



Account / Revue

From commercial racemic fragrances to odour active enantiopure compounds: the ten isomers of irone

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Abstract

This work contains a complete account of the preparation of all the ten enantiopure isomers of irone, the odoriferous principle of orris root oil, by means of enzyme-mediated approaches, starting from commercial racemic *Irone alpha*[®]. **To cite this article:** E. Brenna et al., C. R. Chimie 6 (2003).

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Résumé

Ce travail présente le rapport complet de la préparation des dix isomères énantiopurs de l'irone, le principe odorant de l'huile de racine d'iris, par approches enzymatiques, en partant du mélange racémique du commerce *Irone alpha*[®]. **Pour citer cet article:** E. Brenna et al., C. R. Chimie 6 (2003).

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Keywords: flavour; fragrance; enantiomer; irone; resolution; lipase; odour properties

Mots clés : parfum ; fragrance ; énantiomère ; irone ; résolution ; lipase ; propriétés odorantes

1. Introduction

Nature provides us with most of the finest flavours and fragrances. For decades men have tried to synthesise by themselves these aromatic compounds to fully supply their own needs. Most of these compounds are chiral and some of them show different odour properties according to their absolute configuration [1]. The

cheapest and most direct synthetic routes were those aimed to the preparation of racemates. However, there are remarkable examples of enantioselectivity in odour perception, which made compulsory the synthesis of the sole odour active isomer of a certain odorant. Menthol is an outstanding example.

(–)-L-Menthol (Fig. 1) is one of the most important flavours, with an estimated global market of 5000 tons per year. It is used extensively in pharmaceuticals, cosmetics, toothpastes, and chewing gums. The majority of (–)-menthol, ca. 1800–2300 tons per year, is still

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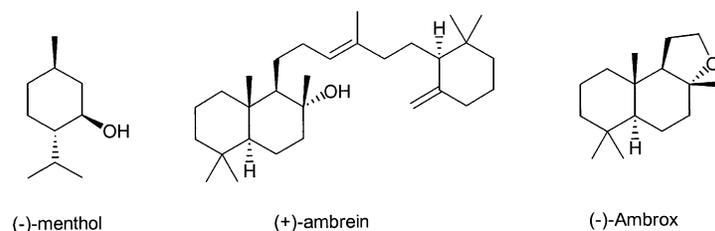


Fig. 1

obtained by freezing the oil of *Mentha arvensis* (from China) to promote the crystallisation of the menthol contained. This natural menthol is then physically separated by centrifugation and subsequent removal of the supernatant liquid, the so-called dementholized corn-mint oil. Residual impurities are due to traces of *Mentha arvensis* oil, and may impart a slight peppermint aroma to the menthol crystals. (–)-Menthol is described as very *cooling, fresh, sweet and minty*, whereas the enantiomer is *fresh, only some cooling, sweet-minty with musty, bitter, phenolic and herbaceous notes* [2]. Great effort has been devoted to the production of the (–)-enantiomer by synthetic routes starting from readily available raw materials. The two major commercial synthetic sources of (–)-menthol are the Haarman–Reimer (1500–2000 tons per year) and Takasago (1200 tons per year) processes [3, 4].

The first one is based on the fractional crystallisation of menthyl benzoate enantiomers promoted by crystal seeds of optically active menthyl benzoate.

m-Cresol and propylene are employed as inexpensive starting materials. The process involves several recycles and leads to a 90% overall yield (Fig. 2).

In the early 1980s, Takasago developed an elegant synthesis of (–)-menthol from myrcene (Fig. 3). Lithium catalysed addition of diethylamine promoted the conversion of myrcene into diethylgeranylamine. This latter is then isomerised to the chiral (3*R*)-citronellal *N,N*-diethyl enamine with 96–99% enantiomeric excess in the presence of Rh^I-(*S*)-binap, with a turnover of more than 400 000. Hydrolysis affords (3*R*)-citronellal of higher optical purity than the one recovered from citronella oil. The conversion of (3*R*)-citronellal into (–)-menthol is performed through a conventional route.

Somewhat different reasons led to the synthesis of the (–)-enantiomer of Ambrox[®] (Fig. 1). In the realm of fragrances, a relevant role is played by ambergris odourants. Ambergris is produced in the gut of the sperm whale, *Physeter catodon* L. It is usually associated with

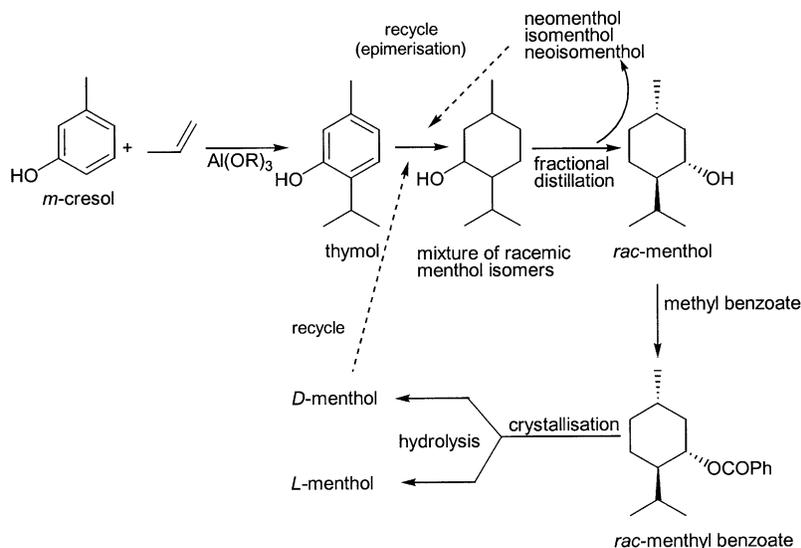


Fig. 2. Haarmann and Reimer process for the synthesis of (–)-L-menthol.

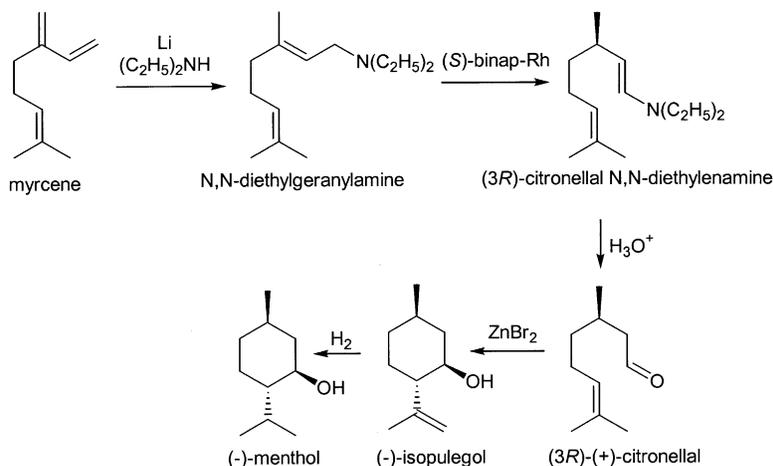


Fig. 3. Takasago process for the synthesis of (–)-menthol.

the beaks of the whale's principal food, the common cuttlefish. It consists of 80% ambrein, a cholesterol derivative that is considered to be a secretion of the whale's gut in response to the constant irritation caused by the sharp beaks of the squid. In the gut of the whale, the ambergris appears as a black, viscous and foul-smelling liquid. On exposure to sun light and air, it quickly oxidises and hardens to give a pleasantly aromatic, marbled, greyish, waxy substance in which the squid beaks are still embedded. When it is warmed it produces a very pleasant, mild, sweet, earthy aroma. From ancient times it has been used as a fixative for rare perfumes since it has the effect of making other fragrances last much longer than they would otherwise.

Since the decline of whaling and the restriction of global whaling operations, ambergris has not been used for some time. With the loss of this material, the perfume industry turned to organic synthesis to prepare

one of the most appreciated ambergris odorant: (–)-Ambrox[®] (Fig. 1).

This latter is characterised by a *persistent, animalic, amber odour*, with an odour threshold of 0.3 ppb. The (+)-enantiomer with its higher threshold value (2.4 ppb vs 0.3 ppb) and accentuated woody note was found to be lacking the *strong and warm animal note* of the (–)-enantiomer [5], and was, therefore, called “poor man's Ambrox” by Firmenich perfumers.

(–)-Ambrox[®] has a complex structure with four stereogenic carbon atoms. It seemed convenient to look for a starting material of natural origin that was an advanced enantiopure intermediate of the whole synthesis. Thus, the most efficient and cheaper access to Ambrox[®] was found to be the oxidative degradation of (–)-sclareol [6], an abundant constituent of clary sage oil (*Salvia sclarea* L.) (Fig. 4). Oxidation of the side chain gives γ -lactones (+)-sclareolide (**1a**) and (–)-iso-

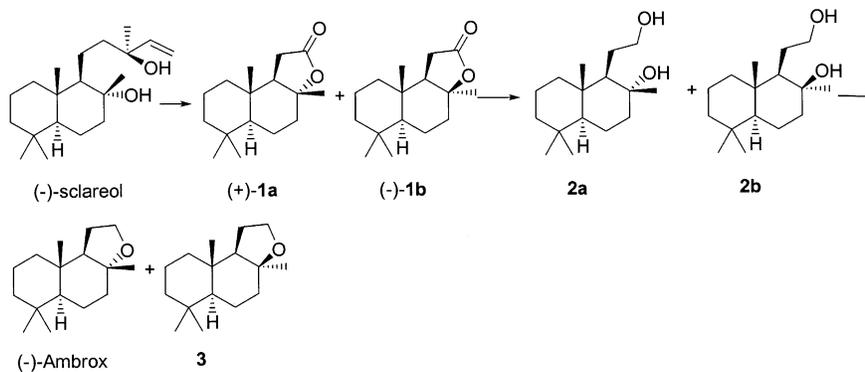


Fig. 4. Synthesis of (–)-Ambrox from (–)-sclareol.

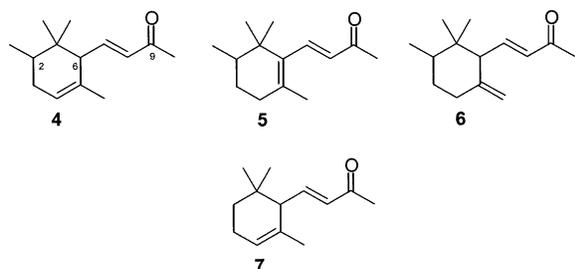


Fig. 5

scclareolide (**1b**), which are reduced to a mixture of diastereoisomeric diols **2a** and **2b**. Cyclisation of these latter derivatives affords (–)-Ambrox and its 8-*epi*-isomer (**3**).

Another great challenge was addressed to organic chemists by irones. In the perfumer's palette, Iris absolute is one of the most expensive products and it is mainly used in luxury perfumes, such as Chanel *No. 19* (1970) and *So pretty* by Cartier (1995). Just to give an insight of the worldwide resonance reached by iris cultivation in the 20th century, we have to take notice that the first multivariate discriminant analysis was performed by R.A. Fisher in 1936 [7] by elaboration of the so called Edgar Anderson's Iris Data [8]. This latter was a set of measurements of four flower parameters (sepal length and width, petal length and width) of 50 plants from each of the three species of iris: *Iris setosa*, *versicolor*, and *virginica*. The multivariate analysis of Fisher allowed the classification of iris species according to the values of these flower parameters. A taxonomic problem related to a practical interest stimulated the optimisation of a procedure, which is nowadays fundamental for data analysis in modern environmental and food science.

Irones **4–6** (Fig. 5) are the odoriferous principle of iris oil. It is well known that freshly harvested iris rhizomes do not contain irones, but their triterpenoid precursors called 'iridals' [9–12]. According to the traditional procedure, decorticated rhizomes are kept in a dry and aerated environment for 2–3 years, then powdered, incubated with diluted sulphuric acid, then steam-distilled to provide the precious 'Orris butter'. The mechanism of the oxidative degradation affording irones from iridals is still unknown. The traditional process is long, troublesome and low yielding; hence the high cost of the essence (butter). Purification of the

essence eliminates the fatty acids and yields the absolute, which is sold at 40 000–50 000 € per kilogram [13].

The odoriferous principle of iris root oil was first isolated by chance by Ferdinand Tiemann, who had started a research in cooperation with Haarmann and Reimer and Laire and co-workers to investigate the components of violet flower oil (*Viola odorata* L., fam. Violaceae). On the assumption that the two oils contained the same odour vector, the more readily available iris root oil was employed for these extensive investigations. In 1893 [14], Tiemann and Krüger isolated via a phenylhydrazone route a ketonic compound to which they assigned the name irone, and the wrong molecular formula $C_{13}H_{20}O$. They thought that irone could be the condensation product of citral with acetone. They performed the reaction in laboratory: base-catalysed condensation of citral with acetone gave a compound, they called pseudoionone, which showed *a strange, but not characteristic odour*. However, when pseudoionone was treated with sulphuric acid in water and glycerine, a ketonic compound was obtained, showing *the typical scent of violets in bloom*. They called it ionone. They concluded that the isolated irone had to be a double-bond isomer of the synthesised ionone.

In 1933, Ruzicka [15] determined the correct elemental analysis of irone ($C_{14}H_{22}O$); then he [16] and Naves [17], independently, found that at least three isomers of irone were present in natural iris oil, their structure being determined as **4**, **5** and **6** (Fig. 5). In 1971, Rautenstrauch and Ohloff [18] completed the structural determination by establishing the stereochemistry of the irone isomers they had isolated from the Italian iris oil (probably from *Iris pallida*) first used by Ruzicka. They found that the oil contained the following four isomers (Fig. 6): (+)-*cis*- α -irone ((+)-**4a**), (+)-*trans*- α -irone ((+)-**4b**), (+)- β -irone ((+)-**5**), and (+)-*cis*- γ -irone ((+)-**6a**). Later on, in the same oil Rautenstrauch and Ohloff [19] were able to detect traces of the *trans*- γ -irone together with some other isomers, probably showing *Z* stereochemistry at the double bond in the side chain.

It has been established that the distribution of irone isomers and enantiomers in different qualities of iris oils depends upon the botanical species of the plant [10, 12, 19, 20]. The average composition [21] of an iris butter prepared from *Iris germanica* is the follow-

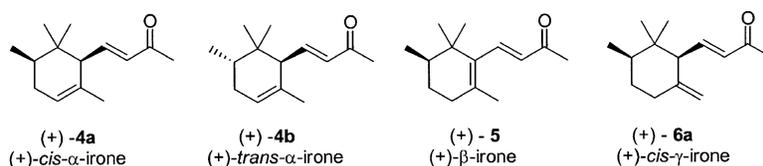


Fig. 6

ing: 0.91% of (+)-*trans*- α -irone (ee = 96%, (+)-**4b**), 61.48% of (–)-*cis*- α -irone (ee = 82%, (–)-**4a**), 0.71% of β -irone (**5**) and 37.60% of (–)-*cis*- γ -irone (ee = 38%, (–)-**6a**). When *Iris pallida* rhizomes are used, the corresponding iris butter contains 4.16% of (+)-*trans*- α -irone (ee = 98%, (+)-**4b**), 34.46% of (+)-*cis*- α -irone (ee = 66%, (+)-**4a**), 0.16% of β -irone (**5**) and 61.26% of (+)-*cis*- γ -irone (ee = 96%, (+)-**6a**).

Commercial racemic irones do not satisfy under the organoleptic point of view the demand of iris fragrance of modern perfumery. The natural extract is still used in perfume compositions. Several enantioselective syntheses have been reported in the literature [22–29] for the enantiomerically enriched irone isomers, but none of them has found practical application. During the last four years we were able to prepare all the ten isomers of irone in enantiopure form by enzyme-mediated approach, starting from the commercial mixture of racemic *cis* and *trans*- α -irone (Irone alpha[®], Givaudan) [30–33]. The synthetic schemes leading to the preparation of all these enantiopure isomers are reviewed in this work.

2. Preliminary investigations

In the aim of using the same approach we had successfully employed to prepare enantiomerically pure (*R*)- and (*S*)- α -ionone (**7**) [34], we submitted Irone alpha[®] to sodium borohydride reduction in methylene chloride/methanol solution, and obtained the four racemic α -irols **8–11** [31] (Fig. 7).

The reduction mixture was submitted to biocatalysed acetylation in *t*-butyl methyl ether solution, in the presence of vinyl acetate as an acyl donor, using two different kinds of enzymic preparations: *Porcine Pancreatic Lipase* (Sigma, Type II), and *Lipase PS Pseudomonas cepacia* (Amano Pharmaceuticals Co.) (Table 1). When PPL was used as a catalyst, a 2:1 mixture of *cis*- and *trans*- α -irol acetates was obtained (24-h reaction time). The main component (60.2%) was enantiopure derivative *ent*-**15**, showing a rather good diastereoisomeric enrichment (*de* = 78%). The unreacted alcohol still contained 4.3% of stereoisomer *ent*-**11** (chiral GC of the corresponding acetates).

Lipase PS-mediated acetylation proceeded rapidly, to afford a nearly equimolar mixture of the four (*9R*)

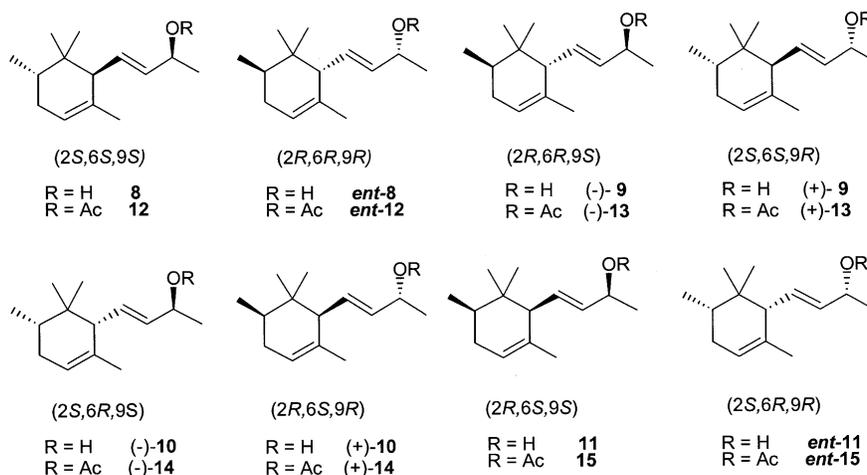


Fig. 7

Table 1
Stereoisomeric composition of the enzyme-produced α -irol acetates.
^a Chirasil DEX CB column.

Enzyme	Acetylated product (area%, chiral GC ^a)	Unreacted alcohol (area%, chiral GC ^a)
PPL	<i>ent</i> - 12 (25.4), (+)- 13 (7.6), (+)- 14 (6.8), <i>ent</i> - 15 (60.2)	8 (16.0), <i>ent</i> - 8 (11.7), (–)- 9 (16.1), (+)- 9 (14.0), (–)- 10 (12.9), (+)- 10 + 11 (25.0), <i>ent</i> - 11 (4.3)
	<i>Cis/trans</i> ratio = 2/1	
	<i>ent</i> - 12 ee = 99%, de = 54%	
	<i>ent</i> - 15 ee = 99%, de = 78%	
Lipase PS	<i>ent</i> - 12 (27.1), (+)- 13 (27.1), (+)- 14 (22.4), <i>ent</i> - 15 (22.4)	8 (26.9), (–)- 9 (27.1), (–)- 10 (22.5), 11 (22.5)
	<i>Cis/trans</i> ratio = 1.2/1	
	<i>ent</i> - 9 ee = 99%, de = 0	
	<i>ent</i> - 12 ee = 99%, de = 0	

diastereoisomers *ent*-**12**, (+)-**13**, (+)-**14**, and *ent*-**15** in enantiopure form. The enzyme neither discriminated between *trans*- and *cis*- α -irols, nor showed any diastereoselection within each single set, *i.e.* *ent*-**12** vs (+)-**13** or (+)-**14b** vs *ent*-**15**. The unreacted material recovered from the reaction was an equimolar mixture of the four enantiopure (9*S*) alcohol stereoisomers **8**, (–)-**9**, (–)-**10**, and **11** (24-h reaction time). We use the prefix *ent* to distinguish the enantiomers of those derivatives for which the sign of the optical rotatory power could not be determined.

The results of these lipase-mediated reactions showed that separation of *cis*- and *trans*- α -irol derivatives had to be accomplished before the enzymic step.

3. Epoxydation of commercial Irone alpha[®] [30]

On the basis of a previous experience on α - and β -ionone epoxides [35], we found that key intermediates for the separation of *trans*- and *cis*- α -irol were the corresponding epoxides. Treatment of *Irone alpha*[®] with 3-chloroperbenzoic acid in methylene chloride gave an oily mixture, containing two main products (\pm)-**16** and (\pm)-**17** (Fig. 8), which could be separated by column chromatography. Derivative

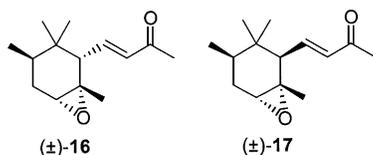


Fig. 8

(\pm)-**17** was obtained in pure form by crystallisation from hexane. X-ray single crystal analysis allowed us to establish that this crystalline material was the diastereoisomerically pure epoxide derivative of *cis*- α -irol bearing the oxirane ring in *anti* relation with the side chain at C(6).

The first eluted fraction of the epoxidation mixture was composed mainly by (\pm)-epoxy *trans*- α -irol **16**, with 5% of the other diastereoisomer of epoxy-*cis*- α -irol, *i.e.* the one obtained by reaction of the peracid on the same side of the substituent at C(6). As a matter of fact, the treatment of this chromatographic fraction with trimethylchlorosilane and sodium iodide in acetonitrile [36] afforded a 95:5 mixture of *trans*- and *cis*- α -irol. The stereochemistry of the epoxy moiety of derivative **16** was determined by ¹H NMR analysis of a suitable derivative.

3.1. *Trans*- α -irols

Substrate (\pm)-**16**, impure of the *cis* derivative, was reduced with sodium borohydride in methylene chloride/methanol to provide, as major components, the two racemic diastereoisomeric alcohols (1:1 ratio) (\pm)-**18** and (\pm)-**19**, which could be separated by column chromatography (Fig. 9). The first eluted alcohol (\pm)-**18** was obtained in pure form by crystallisation of the corresponding 3,5-dinitrobenzoate ester **20** from ethanol. This material was assigned the ring relative stereochemistry depicted in structural formula **20** on the basis of NMR studies.

The 3,5-dinitrobenzoate ester of racemic **19** was an oil. Crude **19** was then esterified with 3,5-dinitrobenzoic acid under Mitsunobu conditions to provide **20**. The two batches of crystalline ester **20** were collected, crystallised from ethanol and hydrolysed under basic conditions to afford (\pm)-**18**. Treatment of (\pm)-**18** with vinyl acetate in *t*-butyl methyl ether in the presence of lipase PS Amano afforded enantiomerically pure acetate (+)-**21** and unreacted alcohol (–)-**18** showing ee = 98% (GC of the corresponding acetate derivative) (Fig. 10). The acetate (+)-**21** was hydrolysed under basic conditions and the derived alcohol was converted into the epoxy ketone (+)-**16** by reaction with MnO₂ in methylene chloride solution. Similarly, alcohol (–)-**18** afforded enantiopure (GC) (–)-**16**. The enantiomeric epoxy derivatives (–)-**16** and (+)-**16** provided upon deoxygenation (–)-*trans*- α -irol **4b**

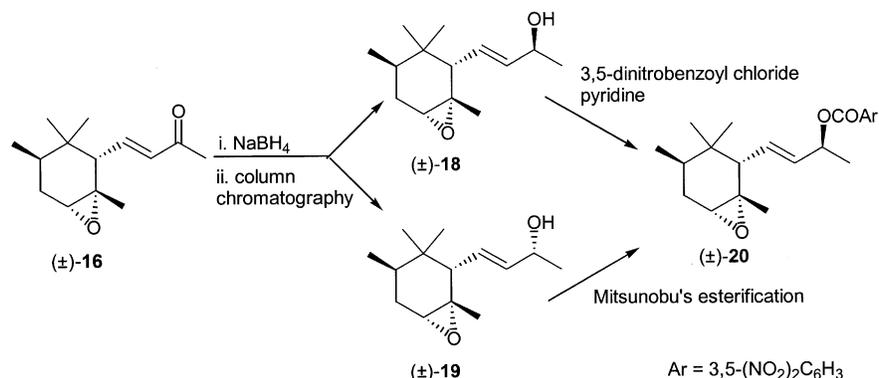


Fig. 9

(chemical purity = 88%, GC; ee = 98%, GC; $[\alpha]_D^{20} = -400$, c 1.05, CH_2Cl_2 , lit. ref. [18] $[\alpha]_D^{20} = -420$, c 0.98, CH_2Cl_2) and (+)-*trans*- α -irone **4b** (chemical purity = 96%, GC; ee = 98%, GC; $[\alpha]_D^{20} = 427$, c 0.95, CH_2Cl_2 , lit. ref. [23] $[\alpha]_D^{20} = 432$, c 2.85, CH_2Cl_2 , 86% ee) in good yields.

3.2. Cis- α -irones

More direct was the access to the enantiomers of *cis*- α -irone. Epoxy-*cis*- α -irone (±)-**17** was reduced with sodium borohydride, to afford a 1:1 mixture of the two diastereomeric alcohols (±)-**22** and (±)-**23**, which could be separated by column chromatography. The second eluted alcohol (±)-**23** was then purified by crystallisation from hexane. In separate experiments (±)-**22** and (±)-**23** were submitted to Lipase PS mediated acetylation in *t*-butylmethyl ether solution in the presence of vinyl acetate, to provide, after column chromatography, acetates (+)-**24** and (+)-**25**, and unreacted alcohols (+)-**22** (ee = 98%, GC of the corresponding acetate derivative) and (–)-**23** (ee = 98%, GC

of the corresponding acetate derivative), respectively (Fig. 11). Acetate (+)-**24** gave upon hydrolysis and oxidation with manganese(IV)oxide the same enantiomer of epoxy-irone **17** obtained by direct oxidation of (–)-**23**. The two samples of (–)-**17** were gathered (ee = 98%, GC) and submitted to deoxygenation with trimethylchlorosilane and sodium iodide in acetonitrile, to give (+)-*cis*- α -irone **4a** (chemical purity = 81%; ee = 98%, GC, $[\alpha]_D^{20} = 117$, c 1.5, CH_2Cl_2 , lit. ref. [18] $[\alpha]_D^{20} = 111$, c 0.92, CH_2Cl_2). Derivative (+)-**17** (ee = 98%, GC), prepared both from acetate (+)-**25** and from alcohol (+)-**22**, was transformed according to the same deoxygenation procedure into (–)-*cis*- α -irone **4a** (chemical purity = 85%; ee = 98%, GC; $[\alpha]_D^{20} = -130$, c 1.55, CH_2Cl_2 ; lit. ref. [23] $[\alpha]_D^{20} = -103.7$, c 0.65, CHCl_3 , 86% ee).

4. Reduction of epoxy *trans*- α -irone and epoxy-*cis*- α -irone [32]

In order to develop a convenient synthetic approach to the other irone isomers, we thought useful to inves-

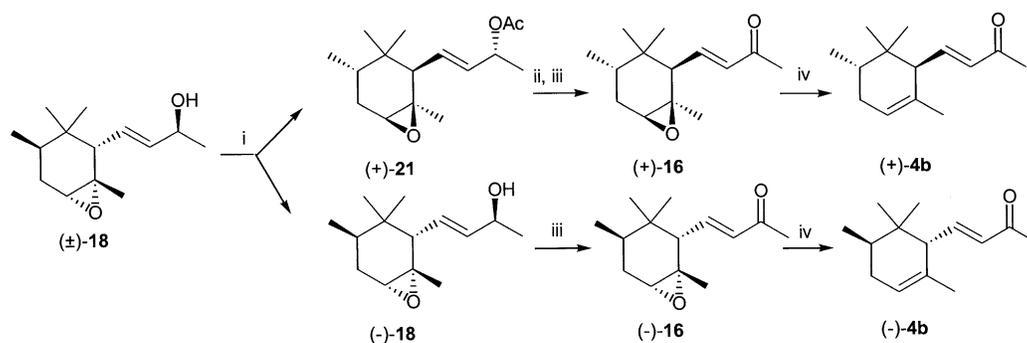


Fig. 10. (i) Lipase PS, *t*-butylmethyl ether, vinyl acetate; (ii) KOH in MeOH; (iii) MnO_2 in CH_2Cl_2 ; (iv) NaI, $(\text{CH}_3)_3\text{SiCl}$, CH_3CN .

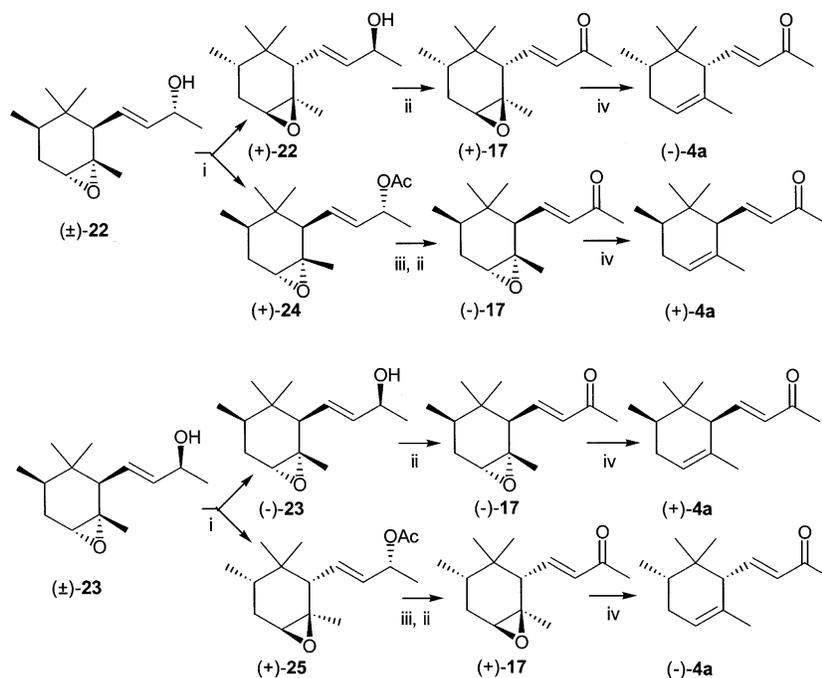


Fig. 11. Lipase PS Amano, vinyl acetate, *t*-butylmethyl ether, column chromatography; (ii) MnO_2 in methylene chloride; (iii) KOH in methanol; (iv) Me_3SiCl , NaI in acetonitrile.

to investigate the reductive opening of the 4,5-oxirane moiety of compounds **16** and **17** and the following dehydration.

4.1. β -irones

Treatment of racemic **16** with LiAlH_4 in refluxing THF afforded diols (\pm)-**26** and (\pm)-**27** in ca. 1:1 ratio. These products crystallised from hexane, once separated by column chromatography. In both compounds, hydride attack at the less substituted carbon atom afforded the tertiary alcohol with the hydroxyl group on the same side of the side chain, through the highly favoured *trans*-diaxial opening [37] of the oxirane ring (Fig. 12).

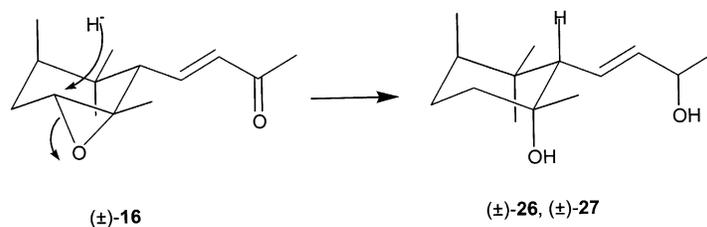


Fig. 12

In enzymic-acetylation experiments, diols **26** and **27** were converted into the corresponding (*9R*) acetate esters (+)-**28** and (+)-**29**, respectively, upon treatment with vinyl acetate in the presence of lipase PS in *t*-butyl methyl ether solution (Fig. 13).

The enantiomeric excesses of acetyl derivatives (+)-**28** and (+)-**29** and of the survived alcohols (–)-**26** and (+)-**27** were inferred from those of their transformation products, i.e. β -irol acetates. (+)-**30**, (+)-**31**, (–)-**30** and (–)-**31**, respectively.

We first investigated the dehydration of tertiary alcohol moiety of allylic monoacetates **28** and **29** by reaction with POCl_3 in pyridine, as it was reported to proceed with high stereo- and regioselectivity [38–42]. At room temperature, monoacetate (+)-**28** reacted

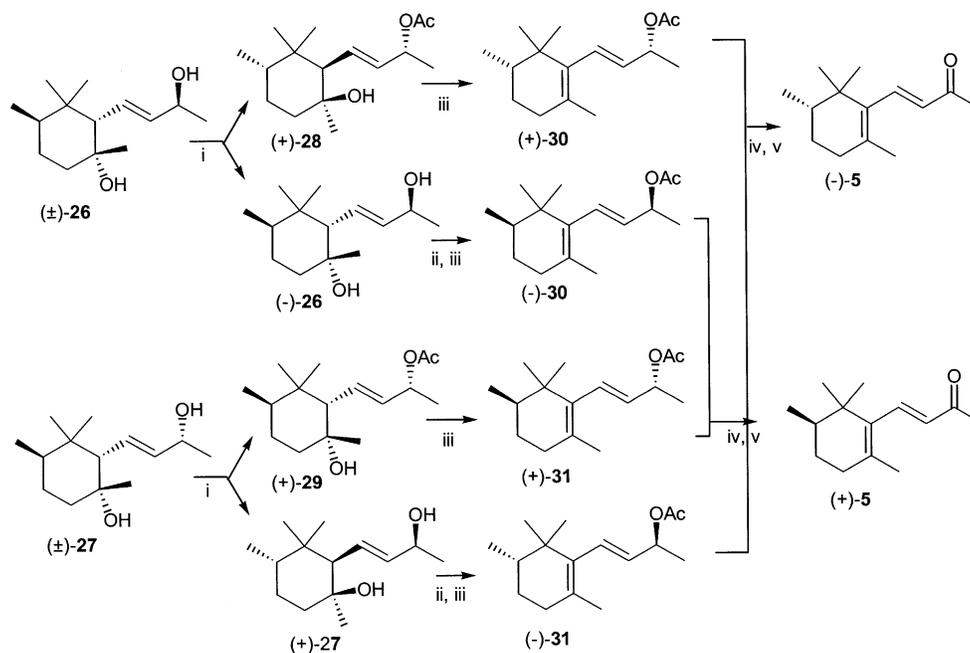


Fig. 13. (i) Lipase PS, *t*-butylmethyl ether, vinyl acetate, column chromatography; (ii) acetic anhydride in pyridine; (iii) phosphorous oxychloride in pyridine; (iv) KOH in methanol; (v) manganese(IV) in methylene chloride.

slowly with POCl_3 , the conversion being complete after one week. β -Irol acetate (+)-**30** (ee = 99% chiral GC) was obtained in 90% yield, impure of 9% of (-)-*trans*- α -isomer. The γ -isomer was not detected by GC/MS amongst the reaction products. The components of the oily mixture could not be separated by chromatography and the same was true for the alcohols obtained by basic hydrolysis. A purification of β -irol from the contaminating *trans*- α -isomer was attempted through fractional crystallisation of the 4-nitrobenzoate ester, obtained as low melting crystals from cold hexane. However, the ratio of the isomers in the mixture recovered from the basic hydrolysis of the nitrobenzoate ester was substantially unaltered respect to that of the starting material.

The sequence described for (+)-**28** was extended to (+)-**29** and to the acetyl derivatives of (-)-**26** and (+)-**27**, observing a similar distribution of isomers (Fig. 13). The (2*R*) diastereoisomeric β -irol acetate esters (-)-**30** and (+)-**31** were pooled, hydrolysed and oxidised with MnO_2 to provide (+)-(2*R*) - β -irone ((+)-**5**) in high yield. Similarly, from (+)-**30** and (-)-**31** the (-)-(2*S*)-enantiomer ((-)-**5**) was obtained. (+)-**5** and (-)-**5** prepared through this synthetic sequence were contaminated by (-)-*trans*- α -irone (9%,

GC-MS) and (+)-*trans*- α -irone (7%, GC-MS), respectively. This made the definition of the enantiomeric purity through optical measurements very difficult.

Reports on the characterisation of the enantiomers of β -irone were scarce. Mixtures highly enriched in β -irone could be obtained upon equilibration of the α - and γ -isomers [18, 43] in basic medium. Product (+)-**5**, containing a minute, but definite amount of (+)-*cis*- γ -irone **6**, obtained via basic isomerisation of the natural extract originally used by Ruzicka for the structural elucidation studies, was assigned a specific optical rotation of +33 in CH_2Cl_2 (calculated value +59) as deduced by Rautenstrauch and Ohloff [18, 43]. Chapuis and Brauchli [23] found $[\alpha]_{\text{D}}^{20} = -65.0^\circ$ for the (-)-enantiomer **5** (86% ee), prepared by synthesis and purified by preparative gaschromatography. Unfortunately, our samples of (+)-**5** and (-)-**5** were contaminated by (-) and (+) *trans*- α -irone, whose specific optical rotations ($[\alpha]_{\text{D}}^{20} = -400$, $c = 1.05 \text{ CH}_2\text{Cl}_2$ and $[\alpha]_{\text{D}}^{20} = +427$, $c = 0.95 \text{ CH}_2\text{Cl}_2$ [30]) were opposite in sign and of much higher numerical value than those of the β -irone enantiomers. Taking into account the chemical composition of the two samples of β -irone [(+)-**5**: 91% β -irone and 9% (-)-*trans*- α -irone,

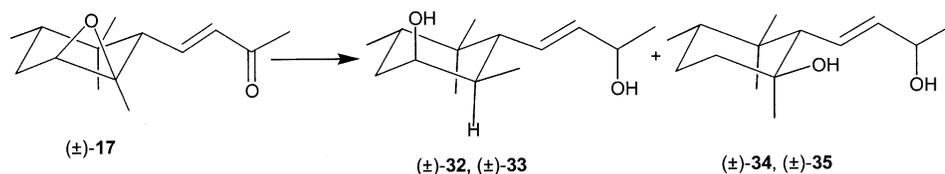


Fig. 14

GC–MS; (–)-**5**: 93% β -irone and 7% (+)-*trans*- α -irone, GC–MS], we determined an extrapolated value of specific optical rotation for enantiopure β -irone of ca. ± 60 from the measured values of $[\alpha]_D^{20} = +17.1$ ($c = 1.45$, CH_2Cl_2) and $[\alpha]_D^{20} = -20.2$ ($c = 2.30$, CH_2Cl_2).

The enantiomeric purity of the two β -irone samples was then assessed by a spectroscopic NMR method based on the use of chiral shift reagents. By this method, the two samples of β -irone (+)- and (–)-**5** were found to be enantiomerically pure

In order to verify whether a different regiochemical course of dehydration could be achieved, we experimented other reaction conditions, besides the concerted elimination of phosphate ester promoted by phosphorous oxychloride and affording β -irone. The tertiary alcohol (\pm)-**29** did not react at room temperature by treatment either with pyridinium 4-toluenesulfonate in ethanol, or with 4-toluenesulfonic acid in toluene, or with thionyl chloride/pyridine in chloroform. However, it was soon converted into a complex mixture of decomposition products, by forcing the reaction conditions.

4.2. *Cis*- α and *cis*- γ -irones

(\pm)-Epoxy-*cis*- α -irone **17** showed a remarkable different behaviour under LiAlH_4 reduction compared to racemic epoxy-*trans*- α -irone **16**. The reduction of (\pm)-**17** was much slower than of (\pm)-**16**, and afforded a ca 3:1 mixture of 4-hydroxy-4,5-dihydro-*cis*- α -irols **32** and **33** and 5-hydroxy-4,5-dihydro-*cis*- α -irols **34** and **35**. Each component of the mixture was separated by column chromatography, the 4-hydroxy-isomers **32** and **33** being the first to be eluted.

This course of epoxide opening could be tentatively explained on the basis of a competition between the hydride attack at the most hindered carbon atom C(5), leading to a highly favoured *trans* diaxial opening in a chair-like transition state, and the reaction at the less substituted carbon atom C(4) (Fig. 14). The stereochemistry thus obtained and shown in the structural

formulas of **32** and **33** was confirmed by ^1H NMR analysis.

Derivatives **32–35** were submitted to enzymic acetylation with vinyl acetate and lipase PS, in *t*-butyl methyl ether solution, to provide (*9R*) acetate esters (+)-**36**, (+)-**37**, (+)-**38** and (+)-**39**, respectively, and the (*9S*) allylic alcohols (+)-**32**, (–)-**33**, (+)-**34** and (+)-**35** (Figs. 15 and 16). Interestingly enough, the enzyme-mediated transesterification of 4,9-diols **32** and **33** afforded only the allylic (*9R*) acetate esters under these conditions.

The (*9R*) 4-hydroxy-4,5-dihydro-*cis*- α -irol acetate esters (+)-**36** and (+)-**37** and the corresponding (*9S*) alcohols (+)-**32** and (–)-**33** were converted into *cis*- α -irone **4a** enantiomers through the convergent sequences outlined in Fig. 15. Treatment of the acetate esters (+)-**36** and (+)-**37** with POCl_3 in pyridine at room temperature provided within a few minutes the diastereoisomeric α -irol acetate esters (–)-**14** (ee = 98%, chiral GC) and **ent-15** (ee = 97% chiral GC). These latter compounds were found to contain 13% (GC–MS) and 28% (GC–MS), respectively, of the corresponding 4-chloro derivatives, obtained by chlorine substitution of the intermediate phosphate ester (Fig. 17). (–)-**14** and **ent-15** afforded (+)- and (–)-*cis*- α -irone **4a** by basic hydrolysis and MnO_2 oxidation, making possible the purification of α -irone from the contaminating 4-chloro-4,5-dihydro-ketone analogue (+)-**40** by column chromatography (Fig. 18). The conversion of diastereoisomeric 4,9-diols (+)-**32** and (–)-**33** into (–)- and (+)-**4a** by the same route required the regioselective chemical acetylation of the allylic alcohol. The attempt based on the use of acetic anhydride and pyridine in CH_2Cl_2 failed: ca. 1:1 mixtures of 4- and 9-mono acetate esters were invariably obtained. However, by inverting the order of application of the reactions of Fig. 15, (+)-**32** and (–)-**33** were straightforwardly converted into (–)- and (+)-**4a**. To this end, (+)-**32** and (–)-**33** were first oxidised with MnO_2 to the enantiomeric 4-hydroxy-4,5-dihydro-*cis*-

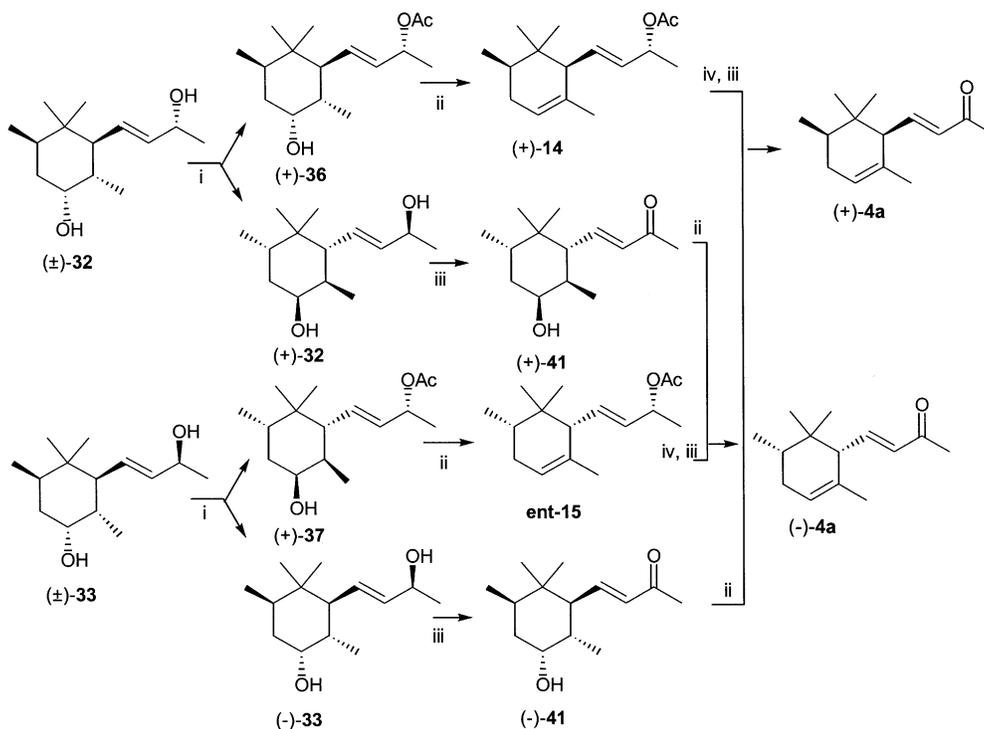


Fig. 15. (i) Lipase PS, *t*-butylmethyl ether, vinyl acetate, column chromatography; (ii) phosphorous oxychloride in pyridine; (iii) manganese(IV) oxide in methylene chloride; (iv) KOH in methanol.

α -irones $(+)$ -41 and $(-)$ -41, then treated with POCl_3 in pyridine.

This sequence would represent a new simple entry to enantiomerically pure forms of *cis*- α -irone from *Irone alpha*[®] via crystalline epoxy derivative **5**. However, there is a drawback in the process. As a matter of fact, the POCl_3 /pyridine treatment of the 4-hydroxy derivatives $(+)$ -36, $(+)$ -37, $(+)$ -41 and $(-)$ -41 produced also the corresponding 4-chloro-4,5-dihydro analogues in small amount by nucleophilic substitution, close to the desired dehydration products with the *cis*- α -irone skeleton. The chlorinated compounds were separated by column chromatography and completely characterised as ketone derivatives $(+)$ -40 (from $(+)$ -36 and $(-)$ -41) and $(-)$ -40 (from $(+)$ -37 and $(+)$ -41). The stereochemistry depicted in the structural formulas of $(+)$ - and $(-)$ -40 was in accordance with the inversion of the configuration at C(4) promoted by the nucleophilic substitution of the phosphate ester with chloride anion (Fig. 17) and was confirmed by ^1H NMR analysis.

The equatorially located chlorine atom could not be eliminated. Various reaction conditions were at-

tempted: $(\text{CH}_3)_3\text{COK}/\text{THF}$, DBU/CHCl_3 and 35% $\text{KOH}/\text{ethanol}$. Thus, the rapid dehydration of $(+)$ -36, $(+)$ -37, $(+)$ -40 and $(-)$ -41 occurring by POCl_3 /pyridine treatment was the result of the *anti* periplanar elimination of the axially located phosphate ester together with the axial hydrogen atom at C(5). The competing substitution of the phosphate ester with chloride anion occurred with inversion of configuration at C(4), and the desired 4,5-dehydrohalogenation became very difficult for stereochemical reasons.

The (9*R*) acetate esters $(+)$ -38 and $(+)$ -39 and the (9*S*) enantiomers, obtained upon treatment with acetic anhydride/pyridine of the alcohols $(+)$ -34 and $(+)$ -35, were also treated with POCl_3 /pyridine. These materials reacted immediately with POCl_3 even at 0 °C, affording, almost quantitatively, mixtures of *cis*- γ -, *cis*- α - and β -irone acetate diastereoisomers in *ca* 45:35:20 ratio. Chiral GC analysis of the reaction mixtures showed conservation of the enantiomeric purity during the dehydration process.

The different regiochemistry of the POCl_3 /pyridine induced dehydration of the tertiary alcohols of the

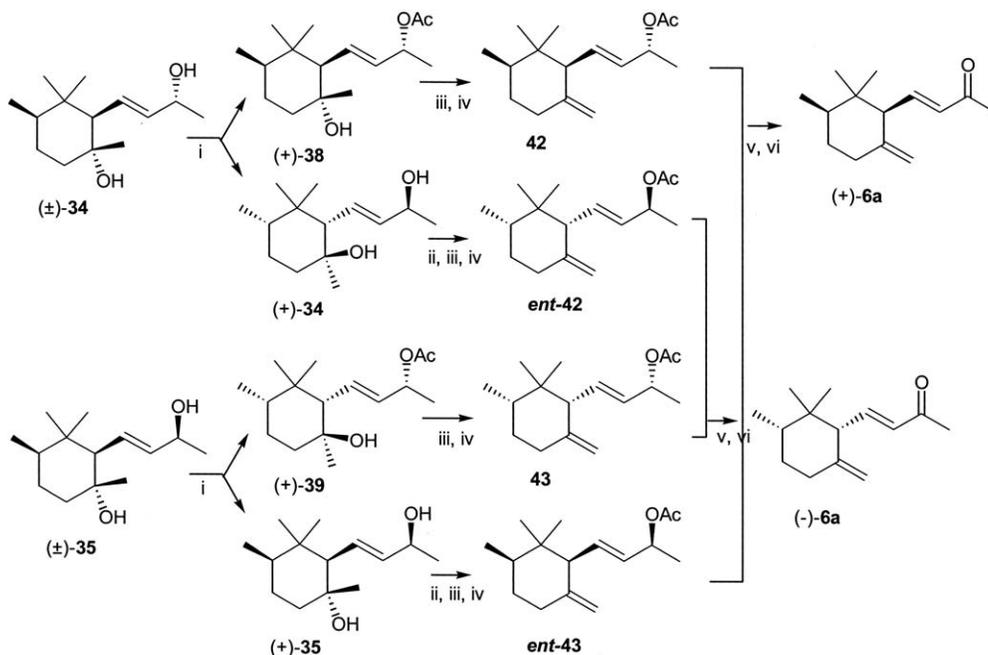


Fig. 16. (i) Lipase PS, *t*-butylmethyl ether, vinyl acetate, column chromatography; (ii) acetic anhydride in pyridine; (iii) phosphorous oxychloride in pyridine; (iv) *hν*, xylene, isopropanol; (v) KOH in methanol; (vi) manganese(IV) oxide in methylene chloride.

trans series (derived from (±)-26 and (±)-27) and of the *cis* series (derived from (±)-34 and (±)-35) could be tentatively explained, taking into account the precise stereochemical requisites of E₂ elimination. Anti-periplanar arrangement of the hydrogen atom and of the other leaving group is required. In such cyclohexane derivatives, this steric condition is best satisfied when the hydrogen atom and, in this case, the phosphate ester are in *trans* diaxial relation. The analysis of the chair conformations reported in Figs. 12 and 14 allowed us to draw the following considerations:

- in derivatives (±)-26 and (±)-27, the hydrogen linked to C(6) and the hydroxylic group at C(5) are in *trans* diaxial relation; thus, dehydration of the corresponding allylic acetates to β-derivatives is highly favoured; traces of *trans*-α-irol acetate

are due to a competing antiperiplanar elimination with the axial hydrogen at C(4);

- in derivatives (±)-34 and (±)-35; the alcohol function at C(5) is equatorial, and is eliminated to give *cis*-γ and *cis*-α derivatives preferentially. The distribution of the dehydration products might suggest a E₁ mechanism [44].

The mixture of *cis*-γ-, *cis*-α- and β-irol acetate diastereoisomers, prepared from (+)- and (-)-38 and (+) and (-)-39, as well as the alcohols obtained by basic hydrolysis, resulted inseparable by SiO₂ column chromatography. Thus, the amount of the γ-isomers present in the various mixtures was increased by photoisomerization, using conditions similar to those described in the synthesis of presiccanochromene A by Nozoe and Hirai [45] from an intermediate similar to our products.

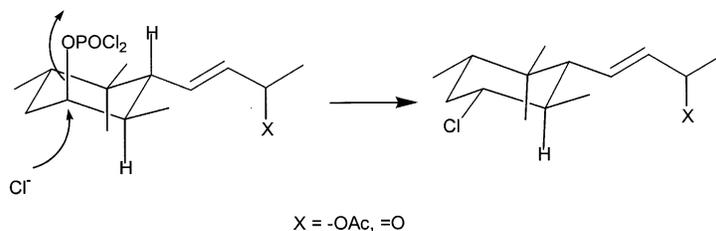


Fig. 17

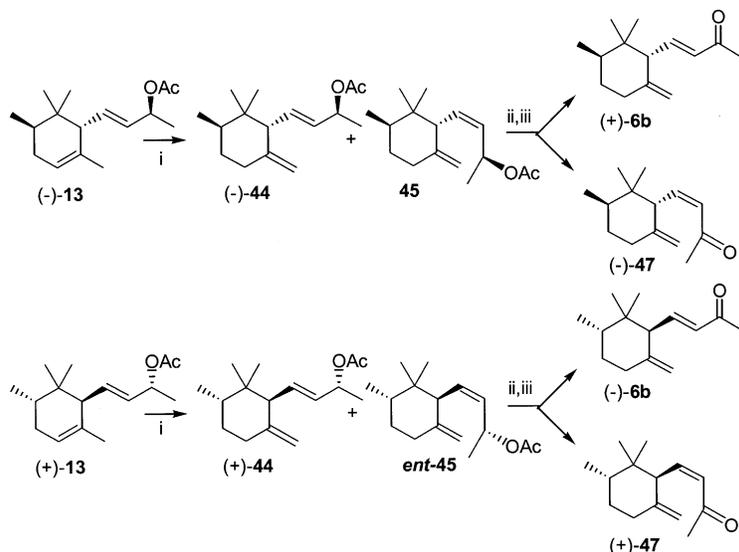


Fig. 19. (i) *hν*, isopropanol, xylene; (ii) KOH, MeOH; (iii) manganese(IV) oxide, CH₂Cl₂, column chromatography.

tively) was detected by GC, and no trace of 7Z isomers, either of the α or of the γ series, was found. Within 27 h from the beginning, *cis*- α -irol acetates had completely disappeared, and ca 30% of 7Z isomers **46** and *ent*-**46b** had formed. The appearance of (7Z)-isomers (5%) was first detected after an irradiation time of 7 h.

The rate constants of the photoisomerisation processes were estimated from the disappearance (5%) of the corresponding α -irol acetate, as measured by GC/MS, using ca 5% of *n*-dodecane as an internal

standard: $k_{\text{trans}} = 2 \times 10^{-6} \text{ s}^{-1}$; $k_{\text{cis}} = 6 \times 10^{-5} \text{ s}^{-1}$. Owing to inherent design limitations of the reactor, constant temperature could not be maintained. A difference of ca. one order of magnitude was observed between the rate constants of *cis* and *trans*- α -irol acetate isomerisation. This could be tentatively attributed to the so-called ‘steric factor’. This process is probably very sensitive to the molecular geometry of the substrate itself, since triplet–triplet transfer generally needs a collision between sensitizer and substrate.

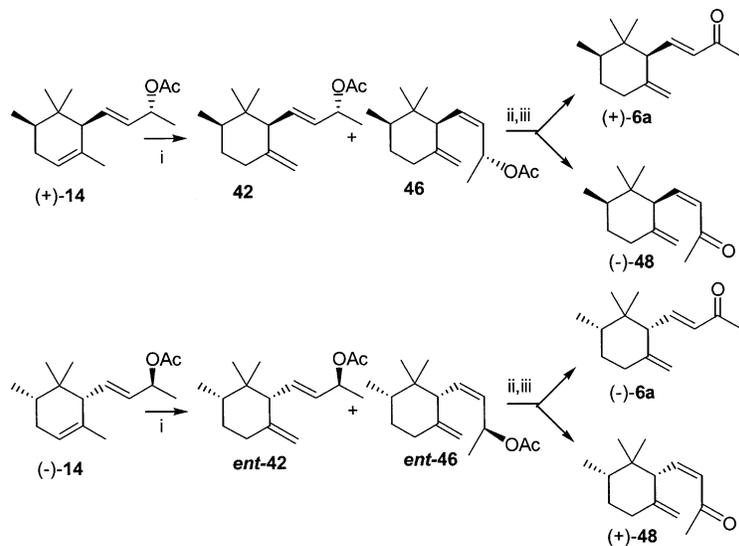


Fig. 20. (i) *hν*, isopropanol, xylene; (ii) KOH, MeOH; (iii) manganese(IV) oxide, CH₂Cl₂, column chromatography.

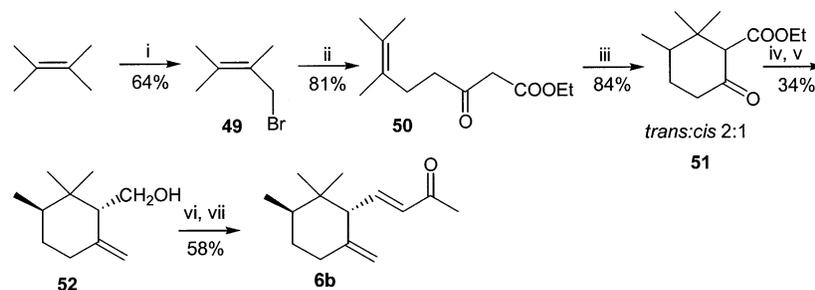


Fig. 21. (i) NBS, CCl_4 , benzoyl peroxide; (ii) ethyl acetoacetate, 1 equiv NaH, THF, then 1 equiv BuLi, 0°C ; (iii) SnCl_4 , CH_2Cl_2 ; (iv) $\text{PPh}_3=\text{CH}_2$, THF, reflux; (v) LiAlH_4 , THF, 0°C ; (vi) ClCOCOCl , DMSO; Et₃N, CH_2Cl_2 ; (vii) $\text{PPh}_3=\text{CHCOCH}_3$, toluene, reflux.

The photoisomerisation mixtures were hydrolysed in methanolic potassium hydroxide, and the corresponding irone derivatives were oxidised in methylene chloride solution in the presence of manganese (IV) oxide. Both in the *trans* and in the *cis* series, 7*E*-irone could be separated from 7*Z* diastereoisomer by column chromatography on SiO_2 . The following products were thus obtained:

- from (–)-13: (7*E*)-(+)-*trans*- γ -irone ((+)-6b): $[\alpha]_{\text{D}}^{20} = 10$, $c = 2.8 \text{ CH}_2\text{Cl}_2$, chemical purity = 74% (GC–MS), with 8% of (–)-*trans*- α -irone; ee = 99% (HPLC); (7*Z*)-(–)-*trans*- γ -irone ((–)-47): $[\alpha]_{\text{D}}^{20} = -43.2$, $c = 1.25 \text{ CH}_2\text{Cl}_2$ (lit. ref. [25]) $[\alpha]_{\text{D}}^{20} = -42$, $c = 0.4 \text{ CH}_2\text{Cl}_2$, ee 76%; chemical purity = 70% (GC–MS);
- from (+)-13: (7*E*)-(–)-*trans*- γ -irone ((–)-6b): $[\alpha]_{\text{D}}^{20} = -16$, $c = 3.0 \text{ CH}_2\text{Cl}_2$, chemical purity = 79% (GC–MS), with 3% of *trans*- α -irone; ee = 99% (HPLC); (7*Z*)-(+)-*trans*- γ -irone ((+)-47): $[\alpha]_{\text{D}}^{20} = 48.7$, $c = 1.32 \text{ CH}_2\text{Cl}_2$; chemical purity = 76% (GC–MS).
- from (–)-14: (7*E*)-(–)-*cis*- γ -irone ((–)-6a): $[\alpha]_{\text{D}}^{20} = -2.54$, $c = 4.9 \text{ CH}_2\text{Cl}_2$; chemical purity = 96% (GC–MS), ee = 99% (GC); (7*Z*)-(+)-*cis*- γ -irone ((+)-48): $[\alpha]_{\text{D}}^{20} = 50.5$, $c = 1.08 \text{ CH}_2\text{Cl}_2$, chemical purity = 90% (GC–MS).
- from (+)-14: (7*E*)-(+)-*cis*- γ -irone ((+)-6a): $[\alpha]_{\text{D}}^{20} = 1.78$, $c = 4.3 \text{ CH}_2\text{Cl}_2$, chemical purity = 93% (GC–MS), ee = 99% (GC); (7*Z*)-(–)-*cis*- γ -irone ((–)-48): $[\alpha]_{\text{D}}^{20} = -48.3$, $c = 1.2 \text{ CH}_2\text{Cl}_2$, chemical purity = 91% (GC–MS).

This approach allowed us to obtain all the eight stereoisomers of *E*- and *Z*-*cis*- and *trans*- γ -irone from commercial *Irone alpha*. However, in the samples of

(+)- and (–)-6b, the presence of α -isomers, showing high values of optical rotatory power of the opposite sign [(+)-4b $[\alpha]_{\text{D}}^{20} = 427$, $c = 0.95 \text{ CH}_2\text{Cl}_2$; (–)-4b $[\alpha]_{\text{D}}^{20} = -400$, $c = 1.05 \text{ CH}_2\text{Cl}_2$] could not be avoided, and strongly affected the values of the measured optical rotatory power. An alternative synthetic path affording enantiopure (+)- and (–)-6b with high chemical purity was thus devised.

This latter approach was based on the resolution of (\pm)-*trans*- γ -irone by enzymic methods. We thus optimised a large-scale synthesis of this racemic material, which is depicted in Fig. 21. Commercial 2,3-dimethyl-2-butene was brominated with NBS in CCl_4 solution, in the presence of catalytic amounts of dibenzoyl peroxide, to give the bromo derivative 49 [47]. This latter was employed as alkylating agent of ethyl acetoacetate to afford 50, according to a general procedure for the alkylation of dianions of β -keto esters, reported in reference [48]. Cyclisation of substrate 50 with SnCl_4 in methylene-chloride solution allowed us to obtain the known cyclic keto ester 51 [49], as a 2:1 mixture of *trans*/*cis* diastereoisomers, which had been previously used in a synthesis of racemic *trans*- γ -irone [50]. This derivative was submitted to reaction with $\text{PPh}_3 = \text{CH}_2$, followed by reduction of the ester function with lithium aluminium hydride, to afford *trans*-6-methyl- γ -cyclogeraniol (52) as a single diastereoisomer (GC–MS, $^1\text{H NMR}$). Oxidation to corresponding aldehyde and condensation with $\text{PPh}_3 = \text{CHCOCH}_3$ completed the synthetic path to racemic *trans*- γ -irone (only 2% of *cis*- γ isomer, GC–MS).

As for the resolution of the so obtained (\pm)-*trans*- γ -irone (Fig. 22), we took advantage of a scheme we had already exploited for α - [34] and γ -ionone [51]. Reduction with sodium boron hydride afforded a 1:1 mixture

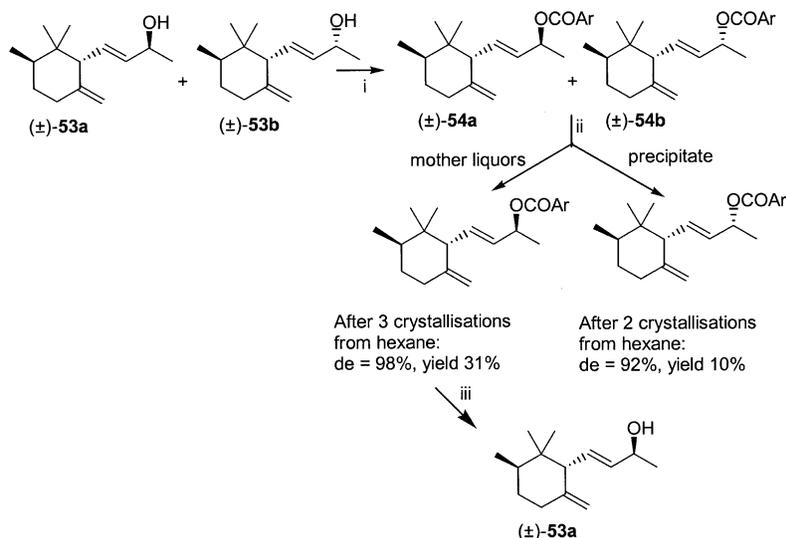


Fig. 22. (i) 4-nitrobenzoyl chloride, pyridine; (ii) fractional crystallisation from hexane; (iii) KOH, MeOH.

of γ -iroles (\pm)-**53a** and (\pm)-**53b**, which were converted into the corresponding crystalline 4-nitrobenzoate esters (\pm)-**54a** and (\pm)-**54b**. These latter derivatives could be separated by fractional crystallisation from hexane. The less soluble diastereoisomer (\pm)-**54b**, obtained as a precipitate from the first crystallisation, was crystallised twice and reached de = 92% (GC–MS). The mother liquors of the first crystallisation, surprisingly enough, afforded diastereoisomer (\pm)-**54a** with de 98% (GC/MS) after four recrystallisations. This latter was the major product, and was then hydrolysed to give (\pm)-**53a**.

After four days, Lipase-PS-mediated acetylation of racemic γ -irol **53a**, in *t*-butyl methyl ether solution in the presence of vinyl acetate, gave γ -irol acetate (+)-**44**, and left unreacted alcohol (+)-**53a** (Fig. 23). Hydrolysis of (+)-**44**, followed by manganese (IV) oxide oxidation afforded (–)-*trans*- γ -irone: $[\alpha]_D^{20} = -81$, $c = 1$, CH_2Cl_2 ; chemical purity 99% (GC–MS); ee > 99% (chiral HPLC). Oxidation of the unreacted alcohol (+)-**53a** allowed to obtain (+)-*trans*- γ -irone: $[\alpha]_D^{20} = 76.4$, $c = 1.2$, CH_2Cl_2 ; chemical purity 99% (GC–MS); ee > 99% (chiral HPLC). The reference values of $[\alpha]_D^{20}$ reported in the literature for (+)- and (–)-**6b** are rather low and not fully comparable: a) (–)-**6b**, ee 70% (NMR with chiral shift reagents), $[\alpha]_D^{20} = -43.3$ ($c = 0.8$, CH_2Cl_2) [24]; b) (–)-**6b**, ee 76% (ee of the starting material), $[\alpha]_D^{20} = -53.4$ ($c = 2.34$, CHCl_3) [23]; c) (+)-**6b**, ee 80% (ee of the starting

material), $[\alpha]_D^{20} = 57$ ($c = 0.33$, CH_2Cl_2) ref. [25]; d) (+)-**6b**, ee 99% (HPLC), $[\alpha]_D^{20} = 59.4$ ($c = 1.2$, CHCl_3) [27].

6. Olfactory evaluations

All the enantiopure samples of irone isomers were submitted to olfactory evaluation and the following results were obtained:

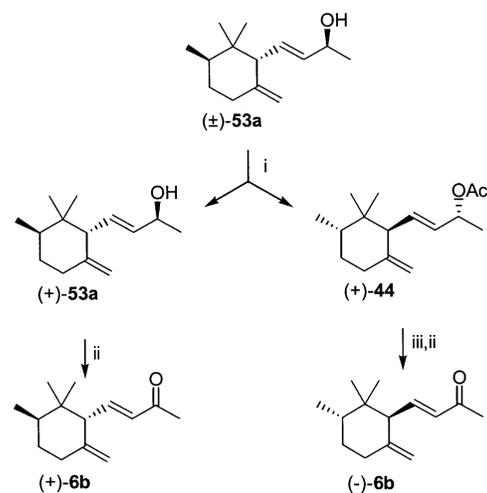


Fig. 23. (i) Lipase PS, *t*-butylmethyl ether, vinyl acetate, column chromatography; (ii) MnO_2 in methylene chloride; (iii) KOH in methanol.

- (+)-**4a**, violet-like, with woody, methylionone undertones; odour threshold (od. thr.) = 100 ppm by triangular test;
- (–)-**4a**, slightly stronger with a distinct ‘orris-butter’ character; od. thr. = 10 ppm by triangular test;
- (+)-**4b**, the weakest of the α -isomers;
- (–)-**4b**, showing a weak violet/wood/red berry character; neither (+)- nor (–)-*trans*- α -irone possesses the characteristic ‘orris’ odour;
- (+)-**6a**, showing a floral, fatty, sweet, and woody odour character, an ionone-type odour with slightly sweet aspects; od. thr. > 100 ng l⁻¹ by GC olfactometry;
- (–)-**6a**, showing a β -ionone-type odour of warm floral-woody tonality; green aspects are present, too. It shows some fruity nuances, reminiscent of pineapples; the odour is linear, and it can be considered a dry-down note; od. thr. 0.75 ng l⁻¹ by GC olfactometry;
- (+)-**6b**, very weak, of a woody odour tonality; od. thr. 113.5 ng l⁻¹ by GC olfactometry;
- (–)-**6b**, not very powerful, but possessing a soft ‘orris-butter’- type of odour; od. thr. 26.35 ng l⁻¹ by GC olfactometry;
- (+)-**5**, possessing a β -ionone-type odour of warm floral-woody tonality with green and anisic aspects; the odour is linear, and the tenacity of the note is good.
- (–)-**5**, having a woody odour with a distinct honey note, that is quite sweet; besides, it shows floral ionone-type facets, and a fruity tonality, but also an unpleasant smoky character; it belongs to the β -ionone-type family, without being very close to β -ionone.

These odour properties, evaluated on isolated samples, resulted to be quite different from those reported by Petrzilka et al. [21] and obtained by chiral GC sniff analyses of the corresponding racemic irones. According to this study, with the exception of *trans*- α -irone, all the other pairs of enantiomers could be distinguished by human nose. In particular, of *cis*- α and *cis*- γ -irone, only the (+)-enantiomers appeared to be odour active, showing also the finest and strongest iris-like notes.

The odour evaluations performed on our samples pointed out that all the irone isomers had different odour properties, and both (–)-*cis*- α - and (–)-*cis*- γ -

irones were found stronger than the corresponding enantiomers. (–)-*Cis*- α -irone was also described to possess a *distinct orris-butter character*.

7. Conclusions

The discovery of irones dates back to more than one century ago: it was in 1893 when Tiemann isolated a ketonic odoriferous fraction from iris root oil. Since then, chemists have devoted plenty of work to establish their chemical constitution, configuration, and distribution according to the various iris species. A blind spot remained in their characterisation: the complete evaluation of the odour properties of all the ten irone isomers in enantiopure form as single isolated compound. This paper is a description of the efforts we devoted to reach this aim.

The great development had by enzyme chemistry during the last century and the commercial availability of enzyme preparations allowed us to successfully face the synthetic problem of irone isomers. We employed commercial racemic *Irone alpha*[®] as a starting material. We always obtained optical activation by lipase mediated kinetic resolution. By means of conventional organic reaction, i.e. epoxidation, reduction, dehydration, and photoisomerisation, we had access to α , β and γ irone skeletons. Thus, we had in our hands samples of enantiopure irones to submit to olfactory evaluation. The results of this study were found quite different from those obtained by Petrzilka by GC-sniffing. We found that (–)-*cis*- α -irone and (–)-*trans*- γ -irone showed the delicate orris-butter character. The odour response of all irone isomers was found to be extremely dependent on the relative and absolute configuration at the two stereocentres at C₂ and C₆.

Acknowledgements

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