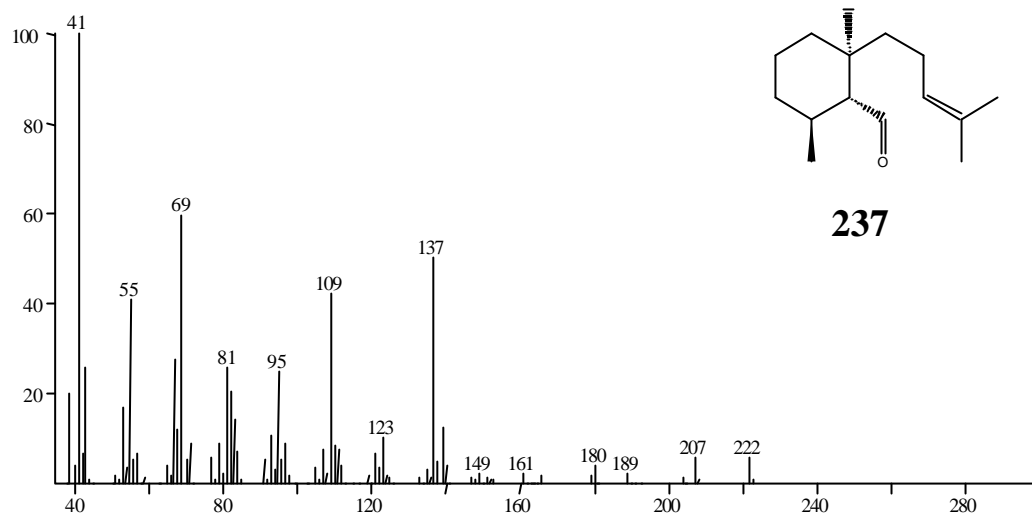


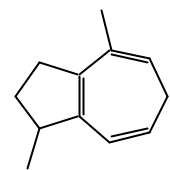
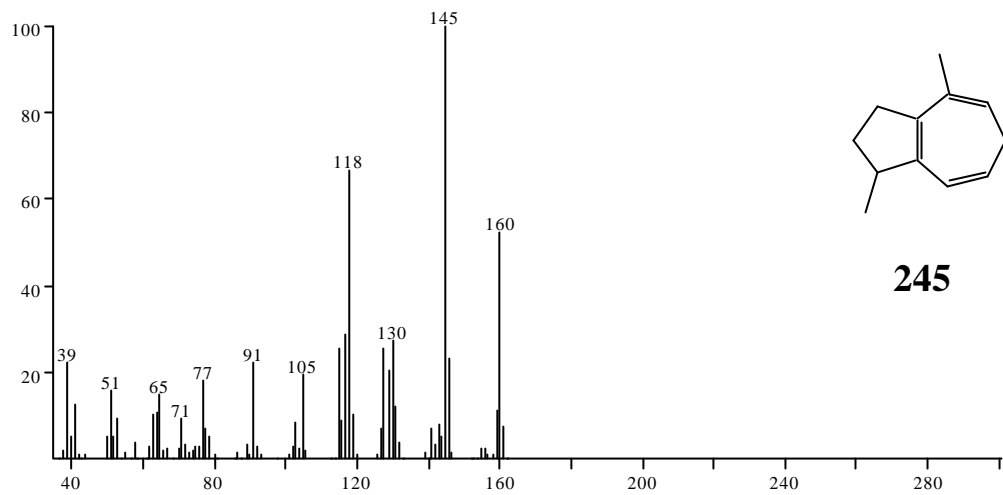
**231**



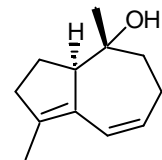
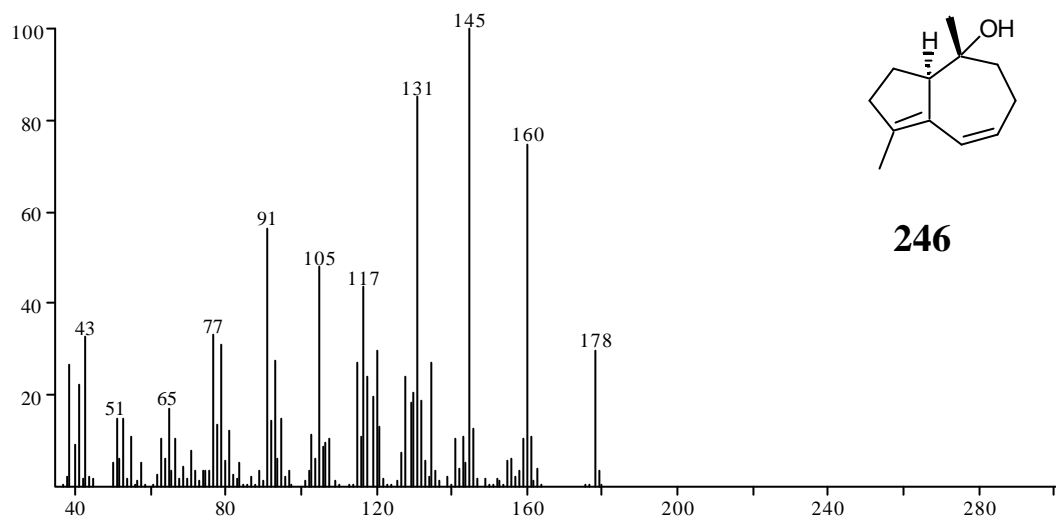
**233**



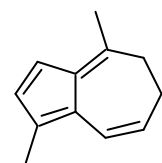
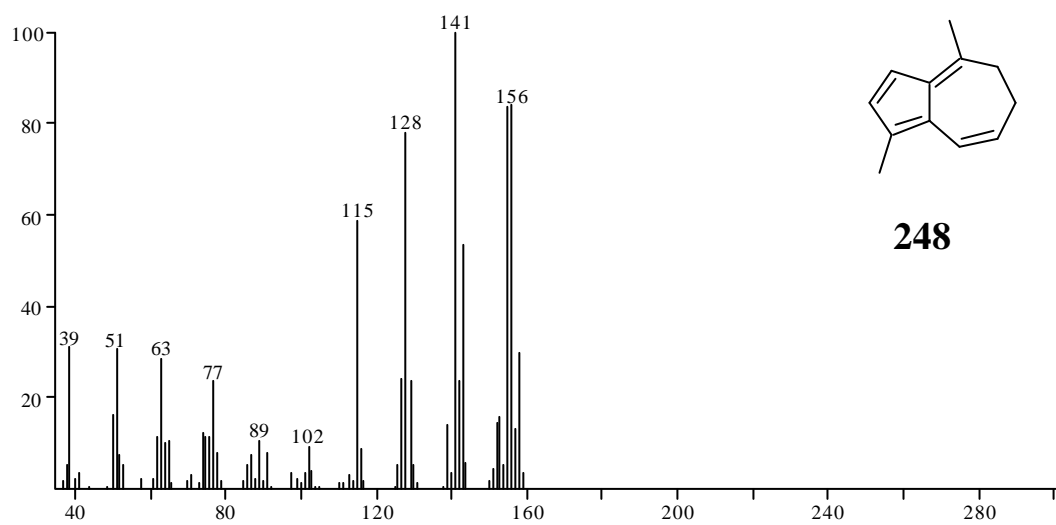




**245**



**246**



**248**

### **Poster Presentation:**

Adio, A. M., König, W. A.; Determination of the Absolute Configuration of a new Sesquiterpenoid and Analysis of the Essential Oil of the Liverwort *Plagiochila asplenioides*. 34<sup>th</sup> International Symposium on Essential Oils (ISEO) Würzburg, Germany, 7-10 Sep. 2003.

Adio, A. M., Paul, C., König, W. A.; Determination of the Absolute Configuration of Sesquiterpenes by Chemical Correlation and Enantioselective Gas Chromatography.

14<sup>th</sup> International Symposium on Chirality, Hamburg, Germany, 8-12 Sept. 2002.

### **Oral Presentation:**

Adio, A. M.; Isolation, Structure Elucidation and Stereochemistry of Sesquiterpene Constituents from the Essential oils of European Liverworts (Hepaticae). Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle/S, Germany, 21. Dec. 2004.

Wilfried A. König, Adewale M. Adio, Claudia Paul, Hailemichael Tesso, Thomas Hackl; Stereochemistry of Germacrene Isomers. Phytochemical Society of Europe, Gargnano, Italy, 5-8 May 2004.

Adio, A. M., König, W. A.; Enantioselective Gas Chromatography as a Tool for Stereochemical studies of Plant Constituents. 13<sup>th</sup> Doktoraden Seminar at Kloster Banz, Germany, 12-14 Jan. 2003.

### **PUBLICATIONS**

Adio, A. M., König, A. W., 2005. Sesquiterpene constituents from the essential oil of the liverwort *Plagiochila asplenioides*. *Phytochemistry* 66, 599-609.

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## **CURRICULUM VITAE**

Name: Adewale Martins Adio

Date of Birth: 9. November 1969

Place of Birth: Ibadan, Oyo State

Status: Married

Nationality: Nigerian

## **INSTITUTIONS ATTENDED**

1981- 1987: West African School Certificate (WASC)

1988- 1992: Bachelor of Science (Industrial Chemistry Option) - 1<sup>st</sup> Class (Hons)

1994- 1995: Master of Science (Biophysical Chemistry)

April 2001: Beginning of Dissertation at the Institute of Organic Chemistry, University of Hamburg. German Academic Exchange Programme Scholarship (DAAD) (Oct. 2000- Dec. 2004)

## **EXPERIENCE**

1999-2001: Lecturer II, Department of Chemistry, University of Ibadan, Nigeria

1997-1999: Assistant Lecturer, Department of Chemistry, University of Ibadan, Nigeria

1997-2000: Part-time Lecturer, Laboratory Technology Training School, University of Ibadan, Nigeria