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Abstract

This paper facilitates a deeper insight into the biological, chemical and toxicological profile, the occurrence, biosynthesis, total synthesis, metabolism and pharmacological relevant aspects of *α*-bisabolol. This sesquiterpene odorant molecule seems to exhibit many significant biological properties: diverse effects on skin, gastroprotection, hepatoprotection, antioxidative, anti-hyperalgesic, anti-edematous, antimicrobial, insecticidal, anti-nociceptive, anti-inflammatory, anti-spasmodic and other activities. As many of these properties had been already determined few decades ago, recently, the anticancer activity of *α-B* has become the focus of intense interest in many studies, *in vitro* and *in vivo* as well.

Considering chemical properties, such as the molecule chirality, mechanisms of activity and the evidenced toxicological profile of this multifaceted terpenoid alcohol, extensive data of numerous scientific studies report on a wide spectrum and safe utilisation of this molecule. By utilising powerful and precise analytical methods in these studies, a detailed pharmacological assessment of *α-bisabolol* significantly indicates on a possible importance of this molecule in the future of therapy. Although this paper allows a more or less detailed insight into the general properties of *α-B,* nevertheless, many new studies still have to be carried out in order to obtain the full overview of this molecule and to enhance its use in therapy.

KEYWORDS: sesquiterpenes, *α-B*, chemical and biological properties

Zusammenfassung

Dieses Werk ermöglicht einen tieferen Einblick in das biologische, chemische und toxikologische Profil, das Vorkommen, die Biosynthese, Synthese, Metabolismus und die pharmakologisch relevanten Aspekte von ***α-B***. Dieses duftende Sesquiterpenmolekül weist viele signifikante biologische Eigenschaften auf: Diverse Effekte auf die Haut, gastroprotektive, hepatoprotektive, antioxidative, antyhyperalgesische, antiödematöse, antimikrobielle, insektizide, antinozizeptive, entzündungshemmende, krampflösende und andere Wirkungen. Da sehr viele dieser Eigenschaften bereits vor einigen Jahrzehnten nachgewiesen wurden, ist jüngst die Antikrebs-Aktivität zum Mittelpunkt von großem Interesse in zahlreichen Studien, *in vitro*, aber auch *in vivo*, geworden.

Wenn man die chemischen Eigenschaften, wie die Chiralität des Moleküls, die Mechanismen der Aktivität und das bewiesene toxikologische Profil dieses vielseitigen Alkohols in Betracht zieht, deuten ausführliche Daten zahlreicher Studien an das breite Spektrum und eine sichere Anwendung des Moleküls. Leistungsstarke und präzise analytische Methoden in diesen Studien und eine detaillierte pharmakologische Begutachtung weisen in signifikanter Weise auf eine große Bedeutung dieses Moleküls für zukünftige therapeutische Anwendungen. Obwohl diese Übersicht einen, mehr oder weniger, detaillierten Einblick über die generellen Eigenschaften von α-B ermöglicht, müssen, dennoch, weitere Studien durchgeführt werden, um einen ausführlichen Überblick über das Molekül zu erhalten und dessen Nutzen in der Therapie zu fördern.

STICHWÖRTER: Sesquiterpen, α-B, chemische und biologische Eigenschaften

Index

Abl- abelson murine leukemia viral oncogene homology

Akt (PKB) - protein kinase B

ALFPs- amplified fragments length polymorphisms

ALR2- aldose reductase

AML- acute myeloid leukemias

AP1- activator protein-1

4AP- 4-aminopyridine

ATP- adenosine triphosphate

B- bisabolol

Bax- bcl-2-like protein 4

Bcl2- B-Cell lymphoma 2

BCR- breakpoint cluster region

Bid- BH3 interacting domain death agonist

CAP- compound-action-potential

Cat- catalase

COSY- correlated spectroscopy

COX- cyclooxygenase

CREB- cAMP response element-binding protein

CYP 450- cytochrome P450

DPPH- 2, 2-diphenylpicrylhydrazyl

EGR1- early growth response protein 1

ESI-MS- electrospray ionisation mass spectrometry

FDA- Food and Drug Administration

GC- gas chromatography

GC-MS- gas chromatography coupled to mass spectrometry

GP- glycogen phosphorylase

GRAS- generally regarded as safe

GSH-px- glutathione peroxidase

HER2- human epidermal growth factor receptor 2

HPLC- high performance liquid chromatography

5HT- 5 hydroxytryptamine receptors

IL6- interleukine 6

LACL- luminol-amplified chemiluminiscence

LC- liquid chromatography

L-NAME- nitroarginine methyl ester

5-LOX- 5-lipoxygenase

LPS- lipopolysaccharide

MDA- malondialdehyde

MIC- minimum inhibition concentration

MIRS- mid-infrared spectroscopy

MITF- microphthalamia-associated transcription factor

MO- mustard oil

MPO- myeloperoxidase

mPTP- mitochondrial permeability transition pore

MS- mass spectrometry

MSH- melanocyte-stimulating hormone

MTT- (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NADPH- nicotinamide adenine dinucleotide phosphate

NCS- isothiocyanate

neu oncogene- transforming gene

NF-kB- nuclear factor-kappa B

NMR- nuclear magnetic resonance

NO- nitric oxide

NOESY- nuclear overhauser effect spectroscopy

NOS- nitrogen-oxide-synthase

OPLS-Da- orthogonal projection to latent structures data

PARP- poly-(ADP-ribose)-polymerase

PCR- polymerase chain reaction

QSAR- quantitative structure-activity relationship

RAPDs- random amplified polymorphic DNAs

ROS- reactive oxygen species

SDH- sorbitol dehydrogenase

SIN1- 3-morpholinosydnoimine

SOD- superoxide dismutase

SPR- surface plasmon resonance

STAT3- signal transducer and activator of transcription 3

TI- therapeutic indices

TKI- tyrokinase inhibitors

TNF-α- tumour necrosis factor α

TPA- 12-O-tetradecanoylphorbol-13-acetate

TRPV1- transient receptor potential vanilloid

CONTENT

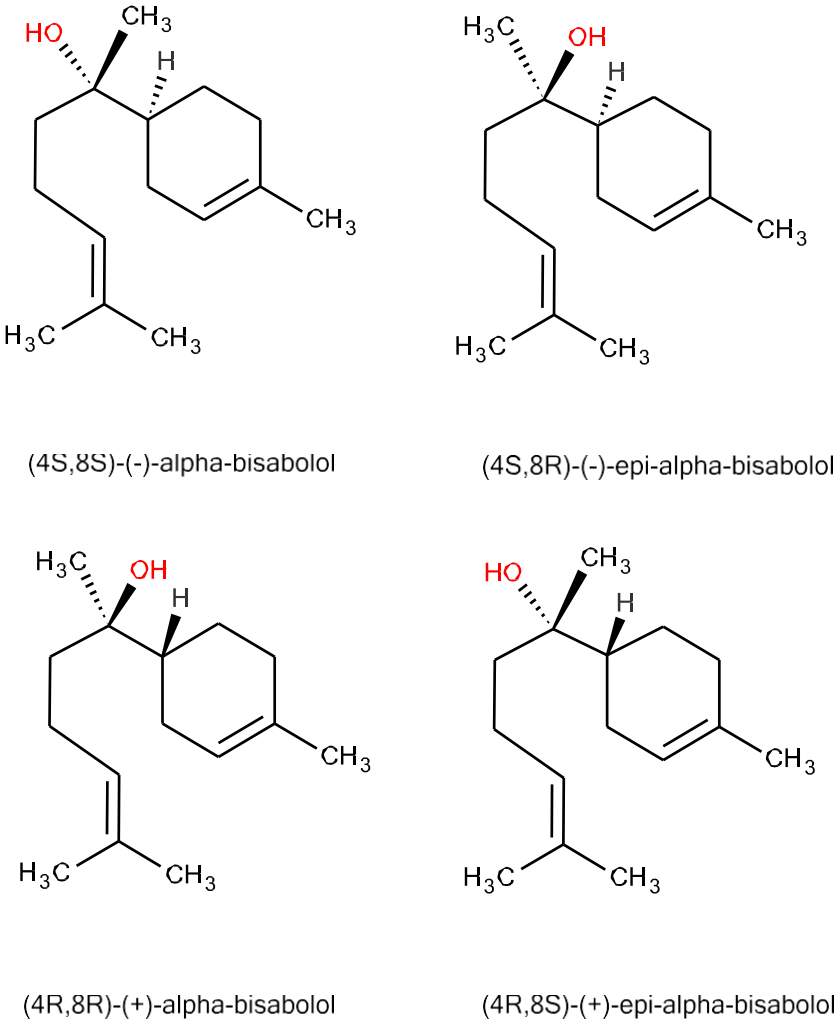
Introduction

The medicinal value of plants has been known and utilised since ancient times. Trials of replacing classic medications and therapeutic aspects by taking advantages of the alternative medicine are emerging constantly. However, information concerning the real human health benefits of natural products is yet seldom available, which is a drawback for their possible valuation. In order to assess the efficacy and safety of products, numerous *in vitro* studies need to be carried out. Although very useful, they are not enough, which requires an extensive observation of them in human or animal models. [1] Many plant-derived compounds have shown a whole spectrum of therapeutic properties. Terpenoids, for example, constitute a class of lipophilic secondary plant metabolites derived from mevalonate and isopentenyl pyrophosphate.[2] Sesquiterpenoids, 15-C-terpenoids, have been the subject of considerable research in recent years and have commonly been identified as active constituents of several medicinal plants used in traditional medicine with a wide spectrum of biological activity. Sesquiterpenic compounds are extensively found in essential oils of several plants and fruits, providing a wide spectrum of aromas, mostly perceived as very pleasant and responsible for the aroma perception of several natural products. These compounds have been used together with medicinal plants and fruits with different health applications. [1]

Bisabolol [1-methyl-4(1,5-dimethyl-1-hydroxhex-4(5)-enyl)-cyclohexene-1] is a natural, unsaturated, optically active, monocyclic sesquiterpene alcohol found in essential oils obtained from a variety of plants, shrubs and trees. When referring to B, *(-)-α-B* is implied. This notion is also explained in further studies. This small molecule, also known as *Levomenol*, has a molecular mass of 222.37 Dalton. Two chiral centers and there exist four stereoisomers in nature (Figure 1). [3, 4] Natural *α-B* consists 97% of the active *(-)-α-B* isomer, whereas synthetic *α-B* contains only 42.5% of the active *(-)-α-B* isomer. [5] Synthetic bisabolol is usually a racemic mixture of the two enantiomers: *(-)-α-B* and *(+)-α-B*. *(+)-α-B* isolated from the essential oil of the balsam poplar,

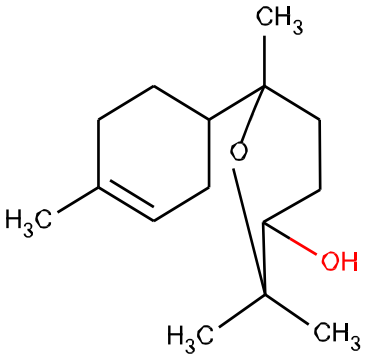
Populus balsamifera Mill. (Salicaceae), shows a lower efficacy than its levorotatory enantiomer. [6]

Figure 1 Four stereo isomers of α-B [7]

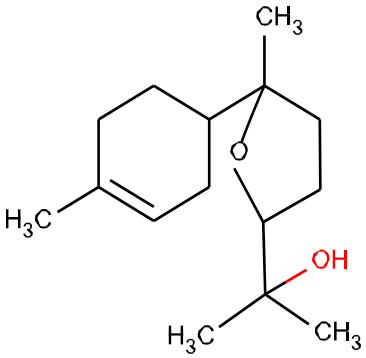


α-B, a colorless liquid, has a relatively low density (0.93) and a boiling point of 153 °C at 12 Torr. B is a very lipophilic substance with a propensity to get oxidised. It is almost insoluble in water, but soluble in ethanol. The oxidation products are mainly B-oxide A and B (Figure 2) [2]

Figure 2 bisabololoxide A and bisabololoxide B [8]



(-)-alpha-bisabololoxide A

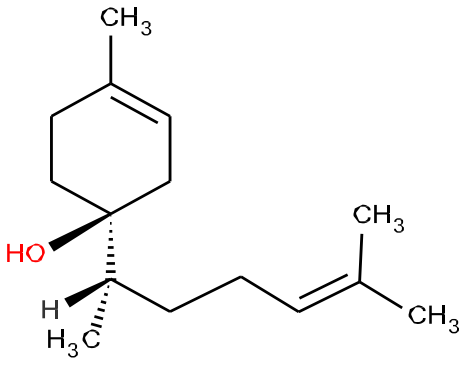


(-)-alpha-bisabololoxide B

First isolated by hydrodistillation in 1951 by Isaac and collaborators from the blossoms of chamomile *(Chamomilla recutita L. or Matricaria chamomilla* L. (Asteraceae)*)*, [2] whose essential oil contains up to 50 %, this highly lipophilic substance seems to be much more than a commercially important aroma chemical. bisabolol, a colourless viscous oil of a delicate floral odor is also comprised in species such as the Candeia tree, native in savannas of Brazil *(Vanillosmopsis erythropappa* (DC.) Sch.Bip. and *Vanillosmopsis arborea* (Gardner) Baker (Asteraceae)*)*, *Zanthoxylum tinguassuiba* L. (Rutaceae) growing in Brazil, *Smyrniopsis aucheri* Boiss. (Apiaceae) growing in Azerbaija, *Salvia runcinata* L.f. and *Salvia stenophylla* Burch. ex Benth. (Lamiaceae), plants indigenous to South Africa. Then also in *Stachys obtusicrena* Boiss. (Lamiaceae) growing in Iran, *Myrcia fosteri* Croat (Myrtaceae) and *Plinia cerrocampanensis* Barrie (Myrtaceae) growing in Panama, also in *Arnica longifolia* D.C.Eaton (Asteraceae), *Aster hesperius*

A.Gray (Asteraceae), *Chrysothamnus nauseosus* (Pall.) Britt. and *Achillea millefolium* L. (Asteraceae), *Lavandula latifolia* Medik. (Lamiaceae) and others. [9, 10, 11] Due to *α-B`s* intoxicity in animals ($LD_{50}$ 13–14 g/kg 4) [12] and it`s non-phototoxic, non-mutagenic and non-genotoxic properties [13], it is widely used in cosmetic formulations, as a cosmetic soothing supplement because of its perceived skin healing properties. It also possesses a variety of biological properties, such as anti-irritant, anti-inflammatory and anti-microbial ones. [14,15] The Food and Drug Administration (FDA) conferred on α-B the GRAS (Generally Regarded as Safe) status and it developed into a desirable ingredient in the formulation of skin care products, such as baby care products, fragrances and pharmaceutical preparations. Recently, this odorant molecule was used as a fragrance ingredient in decorative cosmetics, fine fragrances, shampoos, toilet soaps and in other non-cosmetic products such as cleaners and detergents. [15] It has been commercialized in the supply of fragrance, flavouring and cosmetic active ingredients and raw materials. [10] In many studies, bisabolol, bisabolol-rich oils and a whole diversity of bisabolol-containing plants, were discussed in relation to differential pharmacological properties. In addition, β-B was identified back in 1986. This apple-blossom-like odoured sesquiterpene was first isolated from the essential oil of cotton buds *Gossypium hirsutum* L. (Malvaceae), and afterwards from camphor, corn, vanilla, olibanum and other (Figure 3) [16]

Figure 3 The configuration of β-*B* (16)



(+)-(4R,8R)-beta-bisabolol

Occurrence in Plants

As already mentioned, α-B is a widely occurring natural compound that can be found in a whole variety of plant families, especially in Asteraceae, Lamiaceae. This compound occurs in relatively high levels in these species accounting for 30–90% of the total essential oil composition as determined by gas chromatography .[10] Often bisabolol participates in synergistically effects as one of the biologically most relevant compounds, but it is also not seldom the effective agent. For example, this molecule has been considered to be the main component contributing to the mild anti-inflammatory effect of chamomile. [17]

The quality of chamomile commodities depends on their source of of raw material. Former literature indicated that α-B was mainly obtained by direct distillation of the essential oil of *M. chamomilla* (Asteraceae). [18] As the contents of (-)-α-B and its oxides in the chamomile drug differ considerably according to origin and chemo type, the probable explanation is a genetically fixed relation between their contents. [6] This plant has been reported to reduce the perception of acute pain and has been used for centuries for its medicinal properties. [19] Nevertheless, due to economic reasons, B is currently obtained from the bark of the Candeia tree *(V. erythropappa)*, which

is milled and distilled to produce Candeia oil from which only the efficacy causing isomer *(-)-α-B* is obtained. [20] Identification of α-B as the major component of the essential oil from the heartwood of two populations of *V.pohlii* Baker (Asteraceae) from north-eastern Brazil is in accordance with previous investigations reported for others species of the *Vanillosmopsis* genus, such as *V. arborea*, which furnished 80-90% yield for this compound.[21] The biggest privilege of this oil is that the oil does not get coloured in the end-products and that the allergic risk is evidently smaller than that from chamomile.[22] This small tree grows in the Mata Atlantica rain forest, but harvesting has brought up the necessary of other sources of bisabolol as an important sesquiterpene. An opportunity was given by synthesizing a natural homologue of B, Dragosantol® and Dragosantol 100®. But considering economic aspects, presenting the sage oil of two Salvia species, *S.stenophylla* and *S.runcinata* (Lamiaceae), as a potential, natural source of B could be more promising. [10]

New potential sources of α-B

A chemotaxonomic assessment of two Salvia species indigenous to South Africa is presented and recommended as a potential source of α-B. The essential oil obtained by hydrodistillation of the aerial parts was analysed by GC–MS and mid-infrared spectroscopy (MIRS). The results obtained in the study are consistent with previous reports showing B as the main constituent in both investigated Salvia species. MIRS data mirrored similar anomalies revealed in the GC-MS data, but provided a more reliable, objective technique in the chemotaxonomic evaluation of Salvia species and a quantification of α-B. Since Salvia species are perennial plants, propagations of the chemo taxis with a higher B content may provide a faster and more reliable alternative to the current source of this aroma chemical. [10] Another potential source of α-B could be represented by biotechnological methods, such as *in vitro* seed-germination and cultivation of *S. stenophylla*, whereas the content of α-B was shown to be similar in these microplants (21.0%) in comparison with *ex vitro* plants (20.1%). [23] Recent investigations by GC and GC-MS analysis displayed also a high content of *α-B* (42.8%) in a new species of the genus

Plinia, *Plinia cerrocampanensis* Barrie (Myrtaceae), increasing the interest in this species as a potential industrial source of this oxygenated monocyclic unsaturated alcohol. [24]

Molecular analyses on the genetic diversity and inheritance of (-)-α-B in tetraploid chamomile

The achievement of a higher active compound level by identifying genetic features has been dragging attention by the development of molecular characterization methods. The identification of α-B, along with chamazulene and different flavonoids as active components, led to the development of new chamomile varieties with increased contents of these components in addition to an increased amount of oil. In order to further increase the oil content and the amount of B selected diploid varieties were colchicine treated to generate tetraploid chamomile cultivars. Tetraploids have also the advantage that there is no risk of outcrossing with wild type chamomile leading to lower oil contents in the next generations. Chamomile commodities are highly variable in quality depending on different affects. Molecular PCR-based markers like Random Amplified Polymorphic DNAs (RAPDs) and Amplified Fragments Length Polymorphisms (AFLPs) are used for the assessment of genetic relationships of plant genotypes. For efficient plant breeding, knowledge on the genetic diversity is a prerequisite. Genetic diversity based on RAPD and AFLP was computed using special software. PCR-based DNA-markers provide opportunities for reliably assessing the genetic diversity within and between different accessions. Such markers turned out to be useful tools to distinguish and characterize genotypes rapidly and reliably. For medicinal and aromatic plants only little sequence information is available. Therefore, those markers are the ideal tools since they require no prior sequence information. AFLPs is the more efficient technique in comparison to RAPDs. The amount of bisaboloids is under the influence of environmental effects and genotype-environment interaction. The identification of closely linked molecular markers for these traits will facilitate pre-flowering marked-based selection enhancing breeding of chamomile with high B content. [8]

Biosynthesis

Among the various secondary metabolites, terpenes represent one of the largest and most diverse classes of secondary metabolites, counting over 29.000 known compounds. [25] The enormous diversity of structures is responsible for their diverse functional roles. Sesquiterpenes are formed biosynthetically from three five-carbon isoprene units or are synthesised industrially from monoterpenoid feedstocks. [13] The branched unsaturated diphosphate isoprene units, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), are the universal precursors in the metabolic pathway for terpenoids. During biosynthesis, the active isoprene unit (IPP) has been repeatedly added to DMAPP or a prenyl diphosphate in sequential head-to-tail condensations. Prenyltransferases geranyl pyrophosphate synthase (GPS) and farnesyl pyrophosphate synthase (FPPS) catalyze the condensation of IPP and DMAPP for the formation of GPP and FPP, precursors for monoterpenes and especially sesquiterpenes, respectively. [26] Another, alternative pathway was presented, where IPP, as a precursor for sesquiterpenoids, can be also formed from pyruvate and glyceraldehyde-3-phosphate. For this purpose, ^{13}C glucose was injected in the anthodia of chamomile flowers and a ^{13}C labelling pattern of the isoprene units has been determinated by ^{13}C NMR spectroscopy. ^{13}C enrichment in different positions of the isoprene units at the level of FPP was observed and related to the biosynthetic origin of the same. ^{13}C enrichment of two isoprene units in position 1 and 5 lead to the conclusion that these units were formed via the triose/pyruvate pathway and not the mevalonic acid pathway in the cytoplasm as former hypothesised. The third isoprene showed a specific mevalonic-related labelling in positions 2 and 4, and as the first two isoprenes enrichment in position 5, indicating that this isoprene unit could be arise from both pathways. Further assumptions lead to the conclusion that the biosynthesis of the sesquiterpenoid compounds of chamomile represents a two-step procedure in two different compartments. Still, further studies are required to lighten this hypothesis. [25]

Total Synthesis

Most α-B on the market is currently obtained by synthesis, either from farnesol, a known skin allergen, or from nerolidol and other terpenoid building blocks. The racemic end product contains, despite several cleaning steps, up to 0.5 % farnesol, where again, different methods had been investigated to solve this problem. [22] A method has been established recently, where first an esterification and then a distillative separation of a mixture of α-B and farnesol had been presented. [27]

The racemic mixture of (-)/(+)-α-B by acid-catalysed cyclisation of farnesol or nerolidol furnishes α-B formate and farnesol formate as a by-product. By further saponification, the corresponding alcohols are given. By this acid-catalysed cyclisation and further saponification step, α-B and farnesol are synthesised, with no possibility of distillative separation due to the similarity of the boiling points (α-B 110°C; farnesol 117°C). A new method had to be established in terms of separating farnesol to produce α-B free from farnesol, in one hand, and in the other hand to further use such separated farnesol, especially farnesol derivatives in the fragrance and aroma industry. Therefore, the mixture of α-B and farnesol was treated with:

-an equimolar amount of an ester, based on the amount of farnesol, and

-in the presence of a catalytic amount of an alkali metal and/or alkaline earth metal alkoxide having 1 to 6 C atoms, with selective formation of a farnesol ester

Afterwards, a distillative separation of α-B from the farnesol-ester, was provided. A similar separative method has been observed when α-B- and farnesol-formate were used as the starting substances. [27]

While natural α-B is diastereo- and enantiomerically pure, its synthetic versions are either a racemic mixture (B F) or a mixture of a pair of racemic diastereomers (B rac). All four stereoisomer`s of α-B occur in nature, but racemic α-B has been reported to be only half as active as natural (−)-α-B, suggesting that the (+)-enantiomer has little, if any, biological activity. [28]

The total synthesis of α-B as a chiral compound, was achieved by vanadium complex-catalyzed, asymmetric epoxidation of homoallylic alcohols. A complex based on vanadium triisopropoxide oxide and a α-amino acid-based hydroxamic acid, derived from tert-leucine, was used to obtain sufficient enantioselectivity. It was also observed that the constitution of the epoxided homoallylic alcohol is probably relevant, such as the 3-position, which is strongly recognized by catalysts with a positive effect on the selectivity. [29]

Metabolism

As natural compounds are mostly consumed without any consultations with healthcare professionals, potential herb-drug interactions have gained a lot of interest. Either induction or inhibition of the Cytochrome P450 enzymes play an important role in drug administration and other metabolic activities, such as competition between co-administered drugs, unspecific interactions with proteins, and enzyme induction due to chronic intake. *In vitro* studies about the inhibitory effect of the essential oil of chamomile, showed that α-B, among other constituents, seems to inhibit four selected human CYP450 enzymes (CYP1A2, CYP2C9, CYP2D6, CYP3A4), with CYP1A2 being more sensitive, even *in vivo*. The inhibition of CYP2D6 was very significant (α-B-IC50=2.18 μM). As this odorant molecule also had been reported to possess anti-mutagenic activity by interfering with cytochrome P450, there could be the possibility that it supports the metabolism of drugs and mutagenic agents. [1, 30] More specific, the active constituents of chamomile essential oil were fluorimetically tested in increasing concentrations ranging from 200 to 0.091 μM. α-B and another compound indicated a good activity against CYP2D6, but at a much higher concentration when compared to the positive control quinidine. A 46.11 μM dose of α-B was active against CYP2C9, whereas the B oxides, identified as major constituents, exhibited no efficacy against the four relevant CYP isoforms tested. CYP3A4, the clinically most relevant isoform, was also significantly inhibited. Since these observations were provided by *in vitro* studies, certain major factors of metabolism cannot be related to those *in vivo*. Still, the obtained results could merit further researching to clarify their clinical relevance. [30]

Determination in human blood

In the frame of several studies, α-B´s accurate and precise identification in blood and in other biological fluids and tissues was crucial. As the matter of fact, α-B exhibited different pharmacological properties *in vitro* and *in vivo*, thus the final determination of this odorant sesquiterpene has been essential in order to accomplish more specific data. Although this small molecule reveals a great spectrum of medicinal relevant properties, and the fact that it has been used for hundreds of years in traditional medicine, instrumental methods for α-B analysis in biological samples yet have been recently reported in the literature. As the most optimal methods for measuring α-Bs content in human blood a head space-gas chromatography (HS-GC) coupled with MS and a micro-HPLC coupled with ion-trap MS technique were described. The analysis of α-B with the "head-space" technique coupled with GC-MS proved rapid and highly satisfactory. Although the HS-GC-MS method was characterized as the more rapid and easier one, the mikroHPLC-ESI-MS method, being not dependent on the volatility of the analyst, is potentially more suitable for studying α-B together with its even more polar metabolites and has been proved as a appropriate method for rapid and accurate analysis of this natural product. [31] Furthermore, a new, rapid reversed-phase HPLC method for determination of α-B from essential oil has been examined. The identification and quantification of this sesquiterpene alcohol from the essential oil of *Zanthoxylum tingoassuiba* A. St.-Hil. (Rutaceae) was successfully achieved by this method. Particularly, the absence of interfering peaks, the good symmetry and a tailing factor of 1.06 of the α-B-peak, suggested this method as a precise, accurate, specific and with low detection and quantification limits. Precise identification and quantification methods are obligatory for the improvement and establishment of α-B´s potential as a therapeutic agent of essential oil-based formulations. [32]

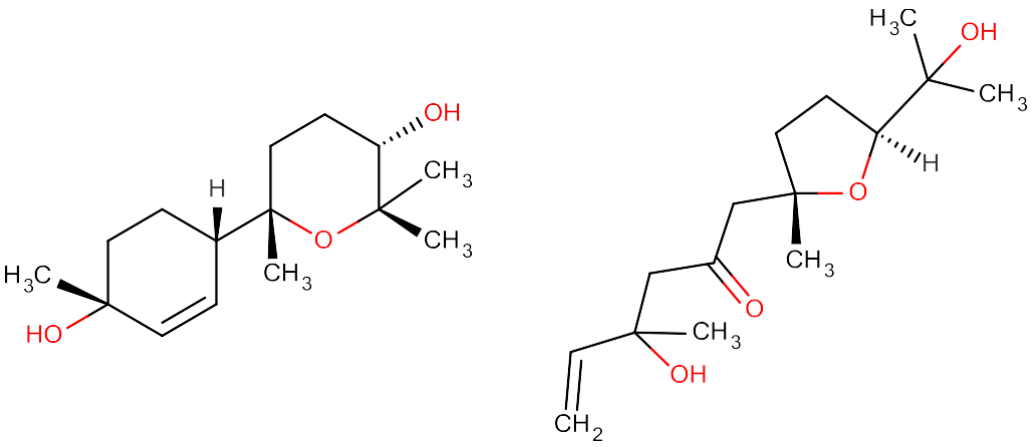
Secondary Oxidative Metabolism

As already mentioned, the broad medicinal use and the big variety of therapeutic properties of German chamomile, *M. chamomilla*, is represented

by the synergistic effect of more than 200 isolated compounds. Compounds such as terpenoids, flavonoids, coumarins, fatty acids, alkaloids, polysaccharides testify of the extraordinary chemical variety of this Asteraceae and make it an interesting theme for a wide number of scientific investigations.

More than 60 sesquiterpenoids have been identified, where α-B, B oxides A and B and α-bisabolone oxide A are used as marker compounds for chemo type identification and authentication of products contained in Matricaria. As already demonstrated, α-B is a high commonly aromatic found in a wide variety of plants, but its furano and pyrano derivates (B oxides) are less common in natural products. As α-Bs broadly spread therapeutic properties such as antibacterial, anticancer etc. and its uses as a marker were clearly observed, an in depth-investigation of the minor compounds has been undertaken, in order to find other possible minor volatile and nonvolatile B-type compounds as authentication markers for Matricaria. A GC-assisted fractionation approach was used in order to find low concentrated B derivatives. A non-polar mixture obtained from partitioning of the flower-head methanol extract was analyzed by GC-MS. As B oxides A and B possess a very distinctive fragmentation pattern, the detection of B oxide-like structures was made easier. As the result of this study, three new compounds were identified (Figure 4).

Figure 4 Hydroxylated derivatives of bisabolol oxides A and B [33]



Compound 1 Compound 2

Compound 1, isolated as a pale yellow oil, was characterised as 9-hydroxyB oxide. ^{13}C NMR spectroscopic data showed 15 signals and the disubstituted olefin was confirmed by ^{1}H-NMR spectroscopy and COSY correlation. Four isolated methyl groups were determined and the new stereo centre (C-9) was confirmed by NOESY experiments. In conclusion, the NMR data of this oxide were very similar to those of B oxide A, suggesting a close structural relationship between the two compounds. The second, new compound isolated as a colourless oil was identified as seco-B oxide B. ^{13}C-NMR spectroscopic data showed once again the presence of 15 signals, of which four were identified as methyl groups. Two additional signals suggested the presence of a tetrahydrofuran moiety, were the structural relationship of the tetrahydrofuran ring with the B-ring of B oxide B was confirmed. The ^{1}H- and ^{13}C-NMR chemical shifts for a further third new compound, showed similarity with the known compound B oxide A glycoside. An interesting new moiety was observed, a D-glucose moiety, which was confirmed by both acid and enzymatic hydrolysis and further using GC-MS-data. Spectroscopic data also confirmed the linkage between the aglycone and the glucose moiety. This new compound was identified as 15- hydroxyB oxide A glycoside, a dark brown, amorphous solid. Thus the two first compounds and an already known, but

never previously reported compound from German chamomile, could be derived from allylic hydroxylation of the parent tepenoid α-B.

Hydroxylated B oxides rarely occur in nature, but some examples have been ascertained. B and its derivatives are characterized by the presence of the A-ring attached to a five-membered or six-membered ether linkage. Similar to B oxide B, compound 2 contains a furano ring, but the presence of the essential six-membered ring A is lacking.

For conclusion, the identification of new minor bisabolane oxidized metabolites could be useful for a better understanding of the biological pathways involved in sesquiterpene synthesis in German chamomile. Furthermore, B derivatives are of interest on account of their biological properties. For example, B oxide A glycoside has been patented for the treatment of diabetes and further evaluation could be of potential interest. It is also worth noting that, because of their high polarity, B glycoside derivatives could occur in aqueous extracts of chamomile (like herbal tea preparations) and may be hydrolysed *in vivo* to release the active aglycon. [33]

Uses

In the last two decades, α-B became a constituent of great interest. Numerous studies have been published and new pharmacological features are still being examined. In studies reported, various medicinal properties and the well explored toxic harmlessness turned this odorant molecule to be recognized as a remarkable compound. (-)-α-B has been widely used as an ingredient in dermatological and cosmetic formulations, such as aftershave creams, hand- and body-lotions, deodorants, lipsticks, sun-care and after-sun products, baby care products and sport creams. This sesquiterpene is rapidly absorbed into human skin, promoting epithelisation and granulation. [6] The use of α-B or α-B-rich oil as an anti-inflammatory agent is ubiquitous, especially due to it`s topical application. [1] This compound also exhibits several other pharmacological properties such as analgesic, antibiotic, antispasmodic, anti-allergic, anti-irritant, anti-pyretic, anti-cholinesterase properties, anticancer activities and ability to enhance transdermal drug permeation. [2,34] It has been also reported that B reduces inflammation in arthtritis, prevents the

development of gastric ulcers, acts against the growth of bacteria and fungi, and promotes burn wound healing and the healing of wounds in general. Cicatrizant activity has also been proven and related to the 1-methylcyclohexene moiety and the tertiary hydroxyl group on C-1 of the side chain as important structural fixtures for the wound-healing enhancement. [35] α-B is also able to reduce injuries associated with the administration of ethanol, the formation of thiobarbituric acid reactive substances and by blocking different ulcerous provided pathologic incidences it has also been defined as gastro protective. [12]

In particular, sesquiterpenes with the hydroxyl group are generally more active in anti-tumour activity. Recently, a selective cytotoxic time and dose-dependent activity was demonstrated against human and murine malignant glioblastoma through mitochondrial damage. An apoptosis-inducing action towards highly malignant human pancreatic carcinoma cell lines has also been demonstrated, even without affecting human fibroblast viability [3, 4, 14, 17]. Considering the inhibitory effect on the transcription factor involved in cancer pathways, namely STAT3, α-B has also attracted considerable attention as an apoptotic and chemo preventive agent. [28]

Recording to the pro-apoptotic effect against BCR-ABL+ leukemic stem cells, α-B could be a candidate for treatment of BCA-ABL+ leukemias to overcome resistance to tyrosine kinase inhibitors, such as Imatinib and Nilotinib alone and to target leukemic cells through BCR-ABL-independent pathways. [36]

The insecticidal activity observed against *Bemisia argentifolli* (Silverleaf whitefly) in a low concentrated application of the oil of *V. pohlii* can be attributed to α-B as the main component. Findings provide evidence for the protection of oxidative stress in erythrocytes and plasma by (-)-α-B which could be further explored for disease associated with ROS generation such as ageing, cancer, artheroscleriosis, neurodegeneration and cardiovascular disorders. [11]

α-B has been shown to enhance the permeability of bacterial cells, such as the halitosis-associated bacterium *Solobacterium moorei*, thereby increasing their susceptibility to antimicrobials. [37]

Considering the extensive outstanding pharmacological properties and recognizing its value, α-B is of big importance in the field of plant-derived constituents.

Effects on the skin

α-B exerts remarkable skin healing properties and has been likely used in many skin formulations, such as lotions, shampoos, even baby care products and many others. The maximum skin level that results from the use of α-B in formulae that go into fine fragrance has been reported to be 0.08%, assuming use of the fragrance oil at levels up to 20% in the final product. [13] It is not only valued for its skin-soothing activities, but also for many pharmacological relevant properties, such as the anti-inflammatory and anti-irritant. [38] As the basic for the evaluation of several studies about α-B´s effect on skin, the cutaneous absorption had to be evidently confirmed as well. Therefore, the cutaneous absorption of it was studied by biochemical incorporation of ^{14}C-acetate into the molecule. After only five hours of application into nude mice, half of the measured radioactivity was represented by intact ^{14}C-labeled B. Upon cutting skin tissue by a cryotome and producing auto radiograms of the same, a fast penetration of α-B into the skin was evidently confirmed. [39] Although skin pigmentation is the major physiological defence against several factors, hyper pigmentation is a distressing problem caused by diverse inflammatory skin disorders, such as chronic inflammation, rubbing of the skin and others. [40] A study reported also the depigmentative activity of this small sesquiterpene alcohol in a treatment of hyper pigmentation, the darkening of an area of skin. This disorder may depend on several factors, such as increased numbers of melanocytes or increased melanogenic enzyme activities, in generally the increase of melanin. [2] The depigmentative effect of α-B was related to the inhibition of melanogenesis by its significant influence on the decrease of the intracellular cAMP level. The CRE, **c**AMP **r**esponse **e**lement, is known to be involved in the α-MSH-induced melanogenesis. α-B managed to inhibit the CRE luciferase reporter activation induced by α-MSH. Therefore, the melanin content induced by α-MSH was also decreased by the application of α-B. α-B indeed reduced the α-MSH

stimulated cAMP production and the CREB phosphorylation, varified by Western Blot analysis, indicating once again the involvement of cAMP in the depigmentative effect of this odorant molecule. On the gene expression level, α-B showed an inhibitory effect on by a-MSH induced MITF, and therefore the tyrosinase gene expression. [41] In another *in vivo* study, a 0.5% α-B containing cream was tested for 8 weeks application, compared to a α-B-free control cream. After only 4 weeks of treatment, almost 105 % decrease of skin colour was evidenced, when compared to the vehicle control. With respect to clinical observations, no significant effects of α-B compared to the vehicle were noticed. Worth of mentioning is, that no adverse side effects were observed, therefore, α-B containing cream was considered as safe for use. All of these data suggest a possible and safe utilization of α-B in hyper pigmentation-related disorders, whether in form of lightening or whitening. [40]. α-B, is a common plant secondary metabolite and has been shown to shorten the healing time in cutaneous burns of guinea pigs exposed to UV light. α-Terpineol and trans-nerolidol were also recorded by The results of the study were clearly defined, the α-B and α-terpineol showed significant *in vivo* cicatrizant activity, while the acyclic isomers were inactive. [35]

The first investigations in the field of natural cicatrizants were described in the study about the tree sap from *Croton lechleri* Mull.Arg. (Euphorbiaceae) - "Sangre de Grado", known in the folk medicine for the healing activities. Further investigations led to "tapsine", an alkaloid which was identified as the active compound of this in plant. Impaired wound-healing should not be considered as a undervalued fact, because it is a significant source of morbidity and may result in severe health-related complications, such as infections and tissue necrosis.

It is often associated with diseases such as diabetes, immunosupression and malnutrition. Compress-made from crushed plant of *Peperomia galioides* Kunth (Piperaceae), except the roots, were applied on cuts and wounds to accelerate healing. This tree dragged the attention in previous phytochemical investigations, such as the one where a prenylated quinone with *in vitro*-antiparasitic activity was isolated from the petroleum ether extract. Characterized as (+)-epi-α-B, this colourless oil has been characterized as the constituent responsible for the *in vivo* cicatrizant activity of *P.galioides*. A few

step chemical analysis led to the isolation of this epimer of α-B. Few chemo-physical properties, such as the optical rotation and NMR spectra of (+)-epi-α-B (anymol) were identical with the ´quinone-like` compound found previously. The great efficacy of natural cicatrizants led to the need of their further exploration. Furthermore, four anymol related structures have been investigated to elucidate the structure-effect relation. [35]

These data lead to a conclusion that the 1-methylcyclohexane unit and the tertiary hydroxyl group of the side chain are important structural features for the wound-healing enhancement. [35] `*In the percutaneous route of drug delivery, the most common approach to alleviate stratum corneum permeability is the concomitant use of penetration enhancers*.` [2] α-B has undergone an *in vitro* study as a possible penetration enhancer of the α-blocking drug Dapiprazole® used in topical eye therapy, in case of mydriasis, miosis or glaucoma. As the transdermal application of this α-blocker showed many advantages, diverse penetration enhancers were tested and α-B increased the permeability coefficient of Dapiprazole® up to 73 times. [2]

(-)-α-Bisabolol-induced gastro protection

Since hundreds of years, chamomile tea is well known by its stomach smoothing effect in folk medicine. Nowadays, stress, inappropriate nutrition and many other factors lead to several disorders, such as ulcer. Ulcer represents an injury of the gastric and duodenal mucosa which occurs when the epithelium is exposed to several potential pathogenic factors. Ethanol induces gastric mucosal lesions by causing membrane damage, exfoliation of cells and erosion. Also, indomethacin, a well established NSAID, increases the incidence of genesis of ulcer in the gastrointestinal tract as one of the side effect of its use in anti-inflammatory treatment. In fact, these two substances are often used as a reliable tool to study the pathogenesis of acute stomach mucosal ulceration. A gastroprotective function of (-)-α-B against ethanol and indomethacin-induced ulcer was demonstrated, related to the capacity to reduce the decrease of GSH amount in gastric mucosa, referring to its antioxidant activity, but not a preventing effect. At least, α-B`s gastro

protective effect could be related with a consequent increase in its bioavailability and strengthening of this defensive factor of gastric tissue. Also, the healing times of ulcers induced by either chemical stress or heat coagulation were significantly reduced by this odorant sesquiterpene. The gastro protective mechanism of α-B cannot be related to a single way process, many other parameters are involved in the gastro protection or ulcer-genesis. For example, prostangladines exhibit a gastro protective activity by decreasing acid secretion, the production of pepsin, and causing vasodilatation by relaxation of vascular smooth muscle. Studies showed that indomethacin administration did not prevent the α-B`s gastro protective activity, an involvement of the prostanglandine production inhibition in the mechanism could be excluded. The same observations were obtained when glibenclamide was administered, suggesting that K+ATP does not appear to be involved in the gastroprotective effect of α-B as well. The results demonstrated that α-B was still able to decrease ethanol-induced ulceration, even when a non-selective competitive inhibitor of NOS-L-NAME, was administered. Therefore, the nitric oxide regulation pathway can be excluded from the involvement with gastro protection of α-B. [1, 42] Reactive oxygen species, namely superoxide anion, hydrogen peroxide, hydroxyl radical, play a big role in the inducing deleterious actions on the gastric mucosal epithelium. The formation of ROS is a constant event of normal metabolism, but under pathological conditions of the gastric mucosa there is an imbalance between formation and degradation of these species by enzymatic and non-enzymatic defence mechanisms. These species are highly damaging to cells, because they interact with DNA, lipids and proteins and they can change their functions. As known, every unreasonable interaction with DNA can lead to mutations and cancer development. Although the human body possesses defence mechanisms against increased ROS values, some limitations are often observed. The defence mechanism of cells of the gastrointestinal tract is represented by enzymes such as the superoxide dismutase, glutathione peroxidase, catalase and then non-enzymatic constituents like vitamin C, carotinoids and others.

For example, the enzyme SOD removes the superoxide anion of the cell environment, the catalase detoxifies and the GSH-px breaks the peroxide hydrogen. GSH is a tripeptide which prevents the cells from interacting with

these cytotoxic species. The acute administration of absolute ethanol was used, to indicate the releasing content of free radicals among other inflammatory agents. The mucosal injury was evaluated by an experienced histologist. The rate of lipoperoxidation in the gastric mucous membrane was estimated by determination of malondialdehyde and the stomach was washed with saline to avoid unwished appearances. After preparation, the absorbance was measured at 532 nm. Furthermore, the activity of the relevant enzymes, such as the myeloperoxidase, SOD and the Cat and the nitrite amount were measured. Animals pre-treated with (-)-α-B showed less macro- and microscopic mucosal damage when compared with the control group treated only with ethanol. This aroma chemical was capable to maintain the integrity of the gastric mucosa against the damaging effects of ethanol. The degree of peroxidation in tissues was measured by determining the amount of MDA, which was evidently increased with augmented apoptosis in gastric tissue of animals subjected to orogastric treatment with absolute ethanol. On the other hand, α-B was able to prevent the increase of the amount of MDA induced by ethanol thus showing the antioxidant activity and gastroprotective ability of this sesquiterpene. It has also been observed, that ethanol increased the production of the SOD enzyme as a part of the endogenous antioxidant system. α-B showed a beneficial role increasing SOD activity and thereby enhances the dismutation of superoxide anion. So, α-B is a compound with gastroprotective activity acting between antioxidant defence systems and this fact may constitute one of the mechanisms of action of this substance. The results show that α-B is able to decrease oxidative stress and an inflammatory event associated with the lesions induced by ethanol. An effect on the nitric oxide and the catalase activity seems to be less important for this effect of α-B. [12] Another study presented the involvement of endogenous prostaglandins, nitric oxide and the activation of K+ATP channels in the gastroprotective role of *M. Recutita* extract, especially α-B. The *M.recutita* extract reduced the gastric damage in all doses tested, and α-B application led to a decrease of gastric damage up to 96%. As a gastroptotective effect of α-B against ethanol-induced mucosal injury has been observed, indomethacin, a non-selective cyclooxygenase inhibitor, and the nitric oxid antagonist N-nitro-L-arginine methyl ester were applied to display the potential gastro protective mechanism

of α-B. Indeed, the gastro protective effect of α-B was impaired after the application of those two remedies, so the importance of endogenous prostaglandins and nitric oxide in the gastro protective effect of α-B was confirmed. Further, by applying the K+ATP-channel blocker, glibenclamide, the view was confirmed that the mechanism actually involves the activation of K+ATP-channels by prostaglandins and nitric oxide. These data are contrary to dose obtained from one of the previous studies mentioned. [42] Nevertheless, the gastro protective effect of α-B is a multifactorial process which is represented by antioxidant and many other activities. [43]

The aqueous extracts and essential oil of chamomile, due to the presence of compounds such as α-B and its oxides, have potential to be effective in relaxing spasms. As investigations showed, α-B turned out to be even more effective (91%) on spasms induced with barium chloride than papaverine, a smooth muscle relaxing drug. An analgesic remedy of big importance is definitely acetylsalicylic acid. One of the most serious side effects of this remedy are gastric complications. An interesting study examined the gastrotoxic influence of acetylsalicylic acid mixed with α-B on rats. α-B in this mixture displayed, once again, a strong protective effect on gastric mucosa. Study data about the oral administration of α-B also showed a protective effect on the gastric mucosa. [1]

In recent years, research groups are constantly trying to find proper agents useful to combat gastric dyspepsia and peptic ulcer. Although anti-secretory agents, such as the proton pump inhibitors are prescribed successfully, their adverse side effects by longer applications, bring up the need for alternative remedies. As several chemical studies already identified α-B as one of the main constituents in the stem bark essential oil of *V. arborea* (VAEO), clarifying the gastro protective mechanism of this essential oil could deepen, at least, the one of α-B. The results showed that at doses of 200 and 400 mg/kg, VAEO prevented hemorrhagic mucosal lesions of the glandular region of the stomach. This effect of VAEO was sensitive over yohimibine, the respective antagonist of α2-receptors, which led to the conclusion that VAEO acts as a agonist of α2-receptors and that this mechanism, at least in part, could explain α-B`s activity as well. Worthy mentioning is, that no similar effects were observed when treatment with glibencamide, L-NAME (nitroarginin

methylester) or indomethacin was involved. These data not only confirm other results, but can also exclude some possible hypotheses and lead to a more specific field in researching the exact mechanism of α-B`s gastro protective activity. [42, 44]

Hepatoprotection of α-B. among other sesquiterpenoids

Sesquiterpenic compounds are related to many medicinal activities, such as anti-inflammatory, anti-carcinogenic, antioxidant and others. Very often, the structure-effect relation is the efficacy-defining one, so for example, oxygenated sesquiterpenes showed higher antioxidant activity. As already mentioned, antioxidant compounds play an important role in protecting cells against damage caused by ROS (reactive oxygen species). These species cause damages of proteins, lipids and the DNA, which contributes to many human diseases. Nevertheless, changes in the permeability of lipid membranes and other membrane functions caused by ROS can lead to major cardiovascular complications and even cancer. These processes can only be stopped by the intervention of free radical scavengers and antioxidants. The mechanism of antioxidant action involves membrane stabilisation and neutralisation of free radicals. Relative hepatoprotection of fifteen sesquiterpenoids, including (-)-α-B, was tested on liver homogenate from rats. Relative hepatoprotection of ascorbic acid was evaluated and used as a positive antioxidant control. In order to assess the molecular structure-activity relationships, sesquiterpenoids were tested at the same molar concentration of 1nM. The relative hepatoprotection activity of the 15 sesquiterpenes was evaluated by observing the malonaldehyde levels in rat liver homogenates for endogenous and induced lipid peroxidation assay. A very low hepatoprotection activity of (-)-α-B was observed in both, endogenous and induced lipid peroxidation assay. The comparison between results obtained from both assays showed that under oxidative stress conditions the sesquiterpenoids hepatoprotection activity was better. The decrease of MDA formation in the induced assay was 150 times better compared to the endogenous assay of α-B. In addition, most of the hepatoprotective drugs belong to the group of free radical scavengers or

antioxidants and their action involves membrane stabilisation and neutralisation of free radicals. The efficacy of the sesquiterpenes can probably be referred to its lipophilic properties. The previously reported inhibition of lipid peroxidation of some sesquiterpenes was confirmed, following the order: farnesol (mix of two isomers) >α-B >(+)-valencene >α-cedrene >(+)-aromadendrene>α-humulene. Also, as already described, (-)-α-B is able to protect the gastric mucous membrane of male Swiss mice against injuries caused by ethanol by reducing lipid peroxidation and increasing the superoxide dismutase activity, which is in agreement with the activity of this compound in the induced lipid peroxidation assay. To get a better picture of the structure-efficacy relations, a QSAR model has been created. This model based on three-dimensional molecular descriptors for the fifteen sesquiterpenoids for the prediction of the relative MDA reduction was developed. According to the obtained models, the most relevant molecular descriptors related to the hepatoprotection properties belong to: the 3D Geometry, Topology and Atom-Weights Assembly, Weighted Holistic Invariant Molecular and Molecule Representation of Structure based on Electron diffraction. The developed models allowed the extraction of relevant information suggesting that sesquiterpenoids possessing more compact molecular structures. Low ramification and less symmetric will be more effective for endogenous hepatoprotection. [45]

Antioxidative properties

As many degenerative serious diseases are related with cellular damage caused by free radicals, the role of anti-oxidants in disease prevention increased enormously the interest in these substances. As many commonly used anti-oxidants of chemical origin exhibit certain toxicity, the possibility of natural compounds to reveal such an activity draws attention to the use of these compounds for anti-oxidative aspects in prevention and treatment. [2] The antioxidative effect of α-B, besides analgesic, anti-arthritis, antibacterial, antipyretic, pesticide activities opens promising therapeutic aspects on ROS generation-associated diseases. [12, 45] The antioxidative property of this odorant molecule was characterized at many points. It has been observed, that

α-B maintains the basal GSH-level of erythrocytes, lowers the MDA level as well, increases the catalase activity of the plasma dose dependently and also increases the superoxide dismutase activity of the blood. All these mentioned parameters or activities are directly or indirectly involved in oxidative stress induction of erythrocytes. The efficacy of α-B was mostly comparable with the one from ascorbic acid which once again proves that α-B is a promising potential antioxidative. [11] The antioxidant activity of α-B in chamomile, as the main compound contributing to the pharmacological properties of this herbaceous plant, was also observed by studying its ability to interfere with the production of reactive oxygen species (luminol-amplified chemiluminiscence) during human neutrophil bursts induced by corpusculate and soluble stimulants and cell-free systems. Human polymorphonuclear neutrophils, reactive oxygen species and inflammatory reactions are closely interrelated. A dose-dependent inhibition of LACL by α-B was observed, at concentrations ranging from 7.7 to 31 lg/mL for *Candida albicans* and N-formyl-methionyl-leucylphenylalanine, respectively. A similar effect was observed in the SIN-1 and H_2O_2/HOCl-systems suggesting B as a means of improving the antioxidant capacity. [1, 46] In contrast, a poor anti-oxidant activity against the DPPH radical has been observed. [2]

Diabetic Complications

Medicinal properties of dietary herbal teas have often been the topic of discussions. Although the medicinal value and an evidently effect of many herbal teas had not been investigated yet, herbal tea remained as one of the most popular part of dietary habits around the world.

One of the mostly consumed single-ingredient herbal teas is chamomile. The major components of its essential oil are (-)-α-B and α-farnesene. High levels of polyphenolic compounds such as flavonoids and coumarins have also been reported. By inhibiting some enzymes, such as the intestinal disaccharidase and the pancreatic α-amylase, the absorption of carbohydrates could be regulated and this effect could be used in oral treatment of noninsulin-dependent diabetes mellitus (type II diabetes). Furthermore, the inhibition of α-glucosidase, the hepatic glycogen phosphorylase and the aldose reductase

could be considered as a potential treatment of this disease. Especially the ALR2-inhibitors could be used in the treatment of many diabetic complications such as cataracts, retinopathies, neuropathies and nephropathies, since this enzyme catalyzes the NADH-dependent reduction of glucose to sorbitol, which is then oxidized to fructose by sorbitol dehydrogenase and accumulates in cells leading to these complications. The ability to inhibit porcine pancreatic α-amylase and rat intestinal maltase and sucrase activities was compared between the nine components in the chamomile hot water extract, among them α-B, where acarbose, a pseudo tetrasaccharide, was used as positive control. The hot water extract of chamomile showed weak inhibitory activity against rat intestinal maltase and sucrase, nevertheless all nine compounds were much weaker inhibitors than acarbose. Essential oils, α-B and α-farnesene, showed no significant inhibition towards any glycosidase tested. A possible way to suppress hepatic glucose production and to impair blood glucose in type II diabetes, is the inhibition of the hepatic glycogen phopshorylase enzyme. The inhibitory effects of chamomile were compared with that of 1,4-dideoxy-1,4-imino-D-arabinitol. Except luteolin and quercetin, no other compound showed obvious inhibition towards the enzyme. High sorbitol levels in rat erythrocytes are positively related with the levels in the lens, sciatic nerve and retina. Sorbitol accumulation was greater when the cells were incubated in high glucose medium, as compared to that in a glucose-free incubation. Polyphenolic substances once again showed better effect than the sesquiterpenes, α-B and α-farnesene. α-B showed no inhibitory activity against ALR2, and furnished no effect on sorbitol accumulation in the cells. The results of the study clearly suggest that chamomile hot water extract has a potential for the treatment of diabetes, because a significant suppressive effect on the blood glucose levels was observed. The daily consumption of chamomile tea with meals could be potentially useful in the prevention of hyperglycemia and diabetic complications, but the effect is not related to the sesquiterpenoid compounds, specifically to α-B. [47]

Anti-hyperalgesic and Anti-oedematous Activities

Since the time of ancient Egypt, Greece and Rome, chamomile has been known for its medicinal values. The flower of Matricaria is a well-known remedy for various gastrointestinal problems and its remarkable activities has been utilized externally in medicine and cosmetics. Essential oil, polyphenolic constituents, as flavonoids and sesquiterpene lactones have been identified as the carrier of the pharmacological properties of the Matricaria flower. According to European Pharmacopoeia, the dried capitula of *M. recutita* should contain a minimum of 4 ml/kg of blue hydrodistilled essential oil and two types of Matricaria oil had been classified when referring to the main components: the (-)-α-B and the B oxides rich oil. Despite the fact that Matricaria preparations have been worldwide used for their anti-inflammatory properties, the effect of Matricaria oil to attenuate pain and oedema has only been partly examined. The anti-nociceptive and antihyperalgesic effect of (-)-α-B has been reported recently, but information about these kinds of effects of B oxides were lacking. The inflammation in the rat paw was induced by intraplantar injection of the pro-inflammatory agents carrageenan, dextran and histamine. As a comparison, ibuprofen and indomethacin were used. Carrageenan was used to examine both, anti-hyperalgesic and anti-oedematous effects of Matricaria oil, while dextran and histamine were used only for testing the anti-oedematous effects. In the carrageenan induced inflammation control group, the prophylactic and a therapeutic scheme were observed. The main constituents were α-B oxide A (21.5%), α-B oxide B (25.5 %) and spiroether, whereas α-B (3.2 %) concentration was lower in this case. The blue aromatic oil of Matricaria reduced dose-dependent hyperalgesia induced by carrageenan in both schemes where the effect dominated in the prophylactic treatment scheme. For the very first time the dose-dependent inhibition of inflammatory hyperalgesia by B oxides rich matricaria oil was observed. It has also been demonstrated that (-)-α-B diminished mechanical hyperalgesia of the mice paw injected with carrageenan and reduced nociceptive behaviour. Although α-B was only presented with 3.2 % in the essential oil which was examined, it is not excluded that this sesquiterpene contributes to the demonstrated effect. It has also been shown that (-)-α-B acts as a COX

inhibitor and reduces neuronal excitability in mice sciatic nerves, probably by irreversible blockade of voltage-dependent sodium channels. In that case, the reduction of hyperalgesia could probably be explained by the reduced neuronal excitability and, in part, by the inhibition of prostanglandine synthesis. The prophylactic treatment of oedema induced by carrageenan and dextran with B oxides rich Matricaria oil showed significant results. A modest reduction of histamine-induced oedema by Matricaria oil has been also demonstrated in this study, probably as result of synergistic (supra-additive) interactions between the active compounds. Previously, a reduction of oedema induced by dextran was only demonstrated for (-)-α-B. [48]

Antimicrobial Properties

When referring to anti-microbial therapeutic aspects the appearance of resistance of several microorganisms to the conventional drugs has to be considered. Many plant derivatives have been evaluated not only for the direct antimicrobial activity, but also as resistance-modifying agents. Compounds of natural origin can be described as modifiers of antibiotic activity for example, because they enhance the activity of specific antibiotics and reverse the natural resistance of specific bacteria to antibiotic used. [49] Thanks to the lipophilic properties of essential oil compounds, especially α-B, this molecule can enter in the lipid bilayer of the cell membrane promoting bacterial cell membrane disruption, allowing better permeation of antibiotics into the bacteria. Therefore, α-B enhances the uptake of antibiotics by means of its membrane permeabilizing activity. The longer the hydrocarbon tail in sesquiterpenes the better the enhancing activity. [1, 2, 49] Due to the lack of additional permeability barriers, this effect was more likely observed on Gram-positive bacteria. [2] α-B, together with nerolidol, displayed an anti-microbial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Candida albicans*. The results showed a higher anti-fungal activity of α-B in comparison with nerolidol and a similar anti-bacterial effect. α-B and other sesquiterpenes were investigated for their ability to increase the susceptibility of *S. aureus* to some conventional antibiotics (ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline and vancomycin). The results showed

that low concentrations (0.5–2 mM) of α-B enhanced the susceptibility of *S. aureus* to the antibiotic tested. [2] The observation that a low concentration (0.5 mM) of α-B modifies the permeability of *S.aureus* and *E.coli* membranes was confirmed in another study. [49] This unsaturated sesquiterpene alcohol was also active against *Bacillus subtilis*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* and inhibits the growth of *B. phlei*. α-B showed also a stronger antibacterial activity when compared to its oxides. [1] The main compound of the essential oil of *V. arborea* was often described as α-B and the insecticide, anti-inflammatory and gastroprotective activities of this oil were also the aim of diverse studies. The obtained data upon investigating the antibiotic activity of the essential oil of *V.arborea* (80.4 % α-B) revealed that this oil influences the activity of antibiotics and could be used in an adjuvant antibiotic therapy against respiratory tract bacterial pathogens. The antibiotic activity of tetracycline and tobramycin was enhanced by the presence of the oil against *S. aureus* and *Proteus vulgaris*. The antibiotic activity of gentamicin was only enhanced against *S.aureus*, not against *P.vulgaris*. [49]

α-B also showed fungistatic activity against *C. albicans*, *Trichophyton mentagrophytes* and *T. rubrum* at a concentration of 0.10 g/l. [1] The antifungal activity of sesquiterpenes including B against *Botrytis cinerea* was examined using the fungal growth inhibition assay and showed a mycelial growth inhibition of about 50 ppm [2]. Dragostanol® represents α-B`s synthetic, racemic mixture. The use of natural compounds as preservatives against microbial growth may have wide application in food and cosmetics preservation. Sensitivity disc assay showed that B and other plant sesquiterpenes sensitized *S. aureus* and *E. coli*. Diseases, such as dermatomycoses as common global infections and others can lead to serious complications, especially among immunocompromised patients. The fungicidal properties of α-B and Dragostanol® against many pathologic species have been observed by reporting on the minimum inhibition concentration. The MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of an antimicrobial after incubation. *Aspergillus niger*, *A.flavus* and *A.fumigatus* cause avian pulmonary mycoses. *A. flavus* also produces the most potent natural hepatocarcinogen known, aflatoxin B1. The fungicidal properties of α-B and Dragostanol®

against these species could be observed, where in case of fungicidal activity against *A. niger*, α-B was the more effective one.

Futhermore, Dragostanol® turned out to be slightly more effective against Fusarium species. Fusarium species, such as *Fusarium oxysporum* and *F.solani* cause wilt and root rot in a number of plants, as well as high mortality in pulmonary mycoses in immune suppressed patients, while *F.verticilloides* produces fumonisins, toxic sphingolipid-like compounds on wheat. These compounds cause liver disease and leuko encephalomalacia in horses and are strongly associated with oesophageal cancer and neural tube defects of humans. α-B and Dragostanol® turned out to be effective against rather germinating than non-germinating conidia, for example the efficacy was higher against germinating *Aspergillus*, *F. verticilloides*, *F. solani*. In contrast to the other tested fungi, significant lethality was observed for both the non-germinated and the germinating conidia *F.oxysporum*. A probable explanation of this observation is related to the conidial outer coating being intact. However, upon an eight-hour incubation, a germination occurs with the result of weakening of the conidial coat. The development of a new generation of broad spectrum antifungals against new targets in the fungal cell without adverse side effects to the host may be required and B is a potential candidate as a treatment for dermatomycoses. α-B inhibited the growth of all isolates of *Trichophyton* and *Microsporon*, on the other side, bactericidal effects of α-B and Dragostanol® were also observed. *P. aerigunosa* is a common, free-living, Gram-negative bacterium in the environment and can be a serious pathogenic threat in hospitals. Both α-B and Dragostanol® proved to be highly lethal to this microbe immediately. This effect was absent when referring to *St. aureus*, but after a two-hour incubation the viability of this bacteria was significantly reduced. *S. aureus* is an opportunistic pathogen causing a range of diseases including minor skin infections as acne and folliculitis. Nevertheless, additional testing is needed for providing α-B and Dragostanol® for the potential use as an inhibitor of bacteria in food samples. A structure-efficacy relationship seems to be crucial in the observed effects. Terpene activity against *S.aureus* may be dependent on the number of carbon atoms in the hydrophobic chain from the hydrophilic groups. In addition, the length of the aliphatic chains of terpene alcohols and the presence of double bonds may

affect the terpene antimicrobial activity against *S.aureus*. No studies were found where the mode of action of α-B against microbes were reported. However, studies have shown that α-B induces apoptosis by decreasing oxygen consumption in human glioma cells by disturbing the structure and function of the mitochondrial permeability transition pore. This effect could be attributed to Bs antifungal and antibacterial properties. It has been proven that α-B, a commercially available sesquiterpene and Dragostanol® are potent fungicides and rapidly and significantly act as bactericides at and below the low concentration of 10 μM. [9]

Considering the fact that about 12 million people suffer from Leishmaniases and 350 million more can be classified in a risk group and that conventional anti-leishmaniasis drugs are related to a high number of adverse effects, there is a urgent need for the establishment of a new therapeutic aspect against this Leishmania genus-induced disease. Conventional anti-leishmaniasis drugs such as antimon, aromatic diamidines, paromycin sulphates, derivatives of amphotericine B and others can be related to undesirable effects such as cytotoxicity and resistance. As previously described, terpenoids, such as α-B, manage to penetrate into the lipid bilayer of cell membrane due to their lipophilic features. The permeability and integrity of cell and mitochondrial membrane structures are thereby modified in order of leading to cell death. α-B showed a positive effect against promastigotes of *Leishmania infatum* at all concentrations tested. In fact, at the highest concentration tested, α-B and pentamidine achieved a total inhibition of *L.infatum* promastigote. So, the effect of α-B was comparable to those of pentamidine, a widely used anti-Leishmaniasis drug. α-B is the only terpenic derivative that can actually act against this flagellate protozoa type and furnishes promising aspects in a new anti-Leishmaniasis treatment with reduced side-effects. [49] The activity of the essential oil from fresh leaves of *P. cerrocampanensis* was evaluated against different bacterial and fungal strains and the larvae from *Aedes aegypti* with a positive effect observed. As α-B has previously been identified as the main constituent of this essential oil, the pharmacological features could be mostly contributed by this sesquiterpene, along with linalool and nerolidol which were less existing in the oil. A strong activity of this oil was observed against *P. aerigunosa, S. aureus* and *Helicobacter pylori*.

A total mortality of *A.aegypti* larvae was observed, when 500 mg/ml essential oil was applied. These larvae act as transmitters of several diseases such as malaria, Dengue fever or yellow fever that even can be fatal. In conclusion, the essential oil of this *Plinia* genus could not only be described as a new, potential source of α-B, than could also serve as a potential industrial resource of new antimicrobial and larvicidal products. [24]

Halitosis is nowadays not rarely occurring, causing not only aesthetic discomfort, but could also indicate on serious disorders, such as rhinopharyngological, oropharyngological, gastrointestinal and other systemic pathologies. Nevertheless, bacterial overgrowth on the dorsum of the tongue is the most common cause for halitosis, but may include other oral causes like gingivitis, periodontitis and dental caries. A great number of products containing antimicrobial substances against the overgrowth-bacteria-induced halitosis exist so far, toothpastes, tongue gels and other. *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Actinobacilli* are some of the bacteria which were isolated from halitosis patients. As recently a proven association between the *Solobacterium moorei*, a Gram-positive, non-sporeforming, anaerobic bacillus was identified, the possible synergistic effect of α-B and tea tree oil (TTO) and the effect of this substances alone on the bacteria have been observed. α-B has been several times reported to exhibit anti-microbial effects, by increasing bacterial susceptibility to antimicrobials and TTO, from *Melaleuca alternifoila* (Maiden and Betche) Cheel (Myrtaceae), was related to antibacterial, antifungal, antiviral and anti-protozoal activities, giving promising indication on the efficacy of these two compounds of natural origin: TTO in concentrations above 0.5% was very potent in killing the halitosis-associated bacterium *S.moorei* cells even after a short incubation time, but α-B turned out to be less efficient, in this case Although 0.1% α-B plus 0.05% TTO had a synergistic effect on *S. moorei*, this effect seemed not to be significant enough. Despite the fact that clorhexidine-containing formulations represent the 'gold standard' in the treatment of halitosis due to the broad antimicrobial spectrum of chlorhexidine digluconate and its substantivity, several adverse effects had been noticed by its application. The further involvement of natural compounds in the preparations used in oral health care, such as thymol, menthol and others

could improve the compliance of patients. Therefore, α-B and the TTO might be promising candidates for the improvement of oral health care product, especially α-B, which could not only be effective on *S.moorei*, but also on microorganisms involved in caries and periodontal diseases. [37]

Another interesting fact is the positive effect of α-B against different microsporum-spezies, which can, for example, cause *tinea capitis* and many other disorders. By reporting on the therapeutic indices of α-B compared to the one of many chemical synthetizited antimycotics, α-B exhibited a better effect. The MIC of α-B against dermatophytes was 50 mg/l, which was far below the LD_{50} tolerable concentration, and the well tolerated 2000 mg/kg dose tested in rats. α-B was superior, when TI were observed, over Griseofulvin, Nystatin, Fluconazole, Itraconazole and Ketoconazole. It is also worth mentioning that the effect of α-B was also observed in single cases. For sure that these single cases cannot be considered as valid for determining the efficacy, but these facts lead to interesting perceptions which could merit further studies to clarify their pharmacological relevance. Three galenic preparations of α-B had been tested, the pure fluid substance, a creme and a α-B containing emulsion. By topical application, microbe induced diseases, such as Oonychomykosis, dermatomykosis, tinea capitis superficialis, lead to astonishing therapeutic results. A special effect was observed when α-B emulsion was applied in form of a hip-bath, especially in the treatment of *C.albicans*-induced vulvovaginitis, supported by staphylococces. [38]

Insecticidal activities

Nowadays, α-B is obtained from the bark of the Brazilian Vanillosmopsis genus, *V. erythropappa* (Asteraceae). Nevertheless, previous reports have indicated the high content of α-B of *Vanillosmopsis* genus in generally thus arousing great commercial and agronomic interests. The percentage composition of the essential oil maintained by hydrodistillation from leaves and heartwood of wild-growing *V. pohlii* plants was analysed and displayed that α-B was the main composition of the heartwood, but not in leaves of *V.pohlii*, where terpenes such as (*E*)-caryophyllene and β-pinene were dominating. An interesting fact is that the chemical composition did not just

differ in the type of plant organ used as also in which area and altitude the species was collected from: The essential oil from leaves of *V.pohlii* collected in Lencois did not show a significant monoterpene percentage, in the one collected in Seabra country monoterpenes were the predominant class. α-B appeared in high amounts in the heartwood of *V.pohlii* regardless of where it was collected. α-B derivatives, such as B oxides, appeared in small, but not insignificant percentage. The B-rich essential oil from heartwood of *V. pohli* showed significant insecticide activity against *Bemisia argentifolii*, the white fly plaque, a common pest in Brazil, in comparison to pure α-B. The fact that higher α-B contents were more effective was also suggested. [21] Furthermore, the use of the essential oil of *V. arborea* as a larvicidal remedy was also investigated. Studies showed that, among ten essential oils tested, the one from *V. arborea*, which is rich in α-B, exerts the highest larvicidal activity. [2]

The anti-malarial activity of α-B against a chloroquine-resistant strain of *Plasmodium falciparum* was tested, compared to nerolidol, using the hypoxanthine incorporation assay. Although α-B exhibited anti-malarial activity, nerolidol seems to be superior in this assay. [1] Thus, many B-containing essential oils exhibited anti-plasmodial activity, for example, the essential oil of *Salvia runcinata* (approximately 60% of α-B) tested against the chloroquine-resistant strain FCR-3 using the 3H-hypoxanthine assay. On other hand, α-B, as a single compound did not exhibit any significant anti-plasmodial activity, suggesting a synergistic effect of the compounds in essential oils. [2]

Anti-nociceptive and anti-inflammatory activities

The anti-nociceptive and anti-inflammatory effects of α-B were the main subject of several studies. The use of α-B or B-rich oils as an anti-inflammatory agent is ubiquitous. The first indication about the anti-inflammatory activity dates from the year 1954 when Janku and Zita noticed a decrease of skin temperature with UV-light treated guinea pigs. [6] For the first time, these effects of α-B were observed in mice ears, rodent`s paws and later in mice cornea. Although the mechanism of this effect has undergone

proper investigations, it still represents an unanswered question. An anti-inflammatory effect of α-B was also observed when hip-endoprothesis inflammation was involved. [38] Observations with *in-vivo* studies confirmed that a pre-treatment with α-B reduces dose-independent a cyclophosphamide and mustard oil-induced nociceptive behaviour in mice. This effect of α-B could be related to its modulative activities of the prostanglandine synthesis. Anti-inflammatory effects similar to the one of dexamethasone and indomethacin, were also confirmed, where croton oil, arachidonic-acid and phenol-induced oedema were significantly reduced by the application of this sesquiterpene. A significant capsaicin-induced oedema reduction could not been observed. These data display the role of α-B as a topically active compound in the attenuation of acute dermatitis induced by the mentioned agents. [50] As α-B was tested as a monosubstance, the essential oil of *V. arborea* ("candeeiro") species, with a high content of this sesquiterpene, was also the subject of an investigation where similar anti-nociceptive and anti-inflammatory effects were studied. Earlier studies indicated that candeeiro is not potentially toxic. Again, an anti-inflammatory effect in the croton-oil, arachidonic acid and phenol-induced dermatitis could be confirmed, but no significant effect was achieved in the capsaicin-activated inflammatory pathway. The possible explanation of this effect, at least in the arachidonic acid-induced oedema, could be the influence of this essential oil on the production of inflammatory eicosanoids. An effect of the essential oil of *V.arborea* against visceral nociception induced by a intraperitoneal cyclophosphamide application aroused great interest, since, despite the advances taken in the mechanisms of visceral nociceptive activities, an effective therapy against the abdominal visceral pain is still lacking. [51] In another model of nociception, on the cornea, the most innerved tissue in the body, a similar anti-nociceptive effect of this oil was observed. Actually, the effect of the oil of this Brazilian tree was observed in a model of corneal pain in mice, induced by a hypertonic, 5 M NaCl solution. This eye irritation could be evidently reduced by the local and oral application of the *V.arborea* oil. In order to get an explanation of the possible mechanism responsible for these activities many drugs have been applied in this model. The pre-treatment of animals with ondasetron, a 5HT3 antagonist, reversed the anti-nociception

caused by the essential oil, suggesting the involvement of this receptor in the mechanism. Prazosin, a α1-receptor antagonist, atropine, a cholinomimetic and a competitive TRPV 1-channel antagonist were applied on the with *V.arborea* essential oil treated eye inflammation and interesting results were obtained. The effect of these substances may indicate the involvement of 5-HT, -1-receptors, muscarinic-receptors, and TRPV1-receptors in the anti-nociceptive mechanism of this essential oil. Once again the probable involvement of α-B in the decrease of prostanglandin synthesis and leukotrienes release could be proposed as a key mechanism of this effect observed. [52] In a further study, α-B efficiently diminished the acetic acid, capsaicin, formalin, and mustard oil-evoked pain-related behaviours. The ability of α-B to suppress the pain-related behaviour against MO-induced visceral nociception could not be related to its effect on primary afferent fibers, but may have a modulatory influence on another membrane receptor, namely the vanilloid receptor. As α-adrenoceptors or K+ATP-channel openers could not be explained as a potential mechanism of the pain-suppressing activity of α-B, further investigations are required to put a light in this field. [53] Similar investigations of the anti-nociceptive and anti-inflammatory effects of this odorant molecule had been done in a model of rodents, where the anti-nociceptive effect of α-B was brought in a relation to the anti-inflammatory activity. Again, the fact that this effect of α-B cannot be connected with a central mechanism was confirmed, when compared with the capsaicin-induced oedema. Instead of a capsaicin-induced oedema model, in this case, a hot plate model showed no effect of α-B on the intensity of licking the paw of rodents, where on the contrary codeine or other central nervous acting showed an effect. The central anti-nociceptive activity of α-B was also denied considering the fact, that this sesquiterpene showed positive effect just in the second inflammatory mediator`s related phase of a formalin-pain-test, but not in the first central regulated one. In contrast, the essential oil of *V. arborea*, suppressed both phases of the formalin-induced-pain model, also compared with the similar active morphine. [52] α-B pretreatment of a carageenan or dextran-induced oedema lead to a significant reduction of edema formation, in comparison with the effect of indomethacin and cyproheptadine. A positive effect was also observed in the 5HT-induced oedema group. Not only protein

extravasations and the amount of TNF-α to the peritoneal cavity induced by carrageenan could be decreased by the application of α-B, but also the blocking of neutrophil migration could be observed, similar to the effect of dexamethasone. These aspects could lead to new promising developments in the area of potential anti-inflammatory remedies when referring to natural products, considering the great fact that the application of those substances of natural origin could not only avoid one of the biggest NSAIDs side effects, such as gastric lesions, but that the therapeutic aspects could instead benefit of the gastro-protective effects of those substances. [18] As with many anti-inflammatory studies represented, α-B was likely brought in comparison with the standard steroidal anti-inflammatory drug-dexamethasone. A study of the possible cytokine production decrease by α-B confirmed once again the anti-inflammatory effect of this sesquiterpene, with no toxic effect observed, which is in agreement with the previous observation that (-)-α-B can inhibit the production of pro-inflammatory cytokines. α-B also showed no effects in form of skin irritations in a rabbit model . Skin diseases are related to an inflammatory process, thus the 12-O-tetradecanoyl-phorbol-13-acetate TPA-induced skin irritation model was used in order to observe the anti-skin inflammative effect of α-B. Lipopolysaccharide and 12-O-tetradecanoyl-phorbol-13-acetate induced production of pro-inflammatory cytokines (TNF-α and IL-6) in macrophage cells as well as in TPA-induced skin inflammation in mice were significantly inhibited by the topical application of α-(-)-B. [42] Not only the cytokine production was influenced in a dose-dependent manner by this odorant molecule, but also the lipid peroxidation rate, which can lead to oxidative stress, and also the actual ear thickness and weight by its application comparable to the effect of dexamethasone. By studying the molecular docking model, α-B and dexamethasone showed also satisfying docking scores with the pro-inflammatory agents TNFα and IL6. As previously demonstrated, a suspicious relation of α-B with pro-inflammatory agents exists and needs to be clarified more explicitly in order to understand the mechanism of its activity. [54] Confirmed by many observations, α-B`s anti-inflammatory activity has to be brought in relation with its influence on pro-inflammatory agents, which was further clarified with the fact that α-B does decrease the LPS-induced production of nitric oxide and prostanglandin

E2 (PGE2) in RAW264.7 cells. NO is a pro-inflammatory mediator which can lead to cytotoxic and oxidative damage effects. It is also a modulator of COX2 activity, which is catalyzing the rate-limiting step in prostanglandin synthesis, along with COX1. The LPS-induced production of NO and PGE2 was significantly reduced by α-B in a dose-dependent manner without no contribution to its cytotoxic effects, indicating to its anti-inflammatory activity. For the first time, α-B`s down regulating effect on the expression of inflammation-associated genes was also demonstrated. α-B exerts the anti-inflammatory effect by down regulating the expression of the inducible nitric oxide synthase-iNOS and COX-2 genes, as evidenced by Western blot and lucifer reporter assays via inhibitory effects on NF-kB and the activator protein-1 promoters. The activation of NF-kB and AP-1 coordinate the induction of many genes encoding inflammatory mediators and these factors present important transcriptional factors which play a critical role in the transcriptional regulation of genes that have been shown to suppress apoptosis and induce many cellular events such as chemo-resistance. NF-kB has been implicated in the regulation of many genes that code for mediators of inflammatory responses, such as the iNOS and COX-2 and AP-1 causes the production of pro-inflammatory mediators. The activation of these NF-kB and AP-1 promoters was significantly reduced by α-B. In conclusion, α-B exerts anti-inflammatory effects by down regulating expression of iNOS and COX-2 genes due to the inhibition of NF-kB abd AP-1 signalling. [15] α-B also appears to be a good 5-lipoxygenase inhibitor and has been widely reported to have a skin soothing action which strongly inhibits 5-LOX *in vitro*. As expected, an essential oil with α-B as the major compound, showed also a significant 5-LOX inhibition, such in the case of the *S. runcinata* oil. [2]

Peripheral nervous blockage

The anti-nociceptive activity of α-B has been extensively described in several studies indicating in its peripheral nervous activities. This suggestion was supported by the fact that a possible central nervous related anti-nociceptive activity of α-B was excluded for sure. Some suspicions on the correlation

between the anti-nociceptive activity of this sesquiterpene and its influence on K+ATP channels could not be explained. Another study focused their aims on the possible association between the anti-nociception-like effect of α-B and the decrease of peripheral nerve excitability. The putative changes on the compound-action potential characteristic were observed to throw a light on the mechanism even more. According to the results obtained, a CAP recording in the absence and presence of α-B, this sesquiterpene was capable to reduce the nervous excitability in a concentration-dependent manner. Considering the fact that some Na+ channel subtypes are widely expressed in sensorial peripheral fiber and are fundamental in pain perception, the activity of α-B could be also related with the blockade of these voltage-dependent channels. Both, depolarization and repolarisation phase of the CAP was observed in order to explain better the involvement of Na+ and K+-channels in this activity of α-B. Compared to a voltage-gated-Na-channel blocker-lidocaine, α-B showed a similar effect on the decrease of the depolarization phase of CAP after an incubation of 30 minutes. The difference between these two sodium channel blockers was displayed when the effect of α-B maintained after the washing out phase, indicating on an irreversible effect of this sesquiterpene. In contrast, α-B and lidocaine did not show any effect on the repolarisation phase of CAP, as expected. A voltage dependent potassium channel blocker 4-aminopyridine showed an effect on the repolarisation, but not on the depolarization phase of CAP, as predicted. Considering the data, a similar effect of α-B and lidocaine has been demonstrated picturing a relation between the α-B`s effect on the reduction of neuronal excitability and Na-channels. On the other hand, an exclusion of the participation of a K-channel in the activity of α-B in the CAP was suggested. [19]

Antispasmodic activity

Since in diverse studies α-B`s broad spectrum of pharmacological relevant features were confirmed, a smooth-muscle relaxing biological activity could be of great interest as well. In dose-dependent manner, α-B relaxed duodenal stripes at a dose of 30-300 μM/l and also endothelium-intact aortic rings and

urinary bladder stripes at a higher dose. Also this sesquiterpene decreased tissue specific spontaneous contractions completely or even increased their amplitude. By *in vivo* administration, α-B managed to weaken significant the activity of the cholinergic agonist carbachol in tracheal rings, but no effect on the decrease of responsiveness of urinary bladder stripes in mice was observed when ifosfamide, a nitrogen mustard alkylating agent, was administered. In conclusion, α-B reduced preferably contractions induced electromechanically. These findings indicate on α-B`s activity as an inhibitor of voltage-dependent Ca^{2+} channels. [55] This assumption was actually confirmed in another experiment, describing a putative Ca^{2+} channel blocking activity of the vasodilatation mechanism of this small odorant molecule. Another aim was to investigate the relaxant effects of potentially bioactive compounds with diverse structures found in chamomile, *in vitro*. It was shown that α-B among other structures exerted a time and dose-dependent relaxant effect in the porcine coronary artery and the splenic artery as well. As at doses of 3 and 10 μM no effects were observed, a dose of 30 μM α-B was effective. In the splenic artery a 80 % relaxation after 90 min was observed. In terms of exploring the mechanism of α-B`s relaxant activity, once again an involvement of the Ca^{2+}-concentration was demonstrated. A removal of extracellular calcium caused a significant inhibition of the effect of α-B. Once again it was demonstrated, that the relaxant effect ofα-B could be contributed due to an inhibition of the calcium influx. K-channel block or phosphodiesterase inhibitory effects by α-B were excluded. [56]

Many other observations actually speak for this fact in a strong way. α-B not only induced a dose dependent hypotension and bradycardia in rats, but also relaxed KCl-induced contractions with pharmacological potency significantly higher than that observed in vessels contracted with phenylephrine. This confirmed the previously reported observations that α-B acts against contractile responses that recruit preferentially voltage-gated Ca^{2+}-channels. The former finding about the independence of α-B`s smooth-muscle-relaxing activity in aortic preparations of the integrity of endothelial layer was expanded by indicating that neither nitric oxide nor prostanglandins release is involved. Furthermore, by taking diverse mechanisms of Ca^{2+}-influx in the cell in account, the authors concluded that α-B`s relaxant effects on vascular

smooth muscles are determined by the inhibition of contractions mediated by voltage-dependent Ca^{2+}-influx. At 100 μM, α-B decreased the contractile force and the Ca-influx in response to a KCl stimulus. *In silico*, α-B interacted with the Ca^{2+}-channel by binding on the β-subunit of this channel, which is important for the voltage dependent activation. Nevertheless, *in vivo* studies would be required to establish this finding. In summary, α-B might act through its ability to inactivate the voltage-dependent Ca^{2+}-channel, probably by an alosteric influence on the mentioned subunit, rather than by direct blocking. [57]

Anticancer activity

One of the biggest fields of research is definitely represented by the one of anticancer substances. Despite proper investigations, one of the biggest problems still remains namely the toxicity over normal healthy cells and many other side-effects evidently observed. The most prominent drugs used in cancer treatments are from chemical origin, where some isolated natural compounds, such as terpenoids, furnished proven and promising perceptions and therapeutic aspects in this area. α-B is known by its remarkable broadly spread therapeutic activities, where the anti-inflammatory effect is dominating, and its nontoxic properties were also evidenced (LD_{50}=13-14 g/kg). The possibility of its cancer preventing and anticancer activities as well, led to promising results in several experiments. The interest of that possible feature of this small odorant molecule grew constantly since the beginning of the20th Century. The effect of α-B in anticancer therapy was observed in many types of human cancer, specifically of those of pancreatic, breast, glioma, leukemia, endothelilal cells and towards malignant tumor cells in general. The aim is mostly clear defined in the trials in order to prove the efficacy of α-B and then to report on the pathway. Also, dose and time-dependent effects were examined yielding astonishing results.

GLIOMA

The inhibition of glioma cell growth and survival may be maintained by α-B by apoptosis-inducing effects. It has been widely reported and accepted that apoptosis is preferred to necrosis as a mechanism of tumour cell killing. This, energy consuming process seems to be genetically programmed and two major routes have been explained, the intrinsic and extrinsic one. Due to α-B`s lipophilic properties, it represents a highly stable structure and overcomes many chemical anti-glioma remedies, such as the most prominent one, carmustine. This anti-glioma drug used in chemical therapy is not able to cause an 100 % cell death of glioma cells. As many other reported drugs showed lower efficacy it might be contributed to their modification in the liver or their incapacity to pass through the blood-brain barrier. Different human glioma cell-lines, as T67 and U87 cell-lines, were used for comparison with rat glioma cell-line C6. The plateau was achieved after 15 hours incubation. The results showed that α-B induced a dose-dependent decrease in cell viability in all 3 cell lines, the human T67/U87 and the C6-cells after a 24hours treatment. Many parameters were evaluated such as the DNA-laddering, the inner transmembrane potential in living cells, the cytochrome c-release, and other important parameters, such as cell viability. The effect of α-B was defined as dose and time-dependent and the dose to cause cell death differed between these cell lines. Nevertheless, α-B kills the cells in the following 24 hours and no effect on viability of normal astro glial cells at a dose of up to 10 μM of α-B was observed. The polymerase cleavage was examined using Western blott analysis and again a dose and time-dependent effect was observed. As carmustine is not able to annihilate glioma cells at a concentration corresponding to LD_{10}, α-B with the capacity of total annihilation of these cells open the discussion whether α-B could be used as an efficient remedy for the clinical treatment of glioma. [4, 17] In fact, the role of mitochondria in the apoptosis-inducing effect of α-B has been either assured or speculated on in many studies. One of the studies [58] set the focus on analyzing the relation between the effect of α-B on mitochondrial damage and the viability of human glioblastoma cell line and human fibroblast as a control group. Especially, the mitochondria permeability transition pore (mPTP) as a possible target of α-B was defined. In addition, cyclosporine A, as an inhibitor of mPTP opening, was also analysed and the relation between Bax and Bcl2,

which are involved in the apoptotic events as well. The results observed were similar to those obtained by studies in which various other cell lines were used. Higher α-B toxicity towards malignant cells in comparison with normal, unchanged cells was observed in each of these studies. The expression of the Bcl-2 proteins in generally and mPTP expression in human glioma T67 cells after the treatment with α-B was examined by Western blotting. The data showed that α-B-induced apoptosis was not related to changes in the amount of cytosolic and mitochondrial Bcl-2 proteins. The oxidative damage was also not relevant. By measuring the oxygen consumption in the T67 cell line and fibroblasts, a decrease has been noticed. Upon adding cyclosporine A, a mPTP blocker, the effect of α-B was abolished. These data indicate that α-B does affect the mPTP. In summary, in T67 cell line apoptosis seems to be caused by a massive mPTP opening with the loss of respiration activity. [58]

PANCREATIC CANCER

An anti-tumour effect of α-B was also examined in human pancreatic cells *in vitro*. As the pancreatic cancer is one of the most malignant cancer types and the prognosis for the patients after the diagnosis is mostly not promising, new therapeutic aspects are constantly being investigated. The anticancer effect was determined by many assays, such as the cell proliferation, viability, colony formation assay. Pancreatic cell lines KLM1, KP4, Panc 1, MCA, Paca 2 and pancreatic epithelial cells were observed. The anticancer effect of α-B was significantly higher on the pancreatic cell lines as on the epithelial pancreatic cells. These results indicate that α-B possesses selective efficacy towards pancreatic cancer cells. α-B indeed increased the expression of PARP which is an important apoptosis mediator and has also been able to reduce the Akt phosphorylation as an crucial step for the full activation of this factor which increases cellular metabolism. Furthermore, α-B increased the expression of the tumor suppressor EGR1 as well. An intragastric α-B application was also examined. With no toxicity observed, the tumour weight of the mice treated with α-B diluted in olive oil was significantly lower than that of the control group. [14, 17]

ANTI-ANGIOGENIC EFFECT

Angiogenesis, the process of new blood vessel formation, represents a multistep process and it has been studied in many *in vivo* and *in vitro* models, especially because the anti-angiogenic therapy is considered as one of the most promising approaches in cancer treatment. The anti-angiogenic effect of α-B has been investigated focusing attention on separate steps of this process: proliferation and viability, chemo invasion and capillary morphogenesis. A strong dose-depend anti-angiogenic effect of α-B on human endothelial cells was observed. At low concentrations, α-B furnished a decrease of proliferation and invasion, on the other hand, at high doses, α-B exerted anti-angiogenic properties. Upon the rising concentration of (-)-α-B the percentage of decrease of the total cell number as compared to a control also increased. Chemo invasion and migration of endothelial cells also decreased by α-B and due to the effect on the *in vitro* capillary morphogenesis the strong dose-depend effect was also confirmed. High doses of α-B offers important perspectives for the treatment of diseases such as cancer, whereas a low dosed therapy could be a promising treatment aspect for diseases such as the post ischemic revascularization. The apoptotic pathway was also involved which was confirmed by observing the cytochrome c release from mitochondria to cytosol, [29] the inactivated procaspase 3, shifting the Bcl-2/Bax balance by Bcl-2 down regulation. One of the biggest problems of anti-angiogenic remedies is the occurrence of side effects. By improving α-B in the anti-angiogenic therapy, many perspectives in further investigations of an anticancer treatment with less side effects could be opened. [4]

BID-INTERACTION

Another aspect observed was the molecular mechanism of α-B`s apoptosis-inducing action including its capacity to interact with Bid, a pro-apoptotic protein. It has been reported that Bid can be recruited in lipid rafts by some apoptosis-inducing agents, playing a crucial role in the formation of an apoptosis-inducing signalling complex. α-B may be that kind of agent. Lipid rafts are small sterol and sphingolipid-enriched membrane domains which play an important role in intracellular protein transport and membrane fusion. Tumour cells seem to contain far more of these lipid rafts on the plasma

membrane than normal cells. Tumour cells have a bigger amount of lipid rafts on their membrane and thus the question appears if the apoptosis-inducing effect of α-B could be related to their binding on lipid rafts and further on the distribution of Bid in tumor cells by its binding on α-B? Indeed, it has been demonstrated that α-B induces a movement of Bid, a member of the pro-apoptotic Bcl2 family, into lipid raft regions. The interaction between α-B and Bid was analysed either by Surface Plasmon Resonance analysis or by intrinsic fluorescence measurement. The binding sites of Bid were also investigated using a special computational mean which ranked the binding sites from most probable to least probable. The binding site for α-B-Bid interaction is demonstrated to be located quite deep within the protein core leading to a total protection of this ligand α-B from environmental influence. For the experimental data, two human pancreatic carcinoma cell lines were cultivated in the presence of α-B and successively the number of live cells was counted. These cells indeed incorporated α-B far more than human fibroblasts did, not only in membrane fractions but also in other fractions such as cytoplasm and nuclei. The amounts of α-B were identified with GC-MS analysis in various fractions. In non treated cells Bid is widely present in low density fractions. Defining targeting lipid rafts as a possible mechanism of selective induced apoptosis in malignant tumour cells, further studies are necessary to declare the exact mechanism in more detail. [3]

LEUKAEMIA

Among a great number of studies about the efficacy and mechanism of α-B`s anticancer activity, an interesting study about the pro-apoptotic activity of this molecule on human acute leukaemia cells displayed once again its various effect on different types of human cancer cells. The pro-apoptotic potential of α-B was tested on three types acute leukaemia cell types: the Philadelphia-negative and positive B acute lymphoid leukaemia`s (Ph-/Ph+B-ALL) and acute myleoid leukaemia`s. It has been proven that α-B has a positive pro-apoptotic effect against these acute leukaemia cells *ex vivo*, primarily against Ph-B-ALL. The Ph+-ALL and AML were also killed, only at higher dose than the Ph-B-ALL. Nevertheless, all cells as a whole were more sensitive to 65 mg α-B than their normal counterpart. A synergistic effect of α-B and the BCR-

ABL tyrosine kinase inhibitor imatinib was also demonstrated. Cells from Ph+B-ALL shifted from 40% cytotoxity with 40 μM α-B alone to 75% with α-B plus imatinib mesylate. [59] A decrease in oxygen consumption in these leukemic cells by α-B was also reported. A red/green fluorescence was used to display the effect, rather a decrease of mitochondrial transmembrane potential by α-B. The decrease of this potential was marked by a loss of red fluorescence and an increase of the green one.

A direct effect on mitochondrial integrity, in this case of leukemic cells could be determined as the mechanism of α-B`s pro-apoptotic effects. According to data accumulated by now, α-B enters cells via lipid rafts [3], directly involves mitochondrial permeability transition pore opening [14] which is responsible for the reduced glutamate/malate supported oxygen consumption and leads to disruption of the mitochondrial membrane potential and programmed cell death. [59]

Some of non-TKI compounds, such as α-B, in combination with conventional TKIs, such as imatinib, dasatinib and nilotinib furnish synergistic effects towards leukaemia cells. These TKIs show high efficacy, especially in chronic myleoid leukaemia in a chronic phase. Nevertheless, more and more resistance observed can be related to the fact that these drugs do not eradicate the primitive BCR-ABL+ leukemic stem cells. By the opportunity of combining α-B with conventional TKIs a reduction of the dose of TKIs, especially imatinib and nilotinib could be possible. The study data indicate that α-B exerts an effect on the viability of BCR-ABL+ cells, shows a slight to strong synergistic effect with TKI, and according to a dose reduction index the decrease of the dose of TKIs when combined with α-B is viably. Previous data demonstrated that α-B enters neoplastic cell more likely than normal cells through lipid rafts. [3] By the fact that those lipid rafts occur mostly on the cellular membrane the HPLC analysis is suited to measure the highest concentration of α-B in those membranes. The loss of plasma membrane integrity was also reported and related to the apoptotic inducing property of α-B. The mitochondrial transmembrane potential was measured in acute leukaemia cells the mitochondrial function was disturbed by this sesquiterpene and also the reduction of oxygen consumption supported the apoptotic process. The preference of α-B to tumour cells is probably due to its lipophilic

properties. In summary, neoplastic BCR-ABL+ cells are significantly more sensitive to α-B than normal cells. Nevertheless, α-B`s anticancer activity is independent from BCR-ABL+, but the combination of α-B and TKI could put a light in the development of strategies to target and eliminate BCR-ABL+ stem cells. [36]

Derivatives, such as α-B-based thiosemicarbazones were evidently identified as core structures of clinically used compounds in chemotherapy, such as Triapine®. Those derivatives were tested on eight different human tumour cell lines: leukaemia (K-562), melanoma (UACC-62), breast (MCF7), breast resistant (NCI-ADR), lung (NCI-460), ovarian (OVCAR), prostate (PCO-3) and colon (HT-29). The concentrations of these derivatives were ranging from 0.25 to 250 μg/ml and doxorubicin was used as a positive control. All derivatives inhibit the growth of the cell lines in a dose-dependent manner. Leukaemia cells showed more sensitivity than the other cells to all derivatives within the range of 0.02-0.22 μM. After the anti-cancer activity of these α-B derivatives was determined a structure-activity relationship was also performed, where the incorporation of the isothiocyanate group into the α-B structure enhanced the pharmacological properties of this sesquiterpene. Although α-B showed cytostatic activity against the leukaemia cells, a low selectivity has been noticed. [60]

BREAST CANCER

Although many therapeutic aspects and remedies have been developed so far, breast cancer represents one of the biggest death causing nowadays. The over expression of the human epidermal growth factor receptor 2 is often a criteria for an individualized treatment of this invasive cancer type. As HER2 is an index of an aggressive form of the tumour, the inhibition of the over expression of this factor could bring up new promising therapeutic aspects. The fact, that terpene families play a key role as chemo preventive agents in induced rat mammary carcinogenesis supported great interest in deeper *in vivo* investigations of α-B`s possible effectiveness on the induction of spontaneous mammary tumours in mice. As this odorant molecule already showed inhibiting effects on the growth of glioblastoma cells, which also

contain *neu* oncogene, a similar effect on breast cancer could be expected as well. Pharmacological studies displayed that this sesquiterpene accumulates preferably in mammary gland and adipocytes. Therefore, α-B has been also investigated in terms of having anticancer effects against this type of cancer. Two different doses have been examined and on Her2/neu transgenic mice the one of 10 mg /mouse of B (via intramammary infusion) has been found to be the optimal one, rather than the 3.6 mg/mouse dose. No adverse effects were noticed at these doses. The effect of α-B after cancer surgical excision has been also examined and as results shown, indeed, a treatment with α-B prolonged the tumour-free weeks after surgery. The number of palpable tumours decreased as well. Several other parameters have been observed, such as the down regulation of the HER 2/neu, EGFR gene, an increase of NK cell cytotoxicity, an increase of the number of T-cells, increase cancer cell death, where α-B administration at the mentioned dose exhibited a positive effect. Especially, the role of α-B as a NF-kB inhibitor could be of great interest, because blocking the NF-kB signalling is directly involved in the oncogenesis. The down regulation of the *neu* gene by α-B leads to subsequent limited proliferation of cancer cells. All of these data allow the conclusion that α-B could be a great remedy in combination with conventional therapies in breast cancer. In fact, α-B could be considered as an adjuvant therapy, especially after the removal of the tumour mass, but further *in vitro* and *in vivo* studies are necessary to clearly define this effect of α-B. [61]

LIVER CANCER

In the row of cancer cell lines interacting with α-B in different pathways, mostly the apoptosis-inducing effect, another aggressive cancer cell lines could be listed, namely different liver carcinoma cell lines. After the cells were treated with different concentrations of α-B (0-20 μM) for 24 hours, the MTT assay seemed to be the proper method for the examination of cell viability once again. The results showed a significant 70 % cell death of the HepG2 human liver carcinoma cell line at a dose of 10 μM. [62]

Using fluorescence microscopy, cells treated with α-B showed not only morphological changes, again in a dose-and time dependent manner, but also

activating effects on initiator and executioner caspases involved in both apoptotic pathways. Especially caspase 8 was characterized as the upstream regulator in α-B-induced apoptosis. The increasing effect of α-B on the expression of p53 and NF-kB was clarified and the suppressing effect on the anti-apoptotic Bcl-2 family members has been confirmed. [29] Not only the anti-cancer activity of α-B was observed, but also the cancer chemo preventive action of this odorant molecule has undergone proper research too. α-B seems to inhibit the elimination of ROS by enhancing the activity of superoxide dismutase and catalase, and therefore leads to cancer cell death. It also inhibits the cell proliferation and expanding cancer cells by inhibiting the activity of ornithine decarboxylase and cathepsin D (up to 28%), as well nitric oxide in a dose-dependent manner. [5]

Table 1 gives a better insight into some of the relevant mentioned anticancer studies of α-B.

Table 1. *Overview of studies on anticancer effects of B/B-containing/based substances*

Subject, cell lines	Compound	Dosage	Investigated parameters	Outcome	Ref.
Pancreatic carcinoma derived cell lines: IO and IM Human fibroblast cells	α-B in absolute ethanol	Initial concentration 250 μM Effective concentration 2 μM	Cell viability Poly (ADP-ribose) cleavage Caspase 3 activation DNA-ladder formation Mitochondrial membrane potential Release of cytochrome C Bid movement to lipid rafts-rich membrane regions	Highly selective activity of α-B towards malignant cell lines, apoptosis mediated Direct interaction with Bid	(3)
Human umbilical endothelial cells (HUVEC)	(-)-α-B	0,1-5 μM	Proliferation /viability of cells Chemo invasion Capillary morphogenesis Release od cytochrome C Caspase 3 activation Bcl-2/Bax ratio	Cell death by cell number reduction at >0,25 μM as a minimal dose Low dose-proangiogenic effect High dose-antiangiogenic effect	(4)
Human pancreatic cell-lines: KLM1, KP4, Panc 1, MIA Paca2 pancreatic epithelial cells: ACBRI515 tumour-bearing mice	α-B in vitro (in vivo-intragastric)	0-6,25 μM α-B in vitro 21-27 mg/mouse in vivo	Proliferation Poly (ADP-ribose) cleavage Akt-phosphorylation EGR1 overexpression	Selective cytotoxic efficacy towards pancreatic cancer cells Reduction of tumour volume in vivo	(14)
Human glioma cell lines: T67 and U87 rat glioma cell line: C6	Soluble fraction of α-B in ethanol	2,5-10 μM	Hypo G1 accumulation Poly (ADP-ribose) cleavage DNA-ladder formation Estimation of mitochondrial- inner transmembrane potential Release of cytochrome C	Efficient and potent cytotoxic effect on human and rat malignant glioma cells	(17)
Human prostate epithelial cells: PZ-HPV-7 human prostate cancer c. LN CaP, DU145, PC-3 HeLa -cervix adeno carc. HT 1080-fibrosarcoma RKO-colon carcinoma T-47 D-breast carcinoma	Aqueous and methanolic extract of chamomile	1000-4000 μ/ml aqueous extr. 100-400 μ/ml methanolic extr.	Cell viability Growth inhibition DNA-ladder formation	Selective anticancer activity	(34)
Primary acute leukaemia cells: BCR-ABL+: K562,LAMA-84, CML-T1 cell lines Acute lymphoblastic leukaemias Peripheral blood mononuclear cells (PBMC)	α-B α-B +imatinib or nilotinib	10-160 μM	Cell viability Synergistic effect –DRI (dose reduction index) α-B concentration in cellular compartments Loss of plasma membrane integrity Mitochondrial transmembrane potential dissipation	Inhibitory effect on BCR-ABL+ cell viability, sparing normal cells A preferentially concentrating in cellular membranes (via lipid rafts)	(36)
HER-2/neu transgenic mice	α-B	3,6 mg/mouse 10 mg/mouse Via intrama-mmary infusion	Kinetic of tumour incidence Reduction of the number of tumour masses NK cell cytotoxicity Number of T-cell (CD4 and CD8) The down regulation of the expression of genes involved in carcinogenesis, angiogenesis -neu gene (Erbb2) -genes involved in the signal transimission of the neu gene	Block of cancer cell cycle or angiogenesis The down regulation of the tested genes expression	(61)

Table 1 Subject, cell lines	Compound	Dosage	Investigated parameters	Outcome	Ref.
Human cell lines: Melanoma UACC-62 Breast MCF-7 Breast resistant NCI-ADR Lung NCI-H460 Prostate PCO-3 Ovarian OVCAR-3 Colon HT-29 Leukaemia K-562	α-B α-B-based thiosemicarba-zones	0,25-250 μg/ml	GI 50 –molar concentration of the compound that inhibits 50 % cell growth TGI-molar concentration of the compound leading to total inhibition of cell growth LC50 value-molar concentration of the compound leading to 50% cell death	α-B GI 50=6,37 μM against K-562 cancer Other cell lines GI>100 μM All of the compounds tested inhibited the growth of the cell lines tested	(60)
Human liver carcinoma cell line Hep G2 Human prostate cancer cell line PC-3 Human cervical carcinoma cell line HeLa Human esophageal-ECA-109	α-B	0-20 μM	Cell viability DNA-ladder formation Sub-G1 peak Poly (ADP-ribose) polymerase cleavage Caspase activation Release of cytochrome C Bcl-2, p53, NFkB, Fas expression	α-B-induced apoptosis α-B-induced apoptosis on Hep G2-cells by the extrinsic and intrinsic apoptotic pathway	(62)

Mutagenicity and genotoxicity

As α-B has been regarded and used as a nontoxic substance in many formulations, the complete toxicological data has yet been observed in an insufficient manner. Although first data in reference to the toxicological aspect of α-B date from around the 80`s, the first ascertained mutagenic and genotoxic observing of this sesquiterpene has been established only recently. As the exposure of cells to diverse mutagenic agents lead to cancer, the certainty of non-mutagenic properties of substances used in therapy seems to be obligatory. Mutagenicity of α-B at doses up to 100 μg/plate in ethanol, was for the first time evaluated with TA100, TA98, TA97a and TA1535 *Salmonella typhi murium*, with or without addition of a metabolic activation system, and further a standard plate and pre-incubation test on rat liver has been done with TA98, TA100, TA1535 and TA1537 strains of the same Salmonella genus. [11, 63] The proof of mutagenicity of this odorant molecule and the criteria for a positive mutagenic response are a clear dose-dependent increase in the number of revertants. As α-B did not cause any increase of revertant colonies over the negative control values for the tester strains, a mutagenic activity of this alcohol in the Salmonella/microsome assay could be excluded. On the other hand, anti-mutagenicity was defined as a decrease of mutagenic-induced increase of the number of revertant colonies. In order to prove the non-toxicity of the non-toxic doses of α-B which had been described previously, a positive anti-mutagenic effect could be also described as a positive toxicity. The previously as non-toxic described doses of α-B did not show any decreases in the number of revertants, nor alterations of background growth, confirming the non-toxicity of the doses. To confirm any anti-mutagenic effect of α-B in generally, the effect of this sesquiterpene was observed against direct-acting and indirect acting mutagens. α-B antagonized the effect of several mutagenic agents, such as aflatoxin B1, and exhibited a dose-dependent reduction of some indirect-acting mutagens. The mechanism of this specific effect has also been investigated. In addition, an inhibition of the activity of hepatic monooxygenases, CYP2B1/2 and CYP1A1, which take part in the conversion of promutagens into their active metabolites, has been reported. [63] Explicitly, data obtained by another study showed, that α-B

exhibited an inhibitory effect on the pentoxyresorufin-o-deethylase and ethoxyresorufin-o-deethylase in rat liver microsomes, which are markers for cytochrome P450 isoforms, especially CYP2B and CYP1A subfamilies. In fact, CYP2B converts aflatoxin B1 into a mutagenic metabolite, and the CYP1A isoform activates several promutagenic factors. Since these both CYP isoforms are considered as activators of promutagenic factors, the inhibitory effects of these isoforms could be considered as the possible mechanism of α-B`s anti-mutagenicity. On the other hand, α-B interferes with cytochrome P450 enzymes indicating that this molecule may be metabolised by these enzymes. [1] 20.0-5000 μg/plate doses of α-B`s mutagenic activity against different Salmonella strains were also tested. At these concentrations, with or without metabolic activation, no mutagenic effect of α-B was observed. [11] The genotoxicity of α-B was tested in the chromosome aberration assay using Chinese hamster V79 cells. For a positive control cells were treated with ethyl methane sulfonate and cyclophosphamide, with and without metabolic activation respectively, and the negative control cells were treated with a vehicle or left untreated. α-B turned out to be negative in this assay, independently from metabolic activation, in other words, this odorant molecule did not show any relevant genotoxic effects. [11] A significant dose-dependent inhibitory effect of α-B at all doses tested, against the clastogenic damage induced by 3 mg/kg of daunorubicin, was noted. After 48-h exposure, the percentage inhibition with 120 and 1200 mg/kg of α-B was 52 and 65%, respectively. [2, 64]

CONCLUSION

α-Bisabolol, a small sesquiterpenic molecule with a big pharmacological relevance, found the way from being a simple odorant and skin effecting agent to become a focus of intense research in many pharmacologically interesting studies.

As earlier presumed that this lipophilic molecule only exhibited a few biological significant activities, nowadays, many scientific studies indicate a more complex spectrum of α-B`s properties. Many significant mechanisms of activities, such as antagonizing oxidative stress, the involvement into the apoptotic pathways, the intrinsic and extrinsic, the antispasmodic activity related to an inhibition of the Ca^{2+}-influx into the cell and many others, indicate the importance of the continuation of, especially, *in vivo* studying about this odorant molecule.

Since about 2010 some research groups offered remarkable observations when referring to α-B`s anticancer activity. Astonishing results were obtained when α-B was compared to common medicines, such as nystatin, ketoconazole, dexamethasone, indomethacine, carmustine and a whole variety of others. A synergistic effect was also observed in some cases, such as the one with the tyrokinase inhibitors, imatinib and nilotinib.

All these, in most cases analytically precise defined results of studies and clearly ascertained safety assessments, indicate α-B`s possible relevance in the future of diverse disease treatment.

REFERENCES

1. Petronilho S., Maraschin M., Coimbra M.A., Rocha S.M. In vitro and in vivo studies of natural products: A challenge for their valuation. The case study of chamomile (Matricaria recutita L.). *Ind. Crops Prod.* **2012**, *40:* 1– 12.

2. Kamatou G.P.P., Viljoen A.M. A Review of the Application and Pharmacological Properties of α-Bisabolol and α-Bisabolol-Rich Oils. *J Am Oil Chem Soc.* **2010**, *87:* 1–7.

3. Darra E., Abdel-Azeim S., Manara A., Shoji K., Marechal J.D., Mariotto S., Cavalieri E., Perbellini L., Pizza C., Perahia D., Crimi M., Suzuki H. Insight into the apoptosis-inducing action of α-bisabolol towards malignant tumor cells: Involvement of lipid rafts and Bid. *Arch. Biochem. Biophys.* **2008**, *476:* 113–123.

4. Magnelli L., Caldini R., Schiavone N., Suzuki H. and Chevanne M. Differentiating and Apoptotic Dose-Dependent Effects in (-)-α-Bisabolol-Treated Human Endothelial Cells. *J. Nat. Prod.* **2010**, *73:* 523–526.

5. Srivastava S., Luqman S. Cancer Chemopreventive action of (-)-α-bisabolol, a sesquiterpene alcohol involves inhibition of cathepsin D and ornithine decarboxylase via tramping nitric oxide. *Nature Precedings* **2012**, *http://dx.doi.org/ 10.1038/ npre. 2012.6902.1* (26.07.2014)

6. Isaac O. Pharmacological Investigations with Compounds of Chamomile I. On the Pharmacology of (-)-α-Bisabolol and Bisabolol oxides (Review). At Hippokrates Verlag GmbH **1979**, *35:* 118-124.

7.Siniazo.Biotechn.SyntheticBiology.Bisabolol.**2013**,*http://www.siniazobiotech.com/technology/synthetic-biology.html* (16.07.2014)

8. Wagner C., Friedt W., Marquard R.A., Ordon F. Molecular analyses on the genetic diversity and inheritance of (-)-α-bisabolol and chamazulene content in tetraploid chamomile (Chamomilla recutita (L.) Rausch.) *Plant Science* **2005**, *169:* 917–927.

9. De Lucca A.J., Pauli A., Schilcher H., Sien T., Bhatnagar D. and Walsh T.J. Fungicidal and Bactericidal Properties of Bisabolol and Dragosantol. *J. Essent. Oil Res.* **2011**, *23:3:* 47-54.

10. Sandasi M., Kamatou G.P.P., Viljoen A.M. An untargeted metabolomic approach in the chemotaxonomic assessment of two Salvia species as a potential source of α-bisabolol. *Phytochemistry* **2012**, *84:* 94–101.

11. Luqman S., Srivastava S. Protective Effect of α-(-)-Bisabolol on Markers of Oxidative Stress in Erythrocytes Subjected to Oxidative Insult. *Planta Med.* **2011**, *77(12).*

12. Moura Rocha N.F., Valente de Oliveira G., Ramos de Araujo F.Y., Vasconcelos Rios E.R., Rodrigues Carvalho A.M., Freire Vasconcelos L., Silveira Macedo D., Gomes Soares P.M., Pergentino De Sousa D., Florenco de Sousa F.C. (-)-α-Bisabolol-induced gastroprotection is associated with reduction in lipid peroxidation, superoxide dismutase activity and neutrophil migration. *Eur. J. Pharm. Sci.* **2011**, *44:* 455–461.

13. Bhatia S.P., McGinty D.,Letizia C.S., Api A.M. Fragrance material review on α-bisabolol. *Food Chem. Toxicol.* **2008**, *46:* 72–76.

14. Seki T., Kokuryo T.,Yokoyama Y., Suzuki H., Itatsu K., Nakagawa A., Mizutani T., Miyake T. Uno M., Yamauchi M. and Nagino M. Antitumor effects of α-bisabolol against pancreatic cancer. *Cancer Sci*. **2011**, *102:* 2199–2205.

15. Kim S., Jung E., Kim J.H., Park J.H., Lee J., Park D.(2011) Inhibitory effects of (-)-α-bisabolol on LPS-induced inflammatory response in RAW264.7 macrophages. *Food Chem. Toxicol.* **2011**, *49:* 2580–2585.

16. Ohloff G., Giersch W., Näf R. and Delay F. The Absolute Configuration of β-Bisabolol. *Helvetica Chimica Act*. **1986**, *69:* 698-703.

17. Cavalieri E., Mariotto S., Fabrizi C., Carcereri de Prati A., Gottardo R., Leone S., Berra L.V., Lauro G.M., Ciampa A.R., and Suzuki H. *a*-Bisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. *Biochem. Biophys. Res. Commun.* **2004**, *315:* 589–594.

18. Moura Rocha N.F.,Vasconcelos Rios E.R., Rodrigues Carvalho A.M., Santos Cerqueira G., De Araújo Lopes A., Moreira Leal L.K.A., Leite Dias M, Pergentino de Sousa D. and Florenço de Sousa F. Anti-nociceptive and anti-inflammatory activities of (-)-α-bisabolol in rodents. *Naunyn-Schmiedeberg's Arch Pharmacol*. **2011**, *384:* 525–533.

19. De M. H. Alves A., Goncalves J.C.R., Santos Cruz J., Araújo D.A.M. Evaluation of the sesquiterpene (−)-α-bisabolol as a novel peripheral nervous blocker. *Neurosci. Lett.* **2010**, *472:* 11–15.

20. Selco. α-bisabolol natural (C) **2009**, *http://www.kinetiktech. com/brochures /pdf/ g fn/Selco-Alpha-Bisabolol-Natural-(C)-Leaflet.pdf* (05.07.2014)

21. De Andrade I.L., S. Bezerra J.N., A. Lima M.A.L., De Faria R.A.P.G., S. Lima M.A., and Andrade-Neto M. Chemical Composition and Insecticidal Activity of Essential Oils from Vanillosmopsis pohlii Baker against Bemisia argentifolii. *J. Agric. Food Chem.* **2004**, *52:* 5879-5881.

22. Spiegel M. α-Bisabolol–Natürlichkeit vs. Nachhaltigkeit. *SOFW-Journal* **2012**, *138:* 1/2.

23. Musarurwa H.T., Van Staden J., Makunga N.P. In vitro seed germination and cultivation of the aromatic medicinal Salvia stenophylla (Burch. ex Benth.) provides an alternative source of α-bisabolol. *Plant Growth Regul.* **2010**, *61:* 287–295.

24. Vila R., Santana A.I., Pérez-Rosés R., Valderrama A., Castelli M.V., Mendonca S., Zacchino S., Gupta M.P., Cañigueral S. Composition and biological activity of the essential oil from leaves of Plinia cerrocampanensis, a new source of α-bisabolol. *Bioresour. Technol.* **2010**, *101:* 2510–2514.

25. Adam K.P. and Zapp J. Biosynthesis of the isoprene units of chamomile sesquiterpenes. *Phytochemistry* **1998**, *48(6):* 953-959.

26. Ajikumar P.K., Tyo K., Carlsen S., Mucha O., Phon T.H. and Stephanopoulos G. Terpenoids: Opportunities for Biosynthesis of Natural Product Drugs Using Engineered Microorganisms. *Mol. Pharmaceutics*. **2008**, *5:* 167–190.

27. Ernst H., Pfaff G., Beck K., Schubert J., Gottwald G., Krause W. Methods for producing bisabolol which is farnesol free or is low in farnesol.: **2010**, US-Patent *2010/0222606 A1*.

28. Taglialatela-Scafati O., Pollastro F., Cicione L., Chianese G., Bellido M.L., Munoz E., Oezen H.C., Toker Z. and Appendino G. STAT-3 Inhibitory Bisabolanes from Carthamus glaucus. *J. Nat. Prod.* **2012**, *75:* 453-458.

29. Makita N., HoshinoY. and Yamamoto H. Asymmetric Epoxidation of Homoallylic Alcohols and Application in a Concise Total Synthesis of (-)-α-Bisabolol and (-)-8-epi-α-Bisabolol. *Angew. Chem. Int. Ed.* **2003**, *42:* 8.

30. Ganzera M., Schneider P., Stuppner H. Inhibitory effects of the essential oil of chamomile (Matricaria recutita L.) and its major constituents on human cytochrome P450 enzymes. *Life Sci.* **2006**, *78:* 856 – 861.

31. Perbellini L., Gottardo R., Caprini A., Bortolotti F., Mariotto S. and Tagliaro F. Determination of alpha-bisabolol in human blood by micro-HPLC–ion trap MS and head space-GC–MS methods. *J. Chromatogr. B.* **2004**, *812:* 373–377.

32. São Pedro A., Detoni C., Ferreira D., Cabral-Albuquerquea E. and Sarmento B. Validation of a high-performance liquid chromatography method for the determination of (-)-*a*-bisabolol from particulate systems. *Biomed. Chromatogr.* **2009**, *23:* 966–972.

33. Avonto C., Wang M., Chittiboyina A.G., Avula B., Zhao J. and Khan I.A. Hydroxylated Bisabolol Oxides: Evidence for Secondary Oxidative Metabolism in Matricaria chamomilla. *J. Nat. Prod.* **2013**, *76:* 1848−1853.

34. Srivastava J.K. and Gupta S. Antiproliferative and Apoptotic Effects of Chamomile Extract in Various Human Cancer Cells. *J. Agric. Food Chem.* **2007**, *55:* 9470–9478.

35. Villegas L.F., Marcüalo A., Martin J., Ferna´ndez I.D., Maldonado H.,Vaisberg A.J. and Hammond G.B (+)-epi-α-Bisbolol Is the Wound-Healing Principle of Peperomia galioides: Investigation of the in Vivo Wound-Healing Activity of Related Terpenoids. *J. Nat. Prod.* **2001**, *64:* 1357-1359.

36. Bonifacio M, Rigo A, Guardalben E, Bergamini C, Cavalieri E. α-bisabolol Is an Effective Proapoptotic Agent against BCR-ABL+ Cells in Synergism with Imatinib and Nilotinib. *Plos One* **2012**, *7(10):* e46674.

37. Forrer M., Kulik E.M., Filippi A., Waltimo T. The antimicrobial activity of alpha-bisabolol and tea tree oil against Solobacterium moorei, a Gram-positive bacterium associated with halitosis. *Archives of Oral Biology* **2013**, *58:* 10-16.

38. Pauli A., Schilcher H. Infektionen, Entzündungen, Wunden: 18 Fallbeispiele zu (-)-α-Bisabolol. *Komplement.integr.Med.* **2009**, 28-32.

39. Hahn B, Hölzl J. Absorption, distribution and metabolism of [^{14}C]-levomenol in the skin. *Arzneimittel-Forschung* **1987**, *37(6):* 716-720.

40. Lee J., Jun H., Jung E., Ha J. and Park D. Whitening effect of α-bisabolol in Asian women subjects. *Int. J. Cosmet. Sci.* **2010**, *32:* 299–303.

41. Niyonsaba F., Hattori F., Maeyama K., Ogawa H., Okamoto K. Mechanisms of depigmentation by α-bisabolol. *J. Dermatol. Sci.* **2008**, *52(3):* 219-222.

42. Moura Rochaa N.F., Teles V.E., Mouraa B.A., Gomes S.M.I., Aquino N.M.R., Vasconcelos R.E.R., De Sousab D.P., Mendes V.S.M., De Franca F.M.M. and De

Sousaa F.C.F. Gastroprotection of (-)-α-bisabolol on acute gastric mucosal lesions in mice: the possible involved pharmacological mechanisms. *Fundam. Clin. Pharmacol.* **2010**, *24:* 63–71.

43. Bezerra S.B., Leal L.K.A.M.,Nogueira N.A.P. and Campos A.R. Bisabolol-Induced Gastroprotection Against Acute Gastric Lesions: Role of Prostaglandins, Nitric Oxide, and KATP+ Channels. *J Med Food* **2009**, *12 (6):* 1403–1406.

44. De O. Leite G., Da Penha A.R., Fernandes C.N., Souza H.H.F., Da Costa J.G.M., Campos A.R. Gastroprotective mechanism of Vanillosmopsis arborea bark essential oil. *Fitoterapia* **2009**, *80:* 77–80.

45. Vinholes J., Rudnitskaya A., Gonçalves P., Martel F., Coimbra M.A., Rocha S.M. Hepatoprotection of sesquiterpenoids: A quantitative structure–activity relationship (QSAR) approach. *Food Chem.* **2014**, *146:* 78–84.

46. Braga P.C., Dal Sasso M, Fonti E., Culici M. Antioxidant activity of bisabolol: inhibitory effects on chemiluminescence of human neutrophil bursts and cell-free systems. *Pharmacology* **2009**, *83:* 110–115.

47. Kato A., Minoshima Y., Yamamoto J., Adachi I., Watson A.A., and Nash R.J. Protective Effects of Dietary Chamomile Tea on Diabetic Complications. *J. Agric. Food Chem.* **2008**, *56:* 8206–8211.

48.Tomić M.,Popović V., Petrović S., Stepanović-Petrović R., Micov A., Pavlović-Drobac M. and Couladis M. Antihyperalgesic and Antiedematous Activities of Bisabolol-Oxides-Rich Matricaria Oil in a Rat Model of Inflammation. *Phytother. Res.* **2014**, *28:* 759–766.

49. Morales-Yuste M., Morillas-Marquez F., Martın-Sanchez J., Valero-Lopez A., Navarro-Moll M.C. Activity of (-)α-bisabolol against Leishmania infantum promastigotes. *Phytomedicine* **2010**, *17:* 279–281.

50. De O. LeiteG., Leite L.H.I., De S. Sampaio R., Araruna M.K.A., De Menezes I.R.A., Da Costa J.G.M., Campos A.R. (-)-α-Bisabolol attenuates visceral nociception and inflammation in mice. *Fitoterapia* **2011**, *82:* 208–211.

51. De Oliveira Leite G., Inocêncio Leite L.A., De Souza Sampaioa R., Andrade Ararunaa M.K., Galvão Rodriguesa F.F., Alencar de Menezesa I.R., Martins da Costaa J.G., Rolim Campos A. Modulation of topical inflammation and visceral nociception by Vanillosmopsis arborea essential oil in mice. *Biomedicine & Preventive Nutrition* **2011**, *1:* 216–222.

52. Inocêncio Leite L.H., De Oliveira Leite G., Silva Coutinho T., Gonçalves de Sousa S.D., Souza Sampaio. R, Martins da Costa J.G., Alencar de Menezes I.R. and Rolim Campos A. Topical Antinociceptive Effect of Vanillosmopsis arborea Baker on Acute Corneal Pain in Mice. *eCAM* **2014**, *2014:* 1-6.

53. De O. Leite G., Fernandes C.N., Alencar de Menezes I.R., Martins da Costa J.G. and Campos A.R. Attenuation of visceral nociception by α-bisabolol in mice: investigation of mechanisms. Leite et al. *Org. Med. Chem. Lett.* **2012**, *2:* 18.

54. Maurya A.K., Singh M., Dubey V., Srivastava S., Luqman S. andBawankule D.U. α-(-)-bisabolol Reduces Pro-inflammatory Cytokine Production and Ameliorates Skin Inflammation. *Curr. Pharm. Biotechnol.* **2014**, *15:* 173-181.

55. De Siqueira R.J.B., Freire W.B.S., Vasconcelos-Silva A.A., Fonseca-Magalhães P.A., Lima F.J.B., Brito T.S., Mourão L.T.C., Ribeiro R.A., Lahlou S. and Magalhães P.J.C. In-vitro characterization of the pharmacological effects induced by (-)-α-

bisabolol in rat smooth muscle preparations. *Can. J. Physiol. Pharmacol.* **2012**, *90(1):* 23-35.

56. Roberts R.E., Allen S., Chang A.P.Y., Henderson H., Hobson G.C., Karania B., Morgan K.N., Pek A.S.Y., Raghvani K., Shee C.Y., Shikotra J., Street E., Abbas Z., Ellis K., Heer J.K. and Alexander S.P.H. Distinct mechanisms of relaxation to bioactive components from chamomile species in porcine isolated blood vessels. *Toxicol. Appl. Pharmacol.* **2013**, *272:* 797–805.

57. De Siqueira R.J.B., Ribeiro-Filho H.V., Freire R.S., Cosker F., Freire W.B.S., Vasconcelos-Silva A.A., Soares M.A., Lahlou S. and Magalhães P.J.C. (-)-α-Bisabolol inhibits preferentially electromechanical coupling on rat isolated arteries. *Vasc. Pharmacol.* **2014**, *63:* 37–45.

58. Cavalieri E., Bergamini C., Mariotto S., Leoni S., Perbellini L.,Darra E., Suzuki H., Fato R. and Lenaz G. Involvement of mitochondrial permeability transition pore opening in α-bisabolol induced apoptosis. *FEBS Journal* **2009**, *276:* 3990–4000.

59. Cavalieri E., Rigo A., Bonifacio M., Carcereri de Prati A., Guardalben E., Bergamini C., Fato R., Pizzolo G., Suzuki H. and Vinante F. Pro-apoptotic activity of α-bisabolol in preclinical models of primary human acute leukemia cells. *J. Transl. Med.* **2011**, *9:* 45.

60. Da Silva A.P., Martini M.V., De Oliveira C.M.A., Cunha S.,De Carvalho J.E., Ruiz A.L.T.G. and Da Silva C.C. Antitumor activity of (-)-α-bisabolol-based thiosemicarbazones against human tumor cell lines. *Eur. J. Med. Chem.* **2010**, *45:* 2987-2993.

61. Costarelli L., Malavolta M., Giacconi R., Cipriano C., Gasparini N., Tesei S., Pierpaoli S., Orlando F., Suzuki H., Perbellini L., Piacenza F., Emanuelli M., Mocchegiani E. In Vivo Effect of α-Bisabolol, a Nontoxic Sesquiterpene Alcohol, on the Induction of Spontaneous Mammary Tumors in HER-2/neu Transgenic Mice. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics* **2009**, *18(9):* 409-418(10).

62. Chen W., Hou J.,Yin Y., Jang J., Zheng Z., Fan H. and Zou G. α-Bisabolol induces dose- and time-dependent apoptosis in HepG2 cells via a Fas- and mitochondrial-related pathway, involves p53 and NFkB. *Biochemical Pharmacology* **2010**, *80:* 247–254.

63. Gomes-Carneiro M.R., Dias D.M.M., De-Oliveira A.C.A.X., Paumgartten F.R.J. Evaluation of mutagenic and antimutagenic activities of α-bisabolol in the Salmonella/microsome assay. *Mutat. Res.* **2005**, *585:* 105–112.

64. Alvarez-Gonzalez I., Uc-Artigas E., Moreno L.M. and Madrigal-Bujaidar E. Inhibitory effect of alpha-bisabolol on the genotoxic damage induced by daunorubicin in mouse. Abstracts. *Toxicol. Lett.* **2006**, *164:* 1–324.

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