ISOLATION AND STRUCTURE ELUCIDATION OF ESSENTIAL OIL CONSTITUENTS

Comparative Study of the Oils of *Cyperus alopecuroides,* *Cyperus papyrus,* and *Cyperus rotundus*

Dissertation for the Fulfillment of the Requirements
for the Degree of *Dr. rer. nat*.

by
Mesmin Mekem Sonwa
from Mbamougong(Cameroon)

Hamburg 2000

ISOLATION AND STRUCTURE ELUCIDATION OF ESSENTIAL OIL CONSTITUENTS

Comparative Study of the Oils of *Cyperus alopecuroides,* *Cyperus papyrus,* and *Cyperus rotundus*

by

Mesmin Mekem Sonwa

ISOLATION AND STRUCTURE ELUCIDATION OF ESSENTIAL OIL CONSTITUENTS

Comparative Study of the Oils of *Cyperus alopecuroide,* *Cyperus papyrus,* and *Cyperus rotundus*

Dissertation for the Fulfillment of the Requirements
for the Degree of Doctor, *Dr. rer. nat*.

by
Mesmin Mekem Sonwa
from Mbamougong (Cameroon)

University of Hamburg
Faculty of Chemistry

The present work was performed from november 1995 to march 1999 at the University of Hamburg, Department of Organic Chemistry, in the laboratory of Prof. Dr. König.

1. Gutachter: Prof. Dr. W. A. König

2. Gutachter: Prof. Dr. K.-H. Kubeczka

Tag der lezten mündlichen Prüfung: 27. 04. 2000

A mes parents bien aimés.

Mercure : «Dis-moi donc ce que tu cherches et ce que tu veux obtenir. Que désires-tu faire?»

L'alchimiste : «La pierre philosophale.»

(Sendivogius, *Nouvelle Lumière de l'Alchimie*)

ACKNOWLEDGEMENT

First and foremost I thank my mentor Prof. Dr. Wilfried A. König for his guidance and support of my work in his laboratory. I took advantage of the occasion he gave me to work under his supervision to learn a great amount during this time. I enjoyed the stimulating and pleasant atmosphere in his research group.

I wish to thank my laboratory mates Yürcel Saritas and Stefan Jung for their great disponibility when I began as newcomer in the laboratory. I should also thank Helmutt Dittmann for stimulating discussion during the course of this work.

I thank the NMR team of the Department of Organic Chemistry for the measurement of NMR spectra. I wish to acknowledge the contribution of Dr. V. Sinnwell in offering valuable suggestions and carrying out of important NMR spectra.

EI-Mass spectra were carried out by Mrs. Annegret Meiners to whom I am thankful. I am most appreciative for the hospitality she showed to me since I arrived in Hamburg to achieve this work.

I thank Mr. Manfred Preuße also for carrying out mass spectra.

I should like to acknowledge the help of Dr. Detlev Hochmuth in solving some computer problems while writing this dissertation.

I am indepted to the Deutscher Akademischer Austauschdienst (DAAD) for the

financial support.

I wish to thank all active collaborators of Professor W. A. König: Christiane Fricke, Angela Rieck, Stephanie Melching, Nils Bülow, Stefan Jung, Martin Pietsch, Ute Warmers, Klaus Scharwächter, Claudia Paul, Kolja Wihstutz, Andre Krüger, Joachim Rösecke, Andrea Arcamone, Hassan Iznaguen, Weichao Wang, Yücel Saritas and Helmutt Dittmann for the pleasant and stimulating atmosphere in the laboratory.

I should thank my friend Jean-Marie Tchoumta and his wife, with whom I shared some hard and nice times during the achievement of this work.

I am finally indebted to my parents who gave all their best for my education.

Table of Contents.

List of Abbreviations.

C:	Carbon.
CD:	Cyclodextrin.
d:	Doublet.
DEPT:	Distortionless Enhancement by Polarization Transfert.
DMS:	Dimethyl sulfide.
DMSO:	Dimethyl sulfoxide.
EI:	Electron ionization.
Fig.:	Figure.
g:	Gramm.
GC:	Gas chromatography.
H:	Proton.
H_a:	Proton in axial position.
H_e:	Proton in equatorial position.
HPLC:	High Performance Liquid Chromatography.
J:	Coupling constant.
m:	Multiplet or meter.
min:	Minute.
ml:	Milliliter.
m/z:	Mass-to-charge ratio.
MS:	Mass spectrometry / or spectrum.
Me:	Methyl (group).
NMR:	Nuclear Magnetic Resonance.
Pe:	Pentyl.
q:	Quadruplet.
s:	Singulet.
Rel. / Int.:	Relative intensity.
Rf:	Ratio of fronts.
t:	Triplet.
TBDMS:	*tert*-Butyl dimethylsilyl.
TBABr:	Tetrabutyl ammonium bromide.
THF:	Tetrahydrofurane.
w:	Weigh.

Cyclodextrin derivatives e.g.: 2,6-Me-3-Pe-γ-CD = Octakis(2,6-di-Omethyl-3-pentyl)-γ-cyclodextrin. The abbreviated form may also be used to named the column in which the cyclodextrin derivative is the stationary phase.

1 The Natural Product Chemistry of Essential Oils.

> «On savait extraire les huiles naturelles et résines, préparer par distillation l'eau de rose et les eaux imprégnées de matières odorantes, spécialement les essences de térébenthine et de genièvre, ces dernières déjà connues des anciens.»
>
> (M. Berthelot, *La Chimie au Moyen Âge I*, Paris, 1893)

The purpose of this chapter is to place this work in a historical context of this field of chemistry. After brief description of ancient folk medecine and fragrance culture it will be continued with an outline of some major conceptual and experimental advances that have been made from the early nineteenth century up to now, and end up in bringing out the raison d'être of our work.

1.1 In the Beginning.

Folk interest in treatments for pains and other deseases, for hallucinogens and fragrance appears to go back to the early ages of humanity. Aromatic

fumigations and fragrances have been in use probably since the discovery of fire. Egyptians and Phoenicians, Jewish, Greeks and Roman and even Mayas and Aztecs possess a fragrance culture of great refinement [1, 2]. The Egyptian art of medecine and perfumery is on top in the civilised antique world. Fragrances are seen as a manifestation of divinity on the earth or as a connection between human beings and the gods, hence the use of aromatic fumigation in daily ritual and during religious ceremonies. But spiritual matters is not the only concern of Egyptians since fragrant products are also used as perfume. Important female guests carry slowly melting fragrant bowls on their head. The hair is treated with balsam [3]. Oils with bactericidal properties are used in the preparation of medical soaps. Such oils are also employed in the art of mummyfication [4, 5]. The use of aromatics spread from Egypt to Israel, Greece, Rome and the whole mediterranean world. In the East, the Vedas, the most sacred books of India and one of the oldest known books codify the uses of perfumes and aromatics for liturgical and therapeutical purposes.

The advent of christianity and the fall of the Roman Empire caused the art and science of fragrance to move into the Arabic world, where it reached an unequalled level of refinement. On their way back from the Holy Land during the Middle Ages, the crusaders introduced in Europe the old art of alchemy. Alchemist, whose experiments were devoted to the discovery of an elixir which would confer infinite longevity, the so-called *elixir vitae* or elexir of life, used all available methods for the purpose of their investigations. Of these methods, one was so effective in bringing about transformations of solid matter into liquids and vapours of totally different character, that through this process alchemists said that they were able to get the *spirit* or quintessencia of whatever material was so affected. These quintessences were for many centuries the basis of most medicines. But during the Renaissance, their use expanded into

perfumery and cosmetics. Indeed, the process used by alchemists was the distillation technique and the quintessencia no other thing as what we know nowadays as essential oil [6].

1.2 The Dawn of Organic chemistry and essential Oil Research.

Toward the end of the eighteenth century, a new era for chemistry were to be born through the works of Lavoisier which culminated in the publication of his book *Traité Élémentaire de Chimie* in 1789. Liberating chemistry from the phlogiston[1] doctrine, Lavoisier restructured chemistry from the fundamental principles, provided it with a new language and fresh goals [8]. The term "organic compound" was coined by Jöns Jacob Berzelius in 1807 to define compounds made by and isolated from living organism. The advent of modern science also marked a new era in the chemistry of essential oils although it was considered as one of the most difficult fields of organic chemistry.

The first[2] reported works on essential oils are those of Houton-Labillardiere in 1818 who did an elementary analysis of turpentine oil and found a ratio of five

[1] - According to Stahl, one of the promotors of the phlogiston theory, the phlogiston is the *terra pinguis* or fatty earth (the ancient unctuous moisture of the alchemists) which produce oily, sulphurous and combustible properties [7]. Lavoisier rejected the phlogiston theory because of its absurdity. To explain some facts, some chemists accepted phlogiston as a principle without weight; yet at other times they had to admit that it did have some weight. In fact the term phlogiston denoted a vague principle which was not properly defined and consequently could be adapted to all kinds of explanations. It could be used to explain why a body was transparent or opaque, white or coloured, caustic or non caustic [8].

[2] - Essential oil research was also done during the phlogiston theory period, but a modern chemist will scarcely understand anything from it. Let us consider the following paragraph from a dissertation of Jenea University in 1765 : *The important constituents of an essential oil are of two sorts: solid and liquid. To the solid belong sulphur or phlogiston, earth and salt.*

carbon atoms to eight hydrogen atoms. More than ten years were to pass before any important contribution appears in the literature again in 1833. This was the works of Dumas [9] who developed an analysis method for essential oils which enabled him to divide them into many groups: Those containing only hydrocarbons (turpentine and citrus oil), those containing oxygenated compounds (camphor and aniseed oil), those containing sulphur (mustard oil) and nitrogen bearing ones. A progress in essential oil research was made when chemists began to allow oils to react with hydrochloric acid. Through such processes, terpene hydrochloride were obtained, some of them crystallized and could be further analysed. The disadvantage of this method is, that very often mixtures of three to four products were considered as pure substances. Using this method of analysis, Berthelot [10] studied turpentine oil and isolated l- and d-pinene, l- and d-camphene and also some sesquiterpene and diterpene hydrocarbons. He also differentiated terpenes which could form monohydrochloride from those forming dihydrochlorides. Nevertheless, the first tentative constitution of a terpene was achieved by Kékulé [11] who proposed a structure for cymol. Two years later Bouchardat [12] synthetised dipentene by polymerisation of isoprene, an important achievement in the study of terpenes. The same year Tilden [13] reported the formation of crystalline derivatives by action of nitrosyl chloride on turpentine oil. However nothing was still known about the constitution of terpenes. In 1877, writing on terpenes, Tilden noted : ... *our knowledge of their properties and constitution is at present extremely imperfect, and some method by which they may be identified and classified is very desirable* [14].

The liquid is constituted of air, fire and water. The presence of solid is deduced from the fact that the oil are inflammable, and all that is inflammable contain either sulphur or phlogiston. ... All coloration is due to sulphur or phlogiston... [9].

This difficult work of identification and classification was achieved by Otto Wallach whose work in the field of terpenes were reported in more than one hundred papers. Forming different types of crystalline derivatives of terpenes he isolated pure compounds and elucidated their structures. In 1887 he clarified the relations between 12 different monoterpenes related to pinene [15, 16]. He also determined the constitution of some sesquiterpenes including cadinene, caryophyllene and clovene. He summarised his work in terpene chemistry in a book form in 1909 and proposed the isoprene rule [15, 17]. Wallach's achievements laid the foundation for future development of terpenoid chemistry and were rewarded by the Nobel prize in 1910.

Taking advantage of the results of his great predecessor, Ruzicka reintroduced the isoprene rule and established it as an instrument for structure elucidation [18, 19]. He was also the first who used systematically the direct and severe method of dehydrogenation in structure elucidation of polyterpenes [19, 20]. He determined the basic structure of many sesqui-, di-, and triterpenes. He also elucidated the structure of lanosterol [21]. This achievement was of great importance, since the facts proving the structure of lanosterol also led Ruzicka to formulate the biogenetic isoprene rule [22, 23]. This rule has become instrumental in the metamorphosis of natural product chemistry into bioorganic chemistry, and forging the link between organic chemistry and biology.

In the context of essential oils analysis some chemists tried to perform partial or total synthesis of natural fragrant products. In the late nineteenth century coumarine [24], heliotropin [25], vanillin [26] and ionone [27] were synthesized. This was the beginning of a new era for modern perfumery [28]. Other early successes in this domain are those of Ruzicka with the total synthesis of fenchone and linalool and the partial synthesis of pinene and thujone [29]. All

these efforts ended up with the appearance of synthetic oils in the market. Together with this development in understanding the nature of essential oils, their domain of utilisation spread from perfumery and cosmetic industry to food and drinks and pharmaceutical industry. Another growing user of essential oils is aromatherapy. With this wide spectrum of uses, all in hygiene, nutrition or human health, an accurate knowledge of essential oils is required. Essential oils are often composed of hundreds of chemical compounds, most of them in very small amount. The biological activity, the scent and flavouring properties of an essential oil may depend on one particular component but most of the time on a complex combination of substances. Therefore, in order to judge the quality of an essential oil, one should take into consideration not only major components, but also those of very low concentration [29]. The enantiomeric composition of the oil is also of great importance in this regard.

1.3 Current Trends in Essential Oil Research.

The rapid advances in spectroscopy, mass spectrometry, and separation and isolation techniques have totally changed the picture of chemical study of essential oils. Gas chromatography has proved to be the most suitable method for separation of essential oil components. The introduction of chiral phases based on cyclodextrins has facilitated the study of the enantiomeric composition of essential oils. The combination of gas chromatography and mass spectrometry (GC-MS) allows rapid identification of essential oil components, provided that these compounds are already known and their mass spectra available in a library. With the advances in NMR spectroscopy, most structure determinations have become quite routine. However, some chemical correlations with known compounds are needed for absolute configuration determination. Partial or total synthesis of the compound is often necessary. Some knowledge of molecular biology is becoming necessary for natural product chemists

to plan research directed towards an understanding of natural products biosynthesis and mechanisms of bioactivity triggered by ligand-receptor interactions. Considering the current essential oil research literature one observes that many essential oil components are still unknown. Therefore the traditional approach of isolation and structure dertermination of single natural products is still of some importance in essential oil research, since it permits at least to build up mass spectra libraries used for rapid identification of essential oil components. This fact just confirms the view of Barton and De Mayo in 1957: *Within the field of sesquiterpenoid chemistry one finds a wide range of oxygenated functions, of ring size, and of mechanistic change. If no other type of organic compound were known, organic chemistry would still be a rich and varied field of investigation* [30].

2 The Subject.

> «Es mußten für alle wirklich voneinander verschiedenen Terpene so scharfe und bestimmte Merkmale der Eigenschaften festgestellt werden, daß eine Erkennung und Unterscheidung der chemischen Individuen möglich wurde.»
> (O. Wallach, *Terpene und Campher*, 2nd edition, 1914)

The increasing importance of essential oils in various domains of human activities including pharmacy, perfumery, cosmetics, aromatherapy, and food and drinks industry has created a great need of reliable method of analysis of essential oils. These requirement have been satisfactorily fulfilled by GC and GC-MS techniques. The last one is the most used for rapid identification of essential oils components and demands the availability of a rich mass spectral library. Such a library can be constituted only after a laborious work of isolation and structure elucidation of essential oils components from different sources. This ambitious project has been one among other task of the research group of Prof. König for many years. Essential oils of various origin have been analysed with the aim to isolate and characterise unknown compounds. The enantiomeric composition of many essential oil constituents has also been studied, and for this purpose essential oils from liverworts have played and important role, since liverworts are known to generate compounds of rare configuration. Essential oils from higher plants and certain herbs were also investigated and in this trend that of *Cyperus alopecuroides* was preliminary analysed by GC-MS. Although the

essential oils of many *Cyperus* species have been formerly analysed by other research groups, the presence of many unknown sesquiterpene hydrocarbons could still be observe. The reason may be that major efforts have been devoted only to identify polar constituents. Another reason may be that no previous work has been reported on *Cyperus alopecuroides.* The fact remains that the presence of unknown sesquiterpene hydrocarbons prompted us to continue our investigation of this essential oil.

In order to compare the chemical composition of the essential oil of *C. alopecuroides* with that of other species of the same genus, we investigated two other plants. The first was *C. papyrus* also collected in Cameroon. Although this second plant share many components with *C. alopecuroides*, there were some differences which justified its investigation. The last essential oil to be studied was that of *C. rotundus* which is produced in China in industrial scale. This oil present a great similarity with that of *C. papyrus*, except some unidentified compounds which needed to be isolated for characterisation.

During the investigation of the three oils our task consisted in:

- GC-MS analysis of the essential oil.

- Isolation of these compounds by chromatographic methods.

- Identification of the isolated products.

- Structure elucidation using NMR spectroscopy and chemical tranformations.

3 Methods and Concepts in the Chemistry of Essential Oils.

> «It is not exaggerating to state that, very frequently, the success or failure of studies with bioactive factors depends solely on whether one succeeds in the isolation; especially when the factors exits in minute quantities or is labile. ...Structural elucidation allows us to advance towards clarification of mode of action, etc.»
>
> (K. Nakanishi, in *Preparative Chromatographic Techniques*, Springer-Verlag, 1986).

3.1 The Concept of Essential oil.

The term essential oil is used to refer to highly volatile substance isolated by steam distillation from an odoriferous plant of a single botanical species. The oil bears the name of the plant from which it is derived [31]. This definition based on the volatility and the process of isolation seems to be unsatisfactory, since many other plant metabolites such as fats, coumarins, anthraquinones, and certain alkaloids are also distillable. A more accurate definition has been proposed by Schilcher [32] and Hegnauer [33] : *Essential oils are products or mixtures of products which are formed in cytoplasm and are normally present in form of tiny droplets between cells. They are volatile and aromatic*. From a chemical point of view *essential oils are mixtures of fragrant substances or mixtures of fragrant and odourless substances. A fragrant substance is a chemically pure compound, which is volatile under normal conditions and*

which owing to its odour can be useful to the society [34].

Essential oils are also known as volatile oils in contrast to fatty vegetable and animal oils, and the mineral oils. Thus a drop of essential oil on a piece of cloth or paper disappears between a few minutes or a few days, depending from the temperature, which is not the case for fatty oils. Essential oils are generally colourless to slightly yellowish when freshly distilled. They are only slightly soluble in water and dissolve fairly well in ethanol and mixe very well with vegetable oils, fats and waxes. The odour of essential oils is similar to that of the portion of the plant from which they are derived, although it may be relatively more intensive. Their specific gravity varies from 0.84 to 1.18. According to the use, essential oils may be classified into three broad classes: a) those used for perfumery, soap and cosmetics; b) those used for flavouring foods and beverages; and c) those used for therapeutic purposes. Classification according to geographical origin is also common since superior types of essential oils are produced in specific geographical areas that have favourable soil and climatic conditions.

3.2 Source and Isolation of Essential Oils.

Almost all odoriferous plants contain essential oils. Depending on the type of the plant, various parts of the plant may be used for isolation of essential oils, e.g. fruits, seeds, buds and flowers, leaves, and stems, roots, bark or wood [33]. The raw material from which essential oils are manufactured may be either fresh, partially dehydrated or dried, but flower oils must be fresh. Many methods are used for the isolation of essential oils.

3.2.1 The Modern Distillation Process.

Steam distillation and hydrodistillation are still in use today as the most

important processes for obtaining essential oils from plant sources.

The steam distillation process consist of a distillation vessel containing the plant material. The steam is perculated through the flask with plants from the bottom and the oil evaporates. The emerging mixture of vaporised water and oil moves through a coil usually cooled with running water, were the steam is condensed. The mixture of condensed water and essential oil is collected and separated by decantation or in rare cases, by centrifugation (fig. 1). If necessary, the oil should be freed from dissolved and suspended water by treatment with anhydrous sodium sulphate. This serves to prevent subsequent hydrolysis of esters and other hydrolisable constituents of the oil, hence helping to preserve its odour and properties.

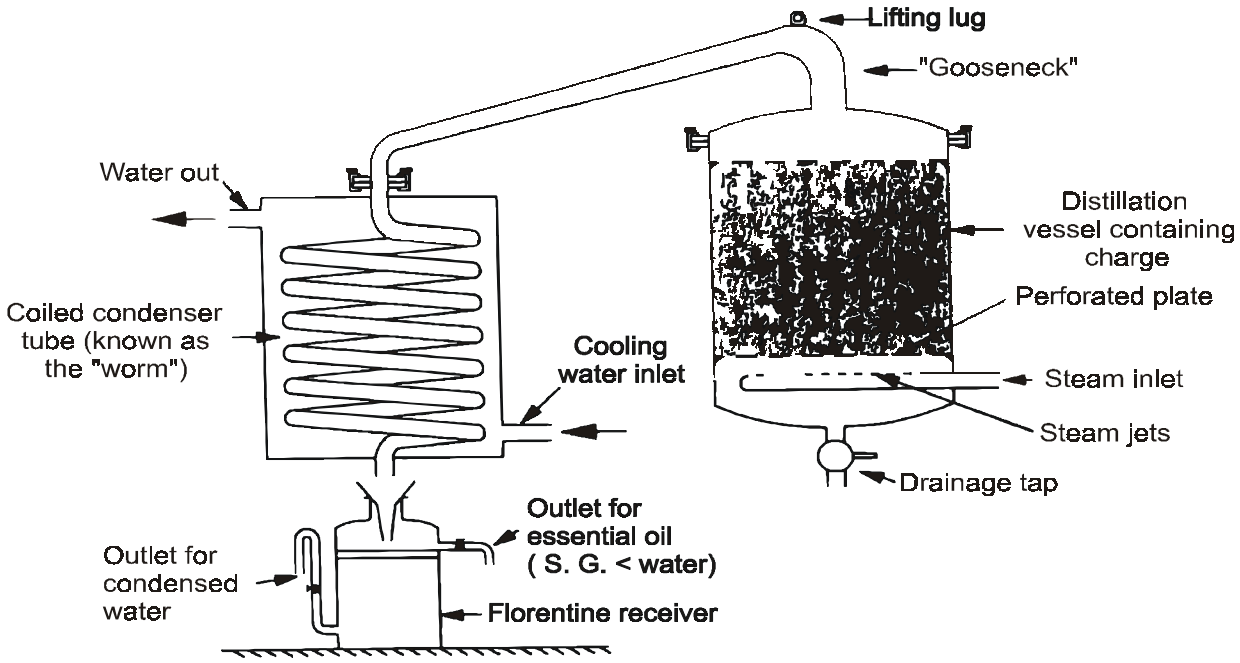


Fig. 1 : Industrial Steam Distillation

In this work, hydrodistillation was used, a process in which the plant material and water are put together in a vessel A (see fig. 2), and the mixture is

allowed to boil. The vaporised mixture of water and essential oil condenses in the trap B and passes through a hexane layer were the oil dissolves and the condensed water returns back into vessel A. The distillation process usually continues for two to three hours (fig. 2).

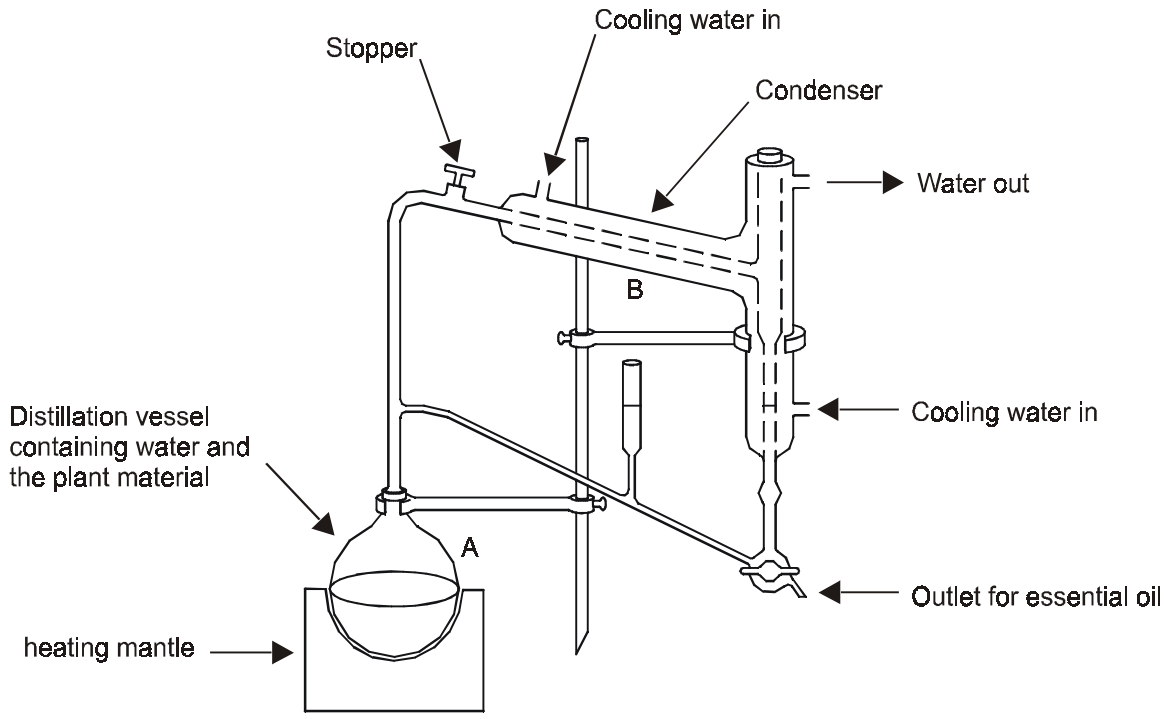


Fig. 2 : Hydrodistillation apparatus.

3.2.2 Maceration and Enfleurage.

These isolation processes are used for delicate flower oils. The flowers are soaked in vegetable oil in a glass jar. After a certain period, the flowers are removed and replaced by fresh buds, where upon further absorption of essential oils into the fat takes place. This process is continued and enfleurage pomade of highly fragrant odour is obtained. The pomade is now repeatedly extracted with alcohol. The alcoholic solutions are then chilled allowing the precipitation of flower wax, which is removed by filtration. The filtrate is submitted to

gentle vacuum distillation to recover the alcohol leaving a residue of enfleurage absolute.

3.2.3 Extraction with Solvent.

This technique is used in order to increase yield of oil, or to extract products that cannot be obtained by any other processes. The plants are immersed in a suitable solvent and the separation is performed by distillation at special temperatures that condense the oil but not the solvent. The products of such solvent extraction are called resinoids or concretes.

Resinoids are products of hydrocarbon extraction of essential oil-bearing resinous plant exudates, such as *bezoin (Styrax tonkinensis)*, *myrrh (Commiphora molmol)* and *labdanum (Cistus ladaniferus)*.

Concretes are liquid, semi-liquid or solid materials, which contain up to and in some cases more than 50 % of odourless fat or wax, together with varying amounts of natural pigment. The treatment of concretes with ethanol affords absolutes [31].

3.2.4 Extraction by Cold Pressing.

The isolation of essential oils by cold pressing or expression is applied only for citrus oils. The outer peel of the fruits is squeezed in presses, and the oil is decanted or centrifuged to separate it from water and cell debris.

3.3 Chemical Analysis of Essential Oils.

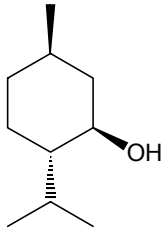
Many methods have been used for studying of the chemical composition of essential oil: IR-spectroscopy, UV-spectroscopy, NMR spectroscopy and gas chromatography [29, 36]. After its introduction as method for the separation of

volatile substances by James and Martin [37], gas chromatography rapidly proved to be an efficient method for the analysis of essential oils [38]. Gas chromatography has three main advantages over other analytical methods; it is very rapid, it has a very high separation capacity and also a very great sensitivity. These may be the reasons which explain the great preference for gas chromatography in essential oils analysis. The complexity of the sample often offers a real challenge. Chromatographic separations allow the quantification of specific compounds that may be indicative of positive or negative quality notes in an oil. Hence it is also a powerful method of detection of clandestine additives such as antioxidants or less expensive components used as diluents. The complexity of many essential oils is such that no single column is able to completely resolve all components. Polymethylsiloxane columns are usually preferred over polar stationary phases for both their longer-lifetime and separation capacity.

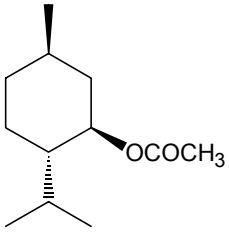
The introduction of highly selective hydrophobic cyclodextrin[3] derivatives as chiral stationary stationary phases brought about the possibility to investigate the

[3] Cyclodextrins are cyclic α-(1 → 4) connected glucose oligomers. According to the number of glucose units three types of cyclodextrins are known: α-, β-, and γ-cyclodextrin corresponding to 6, 7, and 8 glucose units, respectively. They have a torus-shape geometry and the specific dimensions of their cavity are known (see fig. 4). The 6-hydroxy groups are located at the narrow entrance of the cavity while the 2- and 3-hydroxy groups are at the wider opening. The global conformation of cyclodextrins is determined by the α-(1 → 4) -connected glucose units in their $^4C_1$-conformation and the stabilisation is assured by the intramolecular hydrogen bonding forces between the 2- and 3-hydroxy groups. The outer and inner surface of the cyclodextrins display opposite properties which give to this group of compounds its fascinating application possibilities. The outer surface is hydrophilic while the interior cavity is hydrophobic and thus favours the selective inclusion and trapping of non-polar guest compounds. Due to the reactivity of the 2-, 3-, and 6-hydroxy groups, cyclodextrins can be easily O-alkylated and -acetylated. Such derivatizations notably change the macrocyclic conformation of cyclodextrins resulting in a compression of the cavity. This may explain the great difference in enantioselectivity caused by slight changes in the substitution patterns. Capillary columns with cylodextrin derivatives are obtained by coating of the chiral stationary phase onto the glass surface. To expand the temperature range of operation of cyclodextrin derivatives, addition of polysiloxanes OV 1701 has been found advantageous.

enantiomeric composition of chiral constituents of essential oils [39, 40]. Many essential oils such as eucalyptus oil and peppermint oil are used as additives in pharmaceutical preparations. They are used not only for their flavour and fragrant properties but also for their biological activity. Stereochemistry is known to play an important role in biological [41] as well as fragrant [42] properties of molecules.



(-) - Menthol (**1**)



(-)- Menthyl acetate (**2**)

(-)-Menthol (**1**) the main component of peppermint oil is an important ingredient of pharmaceutical formulations for treatment of respiratory infections. Enantioselective gas chromatography allows the investigation of the enantiomeric purity of this product [43]. Moreover enantioselective gas chromatography enables the investigation of the enantiomeric composition of chiral plant volatiles, which may be a fingerprint for the characterisation of essential oils with respect to provenance, phenotype variations and authenticity [44, 45]. It has been proved for instance that the presence of (+)-menthyl acetate in peppermint oil (where the (-)-enantiomer (**2**) is a major product) is an indication of adulteration [46].

3.4 Isolation of Sesquiterpenoids from Essential Oils.

Essential oils are usually very complex mixture of chemical compounds. It has been proved since the beginning of the century that although very complex in composition they are constituted of hydrocarbon and oxygenated terpenoids with 10, 15 and sometimes 20 carbon atoms i. e. monototerpenes, sesquiterpenes and diterpenes. Traces or great amounts of fats can be present in plant extracts, depending on the method of production and the origin (plant) of the oil.

Although GC and GC-MS are the best methods in use for quantitative and qualitative analysis of essential oils, many factors should be considered when using GC for the separation of essential oils. Since essential oils are mixtures of products with a wide range of chemical and physical properties, it is usually reasonable to perform some prefractionations before using gas chromatography. Of course complete essential oils are often chromatographed without any preliminary separation.

3.4.1 GC-Separation of Raw Essential Oils.

Gas chromatography is certainly a very rapid method of separation, since no preliminary operations are required. It is also a method of choice when only a very small quantity of oil is available. However it also presents some shortcomings.

a) First of all, there may be incomplete separation of compounds which could be isolated by preliminary functional group separation, e.g. ketone fractions of an oil.

b) When a complete oil is injected in the gas chromatograph, minor constituents may be too diluted for detection, and if larger amounts are introduced, the major constituents will overload the column and these peaks will mask minor constituents.

c) It can also be time-consuming if our aim is the isolation of a minor constituent. Moreover the overloading of the column leads in the long run to a loss in selectivity of the column.

d) Some oils may contain of high boiling material, and if injected in a column, it will take a very long time at higher temperature to get these compounds

out of the column. This results in poor separation since higher boiling components as ketones will be contaminated with fats. Very often part of the high boiling matter remains in the column and may damage it. See the preparative gas chromatogram of *C. papyrus* (fig. 3).

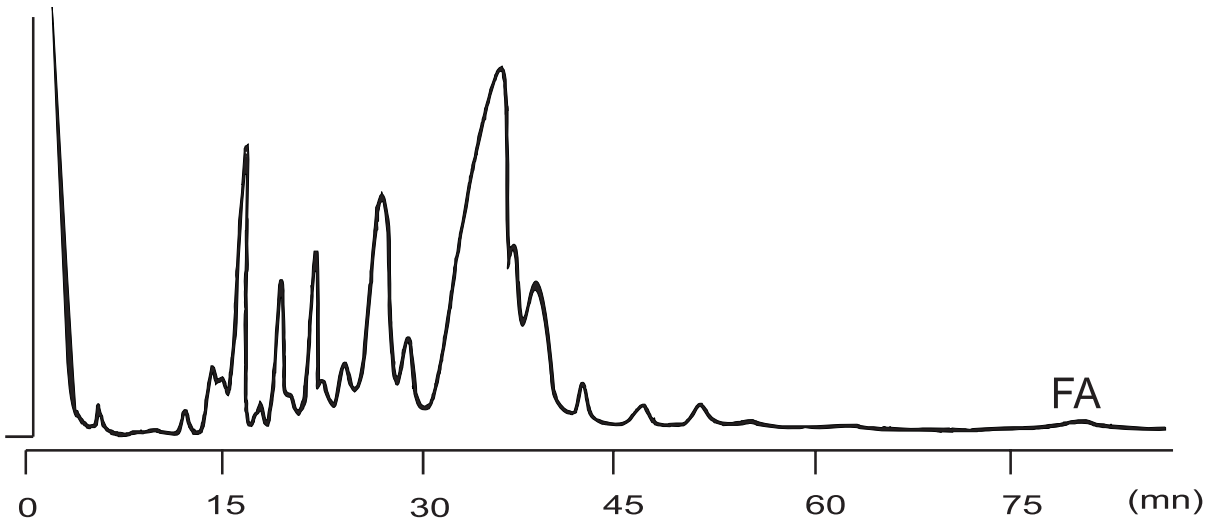


Fig. 3: Gas chromatographic separation of the oil of *Cyperus papyrus*. The column used was coated with a 6T-2,3-Me-β-CD phase. A four ramp temp. program was used. The sample was injected at a temp. of 120° which was kept for ten minutes. The temp. was raised to 140° for fifteen minutes after which it was raised again to 160° and hold for 20 min. The temp. was then raised to 180° for 45 min to allow the fatty acid (FA) to elute from the column.

The above mentioned disadvantages of direct gas chromatographic separation of a complete oil leads to another separation approach.

3.4.2 Combination of GC with other Isolation Techniques.

When greater sample amounts are available, it is undoubtly an advantage to perform some prefractionations of the essential oil before any GC separation. Many methods are used for this purpose: fractionated distillation, dry column flash chromatography and column chromatography at law temperature (-20° to -30° C). Here only the last method was used. Generally, the purpose of a prefractionation is to separate the oil into constituent groups such as

terpenoid hydrocarbons and oxygenated derivatives. If the separation is carefully performed, very good results can be obtained. This means the hydrocarbon part of the oil may be separated in many different fractions and also the oxygenated part.

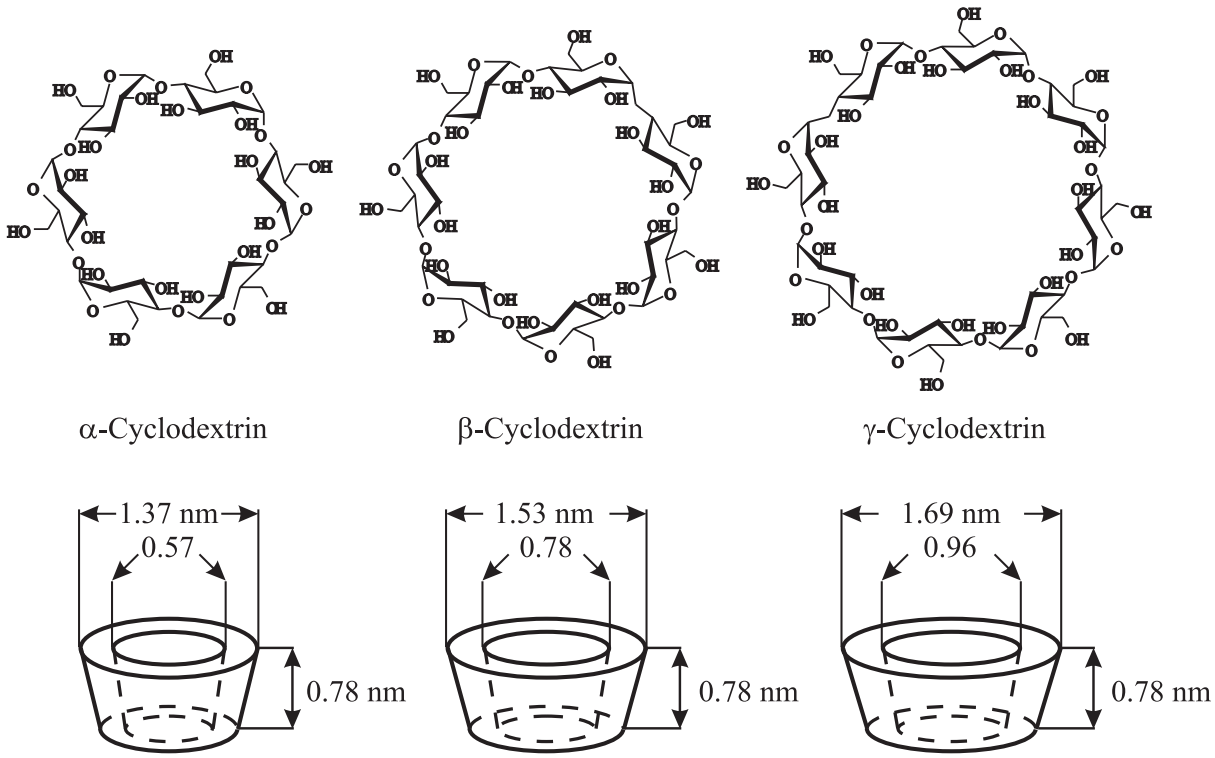


Fig. 4 : Stuctures and dimensions of cyclodextrins.

The subsequent operations depend from the products to be isolated. When very minor components are to be isolated, one may need a second fractionation, using column chromatography over silver nitrate precoated silica gel. The isolation will follow through preparative GC. For some difficult cases, combination of preparative GC with TLC on silver nitrate precoated plates may prove to be successful. In order to use preparative GC to separate a sample, one should first use capillary GC to find out which column (with regard to the stationary phase) is the most appropriate one for the specific purpose. For isolation tasks mostly

modified cyclodextrins as stationary phases were used [47] (fig. 4).

3.5 Terpenes as Chemical Constituents of Essential Oils.

The term *terpene* formerly used to define hydrocarbon isomers of the general formula $C_{10}H_{16}$ contained in turpentine oil was coined by Kekulé in 1866 [48]. Some years later in his efforts to bring order into natural product chemistry of essential oils, Otto Wallach's outstanding works culminated in the proposition of the isoprene rule which was later reintroduced by Ruzicka as a fundamental tool of terpenoid chemistry. The isoprene rule states that terpenes are natural products made up of isoprene units linked together head-to-tail (fig. 5).

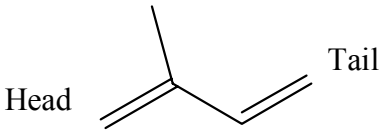


Fig. 5 : Structure of Isoprene (2-methyl-1,3-butadiene).

Further developments in terpenoid chemistry proving that some sesquiterpenes could be obtained by acid catalysed cyclisation of nerolidol or farnesol [49], those dealing with the biogenesis of cholesterol [50], the isolation of many compounds which did not follow the classical isoprene rule, for exemple eremophilone, and mainly Ruzicka's work on the structure of lanosterol [21, 23, 51] constituted the impetus which led him to formulate a more general definition of the isoprene rule. This revised version, which was called the *biogenetic isoprene rule* states: " Terpenes are compounds formed by the combination of isoprene units to aliphatic substances such as geraniol, farnesol, geranylgeraniol, squalene and others of similar kind, and can be derived from these aliphatic precursors by accepted cyclisation, and in certain cases by rearrangement mechanisms" [22, 52]. Taking all these informations into account, Barton and De Mayo proposed that " the best definition of a terpene is that it is a compound whose carbon skeleton is either a) theoritically constructed from isoprenoid units or b) has at some stage in its biogenesis had a carbon skeleton so constructed" [30]. The terpenes are classified according to the number of C_5 units: monoterpenes,

C_{10}; sesquiterpenes, C_{15}; diterpenes, C_{20}; sesterpenes, C_{25}; triterpenes, C_{30}; and tetraterpenes, C_{40}.

Essential oils are complex mixtures containing among others volatile terpenes, which means they are basically constituted of monoterpenes, sesquiterpenes and in some cases of diterpenes. The major group of terpenoids encountered in the essential oils of the *Cyperaceae* are sesquiterpenes.

3.6 Sesquiterpenes.

Sesquiterpenes are for the great majority compounds built up by three isoprene units linked to each other head-to-tail. The isopropyl group of the 2-methylbutane is the head and the ethyl group constitutes the tail. The number of conceivable cyclisations and secondary modifications within the group of sesquiterpenes is impressive. More than 300 hydrocarbon sesquiterpenes have been isolated from plant extracts of diverse origin [53]. They are divided into groups of molecules with the same skeleton (fig. 6).

3.7 Biosynthesis of Sesquiterpenes.

It was said above that the early proposal of Wallach [17] and Ruzicka [51, 52] that terpenes are build up of isoprene units ultimately found mechanistic expression in the proposal by Ruzicka in 1953 [22] of the Biogenetic Isoprene Rule. This was probably one of the most significant contributions in natural product chemistry, since its formulation marked the beginning of the modern era of isoprenoid biosynthetic study. Contemporaneous with Ruzicka's proposal Bloch and Lynen [54] investigated the biosynthesis of cholesterol. These studies led to the isolation and identification of isopentenyl diphosphate (IDP) which become known as the fundamental building block of all isoprenoid natural products.

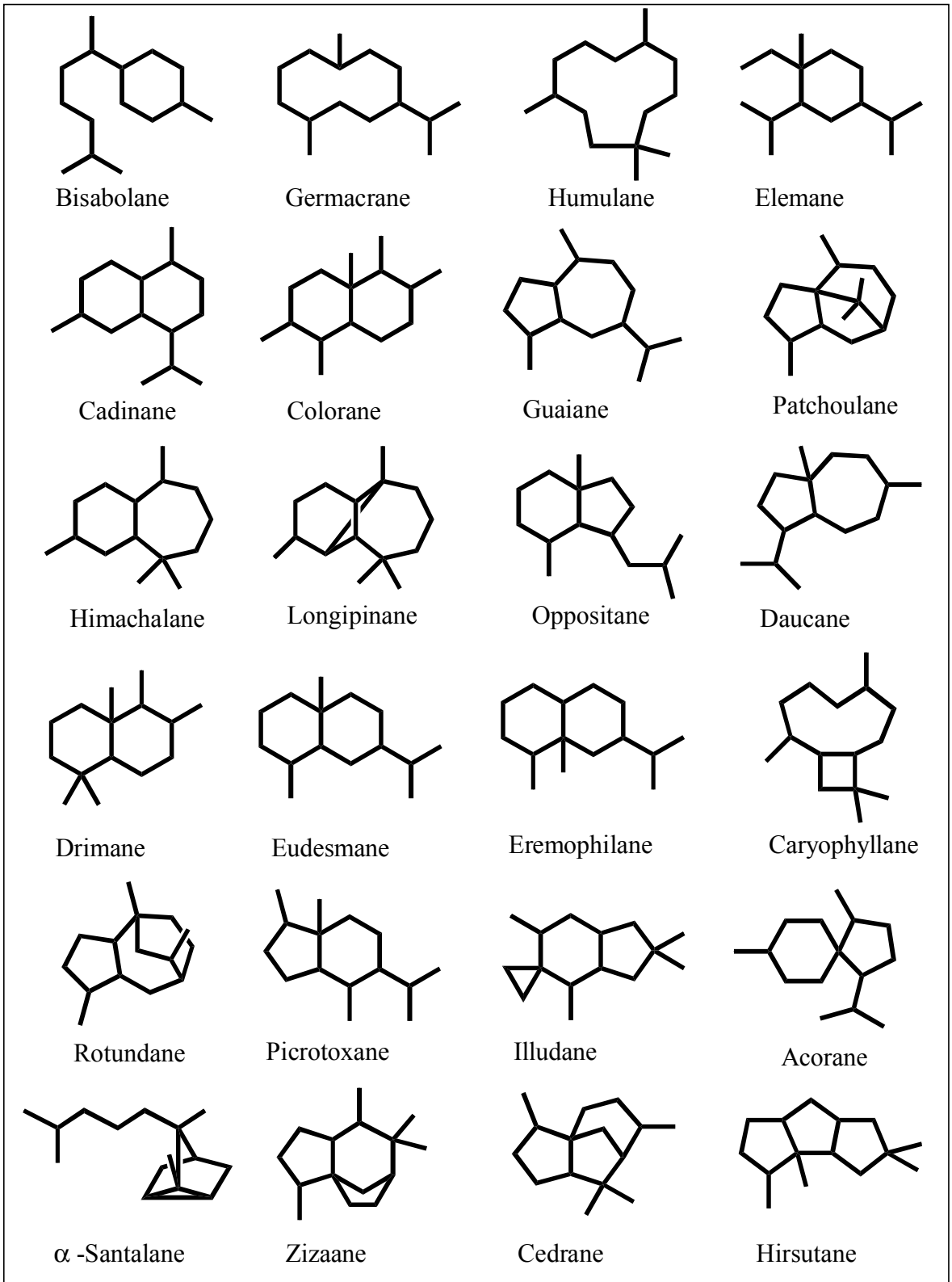


Fig. 6: Selection of Sesquiterpenoid Skeletons.

3.7.1 Biosynthesis of Isopentenyl Diophosphate (IDP).

The discovery that through an enzymatic process mevalonic acid could be converted to squalene [55] definitively established mevalonic acid as the natural precursor of IDP[4]. Acetyl Coenzyme A (acetyl-CoA) was known since 1951 to be the biogenetic precursor of mevalonic acid [56]. The first step in the biosynthesis of mevalonic acid is a biological Claisen condensation of two molecules of acetyl-CoA which yields acetoacetylCo-A. The subsequent formation of hydroxymethylglutaryl-CoA (HMG-CoA) is mechanistically analogous, involving nucleophilic attack of the enolate of an acetyl-S-enzyme derived from acetyl-CoA on the β-ketone moiety of acetoacetyl-CoA. Mevalonic acid is obtained from HMG-CoA by a process requiring two molecules of NADPH. Then follows an ATP dependent formation of 5-diphosphomevalonate, and subsequent coupled dehydration and decarboxy-lation allegedly through the intermediacy of 3-phospho-5-diphosphomevalonate gives rise to IDP (fig. 7).

3.7.2 IDP-Isomerase and Prenyltransferase in Chain Elongation.

The isoprenoid chain elongation is catalysed by a family of enzyme known as prenyl transferases. The discovery [58] of the isopentenyl diphosphate isomerase (IDP isomerase) that catalyses a crucial activation step in the beginning of isoprenoid biosynthesis by converting IDP to its allylic isomer dimethylallyl diphosphate (DMADP) (fig. 8) led to the recognition that Ruzicka's hypothetical

---

4 Mevalonic acid has long been assumed to be the natural precursor for IDP. However, the investigation of the biosynthesis of triterpenoids of the hopanoid series from the bacteria *Zymomonas mobolis* led Rohmer and collaborators [57] to the discovery of a non-mevalonic pathway in which IDP is formed via the glyceraldehyde phosphate-pyruvate pathway. Posibly many other terpenoids are biosynthesised via a non-MVA route.

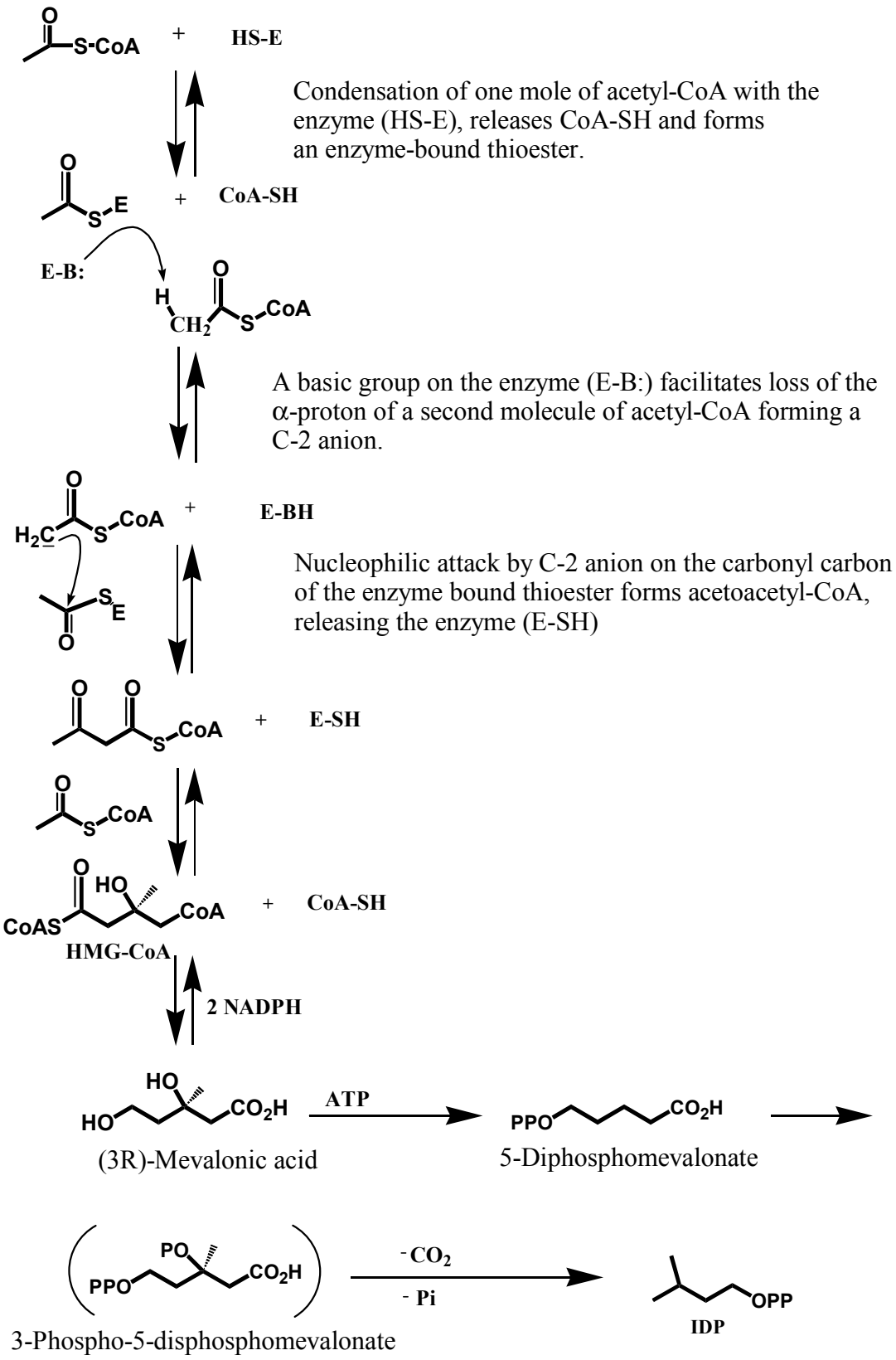


Fig.7: Biosynthesis of Isopentenyl Diphosphate (IDP).

active isoprene [51] is the true biological building block for the astonishing number of isoprenoids in nature. The fundamental chain elongation reaction involves the ionization of an allylic diphosphate substrate and electrophilic attack of the resulting cation on the terminal methylene double bond of the cosubstrate IDP. Deprotonation of the resultant carbocation gives an allylic diphosphate product that has been extended by one isoprene unit. Depending on the particular prenyltransferase, the product itself may be released from the enzyme surface or may function as substrate for one or more additional condensations with isopentenyl diphosphate, leading to the formation of linear isoprenoids with 2, 3, 4 or more isoprene units, up to the million or more that are found in rubber. More than sixteen prenyltranferases with different catalytic functions have been characterised [59].

3.7.3 Biosynthesis of Farnesyl Diphosphate (FDP).

A key-step in the biosynthesis of *trans,trans*-farnesyl diphosphate is the isomerisation of isopentenyl diphosphate (IDP) to dimethylallyl diphosphate (DMADP) (fig. 8). Then follows the IDP chain elongation catalysed by farnesyl diphosphate transferase [60, 61] (FDP transferase). The electropilic CH_2 group of DMADP reacts with the nucleophilic methylene group of IDP and gives geranyl diphosphate which reacts itself with an equivalent of IDP to yield farnesyl diphosphate (fig. 8).

The stereochemistry of the FDP synthesis has been established by Cornforth and Pojack in the course of their study of the biosynthesis of cholesterol [62, 63]. It was been shown that the displacement of the diphosphate moiety from C-1 of both the allylic substrates DMADP and GDP takes place with and inversion of configuration. This result was interpreted in terms of a S_{N2} reaction, implying that the breaking of the C-O diphosphate bond is synchronous with the

formation of the new C-C bond. It was also proved that the electrophilic addition takes place exclusively on the *re* face of the IDP double bond with stereospecific loss of the 2*re* proton, thereby establishing that the formal S_E reaction occurs with net syn stereochemistry [62] (fig. 8).

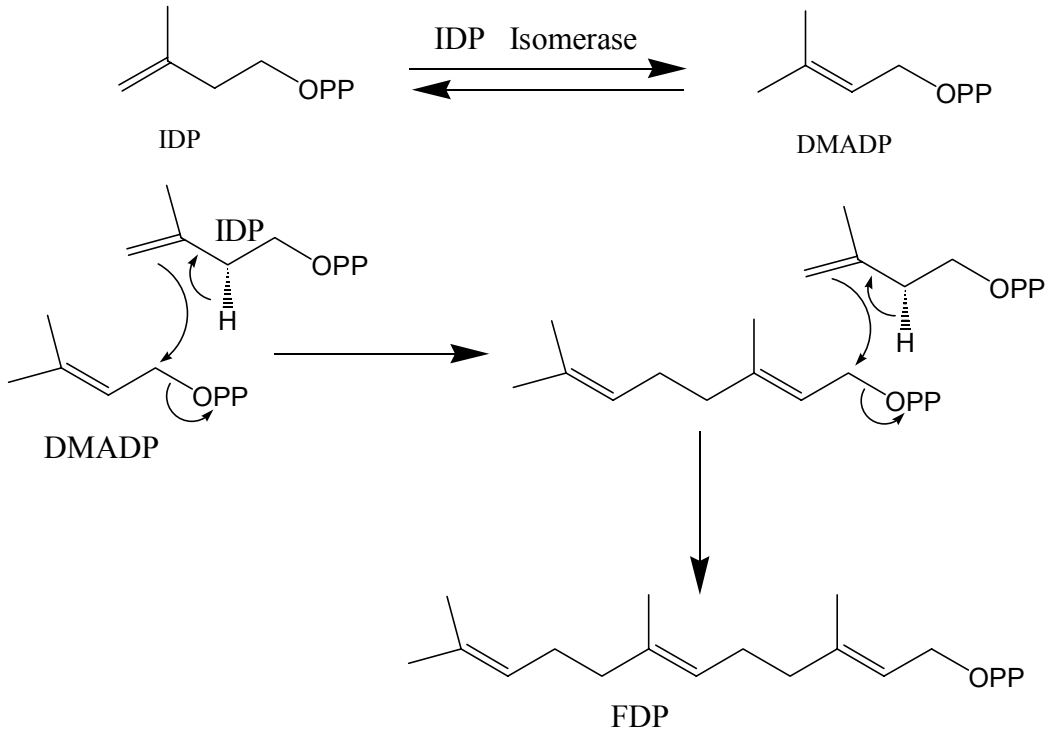


Fig. 8: Biosynthesis of farnesyl diphosphate (FDP).

3.7.4 Sesquiterpene Synthases.

As formulated by Ruzicka's biogenetic isoprene rule, the formation of all cyclic sesquiterpenes can be accounted for by biochemical transformation of farnesyl diphosphate. Natural catalysts mediating these cyclisations are known as sesquiterpene synthases. Up to now, only a small fraction of this family of enzymes has been investigated. However several generalisations have already arised. Sesquiterpene synthases are operationally protein monomers or homodimers of subunit molecular weight of *ca* 40 000 - 70 000. These enzymes,

which are moderatly lipophilic, require no cofactors other than a divalent metal ion, Mg^{2+} almost always being encountered. The apparent Michaelis Constant[5] K_M values for the acyclic substrate, FDP, are generally in the range 0.1 - 10 μM. The isolation of sesquiterpene synthases is difficult, since many appear to be present at relatively low titres in the host organism [64].

3.7.5 Cyclisation of Farnesyl Diphosphate.

It is admitted that the first step in the enzymatic formation of sesquiterpenes is the ionization of *trans,trans* farnesyl diphosphate to the corresponding transoid allylic cation - diphosphate anion pair (fig. 9). The following step consists in electrophilic attack of the allylic cation and subsequent cationic transformations involving further cyclisations and rearrangements, including methyl migrations and hydride shifts, culminated by quenching of the positive charge by loss of a proton or capture of an external nucleophile such as water or the original diphosphate anion. It was recognized early that the direct formation of six-membered rings from the trans allylic disphophate precursor is geometrically impossible. This observation led to the discovery that the geometrical barrier is overcome by initial isomerization of *trans,trans*-FDP to the corresponding tertiary allylic isomer, nerolidyl diphosphate (NDP), which has the necessary conformational flexibility and the required reactivity to allow the cyclisation (fig. 9). Moreover, the cyclisation of FDP or NDP can only occur if the π-orbitals of the relevant double bonds are properly aligned so as to achieve the required geometry for interaction. This condition is met only if the

[5] K_M has a simple operational definition. It can be demonstrated that K_M is the substrate concentration at which the velocity of the enzymatic reaction is half-maximal. Therefore, if an enzyme has a small K_M value, it achieves maximal catalytic efficiency at low substrate concentration.

individual double bonds of the allylic substrate are mutually perpendicular to a common plane. Only a small number of conformations satisfy this condition [65].

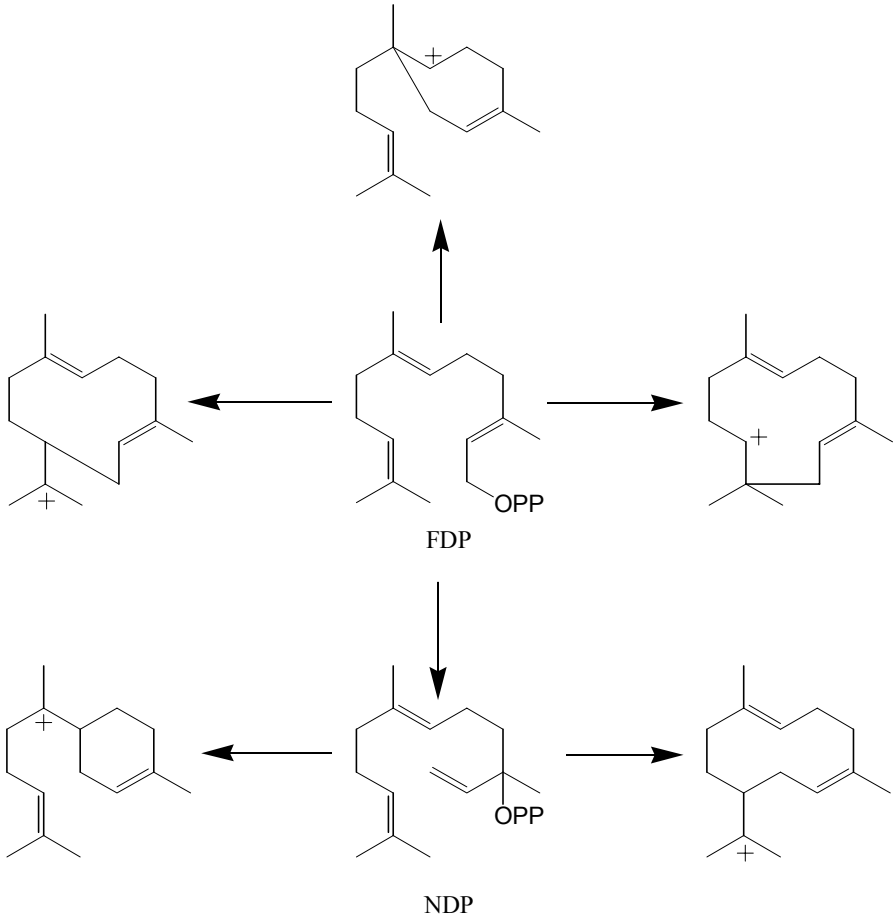


Fig. 9: Cyclisation of farnesyl diphosphate.

3.7.6 Biosynthesis of some Sesquiterpenes of the Cyperaceae.

3.7.6.1 Biosynthesis of Caryophyllene and Humulene.

Humulene and caryophyllene are a type of sesquiterpenes encountered in many essential oil, including that of *C. alopecuroides*. Croteau and collaborators have studied the enzymatic conversion of [1-^{3}H]-FDP to humulene and β-caryophyllene [66]. The process begins with cylisation of FDP to the humulyl cation (**3**) which can either lose a proton from C-9 to form humulene (**4**)

directly, or further cyclise by electrophylic attack on the 2,3-double bond followed by loss of a proton to generate (-)-caryophyllene (**5**) (fig.10).

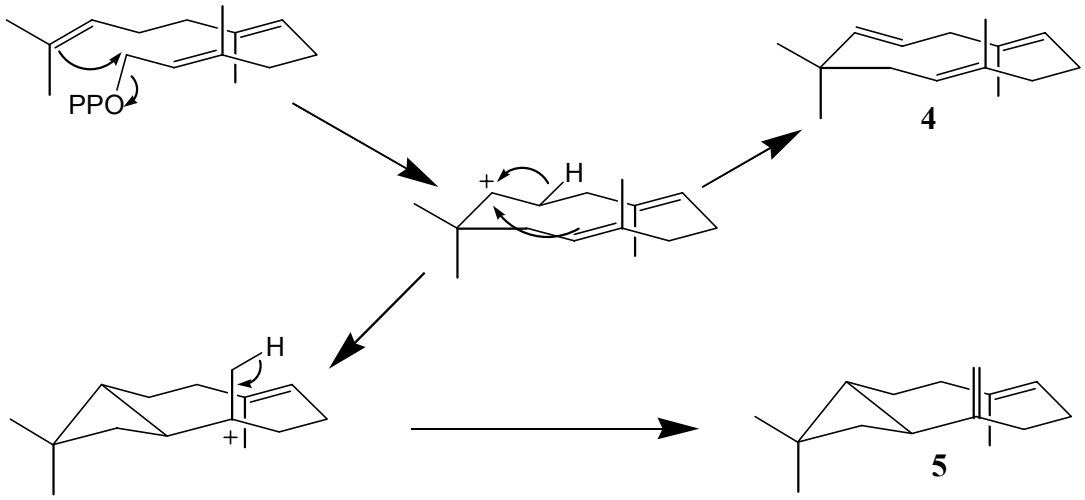


Fig. 10: Biosynthesis of caryophyllene (**5**) and humulene (**4**).

3.7.6.2 Biosynthesis of β-Selinene.

β-Selinene occurs in many essential oils and was also identified in the three investigated *cyperus* species. Although no study of its formation has as yet been published, it is believed that the bicyclic skeleton may be formed by cyclisation of FDP to eudesmane cation (**7**) via germacrene A (**6**). A subsequent deprotonation leads to β-selinene (**8**) (fig. 11).

3.7.6.3 Biosynthesis of δ-Cadinene.

δ-Cadinene is a sesquiterpene hydrocarbone occurring in many essential oils and was also identified it in *C. rotundus* . Its biosynthesis has been studied by Benedict and collaborators [67]. Its formation is believed to start by isomerisation of FDP to nerolidyl diphosphate NDP, followed by cyclisation to the *cis*, *trans*-helminthogermacradienyl cation (**9**). A 1,3-hydride shift leads to the corresponding cation, which upon further cyclisation and subsequent

deprotonation yields δ-cadinene (**10**) (fig. 12).

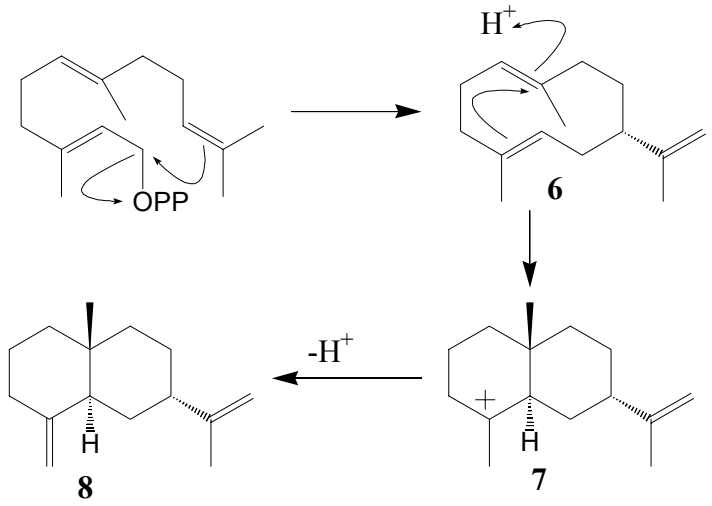


Fig. 11: Biogenesis of β-selinene (**8**).

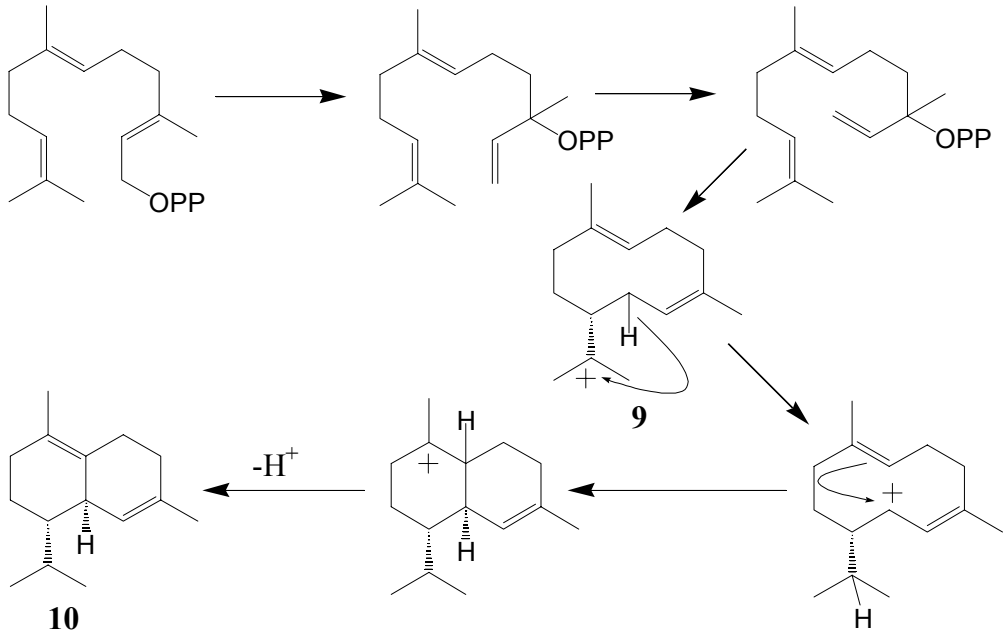
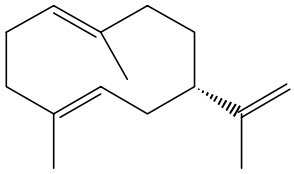


Fig. 12: Biosynthesis of δ-Cadinene (**10**).

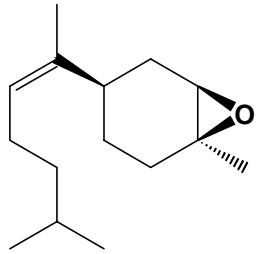
3.8 Functions and Utilities of Sesquiterpenoids.

The isolation and identification of sesquiterpenoids has raised some other questions. Scientist are interested to know why plants, insects, and fungi produce sesquiterpenoids as secondary metabolites. Some sesquiterpenes play

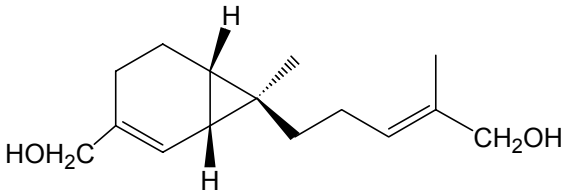
a role as pheromones, i. e. they are responsible for the communication between individuals of the same species. Often they serve as attractants, thus facilitating mating, or in the case social insects as guides to food sources; they can also be of importance for the structure of the community in social insects. Sesquiterpenes are also secreted as defense substances to fight possible predators. They have either unpleasant odour, sometimes being lachrimatory, or they are toxic. Others are known as juvenile hormone or they have growth-inhibitory or growth-regulatory activity. Moreover they may have cytotoxic, antibiotic, antiseptic anthelmintic, sedative, fungistatic or virostatic properties.



(-) - Germacrene A (**11**)



(Z)-α-Bisaboleneoxide (**12**)

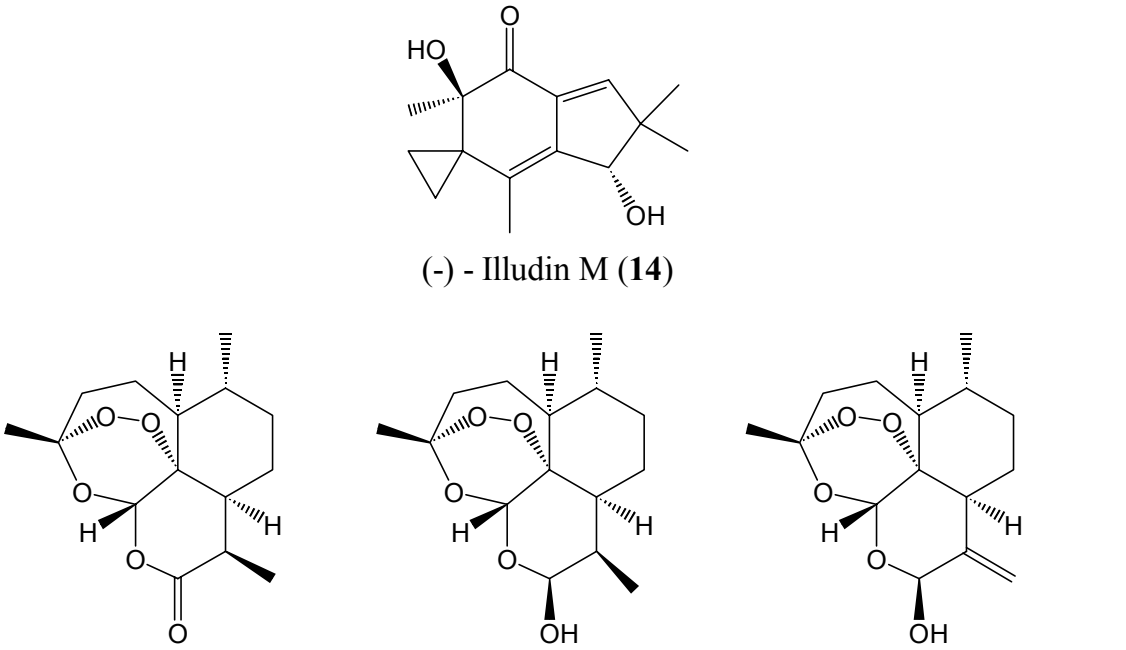


Sirenine (**13**)

Fig. 13: Some Pheromones belonging to the class of Sesquiterpenes.

Therefore sesquiterpenes structures may serve as new prototypes, or templates for synthetic organic chemists to use in the design of potentially superior chemotherapeutic or otherwise biologically active agents. Flavour chemists can also take advantage of sesquiterpenes structures to produce com-pounds with improved fragrance or flavour properties. It has been found that (-)-germacrene A (**11**) is the alarm pheromone of the alfalfa fly *Therioaphis maculata* [68]. (Z)-α-bisaboleneoxide (**12**) is the sexual pheromone of stinking insect of the *Nazara* species [69, 70] (fig. 13). Sirenin (**13**) is also a sex attractant produced by the female of the water mould *Allomyces* [71, 72]. (-)-Illudin M (**14**), isolated from the C*litocybe illudens* has antibacterial and

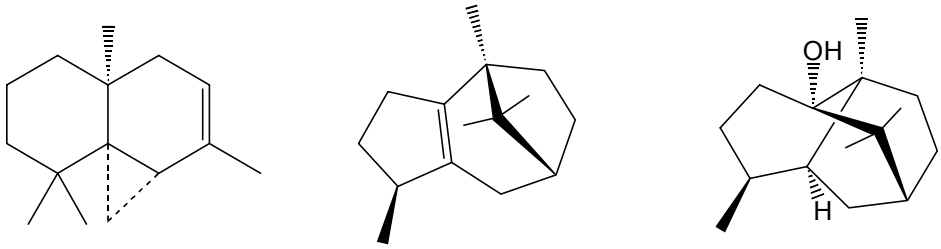
antitumor properties [73, 74]. Some other important molecules which attracted the attention of many medicinal chemists these last years are the antimalarial sesquiterpenoids (+)-artemisinine (**15**), (+)-dihydroqinghoasu (**16**) and (+)-artemisitene (**17**) isolated from the Chinese medicinal plant *Artemisia annua* [75-78] (fig. 14).



(-) - Illudin M (**14**)

Artemisinine (**15**) (+) - Dihydroqinghaosu (**16**) (+) - Artemisitene (**17**)

Fig. 14: Some Sesquiterpenes with important biological activity.



(-)-3-Thujopsene (**18**) (-)-β-Patchoulene (**19**) (-)-Patchoulol (**20**)

Fig. 1: Examples of Sesquiterpenes with fragrant properties.

Sesquiterpenes greatly contribute to the odour and the flavour of essential oils.

For example (-)-3-thujopsene (**18**) [79] is one of the most important fragrant component of the cedar wood oil from *Juniperus virginiana*. β-Patchoulene (**19**) [80] and (-)-patchoulol (**20**) [81] are the major constituents of patchouli oil used in the perfumery for its woody-herbal odour (fig. 15).

3.9 Structure Elucidation of Sesquiterpenoids.

The strategy of analysis of an essential oil begins with a GC-MS study, which allows the identification of components of the oil by comparison of mass spectra of the sample with those of a library. For some doubtful cases, one may need to isolate the products and compare their NMR spectra with those of an authentic sample. The enantiomeric composition is determined by enantioselective gas chromatography. An unknown compound needs a complete structure elucidation. Since sesquiterpenes do not usually crystallize, NMR spectroscopy is always the best method for determination of their structure. The interpretation of ^{1}H-NMR-, ^{13}C-NMR-, DEPT-, HMQC-, HMBC-, and NOESY- spectra usually not only provide the constitution of the molecule, but also the relative configuration. For complex cases, one may need more sophisticated techniques such as phase sensitive COSY and phase sensitive gradient selected HMQC, both of which enable the measurement of scalar coupling of overlapping signals.

3.9.1 Mass Spectrometry in Structure Elucidation.

The electron-impact (EI) mass spectra of many hundreds of sesquiterpene hydrocarbons and oxygenated derivatives of known structures are available as mass spectral librairies [53]. The first step in examining a mass spectrum is usually the determination of the molecular ion peak. Knowing the accurate mass of the molecular ion, it is then easy to obtain the molecular formula and from it the number of double bond equivalents of the molecule. It can be helpful as next to note the major fragment ions and attempt to elucidate the main fragmentation

pathways. The best way in checking such fragmentation pathways is to look for metastable ions. Knowing the molecular weight and the main features of fragmentation of a compound, it may be possible to make a tentative structural assignment.

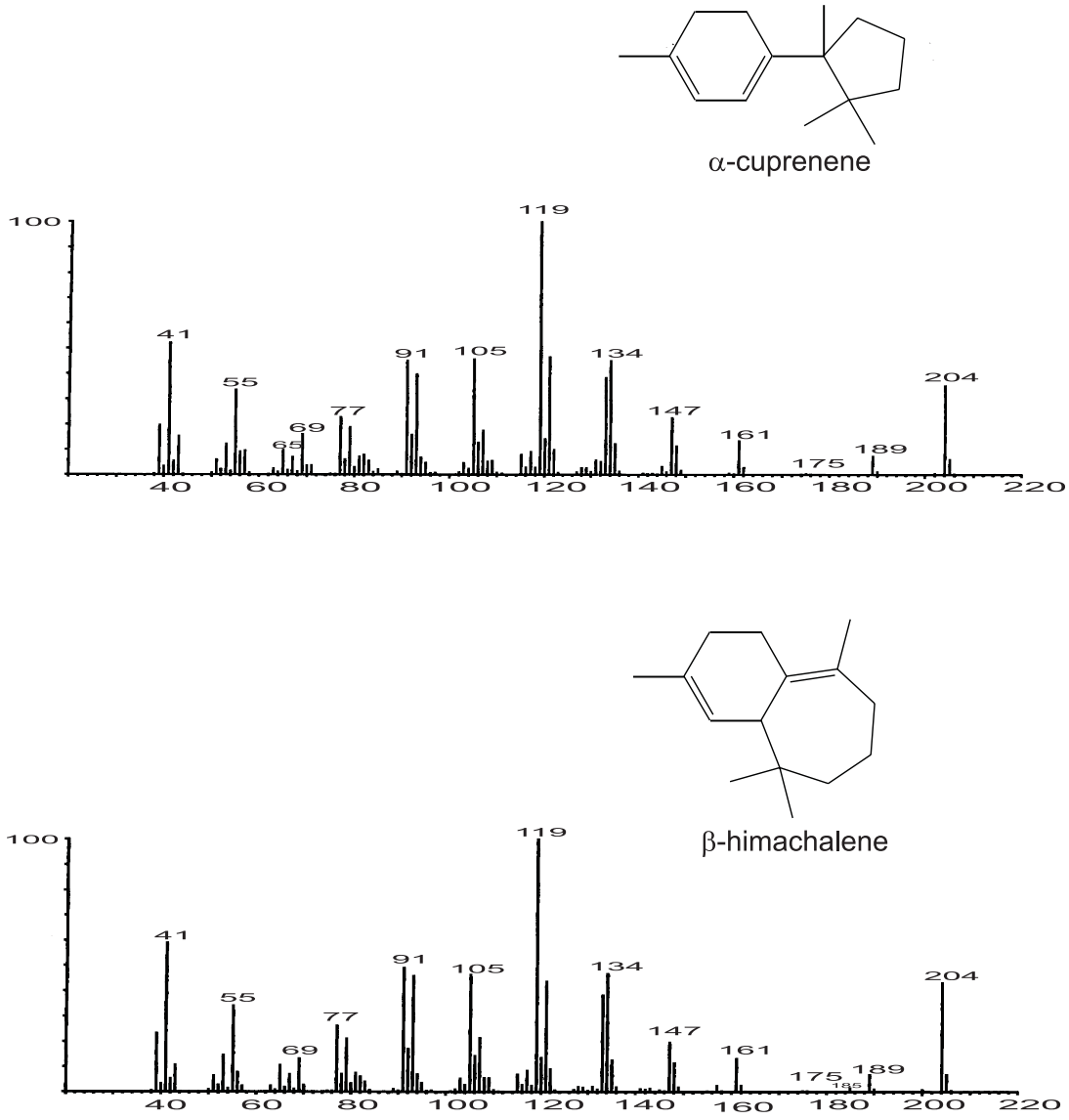


Fig. 16: Mass spectra of α-cuprenene and β-himachalene (EI, 70 eV). Although the two compounds have quite different structures, they exhibit identical mass spectra. This may be explained by initial fragmentation processes as shown in fig. 17.

It has been found that some sesquiterpenes of completly different structures exhibit the same mass spectrum [83], which means that mass spectrometric

fragmentations may not be sufficient in proving a structure. Indeed the primary ionization of two different molecules may lead to the same molecular ion structure and thus result in identical subfragmentations. α-Cuprenene and β-himachalene provide a typical example of such a process (see fig. 16 and 17). Nevertheless, by rapidly disclosing the molecular formula of an unknown compound, mass spectrometry remains a powerful tool in structure elucidation. Its combination with NMR-spectroscopy allows a complete structural assignment.

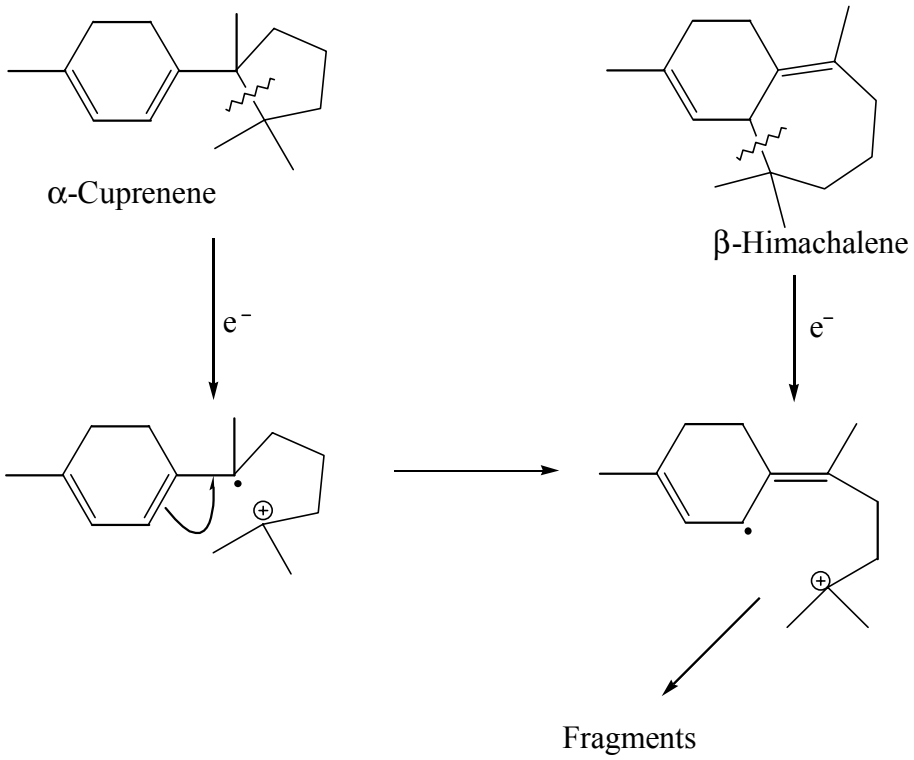


Fig. 17: Ionisation process of α-cuprenene and β-himachalene consecutive to the electron bombardment leading to the same molecular ion structure.

3.9.2 NMR Spectroscopy in Structure Elucidation of Sesquiterpenoids.

NMR-Spectra are spectra of the precession frequence of nuclei with a magnetic moment in a static field. The position of the NMR signal (i. e the resonance frequence) is called the chemical shift δ. The values of the chemical shift δ_H

and δ_C in ^{1}H-NMR and ^{13}C-NMR spectra give informations on partial structures and functional groups contained in the studied molecule. Coupling constants between protons which can be obtained from the fine structure of signals help to identify neighbour protons in building up of partial structures. However, the resolution of one dimensional ^{1}H-NMR spectra is rarely good enough to enable the calculation of all the important coupling constants. Together with the DEPT technique which enables the knowledge of the multiplicity of carbon atoms, two dimensional NMR offer powerful methods for the structure elucidation of natural products [84-88].

3.9.2.1 The Principle of Two-Dimentional NMR Spectroscopy.

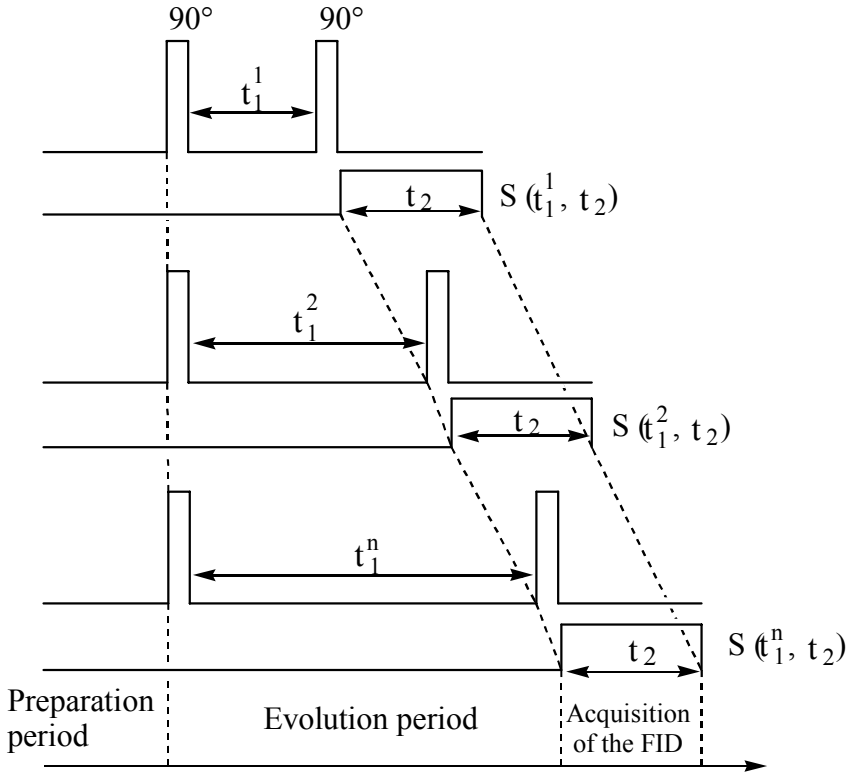


Fig. 18a : Schematic representation series of pulse sequences involved in obtaining a two-dimensional NMR spectrum. Many FIDs are recorded with incremented values of the evolution time t_1 and stored.

Two-dimensional NMR spectroscopy is a spectral method in which the data are collected in two different time domains: acquisition of the FID (t_2), and successively incremented delay or evolution time (t_1). The resulting FIDs are accordingly subjected to two successive sets of Fourier transformations to furnish a two dimensional NMR spectrum. Let us consider the pulse sequence of a 2D NMR experiment (fig. 18a). This experiment can be described by the behaviour of the magnetisation vector M (fig. 18b).

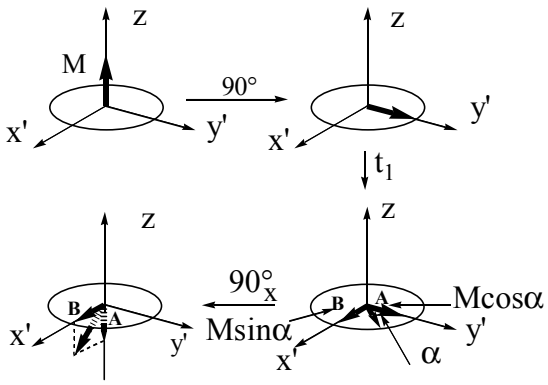


Fig. 18b : Scheme of the behaviour of the magnetisation vector under the effect of the pulse sequence.

The preparation period allows the system to return back to its equilibrium state. The $90_x°$ pulse flips up the magnetisation into the x'y'-plane. During the following period of time t_1, the magnetisation will rotate in the x'y'-plane. After a certain period of time (t_1), the magnetisation would have moved through an angle α, which is proportional to t_1 ($\alpha = 2\pi\nu t_1$). M may be considered as the sum of two vectors : M_x aligned along the x'-axis with the magnitude $M\sin(2\pi\nu t_1)$ and M_y aligned along the y'-axis with the magnitude $M\cos(2\pi\nu t_1)$. The second $90_x°$ pulse at the end of the evolution period rotates the component M_y aligned along the y'-axis onto the z'-axis, leaving behind the last component M_x in the x'y'-plane. The magnitude of both components M_x and M_y will also be reduced to a certain extent because of the relaxation process occuring during t_1. The component M_x which remains in the x'y'-plane after the second $90_x°$ pulse is detected as transverse magnetisation. When the FID is recorded immediatly after the second $90_x°$ pulse, the transverse magnetisation is detected at its

maximum amplitude. However the transverse magnetisation decreases with the time as the magnetisation vector moves away from the y'-axis in the x'y'-plane. It is therefore possible to perform the experiment with discrete changes in the evolution time t_1 and to record many FIDs as a function of t_1.

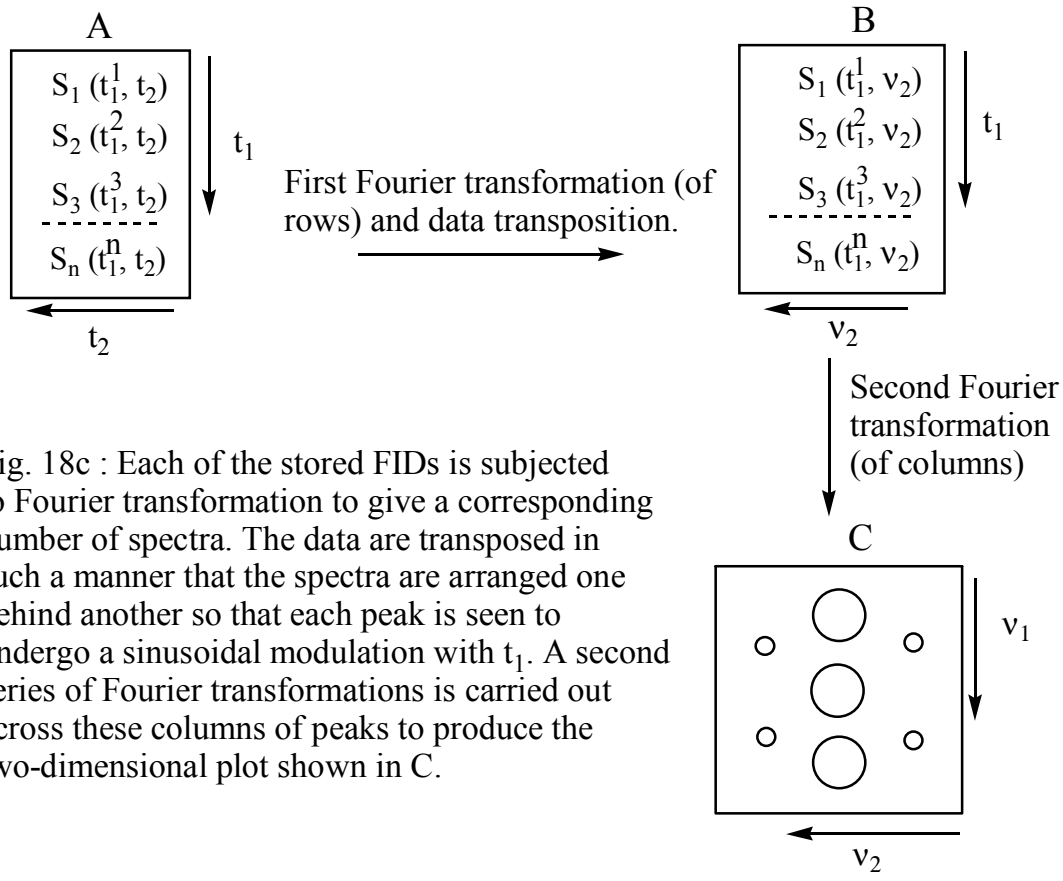


Fig. 18c : Each of the stored FIDs is subjected to Fourier transformation to give a corresponding number of spectra. The data are transposed in such a manner that the spectra are arranged one behind another so that each peak is seen to undergo a sinusoidal modulation with t_1. A second series of Fourier transformations is carried out across these columns of peaks to produce the two-dimensional plot shown in C.

Fourier transformation of each of these FIDs will yield the corresponding 1D NMR spectrum, with the peak appearing at frequency ν_1, except that the amplitude of the peak will oscillate sinusoidally according to $\sin(2\pi\nu t_1)$ in the various spectra obtained. If through the data transposition process the "one-dimensional" spectra are arranged in rows one behind another, and the peak viewed along a column (at 90° to the rows), then the sinusoidally oscillating peak gives the appearance of a pseudo FID, with sinusoidal variation of the peak intensity being evident with respect to the vertical axis (i. e. along t_1). The

second Fourier transformation of this FID with respect to t_1 then generates a two-dimensional NMR spectrum (fig. 18c).

3.9.2.2 Homonuclear Shift-Correlation Spectroscopy (COSY).

The Jeener's experiment (^{1}H-^{1}H-COSY) is the easiest alternative to the old homonuclear proton spin decoupling experiments which were used for determining proton-proton connectivities. The pulse sequence leading to proton-proton COSY spectra is given in fig. 19. In the obtained two-dimensional spectrum, both axes describe the chemical shift of the coupled nuclei. The diagonal signals are those of the normal one-dimensional spectrum and the cross-peaks (off diagonal peaks) tell us which protons are coupled to each other.

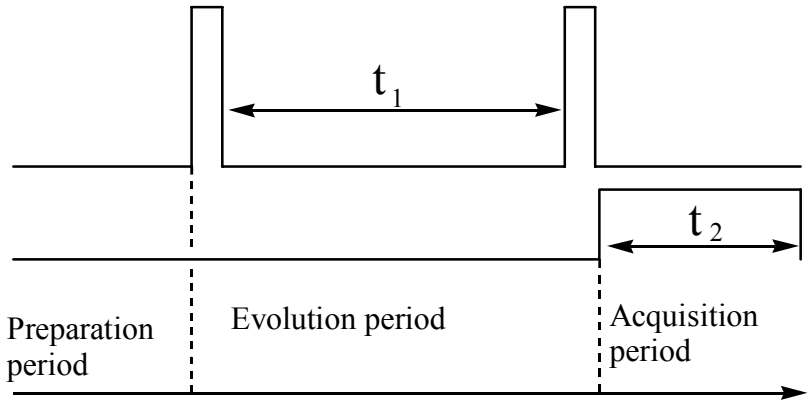


Fig. 19 : The basic COSY pulse sequence : The time axis is divided into a preparation period during which nuclei are allowed to reach an equilibrium state, an evolution period t_1 during which the nuclei interact with each other and finally the magnetisation is detected during the acquisition period t_2.

3.9.2.3 Nuclear Overhauser Spectroscopy (NOESY).

The nuclear Overhauser effect was originally defined as a change in the integrated NMR absorption of a nuclear spin when the NMR absorption of another spin is saturated. Such effects occur when nuclei interact with each other

through space (dipolar or magnetic coupling). These effects, known as nuclear Overhauser effect can only exist for short-range distance (< 5 Å) through space connectivities. This discovery first led to nOe Difference spectra. Later, with the advent of two-dimensional NMR spectroscopy, a slight modification of the proton-proton COSY pulse sequence helped to build up the NOESY pulse sequence (fig. 20) which has a great deal of advantages over the one-dimensional nOe experiments. In the NOESY experiment, all interproton nOe effects appear simultaneously and the spectral overlap is minimised due the spread of spectrum in two dimensions.

In the NOESY spectrum, diagonal signals correspond to the normal one-dimensional proton NMR spectrum, while the off-diagonal cross-peaks represent the nOe interactions between various nuclei.

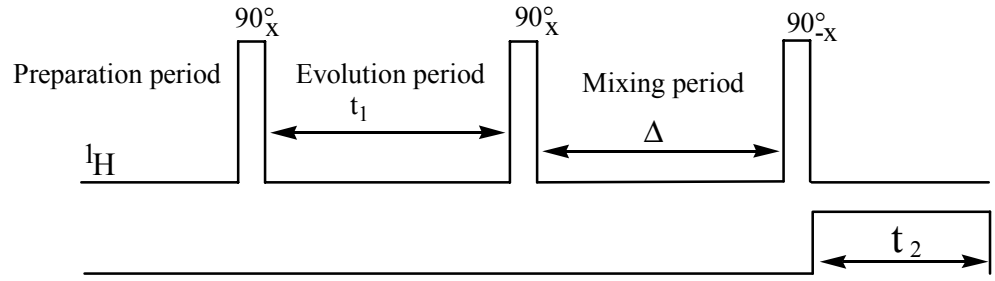


Fig. 20 : NOESY pulse sequence.

3.9.2.4 Heteronuclear Multiple Quantum-Coherence (HMQC).

HMQC is a class of pulse sequences for heteronuclear correlation with detection of protons. It is a highly sensitive procedure for determination of one-bond proton-carbon shift correlations. The HMQC experiment is 16 times more sensitive for ^{13}C than the conventional heteronuclear shift correlated experiment

(^{1}H-^{13}C-COSY). The sensitivity advantage in this experiment is due to the fact that it relies on the equilibrium magnetisations derived from protons. Since this magnetisation is proportional to the larger population difference in proton energy levels (rather than the smaller population difference of ^{13}C energy levels), a stronger NMR signal is obtained. Moreover since the strength of the NMR signal increases with the frequency of observation, a larger signal will be obtained at higher proton observation frequencies than the at the lower ^{13}C observation frequencies (Fig. 21).

3.9.2.5 Heteronuclear Multiple-Bond Correlation (HMBC).

The HMBC technique is a sensitive method for determination of long-range (two- and three-bond) ^{1}H-heteronuclear connectivity, i.e. ^{1}H-^{13}C or ^{1}H-^{15}N connectivity. The pulse scheme of the HMBC experiment is represented in fig. 22. The first 90° (^{13}C) pulse is a low pass J filter, i.e. it suppresses one-bond correlations in the two-dimentional spectrum and allows long-range correlations with smaller J to pass. The second 90° (^{13}C) pulse creates ^{1}H-^{13}C multiple-quantum coherence for the long-range connectivities. The 180° proton pulse effectively removes the effect of the proton shifts from the modulation frequency by interchanging the zero- and double-quantum components. Thus after the final 90° (^{13}C) pulse, the H signals that originate from ^{1}H-^{13}C multiple-quantum coherence are modulated by ^{13}C chemical shifts and homonuclear proton couplings. Signals from protons which do not have a long-range coupling to ^{13}C are removed by phase cycling[6] of the second 90° (^{13}C) pulse (fig. 22).

6 The term phase cycling refers to means by which unwanted signals in an NMR experiment are separated on the basis of their characteristic phase properties, since it is possible to refer to a given class of signals with a given phase with respect to the applied irradiation.

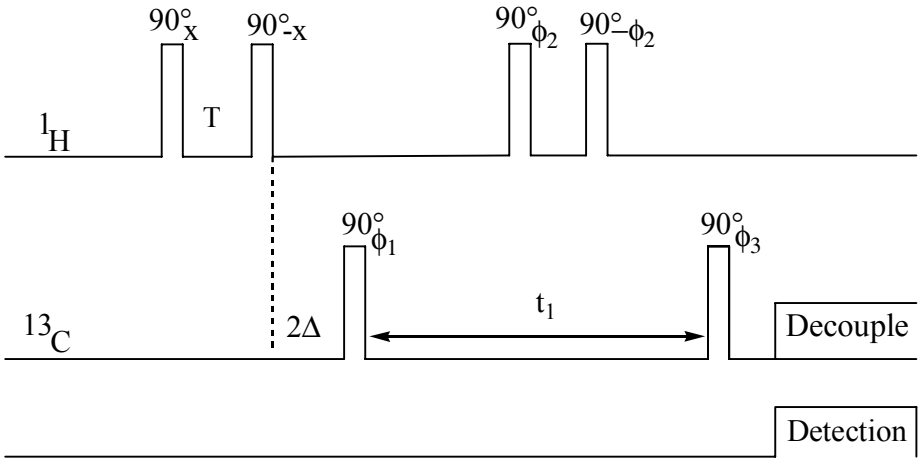


Fig. 21 : Pulse sequence for HMQC incorporating jump-and-return sequences for solvent suppresion.

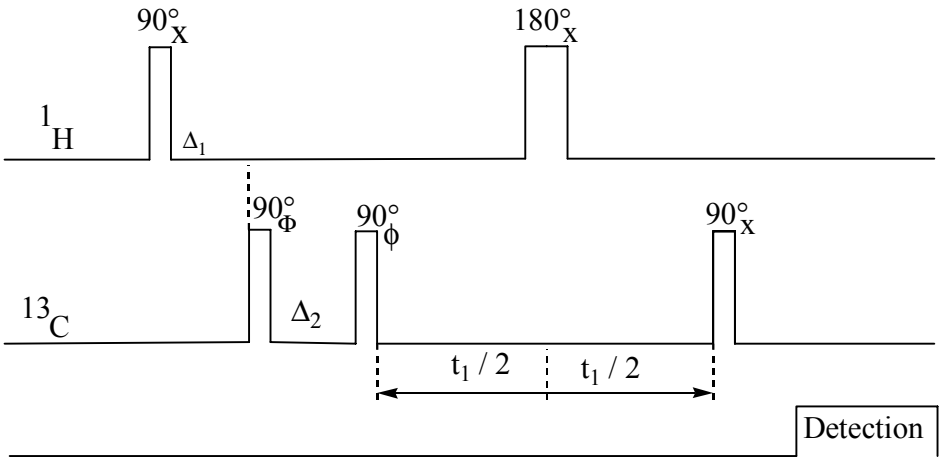


Fig. 22 : Pulse scheme for the acquisition of HMBC spectra based upon the HMQC technique.

3.9.2.6 Phase Sensitive Two-Dimensional Correlated Spectroscopy.

The well-established relationships between dihedral angles and scalar couplings of vicinally bonded protons is a useful tool which can provide structural information on both small and large molecules [89-91]. However the difficulty in calculating the scalar coupling may limit the use of these relationships. The introduction of the NOESY technique helps to over-

come this difficulty in most cases. Nevertheless, it may happen that the proton of interest overlaps in the one-dimensional proton NMR spectrum. In such a situation the NOESY diagram cannot help, and the knowledge of the scalar coupling is needed again. To measure scalar coupling in such conditions, two-dimensional J-resolved NMR spectroscopy [93, 94] were developed and found most use in protein chemistry. However, when many signals are too close to one another, an accurate measurement of coupling constants is impossible through 2D J-resolved spectroscopy. A better alternative is the phase-sensitive experiment [95-99]. In the phase-sensitive COSY technique, the pulse sequences are so selected that the absorption- and dispersion-mode signals are nicely separated; i. e., the diagonal peaks have dispersion shape, and the cross peaks have an absorption shape though they alternate in sense (fig. 23).

When recorded with enough digital resolution, coupling constants can be extracted from the fine structure of the cross-peaks. If we consider the cross-peak between two coupling nuclei A and B which appears at (δ_A, δ_B), its fine structure shows the active coupling (i. e., the coupling between A and B) in an antiphase disposition, but also passive couplings (i. e., the couplings between A an other nuclei) which appear in phase. The way we can take advantage of phase sensitive COSY is shown in fig. 23. In spite of the near coincidence of the chemical shift of A_1 and A_2, the cross-peaks with X_1 and X_2 are well resolved in the plane ν_1-ν_2, so that coupling constants can be measured. That would be impossible in the normal one-dimensional NMR spectrum, or with the 2D J-resolved spectrum were the resonances of A_1 and A_2 overlap.

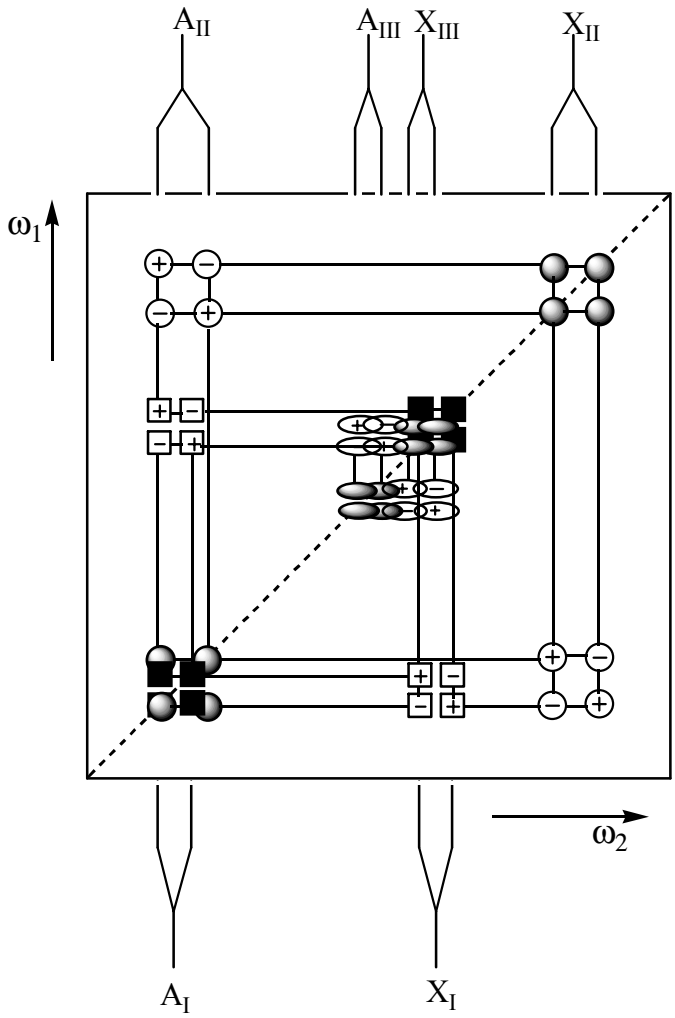
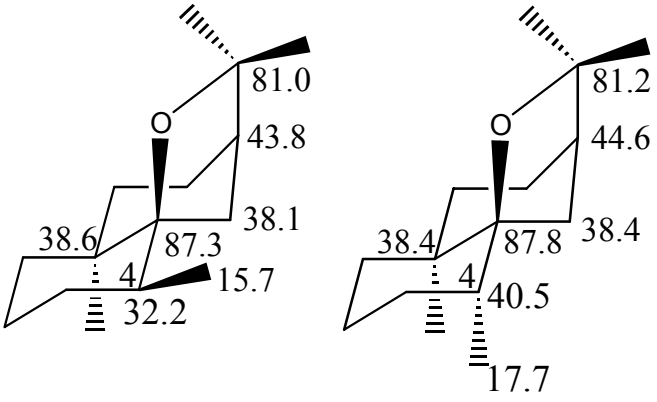


Fig. 23: Schematic diagram of a phase-sensitive COSY spectrum containing three AX spin systems. The diagonal-peaks, which have a dispertion shape, are indicated by filled symbols. The cross-peaks, which have an absorption line shape and alternate in sign, are indicated by open symbols, respectively given by + and -.

3.10 Absolute Configuration determination.

The first optical resolution in the history of chemistry, which was achieved by Louis Pasteur in 1848 led to the recognition of the importance of chirality as well as it raised many other questions. An important step in search for the solutions was made by Joseph A. Le Bel and Jacobus H. van't Hoff who introduced the concept of asymmetric carbon [100]. In the second half of the twentieth century, organic stereochemistry has made such progress that a

more precise knowledge about the dissymetric nature of natural products has become available. It is now possible to investigate the correct enantiomeric composition of bioactive or fragrant natural products. It is even required to clarify the relationship between absolute configuration and bioactivity or odour. These investigations have been made possible by the advances in analytical techniques coupled with the progress in enantioselective synthesis.



21 **22**

Fig. 24 : Stereochemistry of the dihydroagarofurans. Note the significant shift to lower field of C-4 in going from **21** to **22**.

Generally NMR spectroscopy may only give the relative configuration of a molecule. This means that after the elucidation of structure by NMR methods, the question of the enantiomeric identity of the molecule remains to be answered. Of course, it is possible for some particular cases (mainly alcohols and epoxides) through the phenomenon of diastereotopy to use NMR spectroscopy for establishing the relative configuration of a particular assymetric carbon. A typical example is that of the agarofurans (**21**) and (**22**) in which the chemical shift of the carbon C-4 shows a remarkable β-effect and thus allows to distinguish the two diastereoisomers [101] (fig. 24).

Mass spectrometry has also been used to find out the stereochemistry of some particular compounds. For instance, the epimeric sesquiterpenoids with a decalol structure can often be distinguished on the grounds that one of the isomers can undergo a favoured loss of water by a 1,4- or 1,3-elimination process that involves an energically favourable hydrogen usually tertiary or

allylic, cis to the hydroxyl group. The nordrimanes (**23**) and (**24**) [102] provide an example from the trans-fused series, and the decalols (**25**) and (**26**) [103] from the cis-fused.

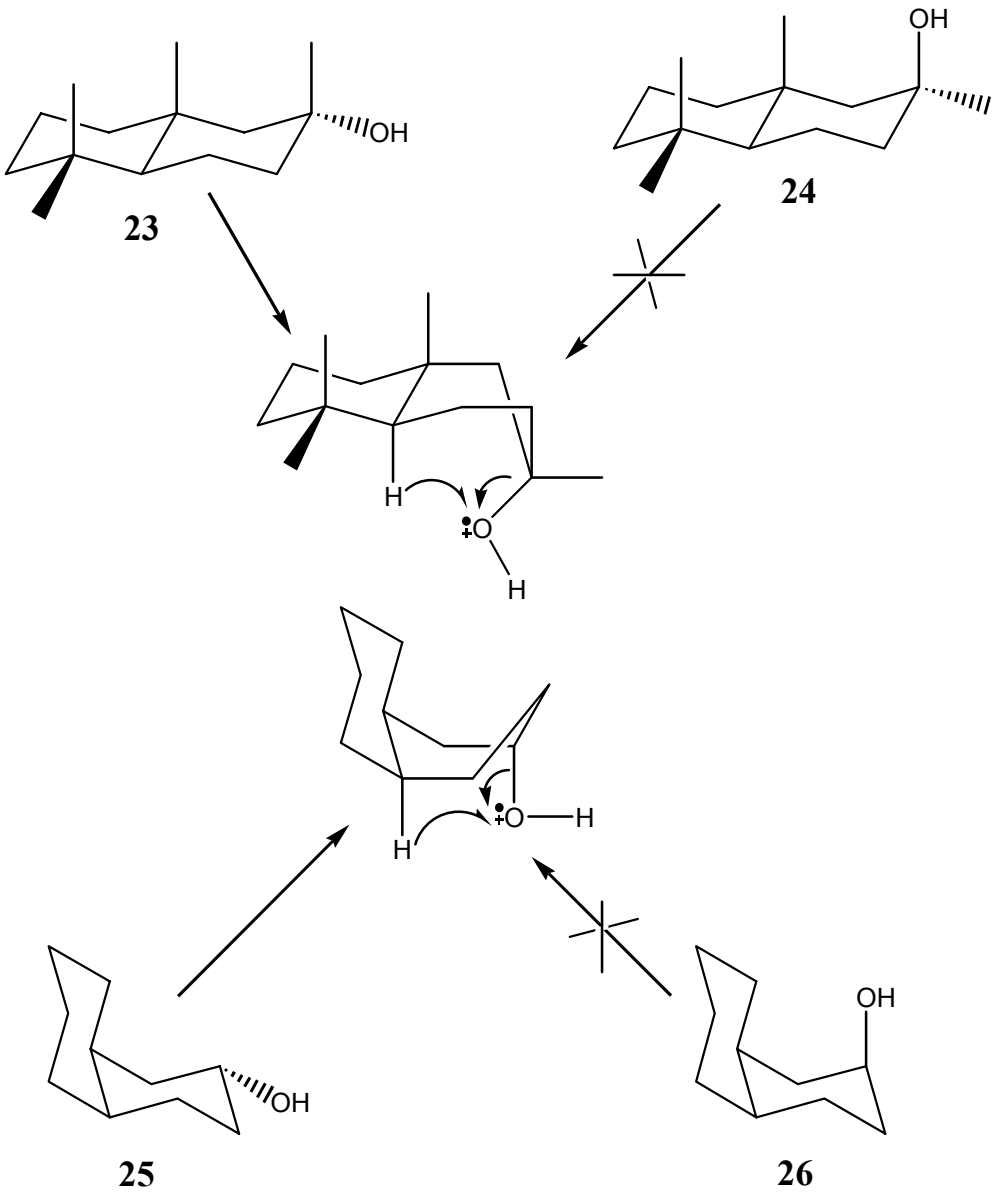


Fig. 25: Mechanism of loss of water showing the disadvantage of axial hydroxyl groups.

The common feature of both pairs of epimers is that the accessibility of tertiary 5α-H for water elimination in the 8α-OH isomers (**23**, **25**). Although requiring a boat conformation, the abundance of the $[M - H_2O]^+$ peak in

these spectra is larger than the corresponding peak in the spectra of their 8β-OH counterparts (**24**, **26**) (fig. 25).

Many methods have been developed for absolute configuration determination. They include chiroptical techniques, NMR with chiral shift reagents, HPLC or GC with chiral stationary phases, or combination of enantioselective synthesis and comparison of synthetic samples with the natural product.

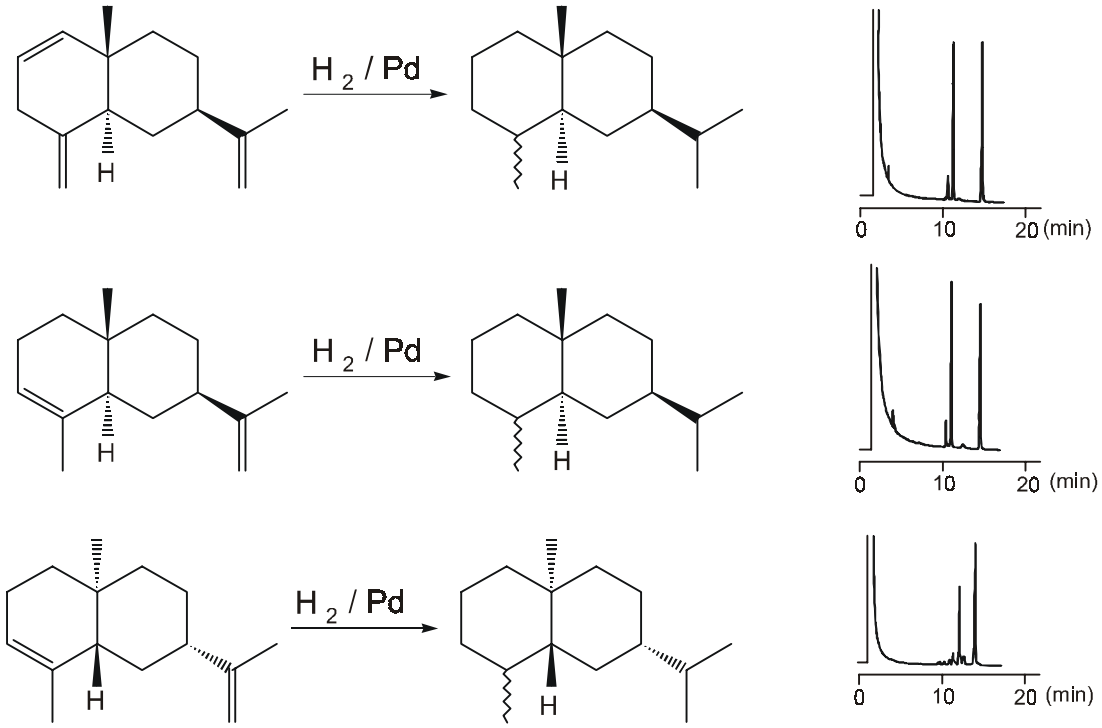


Fig. 26: Total hydrogenation of eudesma-1,4(15),11-triene, (+)- and (-)-α-selinene and gas chromatographic comparison on a 24 m fused capillary column with 6T-2,3-Me-β-CD (50 % in OV 1701, w/w) at 120°C. The diastereomeric hydrogenation products of eudesma-1,4(15),11-triene and (-)-α-selinene have the retention times 11.1 and 14.4 min, respectively while the hydrogenation products of (+)-α-selinene appear at 12.3 and 14.1 min.

However, absolute configurations are usually determined by chemical correlations with authentic sample of compounds of known absolute configuration [104]. Combination of chemical correlation and enantio-selective gas chromatography has proved to be a powerful tool for absolute

configuration determination, as far as volatile compounds are concerned [105]. The eudesmatriene (**27**) was fully hydrogenated yielding two diastereoisomers. Co-injection with a fully hydrogenated sample of (-)-α-selinene (**28**), also consisting of two diastereoisomers, using a cyclodextrin derived chiral stationary phase, proved their identity. In contrary, co-injection with a hydrogenated sample of (+)-α-selinene (**29**) resulted in different retention times for both diastereoisomers, thus establishing the identity of the absolute configuration of the eudesmatriene (**27**) and that of (-)-α-selinene (**28**) [106] (fig. 26).

4 Special Part.

«Man unterschied bekanntlich von jeher in den ätherischen Ölen sauerstofffreie und sauerstoffhaltige Bestandteile.»
(O. Wallach, *Terpene und Campher*, 2nd edition, 1914).

4.1 Botanical Aspect and Economical Importance of the *Cyperaceae*.

The *Cyperaceae* constitute a family of monocotyledons consisting of about 90 genera and 4000 species. They generally look grass-like and are usually found in marshy or aquatic habitats [107] (see fig.27). Although it is a very large family, not many species of the *Cyperaceae* have an economic importance. However, some of them are utilised in various domains of human activities. They may be used as food, medicinal or horticultural plants. Some others are used for weaving or basketwork or building. A few species are used for land stabilisation i.e. to prevent soil erosion. To mention some concrete examples, the tubers of *Cyperus esculentus* are edible and known as chufas or tiger nuts. *Cyperus involucratus* is used as fodder which is grazed by domestic stocks in Kenya. Many people use parts of plants as remedies for a wide variety of ailments, sometimes in conjunction with other plants. In Gabon the rhizomes of *Cyperus articulatus L.* are used in the treatment of migraine [108]. In north-eastern Thailand *Cyperus corymbosus* is used in the production of mats of various

types and sizes. In wet regions used for cultivation of rice, most *Cyperus* species constitute weeds of economic importance to farmers because they have negative benefits. Indeed they are very difficult to eradicate and it has sometime been refered to as the world's worst weed [109].

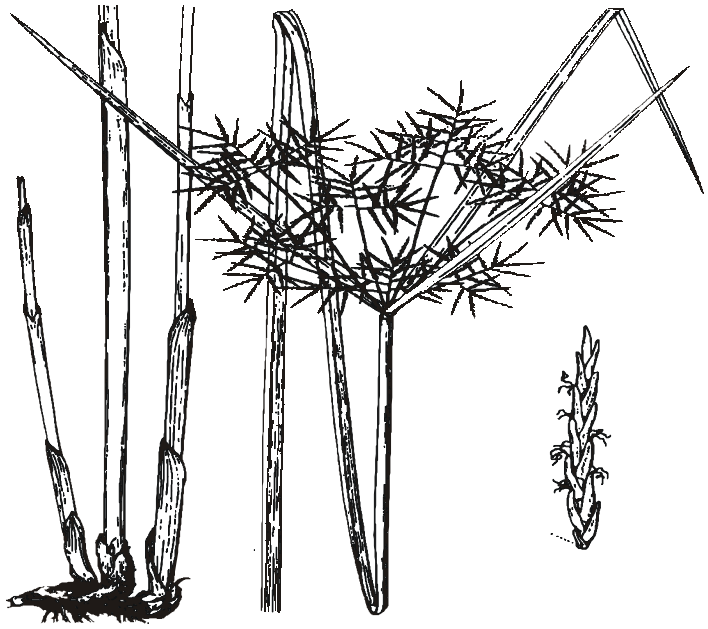


Fig. 27: Scheme of *Cyperus pangorei*. Many members of the genus *Cyperus* look like *C. pangorei* or present comparable patterns.

4.2 The Natural Product Chemistry of *Cyperus* Species.

Many plants of the genus *Cyperus* have been the subject of chemical studies for a long time. Volatile constituents as well as higher boiling compounds have been investigated to such an extend that some generalisation can be done. It has been found that polar secondary metabolites in this genus mainly consist in prenylated quinones such as breviquinone (**29**) [110], cyperaquinone (**30**) [111], scabequinone (**31**) [112] and conicaquinone (**32**) [113]. Some flavonoid quinones have also been isolated from plants of this genus and charaterised, e.g. scaberine (**33**) [110] and breverine (**34**) [110] (fig. 28).

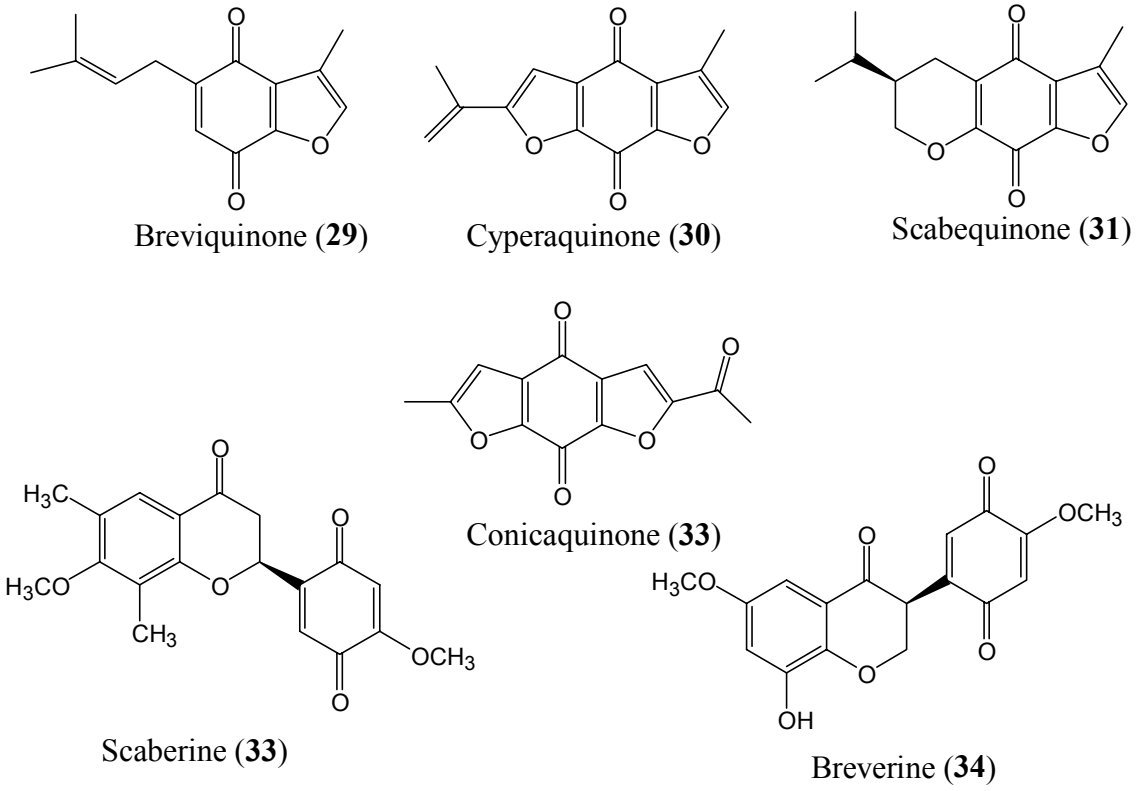


Fig. 28 : Some non-volatile secondary metabolites of *Cyperus* species.

Essential oils from *Cyperus* species are generally constituted of sesquiterpenoids, and traces of monoterpenoids. The main hydrocarbon is always cyperene (**35**) [114], but many other sesquiterpenes of the caryophyllane, eudesmane, patchoulane, and rotundane type are also present as hydrocarbon and oxygenated compounds. Kobusone (**36**) and isokobusone (**37**) have been isolated from *Cyperus rotundus* [115]. From *Cyperus articulatus* Nyasse et al isolated α-corymbolol (**38**), and β-corymbolol (**39**) [117], mandassidione (**40**) and mustakone (**41**) [118]. Corymbolone (**42**) was isolated from the rhizomes of *Cyperus corymbosus* by Garbarino and collaborators [119]. The most investigated species of this genus is *Cyperus rotundus*. The essential oil as

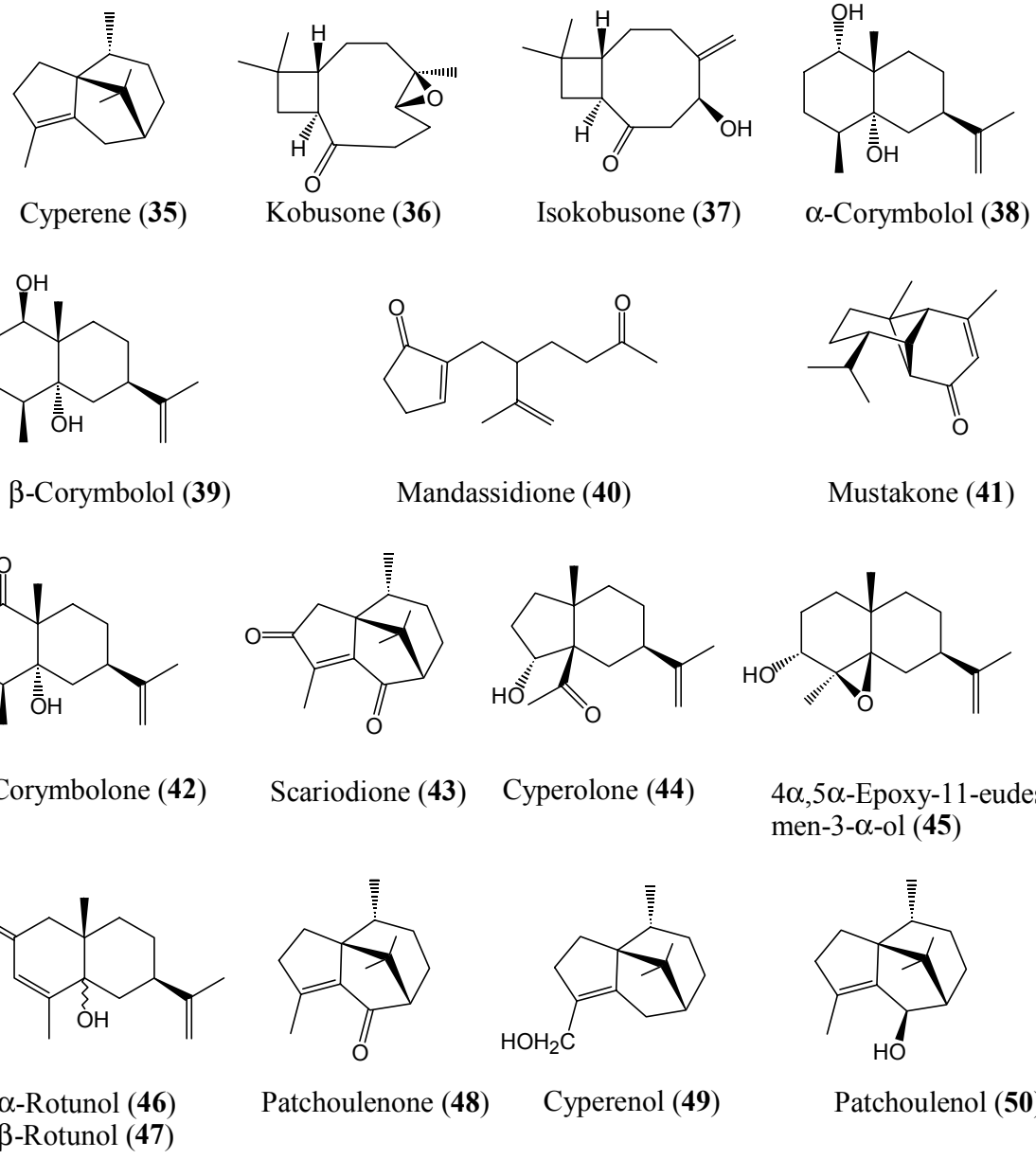
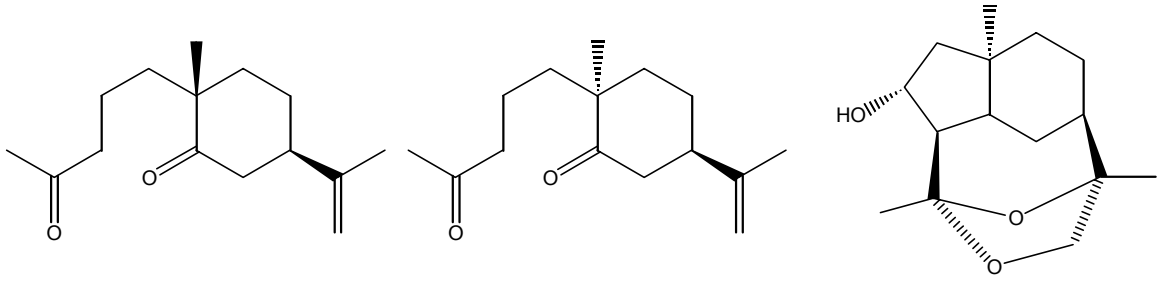


Fig. 29 : Sesquiterpenes from *Cyperus* Species.

well as polar extracts of the rhizomes have been studied, resulting in isolation and characterisation of many compounds, including scariodione (**43**) [121], cyperolone (**44**) [120], 4α,5α-epoxy-11-eudesmen-3-α-ol (**45**) [122], α-rotunol (**46**) and β-rotunol (**47**) [123], patchoulenone (**48**), cyperenol **(49**), and patchoulenol (**50**) [124] (fig. 29). More recently, a Japanese group has isolated

from the dried roots of the same plant two 4,5-secoeudesmanolides **51** and **52** and

a cyclic acetal compound **53** [125] (fig. 30).



51 **52** **53**

Fig. 30 : Sesquiterpenes from the roots of *Cyperus rotundus*.

4.3 Chemical Study of *Cyperus alopecuroides*.

4.3.1 The Plant.

Cyperus alopecuroides is a perennial plant, tufted with short rhizomes. The roots are dusky brown and 1 to 1.5 mm thick. The culm is stout, trigonous, 90 to 140 cm tall, 7 to 12 cm thick and smooth throughout. The leaves are 3 to 5 times shorter than the culm and the cross section is an inversed-W-shaped. They are light green above and pale beneath. The plant is distributed in north and tropical Africa, Madagascar, India, Indochina, Malaysia and northern Australia [116]. No particular ethnobotanic use of the plant has been reported.

4.3.2 Preliminary Study of the Oil of Cyperus alopecuroides.

The rhizomes of the plant were collected in Kollbison in Cameroon and the essential oil was isolated by hydrodistillation. The analysis of this oil by GC (fig. 32) and GC-MS allowed a rapid identification of many sesquiterpene hydrocarbons such as α-cubebene (**54**) [126], α-copaene (**55**) [127], β-caryophyllene (**56**) [128], α-humulene (**57**) [129], cyperene (**35**), rotundene (**58**)

[130], α-selinene (**59**) [131], β-selinene (**60**) [132], calamenene (**61**) [133] and δ-cadinene (**62**) [134] (fig. 32). Yet many other compounds could not be identified (see fig. 31) and needed to be isolated for further investigations.

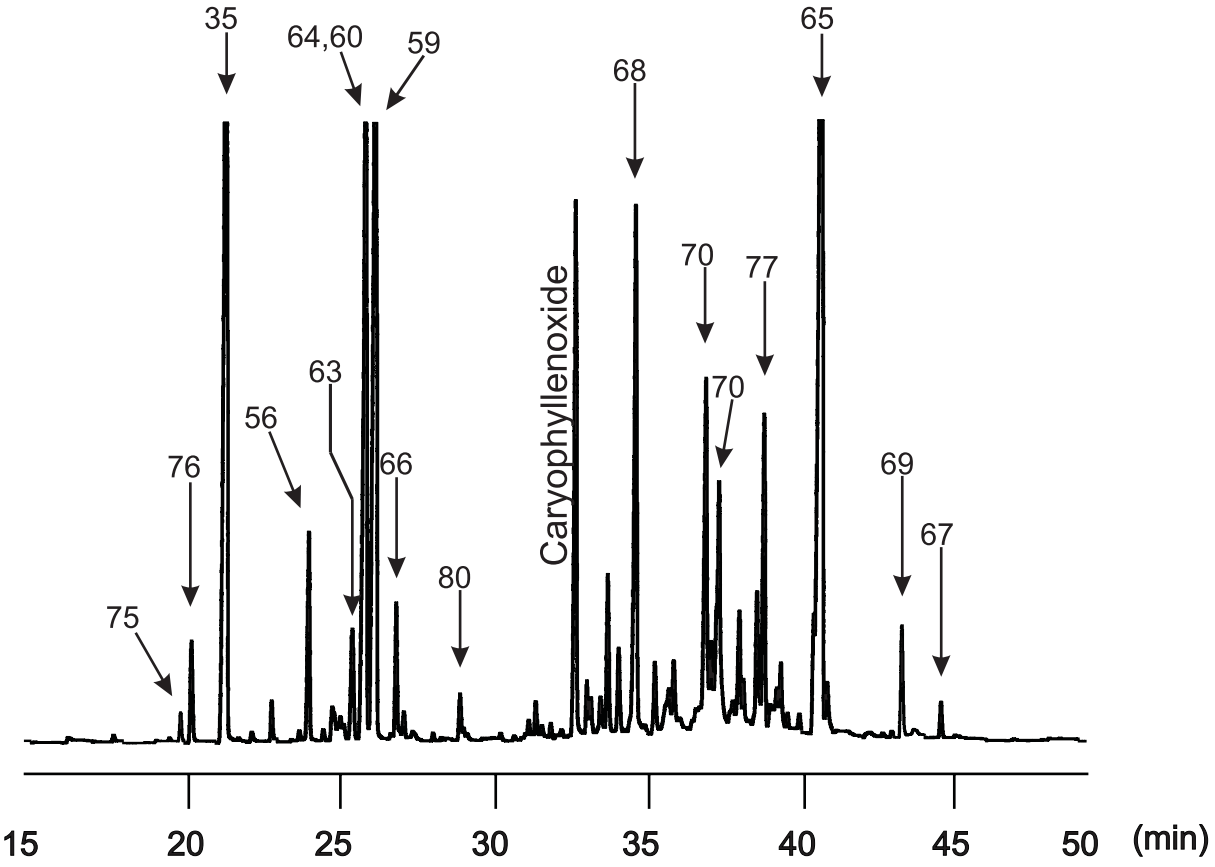


Fig. 31 : Analytical gas chromatogram of ***Cyperus Alopecuroides*** on a 25 m CP Sil 5 column, 50°, 3° /min to 230°C.

4.3.3 Isolation and Characterisation of (-)-Eudesma-1,3,11-triene (63).

The compound **63** was isolated essentially by preparative gas chromatography, using columns of different type. According to its proton NMR spectrum (fig. 33), **63** was obtained as a pure compound. It has the molecular formula $C_{15}H_{22}$ in agreement with its mass spectrum ($[M]^+$, m/z = 202). Because of the small quantity of the isolated product, not all carbon atom signals could be

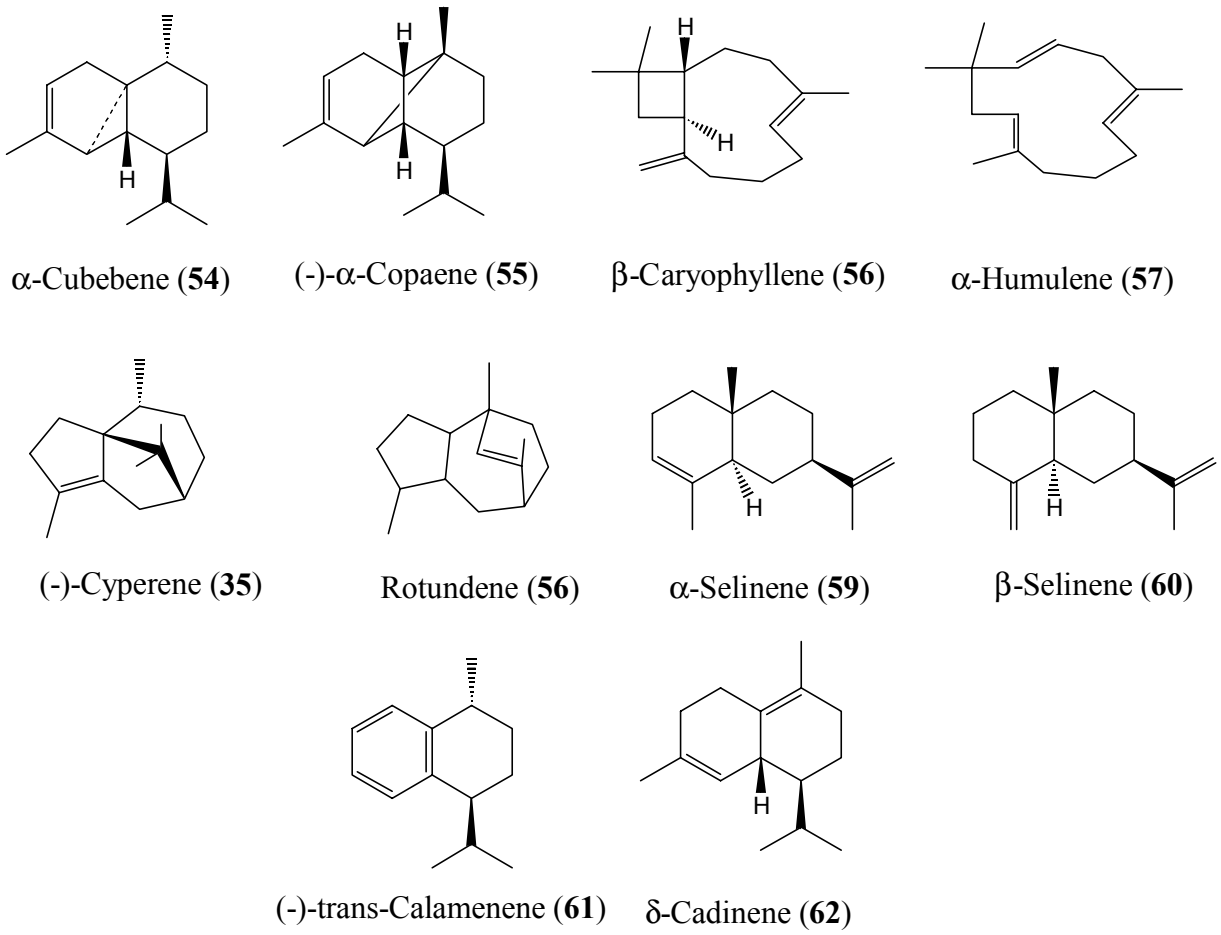


Fig. 32 : Some Sesquiterpene Hydrocarbons of *Cyperus alopecuroides*.

observed in the ^{13}C-NMR spectrum. However, it was obvious that the compound contains three methyl groups, four methylene goups, one being olefinic (δ 110.83) and three methines (δ 44.82, 122.42 and 129.25). In the ^{1}H-NMR spectrum one observes two protons (δ 4.72) in the olefinic region coupling with the olefinic methyl group at δ 1.77 in the ^{1}H-^{1}H-COSY diagram and suggesting the presence of an isopropenyl substituent in the molecule. The olefinic proton at δ 5.72, couples with another olefinic proton at δ 5.66, which again couples to a methylene group at δ 1.96 and δ 2.10, leading to the substructure C-CH=CH-

CH_2-C. Moreover, the value of the coupling constant between the olefinic protons (J = 8.5 Hz) suggests that the substructure belongs to a six-membered ring.

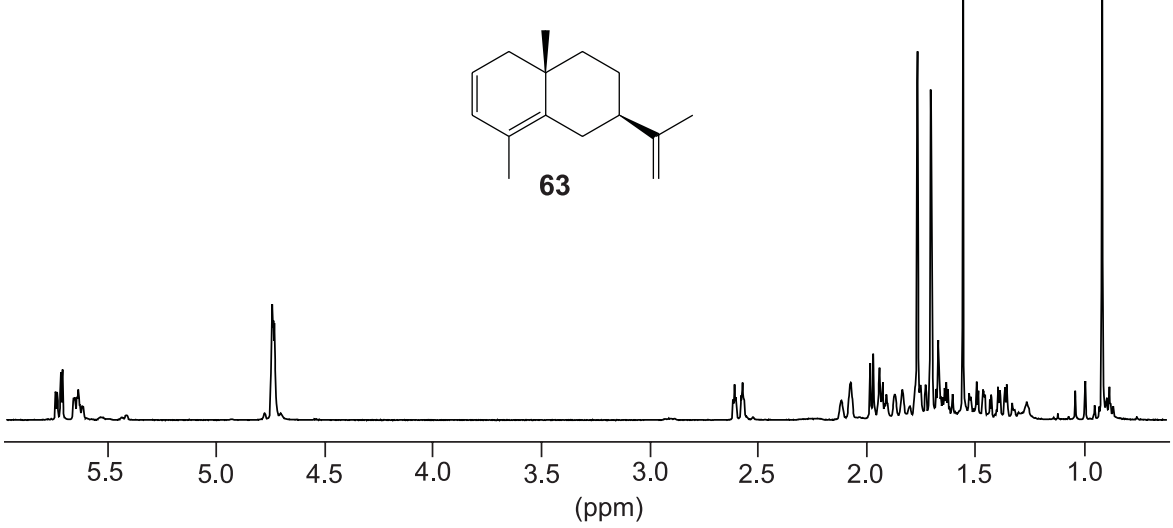


Fig. 33 : Proton NMR-spectrum of (-)-eudesma-2,4,11-triene (**63**) in $CDCl_3$.

The ^1H-^1H-COSY diagram shows that the olefinic methyl group (δ 1.71) couples with a methylene group CH_2-6, which itself couples with a methine proton CH-7 at δ 1.93, giving the substructure CH_3(C)C=C(C)-CH_2-CH(C)-. The angular methyl group (δ 0.98) couples with the methylene CH_2-9 (δ 1.38 and δ 1.68) which itself couples with another methylene CH_2-8 (δ 1.48, δ 1.67) which again couples to the methine proton CH-7, yielding the substructure CH_3-C-CH_2-CH_2-CH-. The combination of the four substructures affords structure **63**. A corroboration for this structure was brought out by a partial synthesis of the compound through reduction [135] and subsequent dehydration of (+)-α-cyperone (**65**) [136] (fig. 35).

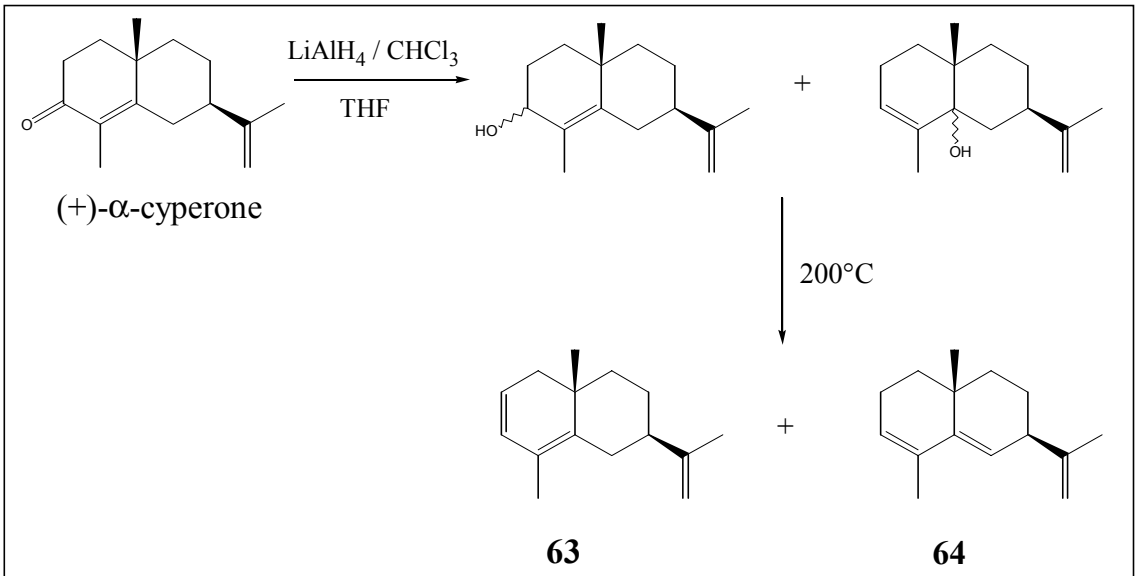


Fig. 34: Generation of (-)-eudesma-2,4-11-triene (**63**) and (-)-eudesma-3,5,11-triene (**64**).

4.3.4 Isolation and Characterisation of (-)-Eudesma-3,5,11-triene (64).

Compound **64**, which was obtained from the same fraction containing **63** was contaminated with β-selinene (**60**). However it could still be characterised, since it was also obtained during the preparation of **63** from (+)-α-cyperone. The molecular formula $C_{15}H_{22}$ is concluded from the EIMS spectrum ($[M]^+$, 202). Except for three quaternary carbon signals, all the other signals appear in the ^{13}C NMR spectrum. The DEPT technique permitted to distinguish three methyl groups, five methylene groups, one being olefinic, and three methine, two of which are olefinic. The ^1H-NMR spectrum (fig. 35), also confirms the structure: two olefinic methyl groups (δ 1.78, CH_3-14) and (δ 1.73, CH_3-13), one angular methyl group (δ 0.99, CH_3-15), four olefinic protons (δ 4.73, H-12; δ 4.77, H-12; δ 5.52, H-3; δ 5.41, H-2;), one allylic methine proton (δ 2.91, H-7) and two allylic methylene protons CH_2-2 (δ 2.26, δ 2.05).

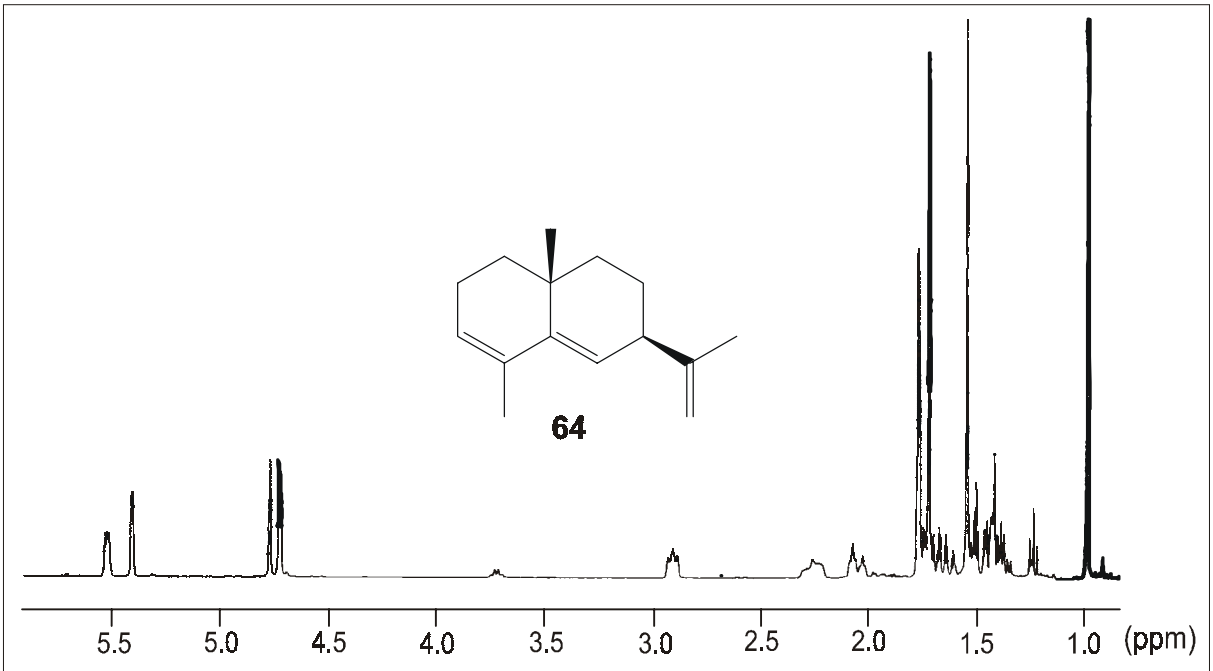


Fig. 35 : Proton NMR-spectrum (-)-eudesma-3,5,11-triene (**64**) in $CDCl_3$.

4.3.5 Isolation and Structure Elucidation of (-)-Eudesma-2,4(15),11-triene **(66).**

Compound **66** was also isolated by preparative gas chromatography from a fraction containing α-selinene (**59**) and β-selinene (**60**) as major components (fig. 36). Further chromatographic treatment allowed the isolation of unknown compound **66** as a pure product. The compound was assigned the molecular formula $C_{15}H_{22}$ ($[M]^+$; m/z = 202). The ^{13}C-NMR and DEPT spectra allow the identification of 15 carbon atoms: two methyl groups, six methylene groups, two of which being olefinic, four methine groups two of which are vinylic and three quaternary carbon atoms, two being olefinic. The ^{1}H-NMR spectrum (fig. 37) shows two protons (δ 4.83) in the olefinic region coupling with the vinyl methyl group at δ 1.68 in the ^{1}H-^{1}H-COSY diagram and therefore suggests the presence of an isopropenyl substituent in the molecule. Further examination of the

^{1}H-NMR spectrum indicates a methylene group (δ 1.88 and δ 1.72) coupling with the vinyl proton (δ 5.58), itself coupled to another vinyl proton (δ 6.19).

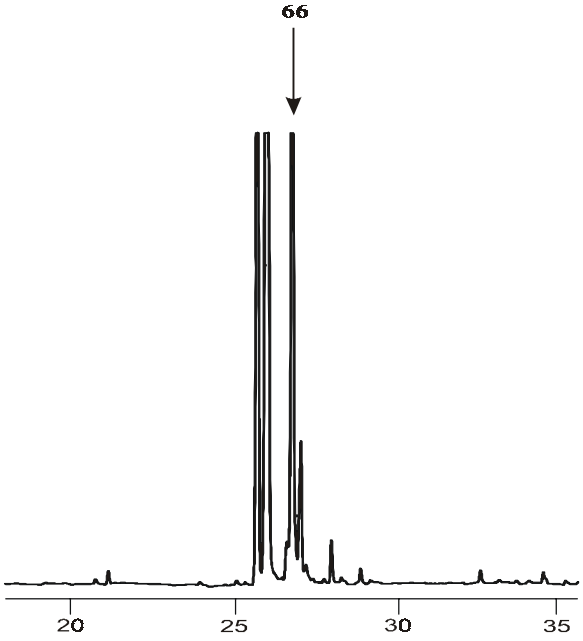


Fig. 36: Analytic chromatogram of the sample containing (-)-eudesma-2,4(15),11-triene (**66**).

Moreover, one can observe in the ^{1}H-^{1}H-COSY diagram coupling correlations of these two vinyl protons with the two methylenic protons at δ 4.87. These informations lead to the substructure -CH_2-CH=CH-C=CH_2. Coupling correlations are also observed between the tertiary methyl group at δ 0.77 and a methylene group (δ 1.09 and δ 1.42) which couples with another methylene group (δ 1.38 and δ 1.50). The last methylene group couples further with the methine proton (δ 2.00), itself coupled to another methylene group (δ 1.29 and δ 1.83) which couples again to the methine proton (δ 1.89), giving the substructure CH_3-C-CH_2-CH_2-CH-CH_2-CH-.

In combining the three substructures, an eudesmane type skeleton was obtained. The stereochemistry of the compound follows from the exploitation of the coupling constant values in the ^{1}H-NMR spectrum. The

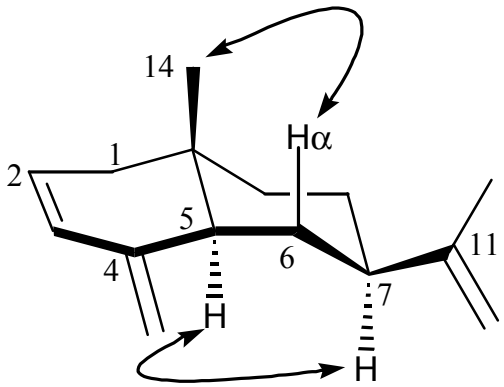


Fig. 38: Important proton-proton spatial correlations from the NOESY diagram of **66**.

trans configuration of the decaline skeleton results from the antiperipla-nar coupling (J = 12.0 Hz) between H_5 and $H_{6\alpha}$ (fig. 38). The proton H_7 exhibits an axial coupling constant (J = 12.0 Hz) showing that the isopropenyl group is equatorial. Moreover NOESY correlations are observed between the methyl group protons H_{15} and $H_{6\alpha}$ and also between H_5 and H_7 (fig. 38). Thus, the structure **66** was assigned to the unknown compound. **66** was also identified as minor product in the formation of **63** and **64** from (+)-α-cyperone (**65**).

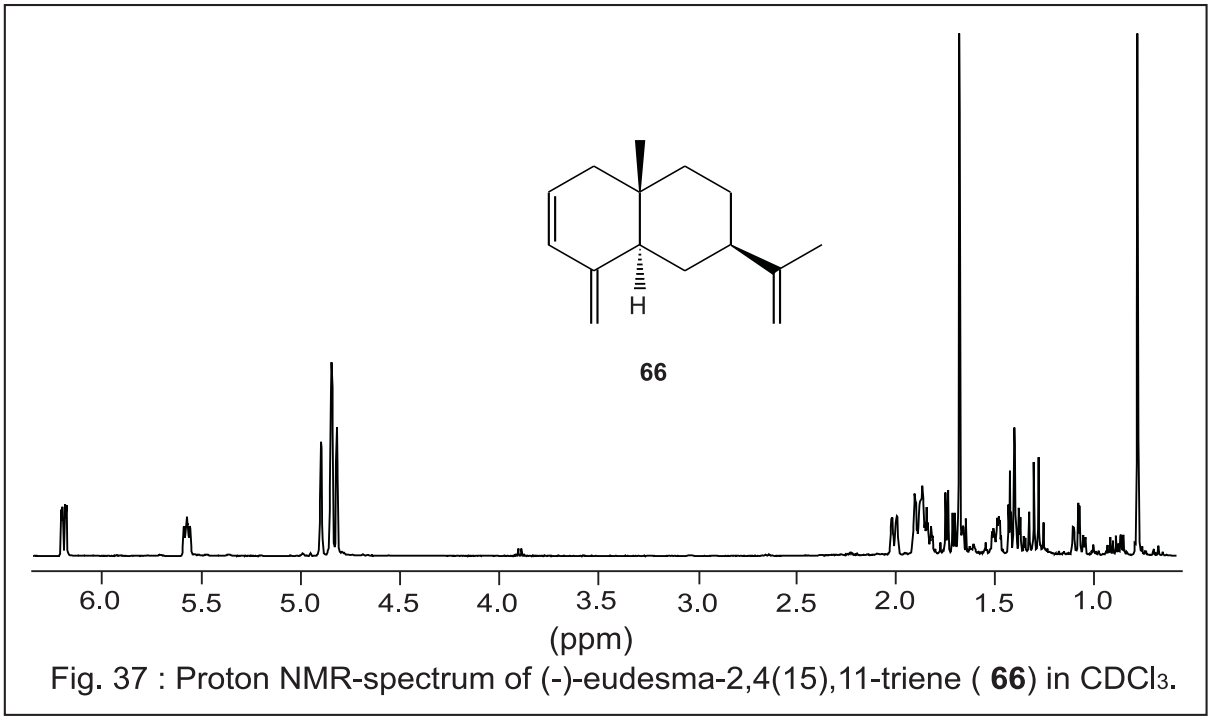


Fig. 37 : Proton NMR-spectrum of (-)-eudesma-2,4(15),11-triene (**66**) in $CDCl_3$.

4.3.6 Isolation and Characterisation of Dolabella-3,7,18-triene (67).

A second sample of the oil of *Cyperus alopecuroides* was analysed, using a combination of column chromatography at low temperature (-20° C) and gas chromatography. From a late hydrocarbon fraction, a compound was isolated with a longer retention time, than sesquiterpene hydrocarbons (fig. 39). The

unknown compound was assigned the molecular formula $C_{20}H_{32}$ in agreement with the molecular ion in its mass spectrum (fig. 40) ($[M]^+$, m/z = 272). The ^{13}C-NMR spectrum shows twenty carbon signals, six being olefinic. From the DEPT spectrum three methyl groups, eight methylenic groups, one being olefinic, four methines two of which are olefinic and four quaternary carbon atoms, three being olefinic, were assigned.

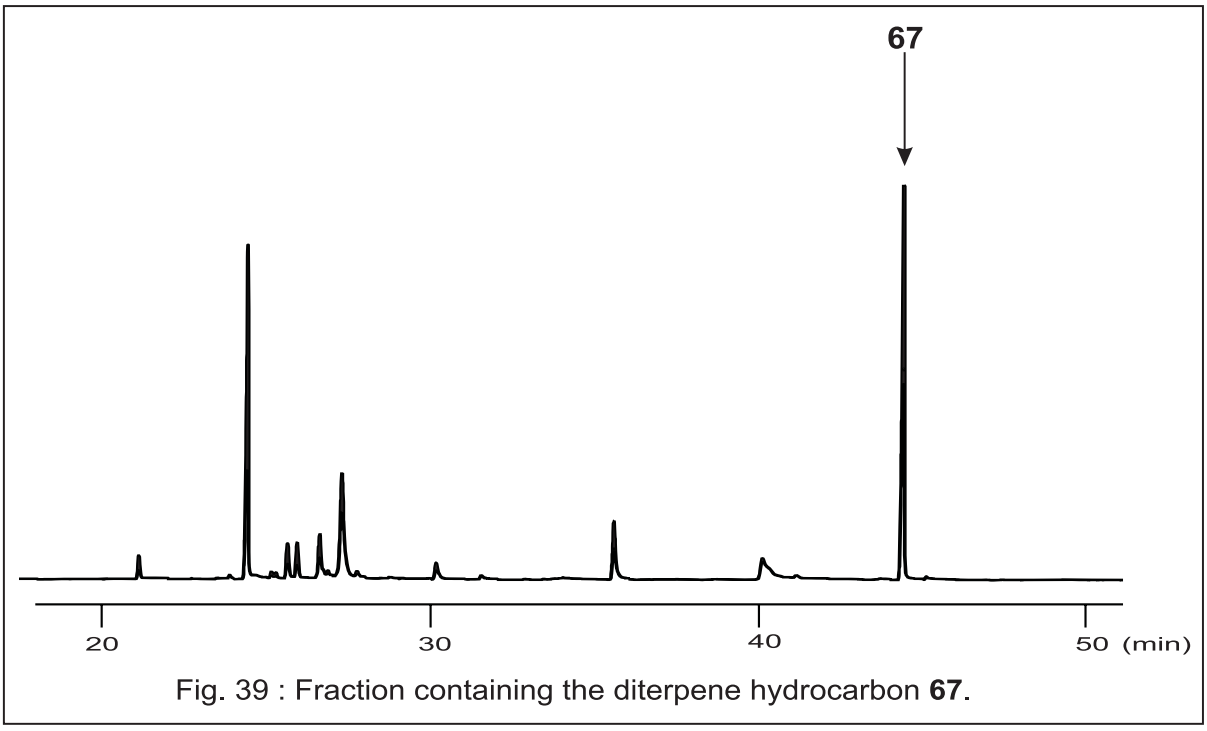


Fig. 39 : Fraction containing the diterpene hydrocarbon **67**.

The interpretation of the ^{1}H-NMR spectrum (fig. 41) and the ^{1}H-^{1}H-COSY diagram allowed the formation of three substructures. First of all the presence of an isopropenyl group was indicated by the coupling correlations between the two vinyl-methylenic protons at δ 4.58 and 4.76 and the olefinic methyl group at δ 1.65. The methine proton at δ 1.69 (CH-11) couples with the methylene group CH_2-10 (δ 1.16, δ 1.27) which couples further with another methylene group CH_2-9 (δ 1.82 and δ 2.07). The same methine proton CH-11 couples with another methine proton CH-12 (δ 2.57) which again coupled to the methylene

group CH_2-13 (δ 1.50, and δ 1.57) which further couples with another methylene group CH_2-14 (δ 1.36 and δ 1.47) giving the substructure -CH_2-CH_2-CH-CH-CH_2-CH_2-.

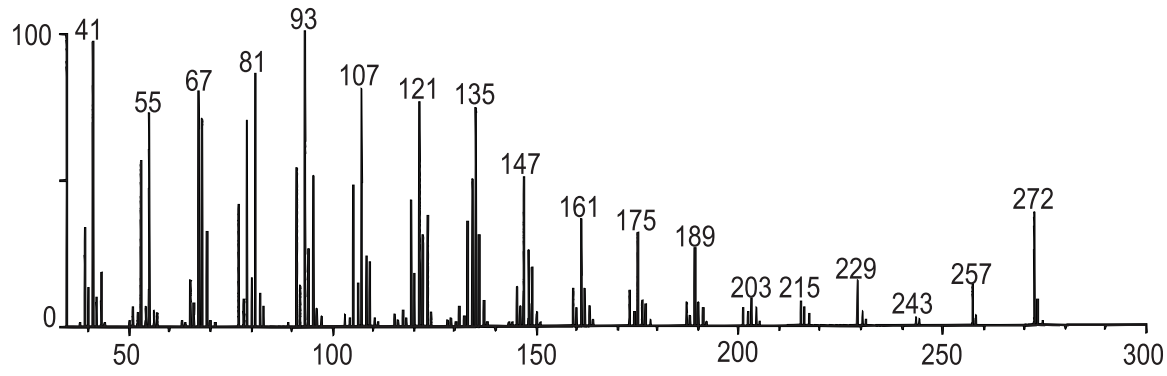
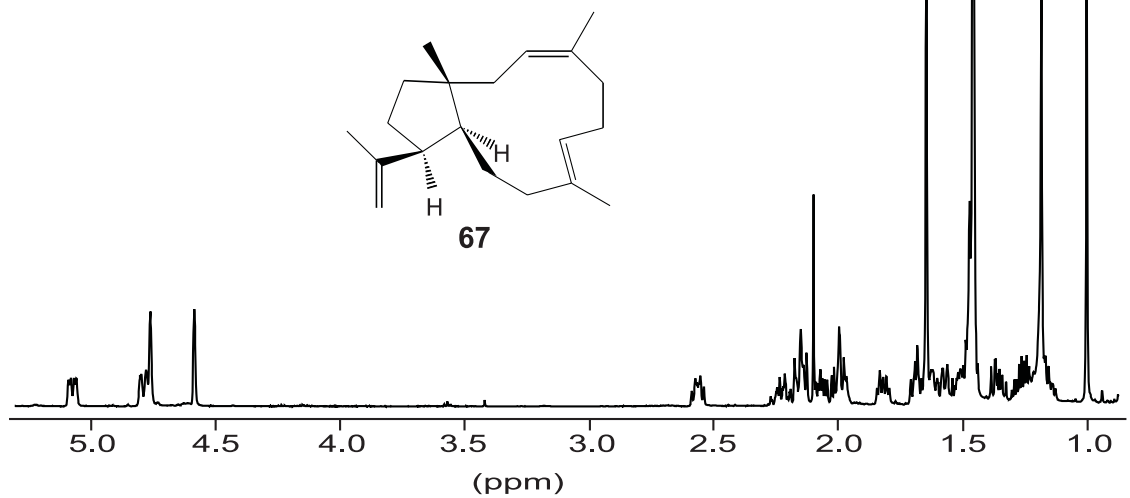


Fig. 40 : Mass spectrum of (-)-dolabella-3,7,18-triene (**67**).



67

Fig. 41 : Proton NMR-spectrum of (-)-dolabella-3,7,18-triene (**67**) in C_6D_6.

The vinylic proton CH-7 (δ 4.79) couples with the methylene group CH_2-6 (δ 2.21 and δ 1.9) itself coupled to the methylene group CH_2-5 (δ 2.01 and δ 2.14)

to give the partial structure -CH_2-CH_2-CH=C. Finally, the methylene group CH_2-2 (δ 1.64 and δ 2.15) couples with the vinylic proton CH-3 (δ 5.07) leading to the substructure -CH_2-CH=C. The combination of the four substructures and subsequent localisation of the methyl groups was achieved with the help of proton-carbon long-range correlations obtained from the HMBC diagram (Table 1). Four sets of spatial interactions between different protons indicated by the NOESY technique helped to establish the relative configuration of the molecule.

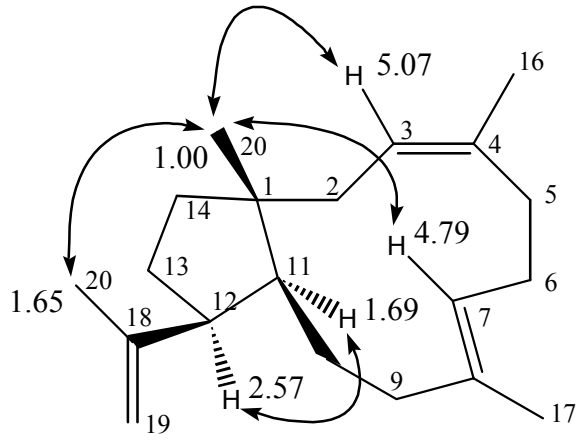


Fig. 42 : Relative configuration of **67** with important NOESY correlations.

The methyl group CH_3-15 shows spatial interactions with the methyl group CH_3-20 as well as with the two vinylic protons H-3 and H-7. Furthermore, the proton H-11 interacts with the protons H-12 and H-7 (fig. 42). The compound **67** is a diterpene with a dolabellane skeleton. Its absolute configuration was not investigated. The structure and chemistry of some naturally occuring dolabellane type diterpenes from molluscs, soft corals, liverworts and higher plants have recently been reviewed [167]. **67** was not yet identified as a natural compound.

Protons	Carbons
H-12	C-13, C-11, C-18, C-19
H-14	C-13, C-1, C-2
H-2	C-12, C-2, C-10
H-3	C-2, C-4, C-16
H-5	C-3, C-4, C-16
H-7	C-6, C-8, C-9
H-11	C-12, C-1, C-2, C-10
H-20	C-12, C-18, C-19
H-16	C-8, C-9, C-7
H-17	C-8, C-9, C-10
H-15	C-14, C-1, C-2, C-11

Table 1: Heteronuclear long-range correlations from the HMBC diagram of **67**.

4.3.7 Isolation and Structure Elucidation of (-)-Eudesma-3,11-dien-5-ol (68).

The alcohol **68** was isolated from the oxygenated fraction of the essential oil of *C. alopecuroides* by combination of preparative gas chromatography and thin-layer chromatography. The pure compound **68** was colourless and was attributed the molecular formula $C_{15}H_{24}O$ by mass spectrometry ($[M]^+$, m/z = 220) (fig. 43) , which means that it has four unsaturations. The ^{13}C-NMR spectrum reveals two double bonds, one of which is characteristic of an isopropenyl group (δ 108.5, C-12, δ 149.4, C-11). The compound **68** has therefore a bicyclic structure. The constitution of the molecule is derived from the interpretation of the ^{1}H-NMR (fig. 44) and ^{1}H-^{1}H-COSY spectra. The two vinylic protons at δ 4.64 coupling with the olefinic methyl group at δ 1.75 confirm the presence of an isopropenyl group in the molecule. The methylene group CH_2-1 (δ 1.21 and δ 1.76) couples with another methylene group CH_2-2 (δ 1.96 and δ 2.13) itself

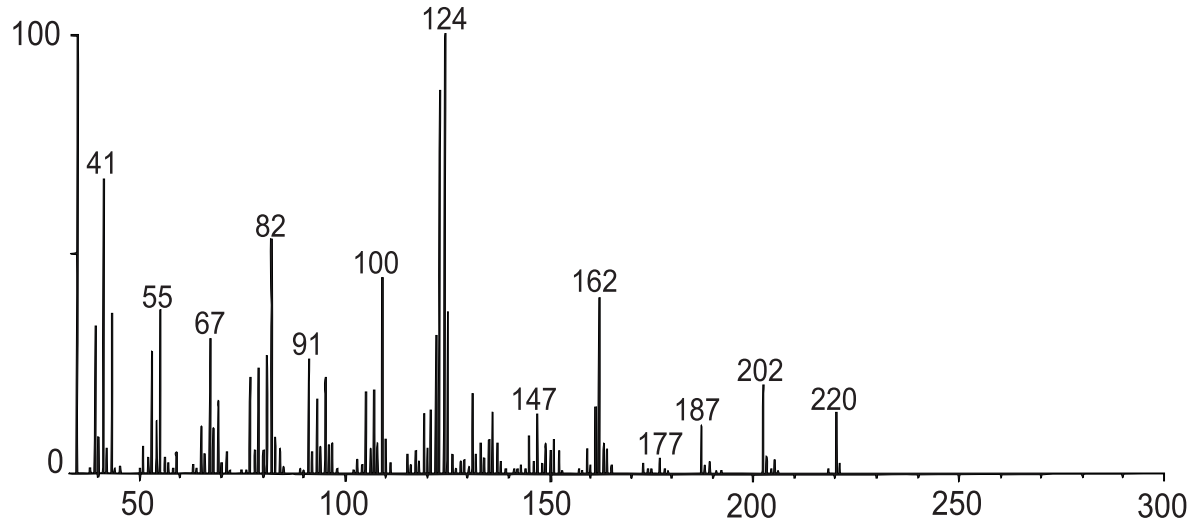


Fig. 43 : Mass spectrum of (-)-eudesma-3,11-dien-5-ol (**68**).

coupled to the vinylic proton CH-3 (δ 5.54) which further couples to the olefinic methyl group CH_3-15 (δ 1.75) giving the substructure CH_2-CH_2-CH=C-CH_3. The methyl group CH-14 shows a long-range coupling with the methylene group CH-9 (δ 1.24 and δ 1.64), which couples again with the methylene group CH-8 (δ 1.45) itself coupled to the methine proton CH-7 (δ 1.72) which couples further with the methylene group CH_2-6 (δ 154) to give the substructure CH_3-C-CH_2-CH_2-CH-CH_2-. The alcoholic nature of the compound was deduced from its mass spectrum which shows a peak for $[M]^+$-H_2O. Futhermore, the ^{13}C-NMR off-resonance spectrum shows a singlet at δ 73.2 proving that it is a tertiary alcohol. A further indication for the tertiary nature of the alcohol was given by the difficulty to perform a phase-transfer catalysed methylation of the alcohol (fig. 45) [138]. The combination of all these informations led to an eudesmane skeleton for compound **68**. A partial absolute configuration of the molecule was deduced from the fact that the dehydration of **68** at higher temperature (200° C) yielded the two hydrocarbons **66** and **64** of known absolute configuration [137] (fig. 46). In the ^{1}H-^{1}H-COSY diagram of **68**, the

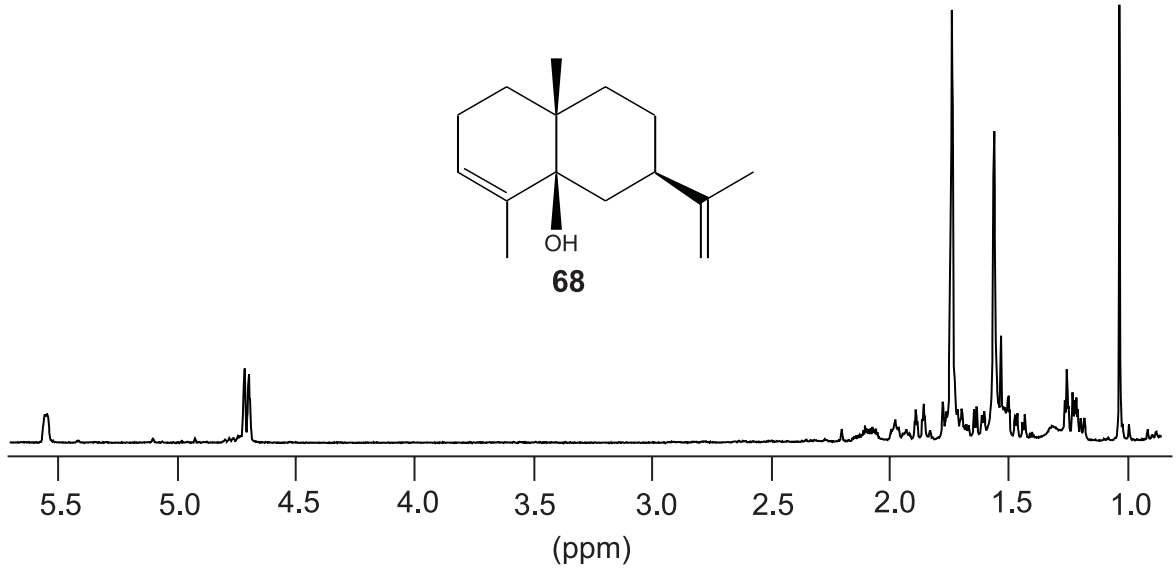
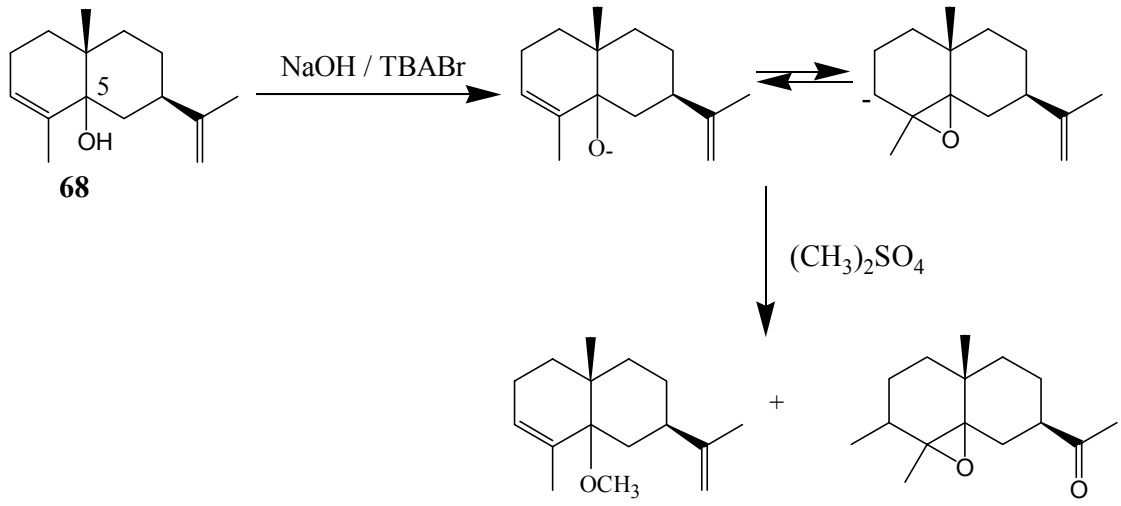


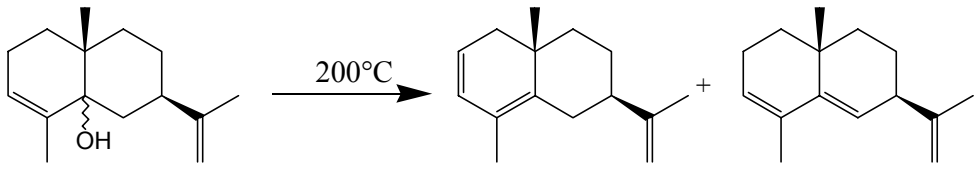
Fig. 44: Proton NMR-spectrum of (-)-eudesma-3,11-dien-5-ol (**68**) in $CDCl_3$.

two methyl groups CH_3-14 and CH_3-15 are long-range coupled to each other. This observation and the known requirements for a ^6J coupling through σ-bond indicate a cis-fusion of the decaline system of **68**. Furthermore, when comparing the mass spectra of **68** with those of α-rotunol (**46**) and β-rotunol (**47**) [123], a great similarity was found between the mass spectrum of **68** and that of β-rotunol in regard to the peaks of $[M]^+$ and $[M]^+$-H_2O. In the mass spectra of **68** and β-rotunol, the relative intensity of the $[M]^+$-peak is almost half of that of $[M]^+$-H_2O, while in the mass spectrum of α-rotunol the relative intensity of the $[M]^+$-peak is only 9% that of $[M]^+$-H_2O. This is explained by the fact that in α-rotunol the elimination of water involves the loss of a tertiary allylic hydrogen, an easier process compared to **68** and β-rotunol were the elimination of water involves the loss of a secondary acidic hydrogen (fig 47.)



68

Fig. 45 : Phase-transfer catalysed methylation of (-)-eudesma-3,11-dien-5-ol (**68**).



68 **64** **66**

Fig. 46 : Dehydration of **68** at higher temperature.

4.3.8 Isolation and Identification of (+)-Eudesma-3,11-dien-2-one (69).

The seventh fraction obtained from the lower temperature column chromatography contained four major peaks (fig. 48). The separation of the fraction by preparative GC yielded three other subfractions, the last being a pure compound. The analysis of the mass spectrum of the of the compound gave the molecular formula $C_{15}H_{22}O$. The proton NMR-spectrum of the compound showed one methyl group at δ 0.70 and two olefinic methyl groups at δ 1.40 and δ 1.60. At δ 4.75 a multiplet characteristic of an olefinic methylene group and at δ 5.90 the signal of a vinylic proton is observed. All these spectral data

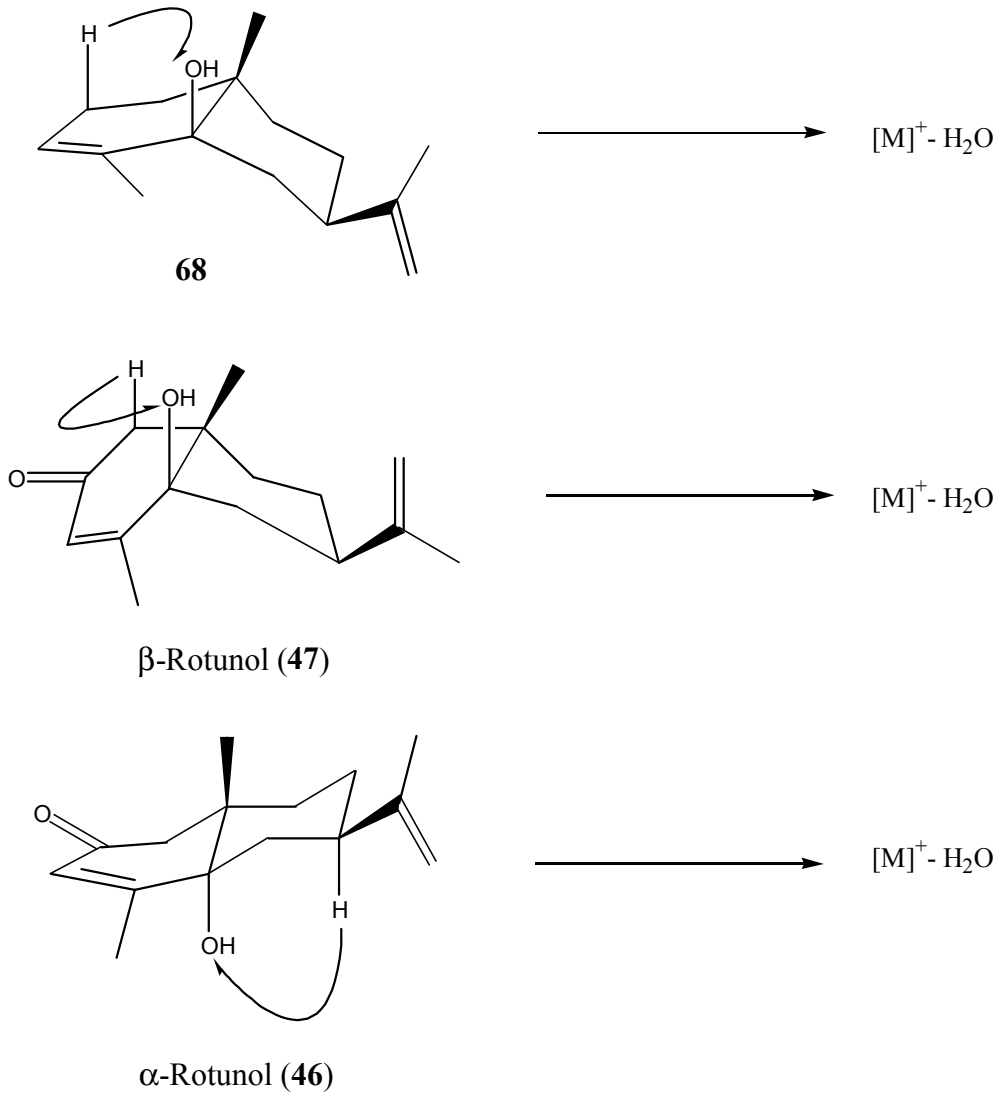


Fig.47 : Comparison of the pathway leading to the $[M]^+ - H_2O$ peak.

correspond to those of (+)-Eudesma-3,11-diene-2-one (**69**) (fig. 49), a compound formerly isolated and described by Demole and Enggist from the fruit of *Citrus paradisi M* [139].

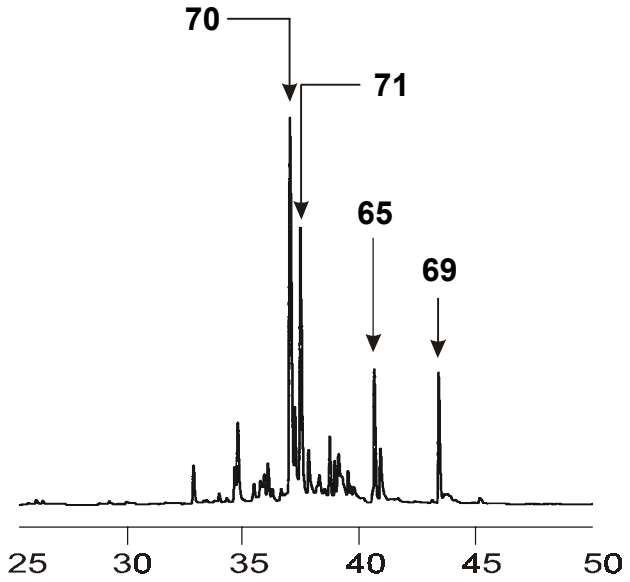
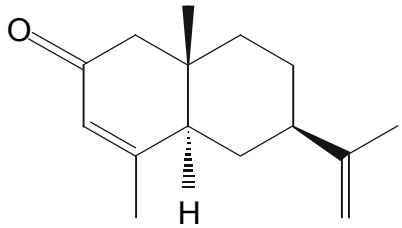


Fig. 48: Gas chromatogram of the fraction containing **69**, **70**, and **71**.



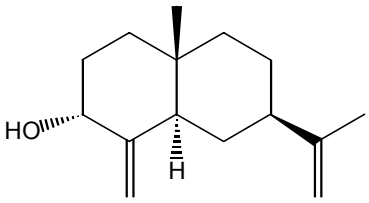
69

Fig.49: (+)-Eudesma-3,11-dien-2-one.

4.3.9 Isolation and Identification of Cyperol (71) *and Isocyperol* (70).

From the oxygenated fraction mentioned above, a subfraction consisting of two compounds was obtained and further separations were done by preparative TLC.

a) Isocyperol.



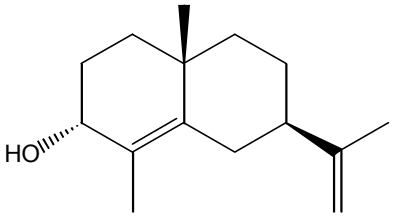
70

Fig. 50: Isocyperol.

To the first purified product the molecular formula $C_{15}H_{24}O$ was assigned in agreement with its mass spectrum ($[M]^+$, m/z = 220). The compound was assumed to be a sesquiterpene alcohol because of the presence of a peak corresponding to $[M]^+$-H_2O in its mass spectrum. From the proton NMR spectra itwas concluded that it is a secondary alcohol since the proton vicinal to the hydroxyl group were found at δ 4.22. The presence of an isopropenyl group is indicated by the signal of two vinylic protons (δ 4,52) showing coupling

correlations with an olefinic methyl group (δ 1.68). An exocyclic olefinic methylene group (δ 4.52, δ 4.88,) was identified. Further the protons H-5 (δ 2.30), H-6a (δ 1.20), H-7 (δ 1.94), H-1a (δ 1.64), H-2e (δ 1.75), and the angular methyl group CH_3-14 at δ 0.70 were detected. The spectral data correspond to the known compound isocyperol (**70**) (fig. 50) which has been isolated from *Cyperus rotundus* [140].

b) Cyperol.



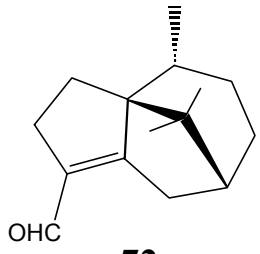
71

Fig. 51: Cyperol

The second substance has almost the same mass spectral pattern as isocyperol and was also assigned the molecular formula $C_{15}H_{24}O$, but in its ^{1}H-NMR spectrum some differences were noted. Further investigation indicated its identity with cyperol (**71**) (fig. 51) [140]. In the proton NMR spectrum the angular methyl group CH-15 appears as singlet at δ 1.10. The two olefinic methyl groups absorb at δ 1.73 and δ 1.76. The proton vicinal to the hydroxyl group H-3 has its signal at δ 4.04, and the two vinylic protons H-12 appear at δ 4.72.

4.3.10 Isolation and Identification of Cyperenal (72).

The isolation of cyperenal (**72**) (fig. 52) was performed by TLC from a fraction containing more than 93% of α-cyperone. The compound was assigned the molecular formula $C_{15}H_{22}O$ (EIMS, [M], m/z = 218). The ^{1}H-NMR spectrum showed many signals similar to those observed for patchoulane sesquiterpenoids (see § 4.6.1). The compound has three methyl groups occuring at δ 0.82 (H-12), δ 0.86 (H-15) and δ 1.02 (H-13). The absence of a fourth methyl group and the



72

Fig. 52: Cyperenal.

presence of an aldehydic proton at δ 9.84 (H-14) confirmed the identity of the compound with cyperenal which has formerly been described as a constituent of the roots of *Cirsium dipsacolepis* by Takano and Kawaminami [141].

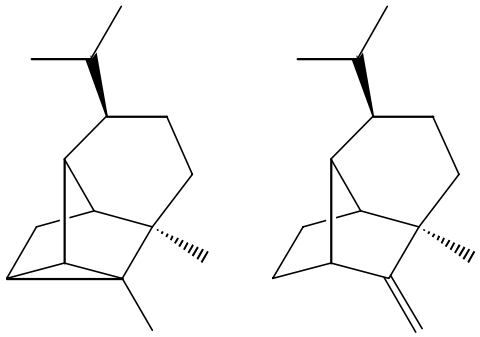
4.4 Chemical Investigation of *Cyperus papyrus*.

4.4.1 The Plant.

Cyperus papyrus is a giant herb, the culm of which ranges from 1 to 5m of height. It is a perennial plant with decumbent and coarse rhizomes. The culm is trigonous, clothed at the base with bladeless sheaths. The plant is distributed in Northern Africa (Egypt, Sudan) and Central Africa (Cameroon, Guinea and Nigeria) and Palestine.

The rhizomes of *Cyperus papyrus* were collected in Yaounde (Cameroon), and dried. Hydrodistillation of the plant material using hexane as collecting solvent yielded a yellow oil.

4.4.2 Preliminary Investigation of the Oil.



73 **74**

Fig. 53: Structures of Cyclosativene (**73**) and Sativene (**74**).

The essential oil of *Cyperus papyrus* first analysed by GC (fig. 54) and GC-MS which allowed the identification of cyclosativene (**73**) [142], α-copaene (**55**), sativene **(74)** (fig. 53) [143], cyperene (**35**) and rotundene (**58**). However, some minor hydrocarbons could not be identified and needed to be isolated for further investigation.

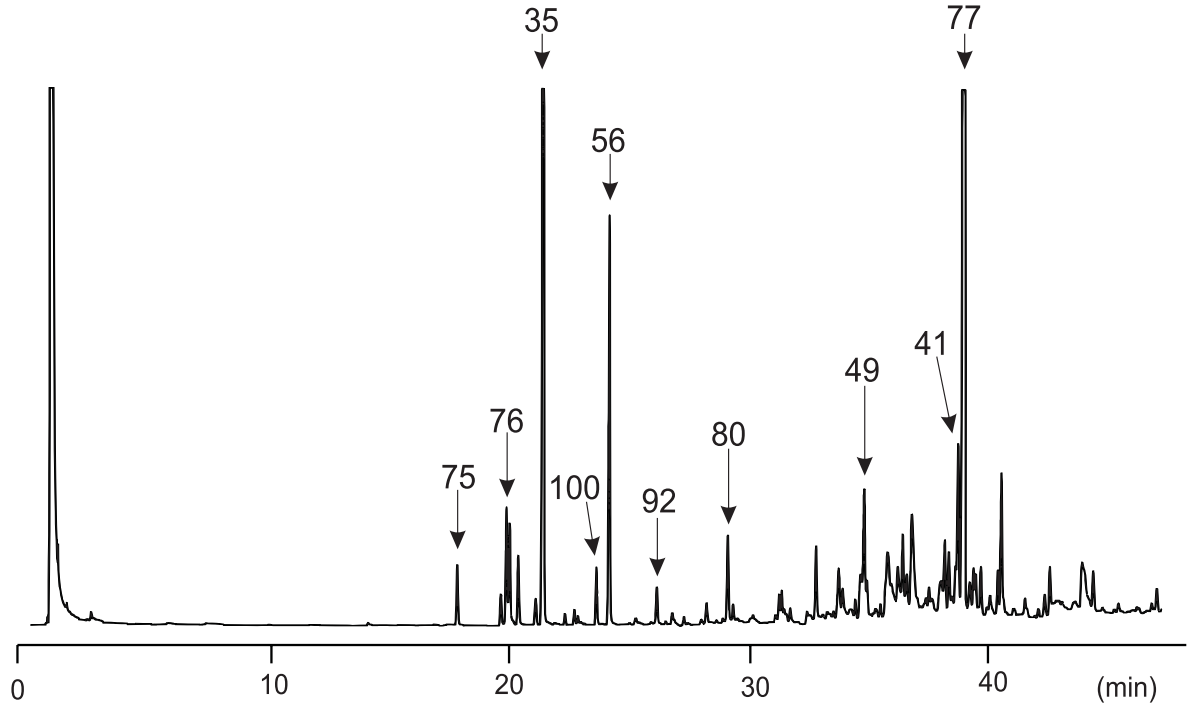


Fig. 54 : Analytical gas chromatogram of the essential oil of *Cyperus papyrus* on a 25m CP-sil 19 CB column. Temp. program: 50°C; 3° / min to 230°C.

4.4.3 Isolation and Characterisation of Cyprotene (75).

Cyprotene (**75)** was isolated from the essential oil of *C. papyrus* by preparative gas chromatography. The compound was assigned the molecular formula $C_{14}H_{24}$ ($[M]^+$, m/z = 192) which is that of a norsesquiterpene. The interpretation of the ^{13}C-NMR, DEPT, HMQC and ^{1}H-NMR spectra (fig. 55) enabled the assignment of all proton and carbon signals. The fourteen carbon atoms were assigned by ^{13}C-NMR and DEPT spectra, two of which being olefinic (δ 115.7 and 145.6), five methyl, three methylene, three methine groups and three quaternary carbon atoms. The ^{1}H-NMR spectrum exhibited three tertiary methyl groups (δ 0.87, 0.90 and 1.04), one secondary methyl group (δ 0.83), one olefinic methyl group

(δ 1.72), and an olefinic proton (δ 5.19). The ^{1}H- and ^{13}C-NMR signal assignments, achieved by ^{1}H-^{1}H-COSY and ^{1}H-^{13}C two-dimensional correlation techniques, allowed the assumption of three substructures *a, b* and *c*. The olefinic proton at δ 5.13 is geminal to the methyl group at δ 1.72 and couples with CH_2-6 which again couples with the methine CH-5, leading to the substructure *a*: CH_3-CH=C(C)-CH_2-C(C)H-.

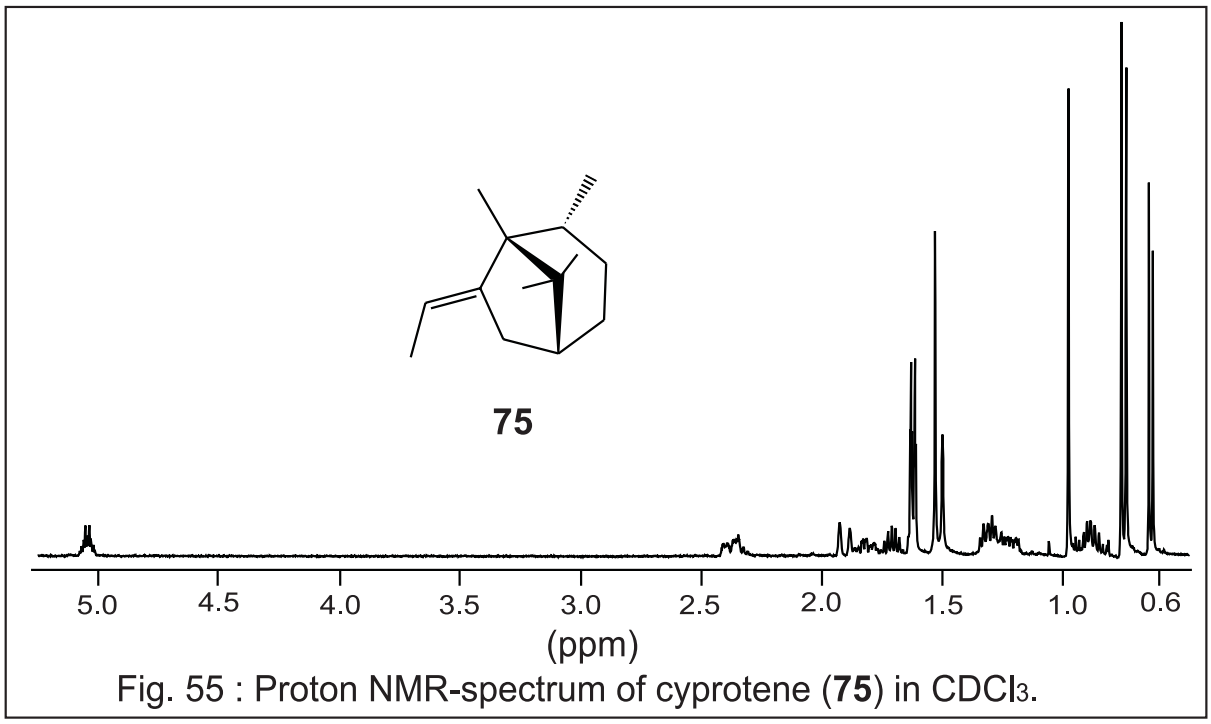


Fig. 55 : Proton NMR-spectrum of cyprotene (**75**) in $CDCl_3$.

The methine proton at δ 1.72 (vicinal to the methyl group at δ 0.83) couples with CH_2-3 connected itself to another methylene CH_2-4 which again couples with the methine CH-5, giving the substructure *b*: CH_3CH(C)-CH_2-CH_2-CH(C)-. The ^{1}H-^{1}H-COSY spectrum shows a coupling correlation between the two methyl groups at δ 0.87 (CH_3-10) and 1.04 (CH_3-11), suggesting that they are geminal groups. Moreover, long-range coupling correlations were observed between these two methyl protons and the two carbon atoms (δ 43.1, C-5 and δ 51.1, C-1) giving the substructure *c:* $(CH_3)_2$C(CH)-C-CH_3. In combining this

information with that of the ^{1}H-^{13}C long range connectivity diagram which shows that the methyl protons (δ 0.90, CH_3-12) were located three bonds from the olefinic quaternary carbon (δ 145.7, C-7) and also three bonds from the tertiary carbon atom (δ 34.7, C-2), structure **75** was proposed for cyprotene. **75** has been isolated previously from the essential oil of *C. rotundus* [144].

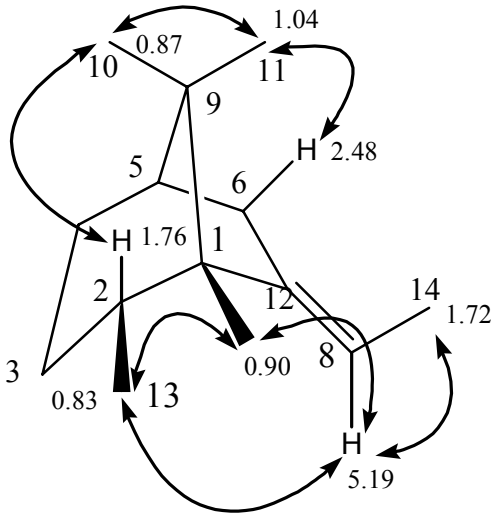


Fig. 56 : Proton-proton spatial correlations from the NOESY diagram of **75**.

The relative configuration was assigned by using two important sets of correlations of the NOESY spectrum (Fig. 56). The olefinic proton H-8 shows strong correlations to the C-13 methyl protons (δ 0.83) and also to the C-12 (δ 0.90) and C-14 (δ 1.72) methyl protons. Furthermore, the methine proton H-2 (δ 1.76) is correlated to the C-10 and C-12 methyl protons δ 0.87 and δ 0.90. **75** is most likely a biogenetic degradation product or precursor of cyperene (**35**), the most abundant sesquiterpene hydrocarbon in most *Cyperus* species. Therefore, the absolute configuration of **35** and **75** is most probably identical.

4.4.4 Isolation and Structure Elucidation of Cypera-2,4-diene (76).

The following unknown compound isolated from the oil of *C. papyrus* was also a sesquiterpene hydrocarbon of molecular formula $C_{15}H_{22}$ (EIMS, $[M]^{+}$, m/z = 202). The ^{13}C-NMR and DEPT spectra permitted the identification of all fifteen carbon atoms along with the presence of four methyl groups, three methylene groups, four methine groups, two of which being olefinic,

and four quarternary carbon atoms, two of which being olefinic, too. The ^{1}H-NMR spectrum (fig. 57) displays some cyperene type pattern, i.e. one secondary methyl group (δ 0.55), two tertiary methyl groups (δ 0.51 and δ 1.12) and an olefinic methyl group (δ 1.73).

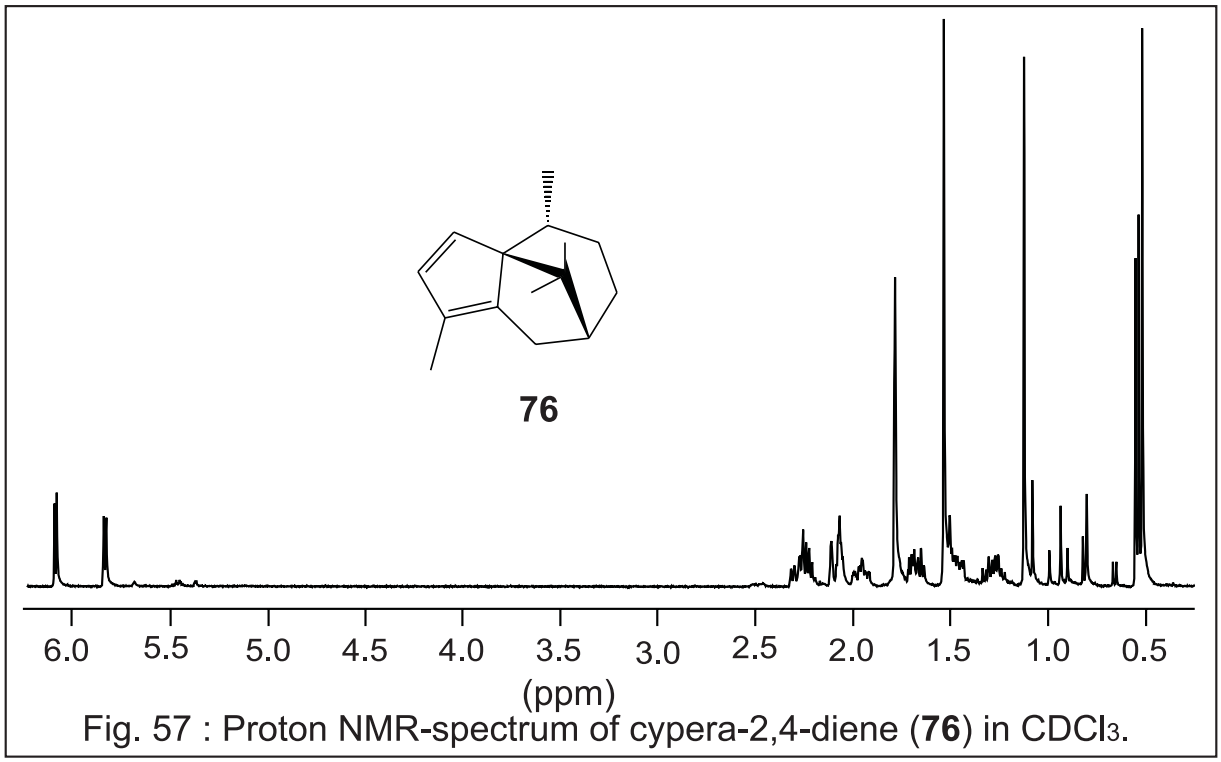


Fig. 57 : Proton NMR-spectrum of cypera-2,4-diene (**76**) in $CDCl_3$.

Important is the presence of two olefinic protons coupling with each other (δ 5.82 and δ 6.09). The value of the coupling constant (J = 5.08 Hz) also suggests the presence of a five-membered ring substructure in the molecule. Thus, structure **76** was derived from the ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-HMQC spectra. The ^{1}H-^{1}H-COSY diagram shows a coupling correlation between the vinylic methyl group (δ 1.73, CH_3-15) and the methylene protons (δ 2.10 and δ 2.30), itself correlated to the methine proton CH-7 (δ 2.08), leading to the substructure $CH_3C(C)=C(C)-CH_2-CH-$. The methine proton CH-10 (δ 2.25) couples with the methylene protons CH_2-9 (δ 1.27 and δ 1.68) which couples with another

methylene CH_2-8 (δ 1.95 and δ 1.68) itself coupled to the methine proton CH-7 (δ 2.08), giving the substructure CH_3-CH(C)-CH_2-CH_2-CH-.

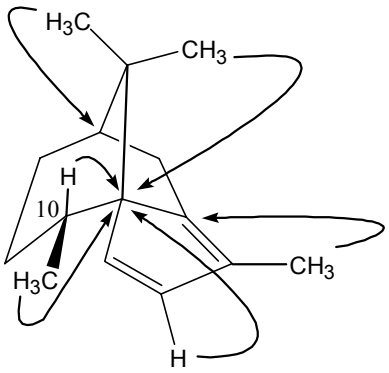


Fig. 58: ^{1}H-^{13}C long-range correlations from the HMBC diagram.

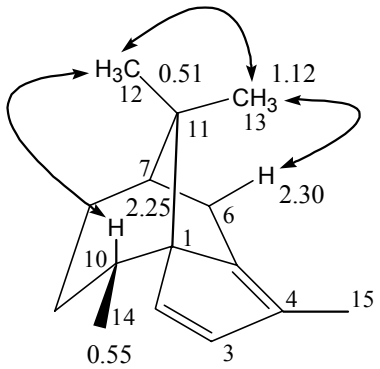


Fig. 59: Proton-proton correlations from the NOESY diagram.

In combining all these informations, structure 7**6** which proved to be in accordance with the HMBC diagram for the ^{1}H-^{13}C long-range connectivities was assigned (Fig. 58). The relative configuration of molecule resulted from spatial interactions shown by the NOESY diagram (fig. 59). The complete structure of **76** was established by its partial synthesis through reduction and subsequent dehydration of the known compound cyperotundone (**77**) [145], (fig. 60). It can be seen in fig. 60 that the dehydration of **78** also yield isopatchoula-3,5-diene (**79**) which has been described in the literature as a constituent of *Cyperus scariosus* [146]. This compound was isolated and identified in the essential oil of *Cyperus papyrus*.

4.4.5 Isolation and Characterisation of Epoxycyperene (80).

Epoxycyperene (**80**) was isolated by preparative gas chromatography from the oil of *C. papyrus*. Its molecular formula $C_{15}H_{24}O$ was derived from its mass spectrum (EIMS, $[M]^+$, m/z = 220). The ^{13}C-NMR spectrum allowed the

identification of the fifteen carbon atoms. The multiplicity was derived from the DEPT spectrum which shows four methyl groups, five methylene groups, two methine and four quaternary carbon atoms. That leads to an elemental composition of $C_{15}H_{24}$, which shows that the product is not an alcohol.

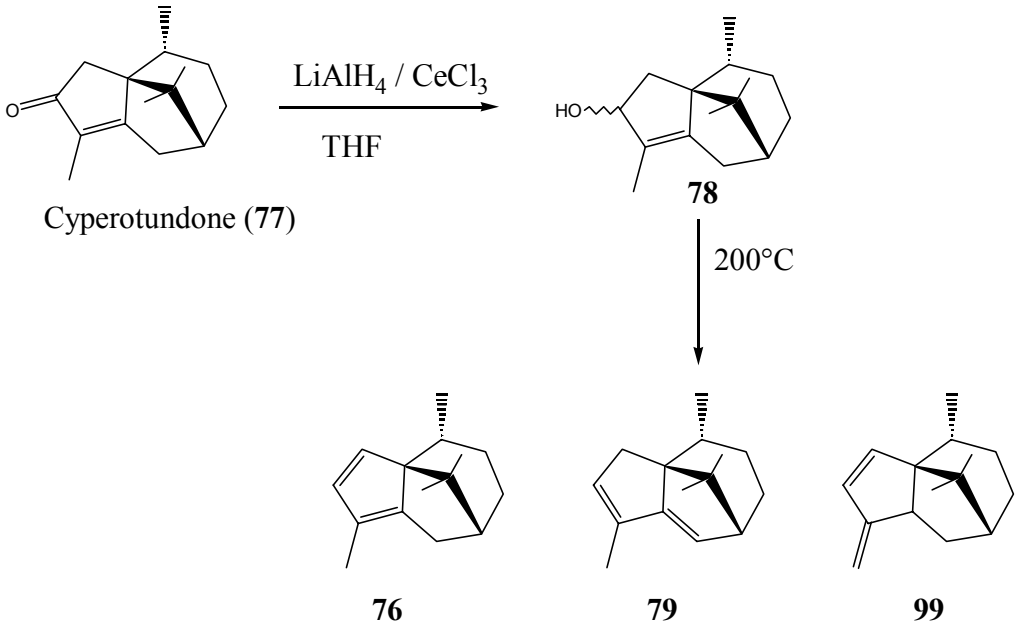


Fig. 60 : Partial synthesis of the patchouladienes **76**, **79**, and **99**.

Moreover, the ^{13}C-NMR spectrum exhibits no carbonyl carbon. Hence, it was to presume that the product is an ether or an epoxide. The ^{1}H-NMR spectrum (fig. 61) displays patchoulane type patterns: one secondary methyl group (δ 1.04), two tertiary methyl groups (δ 0.82 and 0.91) and a fourth methyl group (δ 1.25). Because of the overlapping of the other proton signals, it was not possible to extract more information from the ^{1}H-NMR spectrum. However, the fact that the compound was most likely an epoxide with a patchoulane type skeleton allowed the assumption that the compound is epoxycyperene (**80**). To verify this conclusion, epoxycyperene was prepared by epoxydation of cyperene (**35**) (fig. 62). The product showed identical spectroscopic data (MS and ^{1}H-NMR) as the

isolated compound.

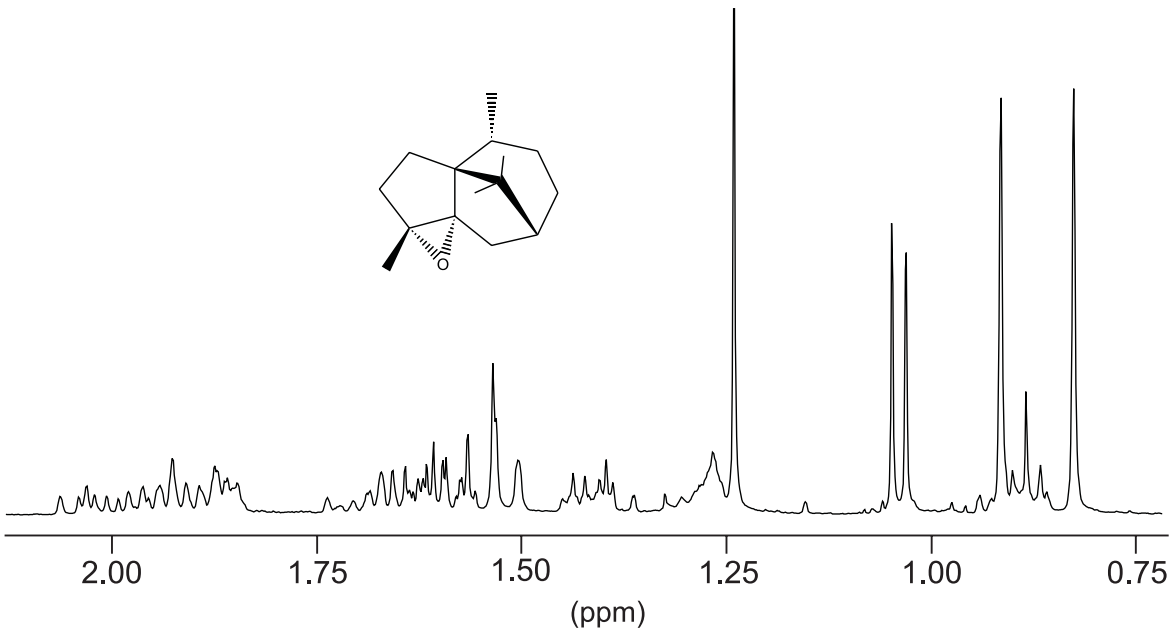


Fig. 61 : Proton NMR-spectrum of epoxycyperene (**80**) in $CDCl_3$.

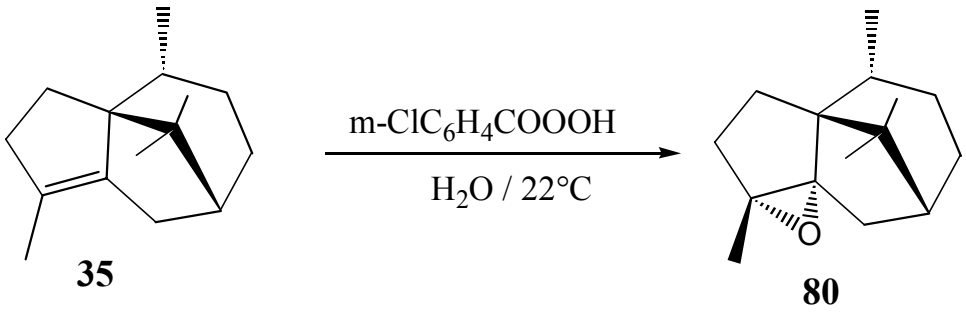


Fig. 62 : Expoxydation of cyperene (**35**).

4.4.6 *Proposition of a Biogenetic Pathway for Cyperene* (35).

Although cyperene (**35**) has been isolated several years ago no study of its biosynthesis has been reported. As major hydrocarbon component of the essential oils of the *Cyperus* species, it would be interesting to know how cyperene is produced in the plant. A biogenetic process is proposed for

cyperene which involves two successive cyclisations of FDP leading to α-guaiene. Subsequent protonation followed by a deprotonation yields the hydrocarbon cyperene (**35**) (fig. 63).

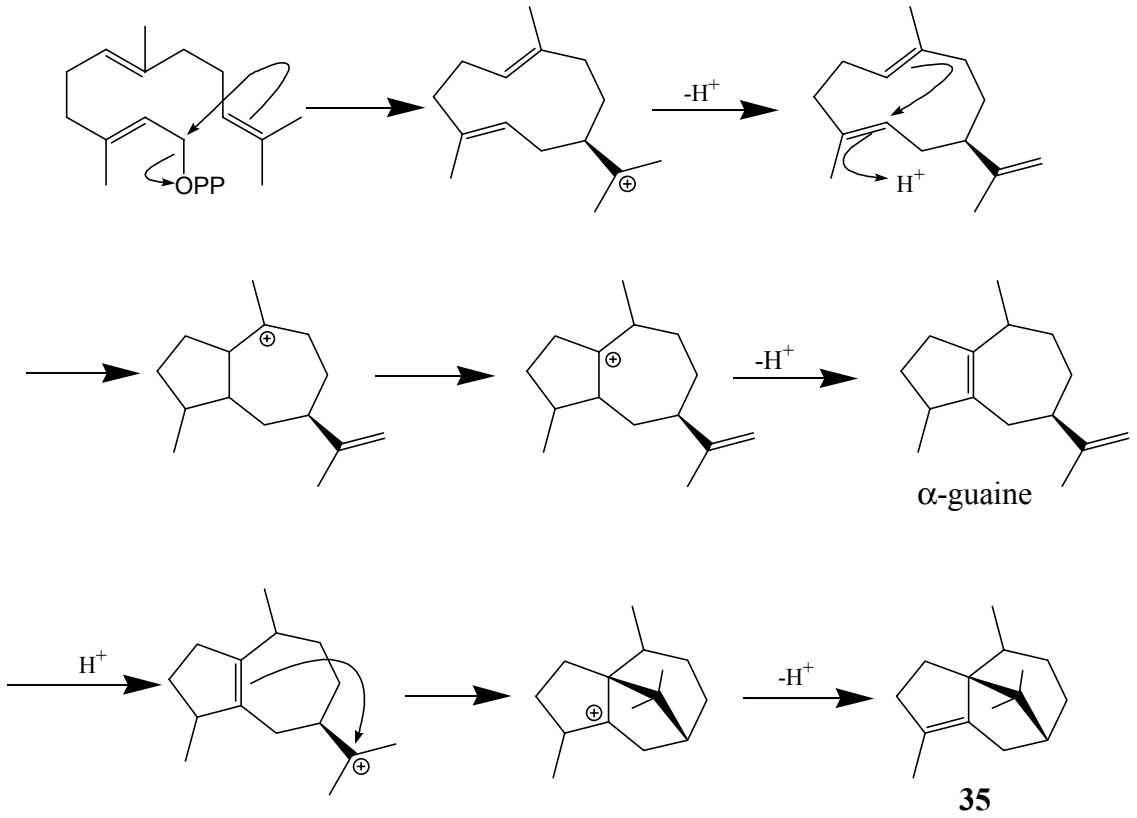


Fig. 63: Proposed biogenetic pathway for Cyperene (**35**).

4.5 Chemical Investigation of *Cyperus rotundus*.

4.5.1 The Plant.

Cyperus rotundus is a perennial plant whose culms are often solitary or a few together and bearing a corn-like enlargement at the base, erect, 10-40 cm tall, slender, 1-2 mm wide triquerous, smooth and leaved at its base. Leaves are few and usually shorter than the culm, blades linear, 2-5 mm wide, flattish, gradually narrowed to acuminate apex; sheaths light brownish, soon disintegrating into brown parallel fibres. It is a cosmopolitan weed seen in tropical, subtropical and temperate regions all over the world. In Asia *C. rotundus* appears among Indian, Chinese and Japanese natural drugs, and in Ceylon the same use is known. To prepare the herbal medicine the tubers with fibrous roots removed by burning are roasted lightly and then ground into powder. Well-dried rhizomes produce a white powder, which is of good quality. This is used as a remedy for spasms or as an emenagogue [147].

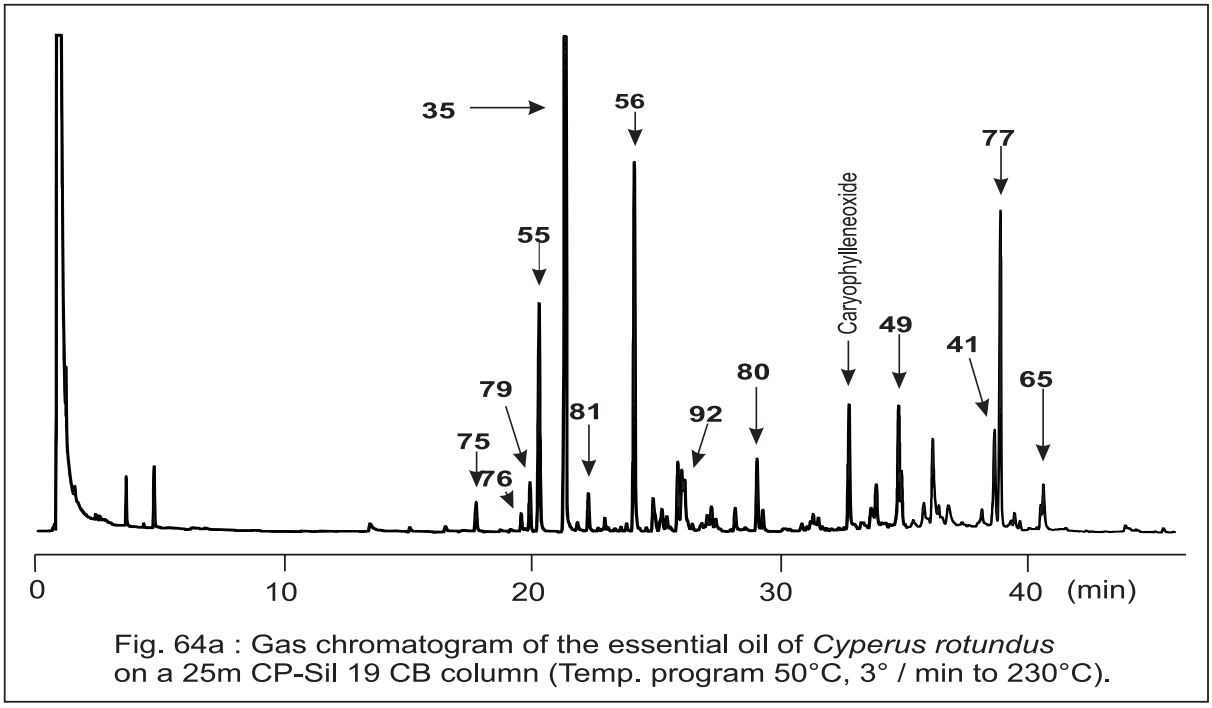


Fig. 64a : Gas chromatogram of the essential oil of *Cyperus rotundus* on a 25m CP-Sil 19 CB column (Temp. program 50°C, 3° / min to 230°C).

4.5.2 Preliminary Study of the Oil.

The essential oil of *Cyperus rotundus* was submitted to GC and GC-MS analysis and many sesquiterpene hydrocarbons were identified.

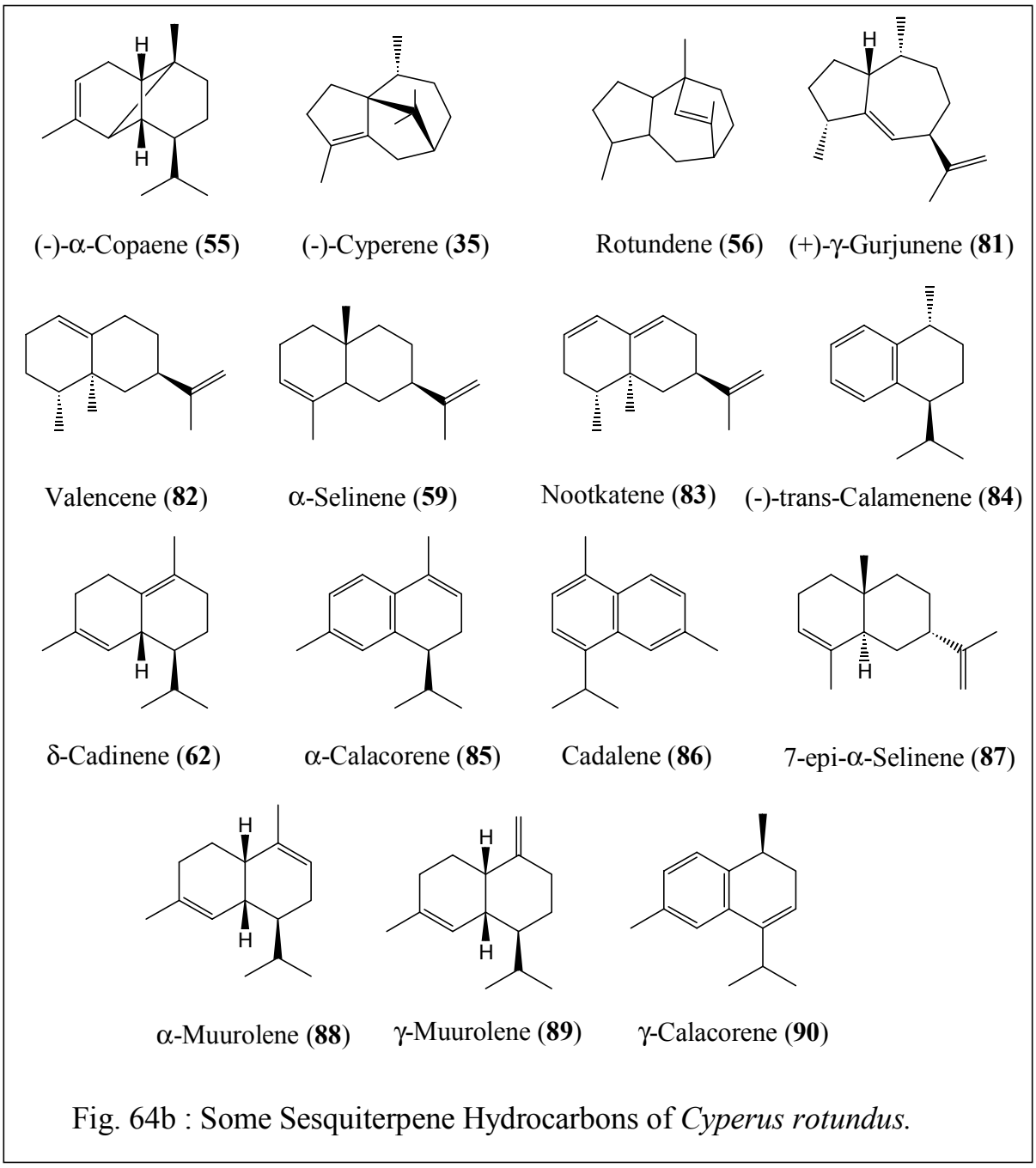


Fig. 64b : Some Sesquiterpene Hydrocarbons of *Cyperus rotundus.*

α-Copaene (**55**), cyperene (**35**), rotundene (**56**), γ-gurjunene (**81**) [148],

valencene (**82**) [149], α-selinene (**59**), nootkatene (**83**) [150], trans-calamenene (**84**) [151], δ-cadinene (**62**), γ-colacorene (**85**) [152], cadalene (**86**) [153], epi-α-selinene (**87**) [154], α-muurolene (**88**) [155], γ-muurolene (**89**) [156], γ-calacorene (**90**) [157]. The decision to continue the investigation of the oil was prompted by the presence of some minor unknown constituents.

4.5.3 Isolation and Identification of (+)-Ylanga-2,4(15)-diene (91).

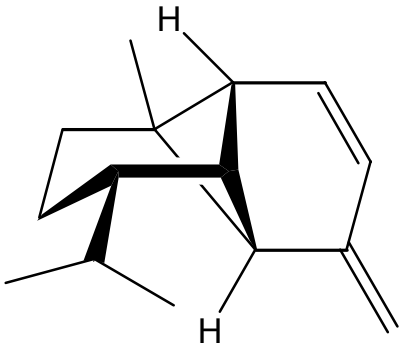


Fig. 65 : (+)-Ylanga-2,4-diene (**91**).

The product identified as **91** (fig. 65) was isolated from a hydrocarbon fraction of the essential oil by combination of preparative TLC with silver nitrate precoated plates and preparative gas chromatography. Since the structure of (+)-ylanga-2,4(15)-diene was established mainly by its similarity to α-copaene **(55**) [154], the spectral data shall be briefly discussed. The compound has the molecular formula $C_{15}H_{22}$ in agreement with its mass spectrum ($[M]^+$, m/z = 202). The ^{13}C-NMR and DEPT spectra permitted the identification the fifteen carbon atoms. The two methyl groups of the isopropyl resdue appear at δ 0.80 and the third methyl group appears at δ 0.94. At δ 6.17 and δ 6.20 the signal of two olefinic protons of H-2 and H-3 are observed. Their coupling constant (J = 8.51 Hz) shows that they belong to a six-membered ring [155]. The protons of the exocyclic methylene group absorb at δ 4.75 and δ 4.77. The signals of the protons H-1 and H-5 are at δ 2.20 and δ 2.23. The large value of their coupling constant (^{4}J = 7.12 Hz) is characteristic of cis protons located on opposite vertices of a cyclobutane ring [156].

4.5.4 Isolation and Structure Elucidation of Isorotundene (92).

Isorotundene (**92**) was also isolated from a hydrocarbon fraction (fig. 66) by combination of preparative TLC over silver nitrate precoated plates and preparative GC.

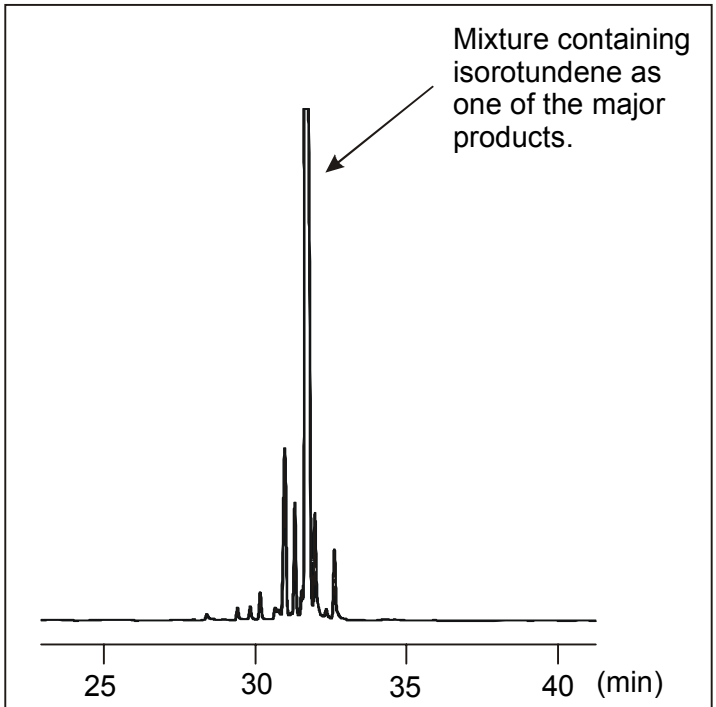


Fig. 66 : Gas chromatogram of the sample containing isorotundene (**92**).

To isorotundene was assigned the molecular formula $C_{15}H_{24}$ (EIMS, $[M]^+$, m/z = 204). The ^{13}C-NMR spectrum shows fifteen carbon signals, two of which are olefinic (δ 107.9 and 151.08). Through the DEPT spectrum we identified two methyl groups, seven methylene groups, four methine and two quaternary carbon atoms. The compound has four unsaturations and only one double bond, therefore it was concluded that it contains three rings. The ^{1}H-NMR (fig. 67) and ^{13}C-NMR signals assignment, achieved by ^{1}H-^{1}H-COSY and HMQC, correlations afforded to three substructures. The methine proton δ 1.99 couples with the methine proton at δ 1.90 which is vicinal to methyl group at δ 0.86. The last methine proton also couples with the methylene group CH_2-3 (δ 1.22 and δ 1.68) itself coupled to another methylene group CH_2-2 (δ 1.46 and δ 1.52) which further couples with the methine proton at δ 1.81 giving the partial structure CH-CH(CH_2)-CH_2-

CH_2-CH-. The methine proton at δ 1.99 also couples with a methylene group CH_2-6 (δ 1.00 and δ 1.70) itself coupled to the methine proton CH-7 (δ 2.52). The proton H-7 also couples with the methylene group CH_2-8 (δ 1.05 and δ 1.71) which again couples with CH_2-9 (δ 1.44 and δ 1.72) to give the second substructure -CH-CH_2-CH-CH_2-CH_2-. The methylene group CH_2-11 (δ 2.14 and 2.52; δ 2.27) and the olefinic one at δ 5.85 couple to each and give the third substructure -CH_2-C=CH_2. The proton-carbon long-range coupling correlations given by the HMBC diagram helped to identify the skeleton of the compound. Long-range coupling correlations were found between the protons CH_2-11, CH_2-13 and the carbon C-7. The protons CH_2-6, CH-5 and CH-4 showed some correlations with the carbon C-1. The combination of all these informations led to a rotundane skeleton with an exocyclic double bond. The relative stereochemistry at the chiral centres C-1, C-4, C-5, C-7 and C-10 was obtained from the NOESY diagram.

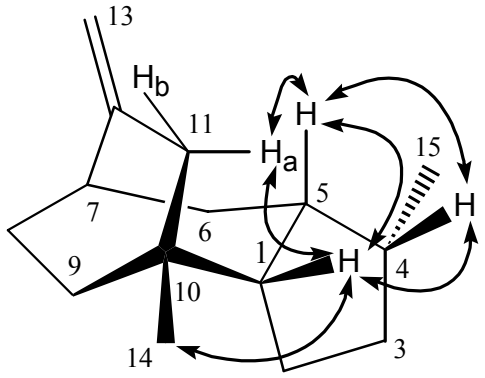
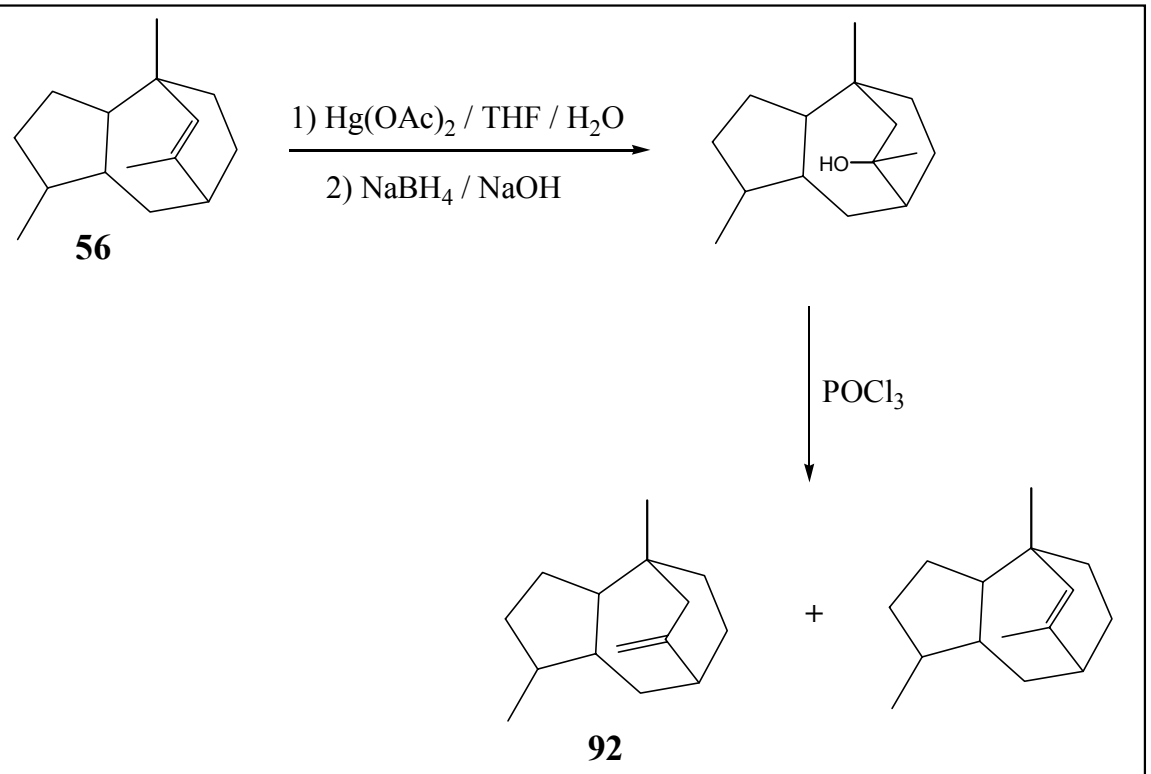


Fig. 67 : Relative configuration of isorotundene (**92**) with the observed NOESY correlations.

The correlations observed between the protons H-1, H-4 and H-5 prove that the three protons are on a same side of the molecule. Furthermore the protons H-1 and H-5 show spatial interactions with the proton H-11a, what is only possible if H-1 and H-5 are located at the same side with the bridge C-11-C-12. Therefore isorotundene has the relative configuration as shown in fig. 67. To verify that the compound really had a rotundane skeleton we performed a partial synthesis of **92** by oxymercuration-demercuration and subsequent dehydration of rotundene (**56**) (fig. 68). The synthesised compound has the same spectroscopic data as the isolated one.



56

92

Fig. 68 : Preparation of isorotundene (**92**) from rotundene (**56**).

4.5.5 Absolute Configuration of Rotundene (56) *and Isorotundene* (92).

Although rotundene (**56)** has been isolated a long time ago its stereochemistry was not known as yet. However, the fact that isorotundene (**92)** could be obtained from rotundene, as we have seen proves that rotundene has the same relative configuration as isorotundene at all its chiral centres. The relative configuration of rotundene could not be derived from its NOESY diagram as the important signals overlapped. In the ^{1}H-NMR spectrum of rotundene (fig. 69) the signals of the protons H_1, H_4, and H_5 are mixed to other signals so that it is hazardous to extract any information from its NOESY spectrum. But in the case of isorotundene (**92**), the exocyclic double bond does not exert any anisotropic effect on the proton H_1 (as observed in rotundene), causing its signal to remain in higher field. So the signal of H_1 and H_5 do not overlap as they do in the

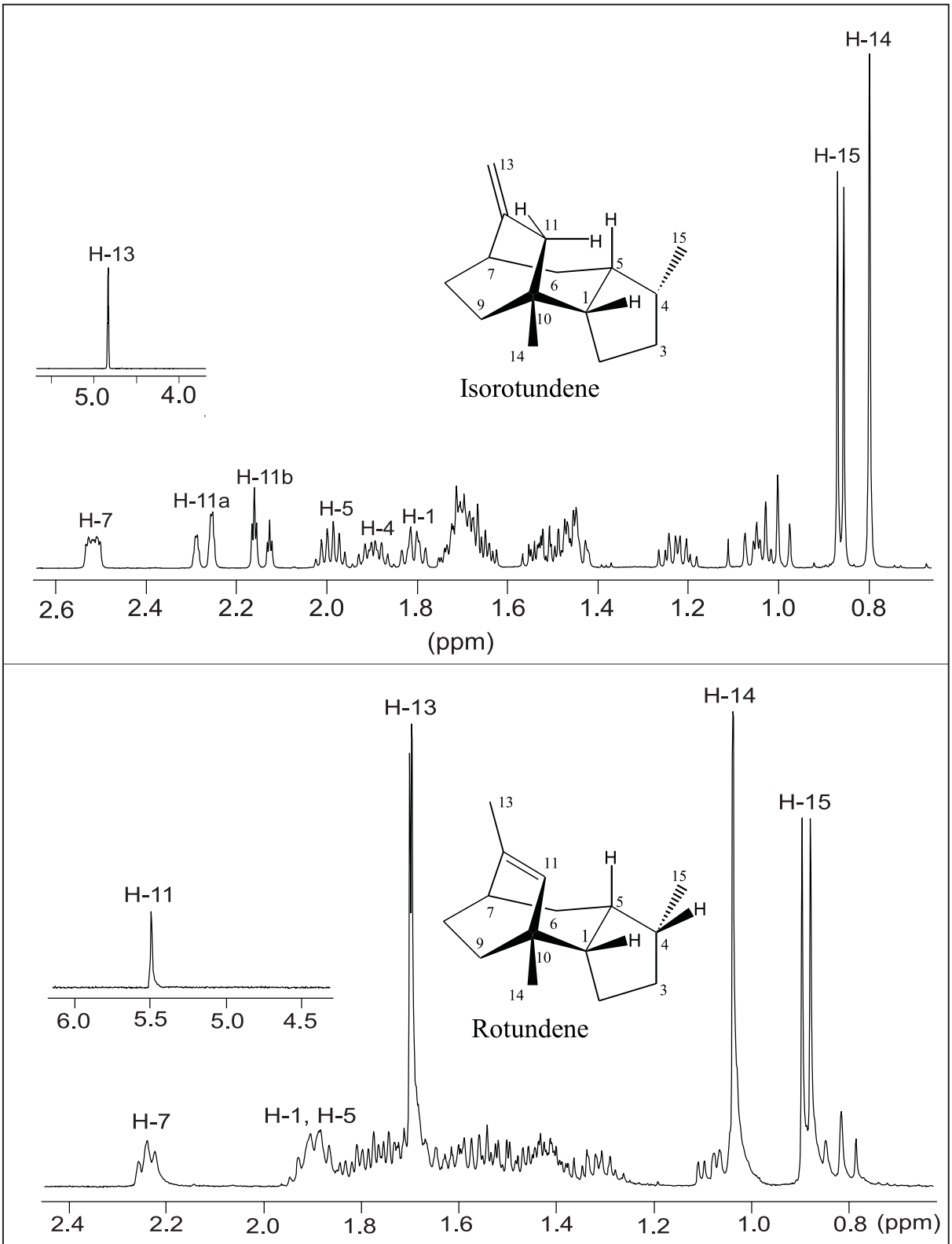


Fig. 69: Comparison of the proton NMR spectra of rotundene and isorotundene. Note the resolution of all the important signals (H-1, H-4, H-5, and H-11) in the spectrum of isorotundene, facilitating the determination of its absolute configuration by the NOESY technique.

spectrum of rotundene (**56**). Moreover, the methylene group CH_2-11 is absent in rotundene, but its presence is decisive for the determination of the relative configuration of isorotundene (**92**) by the NOESY technique. The ^{1}H-NMR spectra of rotundene and isorotundene are compared in fig. 69.

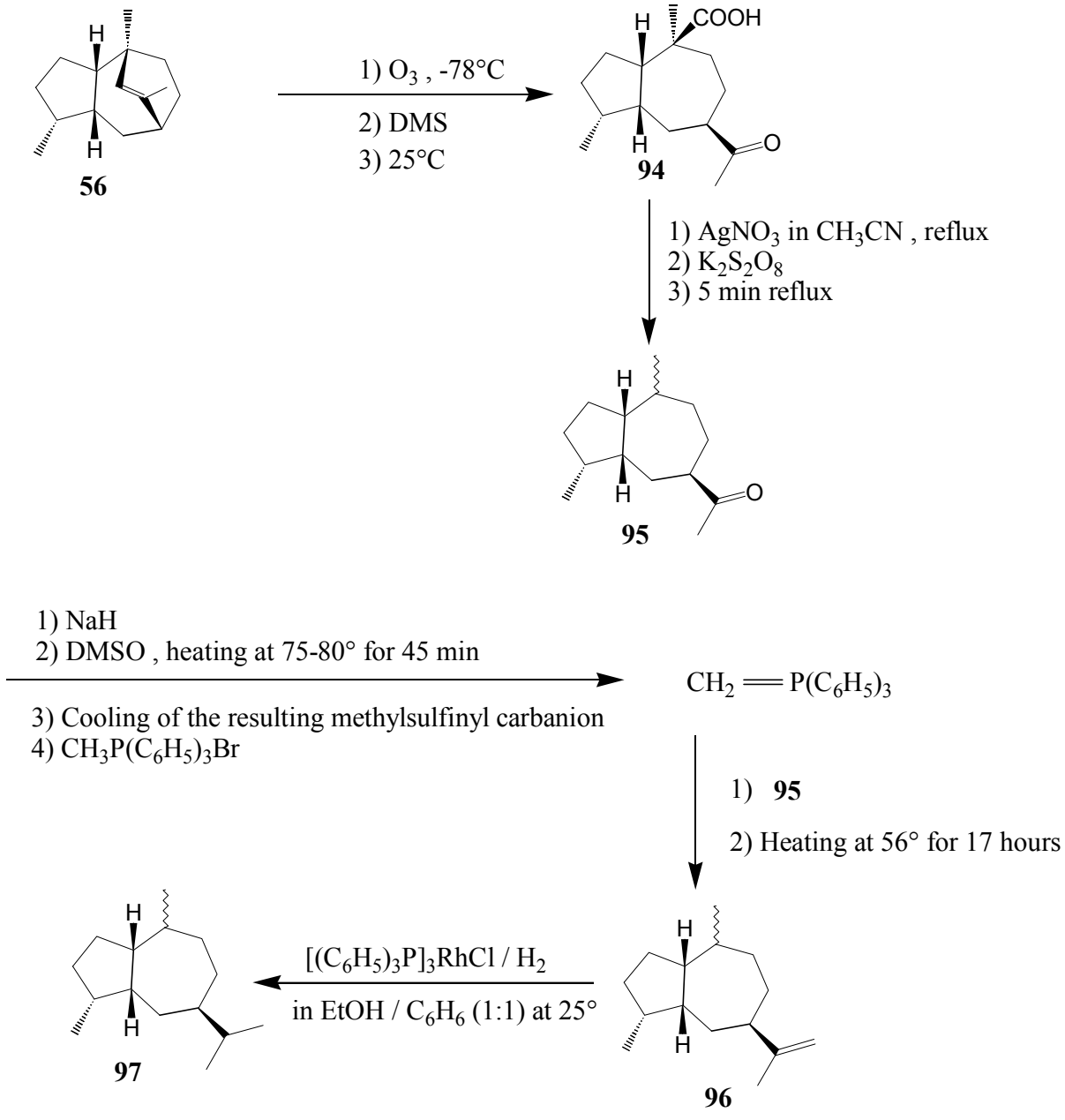


Fig. 70 : Scheme of reactions leading to the hydrocarbon **97**.

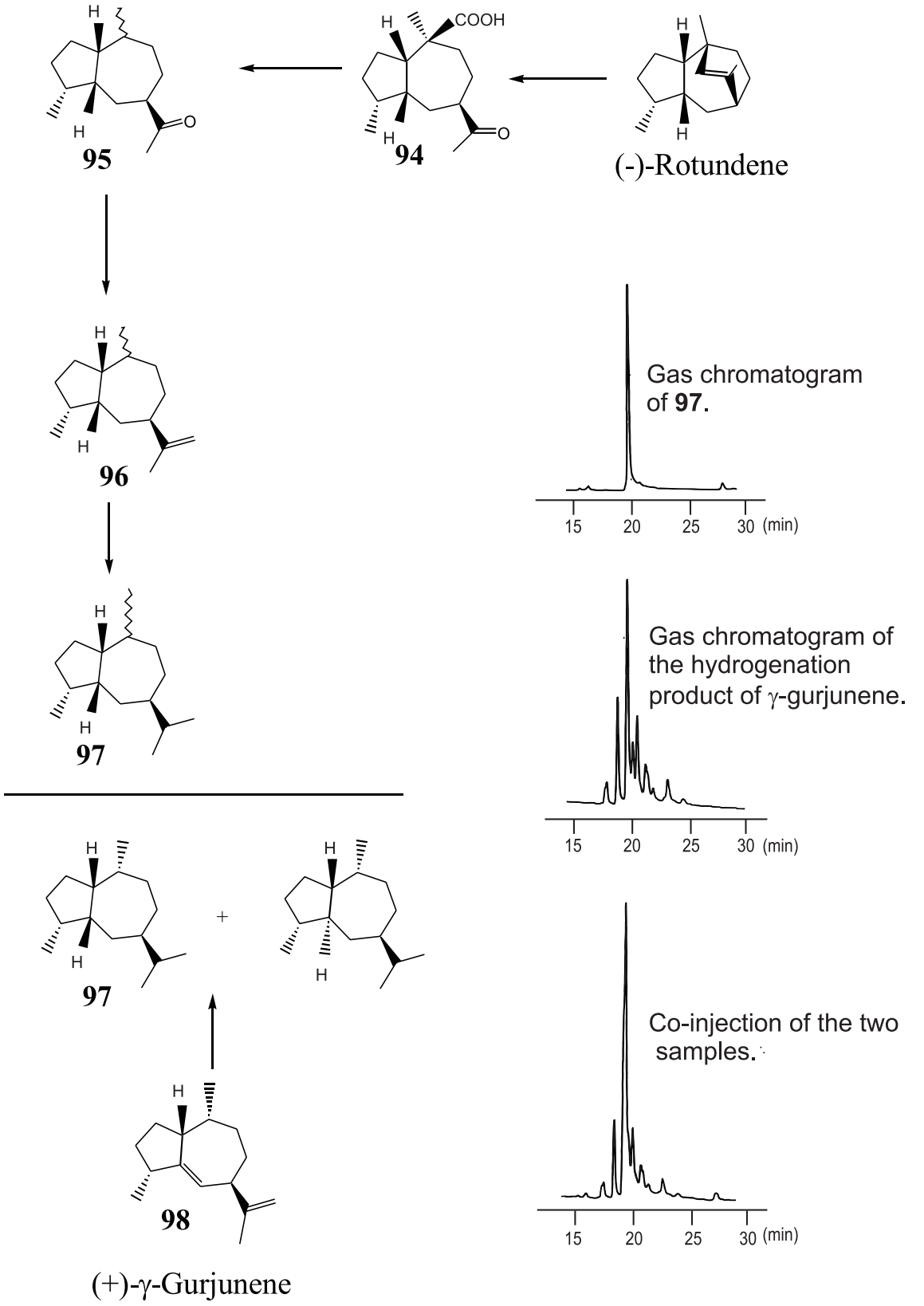


Fig. 71 : Chemical transformations of rotundene and GC comparison with the hydrogenation products of (+)-γ-gurjunene.

In order to determine the absolute configuration of the two compounds a chemical transformation of rotundene was performed (fig. 70) to correlate it with (+)-γ-gurjunene (**98**). Rotundene (**56**) was submitted to ozonolysis followed by reduction with dimethyl sulfide under nitrogen. The desired aldehyde was not stable and oxydised directly to ketoacid **94** which could be isolated. The acid **94** was then decarboxylated [159] to give the ketone **95**. Futhermore, **95** was allowed to react with methylentriphenylphosphine in dimethyl sulfoxide [160] to yield the hydrocarbon **96** which was hydrogenated [161] to give compound **97**. Compound **97** was finally compared by enantioselective gas chromatography with the fully hydrogenated products of (+)-γ-gurjunene (**98**) and proved to have the same retention time with the major hydrogenation product (fig. 71). The co-injection was made in a column which separate (+)- and (-)- γ-gurjunene (fig.72).

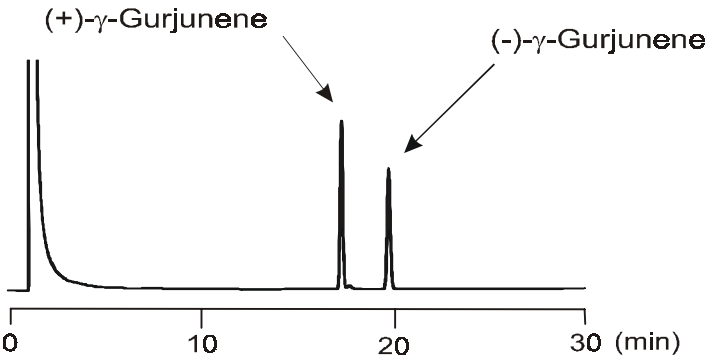


Fig. 72 : Separation of (-)-γ-gurjunene and (+)-γ-gurjunene by capillary gas chromatography. The separation was achieved with 6T-2,3-Me-β-CD at 100°C.

Unfortunately the small quantity of (-)-γ-gurjunene (which was isolated from the liverwort *Marchantia polymorpha*) was insufficient for hydrogenation and comparison with **94**. However, it is very probable that the used column would have separated also the hydrogenation products of the two enantiomers.

Therefore, it is very likely that rotundene has the absolute configuration **93**.

4.5.6 Isolation and Structure Elucidation of Cypera-2,4(15)-diene (99).

The isolation of cypera-2,4(15)-diene (**99**) resulted from the combination of column chromatography and preparative GC. The compound was assigned the molecular formula $C_{15}H_{22}$ (EIMS, $[M]^+$, M/z = 202). The ^{13}C-NMR spectrum combined with the DEPT technique revealed three methyl groups, four methylene groups, one being olefinic (δ 102.84), five methine, two of which are olefinic (δ 133.26 and δ 139.17) and three quaternary carbon atoms one of which is olefinic (δ 148.94).

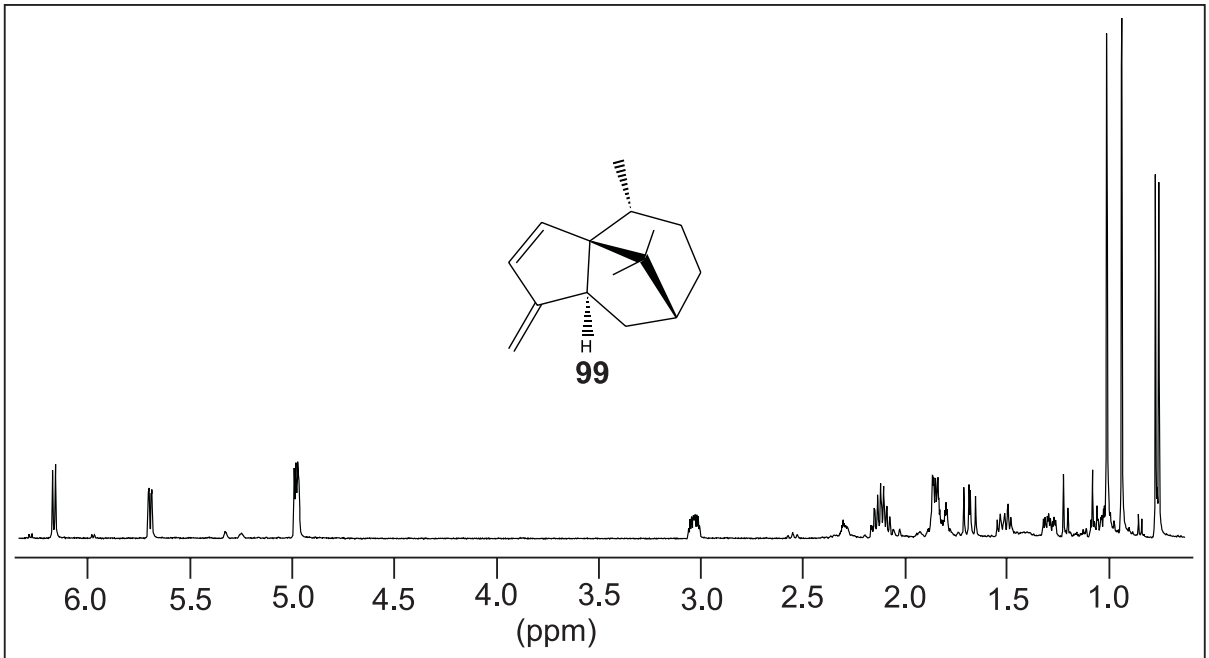
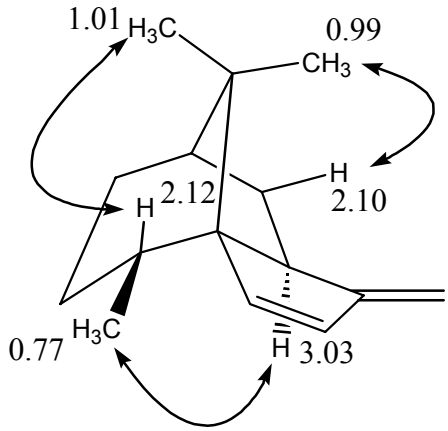


Fig. 73 : Proton NMR-spectrum of cypera-2,4(15)-diene in C_6D_6.

The proton NMR-spectrum (fig. 73) displays some signals common to patchoulane sesquiterpenes, namely the two methyl groups H-13 and H-14 (δ 0.99 and δ 1.01) which are coupled to each other (W-coupling), the methyl group at δ 0.77 appearing as a doublet coupling with the methine proton H-10 (δ 2.12). The vinylic proton H-2 (δ 6.17) couples with another vinylic proton

H-3 (δ 5.69) itself coupled to the olefinic methylene group H-15 (δ 4.98) and also to the methine proton H-5 (δ 3.03) giving the substructure -CH=CH-C(CH)=CH_2. Moreover the value of the coupling constant between H-2 and H-3 (J = 5.60 Hz) indicates that they belong to a five-membered ring. The methine proton H-5 also couples with the methylene group CH_2-6 (δ 1.68 and δ 2.10) which further couples with the methine proton H-7 (δ 1.85) itself coupled to the methylene group CH-8 (δ 1.51 and δ 1.86) which again couples with the methylene group CH_2-9 (δ 1.03, δ 1.29) itself coupled to the methine proton C-10 (δ 2.12). From these results the substructure CH-CH_2-CH-CH_2-CH_2-CH- is derived.

All these informations from the ^{13}C-NMR- and DEPT as well as the ^{1}H-^{1}H-COSY allowed to propose structure **99** for the compound. Its relative configuration resulted from the NOESY diagram which shows NOE correlations between H-5, H-9 and H-14 at one side and between H-13 and H-10 at the other (fig. 74). **99** was formerly found as a by-product during the formation of **76** and **79** from cyperotundone (**77**).



99

Fig. 74 : Important NOESY correlations.

4.5.7 Isolation and Characterisation of Norrotundene (100).

The isolation of **100** was performed by the combination of many processes including CC at low temperature (-20° C), CC over silver nitrate precoated silica, TLC and preparative GC. The compound has the molecular formula $C_{14}H_{22}$ in agreement with its mass spectrum (EIMS, $[M]^+$, m/z =190).

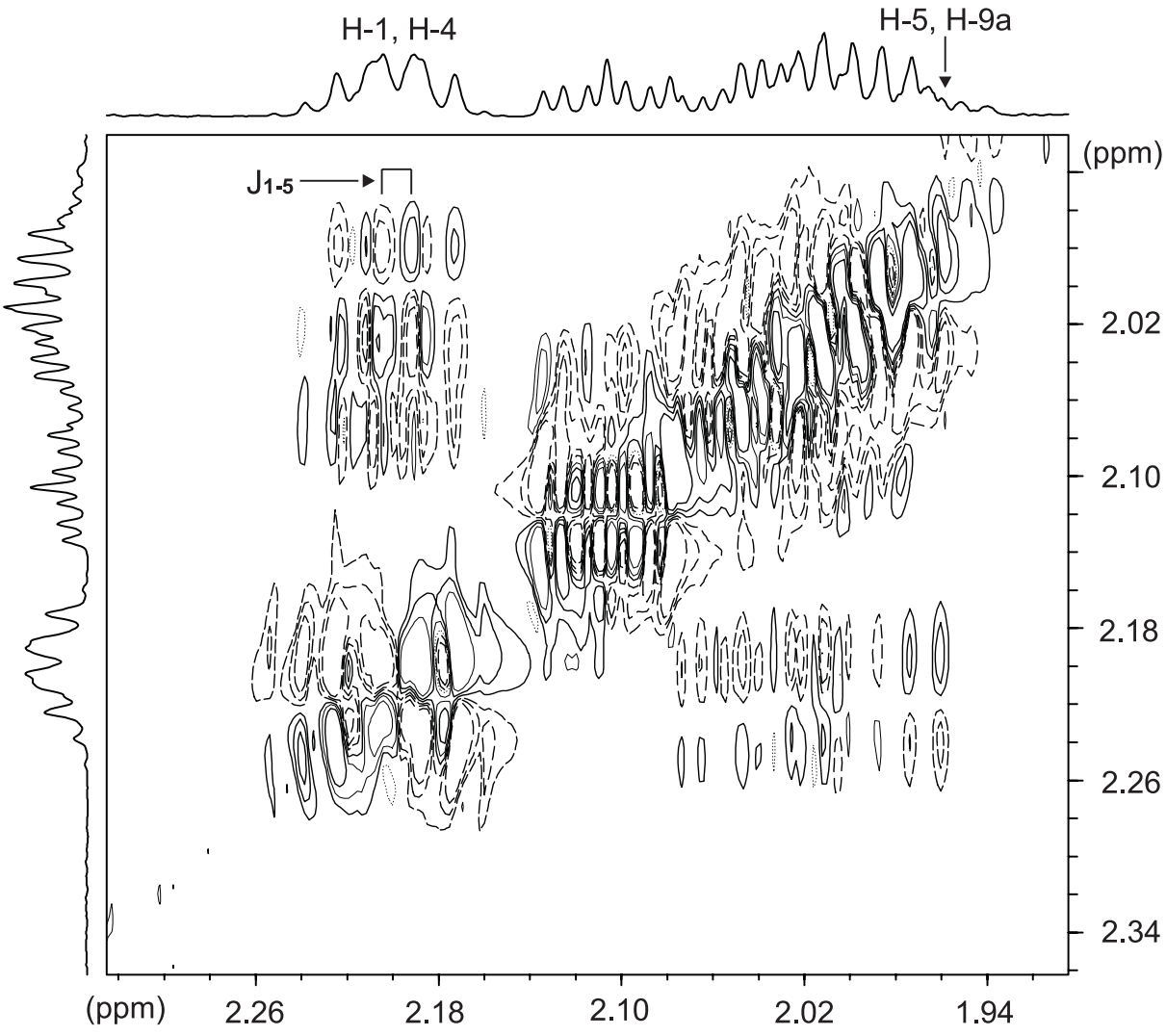


Fig. 75 : Part of the phase sensitive p-p-COSY spectrum of norrotundene. Although the signals of H-1and H-4 the as well as those of H-5 and H-9 overlap, the scalar coupling constant between H-1 and H-5 could be extracted from their cross peak.

The ^{13}C-NMR and DEPT spectra revealed 14 carbon atoms, two methyl groups,

five methylene groups, six methine groups from which two are olefinic (δ 129.15 and δ 139.10) and one quaternary carbon atom. Therefore the compound should be constituted of three rings since it has a total of four unsaturations, one being a double bond. The constitution of the product was derived from interpretation of the ^{1}H-NMR spectrum and the phase-sensitive ^{1}H-^{1}H-COSY spectrum (fig. 75) together with the HMQC and the HMBC diagrams.The methine proton CH-1 (δ 2.20) couples with the methylene group CH_2-2 (δ 1.61 and δ 1.54) which further couples with another methylene group CH_2-3 (δ 1.42 and 1.87) which couples again with the methine proton H-4 (δ 2.10) itself coupled to the methyl group CH_3-14 (δ 0.90) and the methine proton CH-5 (δ 1.80) which further couple with the methine proton H-1 (δ 2.2) and give rise to a five-ring partial structure C_1H-CH_2-CH_2-CH(CH_3)-CH-C_1H-.

Protons	Carbons
H-1	C-2, C-5, C-10
H-5	C-1, C-4, C-6, C-7
H-6	C-4, C-5, C-7, C-12
H-8	C-7, C-9, C-10, C-12
H-9	C-1, C-8, C-10, C-13
H-13	C-1, C-10 C-11
H-14	C-3, C-4, C-5

Table 2: Heteronuclear long-range correlations from the HMBC diagram of norrotundene (**100**).

The methine proton H-5 also couples with methylene group CH-6 (δ 0.95 and δ 1.66) which further couples with the methine proton H-7 (δ 2.56), itself coupled to the methylene group H-9 (δ 1.52 and δ 1.83) to give the substructure CH-CH_2-CH-CH_2-CH_2-. Moreover, the vinylic protons H-11 and H-12 (δ 6.02) are coupled to the methine proton H-7, indicating a partial structure -CH=CH-CH-.

The way the three substructures are linked together was given by the HMBC diagram (table 2).

The relative stereochemistry at the chiral centres of the molecule was not easy to be derived from only the NOESY spectrum. The signals of the two methine protons H-1 and H-4 overlap and hence it is difficult to know which one of them is responsible of the NOE correlation with the proton H-5. However, using the phase-sensitive COSY technique (fig. 75) as well as the gradient selected HMQC method (fig. 76), it was possible to calculate the scalar coupling constant between H-4 and H-5 (J = 5.85 Hz). This value of the coupling constant corresponds (Karplus curve) to an angle of $\Phi = 35°$ between the two protons, which means that they are on the same side of the molecule.

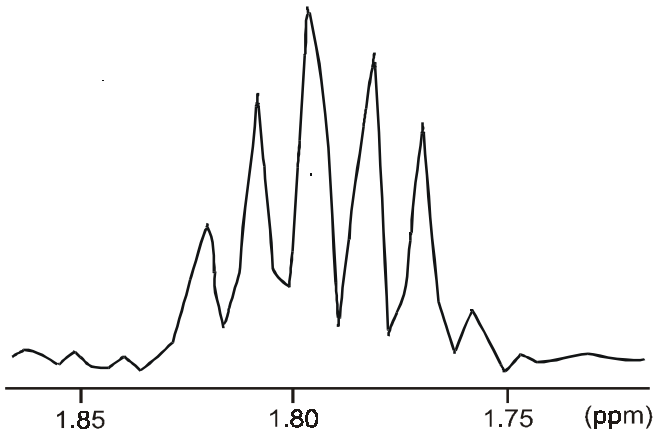


Fig. 76 : Multiplicity of the proton H-5 obtained by the gradient selected HMQC method.

Hence, both of them have spatial interactions with H-1. It remains to investigate the stereochemistry at C-7 and C-10. In the two cases the NOESY diagram could not be of any help because the protons H-1 and H-5 are too far from the vinylic protons H-11 and H-12. The solution came from the ^{1}H-^{1}H-COSY diagram where a long-range coupling correlation is found between the proton

H-1 and one of the H-9 protons. This is a case of ^4J coupling through a σ-bond which requires a particular geometry (W arrangement of the two protons) for the molecule, and this is only possible if the H-1 proton and the bridge C-11-C12 are on the same side of the molecule. Moreover, the axial-axial coupling between H-5 and H-6a is only possible for this stereochemistry of the molecule (fig. 77).

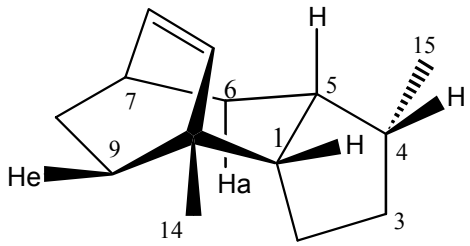


Fig. 77 : Relative configuration of Norrotundene. Note the W-arrangement between H-1 and H-9e, and axial-axial disposition between H-5 and H-6a. These two geometrical patterns which are supported by observed coupling correlations are only possible for this configuration of the molecule.

4.5.8 *Isolation and Structure Elucidation of Cyperadione* (101).

Cyperadione (**101**) was isolated from an oxygenated fraction of *C. rotundus* oil by preparative GC. The compound has the molecular formula $C_{15}H_{24}O_2$ (EIMS, $[M]^+$, m/z = 236). The ^{13}C-NMR- and the DEPT- spectrum indicate that it has four methyl groups, two methine protons and four quaternary carbon atoms, two of which being of carbonyl nature (δ 208.50 and δ 220.25). From the ^{1}H-NMR spectrum (fig. 78) and the ^{1}H-^{1}H-COSY spectrum three substructures were extracted which helped in building up the structure of the product. The methyl group CH_3-14 (δ 0.78) couples with the methine proton CH-10 (δ 2.10) which

couples also with the methylene group CH_2-9 (δ 1.10 and δ 1.68) itself coupled to the methylene group CH_2-8 (δ 1.45 and δ 2.01) which couples further with the methine proton CH-7 (δ 1.90) itself coupled to the methylene group CH_2-6 (δ 1.96; δ 2.48) and thus leading to the partial structure CH_3-CH-CH_2-CH_2-CH-CH_2- . It should be mentioned that the signals of the last methylene group CH_2-6 appearing at lower field indicate that this methylene group is in the proximity of a carbon bearing an oxygen atom.

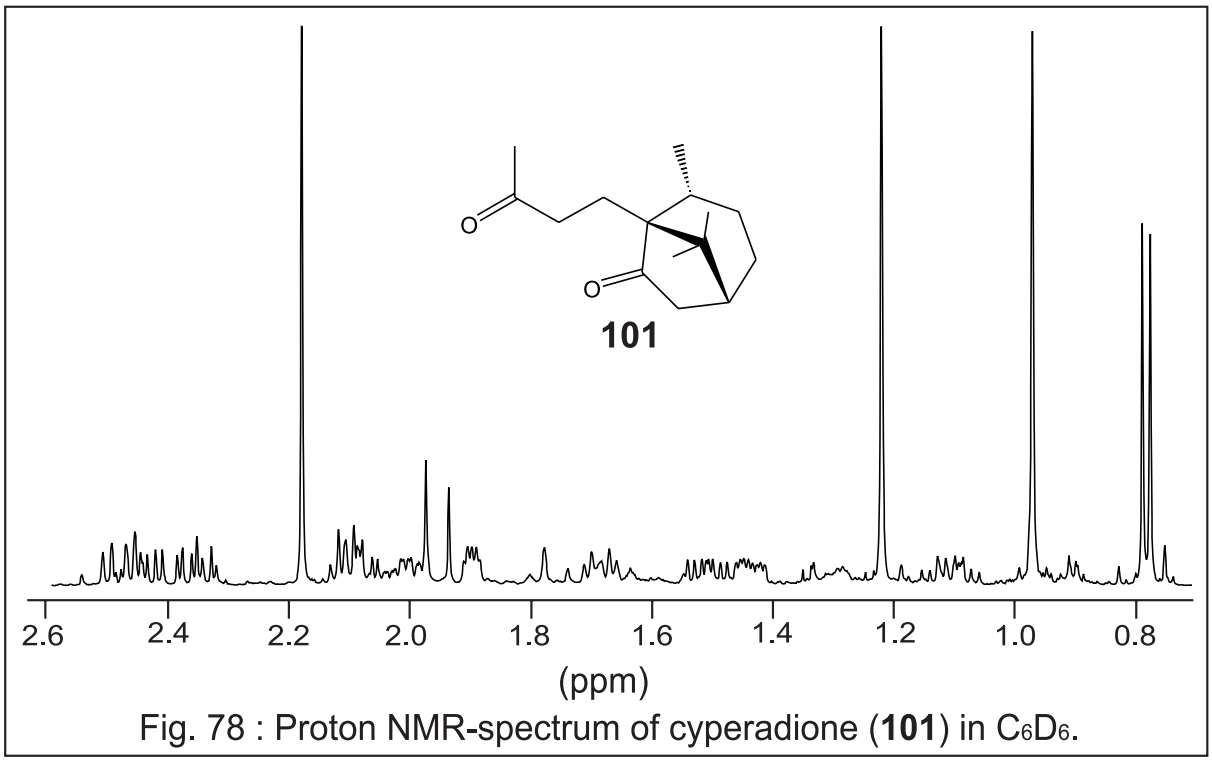


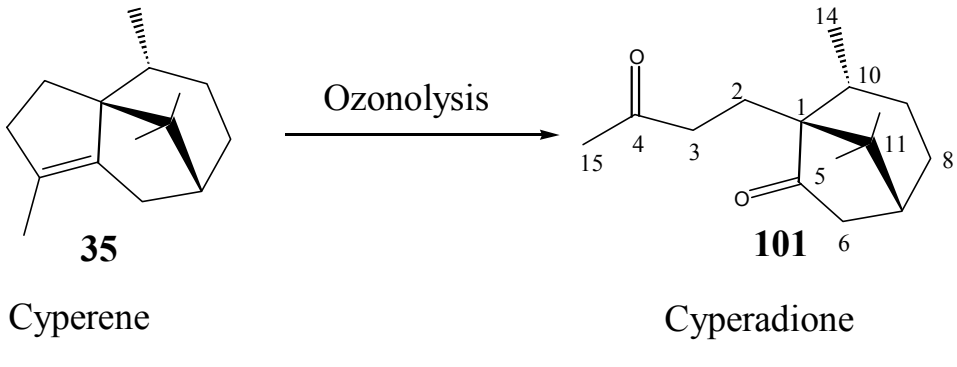
Fig. 78 : Proton NMR-spectrum of cyperadione (**101**) in C_6D_6.

The two methyl groups at δ 0.98 and δ 1.28 appearing as singlet are coupled to each other (W coupling), what indicates that they are linked to the same quaternary carbon atom. This gives the substructure CH_3-C-CH_3. The deshielded methyl group CH_3-15 at δ 2.18 is probably adjacent to a carbonyl function and shows a long-range coupling with the methylene group CH_2-3 (δ 2.35 and δ 2.44) which further couples with the methylene group CH_2-2 (δ 1.50 and

δ 2.11) to give the partial structure CH_3-CO-CH_2-CH_2-. The way the three substructures need to be connected to give the structure of the compound **101** was deduced from the long-range correlations of the HMBC diagram (Table 3). To confirm this structure we performed an ozonolysis of cyperene (**35**) (fig. 79) and the reaction product displayed the same spectral data as the isolated compound.

Protons	Carbons
H-2	C-1, C-3, C-4, C-10
H-3	C-2, C-4
H-6	C-5, C-7, C-8
H-7	C-6, C-8, C-11
H-10	C-1, C-9, C14, C-2
H-12	C-1, C- 7, C-11, C-13
H-13	C-1, C-7, C-11, C-12
H-14	C-1, C-9, C-10
H-15	C-3, C-4

Table 3: Heteronuclear long-range correlations from the HMBC diagram of cyperadione (**101**).



35 Cyperene

101 Cyperadione

Fig. 79 : Ozonolysis of cyperene.

4.5.9 Proposition of a Biogenetic Pathway for Rotundane Sesquiterpenoids.

Rotundene (**56**) is a sesquiterpene occuring in the essential oils of all the *Cyperus* species investigated in this work. Up to now its biosynthesis has not been studied. Therefore, it seems worth to propose a pathway for its biosynthesis since rotundene obviously plays a role in the chemotaxonomy of the *Cyperaceae*. The biosynthesis of rotundene may involves two successive cyclisation processes of FDP leading to a cationic azulane intermediate. An intromolecular electrophilic attack of the cation at the isopropenyl double bond and subsequent deprotonation leads to rotundene or isorotundene (fig. 80).

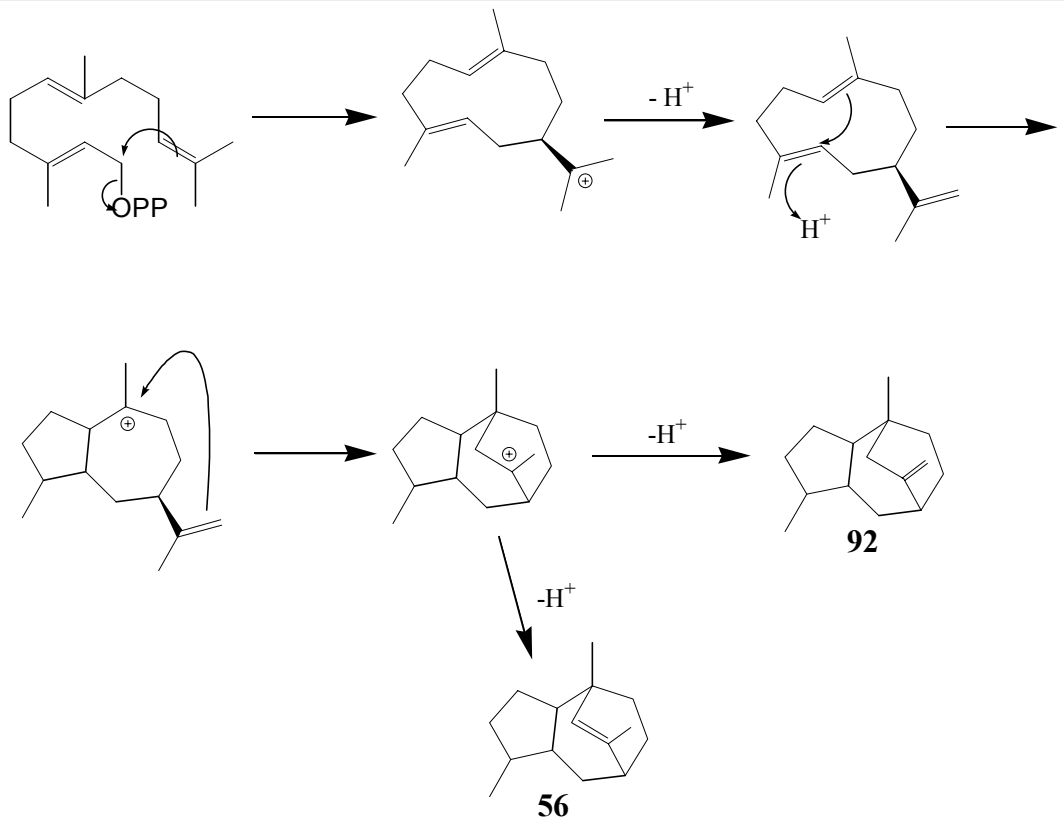


Fig. 80 : Proposition scheme for the biosynthesis of rotundene (**56**) and isorotundene (**92**).

4.6 Conclusion.

4.6.1 Remarks on ^{1}H-NMR of Patchoulane Sesquiterpenoids.

From the essential oil of the *Cyperus* species many sesquiterpenes with patchoulane skeleton were isolated. The structure elucidation of the unknown compounds as well as the identification of known ones by spectroscopic data comparison has proved that some generalisation can be made concerning the ^{1}H-NMR spectra of this type of sesquiterpenes. All the compounds generally have four methyl groups (except cyprotene with five, and compounds were the methyl group CH_3-15 is functionalized, which have only three, e.g. cyperenal), three of them being very characteristic of this group of sesquiterpenes. The two methyl groups CH_3-12 and CH_3-13 always show a strong coupling correlation in the ^{1}H-^{1}H-COSY diagram (W coupling). The methyl group CH_3-13 always appears as a doublet (J = 6 Hz) since it couples with the methine proton H-10. The fine structure of the signal of the methine proton H-10 is also very characteristic for these compounds, since it is always the same in the proton NMR-spectra of all of them (see fig. 55, 57 and 73), and even if it overlaps with another signal it can still be recognized, e. g. in the spectrum of patchoulenone. The fine structure of the signal of H-10 is explained by the conformation of these molecules which is the same in all patchoulane sesquiterpenes (fig. 81). H-10 has an axial-axial coupling with H-9a (J = 12 Hz) and couples further with the methyl group CH_3-14 (three protons, J_1 = 6 Hz) and also with the protons H-9e (J_2 = 6 Hz). This particular fact ($J = 2J_1$ and $J = 2J_3$) leads to the characteristic signal of H-10 (fig. 81).

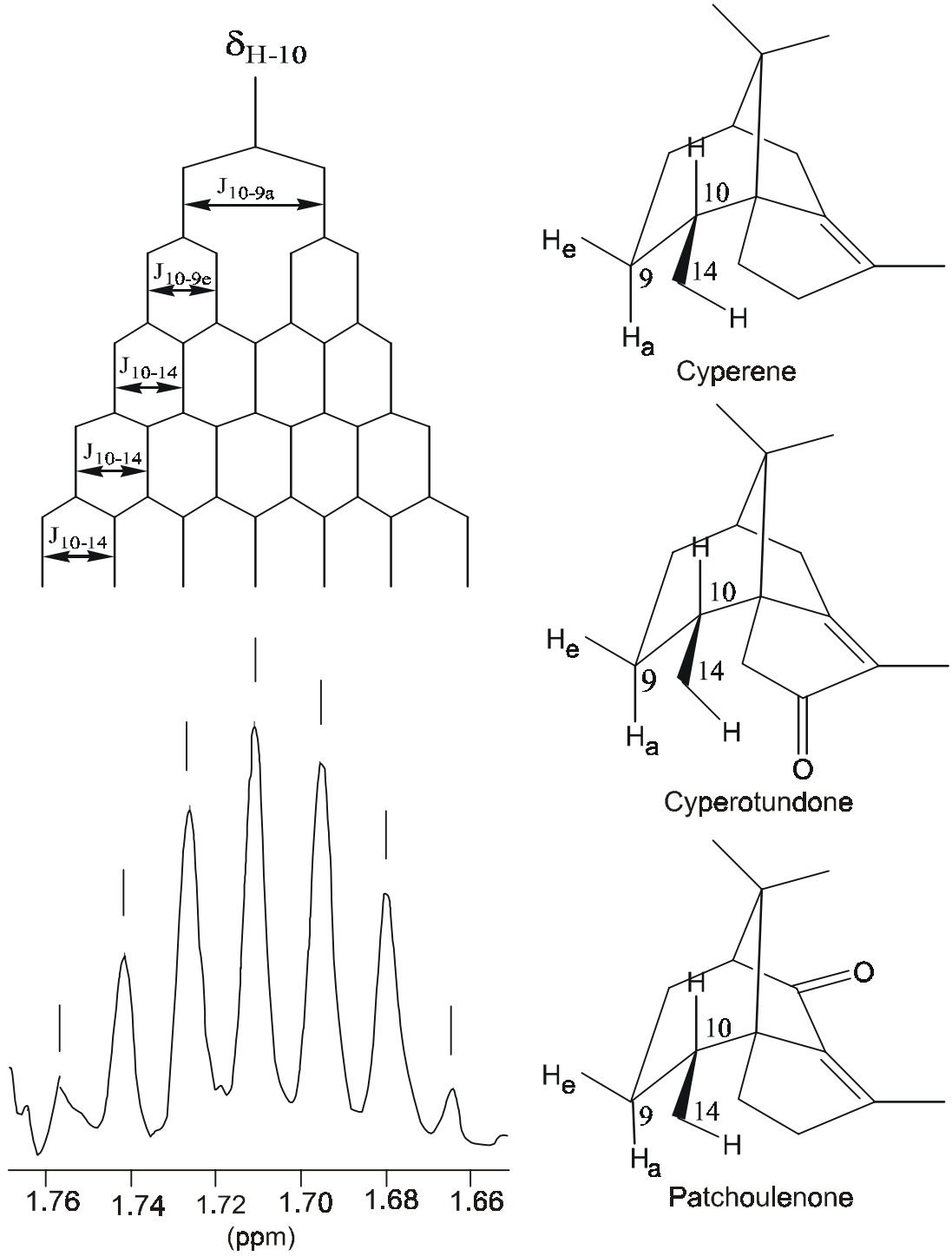


Fig. 81 : Geometry of patchoulane sesquiterpenoids and fine structure of proton NMR signal of H-10. The proton H-10 couples with the three protons of the methyl group H-14 (J = 6 Hz), with the proton H-9e (J = 6 Hz), and with the proton H-9a (J = 12 Hz). The value of the these scalar coupling constants is due to the geometry of the molecule which remains unchanged for almost all patchoulane sesquiterpenoids, thus being responsible for a characteristic pattern of H-10 in this group of compounds.

4.6.2 Comparative Comments on the Chemical Composition of the Three Cyperus Species.

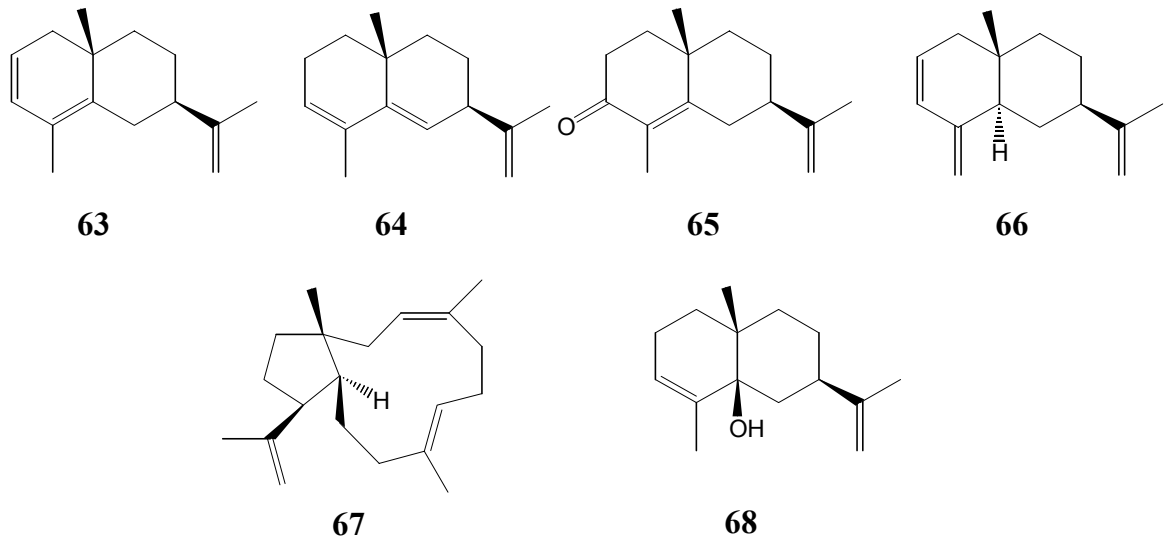
As it has been seen from former work on the essential oils of *Cyperus* species patchoulane, eudesmane, rotundane, and caryophyllane sesquiterpenoids are the most important chemical constituents of this genus. The present investigations also confirme these facts, since all the unknown compounds we isolated are of patchoulane, eudesmane or rotundane nature. However, some differences are observed in the distribution of these products in the different species. All the three species contain compounds of patchoulane, eudemane, and rotundane type, but *C. alopecuroides* contains more eudesmane sesquiterpenoids while *C. papyrus* and *C. rotundus* contain mainly patchoulane sesquiterpenoids. Although cyperene remains the major hydrocarbon in the three species, α-cyperone (eudesmane) is the major oxygenated (ketone) product of *C. alopecuroides* while cyperotundone (patchoulane) is the major oxygenated product in the oil of *C.papyrus* and *C. rotundus*. Moreover, the four new eudesmanes (three hydrocarbons and one major alcohol) were not found in *C. rotundus* and *C. papyrus*. Concerning the new patchoulane compounds, they were also identified by GCMS in *C. alopecuroides*, but their concentrations were so low that they could only be isolated from *C. papyrus* or *C. rotundus*.

A noteworthy particularity of the three investigated species is their tendency to produce norsesquiterpenoids. Two norsesquiterpene hydrocarbons could be identified: cyprotene and norrotundene. Cyprotene was identified in all the three species, although only in a very small amount in the oil of *C. alopecuroides*. Norrotundene was found to be present only in the essential oils of *C. papyrus* and *C. rotundus*.

Summary.

The present dissertation is dealing with the chemical composition of the essential oil of the *Cyperaceae*. Many species of this plant family have economical importance and some are cultivated for utilisation, although their use is often localised in the region were they grow. Their use against various disorders such migraine or abdominal pains or as fumigant and to flavour food is well-known. The chemical composition of the three species *Cyperus alopecuroides, Cyperus papyrus* and *Cyperus rotundus* have been investigated, resulting in the isolation of several new sesquiterpene derivatives of eudesmane, patchoulane and rotundane type.

The investigation of the hydrodistillation products of the rhizomes of *C. alopecuroides* led to the isolation the three eudesmane hydrocarbons **63**, **64** and **66** essentialy by preparative gas chromatography. Their structures were determined by spectroscopic methods and partial synthesis from α-cyperone (**65**). From the hydrocarbon part of the oil the diterpene **67,** whose structure was established only on the base of NMR- and mass spectral data was isolated. Its absolute configuration was not investigated. In order to study the oxygenated part of the essential oil, a combination of many chromatographic techniques including column chromatography at low temperature, preparative thin layer chromatography and preparative GC was necessary. The oxygenated fraction contained many alcohols and ketones which were isolated and identified. One of the alcohols, the eudesmane sesquiterpene **68** is a new compound and its structure was determined by combining mass spectrometry, NMR techniques and chemical transformations (SF. 1). In addition to these new compounds, many other known sesquiterpenoids were identified.



63 **64** **65** **66**

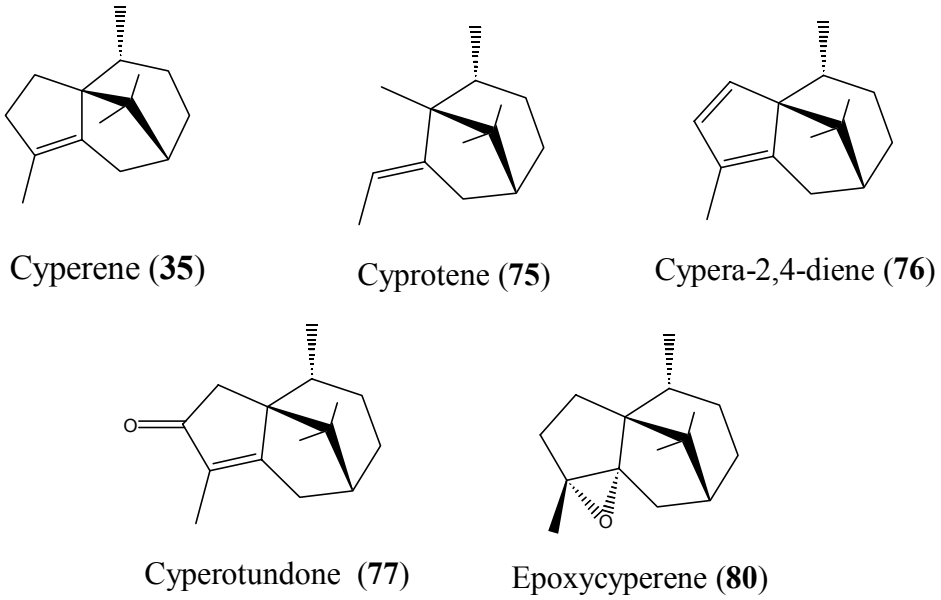
67 **68**

SF 1: Diterpene and sesquiterpenes of *Cyperus alopecuroides*.

Nevertheless some minor compounds were present which could neither be identified by GC-MS nor be isolated. Fortunately, these compounds were also present in relative greater amount in the essential oil of *C. papyrus.*

The hydrodistillation products of the dried rhizomes of *C. papyrus* was submitted to preparative gas chromatography, yielding fifteen fractions. The purification of the first fraction afforded a norsesquiterpene **75** whose structure was determined by one- and two-dimensional NMR techniques. Cyprotene (**75**) is probably a biogenetical degradation product of cyperene (**35**) and therefore both should have the same absolute configuration. Gaschromatographic treatment of the second fraction gave a hydrocarbon which was characterised by NMR methods as cypera-2,4-diene (**76**). This structure was confirmed by chemical correlation with cyperotundone (**77**). The eighth fraction also contained an unknown compound and was purified by preparative GC, yielding an oxygenated sesquiterpene which was characterised as epoxycyperene (**80**) by

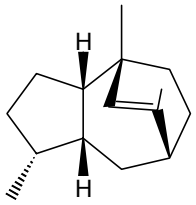
NMR spectroscopy and preparation by epoxydation of cyperene (**35**) (SF. 2).



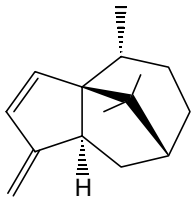
SF 2: Sesquiterpenes isolated from *Cyperus papyrus*.

GC-MS analysis of the essential oil of *C. rotundus* revealed many known sesquiterpenoids. A members of minors hydrocarbons could not be identified. A preliminary fractionation of the oil was perormed to separate hydrocarbons from oxygenated compounds and also to eliminate fatty acids. Separation of the hydrocarbon fraction of the oil was achieved by a combination of column chromatography and thin layer chromatography over silica impregnated with silver nitrate and preparative GC. The first unknown isolated compound was characterised as isorotundene (**92**). Its constitution and relative configuration was established by NMR techniques as well as chemical transformation of rotundene (**56**) to **92**. The preparation of isorotundene from rotundene proved the identity of their relative configurations, and thus opening the way to a study of their absolute configuration. For this purpose, rotundene was submitted to a series of chemical transformations (ozonolysis of the double-bond,

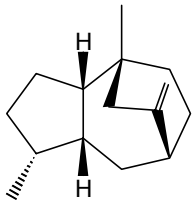
decarboxylation of the resulting keto-acid, olefination of the ketone and finally hydrogenation of the alkene) in order to convert it into a guaiane-type hydrocarbon. The resulting alkane was then correlated with a fully hydrogenated product of (-)-γ-gurjunene (**98**) by enantioselective gas chromatography.



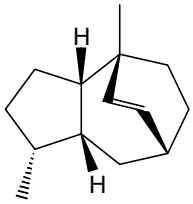
Rotundene (**56**)



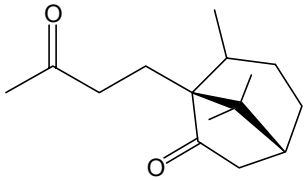
Cypera-2,4(15)-diene (**99**)



Isorotundene (**92**)



Norrotundene (**100**)



Cyperadione (**101**)

SF 3 : Sesquiterpenoids from *Cyperus rotundus.*

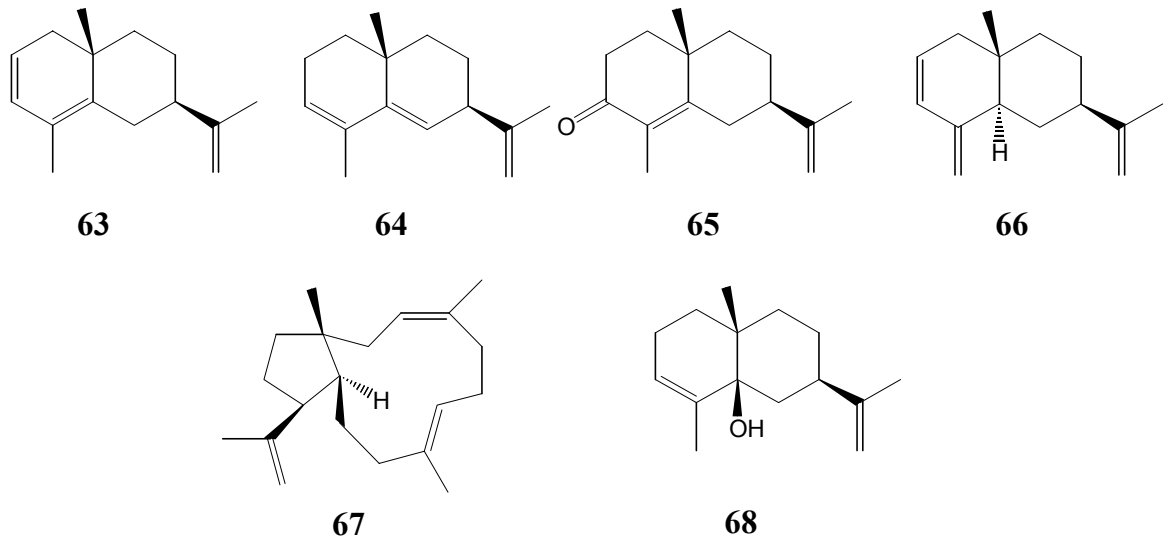
A second sesquiterpene hydrocarbon isolated from the oil of *C. rotundus* was cypera-2,4(15)-diene (**99**). Its structure was determined by NMR techniques and preparation from cyperotundone (**77**). Furthermore, from the hydrocarbon fraction the norsesquiterpene **100** was isolated and its structure determined by NMR spectroscopy. Norrotundene (**100**) is most likely biogenetically related to rotundene (**56**) and thus may have the same absolute configuration. The investigation of the oxygenated part of the molecule led to the isolation and characterisation of cyperadione (**101**). Its structure was determined by NMR

techniques and by its preparation from cyperene (**35**) (SF3). Together with the new compounds, many other sesquiterpenoids were identified in the essential oil of *C. rotundus*.

ZUSAMMENFASSUNG

Das Ziel der vorliegenden Arbeit war es, unbekannte Stoffe aus ätherischen Ölen der *Cyperaceen* zu isolieren und ihre Struktur aufzuklären. Die Familie der *Cyperaceen* umfaßt ungefähr 4000 Arten und 90 Gattungen. Die Mehrzahl der Angehörigen dieser Familie sind Kräuter, die meistens in Sumpfgebieten wachsen. Pflanzen der Gattung *Cyperus* sind für die wohlriechende Note ihrer Wurzeln bekannt. Sie werden in der Naturheilkunde eingesetzt, obwohl ihre Anwendung allerdings oft auf das Gebiet beschränkt ist, in dem sie wachsen. Zu der Gattung *Cyperus* gehören Arten wie *C. papyrus* und *C. giganteus*, dessen Stengel eine Länge von 5 m erreichen können. In Rahmen dieser Arbeit wurden drei Arten untersucht: *Cyperus alopecuroides, Cyperus papyrus* und *Cyperus rotundus*.

Das ätherische Öl von *Cyperus alopecuroides* wurde durch Wasserdampfdestillation der Wurzeln der Pflanze gewonnen. Die Untersuchung der Kohlenwasserstoff-Anteils des Öls führte zur Isolierung von drei neuen Sesquiterpenen der Eudesman-Serie **63**, **64**, und **66** sowie eines Diterpens **67**. Ihre Strukturen wurden mittels NMR-spektroskopischer Methoden und chemischer Umwandlung von α-Cyperon (**65**) aufgeklärt. Die absolute Konfiguration des Diterpens wurde nicht untersucht. Es gelang, aus der Gruppe der oxygenierten Verbindungen, den Alkoholen und Ketonen, einen unbekannten Sesquiterpen-Alkohol zu isolieren. Die Strukturaufklärung erfolgte durch Kombination von Massenspektromerie, NMR-Spektroskopie sowie chemischen Umwandlungen und führte zur Struktur **68** (Abb. 1). Durch GC-MS-Analytik wurde deutlich, daß eine Reihe von nicht isolierbaren unidentifizierten Nebenkomponenten vorhanden sind, die jedoch in relativ großer Menge im ätherischen Öl von *Cyperus papyrus* zu finden sind.



63 **64** **65** **66**

67 **68**

Abb. 1: Diterpene und sesquiterpenes aus *Cyperus alopecuroides*.

Das durch Wasserdampfdestillation gewonnene Öl von *C. papyrus* wurde mittels präparativer Gaschromatographie aufgetrennt. Drei Sesquiterpene, die anhand ihrer Massenspektren nicht identifiziert werden konnten, wurden isoliert, und ihre Strukturen wurden aufgeklärt: Cyproten (**75**), Cypera-2,4-dien (**76**) und Expoxycyperene (**80**). Die Struktur von Cyproten (**75**) sowie dessen relative Konfiguration wurden mittels NMR-Spektroskopie aufgeklärt. Cyproten (**75**) ist wahrscheinlich ein biogenetisches Abbauprodukt von Cyperen (**35**) und besitzt deswegen die gleiche absolute Konfiguration. Die Strukturaufklärung von Cypera-2,4-dien (**76**) erfolgte durch NMR-Spektroskopie und eine von Cyperotundone (**77**) ausgehende partielle Synthese. Weiterhin wurde Isopatchoula-3,5-dien (**79**) identifiziert. **79** ist in der Literatur als Inhaltsstoff des ätherischen Öls von *C. scariosus* beschrieben. Die dritte unbekannte Verbindung wurde mittels NMR-Spektroskopie als Epoxycyperene (**80**) charakterisiert (Abb. 2). Diese Stuktur wurde mittels Vergleich mit dem Epoxydierungsprodukt von

Cyperen (**35**) bestätigt.

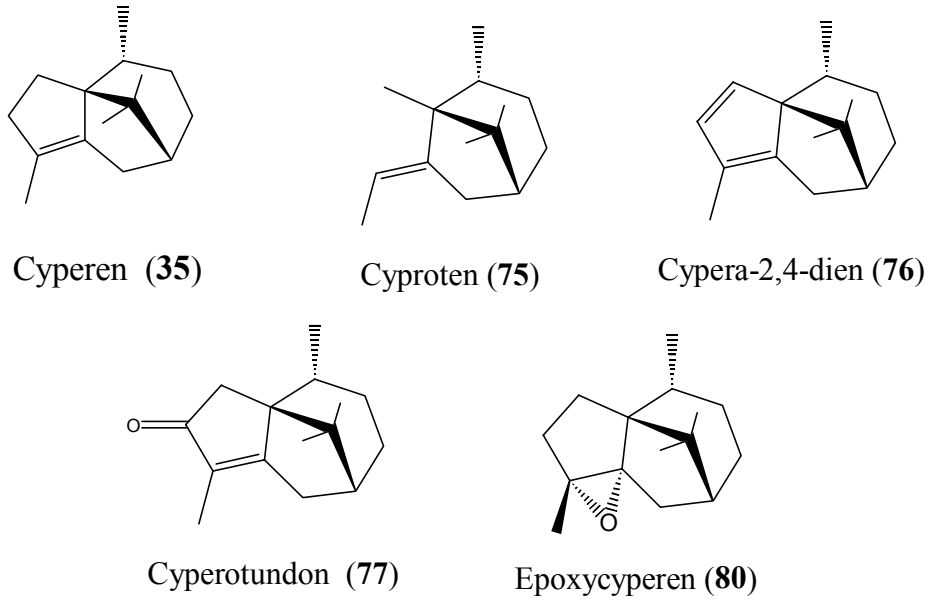
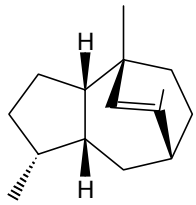


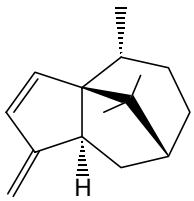
Abb. 2: Sesquiterpene aus *Cyperus papyrus*.

Das ätherische Öl von *C. rotundus* wurde zuerst durch GC-MS analysiert. Anschließend wurden mittels chromatographischer Methoden drei unbekannte Sesquiterpene-Kohlenwasserstoffe isoliert: Isorotunden (**92**), Cypera-2,4(15)-dien (**99**) und Norrotunden (**100**). Die Struktur von Isorotunden (**92**) wurde mittels NMR-Spektroskopie aufgeklärt. Nach der Isolierung und vollständtigen Strukturaufklärung von Isorotunden (**92**) ist klar geworden, daß dies der Schlüssel sein könnte, die relative Konfiguration von Rotunden (**56**) zu beweisen. Demzufolge wurde Rotunden aus *C. rotundus* Öl isoliert. Da die Signale im ^{1}H-NMR-Spektrum überlagert sind, ist es unmöglich, mittels der NOESY-Technik Informationen über die relative Konfiguration des Moleküls zu erhalten. Deswegen ist die Umlagerung von Rotunden (**56**) zu Isorotunden (**92**) ein einfacher Weg zur Ermittlung der Stereochemie von Rotunden (**56**). Zuerst wurde eine Hydroxylierung von Rotunden durchgeführt. Anschließend

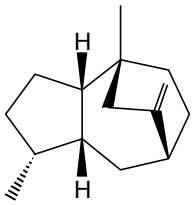
wurde der Alkohol zu Isorotunden (**92**) dehydratisiert. Das aus Rotunden (**56**) hergestellte Isorotunden (**92**) hat dieselben spektroskopischen Daten (^{1}H- , ^{13}C-NMR und Massenspektrometrie) wie die isolierte Probe. Desweiteren wurden beide Proben mittels enantioselektiver GC korreliert, was ihre Identität bestätigte. Rotunden hat demzufolge dieselbe Konfiguration wie Isorotunden an allen chiralen Zentren. Die absolute Konfiguration von Rotunden (und damit auch von Isorotunden) wurde mittels chemischer Umwandlungen und Korrelation mit einer authentischen Probe von (+)-γ-Gurjunene bestimmt.



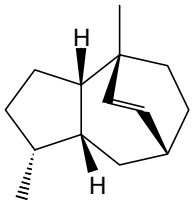
Rotunden (**56**)



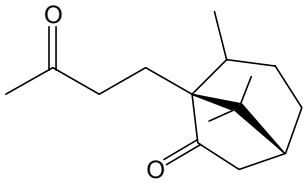
Cypera-2,4(15)-dien (**99**)



Isorotunden (**92**)



Norrotunden (**100**)



Cyperadion (**101**)

Abb. 3 : Sesquiterpene aus *Cyperus rotundus.*

Eine weitere isolierte Substanz wurde mittels NMR-Spektroskopie und chemische Umwandlung von Cyperotundon (**77**) als Cypera-2,4(15)-dien (**99**) charakterisiert. Die dritte unbekannte Substanz mit der Molekülmasse *m/z* = 190 ist auch in *C. papyrus* vorhanden. Die Auswertung ihrer NMR-Spektren ermöglichte es, diese Substanz als das Rotundan-Norsesquiterpen **100** zu

charakterisieren. Norrotunden (**100**) hat wahrscheinlich eine biogenetische Verwandschaft zu Rotunden (**56**) und besitzt deswegen dieselbe absolute Konfiguration wie Rotunden (**56**) und Isorotunden (**92**) (Abb. 3).

Bei der Untersuchung der sauerstoffhaltigen Fraktion wurden Mustakon (**41**) und Patchoulenon (**48**) identifiziert. Es konnte auch ein weiterer unbekannter Stoff mit der Molekülmasse *m/z* = 236 isoliert werden. Seine Struktur ergab sich aus der Auswertung von NMR-Spektren und chemische Korrelation mit Cyperen (**35**). Er wurde Cyperadion (**101**) genannt (Abb. 3).

5 Experimental Part

«The temptation to form premature theories upon insufficient data is the bane of our profession.»

(Sir Arthur Conan Doyle, *The valley of Fear*, 1915)

5.1 Material and Methods.

In order to achieve our purpose of isolation and characterisation of essential oil components, many steps have been necessary each of which involving a specific method.

a) Isolation of essential oil from the plant material.

The essential oils were obtained from the plant by hydrodistillation, using a small laboratory apparatus. (see section 3.2.2 for more details).

b) Capillary gas chromatography.

For analytical purposes or to monitor an isolation process two gas chromatographs were mainly used: an Orion Micromat 412 equipped with two columns (25 m CP-Sil 5 CB and 25 m CP-Sil 19 CB from Chrompack) and a Carlo Erba HRGC equipped with one column (25 m CP-Sil 5 CB from Chrompack). For stereochemical studies or separation purposes some Carlo Erba

instruments, Fractovap 2101 AC, 2150, 4160 and Mega 5300 equipped with enantioselective columns, were used. All these instruments were equipped with split injectors and flame ionisation detectors. Hydrogen (0.5 mbar) was used as carrier gas. The chromatograms were recorded by Merck-Hitachi D-2000 and D-2500 integrators.

c) Preparative gas chromatography.

Preparative gas chromatographic separations were effected using alternatively a Varian 1400 and a Varian 2800 chromatograph. Injectors and detectors were kept at 200° C and 250° C, respectively. The column effluent is split between a flame ionisation detector and the exit port at a 1:400 ratio. Helium was used as carrier gas at flow rate of 240 ml / min. Fractions to be collected were trapped in a bath of liquid nitrogen by sleeving pieces of PTFE tubing (Reichelt, 30 cm x 1.5 mm I. D.) over the column outlet inside the oven through a guide way made out of Swagelock fitting. The chromatographs were equipped with packed columns with different phases depending on the sample to be separated.

2,6-Me-3-Pe-β-CD.

The stainless steel packed column (Silcosteel, Amchro, 2.00 m x 5.3 mm) was prepared by coating chromosorb W-HP (Merck, 100-120 mesh) with 5% (w/w) of a 1:1 (w/w) mixture of heptakis (2,6-di-O-methyl-3-Opentyl)-β-cyclodextrin and polysiloxane OV-1701.

6-Me-2,3-Pe-γ-CD.

This stainless steel packed column (Silcosteel, Amchro, 2.05 m x 5.1 mm) was prepared by coating chromosorb W-HP with 6% (w/w) of a 1:1 (w/w) mixture

of octakis (6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin and polysiloxane PS-086.

6-T-2,3-Me-β-CD.

The stainless steel packed column was prepared by coating chromosorb W-HP (100-120 mesh) with 2.5 % (w/w) of a 1:4 (w/w) mixture of heptakis (6-O-tertbutyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin and polysiloxane SE 52.

SE 30.

The stainless steel packed column was prepared by coating chromosorb W-HP with 10 % (w/w) polysiloxane SE 30.

Preparative chromatograms were registered by a *W+W electronic 312* recorder.

d) Column Chromatography and thin layer chromatography.

Column chromatography was performed using silica gel 60 (60 - 230 mesh) from *Merck*.

Silver nitrate coated silica was prepared by mixing 10 % (w/w) of silver nitrate and silica gel in acetonitril. The solvent was then evaporated under reduced pressure on a rotatory evaporator.

Thin layer chromatography was effected using glass and aluminium plates of silica 60 F_{254} (*Merck*). Silver nitrate coated plates were prepared by immersing the plates in an ethanol-water (4:1) solution of silver nitrate (5 mg $AgNO_3$ in 50 ml of solvent). After 30 min the plates were removed from the solution and

dried in an oven. For detection, an ethanolic solution of sulfuric acid (10 %) was used as a spray reagent.

e) NMR-Spectroscopy.

NMR spectra were recorded using the two instruments Bruker WM 400 (^{1}H : 400 MHz, ^{13}C : 100.62 MHz) and Bruker DRX 500 (^{1}H : 500 MHz, ^{13}C : 125.77 MHz). Tetramethylsilane (TMS) was used as reference signal (δ = 0.00). In the absence of TMS signals of the solvent were used as references. With $CDCl_3$ as solvent the signals of $CHCl_3$ (^{1}H : δ = 7.26 ppm, ^{13}C : δ = 77.7 ppm) served as references. With C_6D_6 as solvent, the signals of C_6H_6 (^{1}H : δ = 7.16 ppm, ^{13}C : δ = 128.7 ppm) served as reference.

f) GC-MS analysis.

Electron impact GC-MS measurements were carried out on a Hewlett-Packard HP 5890 gas chromatograph equipped with a 25 m CP Sil 5 CB capillary column and coupled to a VG Analytical VG 70-250S mass spectrometer. Helium was used as carrier gas.

g) Polarimetry.

Only qualitative optical rotation could be dermined because of the small quantity of isolated product. The measurements were performed using a Perkin-Elmer 243 and a Perkin-Elmer 314 polarimeter at a wavelength of 589 nm.

h) Column chromatography at lower temperature.

Prefractionations of essential oils were performed on a glass column equipped with a condenser and connected to a Kryoflex KF 40 from *Messgeräte-Werk*

Lauda. Ethanol was used as cooling liquid.

5.2 Analysis of *Cyperus alopecuroides*.

The plant material was collected in Kollbison (Cameroon). A voucher specimen is deposited at the National Herbarium of Yaoundé (Cameroon). The essential oil was isolated by hydrodistillation of the air dried rhizomes, using n-hexane as collecting solvent.

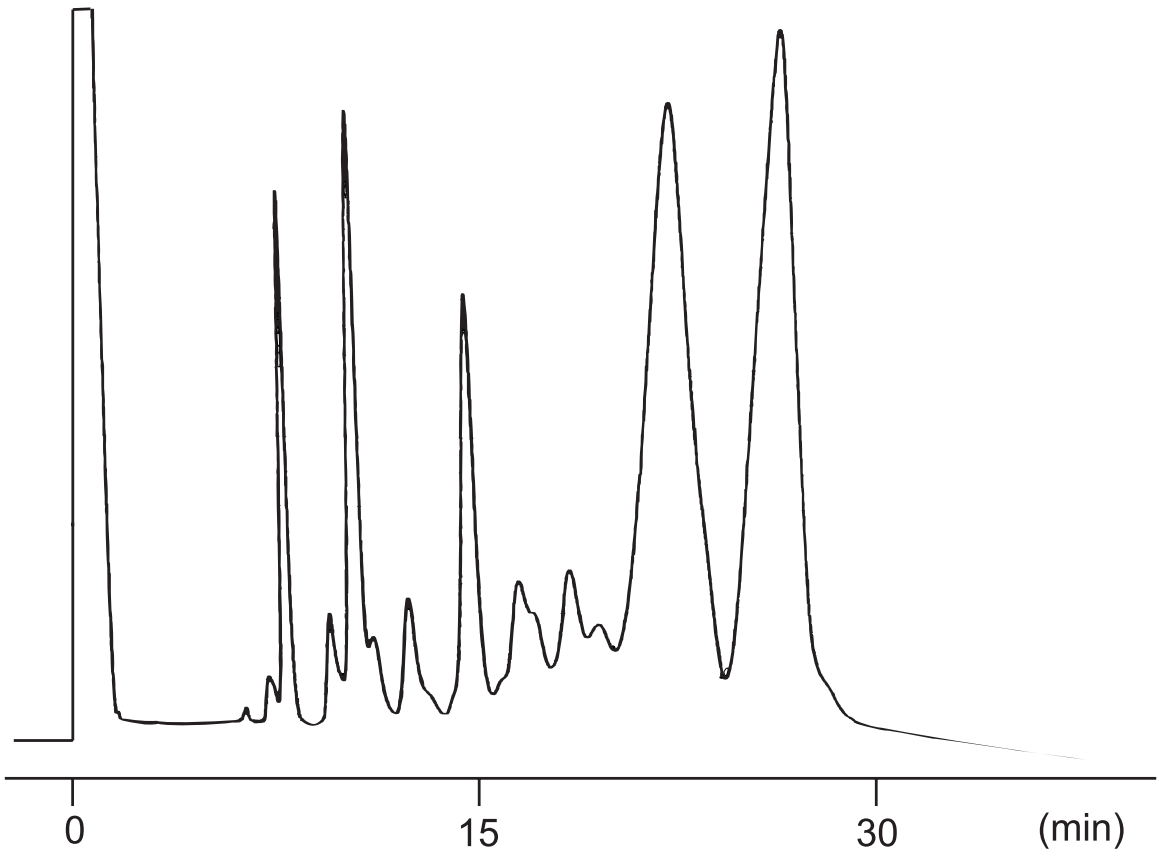


Fig. 82 : Preparative gas chromatogram of the oil of C*yperus alopecuroides* on the 6-Me-2,3-Pe-γ-CD-column.

5.2.1 Isolation of the three Eudesmane Hydrocarbons.

The fractionation of the raw oil was performed by preparative GC on a Varian 2800 instrument equipped with a column with chiral stationary phase

(octakis (6-O-methyl-2,3-di-O-pentyl-γ-CD). A two ramp temperature programme was used: 120° C during the first 25 min and 160° C for the rest of the time. This preliminary separation yielded 20 fractions (fig. 82), the first six ones (CA1 to CA6) being sesquiterpene hydrocarbons followed by oxygenated fractions. Further separations were made using another column with chiral stionary phase (6-O-TBDMS-2,3-di-O-methyl)-β-CD at 120° C. The fraction CA1 and CA2 were analysed by GCMS and consisted of cyprotene and cypera-2,4-diene. However, they were not purified because the quantity was too small. The third fraction contained essentially the major hydrocarbon cyperene. The separation of the fifth fraction CA5 by preparative GC yielded eudesma-2,4,11-triene, eudesma-3,5,11-triene and the known hydrocarbons rotundene and β-selinene. The treament of the sixth fraction CA6 by preparative GC gave two subfractions, one containing α-selinene and β-selinene and the other containing eudesma-2,4(15),11-triene as a pure compound. The oxygenated fractions were not exhaustively investigated.

a) Eudesma-2,4,11-triene (**63**).

^{1}H-NMR (400 MHz): 0.92 (1H, *s*), 1.38 (1H, *dd*, J_1 =12.71 Hz, J_1 = 3.56 Hz), 1.48 (1H, *dd*, J_1 = 12.71 Hz, J_2 = 3.56 Hz), 1.65-1.70 (2H, *m*), 1.71 (3H, *s(br)*), 1.77 (3H, *s(br)*), 1.86 (1H, *d(br)*, *J* = 14.50 Hz), 1.93 (1H, *dt(br)*, J_1 = 6.61 Hz, J_2 = 3.05 Hz), 1.96 (1H, *dd*, J_2 = 17 Hz, J_1 = 6.61 Hz), 2.10 (1H, *d(br)*, *J* = 17 Hz), 2.60 (1H, *dt*, J_1 = 14.50 Hz, J_2 = 2.55 Hz), 4.72 (2H, *m*), 5.66 (1H, *ddd*, J_2 = 8.5 Hz, J_2 = 6.1 Hz, J_3 = 2.03 Hz), 5.72 (1H, *dd*, J_1 = 8.5 Hz, J_2 = 3.05 Hz); MS (EI, 70 eV), m/z (rel. int.): 202(23) (M^+), 187(9), 173(1), 159(9), 145(32), 131(100), 119(43), 105(60), 91(31), 77(18), 67(9), 63(2), 55(14), 51(5), 41(29).

b) Eudesma-3,5,11-triene (**64**).

^{1}H NMR (400 MHz): 0.99 (3H, *s*), 1.36-1.48 (3H, *m*), 1.49-1.56 (1H, *m*), 1.60-1.76 (4H, *m*), 1.78 (3H, *m*), 2.05 (1H, *m*), 2.26 (1H, *m*), 2.91 (1H, *t*, J = 8.14 Hz), 4.73 (1H, *m*), 4.77 (1H, *m*), 5.41 (1H, *m*), 5.52 (1H, *m*); MS (EI, 70 eV), m/z (rel. int.): 202(100) $[M]^+$, 187(91), 173(15), 161(12), 14526), 131(30), 121(41), 115(14), 105(39), 91(39), 77(24), 67(12), 63(4),55(17), 51(8), 41(36).

c) Eudesma-2,4(15),11-triene (**66**).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.77 (*s*, 3H), 1.09 (*ddd*, 1H, $J_1 = J_2$ = 12.61 Hz, J_3 = 5.68 Hz), 1.29 (*q*, 1H, $J_1 = J_2 = J_3$ = 11.98 Hz), 1.38 (*ddd*, 1H, $J_1 = J_2$ = 12.61 Hz, J_3 = 3.78 Hz), 1.42 (*dd*, 1H, J_1 = 12.61 Hz, J_2 = 2.80 Hz), 1.50 (*qt*, 1H, J_1 = 12.61 Hz, J_2 = 5.68 Hz, $J_3 = J_4$ = 2.80 Hz), 1.72 (*dd*, 1H, J_1 = 18.29 Hz, J_2 = 5.59 Hz), 1.83 (*dt*, J_1 = 11.98 Hz, $J_2 = J_3$ = 3.78 Hz),1.88 (*d*, 1H, J = 18.29 Hz), 1.89 (*dd*, 1H, J_1 = 11.98 Hz, J_2 = 3.78 Hz), 2.00 (*dq*, 1H, J_1 = 11.98 Hz, $J_2 = J_3 = J_4$ = 2.80 Hz), 4.83 (*m*, 2H), 5.58 (*qt*, J_1 = 9.66 Hz, J_2 = 5.59 Hz, $J_3 = J_4$ = 2.04 Hz), 6.19 (dd, 1H, J_1 = 9.66 Hz, J_2 = 3.06 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.11 (*q*), 20.98 (*q*), 27.23 (*t*), 29.52 (*t*), 33.73 (*s*), 41.10 (*t*), 43.18 (*t*), 45.24 (*d*), 45.79 (*d*), 108.92 (*t*), 110.03 (*t*), 126.99 (*d*), 130.61 (*d*), 146.50 (*s*), 150.43

(*s*). MS (EI, 70 eV), m/z (rel. int.) : 202(47) $[M]^+$, 187(22), 173(14), 159(30), 145(51), 131(50), 119(53), 105(77), 79(59), 67(36), 53(39), 41(86).

d) Reduction of α*-cyperone and dehydration to* **63** *and* **64**.

The reduction of α-cyperone, which is an α,β-unsaturated ketone, was performed in presence of $CeCl_3$ [135]. Since an anhydrous lanthanide chloride

was not available, $CeCl_3 \cdot nH_2O$ was dehydrated by the following process. Twenty grams of the metal chloride was put into a round-bottomed flask, and 50 ml of water freed thionyl chloride (0.64 mol) was added at room temperature. Evolution of sulfur dioxide and hydrogen chloride began at once. After bubbling stopped, the flask was equipped with a reflux condenser for 2 hours. The condenser was then arranged for distillation, and the excess thionyl chloride was removed *in vacuo*, using a dry nitrogen current. The flask containing the product was transferred immediatly to a vacuum desiccator containing potassium hydroxide and stored for 24 hours to remove the remaining thionyl chloride [157].

30 mg (0.14 mmol) of α-cyperone and 0.14 mmol of $CeCl_3$ were dissolved in THF (3ml) and gave an heterogenous solution since the metal chloride failed to dissolve in THF. After the mixture had been stirred for 5 min, an excess of $LiAlH_4$ (7 mg) was added at room temperature. An evolution of gas followed and the solution was stirred for 12 hours. The solution was then neutralised with diluted HCl, and extracted with diethyl ether (5ml three times). The combined extracts were then dried over magnesium sulfate ($MgSO_4$). Capillary GC and GC-MS revealed the presence of cyperol together with an unidentified alcohol (which proved later to be eudesma-3,11-diene-5-ol) and traces of dehydration products. The dehydration of the mixture of alcohols occurs in the injection port (200° C) of the chromatograph and yielded **63**, **64** and a trace of another hydrocarbon which was recognized later as **66**. However, a small quantity of alcohol could still be isolated.

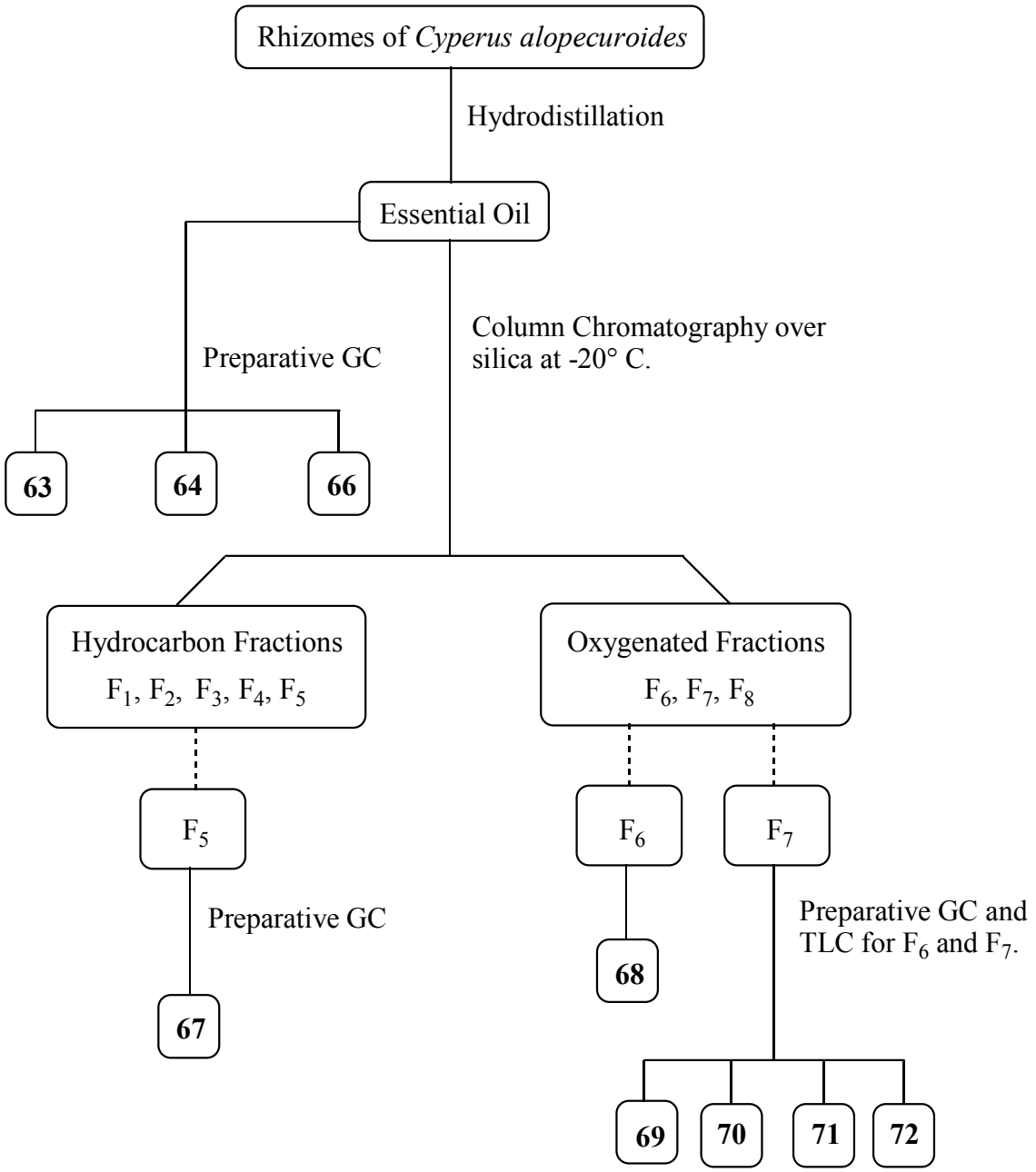


Fig. 83 : Analysis scheme of *Cyperus alopecuroides*.

5.2.2 Isolation of Dolabella-3,7,18-triene (67).

In order to investigate the oxygenated part of the essential oil, it was worthy to begin with a prefractionation of the raw oil (fig. 83) by column chromatography at low temperature (-20° C). From this first separation process eight fractions

were obtained. The four first fractions contained only hydrocarbons. The fifth fraction CA5 also contained only hydrocarbons, but an interesting fact was that one of the hydrocarbons had a very high retention time. GCMS showed that it was a diterpene. Its isolation achieved by preparative GC was very easy since the peak eluted isolated (its retention time is 50 min on a CP Sil 5CB capillary column) far away from other peaks of the sample (see fig. 39, p. 65)

Dolabella-3,7,18-triene (**67**).

^{1}H-NMR (500 MHz, $CDCl_3$) :1.00 (3H, *s*), 1.16 (1H, *m*), 1.27 (1H, *ddd*, J_1 = 19.5 Hz, J_2 = 10.09 Hz, J_3 = 5.04 Hz), 1.36 (1H, *ddd*, J_1 = 13.24 Hz, J_2 = 9.46 Hz, J_3 = 7.54 Hz), 1.44 (3H, *s*), 1.45 (3H, *s*), 1.47 (1H,*m*), 1.50 (1H, *m*), 1.57 (1H, *m*), 1.64 (3H, *s*), 1.64 (1H, *m*), 199 (1H, *m*), 2.01 (1H, *m*), 2.14 (1H, *m*), 2.15 (1H, *dd*, J_1 = 13.24 Hz, J_2 = 11.35 Hz), 2.21 (1H, *m*), 4.59 (1H, *m*), 4.76 (1H, *m*), 4.79 (1H, *b(br)*, *J* = 10.71 Hz), 5.07 (1H, *dd*, J_1 = 11.35 Hz, J_2 = 4.42 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 16.00 (*q*), 17.05 (*q*), 23.90 (*q*), 24.74 (*q*), 24.81 (*t*), 28.89 (*t*), 30.08 (*t*), 38.22 (*t*), 40.28 (*t*), 42.13 (*d*), 42.68 (*d*), 43.76 (*d*), 46.88 (*s*), 51.41 (*d*), 111.53 (*t*), 126.17 (*d*), 127.72 (*d*), 134.33 (*s*), 134.88 (*s*), 147.28 (*s*). MS (EI, 70 eV), m/z (rel. int.) : 272 (22) $[M]^+$, 257 (12), 229 (12), 215 (6), 203 (8), 189 (25), 175 (30), 161(25), 147 (50), 135 (31), 121 (81), 107 (82), 93 (100), 81 (89), 79 (65), 77 (37), 68 (70), 67 (71), 55 (72), 41 (90).

5.2.3 Isolation of (-)-Eudesma-3,11-dien-5-ol (68) *and Cyperenal* (72).

The sixth fraction CAT6 (fig. 84) contained four major peaks consisting of caryophyllenoxide, cyperotundone, α-cyperone and an unknown alcohol. To isolate the unknown compound, the sample was submitted to preparative GC (column 6T-2,3-Me- β-CD). The separation which was monitored by GC and

GC-MS yielded six other fractions.

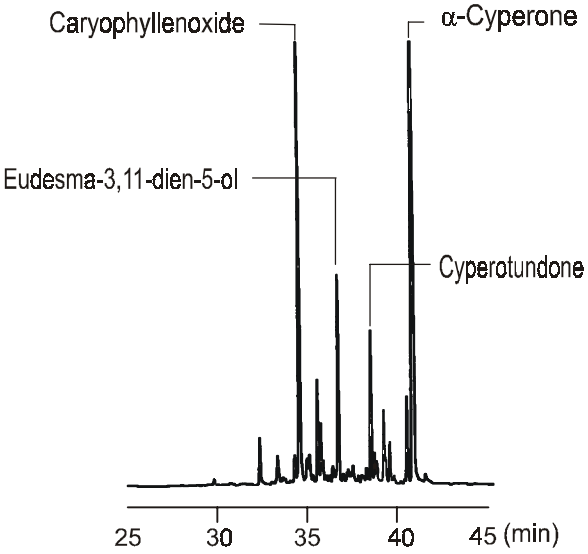


Fig. 84 : Chromatogram of the sixth fraction from the column chromatograhy at -20°C.

The sixth one CAT6.6 contained α-cyperone. The fourth fraction CAT6.4 contained the unknown alcohol as major product, but the intention to purify it by preparative GC was unsuccesfull with all the available columns. Finally preparative TLC with the system chloroform-methanol (10:1) as eluting solvent was used and succeeded in eliminating the most disturbing impurities. The major band of the TLC plates was taken in diethyl ether and after evaporation of the solvent the sample was treated by preparative GC to remove the last impurities and give 3 mg of (-)-eudesma-3,11-dien-5-ol. In treating the sixth fraction CAT6.6 by TLC using the system petroleum ether-ethyl acetate (8:2) it was noticed that near the big band of α-cyperone (Rf = 0.36) two small bands were also present, one of which (Rf = 0.70) was identified as cyperenal.

a) (-)-Eudesma-3,11-dien-5-ol (**68**).

^{1}H-NMR (400 MHz, $CDCl_3$) : 1.08 (3H, *s*), 1.21 (1H, J_1 = 13.88 Hz, J_2 = 6.31 Hz), 1.24 (1H, *dt*, J_1 = 13.56 Hz, $J_2 = J_3$ = 3.15 Hz), 1.45 (1H, *dd*, J_1 = 12.29 Hz, J_2 = 3.78 Hz), 1.52 (1H, *m*), 1.54 (1H, *t*, J = 12.92 Hz), 1.64 (1H, *m*), 1.72 (1H, *m*), 1.75 (6H, *s*), 1.76 (1H, *m*), 1.88 (1H, *dt*, J_1 = 12.92 Hz, $J_2 = J_3$ = 2.20 Hz), 1.96 (1H, *ddddd*, J_1 = 18.8 Hz, J_2 = 6.31 Hz, J_3 = 5.08 Hz, $J_4 = J_5$ = 1.58 Hz), 2.13 (1H, *m*), 4.64 (2H, *m*), 5.54 (1H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$) :

18.85 (*q*), 20.87 (*q*), 22.17 (*q*), 22.28 (*t*), 26.51 (*t*), 31.34 (*t*), 32.78 (*t*), 35.81 (*t*), 36.57 (*s*), 42.85 (*d*), 73.16 (*s*), 108.47 (*t*), 126.47 (*d*), 136.49 (*s*), 149.35 (*s*). MS, (EI, 70 eV), m/z (rel. int.) : 220 (11) $[M]^+$, 202 (27), 187 (17), 177 (2), 162 (34), 145 (17), 131 (32), 124 (100), 123 (92), 109 (42), 91 (30), 82 (60), 67 (27), 55 (39), 41 (66).

b) Cyperenal (**72**).

^{1}H-NMR (400 MHz, C_6D_6): 0.82 (3H, *s*), 0.86 (3H, *d*, J = 6.61 Hz), 1.02 (3H, *s*), 1.15 (1H, *m*), 1.42 (1H, *m*), 1.50-1.62 (2H, *m*), 1.95 (1H, *ddd*, J_1 = 6.62 Hz, J_2 = J_3 = 3.12 Hz, 2.09 (1H, *m*), 2.15 (1H, *s*, J_1 =13.22 Hz, J_2 = 6.61 Hz), 2.35 (1H, *d(br)*, J = 18.90 Hz), 2.77-2.83 (2H, *m*), 9.84 (1H, *s*). MS, (EI, 70 eV), m/z (rel. int.) : 218 (100) $[M]^+$, 203 (76), 185 (25), 175 (40), 161 (17), 147 (32), 133 (40), 119 (35), 105) (48), 91 (56), 79 (39), 67 (34), 55 (26), 41 (22).

5.2.4 Isolation of (+)-Eudesma-3,11-dien-2-one, Cyperol and Isocyperol.

The fraction CAT7 obtained from column chromatography consisted of three major products (see fig. 85).The separation of this fraction by preparative GC (2,6-Me-3Pe-β-CD) yielded four fractions. The fourth fraction CAT7.4 contained the ketone **69** as a pure compound. The second fraction CAT7.2 contained two compounds, cyperol and isocyperol which were separated by TLC using the system chloroform-methanol (10:1) as eluting solvent.

c) Eudesma-3,11-dien-2-one (**69**).

^{1}H-NMR (400 MHz, CDl_3): 0.90 (3H, *s*, H-10), 1.32 (1H, *m*), 1,40-1.70 (5H, *m*), 1.77 (3H, *s*, H-11), 1.91 (3H, *s*), 2.07 (1H, *m*), 2.20 (1H, *d*, J = 16 Hz), 2.27 (1H, *d*, J = 16 Hz), 2.40 (1H, *m*), 4.76 (2H, *m*, H-11), 5.90 (1H, *m*, H-3). MS (EI, 70

eV), m/z (rel. int.): 218(11) $[M]^+$, 203 (3), 176 (26), 133(21), 107 (16), 95 (100), 69 (60), 67 (34), 41 (35),

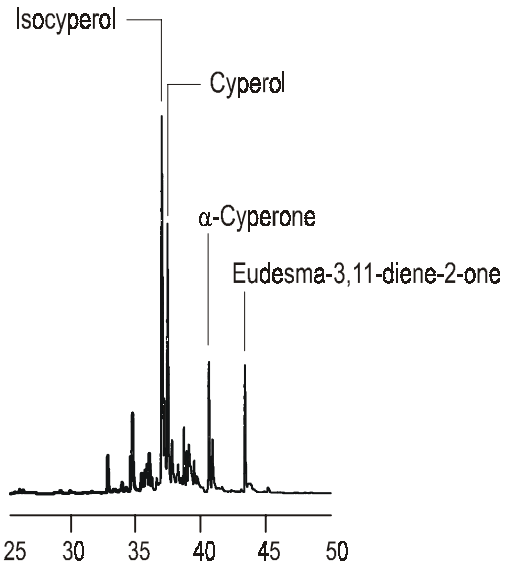


Fig. 85 : Gas chromatogram of seventh fraction from the column at lower temperature (-20°C).

a) Isocyperol (**70**).

^{1}H-NMR (400 MHz, $CDCl_3$): 0.70 (3H, *s*, H-14), 1.20 (1H, *q*, J = 12.61 Hz, H-6a), 1.64 (1H,*ddd*, $J_1 = J_2$ = 13.55 Hz, J_3 = 4.10 Hz, H-1a), 1.68 (3H, *s*, H-13), 1.75 (1H, *dt*, J_1 = 14.19 Hz, J_2 = 4.10 Hz, H-2e), 2.30 (1H, *d*, J = 12.61 Hz, H-5), 4.22 (1H, *m*, H-12), 4.52 (1H, *d*, J = 1.7 Hz, H-15), 4.98 (1H, *t*, J = 1.7 Hz, H-15). MS (EI,70eV), m/z (rel. int.) : 220 (37) $[M]^+$, 202 (33), 187 (31), 177 (13), 159 (18), 145 (29), 138 (100), 131 (52), 124 (55), 105 (47), 91 (50), 72 (39), 67 (39), 55 (42), 41 (73).

b) cyperol **(71)**.

^{1}H-NMR (400 MHz, $CDCl_3$) : 1.10 (3H, *s*, H-15), 1.25-1.40 (2H, *m*), 1.45-1.63 (5H, *m*), 1.73 (3H, *s*), 1.76 (3H, *s*), 1.83-1.95 (3H, *m*), 4.04 (1H, *t*, J = 6.61 Hz, H-3), 4.72 (2H, *m*, H-13). MS (EI, 70 eV), m/z (rel.int.) : 220 (55) $[M]^+$, 205 (36), 202 (29), 187 (31), 177 (13), 159 (24), 145 (33), 138 (77), 131 (52), 124 (45), 105 (60), 91 (61), 67 (47), 55 (57), 41 (100).

5.3 Analysis of *Cyperus papyrus*.

5.3.1 *Isolation of the three Patchoulane Sesquiterpenoids* 75, 76 *and* 80.

The raw essential oil of *C. papyrus* obtained by hydrodistillation of the air-dried rhizomes of the plant was separated by preparative GC (see fig. 3). A four ramp temperature programme was used, starting from 120° and rising up to 180° C. Fifteen fractions (CP1 to CP15) were collected.

The first fraction CP1 was a pure sample of cyprotene (**75**).

The second fraction contained cypera-2,4-diene (**76**) as major product and was further purified using the same column at 110° C to **76** with an acceptable purity.

From the third CP3 fraction we isolated isopatchoula-3,5-diene (**79**) was isolated, using the same process employed for **76**.

Epoxycyperene was obtained from the eighth fraction CP8. All other important fractions contained only known products such as cyperene (in CP4), rotundene (in CP6), (+)-caryophylleneoxide (in CP1), and cyperotundone (in CP13).

a) Cyprotene (**75**).

Crystalline compound, m.p. 22 - 23°C; 1H-NMR (400 MHz): 0.83 (3H, *d*, J = 6.62 Hz), 0.87 (3H, *s*), 0.90 (3H, *s*), 1.04 (3H, *s*), 1.03 (1H, *m*), 1.28 (1H,*m*), 1.38 (1H, *m*), 1.76 (1H, *m*), 1.89 (1H, *tddd*, $J_1 = J_2$ = 13.22 Hz, J_3 = 6.23 Hz, J_4 = 2.54 Hz, J_5 = 1.14 Hz), 1.97 (1H, *d(br)*, J = 18 Hz) 2.46 (1H, *d(br)*, J = 18 Hz), 5.19 (1H, *qt*, $J_1 = J_2 = J_3$ = 6.61 Hz, $J_4 = J_5$ = 2.54 Hz); ^{13}C-NMR (100 MHz): 14.3, 15.4, 16.9, 18.8, 26.7, 28.8, 33.4, 34.7, 37.8, 43.1, 44.1, 51.1,

115.7, 145.7; MS (EI, 70 eV) m/z (rel. int.): 192(39) (M^+), 177(67), 163(11), 149(33), 135(100), 121(78), 107(59), 93(31), 79(21), 67(20), 55(28), 41(56).

b) Cypera-2,4-diene (**76**).

Colourless oil: 1H-NMR: 0.51 (3H, *s*), 0.55 (3H, *d*, *J* = 6.61 Hz), 1.12 (3H, *s*), 1.27 (1H, *d(br)*, J_1 = 12.72 Hz, J_2 = 6.61 Hz) 1.45 (1H, *m*), 1.68 (1H, *m*), 1.73 (3 H, *s(br)*), 1.95 (1H, *tdd*, $J_1 = J_2$ = 12.72 Hz, J_3 = 6.62 Hz, J_4 = 2.55 Hz), 2.08 (1H, *m*), 2.10 (1H, *d(br)*, *J* = 17 Hz), 2.30 (1H, *dd(br)*, J_1 = 17 Hz, J_2 = 6.62 Hz), 5.82 (1H, *d*, *J* = 5.08 Hz), 6.09 (1H, *d*, *J* = 5.08 Hz); ^{13}C-NMR (100 MHz): 13.57, 18.90, 21.75, 24.28, 28.86, 31.80, 73.90, 41.53, 51.06, 133.52, 137.68, 151.55; MS (EI, 70 eV) m/z (rel. int.): 202(32) ($[M]^+$), 187(51), 177(10), 159(100), 145(37), 131(36), 119(54), 105(38), 91(36), 77(21), 69(13), 63(5), 55(20), 51(9).

c) Epoxycyperene (**80**).

^{1}H-NMR (400 MHz): 0.82 (3H, *s*), 0.91 (3H, *s*), 1.05 (3H, *d*, *J* = 7.12 Hz), 1.25 (3H, *s*), 1.38-1.46 (2H, *m*), 1.55-1.70 (5H, *m*), 1.83-2.5 (5H, *m*); ^{13}C-NMR 16.51, 18.66, 1962, 23.78, 25.25, 27.80, 28.22, 29.25, 32.99, 33.91, 42.82, 45.77, 55.58, 67.50, 82.20; MS (EI, 70eV), m/z (rel. int.): 220(7) (M^+), 205(47), 187(8), 177(18), 159(22), 147(23), 133(19), 119(100), 105(32), 91(28), 69(16), 65(8), 55(25), 51(5), 41(56).

5.3.2 Preparation of **76** *and* **79** *from Cyperotundone* **(77).**

Cypera-2,4-diene and isopatchoula-3,5-diene were prepared by reduction and subsequent dehydration of cyperotundone. All the steps were performed as described in the case α-cyperone. In addition to **76** and **79** a trace of compound appeared which was later identified as cypera-2,4(15)-diene (**97**).

5.3.3 *Preparation of Epoxycyperene* (80).

To a stirred dispersion of cyperene (10 mg) in deionised water powdered m-chloroperoxybenzoic acid (excess) was added at 0°C and the mixture was stirred at room temperature for 2 hours. The solution was then neutralized with diluted NaOH and extracted with ether [158].

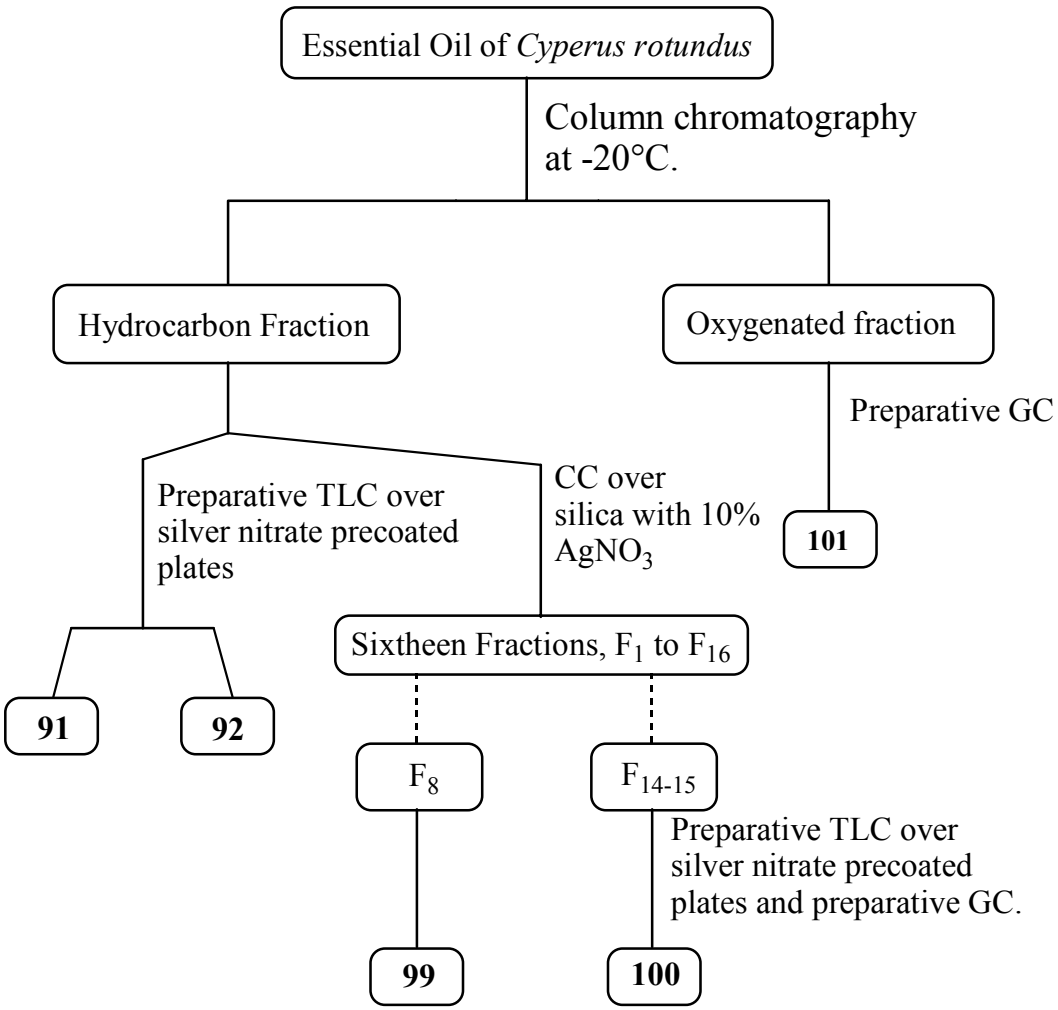


Fig. 86 : Process involved in the investigation of *Cyperus rotundus.*

5.4 Analysis of *Cyperus rotundus*.

The essential oil of *C. rotundus* was separated into two fractions, a hydrocarbon

fraction and an oxygenated one (see fig. 86) The hydrocarbon fraction was divided into two parts which were treated in different ways (also see fig. 86).

5.4.1 Isolation of (+)-Ylanga-2,4(15)-diene (91) *and Isorotundene* (92).

1 g of the hydrocarbon fraction of *C. rotundus* was separated by TLC using petroleum ether as eluting solvent. The aim of this separation was to eliminate the major hydrocarbon (cyperene) from the sample before any other operation. Three bands were obtained, the first two ones containing mainly cyperene and α-copaene. So only the third fraction (Rf = 0.45) was interesting because it contained the unknown products. This fraction was further separated by preparative GC (column 6T-2,3-Me-β-CD, temperature programme : from 110° to 180 C, rate 2°/min). The fifth fraction of the chromatogram contained (+)-ylanga-2,4(15)-diene. The eighth fraction was analysed by GCMS and three major products were present : valencene, α-selinene and an unknown product. This sample, which could not be separated by any available GC-column was submitted to preparative TLC on silver nitrate precoated plates, using petroleum ether as eluting solvent. Two bands were obtained, the second of which (Rf = 0.35) was taken up in petroleum ether and purified by preparative GC. Spectral data analysis resulted in the structure of isorotundene (**92**).

a) (+)-Ylanga-2,4(15)-diene (**91**).

^{1}H-NMR (500 MHz, C_6D_6) : δ 0.80 (6H,dd, J_1 = 6.61 Hz, J_2 = 1.53 Hz), 0.94 (3H,*s*), 1.33-1.61 (5H, *m*), 1.70 1H, *m*), 2.20 (1H, *dd*, J_1 = 7.12 Hz, J_2 = 2.03 Hz), 2.23 (1H, J_1 = 7.02 Hz, J_2 = 1.53 Hz), 2.22 (1H, *m*), 4.75 (1H, *m*), 4.77 (1H, *m*), 6.17 (1H, *dt*, J_1 = 8.51 Hz, $J_2 = J_3$ = 1.58 Hz), 6.20 (1H, *dd*, J_1 = 8.51 Hz, J_2 = 5.36 Hz). ^{13}C-NMR (125 MHz, C_6D_6) :19.94 (*q*), 20.41 (*q*)21.12 (*q*), 22.85 (*t*), 32.95 (*d*), 37.16 (*t*), 39.75 (*d*), 45.94 (*d*), 46.11 (*s*), 50.60 (*d*), 59.85

(*d*), 107.64 (*t*), 128.69 (*d*), 137.65 (*d*), 150.49 (*s*). MS, (EI, 70 eV), m/z (rel. int.) : 202 (17) $[M]^+$, 187 (11), 173 (9), 159 (81), 145 (31), 131 (75), 118 (99), 105 (79), 81 (31), 77 (44), 69 (32), 65 (31), 55 (63), 41 (76).

b) (-)- Isorotundene (**92**).

^{1}H-NMR (500 MHz, C_6D_6), δ 0.80 (3H, *s*), 0.86 (3H, *d*, *J* = 6.61 Hz), 1. 00 (1H, *t*, *J* = 13.24 Hz), 1.05 (1H, *dd*, J_1 = 12.93 Hz, J_2 = 9.46 Hz), 1.22 (1H, *dddd*, J_1 = *13.60* Hz, $J_2 = J_3$ = 11.35 Hz, J_4 = 6.70 Hz), 1.44 (1H, *m*), 1.46 (1H, *dd*, J_1 = 9.46 Hz, J_2 = 3.15 Hz), 1.52 (1H, *m*), 1.70 (1H, *m*), 1.68 (1H, *m*), 1.71 (1H, *m*), 1.72 (1H, *m*), 1.81 (1H, *ddd*, $J_1 = J_2$ = 9.45 Hz, J_3 = 5.90 Hz), 1.90 (1H, *dddd*, J_1 = 13.60 Hz, J_2 = 6.93 Hz, J_3 = 6.61 Hz, J_4 = 6.36 Hz), 1.99 (1H, *dddd*, J_1 = 13.24 Hz, $J_2 = J_3$ =6.36 Hz, J_4 = 5.90 Hz), 2.14 (1H, *dt*, J_1 = 17.02 Hz, $J_2 = J_3$ = 2.52 Hz), 2.27 (1H, *dd*, J_1 = 17.02 Hz, J_2 = 2.21 Hz), 5.85 (2H, *m*). ^{13}C-NMR (100 MHz, C_6D_6) : δ 16.10 (*q*), 25.20 (*t*), 27.53 (*t*), 29.28 (*t*), 31.48 (*t*), 32.28 (*q*), 33.15 (*t*), 34.38 (*s*), 38.25 (*d*), 38.64 (*d*), 41.52 (*d*), 44.36 (*t*), 56.19 (*t*), 107.98 (*t*), 151.08 (*s*). MS (EI, 70 eV), m/z (rel. int) : 204 (33) $[M]^+$, 189 (84), 175 (25), 161 (64), 144 (45), 133 (41), 119 (54), 108 (100), 93 (91), 79 (71), 67 (46), 55 (51), 41 (82).

5.4.2 Preparation of Isorotundene from Rotundene (56).

a) Hydration of Rotundene by Oxymercuration-Demercuration.

In a 50 ml flask, fitted with a magnetic stirrer, 48 mg of mercuric acetate is placed. To this is added 3 ml of water followed by 3 ml of THF. Then 30 mg (0.148 mmol) of rotundene is added. The reaction mixture is stirred for 15 min at room temperature to complete the oxymercuration. Then 3 ml of 3.0 M sodium hydroxyde is added followed by 3ml of a solution of 0.50 M sodium borohydride in 3.0 M sodium hydroxide. Reduction of the mercurial complex is almost instantaneous. The mercury is allowed to precipitate. Sodium

chloride was added to saturate the water layer. The upper layer of THF was separated and purified by preparative GC, giving 26 mg (80 %) of isorotundenol (**93**).

Isorotundenol $C_{15}H_{25}OH$ (**93**):

^{1}H-NMR (500 MHz, C_6D_6) : δ 0.75 (3H, *d*, *J* = 6.61 Hz), 0.76 (3H, *s*), 1.02 (3H, *s*), 1.08-1.54 (12H, *m*), 1.57-1.65 (2H, *m*), 1.75 (1H, *m*), 2.15 (1H, *m*). MS (EI, 70 eV), m/z (rel. int.) : 222 (5), 204 (19), 189 (27), 175 (7), 161 (14), 147 (12), 133 (13), 121 (33), 108 (100), 93 (57), 81 (39), 67 (24), 55 27), 41 (35).

b) Dehydration of isorotundenol.

Phosphoryl chloride (0.5 ml) was added to a pyridine solution (1 ml) of isorotundenol (10 mg). The mixture was stirred for 10 hours and pyridine was then removed by distillation at low pressure. The remaining residue was taken into hexane and separation by gas chromatography yielded 3 mg of isorotundene and 3 mg of rotundene.

5.4.3 Ozonolysis of Rotundene.

A solution of rotundene (50 mg) in dichloromethane (20 ml) was treated at -78° C with a stream of ozone until a persistant blue colour was observed. The solution was then purged with nitrogen for 5 min. Dimethyl sulfide (3 ml) was then added, and washed with ice-cold sodium bicarbonate solution (25 ml), water and brine. The organic layer was dried ($NaSO_4$) and the solvent was evaporated [161]. Purification of the residue by preparative GC gave the keto-acid **94** (45 mg).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.10 (3H, *d*, *J* = 6.61 Hz), 1.30 (1H, *m*), 1.35 (3H, *s*), 1.65-1.92 (9H, *m*), 2.11 (1H, *m*), 2.20 (1H, *m*), 2.29 (3H, *s*), 2.58-2.85 (2H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$) : 16.73, 25.10, 25.66, 27.47, 28.30,

30.07, 31.09, 31.60, 39.64, 41.70, 48.56, 48.64, 50.55, 184.59, 212.01. MS (EI, 70 eV) m/z (rel. int) : 252 (13) $[M]^+$, 234 (7), 206 (15), 191 (11), 163 (36), 149 (7), 133 (10), 121 (18), 107 (25), 95 (68), 81 (43), 67 (22), 55 (28), 43 (100).

5.4.4 Decarboxylation of the Keto-acid **94.**

The keto-acid (40 mg, 0.16 mol) and silver nitrate (1 mg) were dissolved in acetonitrile (6 ml) and water (2 ml) and heated to reflux. To this solution potassium persulfate ($K_2S_2O_8$) (0.32 mg) in water (5 ml) over 15 mn was added. Refluxing was continued another 5min before the reaction mixture was cooled and extracted with a saturated sodium bicarbonate solution (10 ml, three times), dried ($MgSO_4$) [162] and purified by preparative GC to give 14 mg of the ketone **95**.

^{1}H-NMR (500 MHz, $CDCl_3$) : 0.96 (3H, *dd*, J = 6.62 Hz), 1.06 (3H, *s*), 1.40 (1H, *m*), 1.60-1.84 (7 H, *m*), 1.97-2.1 (2H, *m*), 2.13-2.24 (3H, *m*), 2.27 (3H, *s*), 2.44 (1 H, *m*), 2.69 (1H, *dd*, J_1 = 13.87 Hz, J_2 = 6.94 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) : 15.10 (*q*), 21.52 (*q*), 26.09 (*t*), 26.80 (*t*), 27.85 (*q*), 30.26 (*t*), 33.25 (*t*), 37.46 (*t*), 37.99 (*d*), 38.04 (*d*), 46.82 (*d*), 56.70 (*d*), 61.65 (*d*), 207.93 (*s*). MS, (EI, 70 eV), m/z (rel. int) : 208 (5) $[M]^+$, 190 (7), 175 (6), 161 (5), 150(8), 135 (9), 123 (27), 109 (23), 95 (100), 81 (31), 67 (25), 55 (29), 43 (52).

5.4.5 Reduction of the Ketone **95.**

Sodium hydride (0.45 mmol) in a three-necked flask was washed with several portions of pentane to remove the mineral oil. The flask was then equipped with rubber stopper, a reflux condenser and a magnetic stirrer. The system was alternately evacuated and filled with nitrogen; 3 ml of dimethyl sulfoxide was introduced via a syringe and the mixture was heated at 75°-80° C for 45 mn. The resulting solution of methylsulfinyl carbanion was cooled in an ice-water

bath, and 108 mg of methyltriphenylphosphonium bromide in 100ml of warm dimethyl sulfoxide was added. The resulting dark red solution of the ylide was stirred at room temperature for 10 min before 7 mg of the ketone **95** in two ml of dimethyl sulfoxide was added. The reaction mixture was then heated at 56° C for sixteen hours after which the solution was allowed to cool and 10 ml of water was added. The two phases were extracted three times with n-pentane (40 ml). The n-pentane fractions were combined and washed with 10 ml of a (1/1) water/dimethyl sulfoxide solution and then with 30 ml of 50 % saturated sodium chloride solution. The pentane layer was then dried over anhydrous sodium sulfate and purification by preparative GC gave 2 mg of the alkene **96**.

MS (EI, 70 eV), m/z (rel. int) : 206 (12) $[M]^+$, 191 (18), 177 (6), 163 (51), 149 (25), 122 (39), 107 (56), 95 (60), 81 (100), 67 (57), 55 (66), 41 (79).

5.4.6 Hydrogenation of the Alkene **96** *and (+)-γ-gurjunene* **(98).**

a) The alkene **96** (1mg) was dissolved in a mixture of ethanol (1 ml) and benzene (1 mg). The catalyst $((Ph)_3P)_3RhCl$ (1mg) was added and the solution took an orange colour. A stream of hydrogen was then passed through the solution under normal pressure for fifteen minutes. The solvent was removed under reduced pressure and petroleum ether was added, since it does not dissolve the major part of the catalyst [164]. Filtration through a column of alumina afforded the pure alkane **97**. MS (EI, 70 eV), $[M]^+$, m/z = 208.

b) The hydrogenation of (+)-γ-gurjunene (**98**) followed the same procedure as that of the ketone **97** except the catalyst was replaced by nickel on charcoal.

5.4.7 Isolation of Norrotundene and Cypera-2,4(15)-diene **(99).**

The realisation of the above described chemical transformation needed a great amount of rotundene to be isolated from the essential oil of *C. rotundus*.

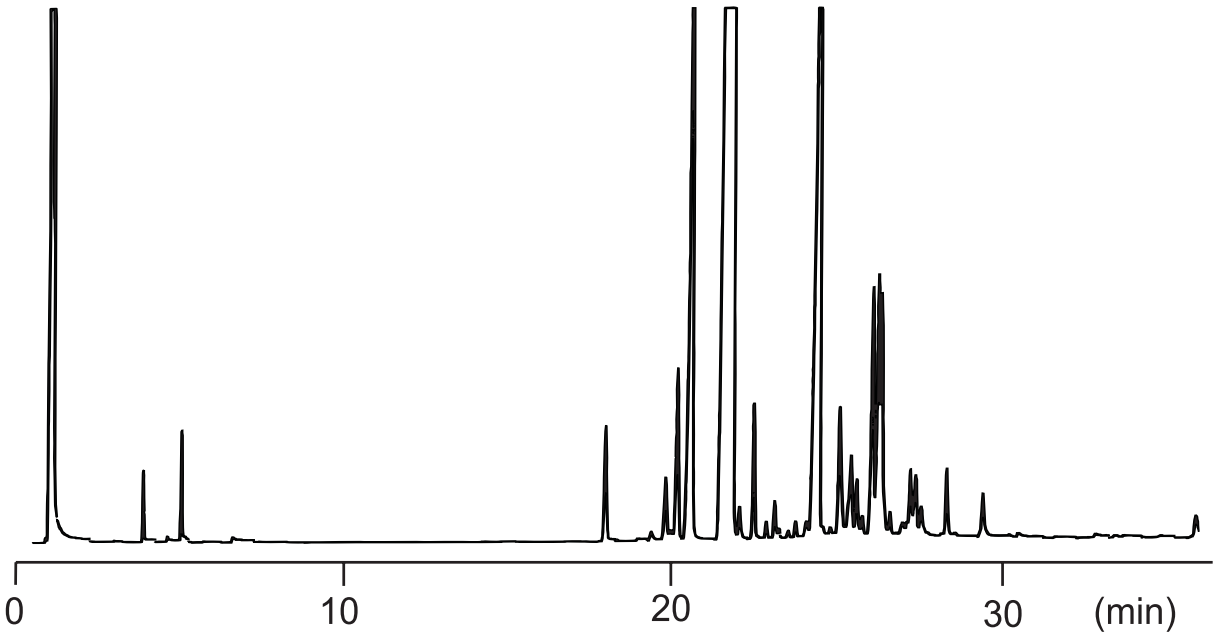


Fig. 87 : Gas chromatogram of the hydrocarbon fraction of the essential oil of *Cyperus rotundus* (CP-Sil 5 CB, 50°C, 3°/min to 230°C).

For the isolation 5 g of the hydrocarbon fraction (see fig. 87) of the oil was separated by column chromatography over silver nitrate precoated silica using a petroleum ether-chloroform gradient system for elution. The eluents from the column were monitored by capillary GC and sixteen fractions were collected. The fractions obtained by elution with petroleum ether (from 1 to 6) contained only known hydrocarbons. The fractions 7 to 10 were obtained by elution with the system petroleum ether-chloroform (7/3) and contained all of the compounds intended to isolate, rotundene. In the GCMS of the eighth fraction it was realised that the sample also contained an unknown minor compound. The separation of this fraction was performed by preparative GC. (Column: 2,6-Me-3Pe-β-CD; temperature programme: 100-140° C, rate 2°/min). The chromatogram displayed five peaks and the fifth contained the pure unknown product which was characterised as cypera-2,4(15)-diene (**99**). The fractions 11 to 15 of the chromatographic column were eluted with the system petroleum ether-chloroform (1/1). All the constituents of the sample 11 to 13 could be identified

by GCMS. But in the fractions 14 and 15 also analysed by GCMS, an unknown product of molecular weight 190 was found.

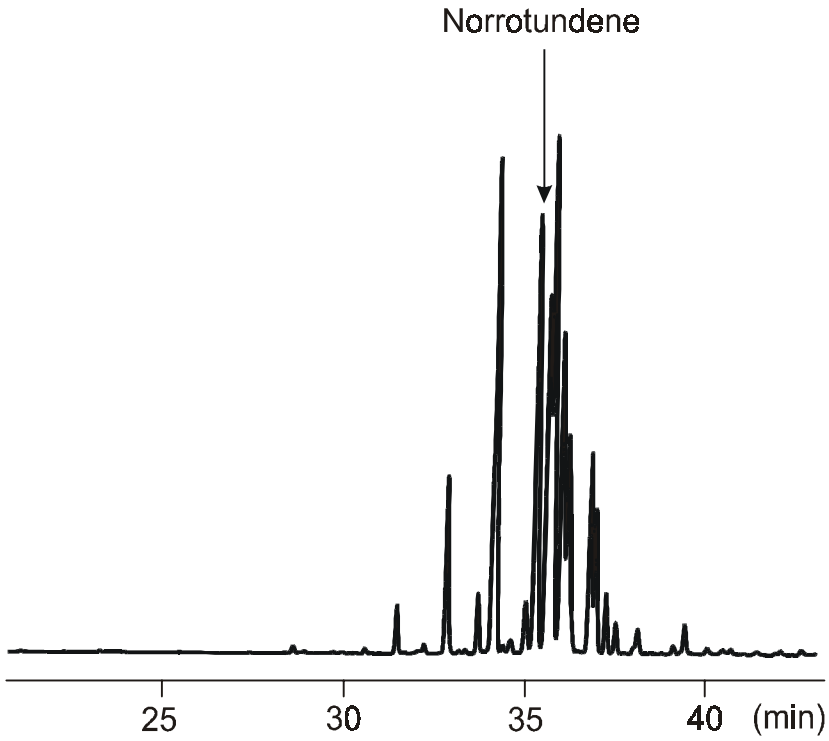


Fig. 88 : Gas chromatogram of the fraction containing norrotundene (**100**).

Since the isolation of this compound by preparative GC was not easy (see fig. 88), first a fractionation of the sample by preparative TLC over silver nitrate precoated plates was performed, using the system petroleum ether-chloroform (7/3) as eluting solvent. Three bands were obtained and the third one, which contained the norsesquiterpene as major product was separated by preparative GC (SE 30, temperature: 125°C)). Spectral data permited the characterization of the compound as norrotundene (**100**).

a) Cypera-2, 4(15)-diene (**99**).

^{1}H-NMR (400 MHz, C_6D_6) : δ 0.77 (3H, *d*, *J* = 6.61 Hz), 0.99 (3H, *s*), 1.01 (3H, *s*), 1.03 (1H, *m*), 1.29 (1H, *m*), 1.51 (1H, *dt*, J_1 = 14.75 Hz, J_2 = 6.11 Hz), 1.85 (1H, *m*), 1.86 (1H, *m*), 2.10 (1H, *m*), 2.12 (1H, *dddd*, J_1 = 13.22 Hz, $J_2 = J_3 = J_4$ = 6.11 Hz), 3.03 (1H, *m*), 4.98 (2H, *m*), 5.69 (1H, *dd*, J_1 = 5.60 Hz, J_2 = 1.02 Hz), 6.17 (1H, *d*, *J* = 5.60 Hz). ^{13}C-NMR (400 MHz, C_6D_6) : 17.32 (*q*), 19.61 (*q*), 26.36 (*q*), 26.64 (*q*), 27.70 (*t*), 28.48 (*s*), 30.16 (*t*), 32.58 (*t*), 44.96 (*d*), 47.66 (*d*), 61.93 (*s*), 102.84 (*t*), 133.26 (*d*), 139.17 (*d*), 148.94 (*s*). MS (EI, 70 eV) m/z (rel. int.) : 202 (83) $[M]^+$, 187 (24), 173 (6), 173 (6), 159 (46), 145 (24), 131 (37), 118 (85), 106 (100), 91 (78), 77 (33), 69 (17), 65 (18), 55 (26), 41 (61).

b) Norrotundene (**100**).

^{1}H-NMR (500 MHz, C_6D_6) : δ 0.95 (1H, *dd*, $J_1 = J_2$ =13.24 Hz), 0.99 (3H, *d*, *J* = 6.62 Hz), 1.13 (3H, *s*), 1.12 (1H, *ddd*, J_1 = 13.86 Hz, J_2 = 11.72 Hz, J_3 = 5.14 Hz), 1.42 (1H, *dddd*, J_1 = 13.50 Hz, $J_2 = J_3$ =11.00 Hz, J_4 = 6.70 Hz), 1.52 (1H, *ddd*, J_1 = 11.72 Hz, J_2 = 9.44 Hz, J_3 = 6.11 Hz), 1.54 (1H, *m*), 1.61 (1H, *m*), 1.66 (1H, *ddd*, J_1 = 13.24 Hz, J_2 = 9.14 Hz, J_3 = 6.36 Hz), 1.80 (1H, *dddd*, J_1 = 13.24 Hz, $J_2 = J_3 = J_4$ = 6.36 Hz), 1.83 (1H, *m*), 1.87 (1H, *m*), 1,91 (1H, *ddd*, J_1 = 13.86 Hz, J_2 = 9.44 Hz, J_3 = 5.00 Hz), 2.10 (1H, *m*), 2.2 (1H,*dddd*, J_1 = 13.50 Hz, J_2 = 6.62 Hz, J_3 = 6.36 Hz, J_4 = 7.00 Hz), 2.56 (1H, *m*), 6.02 (2H, *m*). ^{13}C-NMR (125 MHz, C_6D_6) : 14.73 (*q*), 22.95 (*t*), 28.00 (*t*), 28.67 (*t*), 29.36 (*q*), 30.35 (*d*), 30.54 (*t*), 31.97 (*t*), 35.72 (*s*), 36.32 (*d*), 42.18 (*d*), 50.74 (*d*), 129.15 (*d*), 139.10 (*d*). MS (EI, 70 eV) m/z (rel. int.) : 190 (10) $[M]^+$, 175 (15), 161 (20), 148 (7), 133 (9), 119 (7), 107 (14), 94 (100), 79 (50), 67 (12), 55 (14), 41 (23).

5.4.8 Isolation of Cyperadione (101).

After finishing with the study of the hydrocarbon fractions, we were interested in identifying products contained in the oxygenated part of the oil The oxygenated fraction was then submitted to preparative TLC using the system petroleum ether-ethyl acetate (8/2) as eluting solvent. The second band (Rf. = 0.35) was then taken up in diethyl ether and submited to further separation by preparative GC (column: 6T-2,3-Me-β-CD, 120°-180°C, 2°/mn).

Cyperadione (**101**).

^{1}H-NMR (400 MHz, $CDCl_3$) : δ 0.78 (3H, *d*, *J* = 6.62 Hz), 0.98 (3H,*s*), 1.10 (1H, *dddd*, J_1 = 14.50 Hz, $J_2 = J_3$ = 12.93 Hz, J_4 = 6.62 Hz), 1.22 (3H, *s*), 1.45 (1H, *dq*, J_1 = 13.56 Hz, J_2 = 3.20 Hz), 1.50 (1H, *ddd*, J_1 = 15.44 Hz, J_2 = 11.66 Hz, J_3 = 5.68 Hz), 1.68 (1H, *ddd*, J_1 = 14.50 Hz, $J_2 = J_3$ = 5.99 Hz), 1.90 (1H, *ddd*, J_1 = 6.62 Hz, $J_1 = J_2$ = 3.31 Hz), 1.96 (1H, *d*, *J* = 19.00 Hz), 2.01 (1H, *dddd*, J_1 = 13.56 Hz, J_2 = 12.93 Hz, J_3 = 6.31 Hz, J_4 = 2.68 Hz), 2.10 (1H, *m*), 2.11 (1H, *m*), 2.18 (3H, *s*), 2.35 (1H, *ddd*, J_1 = 16.40 Hz, J_2 = 11.66 Hz, J_4 = 4.41 Hz), 2.44 (1H, *ddd*, J_1 = 16.40 Hz, J_2 = 12.30 Hz, J_3 = 5.68 Hz), 2.48 (1H, *dd*, J_1 = 19.00 Hz, J_2 = 7.25 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 16.76 (*q*), 21.87 (*t*), 22.18 (*q*), 26.42 (*t*), 27.05 (*q*), 28.10 (*t*), 30.03 (*q*), 31.32 (*d*), 38.71 (*t*), 41.79 (*d*), 41.84 (*t*), 42.96 (*s*), 58. 21 (*s*), 208.50 (*s*), 220.25 (*s*). MS (EI, 70 eV), m/z (rel. int.) : 236 (66),221 (89), 207 (7), 193 (25), 179 (63), 161 (17), 150 (20), 135 (22), 123 (22), 107 (25), 95 (19), 81 (20), 67 (23), 55 (27), 43 (100).

6 References.

1. E. Gildemeister, in Die Ätherischen Öle, Erster Band, Verlag von Schimmel und Co. (1910). *Algemeine Geschichte der Ätherischen Öle.*

2. F.-C. Czygan, Pharma. in Uns. Zeit. **10**, 109 (1981). *Ätherische Öle und Duft kulturhistorisch betrachtet.*

3. D. Martinetz and R. Hartwig, Taschenbuch der Riechstoffe, p. 5-15, Verlag Harri Deutsch (1998).

4. G. Ohloff, Irdische Düfte - Himmlische Lust, Insel Verlag (1996).

5. Marcell Lavabre, Aromatherapy Workbook, Healing Arts Press, Rochester, Vermont 05767 (1990).

6. T. Curtis and D. G. William, Introduction to Perfumery, p. 220, Ellis Horwood Limited (1994).

7. E. Farber, The Evolution of Chemistry, A History of its Ideas, Methods, and Materials, p. 72, The Ronald Pree Company, 2nd Edition (1969).

8. W. H. Brock, The fontana history of Chemistry, p. 78-87, Fontana Press (1992).

9. J. B. Dumas, Justus Liebig's Annal. Chem., **34**, 245 (1833). *Über die vegetabilischen Substanzen, welche sich dem Campher nähert und über einige ätherische Öle.*

10. M. Berthelot, Compt.-Rend. Acad. Sci. **55**, 496, 544 (1862). *Nouvelles Recherches sur les camphènes et sur l'isomérie dans les séries alcooliques.*

11. A. Kekulé, Berl. Berichte, **6**, 437 (1873). *Neue Umwandlung des Terpentinöls in Cymol.*

12. G. Bouchardat, Compt.-Rend. Acad. Sci., **80**, 1446 (1875). *Sur la Synthèse d'un Terpilène ou Carbure Camphenique.*

13. W. A. Tilden, Journal of the Chemical Society, **28**, 514 (1875). *On the Action of Nitrosyl Chloride on Organic Bodies Part II. On Turpentine Oil.*

14. W. A. Tilden, Journal of the Chemical Society, **31**, 554 (1877). *Isomeric Nitroso-terpenes.*

15. O. Wallach, Justus Liebig's Annal. Chem., **239**, 1 (1887). *Zur Kenntnis der Terpene und der ätherischen Öle.*

16. L. Ruzicka**,** Journal of the Chemical Society, 1582 (1932). *The Life and Work of Otto Wallach.*

17. O. Wallach, Terpene und Campher, Veit & Co., Leipzig (1909).

18. L. Ruzicka und M. Stoll, Helvetica Chimica Acta, **5**, 923 (1922). *Höhere Terpenverbindungen VII. Konstitution des Eudalins, des Selinens und des α-Santalens. Das Kohlenstoffgerüst der Sesquiterpene.*

19. L. Ruzicka, Helvetica Chimica Acta, **54**, 1753 (1971). *Rolle der Riechstoffe in meinem chemischen Lebenswerk.*

20. L. Ruzicka and J. Meyer, Helvetica Chimica Acta, **4**, 505 (1921). *Über Sesquiterpenverbindungen I. Überführung des Cadinens in einen Naphtalinkohlenwasserstoff.*

21. W. Voser, M.V. Mijovic, H. Heusser, O Jeger, and L. Ruzicka, Helvetica Chimica Acta, **35**, 2414 (1921). *Über die Konstitution des Lanostadienols (Lanosterins) und seine Zugehörigkeit zu den Steroiden.*

22. L. Ruzicka, Experientia, **9**, 357 (1953). *The Isoprene Rule and the Biogenesis of Terpenic Compounds.*

23. L. Ruzicka, Annual Review of Biochemistry, **42**, 1 (1973). *In the Borderland Between Bioorganic Chemistry and Biochemistry.*

24. W. H. Perkin, Ber. Deutsch. Chem. Gesel. Berl., **8**[2], 1599 (1875). *Über die Bildung von Cumarin, Cumarsäure und ähnlicher Säuren.*

25. R. Fittig and W. H. Mielch, Justus Liebig's Ann. Chem. Pharm., **152**, 25 (1869). *Untersuchungen über die Constitution des Piperins und seiner Spaltungsprodukte Piperinsäure und Piperidin.*

26. a) F. Tiemann, Ber. Deutsch. Chem. Gesel. Berl., **9**, 52 (1876). *Über eine Bildungsweise der Vanillinsäure und des Vanillins aus Eugenol, sowie über die Synthese der Ferulasäure.*

b) E. Erlenmeyer, Ber. Deutsch. Chem. Gesel. Berl., **9**, 273 (1876). *Über Vanillinbildung aus Eugenol.*

c) F. Tiemann, Ber. Deutsch. Chem. Gesel. Berl., **24**[2], 2870 (1891). *Über Isoeugenol, Diisoeugenol und Derivate derselben.*

27. F. Tiemann and P. Krüger, Ber. Deutsch. Chem. Gesel. Berl., **26**[3], 2675 (1893). *Über Veilchenaroma.*

28. J. S. Jellinek, Dragaco Report, **3**, 113 (1998). *Die Geburt des modernen Parfums.*

29. K.- H. Kubeczka in Vorkommen und Analytik ätherischer Öle, p. 61, K.-H. Kubezcka (Hrsg), Thieme Verlag, Stuttgart. *Möglichkeiten zur Beurteilung arzneilich verwendeter ätherischer Öle.*

30. D. H. R. Barton and P. de Mayo, Quarterly Review, **11**, 189 (1957). *Recent Advances in Sesquiterpenoid Chemistry.*

31. The New Encyclopedia Britannica, **4**, p. 563, Edited by R. P. Gwinn, 15th Edition (1985).

32. H. Schilcher in Vorkommen und Analytik ätherischer Öle, p. 58, K.-H. Kubeczka (Hrsg.), Thieme Verlag, Stuttgart. *Vorschlag zu einer biologisch orientierten Definition der ätherischen Öle.*

33. R. Hegnauer in Vorkommen und Analytik ätherischer Öle, p. 58, K.-H. Kubeczka (Hrsg.), Thieme Verlag, Stuttgart. *Verbreitung ätherischer Öle im Pflanzenreich.*

34. G. Cohn and F. Riechter, Die Riechstoffe, p. 1, 2nd. Edition (1924).

35. T. Curtis and D. G. William, Introduction to Perfumery, p. 230, Ellis Horwood Limited (1994).

36. H. Farnow, Dragoco Report, **2**, 27 (1968). *Erfahrungen bei der spectrometrischen und gaschromatographischen Untersuchungen von ätherischen Ölen.*

37. A. T. James, and A. J. P. Martin, The Biochemistry Journal, **50**, 679 (1952). *Gas-liquid Partition chromatography: The Separation and microestimation of Volatile Fatty Acids Formic Acid to Dodecanoic Acid.*

38. E. Bayer, G. Kupfer, and H.- H. Reuther, Zeitschrift für Analytische Chemie, **164**, 1 (1958). *Anwendung der Gaschromatographie zur Analyse künstlicher und natürlicher Aromastoffe.*

39. W. A. König, Gas Chromatographic Enantiomer Separation with Modified

Cyclodextrins, Hüthig Buchverlag, Heidelberg (1992).

40. W. A. König, A. Rieck, I. Hardt, B. Gehrcke, K.-H. Kubeczka, and H. Muhle, Journal of High Resol. Chrom., **17**, 314 (1994). *Enantiomeric Composition of the Chiral Constituents of Essential Oils, Part 2 Sesquiterpene Hydrocarbons.*

41. B. Testa, Trends in Pharmacological Science, **7**, 60 (1986). *Chiral Aspect of Metabolism.*

42. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin (1990).

43. W. A. König in Drug Stereochemistry, 2nd Ed. by I. W. Wainer (1993). *The Direct Resolution of Enantiomeric Drugs by Chiral-Phase Gas Chromatography.*

44. W. A. König, A. Krüger, D. Icheln and T. Runge, Journal of High Resol. Chrom. **15**, 184 (1992). *Enantiomeric Composition of the Chiral Constituents of Essential Oils, Part 1 Monoterpene Hydrocarbons.*

45. A. Mosandl, Journal of Chromatography, **624**, 267 (1992). *Capillary Gas Chromatography in Quality Assessment of Flavours and Fragrances.*

46. P. Kreis, A. Mosandl, and H.-G. Shmarr, Dtsch. Apoth. Ztg. **130**, 2579 (1990). *Mentha-Öle: Enantioselective Analyse des Menthylacetats zur Qualitätsbeurteilung von Mentha-Ölen.*

47. I. Hardt and W. A. König, Journal of Chromatography A, **666**, 611 (1994). *Preparative Enantiomer Separation with Modified Cyclodextrins as Chiral Stationary Phases.*

48. A. Kekulé, Lehrbuch der Oganischen Chemie, Band II, p. 437, (1866).

49. L. Ruzicka and E. Capato, Helvetica Chimica Acta, **8**, 259 (1925). *Höhere Terpenverbindungen XXIV: Ringbildungen bei Sesquiterpenen. Totalsynthese des Bisabolens und eines Hexahydro-Cadalins.*

50. J. Würsch, R. L. Huang and K. Bloch, Journal of Biological Chemistry, **195**, 439 (1952). *The Origin of the Isooctyl Side Chain of Cholesterol.*

51. A. Eschenmoser, Chimia, **44**, 1 (1990). *Leopold Ruzicka - From the Isoprene Rule to the Question of the Life's Origin.*

52. L. Ruzicka, Proceedings of the Chemical Society, 344 (1959). *History of the*

Isoprene Rule.

53. D. Joulain, and W. A. König, The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E. B. - Verlag, Hamburg (1998).

54. F. Lynen, H. Eggerer, U. Henning, and I. Kessel, Angewandte Chemie, **70**, 738 (1958). *Farnesyl Pyrophosphat und 3-Methyl-Δ^3-butenyl-1-pyrophosphat, die biologischen Vorstufen des Squalens.*

55. B. H. Amdur, H. Rilling and K. Bloch, Journal of the American Chemical Society, **79**, 2646 (1957). *The Enzymatic Conversion of Mevalonic Acid to Squalene.*

56. F. Lynen, E. Reichert, and L. Rueff, Jutus Liebig's Ann. Chem., **574**, 1 (1951). *Zum biologischen Abbau der Essigsäure, Ihre Isolierung aus Hefe und ihre chemische Natur.*

57. M. Rohmer, M. Seeman, S. Horbach, S. Bringer-Myer and H. Sahm, Journal of the American Chemical Society, **118**, 2564 (1996). *Glyceraldehyde-3-phosphate and Pyruvate as Precursors of Isoprenic Units in an Alternative Non-Mevalonate Pathway for Terpenoid Biosynthesis.*

58. B. W. Agranoff, H. Eggerer, U. Henning, and F. Lynen, Journal of the American Chemical Society, **81**, 1254 (1959). *Isopentenol Pyrophosphate Isomerase.*

59. T. Koyama and K. Ogura in Compr. Nat. Prod. Chem., Vol. 2, by D. E. Cane, Elsevier (1999). *Isopentenyl Diphosphate Isomerase and Prenyltransferase.*

60. N. I. Eberhardt and H. C. Rilling, Journal of Biological Chemistry, **250**, 863 (1975). *Prenyltransferase from Saccharomyces cerevisiae.*

61. L. C. Tarshis, M. Yan, C. Dale Poulter, and J. C. Sacchettini, Biochemistry, **33**, 10871 (1994). *Crystal Structure of Recombinant Farnesyl Diphosphate Synthase at 2.6 Å.*

62. G. Popjak and J. W. Conforth, Biochemistry Journal, **101**, 553 (1966). *Substrate Stereochemistry in Squalene Biosynthesis.*

63. J. W. Conforth, R. H. Conforth, G. Popjak and L. Yengoyan, J. Biol. Chem., **241**, 3970 (1966). *Studies on the Biosynthesis of cholesterol- Steric course of Decarboxylation of 5-Pyrophosphomevalonate and the Carbon to Carbon*

bond Formation in the Biosynthesis of Farnesyl Pyrophosphate.

64. D. E. Cane, Chemical Review, **90**, 1089 (1990). *Enzymatic Formation of Sesquiterpenes.*

65. D. E. Cane, in Compr. Nat. Prod. Chem. vol. 2, Edited by D. E. Cane, Elsevier, (1999). *Sesquiterpene Biosynthesis: Cyclisation Mechanism.*

66. a) R. Croteau, and A. Gundy, Arch. Biochem. Biophys., **233**, 838 (1984). *Cyclisation of Farnesyl Pyrophosphate to Sesquiterpene Olefins Humulene and Caryophyllene by an Enzyme System from Sage (**Salvia officinalis**).*

b) D. E. Cane, Acc. Chem. Res., **18**, 220, (1985). *Isoprenoid Biosynthesis. Stereochemistry of the Cyclisation of Allylic Pyrophosphates.*

67. C. R. Benedict, I. Alchanati, P. J. Harvey, J. Liu, R. D. Stipanovic, A. A. Bell, Phytochemistry, **39**, 326 (1995). *The Enzymatic Formation of δ-Cadinene from Farnesyl Diphosphate in Extracts of Cotton.*

68. W. Francke and S. Schulz, in Compr. Nat. Prod. Chem., Vol. 8, Edited by K. Mori, Elsevier, (1999). *Pheromones.*

69. R. Baker, M. Borges, N. G. Cooke, and R. H. Hebert, Journal of the Chemical Society Chem. Commun., 414 (1987). *Identification and Sythesis of (2)-(1'S,3'E,4'S)(-)-2-(3',4'-Epoxy-4'-methylcyclohexyl)-6-methylhepta-2,5-diene, the Sex Pheromone of the Southern Green Stinkbug, **Nezara viridula (L.)**.*

70. J. R. Aldrich, W. R. Lusby, B. E. Marron, K. C. Nicolaou, M. P. Hoffman, and L. T. Wilson, Naturwissenschaften, **76**, 173 (1989). *Pheromone Blends of Green Stink Bugs and Possible Parasitoid Selection.*

71. L. Machlis, W. H. Nutting, M. W. Williams, and H. Rappoport, Biochemistry, **5**, 2147 (1966). *Production, Isolation, and Characterisation of Sirenine.*

72. K. E. Harding, J. Byron Strickland and J. Pommerville, Journal of Organic Chemistry, **53**, 4877 (1988). *A New Synthesis of (±)-Sirenine and a Physiologically Active Analogue.*

73. W. A. Ayer, and L. M. Browne, Tetrahedron, **37**, 2199 (1981). *Terpenoid Metabolites of Mushrooms and Related Basidiomycetes.*

74. T. C. Mc Morris and M. Andel, Journal of the American Chemical

Society, **85**, 831 (1963). *The Structure of the Basidiomycete Metabolytes Illudin S and Illudin M.*

75. X. Luo, H. J. C. Yeh, A. Brossi, J. L. Flippen-Anderson, and R. Gilardi, Helvetica Chimica Acta, **67**, 1515 (1984). *Configuration of Antimalarials Derived from Qinghaosu: Dihidroqinghaosu, Artemether, and Artesunic Acid.*

76. D. L. Klayman, Science, **228**, 1049 (1985). *Quinghaosu (Artemisinine) : An Antimalarial Drug from China.*

77. N. Acton, and D. L. Klayman, Planta Medica, 441 (1985). *Artemisinine, A New Sesquiterpene Lactone Peroxide from **Artemisia annua**.*

79. a) T. Norin, Acta Chemica Scand., **15**, 1676 (1961). *The chemistry of the Natural Order of Cupressales 40*. The Structure of Thujopsene and Hinokiic Acid.*

b) T. Norin, Acta Chemica Scand., **17**, 738 (1963). *The Configuration of Thujopsene.*

80. G. Büchi, R. E. Erickson, and N. Wakabayashi, Journal of the American Chemical Society, **83**, 927 (1961). *Terpenes. XVI. Constitution of Patchouli Alkohol and Absolute Configuration of Cedrene.*

81. F. Näf, R. Decorzant, W. Giersch, and G. Ohloff, Helvetica Chimica Acta, **64**, 1387 (1981). *A Stereocontrolled Access to (±)-, (-)-, and (+)-Patchouli alkohol.*

82. W. S. Zhou, Pure and Applied Chemistry, **58**, 817 (1986). *Total Synthesis of Arteannuin (Qinghaosu) and Related Compounds*.

83. Y. Saritas, Diplomarbeit, Universität Hamburg (1995). *Die Sesquiterpen-Kohlenwasserstoffe im Lebermoos **Marchantia polymorpha***.

84. G. E. Martin and R. C. Crouch, Journal of Natural Products, **54**, 1- 70 (1991). *Inversed-Detected Two-Dimensional NMR Methods: Applications in Natural Products Chemistry.*

85. M. Hesse, H. Meier, and B. Zeeh, Spektroskopische Methoden in der Organischen Chemie, 5th Edition, Georg Thieme Verlag (1995).

86. J. K. Sanders, and B. K. Hunter, Modern NMR Spectroscopy, Oxford

University Press (1990).

87. E. Breitmaier, Vom NMR-Spektrum zur Strukturformel organischer Verbindungen, 2nd Edition, Teubner-Studienbücher (1992).

88. Atta-Ur-Rahman, and M. I. Choudhary, Solving Problems with NMR-Spectros-copy, Academic Press (1996).

89. M. Karplus, Journal of Chemical Physic, **30**, 11 (1959). *Contact Electron-Spin Coupling of Nuclear Magnetic Moments.*

90 V. F. Bystrov, Progress in NMR Spectroscopy., **10**, 41 (1976). *Spin-Spin Coupling and the Conformational State of Peptide Systems.*

91. W. P. Aue, J. Karhan, and R. R. Ernst, Journal of Chemical Physic., **64**, 4226 (1976). *Homonuclear Broad Band Decoupling and Two-Dimensional J-Resolved NMR-Spectroscopy.*

92. T. S. Chamblee, B. C. Clark,Jr, T. Radford and A. Iacobucci, Journal of Chromatography, **330**, 141 (1985). *General Method for the High-Performance Liquid Chromatochrographic Prefractionation of Essential Oils and Flavor Mixtures for Gas Chromatographic-Mass Spectrometric analysis*.

93. K. Nagayama and WK. Wüthrich, European Journal of Biochemistry, **115**, 653 (1981). *Structural Interpretation of Vicinal Proton-Proton Coupling Constants ^3J-$H^{\alpha}H^{\beta}$ in the Basic Pancreatic Trypsin Inhibitor Measured by Two-Dimensional J-Resolved Spectroscopy.*

94. M. Eberstadt, G. Gemmecker, D. F. Mierke, and H. Kessler, Angewandte Chemie., **107**, 1813 (1995). *Skalare Kopplungen - Ihre Analyse und ihre Verwendung zur Strukturaufklärung.*

95. D. Marion, and K. Wüthrich, Biomed. Biophy. Res. Commun., **113**, 967 (1983). *Application of Phase Sensitive Two Dimensional Correlated Spetroscopy for Measurement of Proton-Proton Spin Coupling constants in Proteins.*

96. J. Keeler, and D. Neuhaus, Journal of Magnetic Resonance, **63**, 454 (1985). *Comparison and Evaluation of Methods for Two-Dimensional NMR- Spectra with Absoption Mode Lineshapes.*

97. Y. Kim, and J. H. Pretegard, Journal of Magnetic Resonance, **84**, 9

(1989). *Measurement of Vicinal couplings from Cross Peaks in COSY Spectra.*

98. A. E. Derome, Modern NMR Techniques for Chemistry Research, p. 209-221, Pergamon Books, 1987.

99. P. Bachman, W. P. Aue, L. Müller, and R. R. Ernst, Journal of Magnetic Resonance, **28**, 29 (1977). *Phase Separation in Two-Dimensional Spectroscopy.*

100. O. B. Ramsay, Nobel Prize Topics in Chemistry - Stereochemistry, p. 81, Edited by J. W. van Spronson, Heyden & Son (1981).

101. A. F. Tomas, and M. Ozaine, Tetrahedron Letters, **20**, 1717 (1976). *The stereochemistry of the Dihydroagarofurans.*

102. I. Wahlberg, A. E. Eklung, T. Nishida, and C. R. Enzell, Acta Chemica. Scand. B, **35**, 307 (1981). *Tobacco chemistry 53*. Two New Nor-Drimanes from Greek Tobacco.*

103. G. Ohloff, N. Näff, R. Decorzant, W. Thommen, and E. Sundt, Helv. Chim. Acta, **56**, 1414 (1973). *Synthesis of Potential Ambra Odorants : 5,5,9-Trimethyldecalyl Derivatives.*

104. C. Chapuis, M. Barthe, B. L. Muller, and K. H. Schulte-Elte, Helv. Chim. Acta, **81**, 153 (1998). *Preparation and Absolute Configuration of (-)-E-α-trans-Bergamotenone.*

105. a) A. Rieck, N. Bülow, and W. A. König, Phytochemistry, **4**, 847 (1995). *An Epoxy-Trinoreudesmane Sesquiterpene from the Liverwort* ***Lophocolea bidentata****.*

b) A. Rieck, N. Bülow, C. Fricke, Y. Saritas, and W. A. König, Phytochemistry, . *(-)1(10), 11-Eremophiladien-9β-ol from the Liverwort* ***Marchantia polymorpha ssp. Aquatica****.*

106. A. O. Oyedeji, O. Ekundayo, M. Mekem Sonwa, C. Fricke, and W. A. König, Phytochemistry, **48**, 657 (1998). *(-)-Eudesma-1,4(15),11-triene from The Essential Oil of* ***Callitris intratropica****.*

107. C. R. Metcalfe, Anatomy of the Monocotyledons, V. *Cyperaceae*, Edited by C. R. Metcalfe, Clarendon Press (1971).

108. C. Inglis, Cyperaceae Newsletter, **4**, 13 (1994). *Checklist of Economically Important Cyperaceae.*

109. D. A. Simpson, Cyperaceae Newsletter, **2**, 10 (1992). *Notes on Cyperaceae in North-Eastern Thailand.*

110. R. D. Allan, R. J. Wells, and J. K. MacLeod, Tetrahedron Letters, 7 (1973). *Flavonone Quinones from **Cyperus** Species.*

111. R. D. Allan, R. L. Correll, and R. J. Wells, Tetrahedron Letters, 4669 (1969). *A new Class of Quinones from Certain Members of the Family Cyperaceae.*

112. R. D. Allan, R. W. Dunlop, M. J. Kendall, and R. J. Wells, Tetrahedron Letters, 3 (1973). *C_{15} Quinones from **Cyperus** Species.*

113. J. K. MacLeod, B. R. Worth, and R. J. Wells, Tetrahedron Letters, 241 (1972). *Synthesis of Benzofuranoid System II. Total Synthesis of Cyperaquinone and Conicaquinone.*

114. B. Trivedi, O. Motl, J. Smolikova, and F. Sorm, Tetrahedron Letters, 1197 (1964). *Structure of the Hydrocarbon Cyperene.*

115. P. Joseph-Nathan, E. Martinez, R. L. Santillan, J. R. Wesener and H. Günther, Organic Magnetic Resonance., **2**, 308 (1984). *Two-Dimensional NMR Studies of Cyperene.*

116. M. D. Dassanayake and F. R. Fosberg, A Revised Handbook to the Flora of Ceylon, V, p.213. Edited by Balkema, Rotterdam (1985).

117. B. Nyasse, R. Ghogumu Tih, B. L. Sodengam, M. T. Martin, and B. Bodo, Phytochemistry, **27**, 179 (1988). *Isolation of α-Corymbolol, an Eudesmane Sesquiterpene Diol from **Cyperus articulatus**.*

118. B. Nyasse, R. Ghogumu Tih, B. L. Sodengam, M. T. Martin, and B. Bodo, Phytochemistry, **27**, 3319 (1988). *Mandassidione and other Sesquiterpenic Ketones from **Cyperus articulatus**.*

119. J. A. Garbarino, V. Gambaro, and M. C. Chamy, Journal of Natural Products, **48**, 323 (1985). *The Structure of Corymbolone, an Eudesmane Sesquiterpenoid Keto-Alcohol from **Cyperus corymbosus**.*

120. V. H. Kapadia, V. G. Naik, M. S. Wadia and S. Dev,

Tetrahedron Letters, 4661 (1967). *Sesquiterpenoids from the Essential Oil of **Cyperus rotundus**.*

121. S. B. Nerali, P. S. Kalai, K. K. Chakravarti, and S. C. Batacharya, Tetrahedron Letters, 4053 (1965). *Terpenoids LXXVII. Structure of Isopatchoulenone, a New Sesquiterpene Ketone from the Oil of **Cyperus scariosus**.*

122. H. Hikino, and K. Aota, Phytochemistry, **15**, 1265 (1976). *4α,5α-Oxidoeudesm-11-en-3α-ol, Sesquiterpenoid of **Cyperus rotundus**.*

123. a) H. Hikino, K. Aota, D. Kuwano, and T. Takemoto, Tetrahedron, **27**, 4831 (1971). *Structure and Absolute Cofiguration of α-Rotunol and β-Rotunol, Sesquiterpenoids of **Cyperus rotundus**.*

b) A. Murai, M. Ono, A. Abiko, and T. Masamune, Bulletin of the Chemical Society of Japan, **55**, 1195 (1982). *Synthesis of α- and β-Rotunols. Revision of the Structure of a Stress Metabolite "1-Keto-α-Cyperone".*

124. S. B. Nerali and K. K. Chakravarti, Tetrahedron Letters, 2447 (1967). *Terpenoids CXVII - Structures of Cyperenol and Patchoulenol Two New Sesquiterpene Alcohols from The Oils of **Cyperus scariosus**.*

125. S. Ohira, T. Hasegawa, K.-I. Hyashi, T. Hoshino, D. Takaoka, and H. Nozaki, Phytochemistry, **47**, 1577 (1998). *Sesquiterpenoids from **Cyperus rotundus**.*

126. S. S. Martin J. H. Langenheim, and E. Zavarin, Phytochemistry, **15**, 113 (1976). *Biosynthesis of Sesquiterpenes in **Hymenaeae** inferred from their Quantitative Co-occurence.*

127. F. Bohlmann, and C. Zdero, Phytochemistry, **17**, 1135 (1978). *Neue Furaneremophilane und andere Sesquiterpene aus Vertreter der Gattung **Euryops**.*

128. E. J. Corey, R. B. Mitra, and H. Uda, Journal of American Chemical Society, **86**, 485 (1964). *Total Synthesis of d,l-Caryophyllene and d,l-Isocaryophyllene.*

129. R. Randriamiharisoa, E. M. Gaydou, and R. Faure, Magnetic Resonance in Chemistry, **24**, 275 (1986). *Carbon-13 NMR Spectra of five Sesquiterpenes Isolated from **Ylang-Ylang** Essential oil.*

130. S. K. Panikar and O. Motl, Tetrahedron Letters, 2121 (1977). *Structures of*

Rotundene and Rotundenol.

131. E. Klein, and W. Rojahn, Tetrahedron Letters, 279 (1970). *(-)-7β,10α-Selina-4,11-dien und (+)-5β,7β,10α-Selina-3,11-dien zwei neue Sesquiterpene der Eudesmanreihe.*

132. a) S. M. Dixit, A. S. Rao and S. K. Panikar, Chemistry and Industry (London), 1256 (1967). *Examination of Essential Oil from* ***Seseli Indicum*** *Wight and Arnold.*

b) B. Maurer and A. Grieder, Helvetica Chimica Acta, **60**, 2183 (1977). *Sesquiterpenoids from Costus Root Oil (****Saussurea lappa*** *Clarke).*

133. a) J. D. Bunko, E. L. Ghisalberti, and P. R. Jefferies, Australian Journal of Chemistry, **34**, 2237 (1981). *(1R,4S)-Calamenene. Chemical Correlation with the Diterpene Analogues of* ***Eremophila****.*

b) S. Melching, N. Bülow, K. Wihstutz, S. Jung, and W. A. König, Phytochemistry, **44**, 1291 (1997). *Natural Occurrence of both Enantiomers of Cadina-3,5-diene and δ-Amorphene.*

134. A. F. Barrero, J. F. Sanchez, J. E. Oltra, J. Altarejos, N. Ferrol, A. Barragan, Phytochemistry, **30**, 1551, (1991). *Oxygenated Sesquiterpenoids from the wood of* ***Juniperus oxycedrus****.*

135. S.-I. Fukuzawa, T. Fujinami, S. Yamauchi, and S. Sakai, Journal Chemical Society Perkin Trans. I, 1929 (1986). *1,2- Regioselective Reduction of α,β-Unsaturated Carbonyl Compounds with Lithium Aluminium Hydride in Presence of Lanthanoid Salts.*

136. a) R. Howe and F. J. McQuillin, Journal of the Chemical Society, 2423 (1955). *The Structure of Cyperone. Part IV. The Synthesis of (Natural) (+)-α-Cyperone, its Enantiomorph, and Epimer.*

b) A. A. Haaksma, B. J. M. Jansen, and A. de Groot, Tetrahedron, **48**, 3121 (1992). *Lewis acid Catalysed Diels-Alder Reactions of S-(+)-Carvone with Silyloxy Dienes. Total Synthesis of (+)-α-cyperone.*

137. M. Mekem Sonwa, W. A. König, K.-H. Kubeczka, and O. Motl, Phytochemistry, **45**, 1435 (1997). *Sesquiterpenes from the Essential Oil of* ***Cyperus alopecuroides****.*

138. A. Merz, Angewandte Chemie, **85**, 868 (1973). *Phasentransfer-katalysierte*

Alkylierung von Alkoholen mit Dimethylsulfat im wäßrigen System.

139. E. Demole and P. Enggist, Helvetica Chimica Acta, **66**, 1381 (1983). *Further Investigation of Grapefruit Juice Flavor Components (**Citrus paradisi** MacFayden). Valencane- and Eudesmane-Type Sesquiterpene Ketones.*

140. H. Hikino, K. Aota, and T. Takemoto, Chemical and Pharmaceutical Bulletin, **15**, 1929 (1967). *Structure and Absolute Configuration of Cyperol and Isocyperol*.

141. S. Takano and S. Kawaminami, Phytochemistry, **27**, 1197 (1988). *Cyperenyl Acetate and Cyperenal from **Cirsium dipsacolepis**.*

142. a) F. Kido, R. Sakuma, H. Uda, and A. Yoshikoshi, Tetrahedron Letters, 3169 (1969). *Minor acidic Constituents of Vetiver Oil Part II. Cyclcopacamphenic and Epicyclocopacamphenic Acids.*

b) S. W. Baldwin, and J. C. Tomesch, Journal of Organic Chemistry, **45**, 1455 (1980). *Total synthesis of dl-Cyclosativene by Cationic Olefinic and Acetylenic Cyclisations.*

143. a) P. de Mayo, R. E. Williams, Journal of the American Chemical Society, **87**, 3275 (1965). *Sativene Parent of the Toxin from **Helminthosporium sativum**.*

b) E. Wenkert, B. C. Bookeser, and T. S. Arrhenius, Journal of the American Chemical Society, **114**, 644 (1992). *Total Synthesis of (±)-α- and (±)-β-Copaene and Formal Total Synthesis of (±)-Sativene, (±)-cis-Sativenediol, and (±)-Helminthosporal.*

144. K.-H. Kubeczka, O. Motl, and V. Formacek, Proceedings of the 20 th International Symposium on Essential Oils. Würzburg, Germany, 1989. *The Structure of Cyprotene, a Novel C_{14} Hydrocarbon Related to Sesquiterpenoids.*

145. a) H. Hikino, K. Aota, and T. Takemoto, Chemical and Pharmaceutical Bulletin, **14**, 890 (1966). *Structure and absolute Configuration of Cyperotundone.*

b) H. Hikino, K. Ito, and K. Aota, Chemical and Pharmaceutical Bulletin, **16**, 43 (1968). *Synthesis of Cyperene, Cyperotundone and Patchoulenone.*

146. Y. Gopichand, P. R. Pednekar, and K. K. Chakravarti, Indian Journal of Chemistry, **16B**, (1978). *Isolation and Characterisation of (-)-a-Selinene and Isopatchoula-3,5-diene from **Cyperus scariosus** Oil.*

147. M. Dassanayake, and F. R. Fosberg, A Revised Handbook to the Flora of Ceylon, V, p. 181, A. A. Balkema / Rotterdam (1985).

148. a) C. Ehret and G. Ourisson, Tetrahedron,**25**, 1785 (1969). *Le γ-Gurjunene, Structure et Configuration. Isomerisation de l'α-Gurjunene.*

b) G. M. König, and A. D. Wright, Journal of Organic Chemistry, **62**, 3837 (1997). *New and Unusual Sesquiterpenes : Kelsoene, Prespatane, Epi-γ-Gurjunene, and T-Cadinthiol from Tropical Marine Sponge **Cymbbastela hooperi**.*

149. a) J. Krepinsky, O. Motl, L. Dolejs, L. Novotny, V. Herout, and R. B. Bates, Tetrahedron Letters, 3315 (1968). *The Structure of Eremophilene, the Sesquiterpenic Hydrocarbon from **Petasites** genus.*

b) G. Majetisch, M. Behnke, and K. Hull, Journal of Organic Chemistry, **50**, 3615 (1985). *A Stereoselective synthesis of (±)-Nootkatene and (±)-Valencene via an Intramolecular Sakurai Reaction.*

150. W. D. MacLeod, Jr, Tetrahedron Letters, 4779 (1965). *The Constitution of Nootkatone, Nootkatene, and Valencene.*

151. K. Adachi, M. Mori, Bulletin of the Chemical Society of Japan, **56**, 651 (1983). *Studies of Aromatic Sesquiterpens. IV. The Synthesis of γ-Calacorene, Calamenene, and 4-Methoxyisocadalene.*

152. F. Bohlmann and C. Zdero, Chemische Berichte, **109**, 2021 (1976). *Über die Inhaltstoffe von **Heterotheca inuloides Cass**.*

153. K. Takashi and M. Takani, Chemistry and Pharmaceutical Bulletin, **24**, 2000 (1976). *Studies on the Constituents of Medicinal Plants XVII. Constituents of **Schizandra nigra** Max. and Their Carbon-13 Nuclear Magnetic Resonance Spectra.*

154. T. A. van Beek, R. Kleis, M. A. Posthumus, and A. Van Veldhuizen, Phytochemistry **28**, 1909 (1989). *Essential Oil of **Amyris balsamifera**.*

155. A. Zabza, M. Romanuk, and V. Herout, Collect. Czch. Chem. Commun., **31**, 3373 (1966). *On Terpenes. CLXXX. The Structure of Two Muurolenes from Turpentine of **Pinus silvestris L**.*

156. C. M. Beecham, C. Djerassi, and H. Eggert, Tetrahedron, **34**, 2503 (1978). *The Sesquiterpenes from the Soft Coral Sinularia Mayi.*

157. A. R. Pray, Inorganic Synthesis, **5**, 153 (1957). *Anhydrous Metal Chlorides*.

158. F. Fringuelli, R. Germani, F. Pizzo, and G. Savelli, Synthetic Communications, **19**, 1939 (1989). *One-Pot Two-Steps Synthesis of 1,2-Diol.*

159. a) H. C. Brown, and P. J. Geoghegam, Jr, Journal of Organic Chemistry, **35**, 18844 (1970). *Solvomercuration-Demercuration. I. The Oxymercuration of Representative Olefins in Aqueous System. A Convenient Mild Procedure for the Markownikov Hydration of the Carbon-Carbon Double Bond.*

b) H. C. Brown, and P. J. Geoghegam, Jr, Journal of Organic Chemistry, **37**, 1937 (1972). *Solvomercuration-Demercuration. III. The Relative Rate of Oxymercuration of Representative Olefins in an Aqueous Tetrahydrofuran System.*

160. J. M. Greenwood, M. D. Solomon, J. K. Sutherland, and A. Torre, Journal of the Chemical Society. (C), 3004 (1968). *A Cyclisation of Humulene.*

161. J. E. McMurry, and G. K. Bosch , Journal of Organic Chemistry, **52**, 4885 (1987). *Synthesis of Macrocyclic Terpenoid Hydrocarbons by Intramolecular Carbonyl Coupling: Bicyclogermacrene, Lepidozene and Casbene.*

162. W. E. Fristad, M. A. Fry, and J. A. Klang, Journal of Organic Chemistry, **48**, 3575 (1983). *Persulfate / Silver Ion Decarboxylation of Carboxylic Acids. Preparation of Alkanes, Alkenes, and Alcohols.*

163. a) E. J. Corey, and M. Chaykovsky, Journal of the American Chemical Society, **84**, 866 (1962). *Methylsufinylcarbanion*.

b) R. Greenwald, M. Chaykovsky, and E. J. Korey, Journal of the American Chemical Society, **28**, 1128 (1963). *The Wittig Reaction Using Methylsulfinyl Carbanion-Dimethyl Sulfoxide*.

164. a) A. S. Hussey, and Y. Takeushi, Journal of the American Chemical Society, **91**, 672 (1969). *Hydrogenation of Cycloalkenes Using Tris(triphenylphosphine)-chlororhodium(I)*.

b) R. E. Harmon, J. L. Parsons, D. W. Cooke, S. K. Gupta, and J. Schoolenberg, The Journal of Organic Chemistry, **34**, 3684 (1969). *Homogeneous Catalytic Hydrogenation of Unsaturated Organic Compounds*.

165. A. P. Marchand, Stereochemical Application of NMR Studies in Rigid

Bicyclic Systems, p. 193. Verlag Chemie International (1982).

166. W. Willker, D. Leibfritz, R. Kerssebaum, and W. Bermel, Magnetic Resonance in Chemistry, **31**, 287 (1993). *Gradient Selection in Inverse Heteronuclear Correlation Spectroscopy*.

167. A. D. Rodriguez, E. Gonzalez, C. Ramirez, Tetrahedron, **54**, 11683 (1998). *The structural chemistry, reactivity, and total synthesis of dolabellane diterpenes*.

Lebenslauf

Persönliche Daten

Name:	Mesmin Mekem Sonwa
Geburtsort:	N'kongsamba (Kamerun)
Geburtstag:	14. Dezember 1966.
Staatsangehörigkeit:	Kamerunisch
Familienstand:	ledig

Schulausbildung

1973-1979:	Grundschule "Ecole Saint-Martin", N'kongsamba.
1979-1986:	Gymnasium "Lycée du Manengouba", N'kongsamba.
Juni 1986:	Abitur (Baccalaureat C)

Studium

Oktober 1986:	Beginn des Physik- und Chemiestudiums an der Universität Yaounde.
1991-1992:	Diplôme D'Études Approfondies en Chimie Organique an der Universität Yaounde.
Januar 1996:	Beginn der vorliegenden Dissertation an der Universität Hamburg.

Berufliche Tätigkeit

1992-1994:	Lehraufträge der Universität Yaounde (Kamerun) im Fachbereich Chemie.
1994-1995:	Lehraufträge der Universität Douala (Kamerun) im Fachbereich Chemie.