CHEMICAL REVIEWS

REVIEW

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Biocatalytic Methods for the Synthesis of Enantioenriched Odor Active Compounds

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1. INTRODUCTION

The occurrence in Nature of the chiral units building up biomolecules in a precise enantiopure form is considered to be a chemical signature of Life,[1] an expression of that superior organization which is necessary to sustain vital processes. Deoxyribose and ribose sugars in the chains of DNA and RNA, and chiral α-amino acids in natural proteins exist only as single enantiomers with a well-defined absolute configuration. This stereochemical uniformity, often defined *homochirality*, is essential to allow those recognition and information processes without which Life can not exist. It is therefore evident that when chiral chemical compounds (i.e., drugs, flavors, fragrances, and agrochemicals) enter in relation with living beings to exert their effect, the efficacy of this interaction largely depends on their absolute configuration, because a stereoselective recognition process takes place.

The request for the really effective enantiomers of chiral compounds is growing increasingly in different fields of human activities, dealing either with pharmaceutically active ingredients or with odorous compounds.[2] The biological effects of *chiral pharmaceuticals* have been widely investigated: it has been established that chirality plays a fundamental role both in the pharmacokinetic and in the pharmacodynamic phase. The first one involves the absorption, the metabolic conversion and the excretion of the drug; the latter consists in the interaction of the bioactive agent with the molecular site of action (receptors, enzymes, etc.) in the target tissue, leading to the observed effect. The consequence is that one enantiomer may be therapeutically active (the "eutomer"), and the other one (the "distomer") may show no or toxic side effects. There are also enantiomers both showing independent beneficial therapeutic value.

1.1. Chiral Odorous Compounds

The same considerations can be applied to chiral odorous compounds. The investigation of their olfactory properties[3] has highlighted that enantiomers of *chiral odorants* (flavors or fragrances) can differ either in odor quality, that is, they can elicit different odor sensations, or in odor intensity. One enantiomer can be more pleasant or more potent than the other one. This is because of the mechanism[4] according to which odors are detected by human nose, based on the interaction of molecules with the olfactory receptors. These latter are seven transmembrane-helix proteins embedded in the plasma membrane of olfactory sensory neurons, which are located in the olfactory epithelium at the back of human nose. The interaction is controlled by the hydrogen bonds between the receptor protein and the polar functional groups of the odorant: these bonds orient the molecule within the binding site. The specificity of this interaction is due to the nonbonding through-space interactions that are responsible for the arrays of receptors that can be bound by a specific odorant. The interaction generates a signal, which is sent through the axon of the olfactory neuron to the corresponding glomerulus in the olfactory bulb located in the

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brain. The perception of thousands of odors by a few hundreds of olfactory receptors results from a combinatorial coding, in which one olfactory receptor recognizes multiple odorants and different odorants are recognized by different combinations of olfactory receptors. The activation of a certain pattern of glomerula is recognized as a specific odor by our brain. Being the mechanism of olfaction based on a molecular recognition, this process can in principle distinguish enantiomers, because it is performed by chiral proteins, made up by L-amino acids.

The physiological mechanism of olfaction is complex and not well understood, however we can give some insights on the mechanism of interactions of two enantiomers according to a very efficacious and explicative description recently proposed by Kraft and Mannschreck.[5] If one enantiomer is perfectly complementary to the binding pocket of the receptor, then the odor of the molecule should be due to that enantiomer, while the other one should be weak or odorless. If neither enantiomer is perfectly complementary to the receptor binding site, and both enantiomers interact with the same receptor but with different affinities, then both enantiomers possess qualitatively similar odors, but quantitatively different intensities, and this is the most common situation. If both enantiomers are not perfectly complementary to the binding site, and the stereogenic element is not differentiated by the receptors (i.e., the stereogenic element is situated outside the binding pocket), then both enantiomers are expected to elicit similar odors of similar, not very high intensity. The rarest case is when both enantiomers of an odorant are complementary to two different receptors and possess completely different odor profiles.

Molecules eliciting odor sensations can be distinguished into three categories: *flavors, fragrances, and pherormones.*

Flavors are of *natural* origin, or can be mixtures of nature-identical and artificial compounds. Natural flavors can be obtained from natural products by extraction procedures, or by synthetic processes which satisfy all the conditions to allow "natural labelling" according to vigent laws. Many flavors are chiral, and they are produced as enantioenriched compounds by Nature. Their pattern of enantiomeric distribution can be employed as fingerprints for the authenticity assessment of natural flavors in food and beverages. Natural bergamot flavor contains (*R*)-(−)-linalool and (*R*)-(−)-linalyl acetate with high enantiomeric excess, while (*R*)-(+)-γ-decalactone is the predominant enantiomer in peach, apricot and strawberry cultivars.[6]

Fragrances are largely compounded with *artificial* ingredients, but some of them are claimed as entirely natural. Artificial compounds can not be found in Nature and they have been synthesized by chemists in search of new odorous molecules. Most of commercial fragrances are chiral, with one or more stereogenic centers, and they are sold as mixtures of racemic stereoisomers. The analysis of the odor properties of the single enantiomers of chiral fragrances is receiving increasing attention, and it is aimed to the identification of the isomers which are effectively responsible for the odor of the fragrance. As a matter of fact, if only the odor active isomers are employed in commercial products, it is possible to elicit the same odor sensation by using a lower quantity of odorant. In this way the toxicological risks connected with the exposure to chemicals added to perfumed articles can be reduced. Recently, the primary fragrance companies have adopted a new politics for the benefit of consumer's health and for environment preservation, and they have started the industrial production of the first enantiomerically enriched fragrances, mainly for captive use. Paradisone (optically active methyl dihydrojasmonate), and Dextro Norlimbanol (optically active 1-(2,2,6-trimethylcyclohexyl)hexan-3-ol) are supplied by Firmenich, while Levosandol (optically active 2-ethyl-4-(2,2,3-trimethylcylopent-3-enyl)but-2-en-1ol) is produced by Takasago.

Pherormones are communication substances secreted by an individual and received by a second individual of the same species, in order to induce a specific reaction such as a special behavior or a developmental process. Their bioactivity depends on chirality, and Mori has identified at least ten different modalities[7] according to which this dependence is shown. For example, there are cases in which only one enantiomer is bioactive, and the opposite enantiomer inhibits the response to the pheromone; different enantiomers or diastereomers of the same substance can be employed by different species; one enantiomer can be active on males, while the other is active on females.

In this review, we will consider the preparation of enantioenriched chiral odorous compounds, in particular flavors and fragrance, by means of biocatalyzed techniques covering literature from 2000. For reference to previous works, see details in refs 3 and 8. Chiral pheromones will not be included: the topic has been extensively reviewed by K. Mori in recent years.[9]

As for natural flavors, the aim is to prepare enantioselectively the best isomer, that is, the natural one, according to methods which can be competitive with or even better than extractive procedures.

As for artificial fragrances, two are the synthetic challenges. The first one is the synthesis of all the stereoisomers of a chiral non-natural odorous compound to evaluate the odor properties of each stereoisomer and identify the real odor vector. The second and resulting need is the development of stereoselective syntheses to prepare the identified odor vector for practical use in commercial products.

1.2. Biocatalytic Methods

The term biocatalytic processes is usually employed to represent transformations of a substrate into a defined target product through one or several enzyme-catalyzed steps. Within this class of procedures it is possible to further distinguish *fermentations*, *biotransformations* and *enzyme-mediated reactions*.[10]

Fermentations and precursor fermentations are the transformations, respectively, of nutrients or raw materials (such as sugar, starch or methanol) and of defined educts (such as amino acids, and fatty acids) into more complex target products by means of living cell systems.

The term *biotransformation* properly indicates the conversion of defined substrates into desired target products using whole cell or resting cell systems. *Enzyme catalysis* is usually employed when crude extracts or partially purified enzyme are used to perform the desired conversion.

The limits between these areas are not strictly fixed: generally biotrasformations and enzyme catalysis are summarized under the term *biocatalysis*.

During recent years bioconversions are becoming key components in the toolbox available to the synthetic chemists for the synthesis of enantioenriched compounds, together with stereoselective chemocatalysis and physical separation techniques (fractional crystallization, chromatographic separation on chiral phases). The driving forces leading chemical industry toward biocatalysis are (i) great competition in an increasingly open market-oriented economy, promoting the search for high yield,

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selective, and shorter process routes; (ii) environmental concerns putting emphasis on the development of clean (or at least less polluting) procedures, characterized by low working temperatures and pressures, and waste reduction. Biocatalysis is best suited to satisfy all these requests.

In addition, an increasing demand of "natural and functional products" from consumers imposes to improve the production of "natural" flavors and bioactive ingredients, and, according to current European laws, flavouring substances obtained by enzymatic or microbiological processes from material of vegetable, animal, or microbiological origin can be labeled as "natural".

The choice between purified enzymes or whole cells is influenced by several factors: the type of reaction, whether there are cofactors to be regenerated or not, and the scale in which the biotrasformation has to be performed.

Integer cell systems can potentially protect the enzyme from shear forces and thus they might extend the enzyme activity half-life in stirred vessels.[11] It has also to be considered that the sequence of enzymatic reactions promoted by integer cell systems may be too complicated to be performed in vitro because it involves many enzymes and cofactors. The great advantage of whole cells is that the addition of cofactors is not required.

Isolated enzymes may be necessary or advantageous in some cases, such as when there are undesirable side reactions involving other enzymatic systems or product degradation, or when the enzyme of interest is commercially available. On the other hand, enzyme purification is often tedious, time-consuming, and expensive.

Most categories of enzymes have been found to be active in organic solvents,[12] not just lipases but also proteases, dehydrogenases, peroxidases, and several others.[10] The main advantages obtained when biocatalitic processes are performed in organic solvents rather than in water are the following ones: (i) increased solubility of nonpolar substrates and products, with a marked enhancement of overall reaction rates; (ii) reversal of thermodynamic equilibrium in favor of synthesis over hydrolysis, allowing the occurrence of reactions usually not favored in aqueous solutions (e.g., transesterification, thioesterification, aminolysis); (iii) changes in the enantioselectivity of the reaction when one organic solvent is changed to another; iv) avoidance of unwanted water-dependent side reactions, especially degradation of common organic reagents; v) elimination of microbial contamination in the reaction mixture.[12b,c]

When either the substrate or the product exhibit inhibition effects on the biocatalyzed reaction or toxicity toward the enzyme itself, their confinement may be necessary. This can be achieved, for example, with the so-called technique of extractive biocatalysis,[13,12d−12g] based on the strategy of integrating the biotransformation with an extractive step, in order to promptly remove the substrate by using either liquid extractants or solid adsorbers, as soon as it is generated in the reaction medium. Organic solvents can be employed in two-phase systems with water,[12d−g] where they act as a substrate reservoir and a product extractant. Other successful process designs include the use of aqueous two-phase systems[13c−e] and the employment of the in situ substrate feed product removal procedure based on the use of adsorbing resins.[13f−h] Specific papers devoted to the successful applications of these procedures are reported in ref 13.

Emerging non conventional media for biocatalytic reactions are ionic liquids,[14] which are considered as environmentally friendly alternatives to volatile, flammable organic solvents. It has been shown that in these solvents many enzymes exhibit very high thermal and operational stability, and excellent selectivity, including substrate, regio- and enantioselectivity.[14a] There are two possible ways of application of ionic liquids in biocatalytic reactions: the hydrophobic ones are employed as substitutes for organic solvents in two-phase systems with water, the hydrophilic ones are used in aqueous two-phase systems.[14b]

In this review, we will consider the application of biocatalyzed techniques, either biotrasformations with whole cell systems or enzyme-mediated reactions, to the preparation of enantioenriched flavors and fragrances, with a distinction between *resolution procedures* and *stereoselective reactions.*

Fermentation processes for the production of natural aromatic molecules, either by de novo syntheses based on normal microbial metabolism or by bioconversion of appropriate precursors compounds (phenyl propanoids, amino acids, and fatty acids) will not be discussed within the topics of this work. Recents reviews of these subjects are reported in ref 15.

2. CHIRAL FRAGRANCES AND FLAVORS BY BIOCATALYZED KINETIC RESOLUTION PROCESSES

In a kinetic resolution process a chiral catalyst or reagent is employed to promote the selective reaction of one enantiomer over the other, to give a mixture of enantioenriched starting material and product, taking advantage of the fact that the two enantiomers are characterized by different reaction rates.[16]

The theoretical yields for such resolutions can not be higher than 50%. However, yields can be improved if the undesired enantiomer can be racemised or somehow converted into the other one. In some cases, it is possible to induce substrate racemisation under the conditions of kinetic resolution, thus a complete conversion of the racemic mixture into one enantiomer is possible. Such processes are known as dynamic kinetic resolutions.[16] Acyl transfer reactions have been widely employed in the context of kinetic resolution, and a wide range of effective biocatalysts (amidases, proteases, esterases, lipases) have been identified. They have been successfully applied for the preparation of enantioenriched amino acids, amides, alcohols, and esters.

Lipases are the most widespread employed. They are readily available in large quantities, because many of them can be produced in high yields by gene expression in an appropriate microorganism, such as a fungi, yeast or bacteria. They do not require cofactors nor catalyze side reactions, and remain active in organic solvents. They catalyze hydrolysis or acylation reactions under mild conditions (common organic solvents, atmospheric pressure, and, usually, room temperature). The handling of lipases is safe for the operator and the environment, and no specific reaction apparatus is needed. In most cases, the enzyme can be recovered and employed again, without loss of activity.

The application of *biocatalytic kinetic resolutions* in the field of chiral fragrances and flavors is herein presented making a distinction between *natural odorous compounds* and *artificial odorants.*

2.1. Natural Odorous Compounds

2.1.1. Menthol, *p*-Menthane, and *p*-Menthene Derivatives. There are still some practical problems in the development of an industrial method for the preparation of natural L-(−)-menthol (**1**, Figure 1) based on the use of biocatalysts. It has been experimented that the esterification or transesterification of racemic menthol in organic solvents mediated by common

commercial lipases or esterases[17] is generally characterized by low esterification rate, and modest enantioselectivity. Accurate screening of enzymes to search systems with excellent selectivity, high specific activity and strong tolerance against high substrate/product concentration is important to satisfy the request of a biocatalyzed industrial process for L-menthol under mild conditions. Some interesting attempts to face this topic are herein presented.

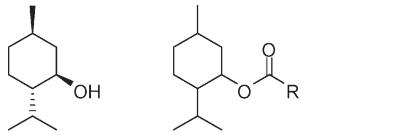
Rac-menthyl benzoate (**2**, Figure 1) is the starting material for the production of natural (−)-menthol in a scale of a thousand tons per year according to the Haarmann-Reimer industrial process.[18] This procedure consists in the preferential crystallization of D-**2**, which is recycled by aromatization and subsequent hydrogenation, and in the recovery of the L-enantiomer, which is submitted to acidic hydrolysis to afford enantiopure L-menthol.

Schmid et al.[19] developed a method to obtain L-menthol by lipase-mediated kinetic resolution of the key industrial intermediate rac-**2** (Figure 1). A screening of 30 commercially available hydrolases showed that some of these enzymes could catalyze the hydrolysis of racemic menthyl acetate and the acylation of racemic menthol with high enantioselectivity. However, only one lipase preparation from *Candida rugosa* (Lipase AY Amano 30) was able to hydrolyze racemic menthyl benzoate, but with an enantioselectivity (E^{20} = 15), which did not allow industrial application. The authors hypothesized that the lack in enantioselectivity was due to opposite selectivity of the various biocatalysts present in the commercial enzyme preparation, so they performed the functional expression of the most prominent *Candida rugosa* isoenzyme LIP1 in *Pichia pastoris* after creating a synthetic gene. They were able to produce a recombinant CRL in large amounts and high purity, composed only by the CRL LIP1 isoenzyme, which exhibited high enantioselectivity (E = 100) in the kinetic resolution of **2**. After 8 h at 40 °C nearly 50% L-(−)-menthol was obtained with an enantiomeric excess (*ee*) value of 99.9%. Recombinant CRL also exhibited high enantioselectivity (E = 100) in the kinetic resolution of menthyl acetate (**3**) and menthyl isovalerate (**4**). No conversion was observed for menthyl anthranilate (**5**): this might be attributed to the presence of the amino functionality in ortho position to the carboxylic group, causing steric hindrance or negative electronic effects.

Recently, a new stereospecific L-menthyl ester hydrolase-producing strain (no. ECU0554) was isolated[21] from soil samples and identified as *Bacillus subtilis*. This latter showed high hydrolytic activity, excellent enantioselectivity and strong substrate tolerance for the production of optically active L-menthol. Several microbial strains were isolated by the authors[21] from soil and those capable of hydrolyzing rac-menthyl acetate (**3**) were rapidly screened. Eight strains showing high conversion (>25%) and excellent optical purity (>94% *ee*) for L-menthol were selected from 265 active ones. The substrate tolerance of these 8 candidate strains was investigated using high concentrations of rac-menthyl acetate. ECU0554 resulted to be the most tolerable strain against a high substrate concentration, it was selected as the best enzyme producer, and it was subsequently identified as *Bacillus subtilis*.

Racemic menthyl esters **2**, **3**, and **6**−**8** (Figure 2) with different acyl groups were synthesized in order to find the optimal substrate for the enzyme in sodium phosphate buffer (pH 7.2, 200 mM) at 30 °C. A remarkable dependence of the hydrolytic activity of *Bacillus subtilis* on the acyl group of the menthyl ester was observed: the increase in the acyl group size led to a dramatic decrease of the enzyme activity.

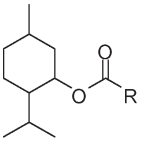
Bacillus subtilis exhibited not only a high hydrolytic activity, but also the best enantioselectivity (E > 200) in the resolution of



L-(-)-**1**

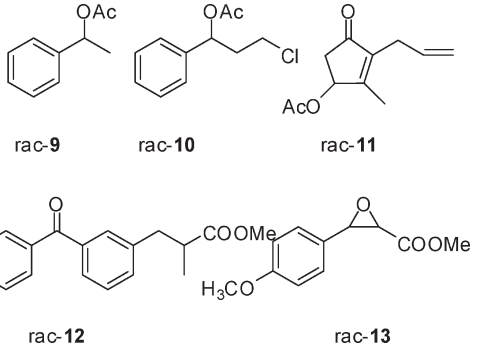
rac-**2** R = Ph
rac-**3** R = CH_3
rac-**4** R = $CH_2CH(CH_3)_2$
rac-**5** R = *o*-aminophenyl

Figure 1



rac-**2** R = Ph
rac-**3** R = CH_3
rac-**6** R = CH_2Cl
rac-**7** R = $CH_2CH_2CH_3$
rac-**8** R = $(CH_2)_2COOH$

Figure 2



rac-**9** rac-**10** rac-**11**

rac-**12** rac-**13**

Figure 3

rac-menthyl acetate (**3**), so this latter was selected as a substrate for the further investigation work.

Several other racemic acetates (**9**−**13**, Figure 3) were also examined using *Bacillus subtilis*, in sodium phosphate buffer (pH 7.2, 200 mM) at 30 °C. The enzyme showed excellent hydrolytic activity (up to 46% conversion within 3 h) and modest enantiomeric excess of (*R*)-product (*ee* = 85%) for racemic compound **11**. Substrates **9** and **10** were converted at moderate rates (40% and 51% conversions within 5 and 12 h) and with poor enantioselectivity (E = 7, 11). No activity was detected for esters **12** and **13**. Thus, the size of the substrate, hindering or preventing its movement into the active site of the enzyme, was found to have a strong influence on the activity of *Bacillus subtilis*.

The effects of pH and temperature on *Bacillus subtilis*-mediated resolution of rac-menthyl acetate were carefully investigated. The activity was low at pH <6.5 or pH > 8.0, and the optimum pH for rac-menthyl acetate was 7.0. The conversion of *Bacillus subtilis*-mediated hydrolysis increased from 32% to 47% with the increase of temperature from 20 to 30 °C, and it did not significantly change from 30 to 40 °C. It rapidly dropped with further increase of the temperature up to 50 °C. Temperature did

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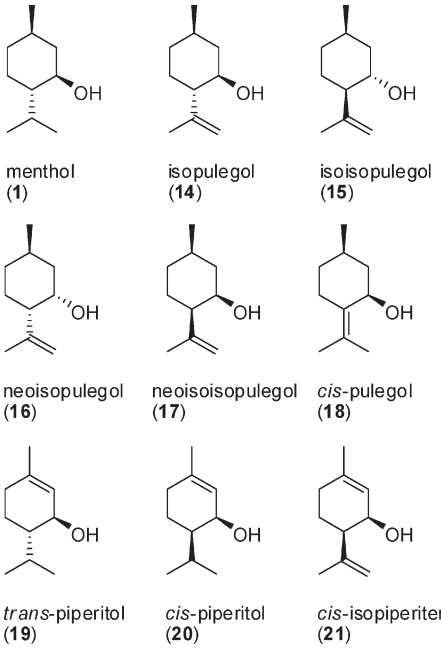

Figure 4

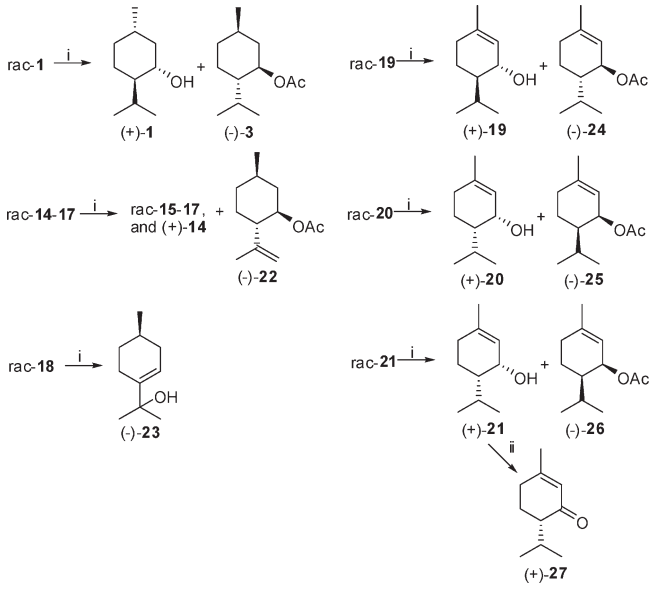

Figure 5. Reation conditions: (i) lipase, MTBE, vinyl acetate; (ii) MnO_2, CH_2Cl_2.

not affect the enantioselectivity remarkably: pH 7.0 and 30 °C were then established as optimal reactions conditions.

Other commercial enzymes were investigated for the same reaction in order to make a comparison with the newly isolated *Bacillus subtilis*. Lipase AS exhibited high hydrolytic activity but low enantiomeric excess of product (*ee* = 52.6%), Lipase MY and CRL exhibited only moderate hydrolytic activities, while other enzymes showed low or even no hydrolytic activity (<10% conversion) toward rac-menthyl acetate. In contrast, *Bacillus subtilis* showed high activity with 49% conversion within 3 h and a much higher enantioselectivity ($E > 200$).

A high substrate/product concentration is beneficial for practical application of an enzymatic process because it will reduce the cost of product isolation. The effect of substrate concentration on the product formation was investigated at a fixed substrate to enzyme ratio: a high substrate concentration up to 500 mM did not produce a decrease of the enzyme activity, thus *Bacillus subtilis* was established to be tolerant against a pretty high concentration of the substrate or the product. More importantly, the optical purity of the produced L-menthol was still above 97% *ee* even at a very high substrate concentration. It was never reported in previous studies that an enzyme or microorganism could tolerate such a high substrate concentration in the preparation of L-menthol via enantioselective hydrolysis of racemic menthyl esters.

To further evaluate the potential of *Bacillus subtilis* for practical use, a preparative scale resolution of rac-menthyl acetate was performed on a 3-g scale. A total amount of 500 mg crude enzyme powder was employed. After 9 h and a conversion of 50%, 39% of the unreacted menthyl acetate, and 42% of enantiopure L-menthol (98% *ee*) were recovered.

A further development of this enzyme will be to immobilize it, in order to increase its long-term operational stability, and to simplify its recovery and reuse. These are indeed fundamental aspects to allow a practical industrial application.

Beside menthol there are other 3-oxygenated monoterpenes of *p*-menthane family which are important natural compounds, and are extensively used in flavor and fragrance industry. Their enantiomeric and diastereoisomeric compositions are essential to establish their organoleptic properties.[3] It is well-known that among the saturated *p*-menthan-3-ol isomers (−)-menthol shows the best organoleptic performance, but recent studies have shown that, while the mixture of isopulegol isomers **14**−**17** (Figure 4) has been used as a perfume ingredient, its main component (−)-isopulegol (**14**) is odorless[22] and can be employed as a cooling agent. The lipase-mediated resolution of compounds **1** and **14**−**21** (Figure 4) was investigated by Serra et al.[23]

Racemic menthol **1** is commercially available, and racemic **14**−**21** were prepared by modifications of known procedures. Racemic *p*-menthan-3-ols were treated with vinyl acetate in *t*-butyl methyl ether (MTBE) solution in the presence of lipases, and the results are herein summarized (Figure 5). *Porcine pancreatic* lipase did not catalyze the acetylation reaction of any of these substrates used, while both *Candida rugosa* lipase and lipase PS were found to be good catalysts for this reaction. Racemic menthol **1** was converted by CRL and lipase PS into (−)-menthyl acetate ((−)-**3**) with good enantioselectivity, though the *ee* of the recovered alcohol was moderate. Hydrolysis of (−)-**3** gave (−)-menthol in good accord with the processes described in the patent literature. The mixture of the isopulegol isomers **14**−**17** was acetylated very slowly and both CRL and lipase PS afforded only (−)-isopulegol acetate **22** with good to excellent enantioselectivity and complete diastereoselectivity. Hydrolysis of acetate **22** gave (−)-isopulegol, the enantiomer appreciated for its cooling effect, with high enantiomeric purity, in a very simple procedure without complex chemical manipulations. Racemic **18** was slowly converted into its acetate but with concomitant isomerization to tertiary alcohol **23** and its acetate. Isolation gave only alcohol **23** with very low enantiomeric enrichment (8% *ee* by PS lipase and 0% *ee* by CRL). *Trans*-piperitol **19** and *cis*-piperitol **20** were converted by lipase PS in enantiopure acetate (−)-**24** and (−)-**25**, respectively, whereas the unreacted (+)-**19** and (+)-**20** showed good enantiomeric purity. The same reactions performed with CRL gave analogous results although with inferior selectivity. Racemic isopiperitenol **21** was converted by CRL and lipase PS to acetate (-)-**26** with

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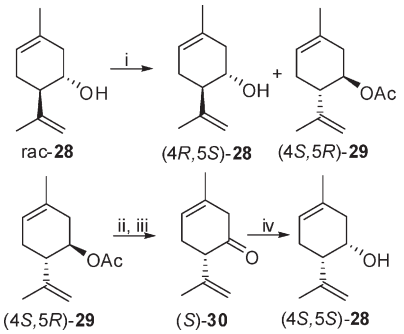

Figure 6. Reaction conditions: (i) lipase PS, MTBE, vinyl acetate; (ii) K_2CO_3, MeOH; (iii) Swern oxidation; (iv) L-selectride, THF.

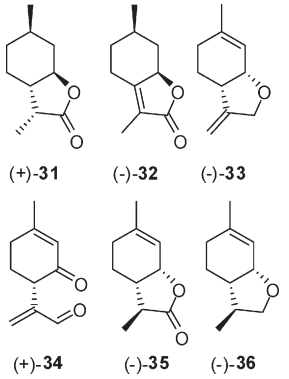

Figure 7

good to excellent enantioselectivity (89 and 99% *ee*, respectively) whereas recovered alcohol (+)-**21** showed moderate to good enantioenrichment by CRL (55% *ee*) and lipase PS (92% *ee*).

The starting material rac-**21** was not a single diastereoisomer (90% of cis derivative): the isolated products showed similar values of diastereoisomeric excess (de), confirming that both lipases were not diastereoselective. The absolute stereochemistry of (+)- and (−)-*cis*-isopiperitenol was assigned by converting (+)-**21** in (+)-isopiperitenone **27** by MnO_2 oxidation.

In a further development of this work, the lipase-catalyzed kinetic resolution of rac-**28** (Figure 6) was also investigated (Lipase PS, MTBE, vinyl acetate).[24] After 2 weeks, acetate (4*S*,5*R*)-**29** (*ee* 98%) and unreacted alcohol (4*R*,5*S*)-**28** (*ee* = 81%) were isolated by column chromatography. Saponification of (4*S*,5*R*)-**29** (K_2CO_3, MeOH) gave natural occurring[25] (4*S*,5*R*)-**28**. This latter was also submitted to Swern oxidation, followed by reduction of the ketone (*S*)-**30** with L-selectride to afford the other natural diastereoisomer[25] (4*S*,5*S*)-**28** without any loss of enantiomeric purity.

There are also monoterpenes of the *p*-menthane family showing O-functionalization in both positions 3 and 9 of the *p*-menthane framework, and most of them are well-known as flavoring ingredients. Some of these compounds were prepared[26,27] in enantioenriched form by lipase-mediated transesterification (Figure 7): 3-hydroxy-*p*-menthan-9-oic acid lactone **31** (found in peppermint oil),[28] mintlactone **32** ((−)-**32** is contained in peppermint oil,[28] while (+)-**32** is a key aroma components of the wood of *Bursera graveolens*[29]), the dienic compound **33** ((−)-**33** is the natural occurring isomer[30]), the structurally related pheromone vesperal **34** ((+)-**34** is the active enantiomer),[31] the odorants wine lactone

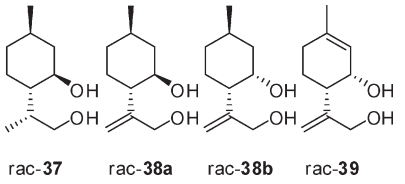

Figure 8

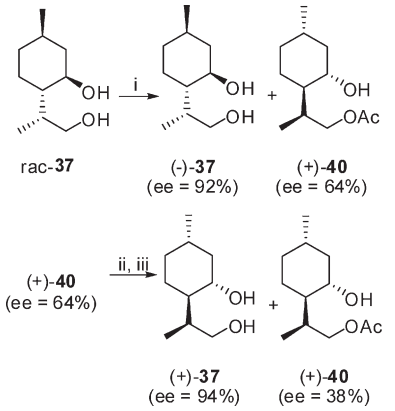

Figure 9. Reaction conditions: (i) lipase PS, MTBE, vinyl acetate; (ii) KOH, MeOH; (iii) CCL, MTBE, vinyl acetate.

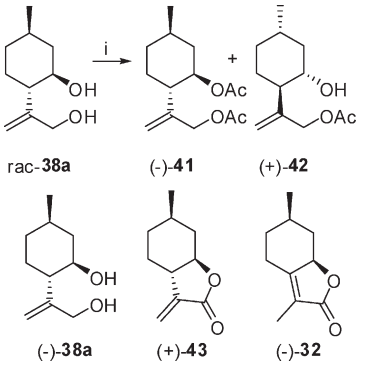

Figure 10. Reaction conditions: (i) lipase PS, MTBE, vinyl acetate.

35, and dill ether **36**. Wine lactone **35** has been recognized as a key flavor in different white wines,[32] and it has been found in orange juice and black pepper.[33] The eight possible isomers of wine lactone were prepared by Guth[34] and the olfactory evaluation showed that natural (−)-**35** is the most-powerful isomer with an odor threshold <0.04 pg/L, whereas the weakest isomers show a threshold >106 pg/L. Similar is the case of dill ether (**36**), the most-important constituent of dill essential oil.[35] The preparation of its eight isomeric forms[36] allowed to establish that (−)-**36** shows a high odor-activity value, and it represents the impact odor component of dill-herb flavor.

It was devised that *p*-menthane-3,9-diols **37**−**39**[26,27] (Figure 8), easily prepared in racemic form from low-cost industrial products, could be used as staring materials for the preparation of *p*-menthane lactones and ethers by known and straightforward methods, involving enzyme-mediated resolution.

Diastereoisomerically pure diols, rac-**37**, rac-**38a**, rac-**38b**, and rac-**39** were prepared by modification of reported procedures,

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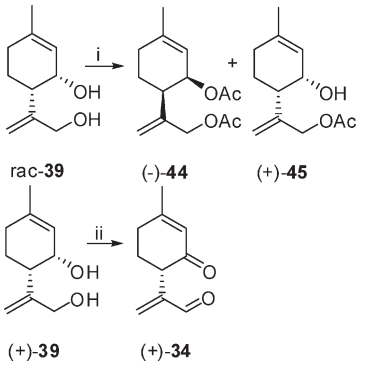

Figure 11. Reaction conditions: (i) lipase PS, MTBE, vinyl acetate; (ii) Dess−Martin periodinane oxidation.

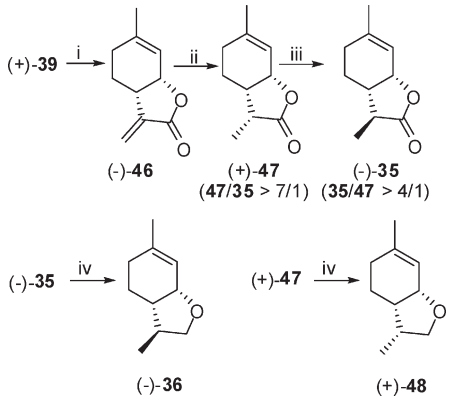

Figure 12. Reaction conditions: (i) BAIB/TEMPO; (ii) Mg, MeOH; (iii) *t*-BuOK, *t*-BuOH; (iv) $LiAlH_4$.

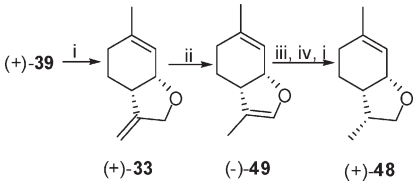

Figure 13. Reaction conditions: (i) 5% HCl, Et_2O; (ii) rhodium hydride catalyst; (iii) 70% $HClO_4$,THF/H_2O; (iv) $NaBH_4$.

and submitted to enzyme-mediated transesterification with vinyl acetate in MTBE solution in the presence of lipase PS, CCL, and PPL.

All the lipases mediated the acetylation of the primary alcohol functions, but when these groups were allylic (rac-**38a**, rac-**38b**, and rac-**39**), the reaction was very fast (less than 36 h), and no enantioselectivity was observed in this step. On the contrary saturated diol rac-**37** reacted slowly, and after four days ca. 50% of the starting diol was acetylated. The enantioselectivity of this step was dependent on the kind of lipase used. Lipase PS showed higher selectivity with a preference for the conversion of the (+)-isomer. PPL showed the lowest selectivity, whereas CCL, converted the (−)-isomer, though with poor selectivity. The monoacetates were not further acetylated by these enzymes, even after long reaction time.

Compounds rac-**38a** and rac-**39** were slowly converted into the enantiomerically pure diacetates (99% *ee*) and to the enantioenriched (92 and 94% *ee*, respectively) monoacetylated derivatives only when lipase PS was used as a catalyst. Compound rac-**38b**, which differed from **38a** only for the *cis* relative configuration at C(3) and C(4), was not converted by any of the enzymes used.

The results of these enzymatic transformations were employed to develop a large-scale method for the preparation of all the enantiomeric forms of diols **37**, **38a**, and **39**, which were then converted into natural products **31**−**36**.

Rac-**37** was treated with lipase PS (MTBE, vinyl acetate) (Figure 9) allowing the reaction to proceed to >50% conversion, to afford unreacted diol (−)-**37** with satisfactory enantiomer purity (92% *ee*), and acetate (+)-**40** showing lower purity (64% *ee*). This latter was hydrolyzed and the crude diol was submitted to CCL-mediated acetylation forcing again the reaction to >50% of conversion. The isolation procedure provided, besides (+)-**40** (38% *ee*), the unreacted diol (+)-**37** with good *ee* (94% *ee*). The oxidation of diols (+)- and (−)-**37** by means of $KMnO_4$/$CuSO_4 \cdot 5H_2O$ in CH_2Cl_2 solution afforded natural lactone (−)-**31** and its enantiomer (+)-**31**, respectively.

Treatment of rac-**38a** (Figure 10) with lipase PS (MTBE, vinyl acetate) gave diacetate (−)-**41** (99% *ee*) and monoacetate (+)-**42** (92% *ee*). Thus, KOH hydrolysis of the acetate moieties provided (−)- and (+)-**38a**, respectively, which were converted into *trans-p*-menthene lactones **43** by a known procedure. The isomerization reaction of the exocyclic C═C bond of **43** was carefully investigated in order to obtain the enantiomeric forms of mintlactone **32**. The authors found that rhodium(I) hydride complex $[RhH(Ph_3P)_4]$ was an efficient catalyst for this kind of isomerization, and it enabled the conversion of (+)- and (−)-**43** into pure (−)- and (+)-mintlactone **32**, respectively, in good chemical yields.

Treatment of rac-**39** (Figure 11) with lipase PS (MTBE, vinyl acetate) gave diacetate (−)-**44** (99% *ee*) and monoacetate (+)-**45** (94% *ee*). KOH saponification provided (−)- and (+)-**39**, respectively. The reaction of the latter diols with a catalytic amount of 5% aqueous HCl solution in Et_2O as a solvent gave ethers (+)- and (−)-**33**, respectively, in very good yields.

The absolute configurations of diols (+)- and (−)-**39** and ether (+)- and (−)-**33** were determined by chemical correlation, taking advantage of the oxidation of (+)- and (−)-**39** with Dess−Martin periodinane to (+)-(*S*)-vesperal ((+)-**34**) and (−)-(*R*)-vesperal ((−)-**34**), respectively (Figure 11).

The enantiomers of diol **39** were employed also for the preparation of odor active isomers of wine lactone and dill ether. The enantiomerically pure (+)- and (−)-**39** were oxidized by reaction with BAIB/TEMPO to afford enantiomerically pure (−)- and (+)-**46** (Figure 12). Reduction of the conjugated C═C bond by means of Mg in MeOH afforded epi-wine lactones (+)- and (−)-**47**, which were then submitted to basic epimerization by treatment with *t*-BuOK in *t*-BuOH to give mixtures enriched in the enantiomers of wine lactone (**35**/**47** >4:1). (−)- and (+)-**35** could be easily purified by column chromatography.

Compounds (−)- and (+)-**35** were reduced by treatment with $LiAlH_4$, and the reaction mixture was quenched directly with an excess of diluted aq. HCl solution (Figure 12). After isolation, natural dill ether (−)-**36**, and its enantiomer (+)-**36** were obtained in chemically and isomerically pure form.

The same procedure was applied to (+)- and (−)-**47** to afford pure epi-dill ether (+)- and (−)-**48**, respectively.

A more-straightforward approach to (+)- and (−)-**48** was also developed (Figure 13). Diols (+)- and (−)-**39** were transformed

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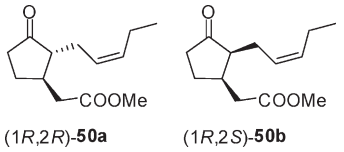

Figure 14

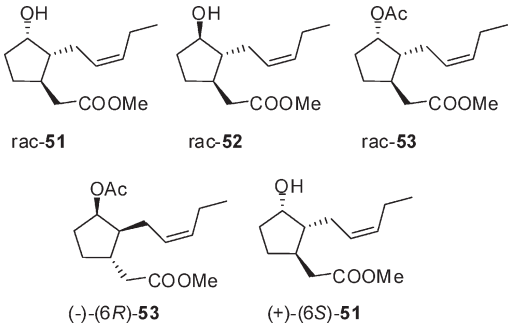

Figure 15

into ethers (−)- and (+)-**33**; isomerization promoted by a rhodium hydride catalyst gave enol ethers (−)- and (+)-**49**, respectively, whose activated C(8)═C(9) double bond was selectively reduced by means of a one pot procedure. This latter involved a hydration step in THF/water solution with a catalytic amount of $HClO_4$, followed by $NaBH_4$ reduction, and cyclization promoted by quenching the reduction mixture with HCl solution. Epi-dill ethers (+)- and (−)-**48** were thus obtained according to a highly diastereoselective procedure in 93 and 94% *de*, respectively, without any chromatographic separation.

2.1.2. Methyl Jasmonate and Methyl Epijasmonate. (−)-(1*R*,2*R*)-Methyl jasmonate ((1*R*,2*R*)-**50a**) and its diastereoisomer (+)-(1*R*,2*S*)-methyl epijasmonate ((1*R*,2*S*)-**50b**) (Figure 14) occur in nature in the proportions of 97:3 as the odorous principle of jasmin flower oil (*Jasminium grandiflorum* L.),[37] whereas their enantiomers are nearly odorless. The odor thresholds of all stereoisomers of methyl jasmonate were determined by Acree et al. in 1985[38] in ethanol solution, according to the triangular text procedure: commercial mixture 90 ng/mL; rac-**50a** 5700 ng/mL; rac-**50b** 13 ng/mL; (1*R*,2*R*)-**50a** >70 ng/mL, (1*R*,2*S*)-**50b** 3 ng/mL.

Conpound (1*R*,2*R*)-**50a** was prepared in enantiopure form by lipase-catalyzed resolution of rac-methyl 7-epicucurbate (**51**) (Figure 15).[39]

Commercially available methyl jasmonate (Jasmoneige, rac-**50** (trans/cis = 20:1)) was treated with DBU in Et_2O to increase the content of the trans-isomer up to >99%, then it was reduced with $NaBH_4$ to afford two diastereomeric alcohols, rac-**51** and rac-**52** (Figure 15). These latter derivatives were separated by column chromatography and submitted separately to enzyme-catalyzed resolution. Both transesterification of rac-**52** in the presence of vinyl acetate or vinyl chloroacetate ($E \approx 2$), and hydrolysis of the corresponding *O*-acetyl derivative (E up to 3.6) were characterized by modest enantioselectivity with all of the enzymes.

The reaction of rac-**51** with vinyl acetate catalyzed by lipase P (Amano) occurred with good selectivity (E = 370), to afford acetate (−)-(6*R*)-**53** (*ee* = 98.6%) and alcohol (+)-(6*S*)-**51** (*ee* = 99.1%). The hydrolysis of the corresponding acetate rac-**53** occurred with (E = 41) in the presence of Lipase P (Amano).

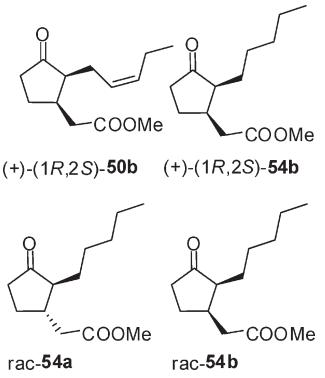

Figure 16

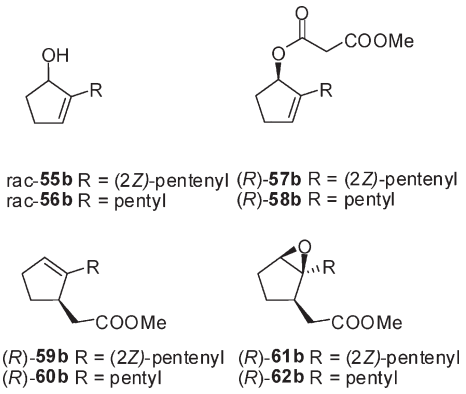

Figure 17

Compound **52**, which could not be employed for efficient kinetic resolution, was converted into rac-**53** by configuration inversion: the combined samples of rac-**53** were submitted to enzymatic hydrolysis. Oxidation of (+)-(6*S*)-**51** afforded the natural enantiomer of methyl jasmonate, while (−)-(6*R*)-**53** was converted into (+)-methyl jasmonate.

Fehr and Galindo reported[40] an efficient, highly enantio- and diastereoselective preparation of the natural enantiomer of methyl epijasmonate (+)-**50b**, in which the optical activation step was based on a lipase - catalyzed kinetic resolution. The same procedure was applied also to obtain (+)-**54b** (Figure 16), the odor active stereoisomer[41] of methyl dihydrojasmonate.

This dihydro derivative was prepared in the course of the structure elucidation of methyl jasmonate, and it was found to show a very pleasant odor. It soon became widely employed in perfumery, with the commercial name of Hedione, a mixture of 90% of rac-**54a** and 10% of the much more powerful diastereoisomer rac-**54b**.

Racemic alcohols **55b** and **56b** (Figure 17) were treated separately with dimethyl malonate at 40 °C under reduced pressure with catalytic amounts of Novozym 435 (immobilized *Candida antarctica* from Novo Nordisk) in the presence of $KHCO_3$ (5 mol %), to afford the corresponding malonates (*R*)-**57b** and (*R*)-**58b** with high enantioselectivity (97 - 100% *ee*) and almost 50% conversion. The unreacted alcohols, (*S*)-**55b** and (*S*)-**56b**, could be racemized under acidic conditions and recycled, to further improve the efficiency of this process. Derivatives (*R*)-**57b** and (*R*)-**58b** were converted by reaction with NaH and trimethylsilyl chloride into the corresponding labile ketene acetals, which

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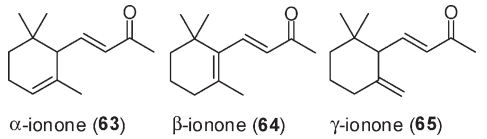

Figure 18

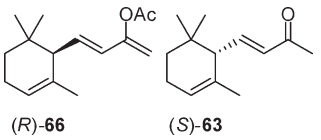

Figure 19

underwent Claisen rearrangement when heated in THF at 50−65 °C, to give methyl esters (*R*)-**59b** and (*R*)-**60b**, respectively. Epoxidation with trifluoroperacetic acid occurred with nearly complete diastereoselectivity, and syn epoxides **61b** and **62b** could be obtained. These derivatives were submitted to acid catalyzed Lewis rearrangement ($BF_3 \cdot OEt_2$) to give (+)-**50b** (*de* = 88%, *ee* = 97%) and (+)-**54b** (*de* = 96%, *ee* = 100%).

2.1.3. Ionones and Derivatives. In 1893 Tiemann and Krüger prepared[42] a mixture of isomeric ionones (**63**−**65**, Figure 18) during their investigations of the odorous principle of violets. Later on it was shown that in *Viola odorata* Linnaeus α-ionone was present in a single enantiomeric form,[43] which was assigned the (*R*) absolute configuration through correlation with manool.[44] α- and *β*-Ionone (**63**, **64**) were also found to make up almost 57% of the headspace of violets in bloom.[45] The odor properties of the enantiomers of ionones is a critical topic: (*R*)- and (*S*)-α-ionone, for example, have been differently evaluated by different perfumers. For an accurate discussion on this matter, see ref 46. The enzyme mediate preparation of the enantiomers of α- and *γ* ionones, and of the corresponding dihydroderivatives has been extensively described and reviewed in the past decade.[3,8]

Takasago has recently patented a biocatalyzed method for the preparation of (*S*)-ionone.[47] The (*S*)-enantiomer is described in this work to exhibit a *unique and strong odor, with fresh juicy green flavor, including a wood-like, cedarwood-like, raspberry-like, and β-like tonalities.*

Rac-α-ionone (**63**) was treated with lithium diisopropylamide in THF at −10 °C to afford the corresponding enolate ion, which was then reacted with acetic anhydride, in order to prepare rac-α-ionone enol acetate (**66**, Figure 19). This latter was submitted to enzyme-mediated hydrolysis in diisopropyl ether solution, in the presence of an acetate buffer (pH = 4.6), using lipase from *Candida antarctica* as a catalyst. After 8 h at 35 °C, a mixture of (*R*)-ionone enol acetate ((*R*)-**66**) and (*S*)-α-ionone was obtained. Distillation allowed the recovery of (*S*)-α-ionone showing *ee* = 66%. The enantiomeric purity of the compound was increased to 95% by submitting the sample again to the same procedure, that is, conversion into the enol acetate and enzymatic hydrolysis.

Dehydrovomifoliol (**67**) and 8,9-dehydrotheaspirone (**68**) (Figure 20) are norterpenoid compounds structurally related to α-ionone. (+)-Dehydrovomifoliol **67** is a natural compound[48] but its relevance is due to its use as a precursor in the well established synthesis of (+)-abscisic acid.[49] Dehydrotheaspirone **68** has recently received increasing attention after its isolation from tobacco,[50] Riesling wine,[51] nectarines,[52] honey,[53] and *Reseda odorata* flowers.[54] Their enantiomeric forms were prepared[55] by kinetic resolutions of diols **69** and **70**.

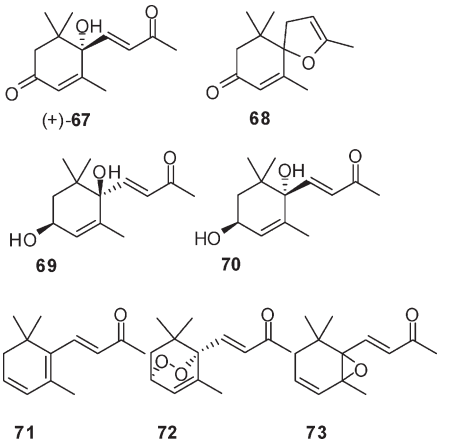

Figure 20

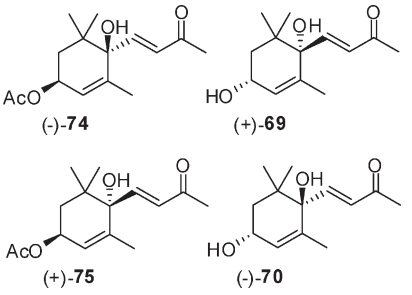

Figure 21

Easily available 3,4-dehydroxy-*β*-ionone (**71**) was treated with oxygen and visible light in the presence of rose bengal as a photosensitizer to provide the stable peroxyderivative **72**, which was reduced with thiourea to give *cis*-3,6-dihydroxy-α-ionone **69**. Oxidation of **71** with *m*-chloroperbenzoic acid (MCPBA) afforded the epoxy-derivative **73**, which rearranged to give a 5:1 mixture of diols **70** and **69**, respectively: crystallization of the crude reaction mixture afforded pure diol **70**.

Kinetic resolution was investigated with vinyl acetate in MTBE solution in the presence of lipases (PS, CRL, and PPL). All the lipases catalyzed the acetylation of the secondary alcohol function regioselectively. Acetylation of *cis*-diol **69** affording (3*S*,6*R*)-(−)-**74** (Figure 21) occurred with an enantioselectivity that ranged from very low for PPL and CRL to moderate values for lipase PS. PPL and lipase PS mediated the conversion of *trans*-diol **70** into (3*S*,6*S*)-(+)-**75** with very low and modest enantioselectivity, respectively. CRL catalyzed the acetylation of diol **70** with opposite enantioselectivity affording (3*R*,6*R*)-(−)-**75**, although with low enantioselectivity. However, both the acetates **74** and **75** resulted to be crystalline and their *ee* could be increased by crystallization. Thus, lipase PS-mediated acetylation of **69** and **70** afforded acetates (−)-**74** and (+)-**75**, and unreacted diols (+)-**69** and (−)-**70**, respectively. After chromatographic separation, the latter diols were converted chemically into the corresponding acetates (+)-**74** and (−)-**75**, respectively. The four acetates were brought to high values of *ee* (96−98%) by repeated crystallizations from hexane/ethyl acetate.

The absolute configuration of the enantiomeric forms of **74** and **75** was assigned by chemical correlation to dehydrovomifoliol. Enantiomerically pure (-)-**74** and (+)-**75** were treated with methanolic KOH and then oxidized with MnO_2 in CH_2Cl_2 to

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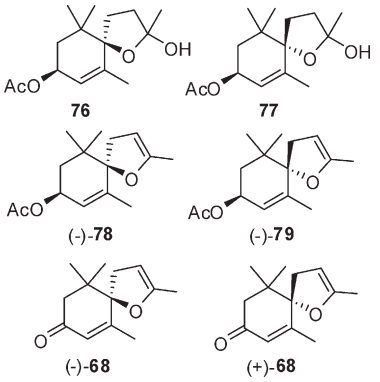

Figure 22

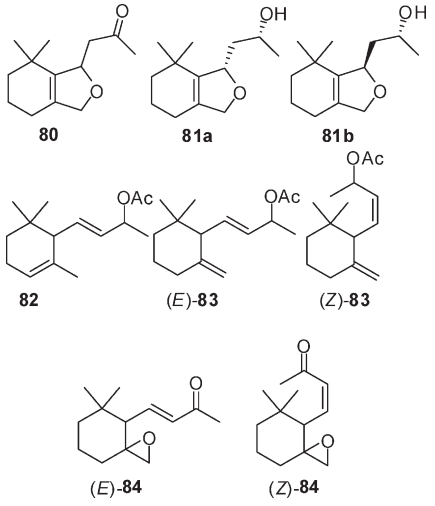

Figure 23

afford (−)-(*R*) and (+)-(*S*) dehydrovomifoliol **67**, respectively. Regioselective hydrogenation of (−)-**74** and (+)-**75** (Figure 22) using Ni Raney as a catalyst afforded hemiacetals **76** and **77**, respectively; as mixtures of diastereoisomers, which were submitted to dehydration with $POCl_3$ and Et_3N to give compounds (−)-**78** and (−)-**79**, respectively. Saponification with methanolic KOH and MnO_2 oxidation afforded dehydrotheaspirone isomers (−)-**68** and (+)-**68**, showing *ee* values of 97% and 98%.

The enantiomerically enriched forms of **68** were evaluated by qualified perfumers (Givaudan Schweiz AG, Fragrance Research). The following results were obtained. (−)-(*R*)-**68**: *woody, dry, cedarwood odor with a green, earthy, tobacco and olibanum inflection and floral-fruity nuances* (odor threshold = 13.7 ng/L air). (+)-(*S*)-**68**: *floral, woody-ambery, powdery, reminiscent of Cetonal with natural fruity, orris-like facets* (odor threshold = 9.8 ng/L air).

Racemic α-ionone was employed as the starting material in the preparation of the enantiomers of the natural C-13 norterpenoids derivatives **80**, **81a**, and **81b** (Figure 23).

They are unusual bicyclic ionone derivatives, formally obtained by oxidation of the C(11) carbon atom and ring closure between positions 7 and 11 of the ionone framework. They were first described in 1977[56] as components of the volatiles of the purple-skinned passion fruit (*Passiflora edulis* Sims), and then they were found in very high amounts in the headspace of the

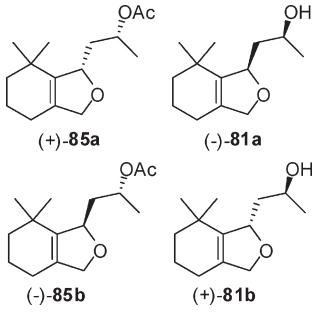

Figure 24

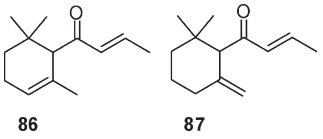

Figure 25

strong ionone floral smelling of the rare terrestrial orchid *Houlletia odoratissima* (Linden)[57] and in the scent of the orchidaceae *Gongora cruciformis* (Whitten and Bennett).[58]

However, the lack of enantiopure reference materials precluded the measurement of the enantiomeric composition of natural extracts. The first preparation of the enantiomers of these compounds was performed by lipase-mediated acetylation,[59] and the odor properties of the enantioenriched samples were evaluated by skilful perfumers.

Racemic **80** was obtained according to the following procedure (Figure 23). Cheap and commercially available α-ionone (**63**) was converted into α-ionol acetate **82**, which was irradiated in quartz vessels with high pressure Hg lamps, to promote the isomerization to a 2:1 mixture of γ-ionol acetates (*E*)-**83** and (*Z*)-**83**, respectively. Acetates (*E*)- and (*Z*)-**83** were saponified, oxidized to the corresponding ketones, and submitted to epoxidation with MCPBA to afford a mixture of four isomers with chemical structure (*E*)- and (*Z*)-**84**. which were treated with sodium methylate in DMF to give **80** regioselectively.

Racemic **80** was reduced with $NaBH_4$ and gave a 70:30 mixture of diastereoisomers **81a** and **81b**, which were separared by column chromatography and then submitted to lipase-mediated kinetic acetylation (Lipase PS, MTBE, vinyl acetate) (Figure 24). Rac-**81a** gave acetate (+)-**85a** (97% *ee*) and unreacted alcohol (−)-**81a** (97% *ee*); rac-**81b** gave acetate (−)-**85b** (99% *ee*), and unreacted alcohol (+)-**81b** (90% *ee*). Acetates (+)-**85a** and (−)-**85b** were saponificated to give alcohols (+)-**81a** and (−)-**81b**, respectively. (−)- and (+)-**80** were then prepared by Py·SO_3 oxidation of (−)-**81a** or (−)-**81b**, and (+)-**81a** or (+)-**81b**, respectively.

The enantiomers of **80**, **81a**, and **81b** were evaluated by qualified perfumers (Givaudan Schweiz AG): both ketonic and alcoholic compounds were characterized by a rather weak odor. Some differences in the odor features were anyway detected, either between diastereoisomers or enantiomers, but neither of them shows a remarkable prevailing odorous note.

2.1.4. Damascones. Ketones α- and γ-damascone (**86** and **87**, Figure 25) possess a typical fruity−flowery rose scent.[60] α-Damascone can be found as the (*S*)-(−)-enantiomer in black tea,[61] and as a trace component in tobacco[62] and in different essential oils.[63] More recently, γ-damascone has been recognized

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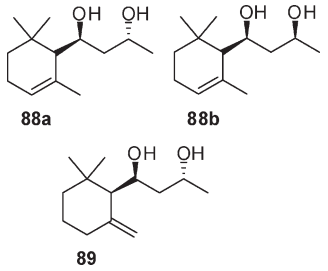

Figure 26

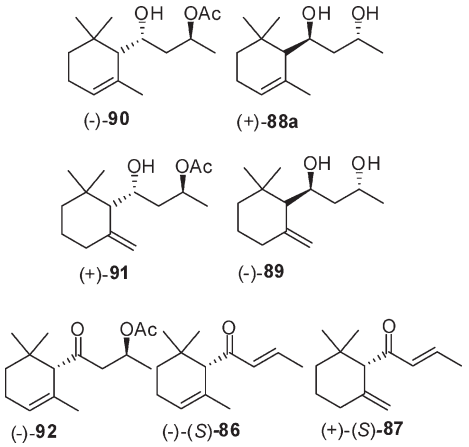

Figure 27

as an original fragrance material,[64] which can favorably complement the α-isomer. The organoleptic properties of the two enantiomers of α-damascone are quite different, especially for the values of the odor thresholds. (*S*)-(−)-damascone (odor threshold = 1.5 ppb) was described[65] as *more floral, reminiscent of rose petals, also having a winy character, without the corn and green apple note found in the (R)-enantiomer* (odor threshold = 100 ppb). Also (*S*)-(+)-γ-damascone showed superior organoleptic properties than those of the (*R*)-isomer. Enzyme-mediated synthesis of the enantiomers of damascone up to 2003 have been already reviewed.[8]

Recently, diols **88** and **89** (Figure 26) were identified as ideal substrates for the enzymic resolution process aimed to the preparation of the enantiomers of α and γ-damascones.

The carbon carbon double bond of the side chain of racemic α-ionone was epoxidised regio and diastereoselectively by means of H_2O_2/NaOH. The epoxide was opened by treatment with aluminum amalgam in THF−H_2O−ethanol at 0 °C, to afford a diastereoisomerically pure hydroxy ketone which was reduced by tetramethylammonium triacetoxyborohydride quantitatively to give diols **88a** and **88b** with good selectivity (anti/syn = 93:7). The purity of compound **88a** could be increased to 99% *de* by chromatography or crystallization from hexane. Diol **89** was then prepared by a photochemical double bond isomerization protocol of the diacetate derivative of diol **88a**.

Both diols **88a** and **89** were submitted to kinetic acetylation procedure using vinyl acetate in MTBE solution, at room temperature (Figure 27).

Complete regioselectivity and high enantioselectivity were observed: diol **88a** gave monoacetate (−)-**90** in 99% *ee* and diol (+)-**88a** in 94% *ee*; diol **89** afforded monoacetate (+)-**91** in 99% *ee* and diol (−)-**89** in 98% *ee*. Oxidation of monoacetate (−)-**90** with the Dess−Martin periodinane reagent afforded acetoxyketone (−)-**92**, that underwent the elimination reaction by DBU treatment in CH_2Cl_2 solution at rt to (−)-(S)-α-damascone **86** without a decrease of enantiomeric excess. (+)-α-Damascone **86** was prepared from diol (+)-**88a**: (+)-**88a** was treated with a slight excess of acetic anhydride in pyridine to give a mixture containing 51% of the corresponding monoacetate (+)-**90**, which was purified by column chromatography. Application of the oxidation−elimination protocol to (+)-**90** afforded (+)-(*R*)-**86**. According to the same procedure monoacetate (+)-**91** and diol (−)-**89** gave (+)-γ and (−)-γ-damascone **87**, respectively.

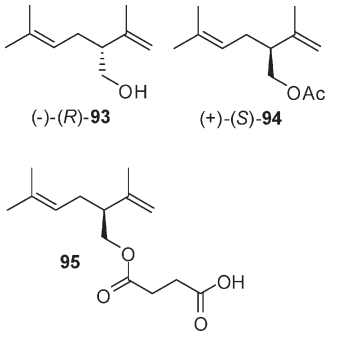

Figure 28

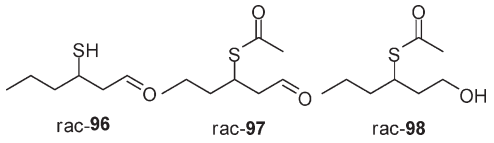

Figure 29

2.1.5. Lavandulol. (*R*)-Lavandulol ((*R*)-**93**) (Figure 28) is an important constituent of lavender oil, and the racemic commercial product is commonly employed in many fragrances and flavors. Several ester derivatives of both (*R*)- and (*S*)-**93** have been identified as sex and aggregation pheromones in two mealybugs, in thrips and in weevils.[66]

Rac-**93** was treated with vinyl acetate in hexane in the presence of PPL[67] to afford (−)-(*R*)-**93** (26% *ee*) and acetate (+)-(*S*)-**94** (80% *ee*), with 35% conversion and $E = 14$. The enantiomeric excess of alcohol (−)-(*R*)-**93** was increased to >99.5% by a second enzymatic acetylation characterized by isolation yield of 26.6%. Enriched (+)-(*S*)-**94** was subjected to PPL-catalyzed hydrolysis to afford pure (+)-(*S*)-**93** (>99.4% *ee*) in 10.8% isolated yield.

The odor evaluation of the two enantiomers confirmed that the odor quality and strength of (*R*)-**93** were superior than those of the unnatural (*S*)-**93** enantiomer and of the racemic material. (*S*)-**93** did not show the original lavandulol-like odor.

Also Zada et al. developed a kinetic resolution of racemic lavandulol using vinyl acetate as the acylating reagent to provide the two enantiomers in good enantiomeric excess.[68] According to them, the drawback of this method was the tedious chromatographic separation on silica gel of the unreacted alcohol from the formed acetate. So they developed a resolution protocol,[69] which avoided chromatography thanks to the use of succinic anhydride as the acylating reagent, and they obtained (*R*)-lavandulol directly in one step with an enantiomeric excess of 96−98%.

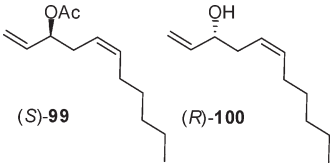

Figure 30

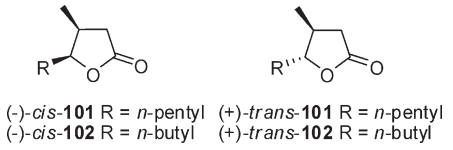

Figure 31

(S)-lavandulol could be obtained by saponification of lavandulyl succinate hemi acid **95** (Figure 28) with aqueous sodium hydroxide. The enantiomeric excess of this enantiomer was only 54−74%, according to the reaction time. Three solvents were tested, MTBE, diethyl ether, and hexane, and two lipases were used, *Porcine pancreas* from Sigma and *Hog pancreas* from Fluka. The conversion rate with *Hog pancreas* was higher than that with *Porcine pancreas* and the enantioselectivity in diethyl ether was higher than that in MTBE.

Attempts to obtain the (*S*)-enantiomer with an enantiomeric excess of more than 74% in one step, by shortening the reaction times or by using an excess of rac-**93**, did not succeed. However, it could be submitted to a second resolution process to increase enantiomeric excess up to 90%.

2.1.6. Miscellaneous. Sulfur-containing flavors are biosynthesized in various plants, especially tropical fruits,[70] and they are very requested by the current market's trends. 3-Mercaptohexanol was first identified in yellow passion fruits[71] and later also described as a volatile constituent of wine.[72] The corresponding aldehyde, 3-mercaptohexanal (**96**, Figure 29), was found in cooked liver as a flavor compound imparting "tropical fruit" type aroma notes.[73] Gas chromatography/olfactometry (GC/O) revealed that this mercaptoaldehyde has a citrus peel note.[74]

Rac-3-Acetylthiohexanal (**97**) was synthesized by Michael addition of thioacetic acid to (*E*)-2- hexenal. 3-Acetylthiohexanol (**98**) was obtained by subsequent reduction with sodium borohydride under controlled pH conditions. Hengel et al.[75] tested commercially available enzyme preparations (15 lipases and one esterase) of various origins (microbial, plant, and mammalian) for their ability to promote the enantioselective hydrolysis of **97**. The best results were achieved by using lipase B from *Candida antarctica* (CAL-B): (*S*)-3-mercaptohexanal (**96**) was obtained with *ee* = 91.1%, leaving unreacted (*R*)-**97** with *ee* =51.1%, *c* = 35.1% and *E* = 36 (*c* conversion, *E* enantiomeric ratio).

When CAL-B was immobilized on a macroporous acrylic resin or *tert*-butyl alcohol was employed as a cosolvent enantioselectivity improved (*E* = 85 and 49, respectively). Modification of the acyl moiety of the substrate was experimented by preparing 3-benzoylthiohexanal, but it had no significant impact on the course of the kinetic resolution. Odor descriptions of the enantiomers of 3-acetylthiohexanal (**97**), 3-acetylthiohexanol (**98**), and 3-mercaptohexanal (**96**) were determined by means of GC/O: rac-**97** *grapefruit, citrus peel, sweet*; (*R*)-**97** *sulfurous, roasted, citrus peel*; (*S*)-**97** *fruity, sweet, grapefruit*; rac-**96** *sulfurous, citrus peel*; (*R*)-**96** *sulfurous, rubberlike*; (*S*)-**96** *green, citrus peel, fruity*. The enantiomers of 3-mercaptohexanol had been already reported to possess the same odor properties.[76]

The sulfur-containing flavors exhibited attractive citrus type notes, and the olfactory properties of the enantiomers were found to be significantly different.

The doubly unsaturated *sec*-acetate ester (*S*)-dictyoprolene ((*S*)-**99**, Figure 30) is the key constituent of the characteristic odor of brown algae belonging to the genus *Dictyopteris prolifera* and *D. membranacea*, resembling the scent of beach.[77] Rac-**99** and the corresponding alcohol **100** were synthesized for lipase-catalyzed resolution[78] starting from the coupling of the Li-anion of 1-heptyne with bromoacetaldehyde diethylacetal, followed by hydrogenation of the triple bond in the presence of Lindlar-catalyst, removal of the acetal protection and addition of vinylmagnesium bromide. Acetylation of rac-**100** gave the corresponding acetate according to literature procedures. The screening of the enzyme-catalyzed transesterification of rac-**100** was performed with several lipases (from *Geotrichum candidum*, *Rhizomucor miehei*, *Candida antarctica*, and *Pseudomonas* sp.) in hexane at room temperature using vinyl acetate as an acyl donor (Figure 30). Only Lipase Amano PS gave (*S*)-**99** with *ee* up to 98% (*E* >200), while low values of enantioselectivities were observed with the other lipases.

To circumvent the classical limitation of kinetic resolution, i.e. the fact that the maximum theoretical yield for each enantiomer is 50%, the lipase-mediated transesterification of rac-**100** was coupled to an in situ inversion of the stereogenic center of the unreacted alcohol (*R*)-**100**, under carefully controlled Mitsunobu conditions, to suppress undesired side reactions, such as elimination and racemisation. Thus, a preparative-scale biotransformation of (*S*)-**99** was optimized by using Lipase Amano PS. Resolution of rac-**100** was terminated at 51% conversion to afford (*S*)-**99** and (*R*)-**100** with *ee* = 91%($E \approx 70$). Without any purification, this mixture was subjected to Mitsunobu inversion to give naturally occurring (*S*)-dictyoprolene with *ee* = 91%. as the sole product in 96% yield.

Quercus lactones (**101** and **102**, Figure 31) could be identified in different types of wood. They are considered to be responsible for the sensory characteristics of wine and other alcoholic beverages,[79] such as whisky, brandy and cognac, in which they are extracted during the period of aging in oak barrels.

Compounds (−)-*cis*-**101** and (+)-*trans*-**101** are called cognac lactones, while of (−)-*cis*-**102** and (+)-*trans* **102** are the so-called whisky lactones.

A chemoenzymatic synthesis of Quercus lactones was developed by atom transfer radical cyclization of optically active α,α-dichloroacetates, prepared in very good enantiomeric excess from allylic alcohols by the use of enzymes.[80]

The enzymatic resolution of 1-octen-3-ol **103** (Figure 32) by transesterification, in the presence of an equimolar amount of vinyl acetate, in diethyl ether solution, using immobilized CAL-B (Novozyme 435) had been already described by Ohtani.[81]

When the reaction was stopped at 37% conversion, the authors isolated ester (*S*)-**104** with 93% *ee* (30% yield), and unreacted alcohol (*R*)-**103** with 46% *ee* (*E* = 28). The hydrolysis of the corresponding racemic acetate **104**, catalyzed by the same lipase, and carried out in phosphate buffer had been also described. Higher *E* (176) had been reported for this reaction with the formation of alcohol (*R*)-**103**, and the recovery of unreacted starting ester (*S*)-**104** in 79% *ee*, by stopping the reaction at 39% conversion.

Taking advantage of these results, Felluga et al.[80] carried out the acylation of racemic alcohols **105** and **103** with vinyl acetate

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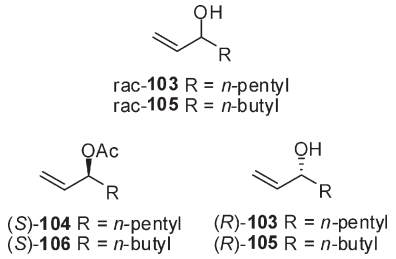

Figure 32

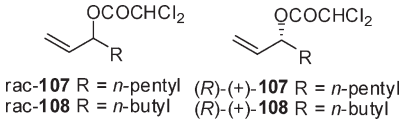

Figure 33

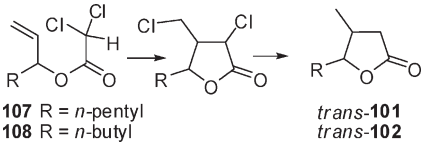

Figure 34

in excess without diethyl ether and observed great enhancement of enantioselectivity: (*R*)-**105** and (*R*)-**103** (*ee* = 93 and 91%) and (*S*)-**106** and (*S*)-**104** (*ee* > 99% for both esters) could be isolated. The hydrolyses of racemic acetates **106** and **104** were performed as described in the literature, with similar results.

The kinetic resolution of dichloroacetates rac-**107** and rac-**108** was also investigated (Figure 33). Hydrolyses of rac-**107** and rac-**108** occurred with a low enantioselectivity. However, the addition of DME as a cosolvent increased the enantiomeric ratio to a value higher than 200: optically pure esters *R*-(+)-**107** and *R*-(+)-**108** could be obtained at 50% conversion.

To overcome the limit of 50% yield of recovery of one enantiomer typical of the resolution procedure, a stereoinversion of protocol based on Mitsunobu esterification was optimized.

The reaction of the crude enzymatic hydrolysis mixtures with DEAD, PPh_3, and dichloroacetic acid afforded dichloroacetate (*R*)-(+)-**107** and (*R*)-(+)-**108**, with the one-pot conversion of the racemic substrates into one single enantiomeric product.

Clean samples of homochiral allyl dichloroacetates (*R*)-(+)-**107** and (*R*)-(+)-**108** were subjected to a atom transfer radical cyclization (TMC-ATRC process) (Figure 34) catalyzed by Cu(I)Cl[bipyridine], affording, after hydrodehalogenation of the chloro substituted intermediates (TBSnH/AIBN at 80 °C in toluene) the corresponding trans diastereoisomers (trans/cis ratio 87/13 and 91/9, respectively). Thus, (+)-trans cognac lactone **101** (yield 53%) and (+)-trans whisky lactone **102** (yield 40%) were obtained, together with a minor amount of the corresponding cis isomers.

2.2. Artificial Odorants

2.2.1. Floral Fragrances. *Non-Natural Ionone Derivatives.* The C(13)-alkyl-substituted α-ionone homologues **109**−**110** (Figure 35) were synthesized[82] to investigate the effect of the

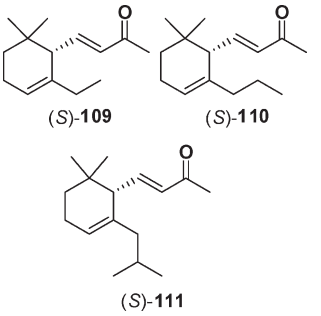

Figure 35

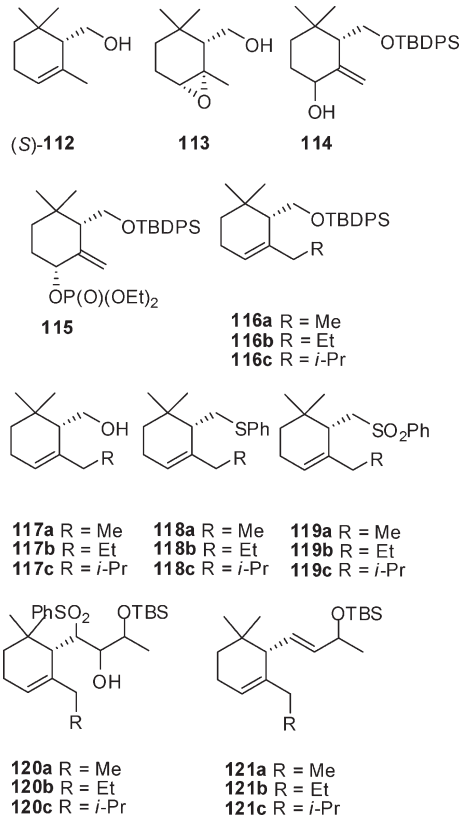

Figure 36

steric hindrance of the substituents of the cyclohexene C=C bond on the olfactory properties of the molecule.

As a matter of fact, it was known[83] that in ionone-type compounds the violet odor depends critically on the presence of a cyclohexene nucleus, carrying at least three methyl groups, two of which should be adjacent to the 3-oxobut-1-enyl chain. An increase of the steric hindrance around C(13) was expected to induce a variation of the odor characteristics compared to that of the parent α-ionone **63**. Derivatives (*S*)-**109**, (*S*)-**110**, and (*S*)-**111** could be prepared starting from (*S*)-α-cyclogeraniol (**112**) according to a lipase-mediated procedure of kinetic resolution.

Phosphoric acid-promoted cyclization of commercial geranic acid, performed on a multigram scale, followed by $LiAlH_4$ reduction afforded **112**, which was submitted to lipase-mediated tranesterification (Figure 36). Four different enzymes were evaluated, and the following values of the enantiomeric ratio (*E*) were obtained: 12.9 for lipase PS (*Pseudomonas cepacia*), 5.8

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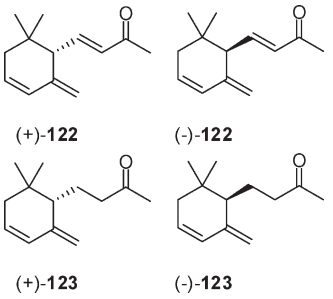

Figure 37

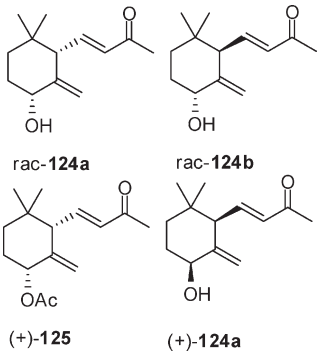

Figure 38

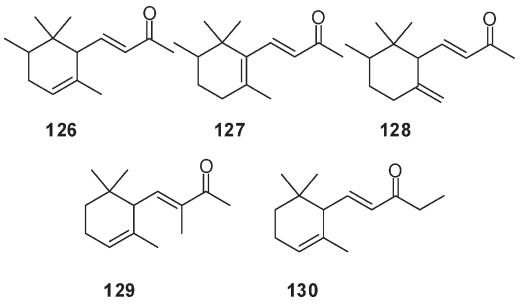

Figure 39

for *Pseudomonas fluorescens*, and 10 for sol−gel immobilized lipase AK, while lipase from *Candida lipolitica* was inactive. A preparative enzymatic resolution of racemic **112** was then performed with 4.5 equiv. of vinyl acetate in hexanes/Et_3N 2.8:1 in the presence of 4 Å molecular sieves. After 5 days at 0 °C, a conversion of 58% was reached, allowing the recovery of (*S*)-**112** (95% *ee*) and the corresponding (*R*)-acetate (68.3% *ee*).

The conversion of (*S*)-**112** into the three 13-alkyl-substituted α-ionones (*S*)-**109**, (*S*)-**110**, and (*S*)-**111** was performed according to the following synthetic sequence (Figure 36).

Epoxidation of (*S*)-**112** with MCPBA in CH_2Cl_2 at 0 °C occurred with high stereoselection and afforded the cis diastereoisomer **113** in 90% yield. This was protected and submitted to a rearrangement procedure by reaction with $Al(OiPr)_3$ in refluxing xylene, to obtain allylic alcohol **114** (85% yield). Alcohol **114** was then transformed into the corresponding diethyl phosphate **115**, which was reacted at −78 °C with an excess of monoalkyl cuprate, prepared from RMgBr (R= Me, Et, *i*Pr) and CuCN·2LiCl to give protected homogeraniols **116a**− **c** in good yields (82− 94%) and complete regioselectivity.

These derivatives were submitted to a *Julia*−*Lythgoe* olefination protocol, in order to add the enone moiety with preservation of the configuration at C(6), and with the stereoselective formation of the C═C bond in the side chain in (E) configuration. Deprotected alcohols **117a**−**c** were converted into the corresponding sulfides **118** under Mitsunobu conditions, and then oxidized to sulfones **119** with H_2O_2 in the presence of catalytic amounts of $(NH_4)2MoO_4$ in $MeOH/CH_2Cl_2$. Subsequent condensation of each sulfone with protected (*S*)-2-hydroxypropanal proceeded smoothly, to give the corresponding hydroxy sulfones **120a**−**c**, which were immediately exposed to 20% Na/Hg alloy to deliver the protected α-ionols **121a**−**c**.

Deprotection, followed by oxidation with Dess-Martin periodinane completed the synthetic sequence to enantiomerically enriched (*S*)-**109**, (*S*)-**110**, and (*S*)-**111**.

The three 13-alkyl-substituted (*S*)-α-ionone homologues were submitted to olfactory evaluation (Givaudan Schweiz AG, Fragrance Research). (*S*)-**109** was described as *a woody-floral, fruity, reminiscent of β-ionone, with a pronounced powdery side*, and it was found to be the most powerful of the three tested ionone derivatives on blotter, with an odor threshold of 0.085 ng/l of air. (*S*)-**110** was found to be *woody-floral, fruity in the direction of ionone, but with a distinct orris inflection, fruitier than (S)-**109**, and more closely reminiscent of raspberry*. (*S*)-**111** was determined *to be the weakest odorant of these three ionones on blotter, slightly ionone-like, in character closer to (S)-**109** than to (S)-**110**.* These results allowed the authors to draw the conclusion that the odor of the three C(13)-alkyl-α-ionone homologues decreased in intensity with the increase of the size of the substituent at C(5), even though, quite surprisingly, the ethyl derivative (*S*)-**109** was found to be more than 30 times more potent than the parent (*S*)-α-ionone (**63**), which has a small Me substituent at C(5). As for the odor character, enhancing the steric hindrance around the cyclohexene C═C bond did not affect significantly the ionone-like tonalities of the series, while it introduced additional distinct notes, like the orris facets displayed by (*S*)-**110**.

The enantiomers of 3,4-didehydroionone derivatives **122** and **123** (Figure 37) were prepared and the corresponding odor properties were evaluated by skilful perfumers.[84]

A 4:1 mixture of racemic diastereoisomers **124a**/**124b** (Figure 38), prepared from racemic α-ionone, was treated with vinyl acetate in MTBE solution in presence of lipase PS and obtained diastereomerically and enantiomerically pure (99% *ee* and *de*) acetate (+)-**125** and a mixture of enantioenriched (+)-**124a** and rac-**124b**.

The mixture (+)-**124a**/rac-**124b** was submitted again to enzymic acetylation to afford (+)-**125** with lower *ee* and a mixture of alcohols which contained a higher amount of rac-**124b** and (+)-**124a** with increased *ee*. Pd-mediated elimination of the acetate group of enantiomerically pure (+)-**125** gave (+)-**122**, which afforded (+)-**123** by C(7)═C(8) bond reduction. According to the same procedure, (+)-**124a**/rac-**124b** was acetylated by Ac_2O and pyridine and then submitted to the above-described reaction sequence, to afford (−)-**122** and (−)-**123** with modest *ee*. The absolute configuration of the enantiomers of **122** and **123** was determined by chemical correlation. All the isomers were submitted to olfactory evaluation (Givaudan Schweiz AG, Fragrance Research) with the following considerations: (*S*)-(+)-**122** *is floral-woody, cetonal-like, slightly fruity, damasconic, sweet, stronger on blotter than the (R)-isomer* (odor threshold 0.080 ng/l of air); (*R*)-(−)-**122** is *fruity-floral, slightly woody* (odor threshold 0.25 ng/l of air); (*S*)-(+)-**123** *is woody-powdery, ionone, slightly fruity-floral, with more pronounced ionone*

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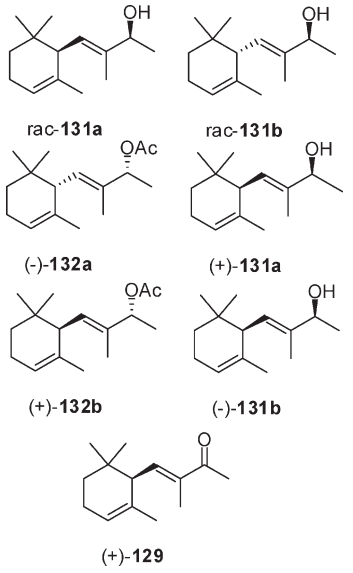

Figure 40

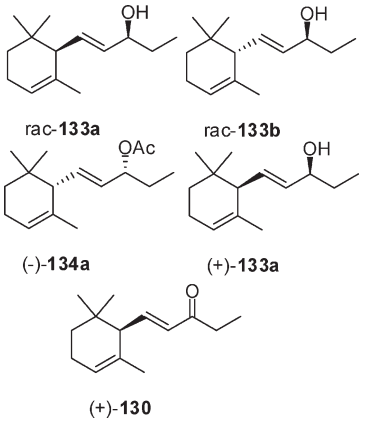

Figure 41

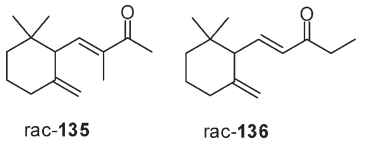

Figure 42

*character than the (R)-isomer, stronger on blotter than (R)-**123*** (odor threshold 92 ng/L of air); (*R*)-(−)-**123** *is fruity, agrestic, floral, ionone- and damascone-like, slightly woody, and saffron-like* (odor threshold 99.4 ng/L of air).

Non-Natural Irone Isomers. In 1947 Ruzicka[85] and Naves,[86] independently, found that at least three isomers of irone were present as odor active components in natural iris oil, one of the most precious perfume ingredients: α- (**126**), *β*- (**127**), and *γ*-irone (**128**) (Figure 39). In 1971, Rautenstrauch and Ohloff[87] were able to establish the stereochemistry of the irone isomers contained in the Italian iris oil (probably from *Iris pallida*) first used by Ruzicka .

The biocatalyzed preparation of the enantiomers of irones has been recently reviewed by the authors.[88] Herein the lipase-mediated preparation of some non natural isomers and derivatives of irones are reviewed.

Compounds **129** and **130** (Figura 39) are constitutional isomers of natural irones, they have not been found in nature, and they are important violet fragrances widely employed in perfumery.

They were first prepared by Tiemann in 1893[89] by reaction of citral with ethyl methyl ketone, followed by cyclization, and put on the market by Firmenich in 1903 under the trade name of Iralia. In 1905, François Coty employed Violettone (α-ionone, **63**) and Iralia to create the great classic *L'Origan*,[90] *a perfume with a warm oriental character*.

The enantiomers of **129** and **130** were prepared by lipase-mediated resolution for the evaluation of the odor properties.[91] Commercial **129** (Isoraldeine 95-Givaudan) was converted by sodium boron hydride reduction into a 1:1 mixture of the two diastereoisomeric alcohols rac-**131a** and **131b** (Figure 40), which were separated by fractional crystallization of the corresponding *p*-nitrobenzoate esters. The relative configuration of the ester derivative of **131a** was established by X-ray diffraction analysis.

Racemic alcohol **131a** (*de* = 98%) and **131b** (*de* = 94%), obtained by saponification of the corresponding esters, were submitted to Lipase PS-mediated transesterification (MTBE, vinyl acetate). After 24 h, acetate (−)-**132a** and alcohol (+)-**131a**, and acetate (+)-**132b** and alcohol (−)-**131b** were recovered as pure compounds from the corresponding reaction mixtures by column chromatography. MnO_2 oxidation of alcohol (−)-**131a**, prepared from (−)-**132a** afforded (−)-**129** (*ee* = 98%), while the reaction of (+)-**131b**, obtained from (+)-**132b**, gave enantiomer (+)-**129** (*ee* = 94%).

Racemic **130** was prepared from commercial α-ionone **63**, through hypobromite degradation to afford the corresponding carboxylic acid, which was reduced as an ethyl ester, oxidized to aldehyde and then submitted to ethyl magnesium bromide addition.

The corresponding α-methylionol was obtained as a 1:1 mixture of the two diastereoisomers rac-**133a** and rac-**133b** (Figure 41), once again separated by crystallization of the corresponding 4-nitrobenzoate esters. The relative configuration of the ester of **133a** was established by X-ray diffraction analysis. Rac-**133a** was submitted to Lipase PS mediated acetylation in the usual conditions to afford acetate (−)-**134a** (*ee* = 98%) and unreacted alcohol (+)-**133a** (*ee* = 93%).

Alcohol (−)-**133a**, obtained by hydrolysis of (−)-**134a**, and its enantiomer (+)-**133a** were converted into (−)- and (+)-**130**, respectively, by oxidation with Mn(IV) oxide.

The absolute configurations of the enantiomers of methyl ionones **129** and **130** were established by chemical correlation.

The sample of enantioenriched methyl ionones have been evaluated by perfumers with the following results. (*R*)-(+)-**129** (*ee* = 94%): *typical dry warm ionone note, irisone, clean tea-like, also on blotter more intense and dryer than (−)-**129*** (odor threshold = 0.079 ng/L air). (*S*)-(−)-**129** (*ee* = 98%): *about ten times weaker floral note in the direction of iris and ionone, with hesperidic—citric elements and sweet fruity damascone-like aspects; more rich and complex in odor than (+)-**129*** (odor threshold = 0.83 ng/L air). (*R*)-(+)-**130** (*ee* = 93%): *woody, powdery, floral odor in the direction of the ionone family, but less powerful and less intense. Dry-down linear, after 4 h woody-ionone, somewhat orris-like, and after 24 h woody-ionone, fruity, raspberry-type* (odor threshold = 0.53 ng/L air). (*S*)-(−)-**130** (*ee* = 98%): *woody, powdery, floral*

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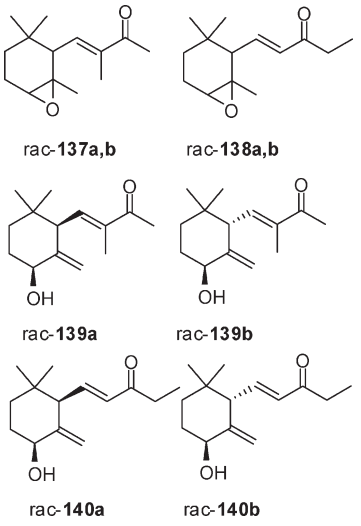

Figure 43

odor in the direction of the ionone family, but less powerful and less intense. Dry-down linear, after 4 h woody-ionone, somewhat orris-like, and after 24 h woody-ionone, only very slightly fruity (odor threshold 0.24 ng/L air).

The enantiomers of compounds **135** and **136** (Figure 42), which are trace components of commercial Iralia, were also prepared for olfactory evaluation.

Compounds **129** and **130** were converted into **135** and **136**, respectively, according to the following procedure (Figure 43). Methyl ionones **129** and **130** were treated separately with MCPA to afford the cis/trans mixtures of epoxides **137a**/**137b** and **138a**/**18b**, respectively, which were submitted to a base-mediated isomerization promoted by an excess of LDA in THF, to afford cis/trans mixtures of allylic alcohols **139a**/**139b** and **140a**/**140b**, respectively.

The allylic alcohols were acetylated, and then the acetate group was reductively removed by treatment with triethylammonium formate and a palladium catalyst to give rac-**135** and **136**, respectively. Allylic alcohols **139** and **140** were chosen as suitable substrates for kinetic resolution. Epoxide **137a** was purified by column chromatography and gave alcohol **139a** upon basic isomerization. This latter was acetylated (MTBE, vinyl acetate, lipase PS) (Figure 44) to afford unreacted alcohol (+)-**139a** (99% *de*, 87% *ee*) and acetate (−)-**141** (99% *de*, 99% *ee*).

The reductive removal of the acetoxy group converted the latter compounds into (+)-**135** (87% *ee*) and (−)-**135** (99% *ee*), respectively. Epoxides **140a** and **140b** could not be separated, thus the 4:1 mixture of alcohols **140a** and **140b** was used in the resolution step, to give (+)-**142** (99% *de*, 99% *ee*) and an inseparable mixture of unreacted alcohols (4*S*,6*R*)-**140a** and racemic **140b** (50% *de*, 85% *ee*). The reductive removal of the acetoxy group converted the latter compounds into (+)-**136** (99% *ee*) and (−)-**136** (65% *ee*), respectively. The absolute configuration of the enantiomers of **135** and **136** was determined by chemical correlation. The olfactory evaluation of these samples (Givaudan Schweiz AG, Fragrance Research) gave the following results. (*S*)-(−)-**135**: *woody-ambery mix odor between methyl ionone and Iso E Super of dry character*. (*R*)-(+)-**135**: *rich and interesting woody-ambery, leather odor with fruity-floral facets in the direction of irone and methyl ionone and additional green accents*. (*S*)-(+)-**136**: *woody-floral odor in the direction of methyl ionone,*

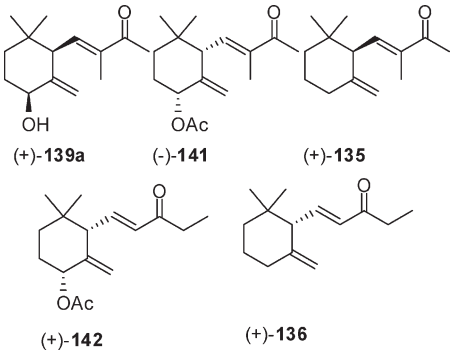

Figure 44

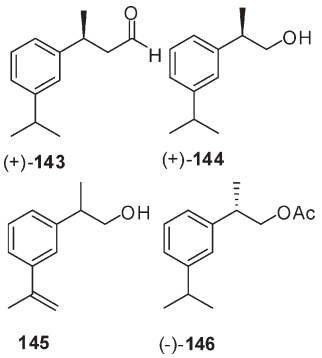

Figure 45

with a fruity-floral violet inclination and facets of orris, but also an oily background. (*R*)-(−)-**136**: *woody odor in the direction of methyl ionone with additional dry, leathery aspects.*

Florhydral. Florhydral (**143**, Givaudan) is a synthetic odorant employed *to convey the fresh marine and ozonic note*. It is described *to have a fine floral, melon odor, with those qualities found in the lily of-the-valley and linden blossom. It is powerful and diffusive, and it can be used to give long lasting fresh and green top notes.*

The successful approach[92] to the enantiomers of **143** was based on the biocatalyzed transesterification of racemic alcohol **144** (Figure 45), prepared starting from the hydroboration reaction of 1,3-diisopropenyl benzene, under controlled reaction conditions, which gave, after quenching of the mixture with $NaOH-H_2O_2$, alcohol **145**. The alkene double bond of **145** was reduced by hydrogenation in the presence of Pd/C in ethyl acetate solution, to afford alcohol **144** in very good overall yield.

Lipase-mediated transesterification of **144** (MTBE, vinyl acetate) was investigated in the presence of PPL, CRL, and Lipase PS. After 24 h, acetate (−)-**146** was obtained with the following *ee* values: PPL *ee* = 81%; CRL *ee* = 50%; Lipase PS *ee* = 33%. PPL-mediated acetylation was investigated for a longer reaction time: after 96 h, acetate (−)-**146** (*ee* = 60%) and unreacted alcohol (+)-**144** (*ee* = 55%) were recovered (*c* = 48%, *E* = 6.8).

The enantiomeric excess of (+)-**144** was increased by submitting it again to the enzymatic transesterification reaction. After 120 h reaction time, alcohol (+)-**144** was recovered with *ee* >99%. Acetate (−)-**146** (*ee* = 60%) was hydrolyzed with KOH in methanol, and the corresponding alcohol (−)-**144** was submitted to enzymatic acetylation. After 48 h, acetate (−)-**146** with *ee* >99% was obtained. The enantiopure alcohols (+)- and (−)-**144** were then converted into enantiopure (+)- and (−)-**143** by

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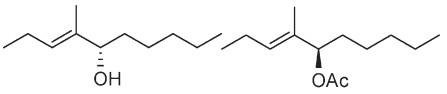

Figure 46

cyanide displacement of the corresponding tosylates, followed by diisobutylaluminum hydride reduction (THF, −10 °C).

The two enantiopure samples of (+)- and (−)-**143** were then submitted to skilled perfumers (Givaudan Schweiz AG, Fragrance Research) for olfactory evaluation and the following descriptions were obtained. (+)-**143**: *floral, watery, green, yet with an acidic touch, even in the dry down note*, in comparison with rac-**143**, it has *a more green, less watery, and more powerful fragrance* (odor threshold = 0.035 ng/L air). (−)-**143**: It shows *a typical Florhydral smell, floral, fresh, green, muguet-like, but more marine, and more plastic* (odor threshold = 0.88 ng/l air). (+)-**143** was found to be the most potent of the two enantiomers of Florhydral, and thus an enzymatic method was optimized to produce it by enantiospecific synthesis (see section 3.2).

Undecavertol. Racemic Undecavertol (**147**, Givaudan, Figure 46) is described *to have a powerful green-floral character, somewhat related to lily of-the-valley, with natural fresh fruity violet leaf and linden blossom aspects.* It is widely employed both in fine and functional perfumery, with successful results in rose and fruity-pear accords. It is characterized by an exceptional strength, so it has to be employed in careful dosage and blending. The commercial product is a 98.5:1.5 mixture (GC/MS) of *trans*- and *cis*-isomers.

Racemic **147**, prepared as a 97.3:2.6 mixture (GC/MS) of trans- and cis-isomers by aldolic condensation of propanal followed by addition of pentyl magnesium bromide, was submitted to lipase-mediated acetylation[93] (MTBE, vinyl acetate) in the presence of three commercial enzymic preparations (lipase PS, PPL, CCL). CCL and PPL produced an acetate derivative **148** (Figure 46) showing very low enantiomeric excess (*ee* =35% and 37%, respectively). The best results were achieved with lipase PS. Lipase PS-mediated acetylation of rac-**147** (6 days) gave acetate derivative (+)-**148** as a 93.5:6.5 mixture of trans- and cis-isomers (GC/MS). This latter was hydrolyzed by reaction with KOH in methanol to afford alcohol (+)-**147** as a 96.5:3.5 mixture of trans- and cis-isomers (GC/MS) with *ee* = 93%.

The unreacted alcohol was treated with lipase PS, in *t*-butyl methyl ether solution, in the presence of vinyl acetate for 10 days. At the end of this period, (−)-**147** was recovered from the reaction mixture by column chromatography, containing 2% of the cis-isomer (GC/MS) and showing *ee* = 75%. The absolute configuration was assigned by chemical correlation. The samples of (+)-**147** (*ee* = 93%) and (−)-**147** (*ee* = 75%) were evaluated by Givaudan perfumers (Givaudan Schweiz AG, Fragrance Research), and the following results were obtained: (*R*)-(+)-**147** *has a typical Undecavertol odor, floral, green, fresh, violet leaves, stronger and greener than the racemic commercial material, with aspects of cucumber and Neofolione (methyl non-2-enoate)* (odor threshold = 0.31 ng/L air). (*S*)-(−)-**147** is *weaker than the enantiomer, fruity-green pinefir balsam note with aspects of tea, not so typical of Undecavertol* (odor threshold = 4.7 ng/L air).

Clarycet and Florol. The lipase mediated acetylation of hydroxy ketones **149** and **150**, simply obtained by aldolic condensation, was the key step for the preparation of all the

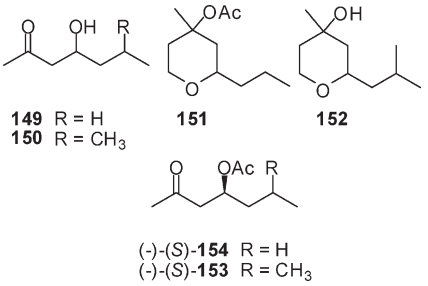

Figure 47

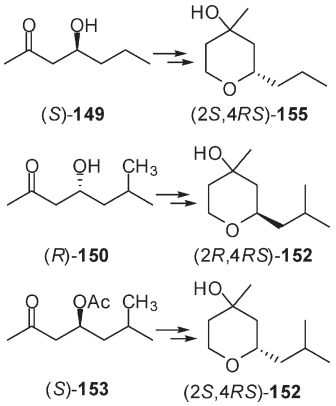

Figure 48

stereoisomers[94] of Clarycet (**151**) and Florol (**152**), two floral odorants which are commercialized as mixtures of two racemic diastereoisomers (Figure 47).

Clarycet (IFF) is described *to have a herbal floral, rosy odor with a dried fruitiness and a suggestion of clary sage.* Florol (Firmenich) *is fresh, soft, with a natural floral note; it can be used in almost all perfume types where it gives elegant floral diffusion without changing the character of the fragrance.*

Lipase PS catalyzed the enantioselective acetylation of derivative **150** to give the corresponding acetate (*S*)-**153** (30% yield) with an *ee* of 95% (Figure 47). A rather slow enrichment of the starting alcohol allowed the recovery of hydroxy ketone (*R*)-**150** with an *ee* of 91% (35% yield). As for derivative **149**, only CCL was found to promote the acetylation with low enantioselectivity, affording acetate (*S*)-**154** (18% yield) with an *ee* of 60%. Further enrichment was achieved by submitting (*S*)-**154** (60% *ee*) to biocatalyzed hydrolysis in H_2O/THF solution at pH 7.8 in the presence of CCL. Alcohol (*S*)-**149** (67% yield) was recovered with an *ee* of 80%. A strong influence of structure on the steric course of lipase acetylation was thus observed, being the branched chain of hydroxy ketone **150** a more favorable structural feature.

Enantiomerically enriched hydroxy ketones (*S*)-**149** (80% *ee*), (*R*)-**150** (91% *ee*), and acetate (*S*)-**153** (95% *ee*) were converted by simple manipulation of functional groups into the corresponding mixtures of cyclic diastereoisomers which could be separated by column chromatography (Figure 48) to afford the following products: (2*S*,4*R*)-**155** (>99% *de*, 80% *ee*, 39% yield), (2*S*,4*S*)-**155** (>99% *de*, 80% *ee*, 31% yield), (2*R*,4*R*)-**152** (>99% *de*, 91% *ee*, 30% yield), (2*R*,4*S*)-**152** (96% *de*, 91% *ee*, 37% yield), (2*S*,4*R*)-**152** (>99% *de*, 95% *ee*, 35% yield), and (2*S*,4*S*)-**152** (>99% *de*, 95% *ee*, 29% yield). Treatment of (2*S*,4*R*)-**155** and

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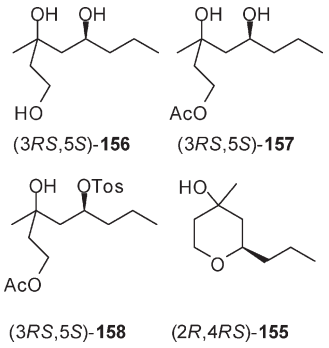

Figure 49

(2*S*,4*S*)-**155** with NaOAc in refluxing Ac_2O gave (2*S*,4*R*)-**151** (76% yield) and (2*S*,4*S*)-**151** (74% yield), respectively.

The CCL-mediated acetylation of rac-**149** was too slow to be synthetically useful, thus a different route to the (2*R*)-configured Clarycet diastereoisomers had to be optimized. The mixture of the two diastereoisomeric triols (3*RS*,5*S*)-**156** (Figure 49), obtained by transformation of the corresponding hydroxy ketone, was treated with lipase PS in MTBE and vinyl acetate to afford the two diastereoisomeric primary monoacetates (3*RS*,5*S*)-**157** (78% yield). Ring closure was promoted with inversion of configuration at C(2) by treatment of (3*RS*,5*S*)-**157** with TsCl in pyridine to give (3*RS*,5*S*)-**158**, followed by saponification with 10% NaOH solution in EtOH: a mixture of the two diastereoisomers (2*R*,4*RS*)-**155** was obtained. The two diasteroisomers were separated by column chromatography (30% and 38% yield) and submitted separately to acetylation in refluxing Ac_2O in the presence of NaOAc to give the two enantiomerically enriched Clarycet isomers (2*R*,4*S*)-**151** (69% yield) and (2*R*,4*R*)-**151** (64% yield).

All the samples were submitted to olfactory evaluation (Givaudan Schweiz AG, Fragrance Research) with the following results. (2*S*,4*S*)-**151**: *Green, fresh, earthy, fruity (sage), dry down odorless.* (2*S*,4*R*)-**151**: *Floral, agrestic, fruity, touch acetic-green-tobacco, dry down slightly fruity, but very weak.* (2*R*,4*R*)-**151**: *Pine, pine oil, terpenic, woody, dry down dusty dirty.* (2*R*,4*S*)-**151**: *Fruity, rosy, rose ketone, good, touch earthy, dry, sweet, woody, sage, dry down floral-sage.* (2*R*,4*R*)-**152**: *Most pronounced and most intense Florol stereoisomer* (odor threshold 1.21 ng/l air), *fresh, soft, sweet, and natural floral odor reminiscent of muguet with some rose oxide side note and earthy nuances.* (2*S*,4*S*)-**152**: *Second intense Florol stereoisomer* (odor threshold 26 ng/l air), *but already much weaker, similar fresh-floral note as the enantiomer, but less sweet and also more linalool-type, more herbaceous, and more earthy in tonality.* (2*R*,4*S*)-**152**: *The second weakest in the series of Florol stereoisomers* (odor threshold 520 ng/l air), *relatively weak, mainly fruity, grape-like, but also reminiscent of linalyl acetate and clary sage oil, with some nuances of dry herbs.* (2*S*,4*R*)-**152**: *Odorless on GC olfactometry* (odor threshold >600 ng/l air), *the weakest of all Florol stereoisomers, very weak in odor, mainly linalool and coumarine like, with some citric and hesperidic nuances.*

As for Clarycet, the single enantiomer (2*R*,4*S*)-**151** has a nice floral odor, which makes it distinguished from the other stereoisomers. A gradual variation of odor intensity was noticed in the four Florol isomers: the two enantiomers of the diastereoisomer bearing the OH group and the isobutyl chain in equatorial positions are decidedly the most intense, and are responsible for the odor of commercial Florol.

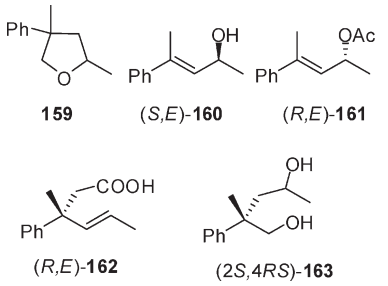

Figure 50

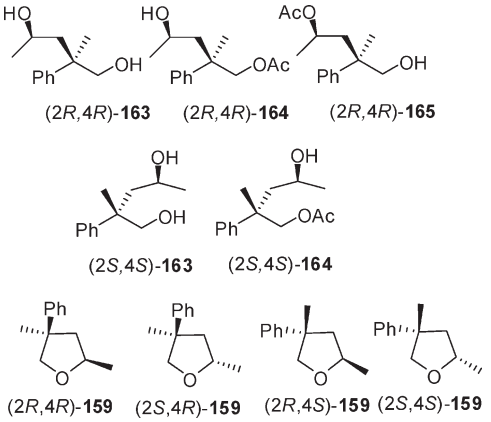

Figure 51

Rhubafuran. The same strategy was employed[94] to prepare the enantiomers of the odorant Rhubafuran (**159**, Figure 50), using as a key intermediate the allylic secondary alcohol (*E*)-**160**. This latter was submitted to lipase-PS acetylation, and after 72 h, acetate (*R*,*E*)-**161** (>99% *ee*, 36% yield) and alcohol (*S*,*E*)-**160** (>99% *ee*, 37% yield) could be isolated by column chromatography. (*R*,*E*)- and (*S*,*E*)-**160** were treated with triethyl orthoacetate in the presence of a catalytic amount of propanoic acid to afford after saponification the two enantiomeric acids (*R*,*E*)- and (*S*,*E*)-**162** (64% and 60% yield).

These latter derivatives were separately converted by simple organic reactions into the corresponding mixtures of diasteroisomeric diols (2*S*,4*RS*)- and (2*R*,4*RS*)-**163**, respectively. Each mixture of diastereoisomers was submitted to column chromatography, and the four isomers of diol **163** were obtained as pure compounds (28−35% yield). Unfortunately, when the diastereoisomerically pure diols **163** were treated with triphenylphosphine and *N*-bromosuccinimide (NBS), or reacted with TsCl and pyridine, mixtures of Rhubafuran diastereoisomers were obtained. An attempt was made to selectively convert the primary OH group to an acetate ester by submitting the four enantiomerically pure stereoisomers of diol **163** to enzyme-mediated acetylation in the presence of lipase PS in separate experiments. The following results were obtained (Figure 51): diols (2*R*,4*S*)-**163** and (2*S*,4*R*)-**163** gave, after 8 days, a 1:1 mixture of the two possible monoacetates, which could not be separated by column chromatography. Diols (2*R*,4*R*)-**163** and (2*S*,4*S*)-**163** gave, respectively, a 1:1 mixture of the two monoacetates (2*R*,4*R*)-**164** and (2*R*,4*R*)-**165** (which could be separated by column chromatography, 35% and 32% yield) and the single monoacetate (2*S*,4*S*)-**164** (44% yield), after 5 days. Reaction with TsCl in pyridine, followed by saponification with 10% NaOH solution in

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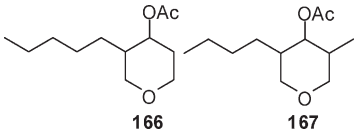

Figure 52

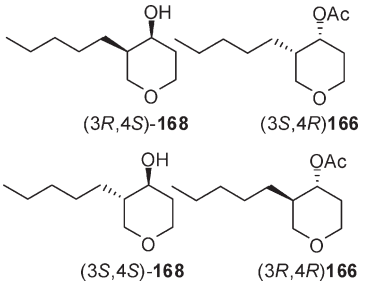

Figure 53

EtOH, afforded the following samples of Rhubafuran: (2*R*,4*R*)-**159** (84% *de*, >99% *ee*, 50% yield from (2*R*,4*R*)-**165**), (2*S*,4*R*)-**159** (66% *de*, >99% *ee*, 56% yield from (2*R*,4*R*)-**163**), (2*R*,4*S*)-**159** (78% *de*, >99% *ee*, 49% yield from (2*S*,4*S*)-**164**). The preparation of the fourth isomer (2*S*,4*S*)-**159** (60% *de*, >99% *ee*, 67% yield) was accomplished by reaction of (2*S*,4*S*)-**163** with NBS and Ph_3P.

The olfactory properties of Rhubafuran samples were thus described (Givaudan Schweiz AG, Fragrance Research). (2*R*,4*R*)-**159**: *Nuts, acidic, animalic, slightly rhubarb*. (2*S*,4*R*)-**159**: *Citric, rhubarb, slightly green, slightly animalic.* (2*S*,4*S*)- **159**: *Grapefruit-like, bitter, cassis, slightly oxane-like and reminiscent of dimethyloctenone, dry down (24 h) bitter, grapefruit, oxane.* (2*R*,4*S*)-**159**: *The most pleasant one, floral, linalool-like, rhubarb and citrus, green, slightly eucalyptus.* (2*R*,4*S*)-**159**, followed by its enantiomer, are the closest to the odor of commercial Rhubafuran.

Jasmal and Jessemal. Other two interesting examples of tetrahydropyranic odorants (Figure 52) are Jasmal (**166**, IFF) and Jessemal (**167**, IFF), which are used in many types of cosmetic products for their pleasant jasmine odor. Their single stereoisomers were obtained by lipase kinetic resolution.[95]

A sample of Jasmal(IFF), containing a 1:1 mixture of cis- and trans diastereoisomers (GC/MS), was hydrolyzed with KOH in EtOH/H_2O to afford the corresponding alcohols (3*RS*,4*SR*)-**168** and (3*RS*,4*RS*)-**168**, respectively, which were separated by column chromatography, and then submitted to kinetic resolution (lipase PS, MTBE, vinyl acetate) (Figure 53). After 9 days, the transesterification of *cis*-**168** afforded acetate (3*S*,4*R*)-**166** (97% *ee*) and unreacted alcohol (3*R*,4*S*)-**168** (92% *ee*).

The enzymatic kinetic resolution of *trans*-**168** gave after 3 days acetate (3*R*,4*R*)-**166** (*ee* >99%) and alcohol (3*S*,4*S*)-**168** (88% *ee*).

The absolute configuration was determined by preparing a Jasmal stereoisomer from L-tartaric acid.

The approach to the synthesis of the stereoisomers of **167** was rather complex. The successful strategy adopted for the preparation of all stereoisomers of **166** could not be used, since the commercial samples of **167** were largely contaminated by **166** and several unknown components (GC/MS). Attention was focused on the preparation of all enantiomers of the diastereoisomers of **167** showing just one trans relationship (Figure 54), that is, (3*R*,4*R*,5*R*)-**167**, (3*S*,4*S*,5*S*)-**167**, (3*R*,4*S*,5*R*)-**167** and (3*S*,4*R*,5*S*)-**167**.

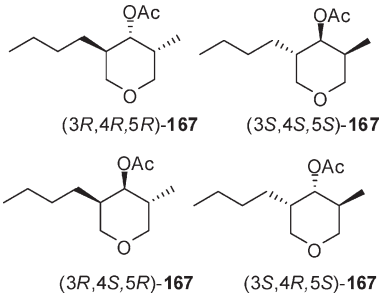

Figure 54

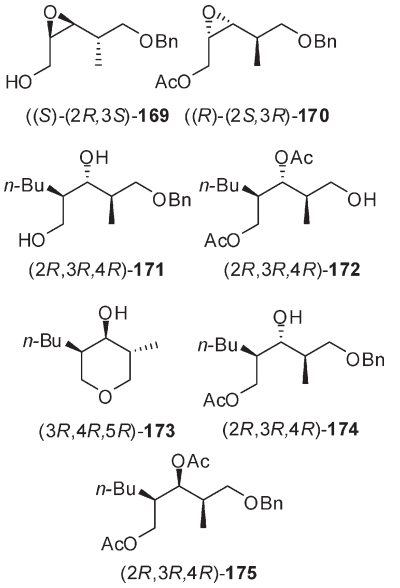

Figure 55

Concerning the synthesis of (3*R*,4*R*,5*R*)-**167** and (3*S*,4*S*,5*S*)-**167**, epoxy alcohol **169** (Figure 55) was chosen as the most suitable building block, because it already contains the C5 skeleton of the final tetrahydropyranol, and it is known in the literature that the nucleophilic attack on this kind of epoxy alcohols should proceed regioselectively affording precursors with the same configuration of the target molecules.

The enzymatic resolution of racemic epoxy alcohol **169**, by acetylation with vinyl acetate in $CHCl_3$ catalyzed by lipase-PS, gave access to both enantiomers of **169** with high *ee* values.

Survived alcohol (*S*)-(2*R*,3*S*)-**169** (*ee* 97%) was obtained after two subsequent resolutions in 20% overall yield, whereas the enantiomer (*R*)-(2*S*,3*R*)-**169** (*ee* 99%) was obtained by hydrolysis of acetate (*R*)-(2*S*,3*R*)-**170**, with K_2CO_3 in MeOH in 27% yield.

Epoxide (*R*)-(2*S*,3*R*)-**169** was converted into (3*R*,4*R*,5*R*)-**167** according to the following route. Reaction with dibutyl cuprate in THF at −40 °C led to the formation of diol (2*R*,3*R*,4*R*)-**171**, which was then transformed, via the corresponding diacetate and cleavage of the benzyl group by hydrogenolysis, into intermediate (2*R*,3*R*,4*R*)-**172**. The iodo derivative of alcohol (2*R*,3*R*,4*R*)-**172** (Ph_3P, 1H-imidazole, and I_2 in CH_2Cl_2) was submitted to ring closure by treatment with MeONa in MeOH to afford tetrahydropyranol (3*R*,4*R*,5*R*)-**173** which was acetylated to give (3*R*,4*R*,5*R*)-**167**. According to the same route, (3*S*,4*S*,5*S*)-**167** was prepared from epoxy alcohol (*S*)-(2*R*,3*S*)-**169**.

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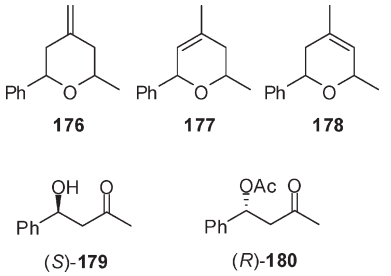

Figure 56

The other two enantiomers, that is, (3*R*,4*S*,5*R*)-**167** and (3*S*,4*R*,5*S*)-**167** were obtained by inverting the configuration of the secondary OH group of diols **171** by a S_N2 reaction. Thus, both diols (2*R*,3*R*,4*R*)- and (2*S*,3*S*,4*S*)-**171** were submitted to regioselective lipase-PS-catalyzed acetylation (MTBE, vinyl acetate) to give monoacetates **174** in almost quantitative yields. Then, the corresponding methanesulfonates were treated with a large excess of CsOAc in DMF at 70 °C, to give diacetates 1**75**. Each diacetate was submitted to the same synthetic pathway described for the preparation of (3*R*,4*R*,5*R*)-**167** and (3*S*,4*S*,5*S*)-**167**, and (3*R*,4*S*,5*R*)-**167** and (3*S*,4*R*,5*S*)-**167** were thus obtained.

The odor evaluation (Givaudan Schweiz AG, Fragrance Research) of the samples gave the following results. (3*R*,4*S*)-**166**: *Weak, floral, acidic.* (3*S*,4*R*)-**166**: *Fruity, floral, and slightly technical.* (3*R*,4*R*)-**166**: *Strong, floral, jasminic, spicy, and slightly acidic.* (3*S*,4*S*)-**166**: *Acidic, metallic, fruity.* (3*R*,4*R*,5*R*)-**167**: *Strong, floral-jasminic, slightly aromatic.* (3*S*,4*S*,5*S*)-**167**: *Very weak, slightly agrestic, fruity-floral.* (3*R*,4*S*,5*R*)-**167**: *The most powerful of this series, floral-fruity, agrestic odor in the direction of jasmine with herbaceous facets.* (3*S*,4*R*,5*S*)-**167**: *Weak, powder-waxy, slightly floral odor, in the direction of jasmine but not pronounced.*

Pelargene. The lipase-mediated transesterification of an hydroxy ketone and of a 1,5-diol intermediate were the key steps[96] in the synthesis of all the isomers of Pelargene (**176**−**178**, Quest International, Figure 56), a floral fragrance with *a powerful odor reminding of the crushed leaves of the Pelargonium plant, with a subtle spicy-floral undertone supporting the main character. Pelargene combines very well with floral notes; it is extremely fiber substantive, and can be used over quite a wide pH range.* Commercial Pelargene is a mixture of the three main regioisomers **176**−**178**: 73.7% *cis*-**176**, 1.7% *cis*-**177**, 17.7% *cis*-**178**, 0.4% *trans*-**177**, 0.8% *trans*-**178**, and 5.7% of unknown constituents.

Lipase PS-mediated acetylation of the hydroxy ketone **179** (Figure 56) gave (48 h) the acetyl derivative (*R*)-**180** (*ee* = 98%), and after prolonged enzyme-mediated acetylation (15 d), the unreacted alcohol (−)-(*S*)-**179** showing *ee* = 90%. The biocatalyzed kinetic resolution of **179** had been already reported by Nair and Joly,[97] together with the assignment of the absolute configuration. They described that, after a 28 h reaction time, *Candida rugosa* lipase afforded (+)-(*R*)-**180** (*ee* >96%) and (−)-(*S*)-**179** (*ee* = 50%).

(*R*)-**180** and (*S*)-**179** were converted separately into derivatives (1*R*,3*RS*,5*RS*)-**181** and (1*S*,3*RS*,5*RS*)-**181**, by β-metallyl magnesium chloride addition and reductive ozonolysis. Treatment of (1*R*,3*RS*,5*RS*)-**181** with lipase PS (MTBE, vinyl acetate) gave a 1:1 mixture of the two diastereoisomeric diacetates (1*R*,3*RS*,5*R*)-**182** (Figure 57). Prolonged lipase PS treatment of the survived alcohol gave a 1:1 mixture of the monoacetates (1*R*,3*RS*,5*S*)-**181**.

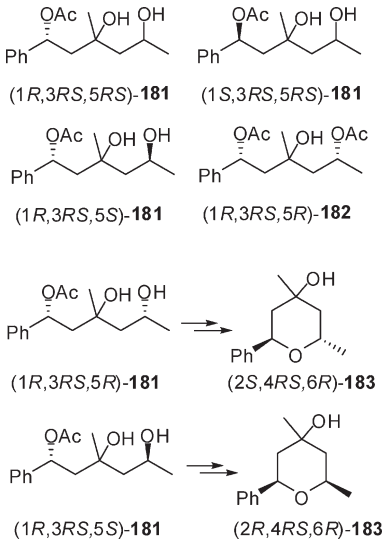

Figure 57

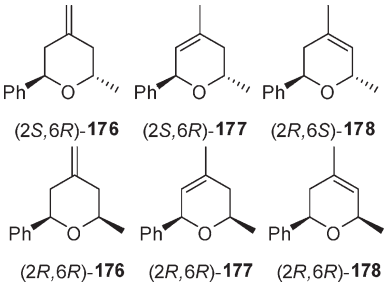

Figure 58

When the mixture of diacetates **182** was submitted to lipase PS mediated saponification (THF/H_2O, pH 7.8), a regioselective hydrolysis occurred and the 1:1 mixture of the two monoacetates (1*R*,3*RS*,5*R*)-**181** was recovered.

The two mixtures of monoacetates, that is, (1*R*,3*RS*,5*R*)-**181** and (1*R*,3*RS*,5*S*)-**181** (Figure 57), were submitted separately to ring closure by treatment with methanesulfonyl chloride (MsCl) in pyridine, followed by exposure to MeONa in MeOH, affording, respectively (2*S*,4*S*,6*R*)- and (2*S*,4*R*,6*R*)-**183**, and (2*R*,4*R*,6*R*)-**183** and (2*R*,4*S*,6*R*)-**183**, which could be recovered as single pure compounds by column chromatography.

Dehydration of the four diastereoisomeric tetrahydropyranols was performed by treatment with $POCl_3$ in pyridine, and after very accurate column-chromatographic purification the following dihydropyran products were isolated (Figure 58): (2*S*,6*R*)-**177** (98% isomeric purity, *ee* 98%), (2*R*,6*S*)-**178** (99% isomeric purity, *ee* 98%), a sample enriched in (2*S*,6*R*)-**176** (39% isomeric purity, *ee* 98%), (2*R*,6*R*)-**177** (94% isomeric purity, *ee* 98%), (2*R*,6*R*)-**178** (97% isomeric purity, *ee* 98%), and (2*R*,6*R*)-**176** (99% isomeric purity, *ee* 98%).

As for the series prepared starting from (*S*)-**179**, the enzymatic acetylation of the mixture of the four stereoisomers (1*S*,3*RS*,5*RS*)-**181** resulted to be too slow to allow practical application. Thus, it was submitted directly to ring closure and chromatographic purification to allow the separation of a mixture of the three tetrahydropyranols (2*S*,4*S*,6*S*)-**183** and (2*R*,4*RS*,6*S*)-**183** from derivative (2*S*,4*R*,6*S*)-**183** (Figure 59).

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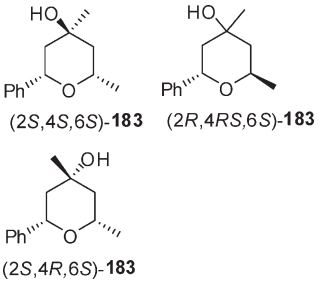

Figure 59

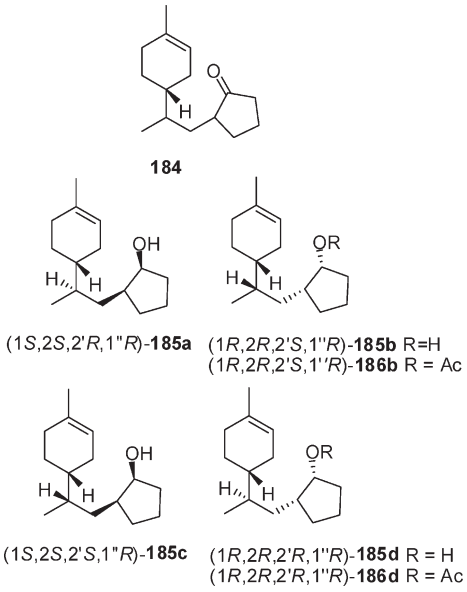

Figure 60

The dehydration reaction of the mixture of (2*S*,4*S*,6*S*)-**183** and (2*R*,4*RS*,6*S*)-**183** required a very careful and laborious chromatographic purification, which allowed the isolation of the following compounds: (2*R*,6*S*)-**177** (92% isomeric purity, *ee* 90%), (2*S*,6*R*)-**178** (94% isomeric purity, *ee* 90%), a sample enriched in (2*R*,6*S*)-**176** (33% isomeric purity, *ee* 90%), (2*S*,6*S*)-**177** (93% isomeric purity, *ee* 90%), (2*S*,6*S*)-**178** (94% isomeric purity, *ee* 90%). Treatment of (2*S*,4*R*,6*S*)-**183** with $POCl_3$ and pyridine gave (2*S*,6*S*)-**176** (97% isomeric purity, *ee* 90%).

The odor properties were thus described (Givaudan Schweiz AG, Fragrance Research). (2*S*,6*R*)-*trans*-**176** (39.5%): *Green, rose oxide like, fruity, orange-type, and somewhat dusty odor, dry down weak, green, fruit.* (2*R*,6*S*)-*trans*-**176** (24.7%): *Harsh, green, technical, pyrazine-type, vegetal odor, with acetic, rose oxide type, floral-rosy facets, dry down green, fruity, and rosy.* (2*R*,6*R*)-*cis*-**176**: *Weak, green, agrestic, and herbaceous odor, with a slightly fruity side, dry down very weak green, fruity.* (2*S*,6*S*)-*cis*-**176**: *Weak, fruity-floral, mushroom-like odor, dry down very weak, fruity floral, somewhat technical.* (2*S*,6*R*)-*trans*-**177**: *Green, petit-grain and orange flower-type pleasant odor, with aspects of neroli oil, dry down linear, but more herbal, buccoxime-like.* (2*R*,6*S*)-*trans*-**177**: *Green, floral odor, with slightly wine- and food-like nuances, and somewhat oily, technical aspects, dry down linear, weak, green, vegetal.* (2*R*,6*S*)-*trans*-**178**: *Green, metallic, rose oxide note, stronger than* (2*S*,6*R*)-*trans*-**177** *and* (2*R*,6*R*)-*cis*-**177**, *dry down green, fruity, buccoxime-like,* the most substantive of the series of enantiomers obtained from (*R*)-**180**. (2*S*,6*R*)-*trans*-**178**: *Strong, green-fruity, fresh, but also somewhat harsh-technical odor, dry down fruity, rose oxide like, with a sweet rosy touch*, the strongest of the series of enantiomers prepared from (*S*)-**179**. (2*R*,6*R*)-*cis*-**177**: *Green, metallic, rose oxide like odor, with fruity, slightly earthy, and potato-like aspects, dry down weak, green, fruity, weaker and less-substantive than* (2*R*,6*S*)-*trans*-**178**. (2*S*,6*S*)-*cis*-**177**: *Rather weak, sharp, green, rose oxide like, stem odor, with a slightly fruity and acetic tonality, dry down very weak, slightly green.* (2*R*,6*R*)-*cis*-**178**: *Fruity, green, metallic odor. dry down green fruity.* (2*S*,6*S*)-*cis*-**178**: *Strong, floral-green rose oxide odor, with a metallic inflection and a typical rose tonality, dry down linear, rose oxide like, floral, rosy.*

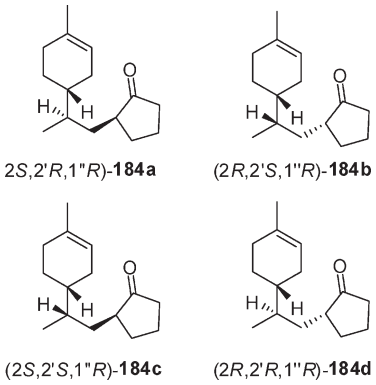

Figure 61

This work gave the authors the chance to highlight the importance of stereochemistry in odor perception. *cis*-**176** is the main component of the commercial Pelargene mixture: both its enantiomers were found to have weak, not interesting odor profiles. (2*S*,6*R*)-*trans*-**177**, (2*R*,6*S*)-*trans*-**178**, and (2*S*,6*S*)-*cis*-**178** are the most-valuable isomers of Pelargene, although they are present in very tiny amount in the commercial product.

From a synthetic point of view, the work shows the great versatility of lipase PS. It was employed for the enantioselective acetylation of hydroxy ketone **179**, to obtain optical activation. It was used for the stereoselective acetylation of monoacetate (1*R*,3*RS*,5*RS*)-**181**, to separate the two (1*R*,5*S*)-diastereoisomers from the (1*R*,5*R*)-isomers. It was also helpful for the regioselective saponification in 5-position of diacetate **182** to afford the monoacetate for the desired ring closure.

Nectaryl. Nectaryl (**184**) is a synthetic fragrance with a pleasant scent of peach and apricot, and it finds several applications as an ingredient of cosmetic formulations and laundry powders.

It was prepared as an equimolar mixture of all four possible stereoisomers by regioselective radical addition of cyclopentanone to the exocyclic double bond of (+)-limonene, catalyzed by a mixture of $Mn(OAc)_2$ and $Co(OAc)_2$ under O_2 atmosphere.

The single stereoisomers of Nectaryl were then obtained[98] according to the following route (Figure 60). The mixture of the four steoreoisomers was reduced with L-selectride in THF at −78 °C to give an equimolar mixture of *cis*-cyclopentanol alcohols **185a**−**d**.

Column chromatography allowed to separate the mixture of (1*S*,2*S*,2′*R*,1″*R*)-**185a** and (1*R*,2*R*,2′*S*,1″*R*)-**185b** (ratio 1:1 by GC) from that containing (1*S*,2*S*,2′*S*,1″*R*)-**185c** and (1*R*,2*R*,-2′*R*,1″*R*)-**185d**. The enzymatic (lipase PS, MTBE, vinyl acetate) acetylation of the two mixtures afforded the following products (Figure 60): acetate (1*R*,2*R*,2′*S*,1″*R*)-**186b** (37% yield, 92% *de*) and unreacted alcohol (1*S*,2*S*,2′*R*,1″*R*)-**185a** (56% yield, 70% *de*)

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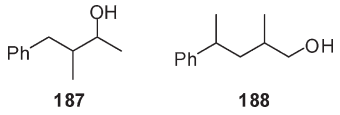

Figure 62

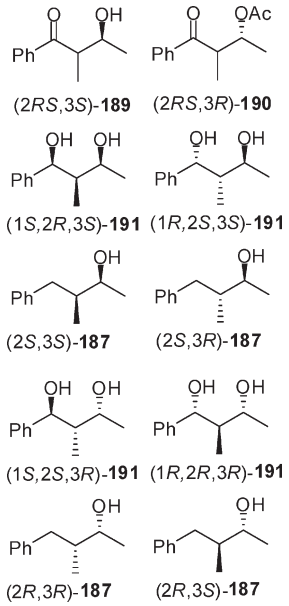

Figure 63

from the first mixture, and acetate (1*R*,2*R*,2′*R*,1″*R*)-**186d** (36% yield, *de* 82%) and unreacted alcohol (1S,2S,2′S,1″R)-**186c** (28% yield, 88% *de*) from the other one.

Dess−Martin oxidation of the diastereoisomerically enriched alcohols gave the corresponding nectaryl stereoisomers (Figure 61): (2*S*,2′*R*,1″*R*)-**184a** (92% yield, *de* 81% by GC), (2*R*,2′*S*,1″*R*)-**184b** (92% yield, *de* 89%), (2*S*,2′*S*,1″*R*)-**184c** (92% yield, *de* 80%), and (2*R*,2′*R*,1″*R*)-**184d** (92% yield, *de* 86%).

All the samples of nectaryl were submitted to professional olfactory evaluation with the following results (Givaudan Schweiz AG, Fragrance Research): Nectaryl (mixture **184a**−**d**), *powerful in the direction of peach and apricot* (odor threshold = 0.354 ng/L air); (2*S*,2′*R*,1″*R*)-**184a**, *rather weak and uncharacteristic fruity-lactonic odor with some additional resemblance of green apple, it does not contribute much to the commercial nectaryl* (odor threshold = 11.2 ng/L air); (2*R*,2′*S*,1″*R*)-**184b**, *powerful, very intense, sweet and dry fruity-lactonic odor in the direction of peach and apricots, with some floral undertone, does contribute much to the overall odor of commercial nectaryl* (odor threshold = 0.094 ng/L air); (2*S*,2′*S*,1″*R*)-**184c**, the same as **184a** (odor threshold = 14.9 ng/L air); (2*R*,2′*R*,1″*R*)-**184d**, the same as **184b** (odor threshold = 0.112 ng/L air).

Muguesia and Pamplefleur. Muguesia (**187**) and Pamplefleur (**188**) (Figure 62) are commercial fragrances (IFF) sold as mixtures of two racemic diastereoisomers. Muguesia is described as *floral, muguet, rose, and minty*, and its usage is suggested in products where aldehydic muguet ingredients are not stable. Pamplefleur is described as *citrus, grapefruit, floral,vetivert, green, and diffusive*, and it is given as *the animal moiety for jasmin types*.

The enzyme-mediated approaches to all the steroisomers of odorants **187** and **188** were optimized and their odor properties were evaluated.[99]

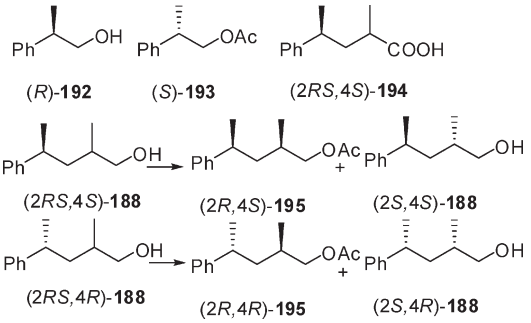

Figure 64

Lipase PS-mediated acetylation of the 1:1 mixture of the two racemic stereoisomers of hydroxy ketone **189** (MTBE, vinyl acetate) gave after 24 h an acetate fraction, which was found to be a 3.1:1 mixture of the two enantiopure (*ee* = 99%) 3R diastereoisomers of derivative **190** (Figure 63). The unreacted alcohol was submitted to prolonged lipase PS-catalyzed transesterification, and at the end of the procedure, the alcoholic fraction resulted to be a 1:1 mixture of the two enantiopure (chiral HPLC) 3S diastereoisomers of compound **189**. The mixture of (2*RS*,3*S*)-**189** was reduced with sodium boron hydride to afford, after column chromatography, diols (1*S*,2*R*,3*S*)-**191** (*de* = 99%, GC/MS) and (1*R*,2*S*,3*S*)-**191** (*de* = 99%, GC/MS). Hydrogenolysis of the two diols gave (2*S*,3*S*)-**187** and (2*S*,3*R*)-**187**.

The mixture of (2*RS*,3*R*)-**190** was reduced with lithium aluminum hydride to afford, after purification on silica gel column, diols (1*S*,2*S*,3*R*)-**191** (*de* = 99%, GC/MS) and (1*R*,2*R*,3*R*)-**191** (*de* = 99%). These latter derivatives were submitted to hydrogenolysis to afford (2*R*,3*R*)-**187** and (2*R*,3*S*)-**187**. A different diastereoselective course was unexpectedly observed in $NaBH_4$ and $LiAlH_4$ reductions.

The absolute configurations of Muguesia stereoisomers was established by chemical correlation. They were evaluated by perfumers with the following results. (2*R*,3*S*)-**187**: *Top note floral, rosy, buttery, rich, slightly green; dry down floral, sweet, lily of the valley, and linalool-like.* (2*S*,3*S*)-**187**: *Top note floral, balsamic, sweet, floral, slightly fruity; dry down floral, cinnamic, balsamic, sweet.* (2*S*,3*R*)-**187***: Top note weak, floral-hesperidic, tea-like; dry down empty.* (2*R*,3*R*)-**187**: *Top note very weak, slightly acidic and agrestic, dry down empty.*

(*R*)- and (*S*)-2-phenyl-1-propanol (**192**) are commercial products, but the need for a large quantity of enantiopure (*R*)- and (*S*)-**192**, to be used as starting materials for the preparation of the four isomers of Pamplefleur, promoted the investigation of a lipase-mediated kinetic resolution of racemic primary alcohol **192** (Figure 64).

Only a few examples of the lipase-mediated kinetic resolution of racemic **192** were reported in the literature. PPL-catalyzed acetylation in water-saturated hexane, in the presence of vinyl acetate, gave after 12 h at 30 °C an acetate derivative and an unreacted alcohol with poor enantiomeric excess (*ee* = 65% and 69%, respectively).[100] Naemura et al. reported that when racemic **192** was treated with Lipase YS at 30 °C in diisopropyl ether solution, with isopropenyl acetate as an acyl donor, after 7 h, a (−)-acetate derivative and a (+)-unreacted alcohol were obtained, with very low optical purity.[101] Better results were described for lipase PS-mediated transesterification of racemic **192** with vinyl 3-(para-substituted phenyl)propanoates in cyclohexane: (*S*)-**193** (*ee* =97−98%) and (*R*)-**192**(*ee* =34−56%) were obtained.[102] Enantiomeric ratios not higher than 2.7 were described when sol−gel encapsulated lipases were employed.[103]

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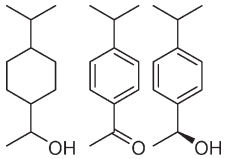

Figure 65

Short reaction times and the use of PPL allowed the authors to recover acetate **193** with *ee* = 92% (Figure 64). The unreacted alcohol was enriched with the (*R*)-isomer (*ee* = 91%, chiral GC) by prolonged PPL-mediated transesterification. (*R*)- and (*S*)-**192** were converted into the corresponding bromo derivatives, which were then submitted to malonic reaction with methylmalonic acid diethyl ester. Saponification and decarboxylation of the reaction mixture allowed the preparation of (2*RS*,4*S*)-**194** and (2*RS*,4*R*)-**194**, which were reduced to alcohols (2*RS*,4*S*)-**188** and (2*RS*,4*R*)-**188**, respectively. Treatment of the two mixtures with PPL (MTBE, vinyl acetate) allowed the recovery, after short reaction times, of acetate (2*R*,4*S*)-**195** (*de* = 84%) and of acetate (2*R*,4*R*)-**195** (*de* = 85%), respectively. PPL treatment of each mixture was then prolonged to increase the de of the corresponding unreacted alcohols: (2*S*,4*S*)-**188** (*de* = 52%) and (2*S*,4*R*)-**188** (*de* = 87%). The catalyzed enantioselective acetylation of the primary function of alcohol **188** was thus employed to separate the two enantiopure diastereoisomers.

The odor evaluation of Pamplefleur isomers were as follows (Givaudan Schweiz AG, Fragrance Research): (2*R*,4*S*)-**188**, *natural fruity odor in the direction of grapefuit and rhubarb, close to gardenol (methyl phenyl carbinyl acetate) and 2,5-dimethyloct-2-en-6-one, slightly metallic*; (2*S*,4*S*)-**188**, *floral-fruity odor in the direction of grapefruit and linalool with earthy, woody, and bitter nuances, also reminiscent of 2,5-dimethyloct-2-en-6-one and of some aspects of vetiver oil*; (2*S*,4*R*)-**188**, *fruity-citric odor, with some harsh, animalic, and slightly woody nuances, also is a bit rubbery*; (2*R*,4*R*)-**188**, *floral-fruity odor in the direction of rhubarb with a touch of grapefruit, reminiscent of gardenol (methylphenyl carbinyl acetate)*.

The Pamplefleur isomers *have a high tenacity on blotter (>24 h), so they are all quite substantive, and each of them contributes to a particular facet of the final odorant, to produce an unique blend.*

On the contrary, the configuration at the carbon atom in position 3 seems to be important in establishing the odor properties of Muguesia: the (3*R*) stereoisomers are weak and completely devoid of odor in the dry down note, while the (3*S*) stereoisomers are the effective odor vectors of commercial Muguesia, and a BY mediated approach to the preparation of this mixture has been optimized (see section 3.2).

Mugetanol. Mugetanol (**196**) is *a floral fragrance with a light herbal, floral note reminiscent of lily of the valley, and typical, natural waxy connotations. Mugetanol is used in lily of the valley fragrances, it is characterized by high stability and it is suitable for a wide range of applications* (Figure 65). Its structure combines a stereogenic center with the possibility of *cis*−*trans*-cycloalkane stereoisomerism, thus allowing four different stereoisomers. In particular, (*S*)-*cis* **196** was found to be characterized by the highest odor intensity.[104]

The chemoenzymatic preparation of the stereoisomers of Mugetanol has been recently reported[105] starting from commercially

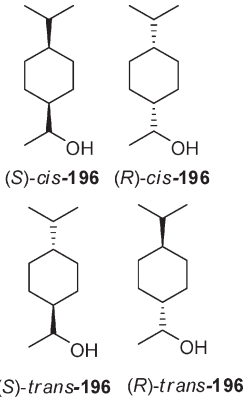

Figure 66

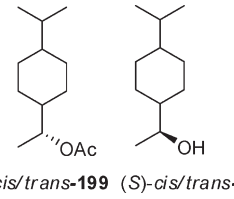

Figure 67

available 4-isopropylacetophenone (**197**), according to three different strategies:

(a) Chemical reduction of ketone **197**, lipase-mediated kinetic resolution of alcohol rac-**198**, and catalytic hydrogenation to afford optically active **196**.
(b) Bioreduction of **197** using alcohol dehydrogenases, followed by chemical hydrogenation.
(c) Catalytic hydrogenation of compound **197** to afford alcohol rac-**196**, followed by lipase-catalyzed kinetic resolution.

$NaBH_4$ reduction of ketone **197** yielded racemic alcohol **198**, which was submitted to lipase-mediated transesterification (Figure 65), at 30 °C using three equivalents of vinyl acetate and *Candida antarctica* lipase B (CAL-B) or *Pseudomonas cepacia* lipase (PSL-C I) as biocatalysts. Both enzymes showed a total preference for the acetylation of the (*R*)-enantiomer, being the reaction rate higher with PSL-C I rather than with CAL-B. Enantiopure (*S*)-**198** (Figure 65), obtained from PSL-C I catalyzed acetylation, was hydrogenated under pressure (80 atm) at room temperature with a catalytic amount of chloro-(1,5-hexadiene)rhodium, in a biphasic medium (THF/hexane/phosphate buffer pH 7.4) in the presence of tetrabutylammonium hydrogen sulfate (TBAHS) as a phase transfer, to afford alcohol (*S*)-**196** as a mixture of cis and trans diastereoisomers (diastereoisomeric excess = 64%).

In a complementary approach for the synthesis of Mugetanol the enantioselective bioreduction of ketone **197** was investigated, using different commercial alcohol dehydrogenases. ADH type T, CP, RS1, and A afforded with complete enantioselectivity (*S*)-**198**, while ADH type LB and PR2 provided enantiopure (*R*)-**198**. The highest conversions were achieved for (*S*)-**198** when ADH A and especially ADH RS1 were employed, while (*R*)-**198** was obtained with the highest conversion using ADH LB.

The catalytic hydrogenation of optically active **198** with H_2 (80 atm), $(1,5\text{-HDRhCl})_2$, and TBAHS at room temperature afforded a mixture of *cis*- and *trans*-(*S*)-**196** (Figure 66).

dx.doi.org/10.1021/cr100289r |*Chem. Rev.* 2011, 111, 4036–4072

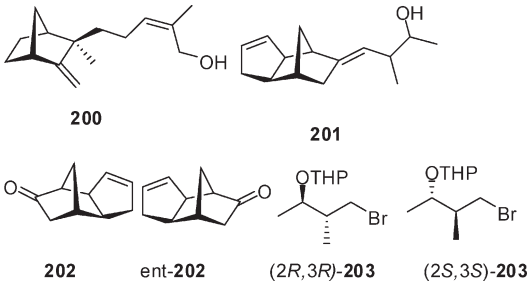

Figure 68

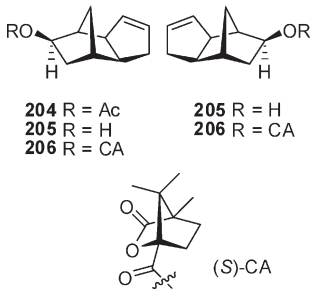

Figure 69

A third attempted approach, based on the catalytic hydrogenation of compound **197** to afford alcohol rac-**196**, to be then submitted to lipase-catalyzed kinetic resolution was not successful: hydrogenation afforded a complex mixture of products which could not be separated by column chromatography. However, some alcohol rac-**196** was recovered and the lipase-catalyzed kinetic resolution could be investigated. PSL-C I catalyzed acetylation afforded acetate (Figure 67) (*R*)-*cis*-**199** with *ee* = 99% and *de* = 15%, and unreacted alcohol (S)-*cis*-**196** with *ee* = 60% and *de* = 16%. CAL-B gave acetate (*R*)-*cis*-**199** with *ee* = 99% and *de* = 25%, and left an unreacted (*S*)-*cis*-**196** alcohol with 99% *ee* and *de* = 22%.

2.2.2. Wood and Balmy Fragrances. East-Indian sandalwood oil (*Santalum album* L.) is one of the oldest ingredients employed in perfumery, and it is still today a highly precious raw material for fragrances. In 1935, Ruzicka and Thomann elucidated the structure of β-santalol (**200**), the olfactorily most important sandalwood-oil constituent.[106] No industrially feasible process has yet been developed for the synthesis of β-santalol, and the perfumers have to use either very expensive natural sandalwood oils or cheaper synthetic substitutes. Thus, the search of new molecules showing sandalwood odor is in continuous progress.

In 2004 Pickenagen[107] described the preparation of the single stereoisomers of a sandalwood odorant (Fleursandol, **201**) by reaction of the enantiomers of ketone **202** with the enantiomers of butanol derivative **203** (Figure 68).

The optical activation step in the synthesis of the tricyclic ketones **202** and ent-**202** was the kinetic resolution of acetate rac-**204** (Figure 69) by treatment with *porcine pancreas* type-II lipase in a phosphate buffer at pH 7 over 11 days. The transformation was stopped when the alcohol ent-**205**/acetate **204** ratio, reached the value of 55:45 (GC). The enantiomer excess of **205**, obtained upon saponification of acetate **204**, and of the unreacted alcohol ent-**205** resulted to be 60% and 48%, respectively.

Enantiomerically enriched alcohol **205** was treated with (−)-(1*S*)-camphanoyl chloride in pyridine, and derivative **206** was brought to diastereoisomeric purity by crystallization from Et_2O. The absolute configuration of compound **206** was determined relative to (−)-(1*S*)-camphanic acid by X-ray diffractometry of a single crystal. Cleavage of ester **206** and oxidation of the resulting alcohol completed the synthetic sequence to **202**. According to the same procedure alcohol ent-**205** was converted into ketone ent-**202**. Crystallization of camphanate derivative (*R*)-**206** was used to increase the enantiomeric excess of the corresponding alcohol.

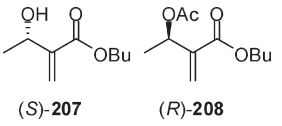

Figure 70

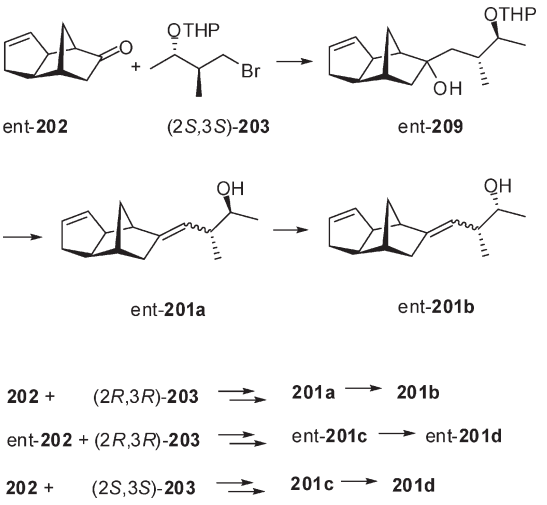

Figure 71

As for the synthesis of alcohols (2*R*,3*R*)-**203** and (2*S*,3*S*)-**203**, rac-**207**, prepared via Baylis−Hillman reaction from acetaldehyde and butyl propenoate with DABCO (1,4-diazabicyclo-[2.2.2]octane) in 95% yield, (Figure 70), was submitted to kinetic resolution with *Pseudomonas* AK lipase and vinyl acetate in hexane. 41% of (−)-(*S*)-**207** (*ee* >99%) and 43% of (+)-(*R*)-acetate **208** (*ee* >98%) were obtained. (−)-(*S*)-**207** and (+)-(*R*)-acetate **208** were transformed into (2*S*,3*S*)-**203** and (2*R*,3*R*)-**203**, respectively, by hydrogenation with rhodium catalyst, to afford stereoselectively the anti isomer, followed by THP protection of the alcohol group, reduction, and bromination.

Enantiomerically pure compound **202** and ent-**202** and (2*R*,3*R*)-**203** and (2*S*,3*S*)-**203** were then assembled to afford the target tricyclic target molecules **201** and ent-**201**.

Lithium Grignard reaction of ketone ent-**202** with bromide (2*S*,3*S*)-**203** gave alcohol ent-**209**, which was dehydrated by reaction phosphorus oxychloride ($POCl_3$) in pyridine, and finally deprotected to give *anti*-alcohol ent-**201a** as a 80:20 mixture of (*E*/*Z*)-isomers (Figure 71). Similar results were observed for **201a** prepared from **202** and (2*R*,3*R*)-**203**; however, for ent-**201c** and **201c** the (*E*/*Z*) ratio changed to 20:80. The *anti*-alcohols **201a** and ent-**201a**, and **201c** and ent-**201c** were then converted into the corresponding *syn*-products **201b** and ent-**201b**, and **201d** and ent-**201d**, respectively, by Mitsunobu reaction and subsequent saponification of the corresponding benzoates.

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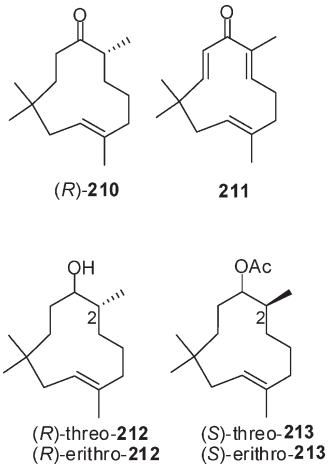

Figure 72

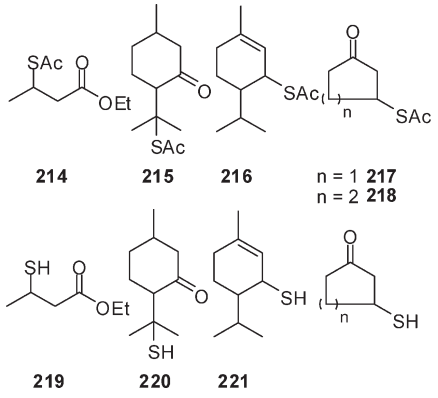

Figure 73

Evaluation of all 16 diastereoisomers (*E*/*Z*)-**201a**−**d** and (*E*/*Z*)-ent-**201a**−**d** by GC olfactometry highlighted that only the (*E*)-isomers contributed significantly to the odor, while the corresponding (*Z*)-isomers were found to be nearly odorless. As for the configuration of the tricyclic skeleton, compounds ent-**201a**−**d** showing the stereochemical sequence (1*R*,2*R*,6*R*,7*R*) of the ring system show the characteristic long-lasting sandalwood odor, while compounds **201a**−**d** were significantly weaker and smelled woody to floral.

Tetrahydrozerumbone **210** (Figure 72) is a powerful balmy fragrance, which can be easily prepared from zerumbone **211**, a monocyclic sesquiterpene found as the major component of the essential oil of wild ginger, *Zingiber zerumbet* Smith.[108]

Racemic tetrahydrozerumbone **210**, prepared by Pd/C hydrogenation of zerumbone **211**, was treated with $LiAlH_4$ in dry Et_2O at 0 °C for 30, to afford a mixture of diastereoisomers, which was separated by silica gel chromatography to afford racemic *threo*-**212** and *erythro*-**212** in 34% and 39% yields, respectively. The relative configuration of racemic *threo*-**212** was determined by X-ray analysis. Lipase-catalyzed kinetic transesterifications of *threo*-**212** and *erythro*-**212** were investigated: the best results were obtained with dry MeitoQLM lipase in THF solution at 35 °C in the presence of isopropenyl acetate. After 24 h and 30 h, repectively, unreacted alcohol (2*R*)-*threo*-**212** and acetate (2*S*)-*threo*-**213** in 99.8% and 91.3% *ee*, and unreacted alcohol (2*R*)-*erythro*-**212** and acetate

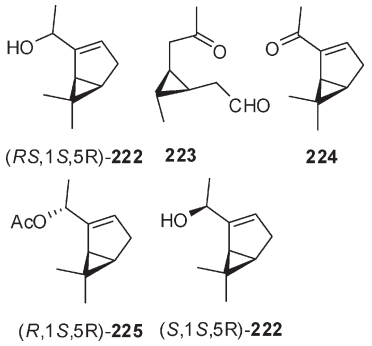

Figure 74

(2*S*)-*erythro*-**213** in 99.4% and 91.2% *ee* were obtained with 52% conversion on both substrates. The absolute configuration of alcohol (2*R*)-*erythro*-**212** was determined by anomalous dispersion X-ray analysis the corresponding 4-chloro-3,5-dinitrobenzoate.

Alcohols (2*R*)-*threo*-**212** and (2*R*)-*erythro*-**212** were oxidized using the Dess−Martin agent to give (*R*)-**210**, while the oxidation of the alcohols recovered from the saponification of actetates (2*S*)-*threo*-**213** and (2*S*)-*erythro*-**213** afforded (*S*)-**210**. Thus, opposite enantioselectivity was observed for lipase-mediated acetylation of the OH groups of *threo*-**212** and *erythro*-**212**. The olfactory properties of the two enantiomers were evaluated: the (*R*) enantiomer was found to show the typical balmy fragrance.

2.2.3. Miscellaneous. A novel method for the preparation of thiol derivatives has been recently developed,[109] consisting of the addition of thioacetic acid to olefins in 80−99% yield, followed by enzymatic solvolysis of the obtained thioesters (18−90%). This approach avoids the handling of H_2S, and it occurs without nonselective formation of rearranged products.

The In(III)-catalyzed (5 mol % $InCl_3$ at 60 °C) hydrothioacetylation of ethyl *trans*-but-2-enoate, (*R*)-carvone, α-pinene, 2-cyclopentenone, and 2-cyclohexenone by means of thioacetic acid yielded the corresponding the Michael-type adducts **214**-**218** (Figure 73) through a conjugate addition. Compound **215** was obtained as a 8:2 mixture of trans and cis diastereoisomers and **216** as a 7:3 mixture of two diastereoisomers.

The enzymatic solvolysis of the thioester group, which was poorly documented in the literature, was then investigated with several hydrolases. Substrate **214** gave the choice to investigate an interesting problem of chemoselectivity, that is, whether or not the thioester function could react faster than the oxoester one. CRL-mediated reaction in aqueous buffer led mainly to hydrolysis of the ester group. When a 3:2 mixture of toluene and ethanol was employed, with ethanol acting as a nucleophile, thiol **219** was obtained with 50% yield and 48% *ee*.

Thioester **215** (1*R*,4*R*)/(1*R*,4*S*) (8:2) was converted into thiol **220** (1*R*,4*R*)/(1*R*,4*S*), (9:1) in the presence of CRL in pH 7 aqueous buffer at 40 °C in low yield (18%), probably as a consequence of the steric hindrance at the carbon atom bearing the thioester group. *trans*-(1*R*,4*R*)-**215** was purified by chromatography over silica gel and submitted to enzyme-catalyzed solvolysis. The fragrant thiol (1*R*,4*R*)-**220**, called mangone, which finds applications in flavouring preparations, was observed after 6 d only in 11% yield.

The enzymatic hydrolysis of thioester **216** (7:3 mixture of diastereomers) was perfomed using 100% w/w CRL in aqueous medium containing 5% v/v DMF, yielding thiol **221** in 27% yield

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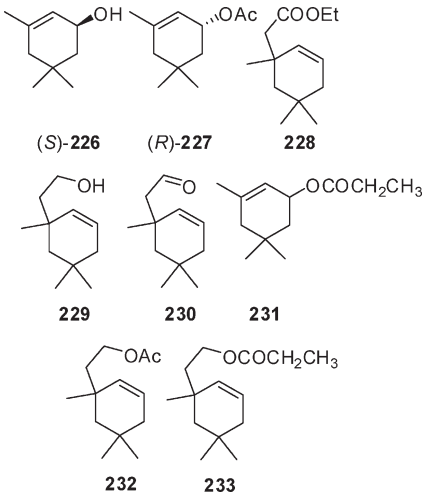


Figure 75

as a 7:3 mixture of diastereoisomers. The conversion increased to 74% after 22 d.

Thioesters **217** and **218** were converted in 62−98% yields into their thiols by CRL, ANL, or AAL in pH 7 and pH 8 aqueous buffer at 40 °C with no enantioselectivity. According to the authors, a necessary evolution of the work had to be the optimization of the biocatalyst, in order to improve the stereopreference of this specific reaction.

The enantiopure stereoisomers of the odorous secondary allylic alcohol **222** (Figure 74), showing a bicyclo[3.1.0]hexane moiety, and the corresponding esters were prepared,[110] starting from (+)-3-carene, a natural, inexpensive, raw material which is widely available a major component of Polish turpentine obtained from *Pinus sylvestris* (L).

Ozonolysis of (+)-3-carene followed by intramolecular aldol condensation of ketoaldehyde **223** afforded compound **224**, which was reduced by lithium aluminum hydride reaction to alcohol (*RS*,1*S*,5*R*)-**222**, as a 85:15 mixture of two diastereoisomers.

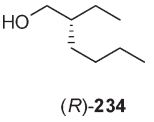
The enzyme-mediated transesterification of allylic alcohol **222** in the presence of lipases from *Burkholderia cepacia*, *Pseudomonas fluorescens*, and *Candida rugosa* was investigated, using vinyl acetate as an acyl donor (Figure 74). The best results were achieved with Amano PS lipase from *B. cepacia* at 37 °C for 24 h: stereoisomer (*R*,1*S*,5*R*)-**225** was obtained together with a mixture of alcohols (*S*,1*S*,5*R*)-**222** (which was not a substrate for this enzyme) and alcohol (*R*,1*S*,5*R*)-**222** in an 89:11 ratio.

Acetate (*R*,1*S*,5*R*)-**225** was reduced with lithium aluminum hydride to afford the secondary alcohol (*R*,1*S*,5*R*)-**222**. This latter was transformed into the corresponding crystalline *p*-nitrobenzoate derivative, whose relative configuration was determined by X-ray analysis.

The enzymatic hydrolysis of (*RS*,1*S*,5*R*)-**225** gave very modest results.

The comparative analysis of the odoriferous properties of alcohols (*RS*,1*S*,5*R*)-**222**, (*R*,1*S*,5*R*)-**222**, (*S*,1*S*,5*R*)-**222** and esters (*RS*,1*S*,5*R*)-**225**, (*R*,1*S*,5*R*)-**225** showed that all these compounds are medium intensive with fruity or herbal-balsamic note.

During an investigation devoted to the syntheses of bicyclic lactones showing a methyl substituted ring, Wawrzeńczyk et al. observed that some of the hydroxyl and acetoxy intermediates they had in their hands showed interesting floral and fruity odors, and they decided to start a careful investigation of odor-structure



(*R*)-**234**

Figure 76

relationship of these compounds.[111] Isophorone was employed as a staring material, and it was reduced by means of $LiAlH_4$ to afford rac-**226** (Figure 75).

This latter was submitted to lipase-mediated acetylation, and nine different commercial lipases were used. CCL and lipase from *Bulkholderia cepacia* resulted to be the most effective catalysts, and they were found to be characterized by opposite enantioselectivity. CCL gave (vinyl acetate, hexane, rt) after 3 days (*S*)-**226** (57%, *ee* = 48%) and (*R*)-**227** (43%, *ee* = 72%). The unreacted alcohol was submitted again to lipase tranesterification, and afforded after 6 days (*S*)-**226** (56%, *ee* = 98%) and nearly racemic **227** (44%). When rac-**226** was submitted to tranesterification in the presence of lipase from *Bulkholderia cepacia*, alcohol (*R*)-**227** showing *ee* = 98% was recovered after prolonged reaction time. The highly stereoselective Claisen rearrangement of (*R*)- and (*S*)-**226** afforded esters (*R*)- and (*S*)-**228** without loss of optical purity. These derivatives were reduced with $LiAlH_4$ to afford (*R*)- and (*S*)-**229** which were further oxidized to the corresponding aldehydes (*R*)- and (*S*)-**230** by means of PCC. The propanoate and butanoate esters of (*R*)- and (*S*)-**226**, and (*R*)- and (*S*)-**229** were also prepared. The odor properties of the enantiomers of all these compounds were evaluated with the following results. Rac-, (*R*)-, and (*S*)-**226** showed *very similar odors, with terpenic, camphor, and borneol note.* Rac-, (*R*)-, and (*S*)-**230** *were very similar, camphoric with less or more marked methanolic notes.* The odor of butanoate esters were definitely unpleasant. (*S*)-**228** was describes as *medium-intensive, fruity with plum note,* and the enantiomer (*R*)-**228** was *as well medium-intensive, floral-fuity with quince note.* (*S*)-**229** was found to be *more intensive than the racemate, fruity with nmentholic notes,* while the enantiomer (*R*)-**229** *was weak-intensive, fruity woth pear note.* (*S*)-**227** was *weaker than the enantiomer, fruity with forest berry note,* while the enantiomer (*R*)-**227** showed *a blueberry note.* (*S*)-**231** was *similar to the racemate, fruity with freshly collected blackcurrant note,* while the enantiomer had *a forest berry note.* (*S*)-**232** was found to be *medium-intensive, airy, sweet with pear note, more fruity than the rac-232;* (*R*)-**232** was *weak-intensive, fruity with pear note.* (*S*)-**233** *was found to have a pleasant fruity odor with a cherry note, while the enantiomer possessed a fruity odor with fresh planed board note.*

2-Ethylhexanol (**234**, Figure 76) is one of the most widely used yet least expensive racemic industrial products.[112]

The racemic material is characterized by an intensive floral, earthy smell, which is different than that of the each single enantiomer. The *R*-enantiomer is described to be *heavy, earthy, and slightly floral*, whereas the *S*-enantiomer shows *a light, sweet floral fragrance*.[113]

The (*R*)- and (*S*)-enantiomers of 2-ethylhexanol were prepared by lipase-catalyzed transesterification of rac-**234** involving lipase from *Pseudomonas* species (PSL), vinyl acetate as acyl donor, and chromatographic separation of products.[114] A larger-scale preparation procedure has been recently optimized employing vinyl laurate as an acyl donor: the unreacted alcohol and the laurate ester could be separated by fractional distillation. *S*-**234** could be recovered with optical purity >90% by sequential kinetic resolution.[115]

dx.doi.org/10.1021/cr100289r |*Chem. Rev.* 2011, 111, 4036–4072

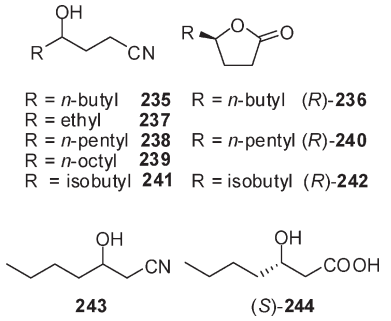


Figure 77

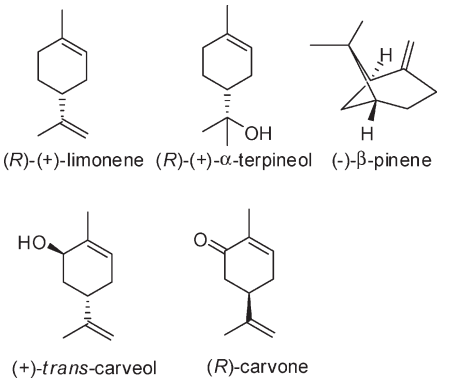


Figure 78

Many components of natural flavors and fragrances are γ-butyrolactones, and they can be produced in one-step process by hydrolysis of 4-hydroxynitriles to the corresponding hydroxyacid and subsequent lactonization. Microbial hydrolysis of hydroxynitriles has been investigated[116] by using nitrilases as biocatalysts, both on straight and branched chain substrates. The reactions could be performed in milder, and more environmentally friendly conditions (pH 6 or 7 and temperatures ranging from 15 to 35 °C) than typical chemical hydrolysis.

The ability to convert 4-hydroxyoctanenitrile (**235**, Figure 77) enantioselectively into the corresponding lactone **236** was first investigated with six commercially available nitrilases.

NIT 1001 gave a racemic product; NIT 1002 and 1003 produced the (*R*)-enantiomer with *ee* = 32% and 52%, respectively, whereas NIT 1004, 1005, and 1006 were selective for the (*S*)-enantiomer (*ee* = 42%, 10%, 20%, and 20%, respectively).

Nitrilases NIT 1002, 1003, and 1004 seemed to be the most promising, hence the authors tried to enhance the selectivity of these enzymes by decreasing the temperature from 35 to 30 °C and the pH from 7 to 6. Substrates **237**−**239** were also considered.

The highest enantioselectivity was observed with substrate **238**, in the presence of NIT 1002, at 30 °C and pH = 7: after a reaction time of 11.5 h lactone (*R*)-**240** was obtained with a conversion of 42% and an *ee* = 70%. A further decrease of temperature to 15 °C actually decreased the *E*-value and prolonged the reaction times.

The reaction was then studied on a branched substrate, 4-hydroxy-6-methylheptanenitrile (**241**).

No reaction was observed with NIT 1001 and 1006, while the best results were obtained with NIT 1004: (*R*)-**242** was recovered after 2 h with *ee* =70% and a conversion 27.5%.

The investigation was completed by considering also β-hydroxynitrile **243** to observe the effect of setting the stereogenic center closer to the reacting nitrile group. No spontaneous lactonisation was expected in this case because of steric effects. An improvement of enantioselectivity was obtained with NIT 1002, which afforded (*S*)-**244** with *ee* = 82%, after 5 h and a conversion of 13%.

More selective enzymes will be developed, but the conversion can be of potential use for of these kinetic resolutions.

3. CHIRAL FRAGRANCES AND FLAVORS BY BIOCATALYZED STEREOSELECTIVE REACTIONS

The practical applications of biocatalyzed stereoselective reactions are still modest, in spite of the Earth's diversity of useful microorganisms and the wide spectrum of reactions they can trigger. Active biocatalysts can be obtained by screening a broad variety of microorganisms, which are widespread in Nature. Fungi have traditionally been one of the most studied whole cell systems for microbial natural product isolation and also for biotransformation reactions.[117] Bioconversions are usually chemo-, regio-, and stereoselective, they allow the avoidance of protection−deprotection steps, and they can afford novel and useful products that are difficult or impossible to be obtained through conventional chemical procedures. Some limitations encountered in the past in the practical applications of bioconversions, such as enzyme availability, substrate scope, and operational stability, can be overcome by recent scientific progress in genomics, directed enzyme evolution, and the exploitation of biodiversity.

It is possible to find enzyme catalysts for most of the reactions of organic chemistry,[118] including the formation of C−C bonds, oxidation, and reduction reactions. The preparation of odorous compounds by enzyme-mediated oxidations, mainly stereoselective hydroxylations, and reductions, mainly by baker's yeast (BY) reductions of activated carbon carbon double bonds, are herein reported. Microbial ester formation or hydrolysis have been not considered.[119]

3.1. Stereoselective Oxidations

3.1.1. Terpene Oxidations. Terpene hydrocarbons and their oxyfunctionalized derivatives, the so-called "terpenoids", are among the most frequently investigated substrates of microbial oxidations. Oxofunctionalization of the often odorless precursor terpene hydrocarbons by plants and microorganisms occurs predominantly at allylic positions of the carbon skeleton. The advantage is also that terpenes are very attractive renewable feedstock for sustainable chemical synthesis,[120] because of their wide availability, well-defined absolute configuration, and high reactivity.

The key challenge is the development of highly selective and productive methods to convert terpenes in useful products. While chemical methods suffer from poor selectivity, biotransformations are a useful alternative and provide the additional advantage of producing natural identical aroma and flavor compounds. Over the years, biotransformations of terpenes have been intensively investigated and also recently reviewed.[121] Thus, only some recent examples are herein reported to convey a general idea of the practical applications of this kind of reactions. A selection was made of those stereoselective reactions transforming one starting material in mainly one product.

(*R*)-(+)-α-terpineol (Figure 78) has *a floral, typically lilac odor* while (*S*)-(−)-α-terpineol has *a characteristic coniferous odor*.

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α-Terpineol is one of the most commonly used fragrance compounds, and it is mostly produced chemically, starting from pinene or crude turpentine oil by acid hydration to terpine, followed by partial dehydration. In this way, α-terpineol is commercially available at relatively low price.[122] Thus, the implementation of microbial process requires high yields of α-terpineol to be competitive compared to the chemical transformation.

The bioconversion of limonene to α-terpineol as the main end product has been described using a wide range of microorganisms as catalyst: *Pseudomonas sp.*,[123] *Penicillium digitatum*,[124] and *Pseudomonas gladioli*[125]

In 2007 a study appeared in the literature[126] using two relevant Brasilian industrial wastes (orange essential oil and cassava wastewater) in a biotechnological process for the transformation of (*R*)-limonene into (*R*)-α-terpineol.

The composition of the orange essential oil was determined by GC-MS and (*R*)-(+)-limonene was found to account for more than 94% of the total content. Cassava wastewater was shown to be a suitable substrate for mycelial growth, leading to good, rapid growth with all the fungal strains tested (*Penicillium sp*. 2025, *Aspergillus sp*. 2038, *Fusarium oxysporum* 152B), reaching, for example, 29.4 g/L (dry weight) after 3 days of growth with *Penicillium sp*. 2025. The best (*R*)-(+)-α-terpineol yields were achieved when the strains were grown in cassava media and the mycelia then transferred to a new flask containing mineral medium and orange essential oil as the sole C- and energy source. One of the strains tested, *Fusarium oxysporum* 152B, converted (*R*)-(+)-limonene to (*R*)-(+)-α-terpineol, yielding nearly 450 mg/L after 3 days of transformation with a low amount (10 mg/L after 96 h) of perillyl alcohol as a by product. Growth in the presence of a solution of 1% orange essential oil in decane did not increase the transformation yields.

An investigation of the biohydroxylation of (*R*)-(+)-limonene into *trans*-carveol (Figure 78) was performed.[127] Carveol is a useful and valuable fragrance and flavor additive, and it is prepared in the *cis*-form[128] or as a mixture of trans- and cis-forms[129] by reduction of (*S*)-(+)-carvone which is chemically produced from (*R*)-(+)-limonene.

Accurate screening of microorganisms showed that *Cellulosimicrobium cellulans* EB-8-4 was a powerful catalyst for the regio- and stereoselective allylic hydroxylation of (*R*)-(+)-limonene to (+)-*trans*-carveol. Cells of strain EB-8-4 were easily obtained by growing on ethylbenzene and showed a specific hydroxylation activity of 4.0 U/g cdw (cell dry weight), and accepted 62 mM (*R*)-(+)-limonene without inhibition. The hydroxylation was probably catalyzed by a nicotinamide adenine dinucleotide (NADH)-dependent oxygenase involved in the degradation of the aromatic ring during cell growth. 13.4 mM of (+)-*trans*-carveol were obtained by biohydroxylation of (*R*)-(+)-limonene with resting cells of *C. cellulans* EB-8-4, thus being 11 times higher than that obtained with the best biocatalyst known thus far. High conversion and high yield were obtained in the transformation of 11.6 mM of (*R*)-(+)-limonene in a closed shaking flask, giving 10 mM of (+)-*trans*-carveol, and 0.30 mM of carvone as the only byproduct.

The bioconversion of *β*-pinene (Figure 78) to α-terpineol has been hardly described in the literature. Recently, a detailed study related to the bioconversion of (−)-*β*-pinene and *R*-(+)-limonene into the corresponding oxygenated compounds has been reported.[130]

400 microorganisms were tested for their ability to bioconvert the substrates, but no one was found to be able to convert R-(+)-limonene and 4 were able to bioconvert (−)-*β*-pinene into oxygenated monoterpenes. The metabolites recovered were α-terpineol (2857 mg/L) and fenchol (traces) for *Aspergillus niger* ATCC 16404, α-terpineol (688 mg/L) for *A. niger* ATCC 9642, α-terpineol (172 mg/L) for *A. niger* ATCC 1004, and α-terpineol (24 mg/L) and *trans*-pinocarveol (traces) for *Penicillium camembertii* ATCC 4845. After screening and optimization experiments, the best experimental condition for bioconversion of (−)-*β*-pinene to α-terpineol was established using *A. niger* ATCC 16404 at 35 °C without addition of vitamin solution, yielding a conversion in α-terpineol of 15494 mg/L.

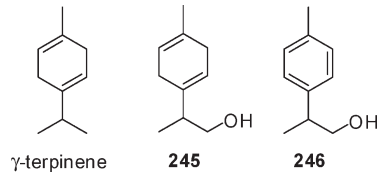

Figure 79

γ-Terpinene usually is a minor constituent of essential oils, but it accounts, for example, for 10−20% of the total oil in some citrus peel oils or in Spanish origanum.[131] Because of its low aroma value and chemical instability, it is removed as a waste in citrus oil processing. Berger et al. reported[132] the identification of odor-active *γ*-terpinene biotransformation products from submerged cultures of a *Stemphylium botryosum* wild strain.

During an extended screening for terpene tolerant and transforming microorganisms, it was found that a *Stemphylium botryosum* wild strain from sage (*S. officinale*) could tolerate and even grow at the aqueous saturation concentration of *γ*-terpinene and generate oxyfunctionalized transformation products. The two major products were identified to be the monoterpene alcohol **245** and its oxidation product **246** (Figure 79). The monoterpene alcohol **245** showed *a distinguished herbaceous odor, reminiscent of dill,* with a flavor threshold of 5 *μ*g in air (sniff port of a GC-O).

The two compounds were isolated from the fermentation broth by continuous liquid/liquid extraction with pentane diethyl ether (1:1.2 v/v), concentrated and isolated by preparative GC. Their structures were determined on the basis of ^{1}H-, ^{13}C NMR data, chiral GC, and low- and high-resolution mass spectrometry.

Compound **245** appeared to be the result of an usual regiochemical course for the oxygenation reaction of *γ*-terpinene with hydroxylation of the terminal methyl group.

The introduction of the hydroxy group at the nonactivated position of the terminal methyl group (C9) of the isopropyl moiety produced a new stereogenic center: compound **245** showed an *ee* value of 74%. The enantioselectivity of the reaction affording **245** was considered to be another indication of the enzymatic nature of the reaction. The $[\alpha]_D$ value and the absolute configuration of **245** were not determined due to the small quantity of compound available. Therefore, the preferential oxidation of one of the two prochiral methyl groups on the isopropyl moiety could not be allocated. During cultivation compound **245** was further oxidized to **246**,whose identity and stereochemistry was already known.[133] Compound **246** showed *ee* = 70%, almost identical to that shown by the parent compound **245**.

3.1.2. Miscellaneous. Carvone is a common terpenoid showing one stereogenic center: (*R*)-carvone (Figure 78) has *a spearmint aroma* and (*S*)-carvone has a typical *caraway odor*. Both carvone enantiomers are natural flavors widely used by the flavor

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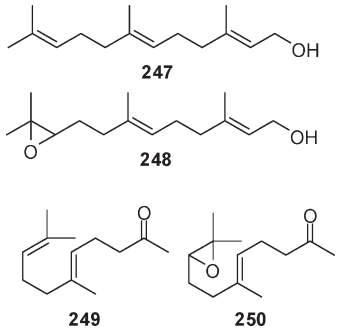

Figure 80

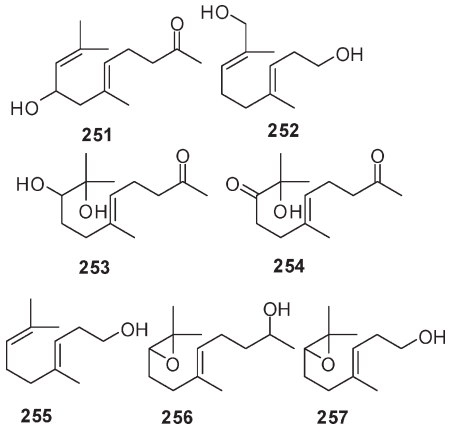

Figure 81

and fragrance industry. The essential oils containing these enantiomers are obtained primarily from caraway (*Carum carvi*), dill (*Anethum graveolens*), and spearmint (*Mentha spicata*) plants according to conventional extraction methods. The oil yield and composition (or quality) depend largely on the quality of the plant and the extraction method used.

The production of carvone by microbial biotransformation offers several advantages over agricultural methods, including the ability to overcome problems connected with seasonality, and the modest essential oil content of certain plants. A single-step microbial biotransformation is possible by using the whole cells of *Rhodococcus erythropolis* DCL14, which show carveol dehydrogenase activity,[134] when grown on limonene as the sole carbon source. The biotransformation substrate is a mixture of *cis*- and *trans*-carveol isomers: the trans-isomer is stereoselectively converted into (*R*)-carvone, and isomerically enriched *cis*-carveol is left unreacted.[135]

The major limitation of this biotransformation system is that carvone and *cis*-carveol become toxic to the cells and inhibit the biotransformation rate.

In a recent work,[136] the biotransformation by *Rhodococcus erythropolis* DCL14 was carried out in a 3 L two phase partitioning bioreactor with an immiscible liquid second phase, to improve the reactor performance achieved in a single aqueous phase system. The purpose of the second liquid phase was to minimize biotransformation rate inhibition due to the accumulation of the toxic components. 1-Dodecene was chosen as the solvent for this application because it is biocompatible, nonbiodegradable and has a great affinity for carvone relative to the other solvents tested. However, when 1-dodecene was used the extremely hydrophobic *R. erythropolis* DCL14 generated an emulsion with the organic solvent with significant sequestering of the cells into the organic phase and decreasing of substrate conversion. Silicone oil was thus added to prevent emulsification and sequestration of cells in the nonaqueous phase. In these conditions the system was able to convert approximately 21/2 times more carveol than a benchmark single aqueous phase system before substrate/product toxicity caused the biotransformation to stop.

Isoprenoids are widespread in the Nature, and they are characterized by specific odors making some of them of considerable industrial value in the flavors and perfumery industries.

Total synthesis of these compounds and their derivatives is often difficult, so their production by biotransformation of natural compounds is an interesting alternative way. The transformation of farnesol (**247**), its epoxide (**248**), geranylacetone (**249**) and 9,10-epoxygeranylacetone (**250**) (Figure 80) by four fungal strains (*Fusarium culmorum, Botrytis cinerea, Rhodotorula rubra, Rhodotorula marina*), selected among a total number of 20, was investigated[137] in the aim of obtaining odoriferous oxiderivatives.

Farnesol (**247**) was converted by *F. culmorum, B. cinerea, R. rubra*, and *R. marina* into the oxiderivatives of geranylacetone. The biotransformation of farnesol with *F. culmorum* proceeded very fast: two days of incubation gave a mixture containing 2% of substrate, 40% of **250** and 58% of (−)-8-hydroxygeranylacetone (**251**) (Figure 81). *R. rubra* gave only diol **252** (98%) after 2 days incubation, whereas in experiment with *R. marina* after 4 days incubation 51% of **252** was obtained. *B. cinerea* converted substrate **247** very fast giving 93% of 9,10-dihydroxygeranylacetone (**253**) after 2 days incubation.

The process of biotransformation of epoxyfarnesol proceeded in a different way. After 4 days of incubation with *F. culmorum* three products were obtained: 45% of **250**, 23% of **253**, and 32% 10-hydroxy- 6,10-dimethylundec-5-en-2,9-dione (**254**). *B. cinerea, R. rubra*, and *R. marina* gave only product **253**, and after 4 days no substrate was observed in the mixtures.

As for geranyl acetone, it was converted by *F. culmorum* into three products (**251**, **253**, and **254**). *R. rubra* and *R. marina* gave only alcohol **255**. In the case of *R. marina* after 2 days the conversion of substrate into alcohol **255** reached about 40% and during the process it increased to 100%. The formation of homogeraniol **255** was very interesting from the practical point of view because this compound was characterized by *very intensive, fruity odor with well-marked citrus note*. The transformation of geranylacetone (**249**) with *B. cinerea* afforded compound **253** after 4 days and it was the only product detected.

The last substrate, 9,10-epoxygeranylacetone (**250**), was transformed by *R. marina* into rac-9,10-epoxy-6,10-dimethylundec-5-en-2-ol (**256**) and (+)-7,8-epoxy-4,8-dimethylnona-3-en-1-ol (**257**). *R. rubra, B. cinerea and F. culmorum* gave the same product 9,10-dihydroxygeranylacetone with the predominance of enantiomer (+)-**253**. The most effective strain was *B. cinerea* which converted the substrate in 90% after 2 days incubation.

The results of biotransformation of compounds **247**-**250** allowed also the authors to draw proposals on their biodegradation pathways in the culture of fungal strains studied.

Ambergris is a pathological metabolite of sperm whales (*Physeter macrocephalus* L.), and it is probably secreted as a cover for the injuries incurred in their intestines as a result of food intake. It has been described[138] as possessing an *exotic-woody, incense-like, earthy, camphoraceous, tobacco-like, musk-like odor, which also smells of the ocean.* Its main component is the odorless derivative ambrein. The odor of ambergris is thus due to components that are generated from ambrein by exposure to

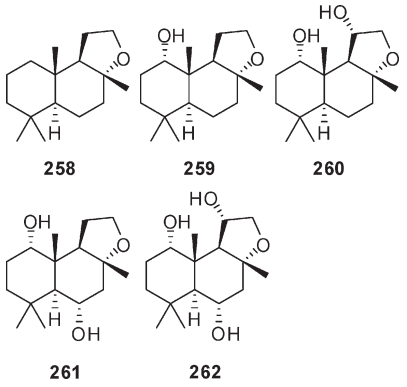

Figure 82

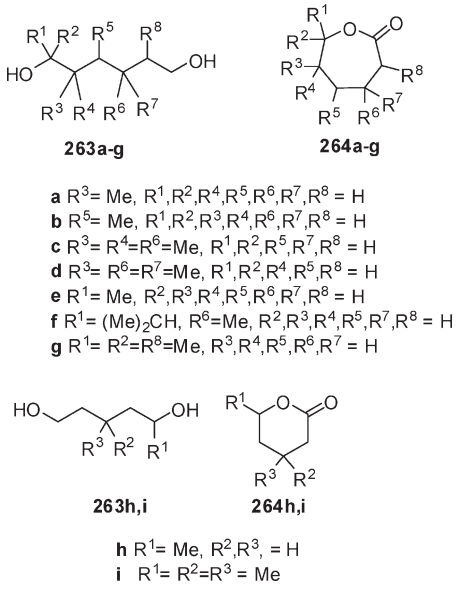

Figure 83

air and sunlight, after ambergris is expelled by the whale. Ambrox (**258**, Figure 82) is one of the most important ambergris-like artificial odorants. For biocatalyzed preparations of amber odorants up to 2003, see ref 8.

In 2006, a patent described[139] the preparation of mono-, di-, and trihydroxylated ambrox derivatives by microbial fermentations. The introduction of the OH groups was found to enhance the odoriferous properties of the products which were described to be *clearly distinct from those of the parent compound, showing a woody note combined with an intense fruity note.* Fermentation was conducted using *Fusarium lini,* and the four hydroxy (**259**−**262**, Figure 83) derivatives were isolated and fully characterized, resulting to be also enantiomerically single compounds.

The enantioselective oxidation of acyclic racemic 1,5 and 1,6-diols **263a**−**i** promoted by horse liver alcohol dehydrogenase (HLADH) was investigated[140] to obtain enantiomerically enriched odorous δ- and ε-lactones.

Commercially available NAD^+-dependent HLADH was known to be an effective catalyst in the oxidation of only one selected hydroxy group in a polihydroxylated molecule,[141] and in the stereospecific oxidation of only one enantiotopic hydroxy group of meso diols.[142]

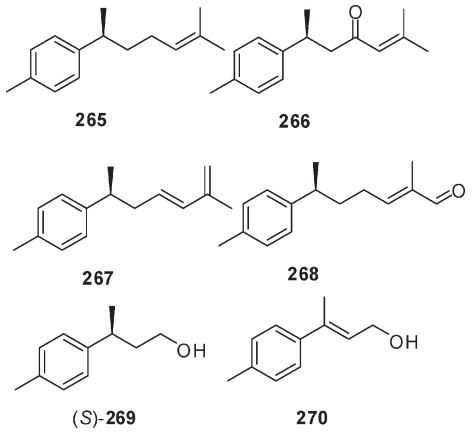

Figure 84

The reactions were carried out in a buffer at pH 9.0 using FMN as the effective agent in the recycling process of the catalytic amounts of NAD^+ coenzyme used. Diols **263a**−**i** were synthesized in good yields by reduction of the corresponding lactones and could be divided in four groups: primary−primary 1,6-diols **263a**−**d**, primary−secondary 1,6-diols **263e**,**f**, primary−tertiary 1,6-diol **263g**, and primary−secondary 1,5-diols **263h**, **i**.

No enantioselectivity was observed in the transformations of primary−primary 1,6-diols **263a**−**d** into the corresponding lactones **264a**−**d**. Regioselective oxidation of primary−secondary 1,6-diols **263e**, **f** and 1,5-diols **263h**, **i** afforded the corresponding enantiomerically enriched ε-lactones **264e**, **f** and δ-lactones **264h**, **i**. ε-Lactones **264e**, **f** were formed with higher enantiomeric excesses (*ee* = 85−99%), **264f** is (+)-(4*S*,7*R*)-menthodiolactone (*ee* = 85%), and it was obtained as the main product of the reaction mixture. Enzymatic oxidation of primary−tertiary 1,6-diol **263g** did not give any lactone product.

3.2. Stereoselective Reductions

The monocyclic aromatic sesquiterpenes of the bisabolane family are constituents of a large number of essential oils, and most of them are characterized by a benzylic stereogenic center. This structural feature is the main reason for the difficulty which is usually met in preparing the single enantiomers of these compounds.

The bisabolane sesquiterpenes (*S*)-(+)-α-curcumene (**265**, Figure 84) and (*S*)-(+)-*ar*-turmerone (**266**) are constituents of many essential oils and have been recognized as flavor components of the *Zingiber*[143] and *Curcuma*[144] species. The structurally related compounds (*S*)-dehydrocurcumene (**267**) and (*S*,*E*)-nuciferal (**268**) show the same absolute configuration and were isolated from vetiver oil[145] and from the wood oil of *Torreya nucifera*,[146] respectively. They can be prepared using **269** as a common key intermediate.

An interesting approach was developed[147] to prepare it, based on the enantiospecific reduction of the activated C=C double bond of allylic alcohol **270** by fermenting BY.

Allylic alcohol **270** was easily obtained by Horner−Wadsworth−Emmons reaction of *p*-methylacetophenone with triethyl phosphonoacetate and sodium hydride, followed by DIBAH reduction of the resulting ester. Although β-aryl-β-methyl-α,β-unsaturated alcohols were not described in the literature as substrates for this kind of reaction, Serra et al. found that BY was able to reduce **270** with high enantioselectivity to give the saturated alcohol bearing the benzylic stereogenic center showing *S* configuration.

dx.doi.org/10.1021/cr100289r |*Chem. Rev.* 2011, 111, 4036–4072

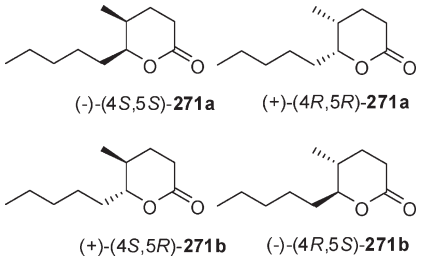

Figure 85

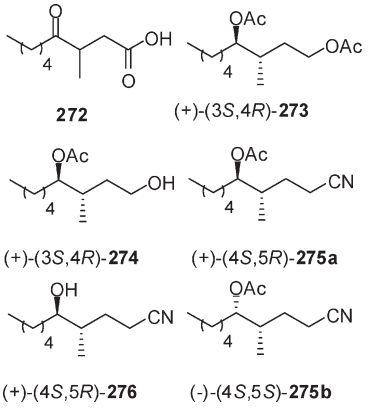

Figure 86

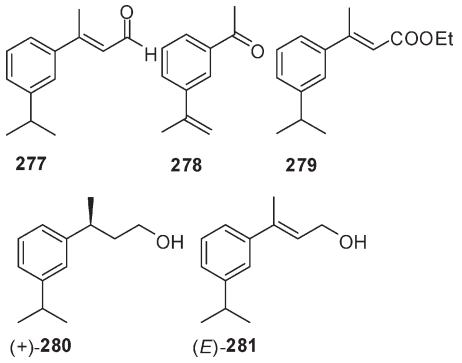

Figure 87

Compound **269** was obtained in about 20% isolated yield with *ee* > 95% after reaction of **270** with fermenting BY for 3−5 days at room temperature.

The reaction was also performed in the presence of a nonpolar resin with the same results in terms of yields and *ee* (21%, *ee* >95%), but with the advantage to use a high concentration of substrate (5 g L^{-1}) and a very simple workup procedure.

The transformation of (*S*)-**269** into the bisabolane derivatives was then achieved by accurate manipulation of the substrate.

The ability of baker's yeast to enantioselectively reduce double bonds activated by the presence of carbonyl groups was exploited by Fuganti et al. in the past for the preparation of the single most odorous stereoisomers of fragrant molecules, such as rose oxide,[148] and Aerangis lactone.[149]

cis-Aerangis lactone **271a** (Figure 85) was described by Kaiser as the main odor component of the African "moth orchids" *Aerangis confusa* J. Stewart and *Aerangis kirkii* (Rolfe) Schltr.[150]

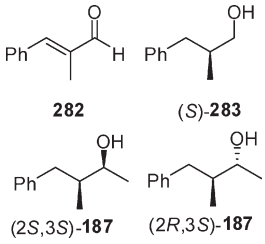

Figure 88

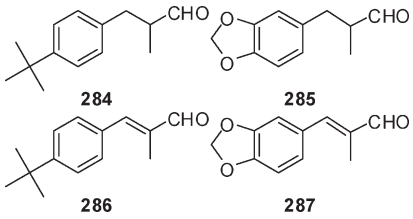

Figure 89

Racemic *cis*-**271a** and *trans*-**271b** were synthesized and separated by preparative GC, in order to evaluate their odor.[151] The *cis* isomer was found to have *a very pleasant and room-filling odor, reminiscent both of the smell of tuberose and gardenia, and of the fragrance of caramel, condensed milk and coconut*. The *trans isomer exhibited similar, but somewhat weaker, olfactory properties*. Mosandl, Kaiser et al.[152] obtained samples of all four stereoisomers of Aerangis lactone, (4*S*,5*S*)- and (4*R*,5*R*)-**271a** and (4*S*,5*R*)- and (4*R*,5*S*)-**271b** as single stereoisomers, and determined their absolute configuration by chemical correlation. By means of enantioselective multidimensional capillary GC the authors found that (4*S*,5*S*)-(−)-*cis*- **271a** was *the sole stereoisomer of Aerangis lactone present in the scent of living, white flowering orchids (Aerangis confusa), and that its* was *typical for the lactonic odor of A. confusa and A. kirkii, and identical to the olfactory qualities of natural Aerangis lactone.* Its enantiomer (4*R*,5*R*)-(+)-**271a** was found *to be reminiscent of δ-decalactone and cocos, and with a fragrance intensity much lower than* (4*S*,5*S*)-**271a**.

In 2001, a stereoselective approach to the synthesis of (+)-**271a** was developed[153] within a work devoted to the investigation of BY mediated reductions of 1,3-, 1,4-, and 1,5-ketoacids.

BY reduction of 1,4-keto acid **272** was found to afford with complete enantioselectivity and diastereoselctivity *trans*-cognac lactone (+)-*trans*-**101**, which was reduced with lithium aluminum hydride, and gave, after treatment with acetic anhydride in pyridine, diacetate (+)-(3*S*,4*R*)-**273** (Figure 86).

This latter was submitted to regioselective hydrolysis in water−THF in the presence of *Porcine pancreatic* lipase (pH = 7.8) and mono alcohol mono acetate (+)-**274** was thus recovered. Tosylate derivatization and cyanide treatment converted (+)-**274** into nitrile (+)-**275a**. Basic hydrolysis of (+)-**275a** in ethylene glycol with 10% KOH gave directly (+)-*trans*-**271b**, after acid workup.

The configuration of C(5) in (+)-**275a** was inverted via acetate displacement: hydrolysis of (+)-**275a** with KOH in methanol gave alcohol (+)-**276** which was converted into the corresponding tosylate derivative. This latter was treated with sodium acetate in DMF, to afford nitrile (−)-**275b** (*de* = 73%), thus showing no complete configuration inversion. Nitrile (−)-**275b** was submitted to basic hydrolysis to afford natural Aerangis lactone (−)-**271a** (*ee* = 99%, *de* = 70%).

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More recently, BY fermentation was employed to prepare the strongest enantiomer of Florhydral ((+)-**143**) and the two best isomers of Muguesia (2*R*,3*S*)- and (2*S*,3*S*)-**187**, after an accurate screening of the odor properties of all the stereoisomers obtained via a lipase-mediated kinetic resolution approach, as it has been explained in section 2.2.1.

Unsaturated aldehyde **277** (*E*/*Z* = 5:1) (Figure 87) was prepared in a synthetic procedure[92] which started from the ozonolysis of 1,3-diisopropenylbenzene, followed by PPh_3 quenching, to afford unsaturated ketone **278**. Subsequent hydrogenation and condensation with (triphenyl-λ^5-phosphanylidene)acetic acid ethyl ester gave unsaturated ester **279** (*E*/*Z* = 10:1), which afforded aldehyde **277** by Red-Al reduction, and MnO_2 oxidation.

After 48 h of BY fermentation, complete consumption of (*E*)- and (*Z*)-**277** was observed, and the alcohol fraction consisted (GC/MS) of saturated alcohol **280** (49%), unsaturated alcohol (*E*)-**281** (30%) and (*Z*)-**281** (21%). This product mixture was treated with MnO_2 in refluxing methylene chloride, to give, after removal of the unsaturated aldehyde by column chromatography, saturated alcohol (+)-**280** (*ee* = 97%). The high enantiomeric excess of (+)-**280**, in spite of the presence of (*Z*)-**277**, could be justified on the basis that only the double bond of intermediate (*E*)-**281** was microbially reduced.

Derivative (+)-**280** was oxidized with PCC to afford (+)-**143**, the most potent enantiomer of Florhydral.

The evaluation of Muguesia samples (**187**) by professional perfumers had shown that the two 3*S* stereoisomers had the most interesting odor properties, thus a BY approach to the mixture of these two (3*S*) isomers was optimized (Figure 88).[99]

BY reduction of unsaturated aldehyde **282** gave enantioenriched (*S*)-**283**, which was oxidized according to the Swern procedure and treated with methylmagnesium chloride in diethyl ether, to afford a 1:1 a mixture of the two enantiopure (3*S*)-**187**.

Lilial (**284**, Figure 89) is one of the most widely employed muguet type odorant. It shows *a very powerful, fresh, floral note, reminiscent of lily of the valley, lindlen blossom, and cyclamen.*[154] (*R*)-(−)-lilial[155] (*ee* = 95%) was described *as stronger and more aggressive than the racemate, top scent a little more watery*. (*S*)-(+)-Lilial (*ee* = 93%) was found a *little softer and less expressive than the racemate.* Thus (−)-Lilial was described as the most intense, but both enantiomers smelled of lily of the valley. Helional (**285**, Figure 89) is described to be *floral, green, aldehydic with top notes of ozone and new mown hay.*

The stereoselective bioredution of methylcinnamaldehydes **286** and **287** was investigated[156] using cloned and overexpressed enoate reductases, to give the enantioenriched Lilial and Helional enantiomers. (*R*)-Enantiomers were obtained using old yellow enzyme homologue YqjM from *Bacillus subtilis* and 12-oxophytodienoic acid reductase isoenzyme OPR1 from tomato: the enantiomer excess values were very modest, not higher than 53%.

The (*S*)-enantiomers of aldehydes **284** and **285** were the reduction products when isoenzyme OPR3, nicotinamide 2-cyclohexene-1-one reductase NCR from *Zymomonas mobiliz*, and yeast Old Yellow Enzymes (OYEs) 1−3 were employed as catalysts in a biphasic aqueous−organic system containing *t*-BuOMe as a cosolvent (20% v:v): *ee* values up to 97% were obtained.

4. CONCLUSIONS

In this Review, several odorous molecules prepared as single stereoisomers by biocatalytic methods have been described. These odorants belong to very different functional classes of organic chemistry, even if most of them share the presence of OH groups for lipase-mediated tranesterification. Moreover, those compounds, which are chiral because of the presence of stereogenic carbon atoms with oxygenated substituents (alcohols, ethers), are quite difficult to prepare in enantioenriched form by abiological methods. The richness of structural features that can be tolerated by enzymes is clearly shown in this work.

The enzymatic procedures herein described highlight the high stereoselectivity, and regioselectivity that can be achieved by using these methods, with the advantage of mild reaction conditions that do not require special expensive reagents or extreme working temperatures. The avoidance of complex reagents or catalysts reduces the appearance of off-flavors in the samples of the final products because of the presence of chemical impurities.

Simple tricks have been employed to increase yield, and compensate for low conversions: enzymes have been reused, substrates have been submitted to configuration inversion according to chemical methods, long reaction times have been adopted, and serial enzyme treatments have been performed.

The plethora of enantioenriched non-natural odorants prepared according to these methods have been properly submitted to odor evaluation to single out the best isomer, and to create a rich database for the investigation of structure−odor relationships. The concept of chiral switch, which is well established in pharmaceutical research, it is now becoming a leading idea also in the field of chiral odorants, in the view of limiting the amount of chemicals which go in touch with living beings or which are inevitably dispersed in the environment. The enzyme-mediated approach resulted to be successful to obtain samples for a first screeening of the odor properties of all the possible stereosiomers, and also for the enantioselective synthesis of the real odor vectors.

Several bioconversions, other than lipase acetylations and redox reactions, are still to be explored for a more fruitful exploitation of these procedures in the field of flavors and fragrances. The chemical variety of odorants can match with the wide biodiversity of the microoganisms producing enzymes.

APPENDIX

An essential step in the complete characterization of chiral flavors and fragrances prepared by biocatalytic methods is the determination of their enantiomeric excess. The enantiomeric recognition of odorous compounds is a relevant tool not only for the correlation of stereochemistry to odor, but also for the quality control of natural samples, aimed to the detection of fraud and adulteration, for the investigation of the biosynthesis of a compound, and also for the definition of the geographic origin of natural essential oils.[157]

Classical analytical methods for the determination of enantiomeric excess are based on the use of chiral phases for GC and HPLC analyses, and on the development of chiral derivitizing or solvating agents for NMR spectroscopy based procedures. As far as volatile compounds are concerned, high efficiency separation, sensitivity and selectivity are usually obtained by gas chromatography, thus the enantiomeric composition of flavor and fragrance samples is most commonly determined by this technique.[157,158] For recent reviews on HPLC and NMR methods, see refs 159 and160, respectively.

Chiral stationary phases for enantioselective GC analysis (CSPs) are obtained by combining the so-called "chiral selector" with a conventional stationary phase. Initially, the chiral selectors were used as non volatile neat liquids or as solutions in squalane or polysiloxanes, respectively. Subsequently, the chiral selectors

were chemically linked to polysiloxanes. in order to combine chemical selectivity with chromatographic efficiency and improved temperature stability.[161]

Cyclodextrin derivatives[162] (CDs) are the most widely used chiral selectors for flavor and fragrance analysis.[157,158a] A universal CD derivative for the separation of the most relevant racemic compounds in this field has not yet been developed, most likely as a consequence of the intrinsic mechanism of chiral recognition in gas chromatography. This is based on a host−guest interaction of each enantiomer of a racemate with the CD selector, and separation depends on the rather small difference in the energy of interaction between each enantiomer and the CD chiral selector.[163] At least two columns coated with different CD derivatives should be available to have the chance to separate about 80% of the most common racemates in the flavor and fragrance field.

The most effective CD derivatives are nowadays those belonging to the so-called second generation, consisting of β-cyclodextrins substituted in position 6 (i.e., the narrow side of the CD) with a bulky group (*tert*-butyldimethylsilyl- or *tert*-hexyldimethylsilyl-) and with alkylated and acylated groups in positions 2 and 3(mainly methyl, ethyl and acetyl) of its wide side.[164]

The determination of the enantiomeric excess of the chiral components of complex natural samples often requires a two-dimensional approach. Two complementary strategies have been developed.

The first one takes advantage of a second dimension *in separation*. A peak eluting from a first column is online injected into a second column: this operation can be realized according to two different instrumental devices, leading to the distinction between conventional heart-cut GC−GC,[165] and comprehensive GC×GC.[166]

It is possible to have a second dimension also *in identification*. In this case, an enantiomer is selectively isolated in the chromatogram by spectroscopic detection (usually MS) in single- or multiple-ion monitoring-MS (SIM−MS) through a careful choice of suitable diagnostic ions characterizing the investigated enantiomers.[167]

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BIOGRAPHIES

Elisabetta Brenna received her laurea (1989) and PhD (1993) in chemistry from the University of Milan. In 1996, she became Assistant Professor at Politecnico di Milano, where she is now Associate Professor. Her main scientific interests are the enzyme-mediated synthesis of the single enantiomers of chiral biologically active compounds, and the use of SNIF NMR technique for tracing back the synthetic history of active pharmaceutical ingredients and flavors. She is author of more than 120 publications on international journals.

Claudio Fuganti, after two years' postdoctoral fellowship with Professor A. R. Battersby at Liverpool, spent his career at the Politecnico di Milano where he has been Professor of Organic Chemistry since 1980. His main research interest is in the use of enzymes in organic synthesis and in the biogeneration of aroma chemicals. He is the author of over 300 publications and 30 patents.

Francesco G. Gatti graduated in Chemistry at the University of Pavia in 1995 under the supervision of professor L. Fabbrizzi. He obtained his PhD at the University of Warwick (U.K.) under the supervision of Prof. D. Leigh in 2001, working on the synthesis of interlocked molecules. From September 2001, he worked on the synthesis of peptido mimics under the supervision of Prof. Scolastico at University of Milan. In 2003, he was appointed as an assistant professor at Politecnico of Milano, where he joined the research group of Prof. Fuganti. In the last years he synthesised fragrances, natural products and drugs in their enantiomerically pure forms by means of enzyme resolutions and bakers's yeast mediated reductions of pro-chiral double bonds. He is author of more than 40 articles on international journals including *Nature* and *Science*, and a chapter book.

Stefano Serra, born in Pavia (Italy) in 1970, received his laurea (1995) from the University of Pavia, working on the synthesis of natural products. In 1996, he joined the group of Professor Fuganti, and in 2000, he received his PhD degree from the University of Milano. In 2001 he became researcher of the National Research Council (CNR). His main scientific activities are devoted to the enantioselective synthesis of chiral compounds and the development of new synthetic methods.

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