Acetylation of Racemic *cis*- and *trans*-α-Irols, Mediated by *Porcine Pancreatic Lipase (PPL)* − A New Route to (−) and (+)-*cis*-α-Irone

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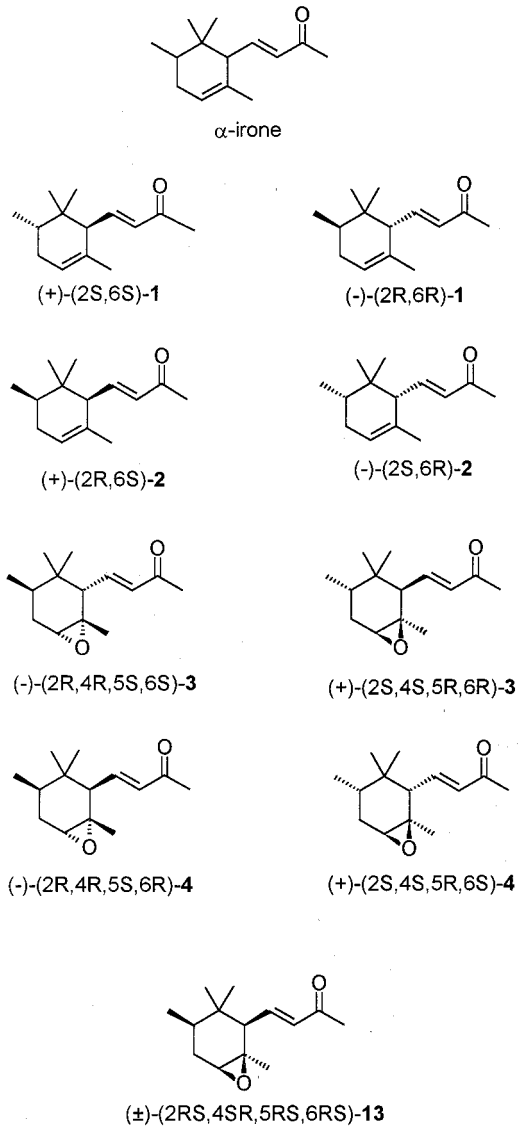
The mixture of the four racemic stereoisomers of α-irol (**5**–**8**) was submitted to PPL-mediated acetylation; *cis*-α-irol **8b** was converted more quickly than any other diastereoisomer and in enantiopure fashion. By chance, this latter derivative was the precursor of (–)-*cis*-α-irone, the stereoisomer showing the strongest *Orris* butter character. Suitable manipulation of the material left unchanged by PPL provided (+)-*cis*-α-irone. PPL's mode of transformation of epoxy *trans*- and *cis*-α-irols was investigated, in order to ascertain the effect of chemical structure on the discriminating properties of this enzyme.

Introduction

Orris root oil is one of the most valued raw materials in the flavour and fragrance industry, and is obtained by a complex procedure from the dried, peeled rhizomes of various *Iris* species.[1] It is mainly composed of *cis*-α- and *cis*-γ-irone, with minor quantities of *trans*-α- and β-irone. (The last two isomers are probably produced from the first two during the extractive processing.) The sensory response to each irone isomer is different, and depends upon its absolute configuration, and the isomeric distribution varies with the botanical species of the plant.[2] Despite the high selling price of the *Iris* essential oil, which a few years ago reached a peak of 120,000 DM/kg,[1c] of the known synthetic entries to the single enantiomers of *cis*-α- and *cis*-γ-irones (the key constituents of the mixture) none seems of interest practically.[3] Synthetic substitutes for the *Iris* essential oil do exist, consisting of racemic mixtures of *trans*- and *cis*-α-irone and impure β-isomer, and with varying content of the most highly prized *cis* diastereoisomer. These products cost tenths of the price of the natural extract, but hardly match its fragrance and finesse.

Recently, we succeeded in the preparation of the single enantiomers of *trans*- (**1**) and *cis*-α-irone (**2**),[4] using a chemoenzymatic sequence that we had previously developed for the resolution of α-ionone,[5] and 4,5-epoxy-α-ionone.[6] We submitted the four enantiopure stereoisomers of α-irone to olfactory appraisal and discovered that (−)-*cis*-**2** exhibited the finest *orris butter* character.

A preliminary derivatisation of commercial racemic α-irone (**1**/**2** = 55:45), by conversion into the 4,5-epoxy derivatives, was necessary in order to apply our synthetic scheme. Kinetic resolution by acetylation, mediated by Lipase PS,



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of suitable epoxy *trans*- and *cis*-α-irol derivatives, followed by manganese(IV) oxide oxidation, allowed us to obtain enantiopure epoxy-α-irones (+)- and (−)-**3** and (+)- and (−)-**4**. However, the deoxygenation reaction, converting these derivatives into the corresponding (+)- and (−)-**1** and (−)- and (+)-**2**, introduced varying amounts of chemical impurities, which could be separated from the desired α-irones only with difficulty.

Thus, in order to overcome this drawback of the introduction and removal of the oxygen atom onto and from the α-irone skeleton, however necessary to achieve sufficient physical differentiation between the *trans* and the *cis* series, the direct lipase-mediated acetylation of the mixture of the four racemic α-irols **5**−**8** was investigated (enantiomers are labelled **a** and **b** wherever sign determination has not been possible).

Discrimination in the acetylation of *cis*- and *trans*-α-irols was observed only in PPL-mediated reactions; *cis*-α-irol **8b** was converted more rapidly than any other diastereoisomer, and in enantiopure manner. By chance, the derivative thus obtained was the precursor of (−)-*cis*-α-irone, the most precious of the α-irone stereoisomers. Suitable manipulation of the alcohol recovered unchanged from enzymatic acetylation provided (+)-*cis*-α-irone. No interesting synthetic access to the less valuable *trans* derivatives was found by this new approach. On account of the good diastereoselectivity shown by this enzymatic reaction, and for the sake of completeness, the mode of the transformation by PPL of epoxy *trans*- and *cis*-α-irols was investigated, in order to ascertain the effect of chemical structure on the discriminating properties of this enzyme. We report on the results of this research here.

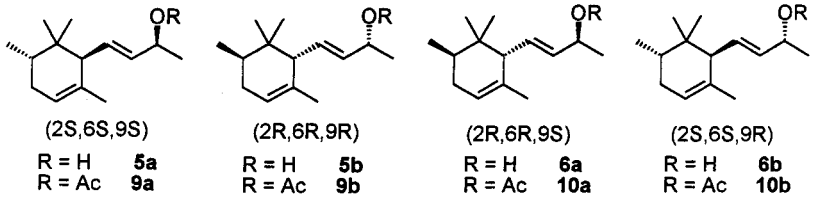
Results and Discussion

Enzyme-Mediated Acetylation of α-Irols

Preparation of (−)- and (+)-*cis*-α-Irone: *Irone alpha*®, a commercially available 55:45 mixture of racemic *trans*- and *cis*-α-irone (**1** and **2**) with 5% of β-isomer, was reduced with sodium borohydride in dichloromethane/methanol (2:1) solution, to afford the four racemic α-irols **5**−**8**.[4] Chiral GC analysis (see Experimental Section) of the corresponding acetate derivatives gave eight peaks under suitable conditions, using a Hydrodex-β-PM column (Figure 1a). Routine chiral GC analyses, however, were performed on a Chirasil DEX CB column, total analysis time being much shorter (40 min vs. 100 min), even though only seven peaks could be detected in the GC trace of α-irol acetate derivatives [the relative intensities were in accordance with the overlapping of two peaks (**11b** + **12a**)].

The reduction mixture was submitted to biocatalysed acetylation in *tert*-butyl methyl ether solution, in the presence of vinyl acetate as an acyl donor, using two different types of enzymatic preparations: *Porcine Pancreatic Lipase* (Sigma, Type II), and *Lipase PS Pseudomonas cepacia* (Amano Pharmaceuticals Co.). The stereoisomeric compositions of the resulting acetate derivatives are reported in Table 1. All the enantiomeric and diastereoisomeric excesses were determined by chiral GC analysis (Chirasil DEX CB column, see Experimental Section). For the sake of simplicity, throughout the whole work diastereoisomeric excess will be used only to express the ratio between diastereoisomers showing the same relative stereochemistry (*cis* or *trans*) at C^2 and C^6. We will refer to the ratio between *cis* and *trans* derivatives as ''*cis/trans* ratio''.

***Trans*-α-Irols**



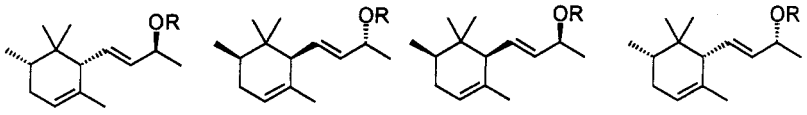
(2S,6S,9S) R = H **5a** R = Ac **9a**

(2R,6R,9R) R = H **5b** R = Ac **9b**

(2R,6R,9S) R = H **6a** R = Ac **10a**

(2S,6S,9R) R = H **6b** R = Ac **10b**

***Cis*-α-Irols**



(2S,6R,9S) R = H **7a** R = Ac **11a**

(2R,6S,9R) R = H **7b** R = Ac **11b**

(2R,6S,9S) R = H **8a** R = Ac **12a**

(2S,6R,9R) R = H **8b** R = Ac **12b**

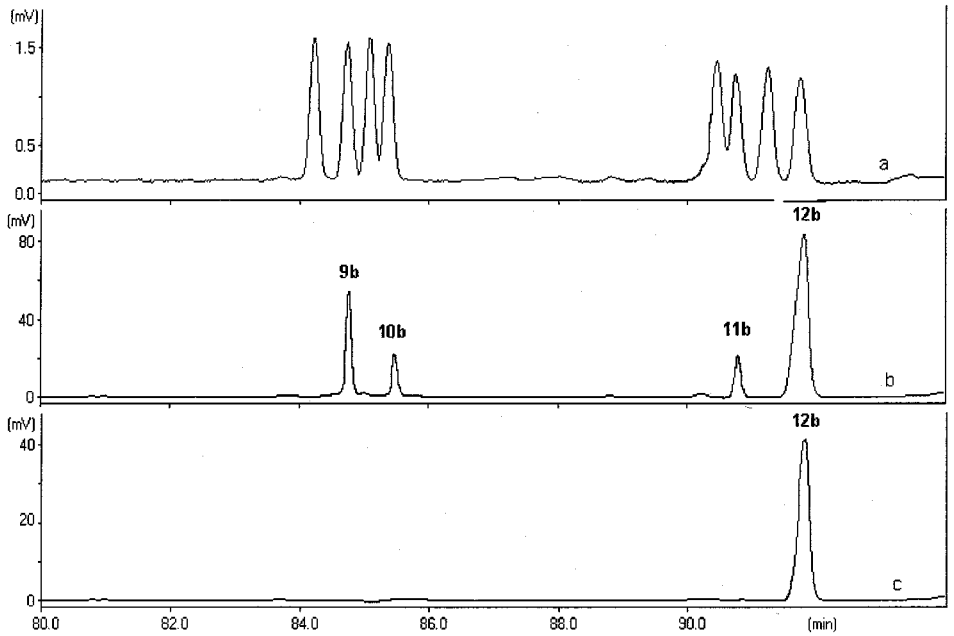


Figure 1. a) GC analysis of the eight α-irol acetate stereoisomers; b) GC analysis of the PPL-acetylated product; c) GC analysis of acetate **12b** (Hydrodex-β-PM column)

Table 1. Stereoisomeric composition of the enzymatically produced α-irol acetates

Enzyme	Acetylated product (area%, chiral GC[a])	Unreacted material (area %, chiral GC[a])
PPL	**9b** (25.4), **10b** (7.6), **11b** (6.8), **12b** (60.2) *cis*/*trans* ratio = 2/1 **9b** *ee* = 99%, *de* = 54% **12b** *ee* = 99%, *de* = 78%	**5a** (16.0), **5b** (11.7), **6a** (16.1), **6b** (14.0) **7a** (12.9), **7b** + **8a** (25.0), **8b** (4.3)
Lipase PS	**9b** (27.1), **10b** (27.1), **11b** (22.4), **12b** (22.4) *cis*/*trans* ratio = 1.2/1 **9b** *ee* = 99%, *de* = 0 **12b** *ee* = 99%, *de* = 0	**5a** (26.9), **6a** (27.1), **7a** (22.5), **8a** (22.5)

[a] Chirasil DEX CB column

The stereochemical assignment of the enzymatically acetylated derivatives **9b**, **10b**, **11b**, and **12b** was based on the following considerations:

i) we assumed that the four stereoisomers preferentially acetylated by lipases were of (*R*) configuration at C^9, as we had already verified in a previous work[5] for enzyme-mediated esterification of α-ionol, by means of chemical correlation;

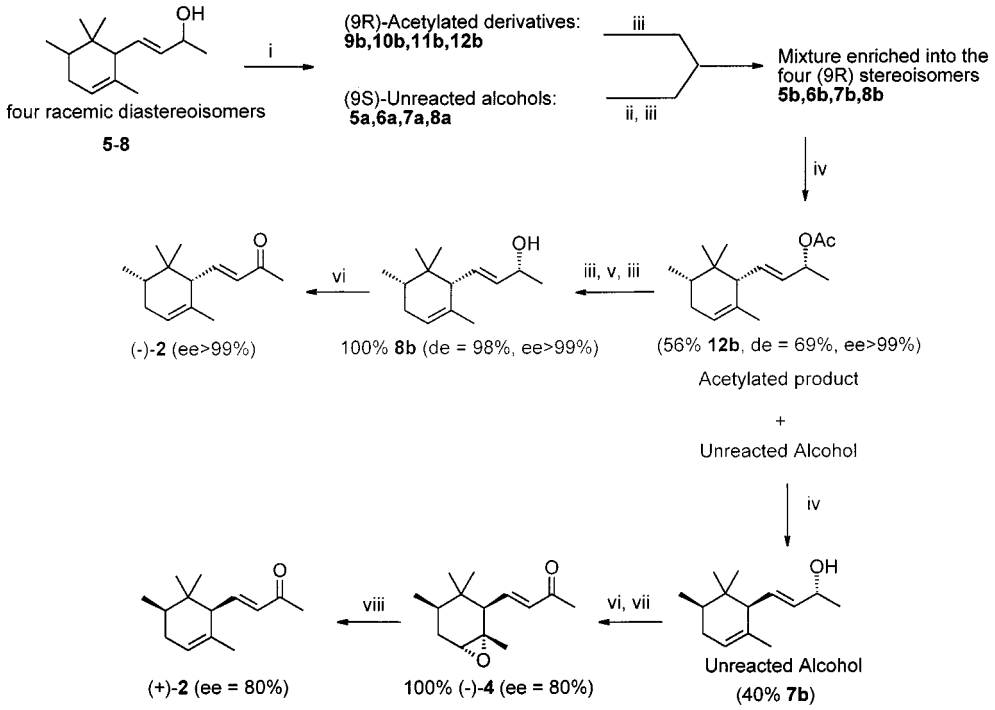
ii) the acetate derivative recovered from the PPL-mediated reaction was hydrolysed and then oxidised with manganese(IV) oxide, to give a sample of α-irone composed (chiral GC[4]) of (−)-*trans*-α-irone 25.2%, (+)-*trans*-α-irone 8.3%, and *cis*-α-irone 67.0%. Thus, the (2*R*,6*R*) configuration was assigned to the most abundant *trans*-acetate stereoisomer **9b**, and the (2*S*,6*S*) configuration to the other *trans*-acetate **10b**;

iii) at the end of the synthetic sequence described below, derivative **12b** afforded (−)-*cis*-α-irone. This result allowed us to conclude that stereoisomer (2*S*,6*R*,9*R*)-**8b** was the one preferentially acetylated by PPL.

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 **12b**, showing a rather good diastereoisomeric enrichment (*de* = 78%.). The recovered unchanged alcohol still contained 4.3% of stereoisomer **8b** (chiral GC on the corresponding acetates).

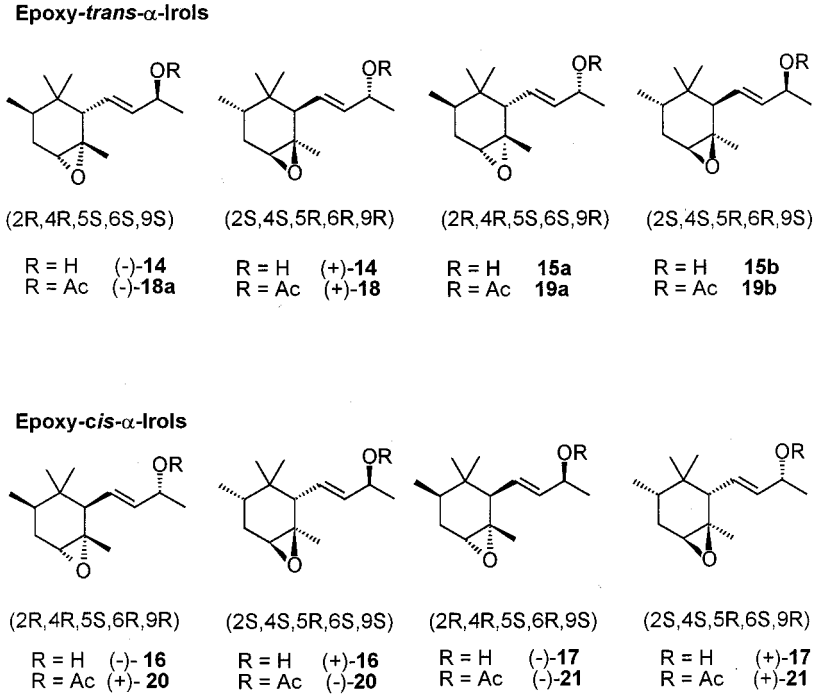
Acetylation mediated by Lipase PS proceeded rapidly, to afford a nearly equimolar mixture of the four (9*R*) diastereoisomers **9b**, **10b**, **11b**, and **12b** in enantiopure form. The enzyme neither discriminated between *trans*- and *cis*-α-irols, nor did it show any diastereoselection within each single set (i.e. **9b** vs. **10b**, or **11b** vs. **12b**). The unchanged material recovered from the reaction was an equimolar mixture of the four enantiopure (9*S*) alcohol stereoisomers **5a**, **6a**, **7a**, and **8a** (24 h reaction time).

The results of these two biocatalysed experiments were usefully combined as shown in Scheme 1. α-Irol (four racemic stereoisomers, **5**−**8**) was submitted to acetylation mediated by Lipase PS; the acetylated product (**9b**, **10b**, **11b**,



Scheme 1. Lipase PS, *tert*-butyl methyl ether, vinyl acetate, column chromatography; ii. 4-nitrobenzoic acid, diisopropyl azodicarboxylate, triphenylphosphane, THF; iii. KOH, MeOH; iv. PPL, *tert*-butyl methyl ether, vinyl acetate, column chromatography; v. 4-nitrobenzoyl chloride, pyridine, dichloromethane; two crystallisations from hexane; vi. manganese(IV) oxide, dichloromethane; vii. *m*-chloroperbenzoic acid, chloroform, column chromatography; viii. NaI, chlorotrimethylsilane, THF

and **12b**; 48% chemical yield**)** and the unchanged material (**5a**, **6a**, **7a**, and **8a**; 44% chemical yield) were separated by column chromatography. The unchanged material was converted into the 4-nitrobenzoate ester derivative according to Mitsunobu's procedure (74% chemical yield), in order to perform the inversion of the configuration at C[9]. The alcohol recovered from saponification of this benzoate derivative (96% chemical yield) showed the following composition by chiral GC analysis of the corresponding acetates: **5a** (5.3%), **5b** (21.8%), **6a** (5.4%), **6b** (21.7%), **7a** (4.5%), **7b** + **8a** (22.3%), **8b** (18.1%). Thus, no complete inversion of the configuration was observed. This material could be combined with the alcohol obtained upon hydrolysis of the enzymatically acetylated product to give a mixture of α-irols



highly enriched in the four (9*R*) diastereoisomers **5b**, **6b**, **7b**, and **8b**. We took advantage of the clear-cut separation of (9*R*) and (9*S*) diastereoisomers assured by Lipase PS, and of their relative conversion by Mitsunobu esterification, in order to enrich the starting mixture in the stereoisomers efficiently transformed by lipases, and hence reduce the unconverted waste material.

PPL-catalysed reaction of this mixture provided an acetylated product (37% chemical yield) containing 56% of the enantiopure stereoisomer **12b** (*de* = 69%, Scheme 1, Figure 1b). 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** (51% chemical yield from **12b**) with high diastereoisomeric purity {$[\alpha]_D^{20} = -56$ (c = 1.02, CH_2Cl_2); *ee* = 99%, *de* = 98%; Figure 1c}. Oxidation of **8b** with manganese(IV) oxide in dichloromethane afforded enantiopure (−)-*cis*-α-irone (82% chemical yield, 96% chemical purity) showing $[\alpha]_D^{20} = -118$ (c = 0.95, CH_2Cl_2) (ref.[4] $[\alpha]_D^{20} = -130$ (c = 1.55, CH_2Cl_2), chemical purity = 85%, *ee* = 98%; ref.[3a] $[\alpha]_D^{20} = -103.7$ (c = 0.65, $CHCl_3$), 86% *ee*).

As for the preparation of *cis*-(+)-α-irone, its potential precursor **7b** was converted very slowly by PPL, and mostly remained in the unchanged material. Two subsequent PPL-mediated acetylations left an unchanged alcohol which, upon manganese(IV)oxide oxidation, gave an α-irone sample of the following composition: (−)-**1** (19%), (+)-**1** (34%)**,** *cis*-α-irone (45%). Separation of *trans* and *cis* diastereoisomers could be achieved only by conversion into the epoxide derivatives. Column chromatography allowed us to recover a sample of (−)-**4** {*ee* = 80%, GC; $[\alpha]_D^{20} = -12$ (c = 1.35, CH_2Cl_2)} with no trace of *trans* derivatives; upon deoxygenation this gave (+)-*cis*-α-irone {$[\alpha]_D^{20} = 87$ (c = 1.4, CH_2Cl_2); ref.[4] $[\alpha]_D^{20} = 117$ (c = 1.5, CH_2Cl_2), chemical purity = 81%; *ee* = 98%; ref.[7] $[\alpha]_D^{20} = 111$ (c = 0.92, CH_2Cl_2)}.

Effect of Chemical Structure on the Steric Course of PPL-Mediated Reactions

Acetylation of Epoxy-α-irols: Commercial *Irone alpha* was submitted to epoxidation under different reaction conditions (magnesium monoperphthalate in aqueous ethanol; *m*-chloroperbenzoic acid in chloroform; *m*-chloroperbenzoic acid in toluene). Only three racemic epoxy-α-irone diastereoisomers could be detected: (±)-**3**, (±)-**4**, and (±)-**13** (see ref.[4] for the structural assignment). The use of *m*-chloroperbenzoic acid (*m*-CPBA) in chloroform allowed us to obtain the most favourable ratio between the two epoxy-*cis*-irone diastereoisomers ((±)-**4**/(±)-**13** = 2.5, GC). The composition of the crude epoxidation mixture (*m*-CPBA, $CHCl_3$) was determined by GC analysis:[4] (±)-**3** (58%), (±)-**13** (12%), (±)-**4** (31%). Epoxy-*trans*-α-irone (±)-**3** could only with difficulty be separated from epoxy-*cis*-α-irone (±)-**13** by column chromatography: the best sample of (±)-**3** contained 20% of (±)-**13** {^{1}H NMR: δ_H = 3.11, m, C(4)H in (±)-**3**; δ_H = 3.07, d, J = 6 Hz C(4)H in (±)-**13**}. Epoxy-*cis*-α-irone (±)-**4** could be purified by recrystallisation from hexane.[4]

The steric course of PPL-catalysed acetylation was investigated on the following three substrates:

A) a sample enriched (80%, GC) in the two racemic epoxy-*trans*-α-irol diastereoisomers (±)-**14** and (±)-**15**, obtained by $NaBH_4$ reduction of an 8:2 mixture of (±)-**3** and (±)-**13;**

B) a 1:1 mixture of the two racemic epoxy-*cis*-irols (±)-**16** and (±)-**17**, prepared starting from crystalline (±)-**4**;

C) an approximately equimolar mixture of epoxy *trans*- and *cis*-α-irol stereoisomers, prepared by reduction of a 1.3:1 mixture of (±)-**3** and (±)-**4** containing 9.5% of (±)-**13**. The results are reported in Table 2.

For studies of this type, we did not perform any chromatographic separation either of the mixture (±)-**14/**(±)-**15**, or of the mixture (±)-**16/**(±)-**17**. These separations are indeed possible though, and have already been reported in ref.[4]

The following considerations could be drawn from the analysis of these latter enzymatic reactions:

i) in the acetylation reaction, PPL showed greater preference for epoxy-*cis*-α-irols than for *trans*-stereoisomers: (+)-**21** was the main component (59%) of the acetylated product of sample C;

ii) high diastereoselectivity was observed in the PPL-mediated esterification of sample B (epoxy-*cis*-α irols only): (+)-**21** was produced with a *de* = 82%. The diastereoisomeric purity decreased when a *trans*/*cis* mixture was used (sample C (+)-**21** *de* = 65%);

Table 2. Stereoisomeric composition of the enzymatically produced 4,5-epoxy-α-irol acetates

Sample	Acetylated products (Area%[a])	Chemical yields (%), reaction time (h)
40% (±)-**14** 40% (±)-**15**	(+)-**18** + **19a** (2 overlapping peaks, 73); derivatives of (±)-**13** (27)	22, 24
50% (±)-**16** 50% (±)-**17**	(+)-**20** (9)**,** (+)-**21** (91) (+)-**21**: *ee* > 99%, *de* 82%	34, 24
26% (±)-**14** 26% (±)-**15** 19% (±)-**16** 19% (±)-**17**	(+)-**18**+**19a** (2 overlapping peaks, 21);(+)-**20** (12.3); (+)-**21** (59); derivatives of (±)-**13** (7.5). *cis*/*trans* ratio: 3.7/1 (+)-**21**: *ee* > 99%, *de* 65%	30, 24

[a] Chirasil DEX CB column

iii) PPL-catalysed acetylations of α-irols (*cis*/*trans* mixture) and epoxy-α-irols (*cis/trans* mixture, sample C) showed a very similar steric course: **12b** and (+)-**21** were the main components of the two acetylated mixtures, respectively (**12b** *de* = 78%, *cis/trans* ratio 2:1, (+)-**21** *de* = 65%, *cis/trans* ratio 3.7:1);

iv) the mixture of epoxy-α-irones obtained upon saponification and manganese(IV) oxide oxidation of the acetylated product of sample A showed the following composition: (−)-**3** (51.2%), (+)-**3** (13.7%), (+)-**4** (26.7%), (−)-**4** (8.2%). It could be inferred that **19a** and (+)-**18** were produced by PPL in a 3.7 ratio (**19a** *de* = 58%).

Conclusions

This study on the lipase-mediated acetylation of α-irols and their corresponding epoxy derivatives has confirmed known behaviour; i.e. reactions catalysed by Lipase PS are enantiospecific but not diastereoselective at all, while the best diastereoselection values are obtained when PPL is used as a catalyst.[4,5,6,7] In this context, however, it is interesting to remind oneself that, in the *Timberol* series,[8] lipase PS displayed 100% diastereoselectivity associated with enantiospecificity for conversion from the allylic alcohol to the saturated counterpart.

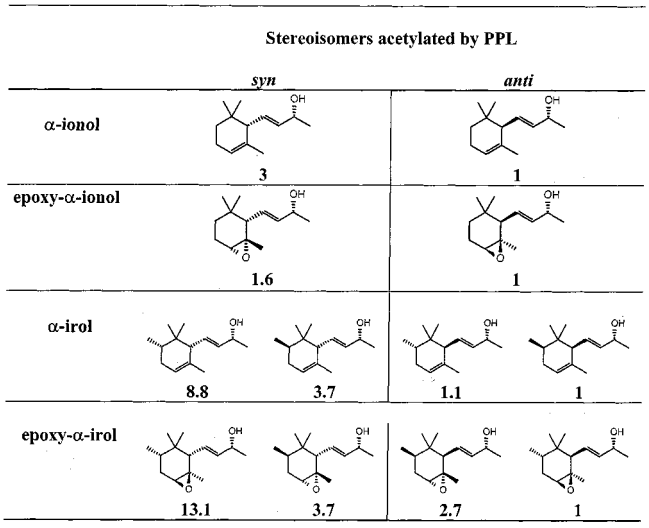
Comparing the data (Table 3) collected for α-ionol,[5] epoxy-α-ionol,[6] α-irol (this work), and epoxy-α-irol,[4] we have observed that the diastereoselection values shown by PPL are strongly influenced by subtle structural modifications, even far away from the reacting centre. First of all, the (9*R*) diastereoisomers with a *syn* relationship between the side chain and the hydroxyl group are those preferentially acetylated by PPL in all four series. When a methyl group is added at C(2) of the non-terpenoid skeleton (α-irol and epoxy-α-irol), the preference for the *syn* diastereoisomers increases (*syn*/*anti* ratio: 3:1 for α-ionol, 1.6:1 for epoxy-α-ionol, 5.9.1 for α-irol, 4.5.1 for epoxy-α-irol). Moreover, of the possible *syn* diastereoisomers, those bearing the methyl group at C(2) on the same side of the side chain are converted more quickly (**8b** and (+)-**17**).

It seems that the presence of the epoxide ring does not definitely alter the steric course of these enzymatically mediated acetylations.

The striking difference in the rate of transformation of **8b** out of the four (9*R*) stereoisomers of the mixture **5**−**8** is highly useful from the synthetic point of view. As a matter of fact, it makes access to (−)-**2** much easier and more direct than that described for (*S*)-α-ionone,[5] in spite of the presence of eight rather than four stereoisomers in the starting mixture.

The combination of an enantiospecific transformation, mediated by lipase PS, of the mixture **5**−**8** and a Mitsunobu esterification, followed by diastereoselective acetylation catalysed by PPL and purification by recrystallisation of the 4-nitrobenzoate derivative of **8b**, allowed us to isolate from commercial *Irone alpha*® (−)-*cis*-α-irone, possessing the most elegant *Orris* butter sensory character,[4] through admittedly long, but simple, chemical operations. More complicated, but similarly workable, was the recovery of (+)-**2** by means of derivatisation of the products surviving the enzymatic acetylation.

Table 3. Composition of PPL-acetylated products in α-ionol, epoxy-α-ionol, α-irol, and epoxy-α-irol series (chiral GC)



Experimental Section

Irone alpha® (55:45 **1/2**) was purchased from IES (Allauch, France). Lipase PS *Pseudomonas cepacia* (Amano Pharmaceuticals Co., Japan) was employed in this work. − Chiral GC analysis of α-irol acetates was performed on a Hydrodex-β-PM 50m × 0.25 mm column (Superchrom), using the following temperature program: 70 °C (3′) − 1 °C/min − 150 °C(10′) − 1 °C/min − 160 °C(1′)-10 °C/min − 200 °C(1′); t_R = 84.21, 84.72, 85.07, 85.35, 90.43, 90.72, 91.22, 91.73. The enantiomeric and diastereoisomeric excesses for all the substrates were determined by chiral GC analysis on a Chirasil DEX CB, 25m × 0.25 mm (Chrompack) column, installed on a DANI HT 86.10 gas chromatograph, with the following temperature program: 70 °C (3′) − 3.5 °C/min − 140 °C − 8 °C/min − 180 °C (1′). Hydrogen was employed as a carrier gas. Mass-detection limit for chiral GC analyses was *ca.* 10 ng for an injected volume of 1 μL. − ^{1}H NMR spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise stated, on a Bruker AC-250 spectrometer (^{1}H; 250 MHz). The chemical shift scale was based on internal tetramethylsilane. *J* values are in Hz. − Optical rotations were measured on a Jasco DIP 181 digital polarimeter. − Microanalyses were determined on a 1106 Carlo Erba Analyzer. − TLC analyses were performed on Merck Kieselgel 60 F_{254} plates. All the chromatographic separations were carried out on silica gel columns. − Syntheses and characterisation of 4,5-epoxy-α-irones (±)-**3**, (±)-**4**, and (±)-**13** and of the corresponding 4,5-epoxy-α-irol derivatives are reported in ref.[4]

(2*RS*,6*RS*,9*RS*)-, (2*RS*,6*RS*,9*SR*)-, (2*RS*,6*SR*,9*RS*)-, and (2*RS*,6*SR*,9*SR*)-α-Irols (5−8): Irone alpha® (50.0 g, 0.243 mol) was reduced with $NaBH_4$ (13.7 g, 0.360 mol) in dichloromethane/methanol (2:1; 500 mL) solution at 0 °C. After the usual workup, a mixture of the four racemic diastereoisomers **5**−**8** was obtained (47.9 g, 95%) (found C 80.67, H 11.65; $C_{14}H_{24}O$ calcd. C 80.71, H

11.61); δ_H 5.47 (m, 3 H, vinylic hydrogens), 4.32 (m, 1 H, C(9)H), 2.4−1.4 (m, 7 H,), 1.28 (m, 3 H, CH_3CHOH), 0.9−0.6 (m, 9 H, 2C(1)Me and C(2)Me. Chiral GC analysis of the corresponding acetate derivatives: t_R/min **9a**, 19.74 (13%); **9b**, 19.98 (12%); **10a**, 20.12 (12%); **10b**, 20.36 (13%); **11a**, 21.50 (12%); **11b** + **12a**, 21.66 (22%); **12b**, 21.93 (12%).

General Procedure for Enzyme-Mediated Acetylations: A mixture of α-irols (**5−8**) (10.0 g, 0.0481 mol), lipase (10.0 g), and vinyl acetate (40 mL) in *tert*-butyl methyl ether (150 mL) was stirred at room temperature for 24 h. The residue obtained upon evaporation of the filtered reaction mixture was chromatographed, eluting with hexane→hexane:ethyl acetate (1:1). The first eluted fractions provided the acetate derivative, to be analysed on a chiral GC column. The last eluted fractions afforded the unchanged starting material, a sample of which was acetylated by treatment with acetic anhydride and pyridine to perform chiral GC analysis. This procedure was employed for the enzymatic acetylations of α-irols and of epoxy-α-irols (samples A, B, C). Detailed results are reported in Table 1 and 2.

Mitsunobu Esterification of the Unchanged Alcohols 5a,6a,7a,8a Recovered from Lipase PS Acetylation of α-Irols 5−8: A solution of the four (9*S*) alcohol diastereoisomers **5a**, **6a**, **7a**, and **8a** (10.0 g, 0.0481 mmol, chiral GC analysis of the corresponding acetates: t_R/min **9a**, 19.74; **10a**, 20.12; **11a**, 21.50; **12a** 21.66) and triphenylphosphane (13.6 g, 0.0519 mol) in tetrahydrofuran (70 mL) was dropped into a solution of diisopropylazodicarboxylate (10.5 g, 0.0519 mmol) and 4-nitrobenzoic acid (8.68 g, 0.0519 mol) in tetrahydrofuran (100 mL). The reaction mixture was stirred at room temperature for 12 h, then concentrated under reduced pressure to give a residue, which was chromatographed, eluting with hexane→hexane/ethyl acetate (9:1). The first eluted fractions gave the desired 4-nitrobenzoate ester derivatives (12.7 g, 74%) (found C 70.59, H 7.57, N 3.99; $C_{21}H_{27}NO_4$ calcd. C, 70.56, H 7.61, N 3.93); chiral GC analysis of the corresponding acetates: *t*/min **9a,** 19.74 (5.3%); **9b**, 19.98 (21.8%); **10a**, 20.12 (5.4%); **10b**, 20.36 (21.7%); **11a**, 21.50 (4.5%); **11b** + **12a**, 21.66 (22.3%); **12b**, 21.93 (18.1%). δ_H 8.22 (m, 4 H, aromatic hydrogens), 5.8−5.5 (m, 3 H), 5.5−5.3 [m, 1 H, C(4)H], 2.4−1.1 (m, 10 H), 1.0−0.6 [m, 9 H, 2C(1)Me + C(2)Me].

(2*S*,6*R*,9*R*)-*cis*-α-Irol Acetate (12b): The 4-nitrobenzoate ester (12.0 g, 0.0338 mol) recovered from the Mitsunobu reaction was hydrolysed with potassium hydroxide (85%, 3.36 g, 0.0510 mol) in methanol (50 mL) to give a mixture of (9*R*)-α-irol diastereoisomers (6.79 g, 96%) which was combined with the alcohol (8.32 g) obtained by saponification (KOH, methanol) of the Lipase PS acetylated product (10.0 g). The ^{1}H NMR spectrum was in accordance with that of the mixture of the four racemic diastereoisomers (**5−8**). This mixture of α-irols enriched in the (9*R*) diastereoisomers **5b**, **6b**, **7b**, **8b** (15.0 g, 0.0721 mol) was submitted to PPL acetylation according to the described procedure. The acetylated product (6.66 g, 37%) contained 56% (GC) of acetate **12b** (chiral GC analysis t_R/min 21.93; *ee* > 99%, *de* = 69%) (found C 76.79, H 10.42; $C_{16}H_{26}O_2$ calcd. C 76.75, H 10.47%). A second PPL acetylation of the unchanged material (10.3 g, 0.0495 mol) left an alcoholic mixture (7.21 g, 0.0347 mol) enriched in stereoisomer **7b** (40%, chiral GC analysis of the corresponding acetate derivative: t_R/min 21.66.). After the diastereoisomeric enrichment, described below, acetate **12b** showed the following ^{1}H NMR spectrum: δ_H 5.55−5.40 (m, 3 H, vinylic hydrogens), 5.36 (m, 1 H, C(9)H), 2.33 (m, 1 H), 2.03 (s, 3 H, CH_3CO), 1.90 (m, 1 H), 1.70 (m, 1 H), 1.51 (m, 3 H, C(5)Me), 1.44 (m, 1 H), 1.33 (d *J* = 6.5, 3 H, CH_3CHOAc), 0.85 [d *J* = 7, 3 H, C(2)Me], 0.84 [s, 3 H, C(1)Me], 0.62 [s, 3 H, C(1)Me].

(−)-(2*S*,6*R*,9*R*)-*cis*-α-Irol (8b): The acetylated product (6.60 g, 0.0264 mol) containing 56% (GC) of acetate **12b** was saponified (KOH, methanol) and converted into the corresponding 4-nitrobenzoate ester by reaction with 4-nitrobenzoyl chloride (7.05 g, 0.0384 mol) in dichloromethane (50 mL) in the presence of pyridine (10 mL). After the usual workup, the benzoate ester was crystallised twice from hexane (5.01 g, 54%): m.p. 77 °C; $[\alpha]_D^{20}$ = −92 (*c* = 1, CH_2Cl_2). − δ_H = 8.23 (m, 4 H, aromatic hydrogens), 5.70−5.55 (m, 3 H), 5.44 [m, 1 H, C(4)H], 2.36 (m, 1 H), 1.90 (m, 1 H), 1.70 (m, 1 H), 1.50 (m, 7 H), 0.85 [s + d *J* = 6.5, 6 H, C(1)Me + C(2)Me], 0.63 [s, 3 H, C(1)Me]. This benzoate ester was saponified (KOH, methanol) to afford (2*S*,6*R*,9*R*)-*cis*-α-irol **8b** (2.65 g, 91%): $[\alpha]_D^{20}$= −56 (*c* = 1.02, CH_2Cl_2); *ee* >99%, *de* = 98%, chiral GC of the corresponding acetate derivative (t_R/min 21.93); δ_H = 5.57 [dd *J* = 15.2 and 6.5, 1 H, C(8)H], 5.43 (dd + m *J* = 15.2 and 10.3, 2 H, C(7)H + C(4)H], 4.33 [quintuplet *J* = 6.5, 1 H, C(9)H], 2.34 [d *J* = 10.3, 1 H, C(6)H], 1.90 (m, 1 H), 1.70 (m, 1 H) 1.54 [m, 3 H, C(5)Me], 1.45 (m, 1 H), 1.29 (d *J* = 6.5, 3 H, CH_3CHOH), 0.85 [d + s *J* = 7, 6 H, C(2)Me + C(1)Me], 0.63 [s, 3 H, C(1)Me].

(−)-(2*S*,6*R*)-*cis*-α-Irone (2): (−)-*cis*-α-Irol (**8b**) (2.50 g, 0.0121 mol) was oxidised with manganese(IV) oxide (1.5 equiv.) in dichloromethane (30 mL) to afford (−)-*cis*-α-irone (2.02 g, 82%): $[\alpha]_D^{20}$= −118 (*c* = 1.02, CH_2Cl_2); chemical purity after bulb-to-bulb distillation 96% (chiral GC analysis: t_R/min = 22.77); ^{1}H NMR: δ = 6.65 [dd, *J* = 15.7, 1 H, C(7)H], 6.12 [d, *J* = 15.7, 1 H, C(8)H], 5.52 [m, 1 H, C(5)H], 2.55 (m, 1 H), 2.28 (s, 3 H, CH_3CO), 1.5−1.8 (m, 2 H), 1.53 [m, 3 H, C(5)Me], 1.46 (m, 1 H), 0.88 [d, *J* = 6, 3 H, C(2)Me], 0.86 [s, 3 H, C(1)Me], 0.71 [s, 3 H, C(1)Me].

(−)-(2*R*,4*R*,5*S*,6*R*)-4,5-Epoxy-4,5-dihydro-α-irone (4): The alcohol mixture (7.20 g, 0.034 mol) enriched in stereoisomer **7b** (40%, chiral GC analysis of the corresponding acetate derivative) was oxidised with manganese(IV) oxide (1.5 equiv.) in dichloromethane (50 mL). The resulting α-irone (5.77 g, 0.0280 mol, 61:39 *trans/cis* ratio) was treated with *m*-chloroperbenzoic acid (75%, 9.40 g, 0.0414 mol) in chloroform (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, then poured into water. The organic phase was washed with a saturated solution of sodium hydrogen carbonate, dried with sodium sulfate and concentrated under reduced pressure. The residue was chromatographed eluting with hexane/ethyl acetate (9:1). The last eluted fractions gave, after recrystallisation from hexane, (−)-epoxy-*cis*-α-irone **4** (2.05 g, 33%) (*ee* = 80%, chiral GC analysis t_R/min (−)-**4** 25.41, (+)-**4** 25.78 min); $[\alpha]_D^{20}$ = −12 (*c* = 1.35, CH_2Cl_2); the ^{1}H NMR was in accordance with that of (±)-**4**.

(+)-(2*R*,6*S*)-*cis*-α-Irone (2): Chlorotrimethylsilane (1.63 g, 0.0150 mol) was added dropwise to a solution of sodium iodide (4.50 g, 0.0300 mol) in dry acetonitrile (10 mL) under nitrogen. After a few minutes, a solution of epoxy-α-irone (−)-**4** (2.00 g, 0.00971 mol) in acetonitrile (5 mL) was added. After stirring at room temperature for 30 min, the reaction mixture was poured into a 4 N solution of sodium thiosulfate, and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated under reduced pressure to give a residue, which was chromatographed on a silica gel column, eluting with hexane. The first eluted fractions gave (+)-*cis*-α-irone **2** (1.17 g, 63%,) showing, after bulb to bulb distillation, chemical purity = 79%, *ee* = 80% (chiral GC analysis t_R = 22.69 min) and $[\alpha]_D^{20}$ = 87 (*c* = 1.5, CH_2Cl_2); the ^{1}H NMR spectrum was in accordance with that of the (−)-enantiomer.

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