Applications of biocatalysis in fragrance chemistry: the enantiomers of α-, β-, and γ-irones

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The history of iris extracts, and of the isolation and enzyme-mediated synthesis of their odoriferous principle, the "irones", will be used to describe the improvement brought about by chemistry and biocatalysis in the development of natural fragrances. In particular, this *tutorial review* will discuss how the progress in the field of enzyme chemistry allowed the optimisation of accelerated procedures for the preparation of natural irone extracts, and the synthesis of all the ten isomers of irone, starting from commercial irone alpha.

Introduction

For centuries Nature has been providing us with useful molecules for the preservation and restoration of our health and for our wellbeing. The evolution of organic chemistry has increased our synthetic ability, serving to supply scarce or inaccessible natural products and allowing for the conversion of natural biologically active products into derivatives better suited for our needs. Fragrance chemistry is one of the fields that have witnessed the improvement in the quality and quantity of newly prepared compounds brought about by the development of organic synthetic methods.

Odorous molecules, as well as drugs, have to interact with human beings, and, if they are chiral, their mode of interaction will depend on their absolute configuration.[1] It may happen that the enantiomers of a chiral odorant show different odour properties and/or different odour thresholds. This fact has promoted great interest in applying synthetic techniques for enantio- and diastereo-control in the synthesis of chiral fragrances.[2,3] Enzymes are important tools for the preparation of single enantiomers: they show broad substrate tolerance, they are easy to handle and are considered environmentally benign. The optimisation of synthetic procedures to the most odorous stereoisomers of chiral odorants has important implications in everyday life, because of the huge amount of odorous chemicals added to products for household and personal care.

The history of iris extracts, and of the isolation and enzyme-mediated synthesis of their odoriferous principle, the "irones", is an interesting example of involvement of chemistry and biocatalysis in the world of fragrances, and it will be described in detail in this tutorial review.

Historical notes

When Catherine de' Medici married Henry II, she introduced Florentine customs into France. She had in her entourage two artisans, Tombarelli and Renato Bianco, who were skilful in the crafts of perfumes and poisons.[4] Iris was the symbol of Florence and Catherine's favourite flower, and Italian popular tradition tells that the delicate fragrance of the so called 'Queen's water' was due to the scent of iris extracts. According to the traditional recipe, decorticated iris rhizomes (orris roots) were kept in a dry and aerated environment for 2–3 years, then powdered, incubated with diluted sulfuric acid, and steam-distilled to provide the precious 'orris butter'. Purification of the essence to eliminate fatty acids yielded the "orris absolute".

In the fields around Florence *Iris florentina* was extensively cultivated, and at the end of the nineteenth century the production of dried iris rhizomes was a major industry in Italy. Large

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portions were used by well-known distillers in Florence, but considerable amounts were also exported to other countries. At the same time chemists started intensive work to isolate and characterise the odoriferous principles of natural extracts.

Isolation of irones from Iris extracts

At the University of Berlin Tiemann and Krüger were investigating orris root oil. In 1893 they could isolate[5] the phenylhydrazone of a pleasant-smelling ketonic compound to which they assigned the name "irone", and the wrong molecular formula $C_{13}H_{20}O$. In 1933, Leopold Ruzicka[6] corrected the elemental analysis of irone ($C_{14}H_{22}O$); then he[7] and Naves,[8] independently, found that at least three isomers of irone were present in natural iris oil: α- (**1**), β- (**2**), and γ-irone (**3**) (Fig. 1). In 1971, Rautenstrauch and Ohloff[9] were able to establish the stereochemistry of the irone isomers contained in the Italian iris oil (probably from *Iris pallida*) first used by Ruzicka (Fig. 2): (+)-*cis*-α-irone ((+)-**1a**), (+)-*trans*-α-irone ((+)-**1b**), (+)-β-irone ((+)-**2**), and (+)-*cis*-γ-irone ((+)-**3a**). Later on, in the same oil, they could detect[10] traces of *trans*-γ-irone together with some other isomers.

In 1970 the perfumer Henri Robert composed Chanel No. 19, the last perfume to be selected by Gabrielle ("Coco") Chanel in person. The perfumer harnessed the green notes in his formula by incorporating the very expensive iris extract, thus bringing a feminine touch to No. 19.

Biogenesis of irones

Investigations on the biogenesis of irones established that they are derived from the oxidative degradation of methylated bicyclic triterpenoids, the cycloiridals (Fig. 3). These latter derivatives were found together with the monocyclic iridals in roots and rhizomes of several iris species.[11] The irone moiety is formed by transfer of a methyl group to the terminal double bond of an open-chain iridal and subsequent cyclisation.[12] The

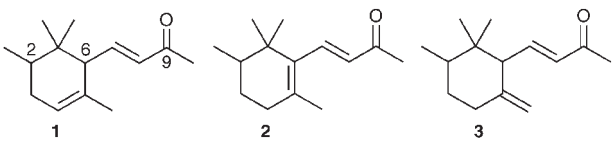


Fig. 1

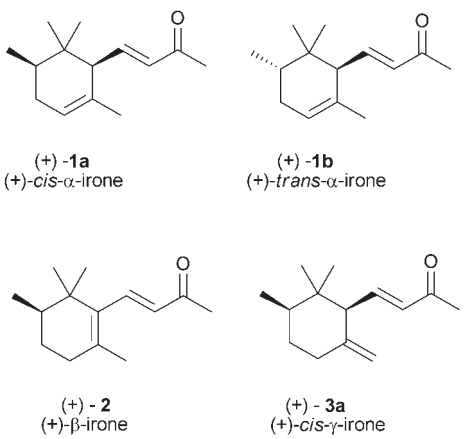


Fig. 2

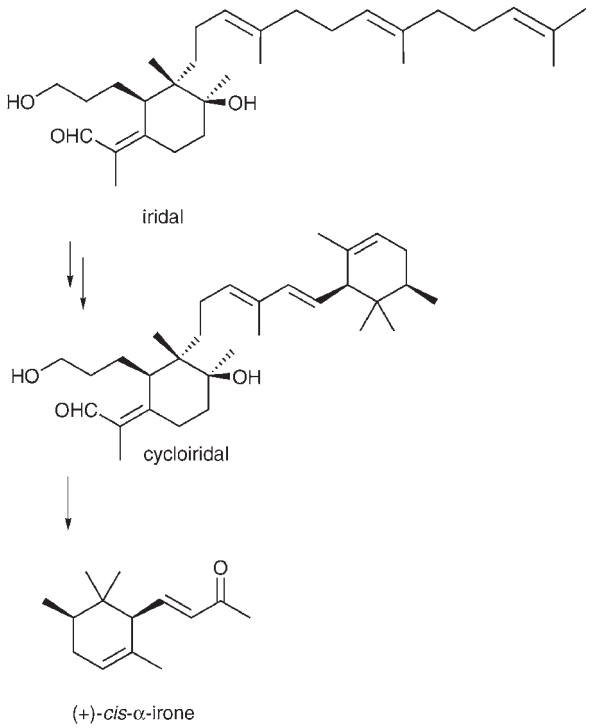


Fig. 3

reaction does not lead to the formation of a single irone isomer, but to a mixture of the three regioisomers α, β, and γ (**1–3**). The relative configuration at the two stereogenic centres is mainly *cis*, and *trans* isomers have been found only in minor amounts. It has been observed that iris oils of different origins show different enantiomeric composition of the three regioisomers. The dextrorotatory irones are found in the oil of Italian *Iris pallida* varieties and the laevorotatory enantiomers in that obtained from Moroccan *I. germanica*.[13]

Synthetic irones

The most useful synthetic approach to the production of racemic irones is the acid catalysed cyclisation of methyl-3-pseudo ionone (Fig. 4),[14] affording mainly the two diastereoisomers **1a** and **1b** of α-irone, and a minor quantity of the β-regioisomer **2**. Irone Alpha is the trade name of the commercial product sold by Givaudan and it is described as having a rich, floral and natural character, and constituting an important element in orris and violet compositions, as well as being useful when an exotic nuance is required, being

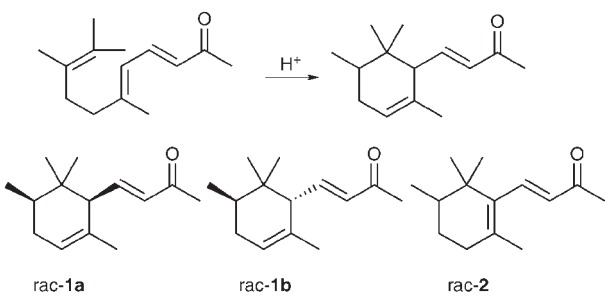


Fig. 4

extremely diffusive and giving volume and tenacity to compositions.[15]

Organic chemists have also investigated several syntheses to obtain enantiomerically enriched irone isomers, according to two main approaches. The first one was the development of accelerated methods to obtain irones from decorticated rhizomes by means of micro-organisms. The second one was the optimisation of enantioselective procedures to obtain the single enantiomers of irone isomers.

Obtaining irones from rhizomes by enzymatic routes

In 1991, Elf Aquitaine patented an enzymatic process for obtaining irones.[16] The terpene precursors were extracted from crushed or ground rhizomes with ethanol or methanol, and the extraction residue was submitted to enzymatic oxidation. This second step was performed in water in the presence of a surfactant or a co-solvent to improve the dispersibility of the hydrophobic molecules in the aqueous medium. Oxidation was carried out either with soy bean lipoxidase in an oxygen atmosphere or with horseradish peroxidase in the presence of hydrogen peroxide. After 48 and 60 h, respectively, the irones were recovered by steam distillation. Typically, 1.8–2.2 g and 0.450–0.680 g of irones were obtained per kilo of dry weight of starting rhizomes, according to the two different enzymic compositions. The addition of linolenic acid in the soy bean lipoxidase method increased the yield in the corresponding irones. The method did not require decortication of rhizomes, necessary if maturation in air is to occur, nor prolonged storage. The precursors were extracted not long after harvesting and the employment of enzymic rather than chemical oxidation allowed the use of the obtained irones in food applications.

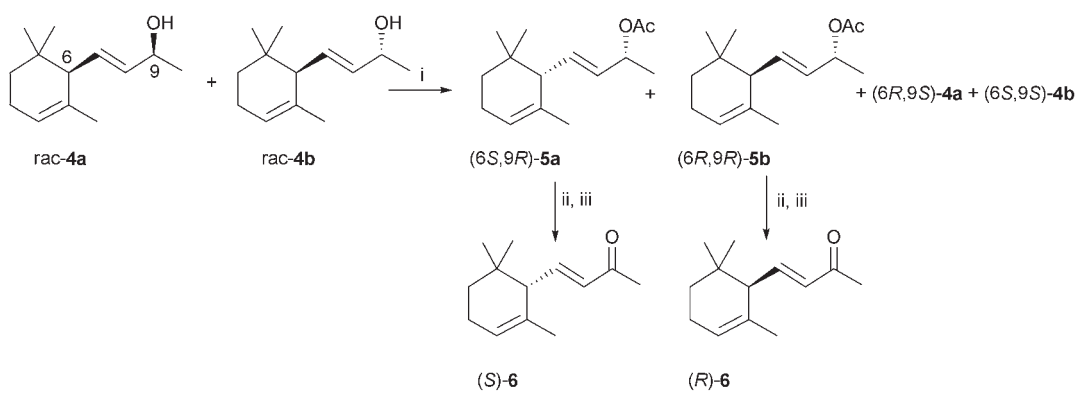
In 2001, we determined[17] the configuration of the irone isomers generated from fresh Italian iris rhizomes by this accelerated enzymatic procedure. Four batches of fresh iris rhizomes (*Iris pallida*) were cut into slices and extracted. The residue was suspended in water in the presence of soy bean flour as a source of lipoxidase and of linoleic acid, in an oxygen atmosphere. After 24 h steam distillation allowed the recovery of an odorous oil, which was chromatographed on a silica gel column to isolate the fraction containing irone isomers. The four irone samples thus obtained were submitted to GC and HPLC analysis, in order to establish the regioisomer distribution (α, β, γ) and the stereoisomer composition. The average composition of the irone oil produced by the enzymatic method employed was as follows: 4% of (+)-*trans*-α-irone (ee 99%), 0.6% of (−)-*trans*-γ-irone (ee 99%), 31.6% of predominantly dextrorotatory *cis*-α-irone, 49.8% of (+)-*cis*-γ-irone (ee 99%), traces of β-irone, and 13.8% of other unidentified components. This composition was found to be quite similar to the one reported for natural *Iris pallida* butter obtained *via* the classical method.[18] In these extracts, we found only the laevorotatory *trans*-γ-irone, which might derive from partial isomerisation of the corresponding (+)-*trans*-α-isomer.

In 1989 Roure S.A. patented[19] another method for the production of a mixture enriched in *cis*-γ-irone by bioconversion. The process consisted in the treatment of iris rhizomes, parts of these rhizomes, iris extracts, or iris wastes with two bacterial strains, in the presence of a plant cell culture medium. *Serratia liquefaciens* and *Pseudomonas maltophilia* were isolated from orris rhizomes (*Iris pallida*) which were cultured on Murashige and Skoog medium. Incubation of orris rhizomes with these strains led to the production of irones. After eight days, irone content reached 1 g per kg of dry rhizome, whereas only 400 mg per kg were obtained by the traditional procedure using rhizomes stored for three years.

Obtaining single irone stereoisomers by enzymatic routes

Enzyme-mediated preparation of the enantiomers of *cis*-α and *trans*-α-irone

Ten years ago we started a program aimed at the synthesis of the single stereoisomers of the three regioisomeric irones in order to evaluate the odor properties of each single compound. We chose as a starting material Irone Alpha, which is a mixture of 43% *cis*-α-irone (**1a**), 52% *trans*-α-irone (**1b**), and 5% of the β isomer (**2**) (Fig. 4). Working on α-ionone,[20] we could establish that Lipase PS-mediated transesterification of α-ionols **4a** and **4b** (Scheme 1), in *tert*-butyl methyl ether solution, in the presence of vinyl acetate, gave a 1 : 1 mixture of the two (9*R*)-α-ionol acetates **5a** and **5b**. We succeeded in separating the two racemic stereoisomers **4a** and **4b** prior to enzyme-catalysed acetylation by fractional crystallisation of the corresponding *p*-nitrobenzoates. So, we could obtain



Scheme 1 i. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; ii. KOH, MeOH; iii. MnO_2, CH_2Cl_2.

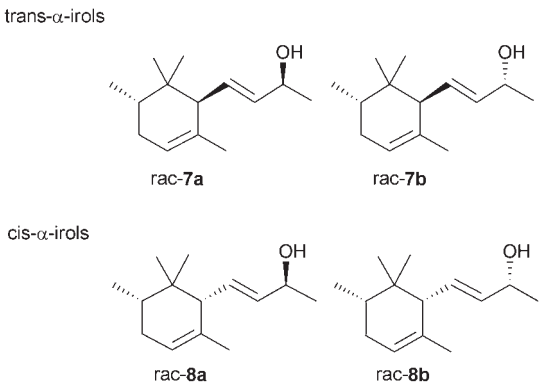


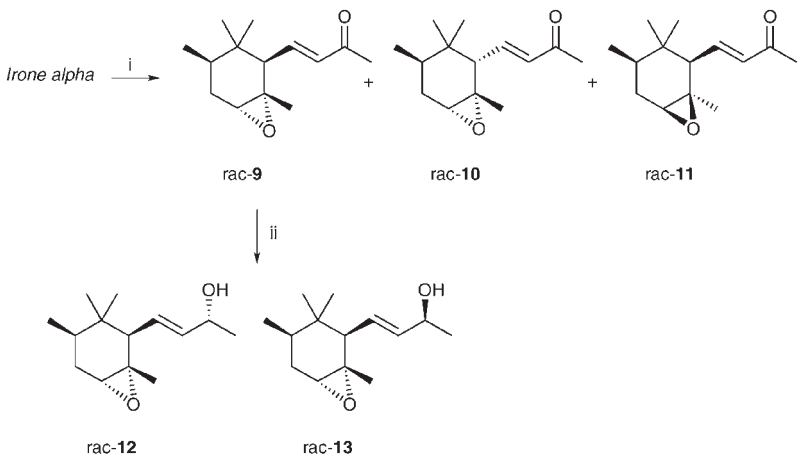
Fig. 5

(6*S*,9*R*)-**5a** and (6*R*,9*R*)-**5b** as single enantiopure stereoisomers, and convert them into (*S*)- and (*R*)-α-ionone **6**, respectively. We decided to follow the same strategy with α-irone, being aware of the increased complexity generated by the additional stereogenic centre.

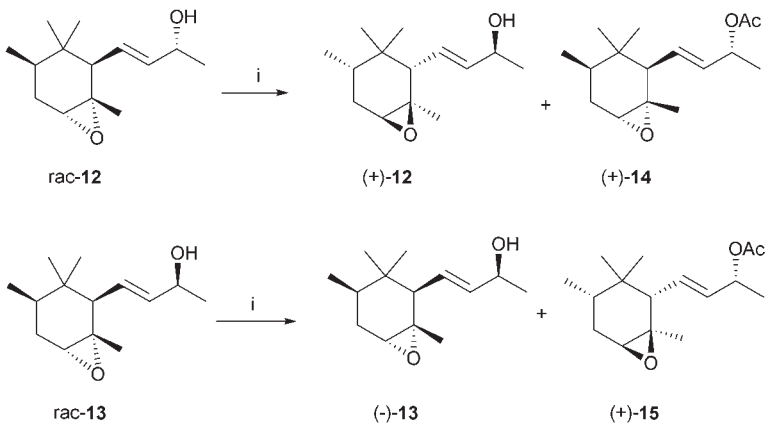
We performed some preliminary experiments of lipase-mediated transesterification of the mixture of the four racemic α-irols **7a**, **7b**, **8a** and **8b**, obtained by $NaBH_4$ reduction of commercial Irone Alpha (Fig. 5). The use of Lipase PS gave a nearly equimolar mixture of four enantiopure stereoisomeric acetates (chiral GC), presumably the four possible 9*R* acetates. The enzyme neither discriminated between *trans* and *cis*-α-irols, nor showed any diastereoselection within each single set, *i.e.* **7a** *vs.* **7b**, or **8a** *vs.* **8b**. In order to exploit this same procedure we had to find a way to separate α-irone derivatives showing *cis* and *trans* arrangement of the two substituents on the cyclohexenyl ring. A series of lucky circumstances facilitated the achievement of this goal.[21]

We found that the key step to achieve this separation was the epoxidation of the internal double bond. Treatment of Irone Alpha with 3-chloroperbenzoic acid in CH_2Cl_2 afforded a mixture (Scheme 2), containing mainly two products which were separated by column chromatography. The last eluted compound crystallised nicely from hexane, and X-ray single crystal analysis allowed us to establish that it was the diastereoisomerically pure epoxide derivative of *cis*-α-irone, bearing the oxirane ring *anti* to the side chain at C(6), *i.e.* compound *rac*-**9**. The first eluted fraction was composed mainly of the epoxy derivative of *trans*-α-irone *rac*-**10**, with 20% of the other diastereoisomer of epoxy-*cis*-α-irone, *i.e.* derivative *rac*-**11**. The relative configuration of compound **10** was determined by ^{1}H-NMR analysis of a suitable derivative.

Epoxy-*cis*-α-irone *rac*-**9** was reduced with $NaBH_4$ to afford a 1 : 1 mixture of the two diastereoisomeric alcohols *rac*-**12** and *rac*-**13**, which could be separated by column chromatography.



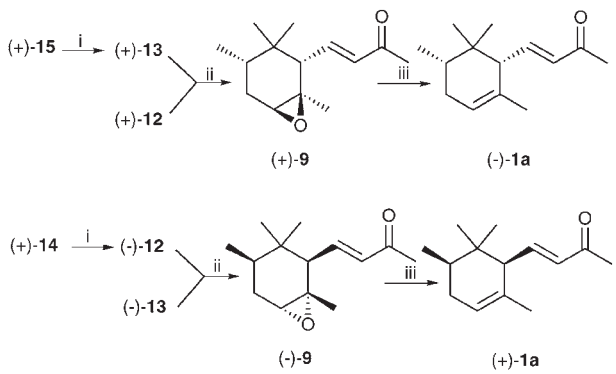
Scheme 2 i. 3-Chloroperbenzoic acid, CH_2Cl_2; ii. $NaBH_4$, CH_2Cl_2–MeOH.



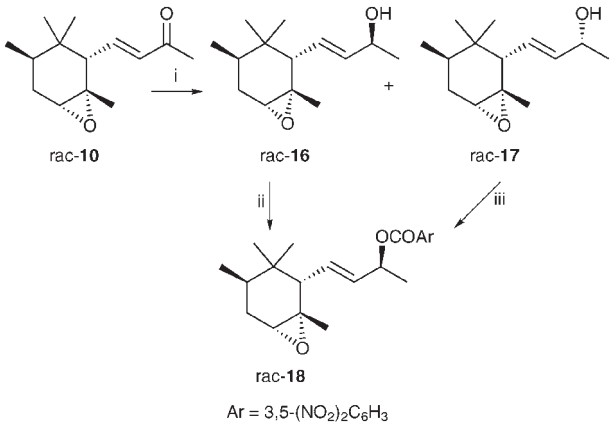
Scheme 3 i. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; column chromatography.

rac-**12** and *rac*-**13** were treated separately with lipase PS (Scheme 3) in *tert*-butyl methyl ether solution in the presence of vinyl acetate to afford, after column chromatography, acetates (+)-**14** (ee 98%, chiral GC) and (+)-**15** (ee 98%, chiral GC), respectively, and unreacted alcohols (+)-**12** (ee 98%, chiral GC of the corresponding acetate derivative) and (−)-**13** (ee 98%, chiral GC of the corresponding acetate derivative), respectively (enantiomer ratio[22] = 461, conversion = 0.5 for both the kinetic resolutions). The two batches of epoxy ketone (+)-**9** (Scheme 4), obtained by MnO_2 oxidation of (+)-**12** (left unreacted by Lipase PS esterification), and of (+)-**13** (recovered by saponification of (+)-**15**) showed the same enantiomeric purity (ee = 98%). They were combined and submitted to deoxygenation with trimethylchlorosilane and NaI in acetonitrile, to afford (+)-*cis*-α-irone (−)-**1a**. The same procedure led us to prepare (+)-**1a**, from (−)-**13** and (+)-**14**. The Lipase PS-mediated enantiospecific acetylation of the diastereoisomeric alcohols *rac*-**12** and *rac*-**13** allowed us to convert both unreacted alcohols and acetates into (+)- and (−)-**1a**, through a formal intermediate resolution of epoxy-α-irone **9** in 49% overall yield.

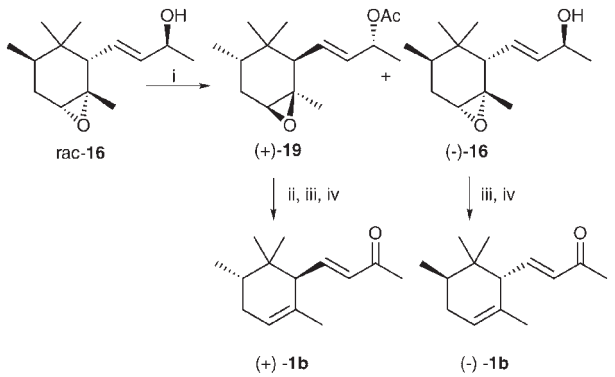
Epoxy-*trans*-α-irone *rac*-**10** was contaminated with the *cis* derivative *rac*-**11**. $NaBH_4$ reduction gave a mixture containing as major components alcohols *rac*-**16** and **17** (Scheme 5), which were chromatographed on a silica gel column. The first eluted alcohol *rac*-**16** was recovered in pure form by crystallisation of the corresponding 3,5-dinitrobenzoate *rac*-**18** from ethanol. This material was assigned the shown relative configuration by NMR experiments. Diastereoisomer *rac*-**17** gave an oily dinitrobenzoate ester, so it was esterified under Mitsunobu conditions, to afford *rac*-**18**. Crystalline ester *rac*-**18**, recovered both from *rac*-**16** and from *rac*-**17**, was employed as a starting material to prepare (+)- and (−)-**1b** (Scheme 6). Saponification of *rac*-**18** afforded diastereoisomerically pure *rac*-**16**, which was submitted to Lipase PS-catalysed acetylation in the usual conditions, to afford acetate (+)-**19** and unreacted alcohol (−)-**16** (E = 461, c = 0.5). Acetate (+)-**19** and alcohol (−)-**16** were then converted into (+)- and (−)-*trans*-α-irone, respectively, by oxidation of the OH group in position 9, followed by deoxygenation with chlorotrimethylsilane and NaI in acetonitrile.



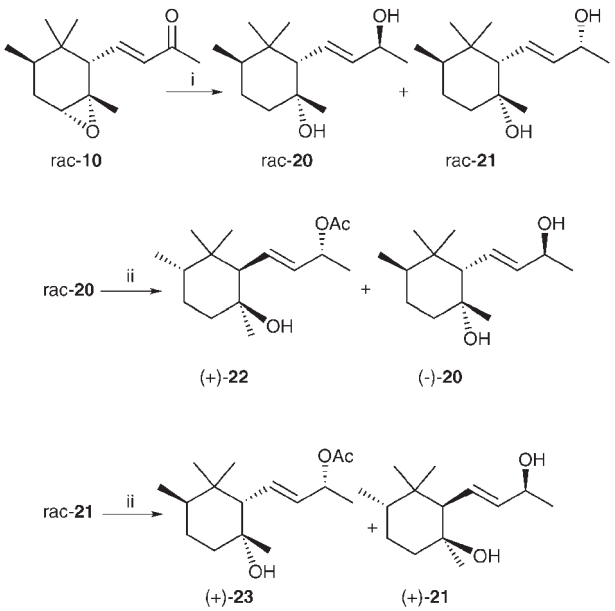
Scheme 4 i. KOH, MeOH; ii. MnO_2, CH_2Cl_2; iii. Trimethylchlorosilane, NaI, CH_3CN.



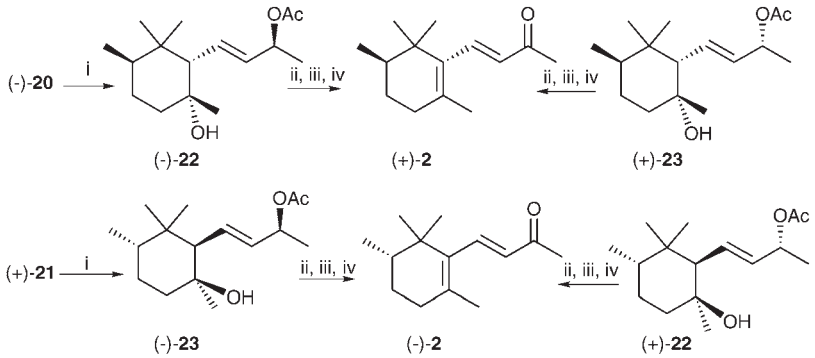
Scheme 5 i. $NaBH_4$, CH_2Cl_2–MeOH; ii. 3,5-$(NO_2)_2C_6H_3COCl$, pyridine; iii. Mitsunobu's esterification.



Scheme 6 i. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; column chromatography; ii. KOH, MeOH; iii. MnO_2, CH_2Cl_2; iv. trimethylchlorosilane, NaI, CH_3CN.



Scheme 7 i. $LiAlH_4$, THF; ii. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; column chromatography.



Scheme 8 i. Ac_2O, pyridine; ii. $POCl_3$, pyridine; iii. KOH, MeOH; iv. MnO_2, CH_2Cl_2.

Enzyme-mediated preparation of the enantiomers of β-irone and *cis*-γ-irone

Epoxy-α-irones **9** and **10** were also the key intermediate in the preparation of β- and γ-irone enantiomers by means of the reductive opening procedure of their oxirane moiety.[23]

$LiAlH_4$ reduction of epoxy *trans*-α-irone *rac*-**10** afforded a 1 : 1 mixture of diols *rac*-**20** and *rac*-**21** (Scheme 7). They could be separated by column chromatography, and their relative configuration was established by NOE experiments. The two diols were submitted separately to Lipase PS mediated acetylation, to afford, respectively, (+)-**22** (98% ee) and (−)-**20** (98% ee), (+)-**23** (98% ee) and (+)-**21** (98% ee) (E = 461, c = 0.5 for both kinetic resolutions). Unreacted alcohols (−)-**20** and (+)-**21** were acetylated and the four acetate derivatives, (+)- and (−)-**22** and (+)- and (−)-**23**, were dehydrated with $POCl_3$ in pyridine. After saponification and MnO_2 oxidation (Scheme 8), (+)-**2** (98% ee) was obtained from (−)-**20** (through acetate (−)-**22**) and (+)-**23**, while (−)-**2** (98% ee) was prepared from (+)-**21** (through (−)-**23**) and (+)-**22**.

When epoxy *cis*-α-irone *rac*-**9** was submitted to $LiAlH_4$ reduction, a *ca.* 3 : 1 mixture of 4,5-dihydro-4-hydroxy-*cis*-α-irols (*rac*-**24** and *rac*-**25**), and 4,5-dihydro-5-hydroxy-*cis*-α-irols (*rac*-**26** and *rac*-**27**) (Fig. 6) was obtained. The four components of the reduction mixture could be separated by column chromatography. The following considerations were made to explain the different course of the reduction of the two epoxide derivatives **9** and **10**. According to X-ray analysis, derivative *rac*-**9** shows the oxirane ring *anti* to the side chain at C(6). Thus, there should be competition between the hydride attack at the most-hindered C-atom C(5), leading to a highly favored transdiaxial opening in a chair-like transition state, and the reaction at the less substituted C-atom C(4). The resulting configurations of the favored *rac*-**24** and *rac*-**25** were confirmed by ^{1}H-NMR analysis.

Derivatives *rac*-**26** and *rac*-**27** were envisaged as suitable precursors of *cis*-γ-irone enantiomers. They were submitted to lipase PS-mediated acetylation in *tert*-butyl methyl ether solution, in the presence of vinyl acetate, to provide (9*R*)-acetate esters (+)-**28**, and (+)-**29**, respectively, and the (9*S*)-allylic alcohols (+)-**26**, and (+)-**27** (Scheme 9).

The (9*R*)-acetate esters (+)-**28** and (+)-**29** and their (9*S*)-enantiomers (obtained upon treatment with Ac_2O–pyridine of the alcohols (+)-**26** and (+)-**27**) were treated with $POCl_3$ in pyridine at 0 °C (Scheme 10), affording almost quantitatively mixtures of *cis*-γ, *cis*-α, and β-irol acetate diastereoisomers in a *ca.* 45 : 35 : 20 ratio. These mixtures were enriched in the *cis*-γ-stereoisomer by photoisomerisation, with concomitant formation of the (7*Z*)-isomer of *cis*-γ-irol acetate. After saponification and MnO_2 oxidation, the desired (7*E*)-*cis*-γ-irone enantiomers were separated from the corresponding (7*Z*) diastereoisomers by column chromatography. According to this sequence, (+)-**28** and the acetate derivative of (+)-**27** were converted into (+)-*cis*-γ-irone (**3a**, ee = 97%), while (+)-**29** and the acetate derivative of (+)-**26** afforded (−)-*cis*-γ-irone (**3a**, ee = 97%).

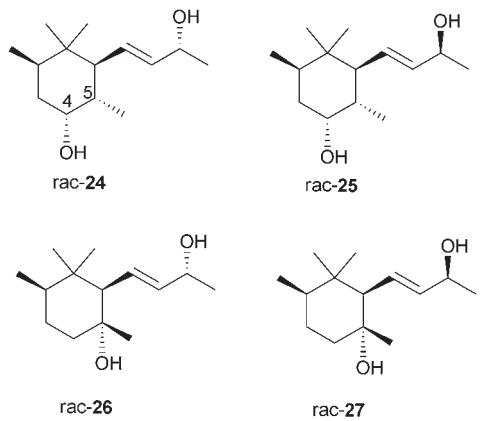
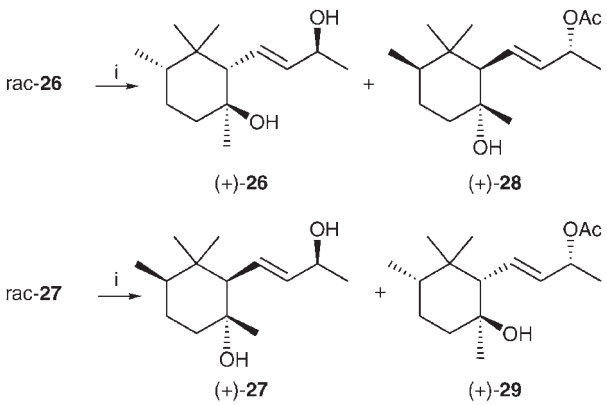
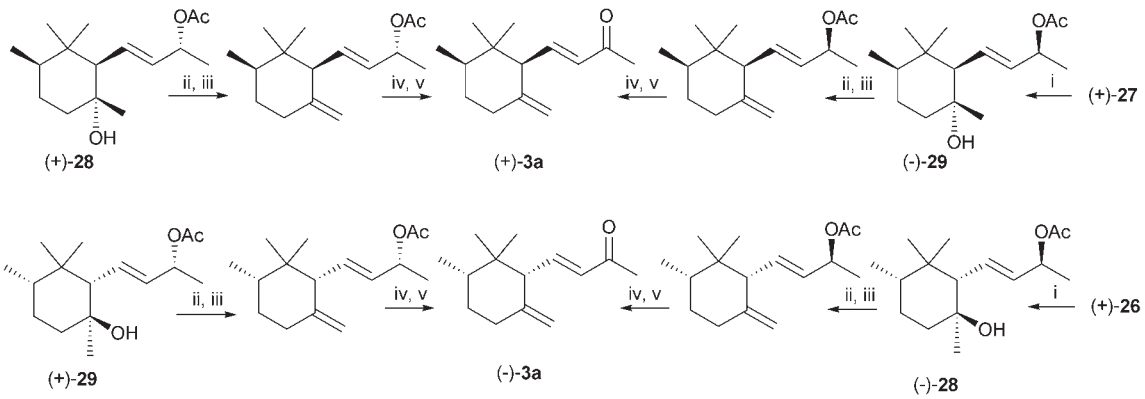


Fig. 6



Scheme 9 i. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; column chromatography.



Scheme 10 i. Ac_2O, pyridine; ii. $POCl_3$, pyridine; iii. *hν*, xylene–isopropanol; iv. KOH, MeOH; v. MnO_2, CH_2Cl_2.

Enzyme-mediated preparation of the enantiomers of *trans*-γ-irone

We exploited the photo-induced shift of the endocyclic double bond to convert the enantiomerically pure acetate derivatives of *trans*-α-irols into the corresponding γ regioisomers.[17] Epoxy *trans*-α-irols (+)- and (−)-**16** were deoxygenated by reaction with Zn and NaI in AcOH at room temperature, to give (+)-and (−)-**7b**, respectively (Fig. 7). Acetylation of these derivatives in pyridine and Ac_2O, followed by isomerisation in iPrOH with 10% of xylene gave, respectively, 1 : 1 mixtures of derivatives (+)- and (−)-**30a** and of the corresponding (7*Z*) diastereoisomers. Saponification and MnO_2 oxidation, followed by purification by column chromatography, allowed the isolation of (−)- and (+)-*trans*-γ-irone **3b** and the corresponding (7*Z*) isomers (+)- and (−)-**31**.

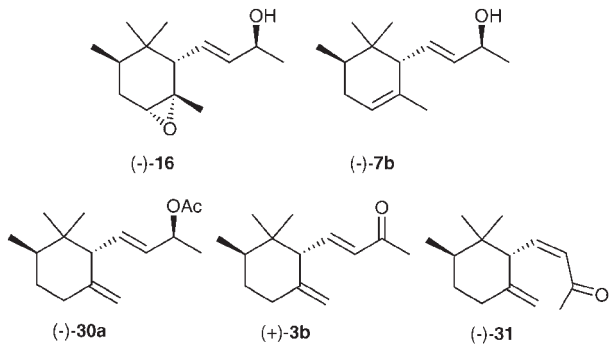
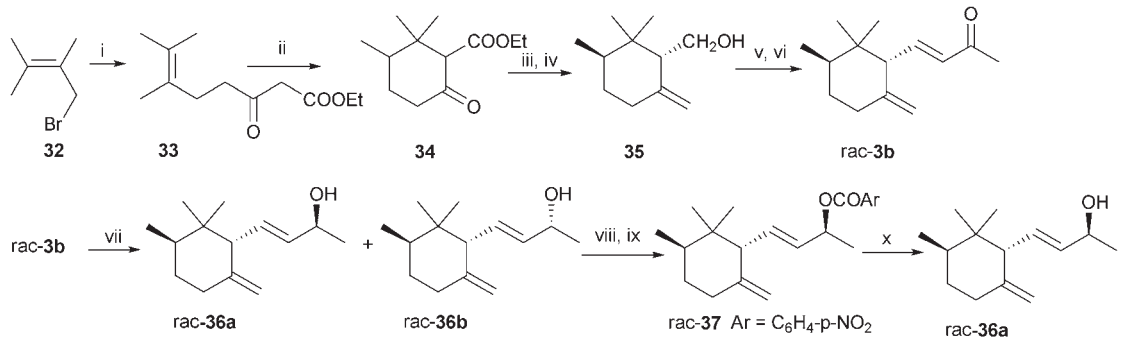


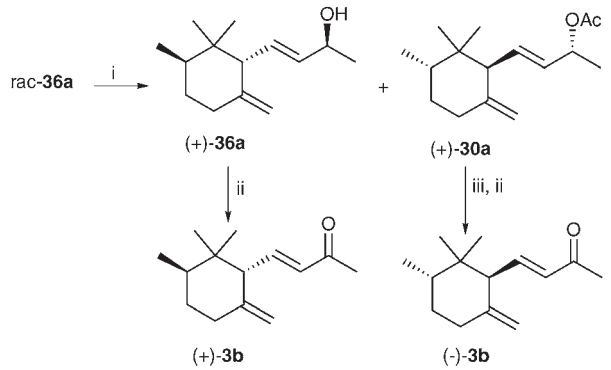
Fig. 7

We optimised also another biocatalysed route to the enantiomers of *trans*-γ-irone, based on the resolutions of the corresponding racemic irols. *rac*-**3b** was prepared starting from bromination of commercial 2,3-dimethylbut-2-ene with *N*-bromosuccinimide in CCl_4 solution (Scheme 11), in the presence of catalytic amounts of dibenzoyl peroxide, to give bromo derivative **32**. This latter was employed to alkylate ethyl acetoacetate to afford **33** which in turn was submitted to cyclisation with $SnCl_4$ in CH_2Cl_2 to afford keto ester **34** as a 2 : 1 mixture of *trans* : *cis* diastereoisomers. The mixture was reacted with $PPh_3{=}CH_2$, followed by reduction of the ester function with $LiAlH_4$, to give **35** as a single diastereoisomer. Oxidation to the corresponding aldehyde and condensation with $PPh_3{=}CHCOCH_3$ completed the synthetic path to racemic *trans*-γ-irone **3b** (only 2% of *cis*-γ isomer, GC-MS).

rac-*trans*-γ-Irone was reduced with $NaBH_4$ to afford a 1 : 1 mixture of *trans*-γ-irols **36a** and **36b**, which were converted into the corresponding crystalline 4-nitrobenzoates (Scheme 11). Fractional crystallisation from hexane allowed us to isolate diastereoisomer *rac*-**37** with a de of 98%, which was hydrolysed to *rac*-**36a**. Lipase-PS-mediated acetylation of racemic *trans*-γ-irol **36a** in *tert*-butyl methyl ether in the presence of vinyl acetate gave (+)-*trans*-γ-irol acetate **30a** (ee = 99%), and left unreacted alcohol (+)-**36a** (ee = 99%) (E = 1057, *c* = 0.5) (Scheme 12). Hydrolysis of (+)-**30a**, followed by manganese(IV) oxide oxidation, afforded



Scheme 11 i. CH_3COCH_2COOEt, NaH, THF; then BuLi, 0 °C; ii. $SnCl_4$, CH_2Cl_2; iii. $PPh_3{=}CH_2$, THF, reflux; iv. $LiAlH_4$, THF, 0 °C; v. ClCOCOCl, DMSO; Et_3N, CH_2Cl_2; vi. $PPh_3{=}CHCOMe_3$, toluene; vii. $NaBH_4$, CH_2Cl_2–MeOH; viii. *p*-NO_2-C_6H_4COCl, pyridine; ix. crystallisation from hexane; x. KOH, MeOH.



Scheme 12 i. Lipase PS, vinyl acetate, *tert*-butyl methyl ether; column chromatography; ii. MnO_2, CH_2Cl_2; iii. KOH in MeOH.

(−)-*trans*-γ-irone **3b** (ee = 99%). Oxidation of the unreacted alcohol (+)-**36a** allowed us to obtain (+)-*trans*-γ-irone **3b** (ee = 99%).

Olfactory evaluation of irone enantiomers. Enzyme-mediated preparation of (−)-*cis*-α-irone

All the samples of irone enantiomers were evaluated by professional perfumers (Robertet S.A. Grasse, France; Givaudan Schweiz, Dübendorf, Switzerland), and their odour properties were reported extensively in the corresponding papers. The odour descriptions obtained on these isolated samples were found quite different from those reported by Petrzilka *et al.*[18] and obtained by GC sniffing analysis on a chiral GC column of racemic irones.

The odour analysis of the single enantiomers showed the ability of the human nose to distinguish all the ten irone isomers, (−)-*cis*-α and (−)-*cis*-γ-irones being stronger than their enantiomers. (−)-*cis*-α-Irone showed the finest and strongest iris-like notes, and also the less powerful *trans*-γ-irone was appreciated for its soft "orris butter" type of odour.

We optimised a biocatalysed procedure for the preparation of (−)-*cis*-α-irone, taking advantage of the different diastereoselectivity showed by Lipase PS and PPL in the acetylation of α-irols.[24]

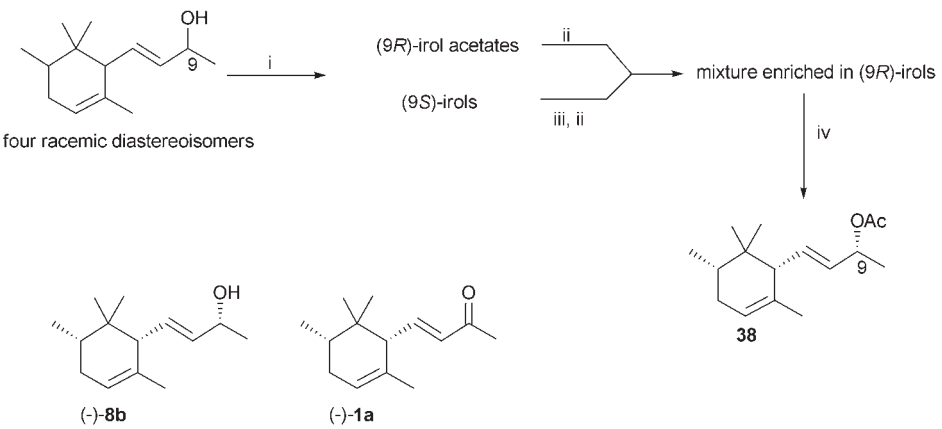
α-Irols (four racemic stereoisomers, **7a,b** and **8a,b**, Scheme 13) were submitted to Lipase PS-mediated acetylation; the acetylated product was found to be a 1 : 1 : 1 : 1 mixture of the four possible (9*R*)-α-irol acetates. The unreacted alcohol, *i.e.* a 1 : 1 : 1 : 1 mixture of the (9*S*)-α-irol stereoisomers, was converted into the 4-nitrobenzoate ester derivative according to Mitsunobu's procedure, in order to perform the inversion of configuration at C9. Thus, a mixture enriched in (9*R*)-α-irol stereoisomers, obtained by combining the saponification products of the acetate derivatives and of the *p*-nitrobenzoates, was submitted to PPL-mediated acetylation, to afford an acetylated product containing 56% of the enantiopure stereoisomer **38** (de = 69%). Two subsequent recrystallisations from hexane of the 4-nitrobenzoate ester straightforwardly prepared from the alcohol recovered by hydrolysis of this enzymatically acetylated product gave, after saponification, enantiopure alcohol (−)-**8b** (ee = 99%) with high diastereoisomeric purity (de = 98%). Oxidation of (−)-**8b** with manganese(IV) oxide in dichloromethane afforded enantiopure (−)-*cis*-α-irone.

Conclusions

Progress in the field of enzyme chemistry has allowed the optimisation of accelerated procedures for the preparation of natural irone extracts, and the preparation of all the ten isomers of irone starting from commercial Irone Alpha.

The review shows at a glance the high potential of enzyme-mediated reactions in this field of fragrance chemistry. A simple enzymatic oxidation allowed to the troublesome and lengthy manipulations of orris roots, such as decortication and prolonged storage, to be by-passed, producing less expensive iris extracts.

The kinetic resolutions of secondary allylic alcohols, showing a molecular skeleton structurally related to irone, proceeded with high enantioselectivity, and were the key steps of all these preparations. The biocatalysed resolution was coupled with simple organic reactions (epoxidation, reduction, photoisomerisation) to control diastereoisomer and regioisomer composition. Lipases allowed all the ten irone isomers to be obtained in enantiopure form as single isolated compounds for a complete evaluation of the odour properties, more than one century after the first isolation of the ketonic



Scheme 13 i. Lipase PS, *tert*-butyl methyl ether, vinyl acetate; column chromatography; ii. KOH, MeOH; iii. 4-Nitrobenzoic acid, diisopropyl azodicarboxylate, triphenylphosphine, THF; iv. PPL, *tert*-butyl methyl ether, vinyl acetate; column chromatography.

odoriferous fraction from iris root oil. This study highlighted the best enantiomer, for which we optimised a biocatalysed approach, based on the different diastereoselectivities showed by Lipase PS and PPL in the acetylation of these substrates.

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