Enzyme-mediated synthesis of (*R*)- and (*S*)-α-ionone

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Diastereoisomeric enrichment through fractional crystallisation of 4-nitrobenzoate derivatives of α-ionol, and enantioselective enzyme-mediated reactions of α-ionol and α-ionol acetate, were usefully combined to optimise two different procedures to enantiopure (*S*)- and (*R*)-ionone.

Introduction

α-Ionone **1** entered the realm of flavours and fragrances over one century ago, when, after its discovery as a fragrant component of violet flowers by Tiemann,[1] it became accessible on a large scale by chemical synthesis from citral.[2] Many years later it was shown that in *Viola odorata* Linneaus α-ionone was present in the (+)-enantiomeric form,[3] which was assigned the (*R*) absolute configuration through correlation with manool.[4] The relative sensitivities for both enantiomers of α-ionone were found to diverge widely for different flavorists.[5,6] (*R*)- and (*S*)-**1** show violet and, respectively, woody-like taste, whereas the threshold values appeared in the 0.5–5 and, 20–40 ppb range, respectively.[6,7]

Despite the marked difference in the flavour response of the two enantiomers, α-ionone **1** is used in industrial formulations in the racemic modification. This circumstance might also be due to the fact that (*R*)-α-ionone is a rather inaccessible material. The first historically important separation[8] of the enantiomers of α-ionone **1**, applied also for the preparation of enantiomer (*R*)-**1** used as a starting material in the synthesis of a variety of natural carotenoids,[4] is based on a classical optical resolution through fractional crystallisation of the *l*-menthylhydrazide[9] of the racemic ketone. Reportedly, over 20 recrystallisations are required to obtain isomer (*R*)-**1** (~1% yield) and isolation of the (*S*) material is similarly laborious. A straightforward four-step entry to the enantiomers of α-ionone **1** from (*R*)- and (*S*)-α-damascone in 48% yield through a new enone transposition has been reported fairly recently.[6] The starting materials were produced, in turn, from racemic damascone *via* enantioselective protonation of the corresponding enolate.[10] Despite its elegance, the above method of synthesis requires several steps and/or delicate reaction conditions starting from a rather precious material such as racemic α-damascone. Even more laborious is the procedure leading from (*S*)-(−)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone to isomer (*R*)-**1** [85% enantiomeric excess (ee)].[11]

In the light of the above considerations we thought it worthwhile to explore direct entries to ionones (*R*)- and (*S*)-**1** through enzymic kinetic resolution of the easily accessible α-ionol **2**. Indeed, the structural similarity between allylic alcohol **2** and the aromatic allylic methyl alcohol recently resolved in the two enantiomeric forms by lipase-mediated kinetic esterification[12] induced us to explore the preparation, by these means, of the enantiomers of the single diastereoisomers of compound **2**, in the expectation of subsequently obtaining from these latter derivatives the target ionone enantiomers (*R*)- and (*S*)-**1** by MnO_2 oxidation.

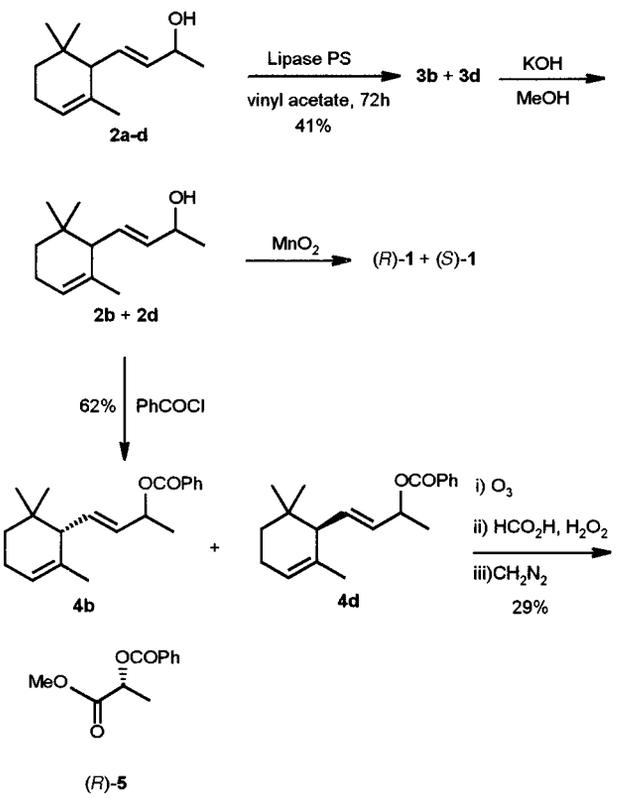
We report herein on the results obtained.

Results and discussion

Commercial α-ionol **2** is a 1 : 1 mixture of two racemic diastereoisomers. The same composition was obtained when α-ionone **1** was reduced either with $LiAlH_4$ in THF at −70 °C or with $NaBH_4$ in propan-2-ol at −15 °C. The diastereoisomeric as well as the enantiomeric composition of α-ionol **2a–d** could be best determined by gas-chromatographic analysis of the corresponding acetate esters on a permethylated β-cyclodextrin-based column. Under suitable conditions, four baseline-separated peaks (Fig. 1a) were obtained for the two racemic diastereoisomers **3a–d** (four stereoisomers).

Preliminary enzymic esterification experiments on commercial α-ionol **2**, in the presence of lipase PS (*Pseudomonas cepacia*), using vinyl acetate as an acetate donor in *tert*-butyl methyl ether (TBME) solution, provided a 1 : 1 mixture of enantiomerically pure acetates **3b**(*S*,*R*) and **3d**(*R*,*R*) (GC analysis, second and fourth peaks). Acetylation with Ac_2O–pyridine of the alcohol derivative, recovered from the enzymic esterifi-

cation and separated from substrates **3b**(*S*,*R*) and **3d**(*R*,*R*) by column chromatography, gave enantiomerically pure diastereoisomers **3a**(*S*,*S*) and **3c**(*R*,*S*) in a 1 : 1 ratio, as was shown by GC analysis (first and third peaks). The assignment of the absolute configuration to isomers **3b** and **3d** was performed through the chemical correlations shown in Scheme 1, following the



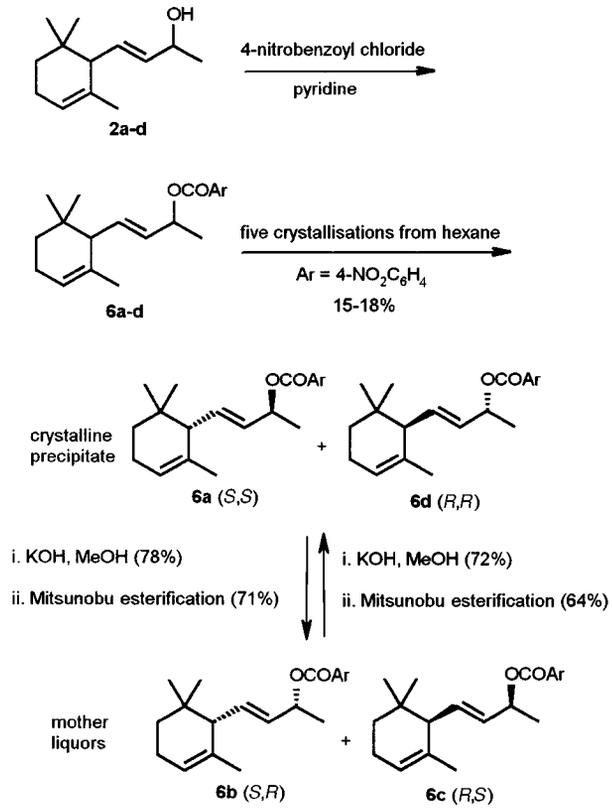
Scheme 1 Determination of the absolute configuration of acetate derivatives **3b** and **3d** obtained upon Lipase PS-catalysed acetylation of compounds **2a**–**d**.

same procedure as described for the structural determination of (+)-3-oxo-α-ionol isolated from tobacco leaves.[13]

First, in order to determine the absolute configuration of C-9, enzyme-generated diastereoisomers **3b** and **3d** were converted into the corresponding alcohols **2b** and **2d**, by treatment with methanolic potassium hydroxide. A certain amount of the mixture of alcohols **2b** and **2d** was submitted to benzoylation, to give ester derivatives **4b** and **4d**. The mixture of these latter substrates provided enantiomerically pure methyl (*R*)-*O*-benzoyllactate **5** on ozonolysis, oxidative work-up and diazomethane treatment. This result allowed us to assign (9*R*) configuration to both parent compounds **3b** and **3d**. The remaining mixture of alcohols **2b** and **2d** was oxidised with MnO_2 to afford α-ionone **1** devoid of optical activity (1 : 1 mixture of (*R*)- and (*S*)-enantiomers by GC analysis). These results clearly indicated that the esterification of alcohols **2a**–**d**, mediated by lipase PS, had provided a 1 : 1 mixture of the two enantiomerically pure (9*R*) ionol acetates, showing opposite configurations at C-6.

In the light of these observations, it appeared necessary to separate the two racemic diastereoisomers of α-ionol, isomers **2a**,**d**(*RS*,*RS*) and **2b**,**c**(*RS*,*SR*), in order to obtain α-ionones (*R*)- and (*S*)-**1**. Two procedures were devised to this end.

The first was based on the fractional crystallisation of ester derivatives of α-ionol. Unfortunately, among the several known esters of α-ionol (3,5-dinitrobenzoate and 4-bromobenzoate derivatives) only the 4-nitrobenzoate derivative was found to be a crystalline material.[14] We thus relied on the fractional crystallisation of the 4-nitrobenzoate esters **6a**–**d** for the separation of the two diastereosiomers of α-ionol (Scheme 2).



Scheme 2 Separation of the two racemic diastereoisomers of nitrobenzoate **6a**–**d**.

Five crystallisations from hexane provided the racemic diastereoisomers **6a**,**d**(*RS*,*RS*) in ~15–18% yield. GC of the corresponding acetate derivatives showed that these two enantiomers corresponded to the first and last peak of the series of four.

The other racemic diastereoisomer **6b**,**c**(*RS*,*SR*) could not be recovered in a pure state from the mother liquors of the hexane crystallisations. Therefore, it was obtained from diastereoisomeric racemate **6a**,**d**(*RS*,*RS*) upon saponification with methanolic potassium hydroxide, followed by Mitsunobu esterification (Scheme 2) (second and third peak in the GC analysis of the corresponding acetates).

The material recovered from the mother liquors and enriched in the more soluble diastereoisomer **6b**,**c**(*RS*,*SR*) was not wasted at all. It was hydrolysed with KOH–MeOH, the alcohol was submitted to Mitsunobu esterification,[15] and the product was then crystallised from hexane to afford isomers **6a**,**d**(*RS*, *RS*). ^{1}H NMR studies were particularly useful in determining the diastereoisomeric composition of the esters during the crystallisation sequence. Indeed, the two singlets, relative to the methyl groups at position 1 of the framework of the two diastereoisomers, appeared as baseline-separated peaks, thus allowing a precise analysis.

The two diastereoisomers of α-ionol were recovered from esters **6a**,**d**(*RS*,*RS*) and **6b**,**c**(*RS*,*SR*), respectively, upon treatment with methanolic potassium hydroxide, and were submitted separately to enzyme-mediated acetylation.

Lipase PS-mediated esterification of α-ionols **2a**,**d**(*RS*,*RS*) gave enantiomerically pure acetate **3d**(*R*,*R*) (fourth peak in GC analysis), and unchanged alcohol **2a**(*S*,*S*), showing high enantiomeric purity as was shown by GC analysis of the corresponding acetate derivative (first peak).

Enantiomerically pure acetate **3b**(*S*,*R*) (second peak) and alcohol **2c**(*R*,*S*) (third peak in GC analysis of the acetate derivative) were obtained upon Lipase PS-mediated esterification of racemic diastereoisomer **2b**,**c**. Basic hydrolysis of enzyme-generated acetates **3d**(*R*,*R*) and **3b**(*S*,*R*) provided

Table 1 Qualitative studies of enzyme-mediated acetylation of ionols **2a–d**

Enzyme	**3b**:**3d** ratio (GC)	ee % **3b** (GC)	ee % **3d** (GC)
PPL	3:1	90	64
CCL	1:1.15	72	79
Lipase PS	1:1	99	99

enantiomerically pure alcohols **2d**(*R*,*R*) and **2b**(*S*,*R*), which were straightforwardly oxidised with freshly prepared MnO_2[16] to (*R*)- and (*S*)-α-ionone **1**, showing 98% and 97% ee (GC), and $[\alpha]_D^{20}$ 420† and −418 (*c* 1, $CHCl_3$) {lit.,[6] (*R*)-ionone ee 98%, $[\alpha]_D^{20}$ 407, *c* 0.04, $CHCl_3$; (*S*)-ionone ee > 99%, $[\alpha]_D^{20}$ −431, *c* 0.035, $CHCl_3$} in ~35% yield from the single racemic diastereoisomer. Oxidation of the alcohols recovered from the two enzymic esterifications provided (*S*)- and (*R*)-**1** ionones showing 87–93% ee.

In the alternative procedure the separation of the two diastereoisomers of α-ionol, to be used as precursors of enantiopure α-ionone **1**, was performed after the enantiomeric enrichment of the same two stereoisomers by lipase-mediated reactions.

To this end, qualitative studies of enzyme-catalysed acetylation of alcohols **2a–d** in TBME solution, in the presence of vinyl acetate as an acylating agent, were performed also with other lipases [porcine pancreas lipase (PPL) and *Candida cylindracea* lipase (CCL)] (Table 1). PPL-mediated reaction gave a 3:1 mixture (de‡ = 52%, GC) of acetates **3b**(*S*,*R*) (ee = 90%, GC) and **3d**(*R*,*R*) (ee = 64%, GC), while CCL catalysis afforded the same two diastereoisomers in a low enantiomeric enrichment (ee = 72% and 79% respectively) in a nearly 1:1 ratio [de 7% of **3d**(*R*,*R*)]. On the other hand, as we have previously described, Lipase PS led to total enantioselectivity with no diastereoselectivity at all.

We tried to take advantage of the good diastereoselectivity assured by PPL in the reverse reaction of hydrolysis of the two racemic diastereoisomers of acetates **3a–d** in water, keeping the solution pH at 7.5 by means of the progressive addition of 0.5 M NaOH. The stereoisomer **3b**(*S*,*R*) was preferentially hydrolysed under these reaction conditions to give alcohol **2b**(*S*,*R*) showing ee 98% and de 55% (GC). The gas chromatogram of the surviving acetate (Fig. 1b) clearly showed the almost complete consumption of the (*S*,*R*) stereoisomer (three peaks survived). The alcohol, recovered from the saponification of this residual acetate, was submitted to lipase PS-mediated acetylation. The gas chromatogram of the resulting acetate is shown in Fig. 1c (two peaks survived): the acetate **3d**(*R*,*R*) was thus obtained in 99% ee and 66% de. These two subsequent enzyme-catalysed reactions afforded an enantiomerically pure acetate **3d**(*R*,*R*) which was to be brought to diastereoisomeric purity. This goal was reached by means of two crystallisations of the corresponding 4-nitrobenzoate derivative from hexane: the gas chromatogram of the acetate prepared from the crystalline ester is shown in Fig. 1d (one peak survived). The oxidation of the corresponding alcohol (6*R*,9*R*)-**2** with MnO_2 gave enantiomerically pure (*R*)-ionone (GC).

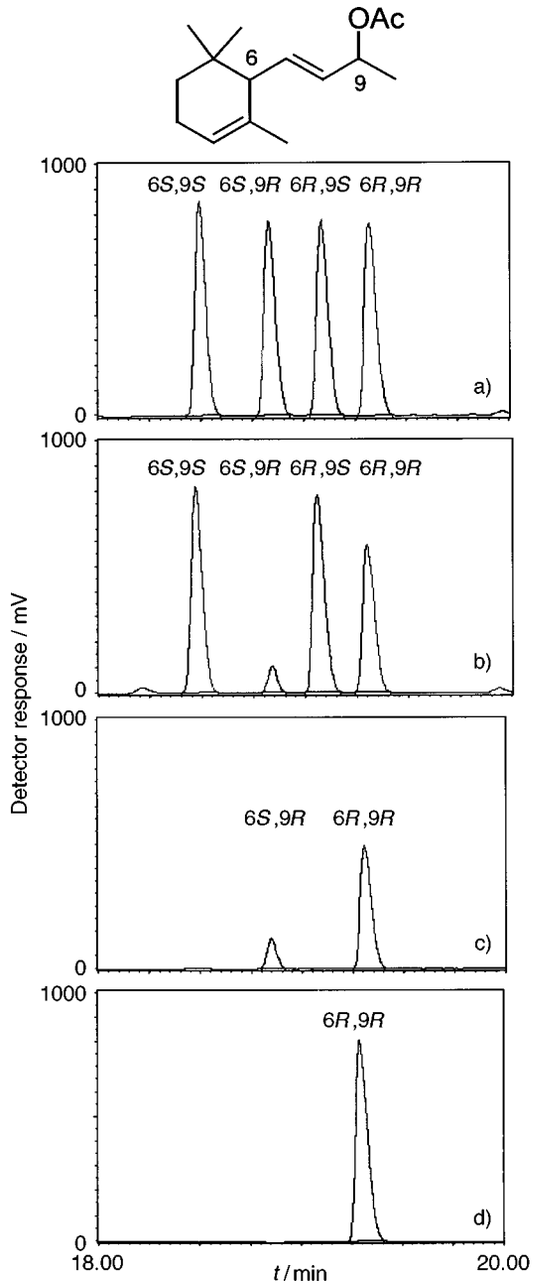


Fig. 1 GC analyses of acetate derivatives **3a–d**: (a) 1:1 mixture of the two racemic diastereoisomers of compound **3**; (b) mixture of stereoisomers recovered from PPL-mediated hydrolysis; (c) acetate derivatives produced by Lipase PS-mediated acetylation of α-ionol recovered from the residual acetate of the preceding hydrolysis; (d) acetate **3d**(*R*,*R*) after diastereoisomeric enrichment.

Conclusions

A successful combination of simple chemical and enzymic methods allowed us to prepare extremely valuable (*R*)- and (*S*)-α-ionone using inexpensive racemic α-ionone as a starting material. Two different approaches have been devised by interchanging the application of traditional techniques of fractional crystallisation and enzyme-mediated reactions. Fractional crystallisations, from hexane, of 4-nitrobenzoate derivatives of α-ionol were successfully used in both synthetic paths to achieve diastereoisomeric purity, while optical activation was assured by enantioselective enzyme-mediated acetylation of α-ionol and hydrolysis of α-ionol acetate.

Experimental

The following enzymes were employed in this work: CCL (Sigma, Type VII, 900 U mg^{-1}), PPL (Sigma, Type II), and Lipase PS (*Pseudomonas cepacia*) (Amano Pharmaceuticals Co., Japan). Enantiomeric and diastereoisomeric excesses (ees and des) were determined by chiral GC analysis on a Chirasil DEX CB, 25 m × 0.25 mm (Chrompack), using a DANI HT 86.10 gas chromatograph; 70 °C (1 min)—3.5 °C min^{-1}—140 °C (6 min)—8 °C min^{-1}—180 °C (1 min): (6*S*,9*S*)-**2** t_R 18.50 min, (6*S*,9*R*)-**2** t_R 18.83 min, (6*R*,9*S*)-**2** t_R 19.09 min, (6*R*,9*R*)-**2** t_R

† $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}.
‡ de = diastereoisomeric excess.

19.32 min, (*S*)-**1** t_R 17.55 min, (*R*)-**1** t_R 18.13 min. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions at rt unless otherwise stated, on a Bruker AC-250 spectrometer (250 MHz ^{1}H; 62.9 MHz ^{13}C). 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. TLC analyses were performed on Merck Kieselgel 60 F_{254} plates. All chromatographic separations were carried out on silica gel columns.

(6*RS*,9*RS*)- and (6*RS*,9*SR*)-α-Ionol 2

A 1 : 1 mixture of the two racemic diastereoisomers of α-ionol **2** was obtained upon $NaBH_4$ reduction of α-ionone **1** in propan-2-ol at −15 °C (82% yield), and on treatment with $LiAlH_4$ in THF at −40 °C (75%) (Found: C, 80.3; H, 11.45. $C_{13}H_{22}O$ requires C, 80.36; H, 11.41%); δ_H 5.45 (6H, m, olefinic hydrogens), 4.30 (2H, quintet, *J* 6.1, 2 × *CH*OH), 2.08 [2H, d, *J* 8.3, C(6)H], 1.99 [4H, m, 4 × C(3)H], 1.58 [6H, m, 2 × C(1)Me], 1.55–1.35 [2H, m, 2 × C(4)H], 1.27 (6H, m, 2 × CH_3CHOH), 1.22–1.08 [2H, m, 2 × C(4)H], 0.88 [6H, s, 2 × C(5)Me], 0.80 [3H, s, C(5)Me of one diastereoisomer] and 0.82 [3H, s, C(5)Me of other diastereoisomer].

(6*RS*,9*RS*)- and (6*RS*,9*SR*)-α-Ionol acetate 3

Treatment of the above described mixture (0.01 mol) with acetic anhydride (0.02 mol) in pyridine (20 cm^3) gave a 1 : 1 mixture of the two racemic diastereoisomers of α-ionol acetate **3** (Found: C, 76.3; H, 10.2. $C_{15}H_{24}O_2$ requires C, 76.23; H, 10.23%); δ_H 5.6–5.2 (4H, m, olefinic hydrogens and *CH*OAc), 2.10–0.90 [6H, m + s, C(6)H, 2 × C(3)H + CH_3CO_2], 1.56 [3H, m, C(1)Me], 1.38 [1H, m, C(4)H], 1.28 (3H, 2 d, *J* 6.1, CH_3CHOAc of both diastereoisomers), 1.15 [1H, m, C(4)H], 0.88 [3H, s, C(5)Me] and 0.81 [3H, s, C(5)Me].

General procedure for enzyme-mediated acetylations

A 1 : 1 mixture of (6*RS*,9*RS*)- and (6*RS*,9*SR*)-**2** (10 g, 0.04 mol), lipase PS (10 g), and vinyl acetate (40 cm^3) in TBME (150 cm^3) was stirred at rt for 72 h. The residue obtained upon evaporation of the filtered reaction mixture was chromatographed with gradient elution, with hexane ⟶ hexane–ethyl acetate (1 : 1). The first eluted fractions provided a 1 : 1 mixture (4.16 g, 44%) of (6*S*,9*R*)-**3** (ee > 99%, GC) and (6*R*,9*R*)-**3** (ee > 99%, GC). The last eluted fractions gave a 1 : 1 mixture (3.20 g, 41% recovery) of (6*S*,9*S*)-**2** (ee > 99%, GC) and (6*R*,9*S*)-**2** (ee > 99%, GC). The same procedure was followed for the preliminary studies of PPL- and CCL-mediated acetylations, to provide acetates **3** showing the ees and des reported in the text.

Determination of the absolute configuration at C-9 in acetate derivatives 3 obtained from alcohols 2 by Lipase PS-mediated acetylation

The 1 : 1 mixture of the two diastereoisomeric acetates obtained in lipase-mediated acetylation of alcohol **2** (4.0 g, 0.017 mmol) was saponified with potassium hydroxide (1.14 g, 0.020 mol) in refluxing methanol (40 cm^3). A portion of this alcohol was oxidised with manganese(IV) oxide in methylene dichloride solution, to give racemic α-ionone (GC). The remaining alcohol was submitted to the following sequence of chemical correlation to determine the configuration at C-9.

Benzoylation. The alcohol **2** (2.50 g, 0.013 mol) was treated with a solution of benzoyl chloride (2.67 g, 0.019 mol) in pyridine (20 cm^3). The reaction mixture was poured into ice, and treated with 10% HCl. The organic phase was separated, washed first with saturated aq. sodium hydrogen carbonate, then with water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography with hexane as eluent. The first eluted fractions gave a 1 : 1 mixture of benzoates (6*R*,9*R*)-**4** and (6*S*,9*R*)-**4** (2.40 g, 62%) nearly devoid of optical activity in chloroform (Found: C, 80.6; H, 8.65. $C_{20}H_{26}O_2$ requires C, 80.50; H, 8.78%); δ_H 7.96 (4H, d, *J* 7.9, 4 × Ar *o*-H), 7.47 (2H, t, *J* 7.9, 2 × Ar *p*-H), 7.36 (4H, t, *J* 7.9, 4 × Ar *m*-H), 5.51 [6H, m, 2 × C(7)H, 2 × C(8)H + 2 × *CH*OCOPh], 5.33 [2H, m, 2 × C(2)H], 2.04 [2H, d, *J* 7.3, 2 × C(6)H], 1.92 [4H, m, 4 × C(3)H], 1.57 [6H, m, 2 × C(1)Me], 1.37 [8H, 2 d, *J* 6.1, +m, 2 × CH_3CHOCOPh + 2 × C(4)H], 1.09 [2H, m, 2 × C(4)H], 0.82 [3H, s, C(5)Me], 0.80 [3H, s, C(5)Me], 0.73 [3H, s, C(5)Me] and 0.74 [3H, s, C(5)Me].

Ozonisation, oxidative work-up and diazomethane treatment. The two diastereoisomeric benzoyl derivatives (2.40 g, 8.05 mmol) were ozonised in methanol solution at −78 °C for 15 min. The reaction mixture was allowed to reach rt and the solvent was removed under reduced pressure, to give a residue to which was added a mixture of formic acid (20 cm^3) and 35% hydrogen peroxide (3 cm^3). The reaction mixture was refluxed for 30 min, concentrated *in vacuo*, diluted with water and extracted with diethyl ether. The ethereal solution was treated with diazomethane, and concentrated under reduced pressure to give a residue, which was purified by column chromatography with hexane as eluent, to give enantiopure *methyl (R)-O-benzoyllactate* **5** (0.485 g, 29%) (Found: C, 63.6; H, 5.8. $C_{11}H_{12}O_4$ requires C, 63.46; H, 5.81%); $[\alpha]_D^{20}$ −13.7 (*c* 4.5, $CHCl_3$); δ_H 8.10 (2H, d, *J* 7.5, Ar *o*-H), 7.59 (1H, t, *J* 7.5, Ar *p*-H), 7.46 (2H, t, *J* 7.5, Ar *m*-H), 5.34 (1H, q, *J* 7.5, *CH*OCOPh), 3.78 (3H, s, CO_2CH_3) and 1.64 (3H, d, *J* 7.5, CH_3CHOCOPh).

First procedure to (*R*)- and (*S*)-α-ionone

(6*RS*,9*RS*)-α-Ionol 2a +2d. *(a) Fractional crystallisation of α-ionol 4-nitrobenzoate esters.* A solution of α-ionol (50 g, 0.26 mol), 4-nitrobenzoyl chloride (70.50 g, 0.38 mol) and pyridine (40 cm^3) in methylene dichloride (400 cm^3) was stirred at rt for 2 h. The reaction mixture was poured into ice and treated with 10% HCl; the organic layer was separated, washed first with saturated aq. sodium hydrogen carbonate, then with water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography with hexane as eluent. The first eluted fractions gave a 1 : 1 mixture of (6*RS*,9*RS*)- and (6*RS*,9*SR*)-4-nitrobenzoate esters **6** (63.32 g, 71%) (Found: C, 69.9; H, 7.4; N, 4.1. $C_{20}H_{25}NO_4$ requires C, 69.95; H, 7.34; N, 4.08%); δ_H 8.20 (8H, m, ArH), 5.60 [6H, m, 2 × C(7)H, 2 × C(8)H + 2 × *CH*OCOAr], 5.40 [2H, m, 2 × C(2)H], 2.12 [2H, d, *J* 8.3, 2 × C(6)H], 2.00 [4H, m, 2 × C(3)H], 1.58 [3H, m, C(1)Me of one diastereoisomer], 1.52 [3H, m, C(1)Me of the other diastereoisomer], 1.42 (6H, 2 d, *J* 6.1, 2 × CH_3CHOCOAr), 1.40–1.30 [2H, m, 2 × C(4)H], 1.05–1.20 [2H, m, 2 × C(4)H], 0.89 [3H, s, C(5)Me of one diastereoisomer], 0.87 [3H, s, C(5)Me of the other diastereoisomer], 0.79 [3H, s, C(5)Me of one diastereoisomer] and 0.81 [3H, s, C(5)Me of the other diastereoisomer].

Five crystallisations from hexane afforded (6*RS*,9*RS*)-α-ionol 4-nitrobenzoate ester (**6a** and **6d**) (16 g, 18%) with de > 99% (NMR and GC of the corresponding acetate derivatives): mp 65–67 °C; ν_{max}/cm^{-1} 1720 (CO); δ_H 8.23 (4H, m, ArH), 5.60 [3H, m, C(7)H, C(8)H and *CH*OCOAr], 5.42 [1H, m, C(2)H], 2.12 [1H, d, *J* 8.0, C(6)H], 1.99 [2H, m, 2 × C(3)H], 1.57 [3H, m, C(1)Me], 1.50–1.35 [4H, d, *J* 6.1, +dt, *J* 13.5 and 8.0, CH_3CHOCOAr + C(4)H], 1.17 [1H, dt, *J* 13.5 and 5.2, C(4)H], 0.87 [3H, s, C(5)Me] and 0.79 [3H, s, C(5)Me]; δ_C 20.6, 22.8, 27.0, 27.4, 31.5, 32.0, 54.0, 73.2, 121.5, 123.5, 130.4, 130.6, 133.4, 135.2, 136.3, 150 and 164.

(b) Saponification. (6*RS*,9*RS*)-α-Ionol 4-nitrobenzoate ester **6a/d** (16.0 g, 0.047 mol) was saponified with potassium hydroxide (3.14 g, 0.056 mol) in refluxing methanol (100 cm^3). After

the usual work-up, ionol (6*RS*,9*RS*)-**2** (7.11 g, 78%) was obtained: de > 99% (GC analysis of the corresponding acetates: first and fourth peak); δ_H 5.45 (3H, m, olefinic hydrogens), 4.30 (1H, quintet, *J* 6.1, *CH*OH), 2.08 [1H, d, *J* 8.3, C(6)H], 1.99 [2H, m, 2 × C(3)H], 1.57 [3H, m, C(1)Me], 1.42 [1H, dt, *J* 13.1 and 8, C(4)H], 1.27 (3H, d, *J* 6.1, CH_3CHOH), 1.16 [1H, dt, *J* 13.1 and 5, C(4)H], 0.89 [3H, s, C(5)Me] and 0.82 [3H, s, C(5)Me].

(6*RS*,9*SR*)-α-Ionol 2b + 2c. *(a) Esterification of (6RS,9RS)-2 under Mitsunobu conditions.* A solution of ionol (6*RS*,9*RS*)-**2** (5.0 g, 0.026 mmol) and triphenylphosphine (4.45 g, 0.017 mol) in THF (45 cm^3) was dropped into a solution of diisopropyl azodicarboxylate (3.43 g, 0.017 mmol) and 4-nitrobenzoic acid (2.84 g, 0.017 mol) in THF (30 cm^3). The reaction mixture was stirred at rt for 12 h, then was concentrated under reduced pressure to give a residue, which was chromatographed with gradient elution with hexane ⟶ hexane–ethyl acetate (9 : 1). The product obtained from the first eluted fractions was (6*RS*,9*SR*)-α-ionol 4-nitrobenzoate **6b** and **6c** (de > 99%, NMR and GC of the corresponding acetate derivatives) (6.33 g, 71%): mp 62–64 °C; ν_{max}(Nujol)/cm^{-1} 1720 (CO); δ_H 8.22 (4H, m, ArH), 5.60 [3H, m, C(7)H, C(8)H + *CH*OCOAr], 5.42 [1H, m, C(2)H], 2.12 [1H, d, *J* 8.3, C(6)H], 1.99 [2H, m, 2 × C(3)H], 1.56 [3H, m, C(1)Me], 1.47 (3H, d, *J* 6.1, CH_3CHOCOAr), 1.39 [1H, dt, *J* 13.5 and 8.0, C(4)H], 1.17 [1H, dt, *J* 13.5 and 5.2, C(4)H], 0.89 [3H, s, C(5)Me] and 0.81 [3H, s, C(5)Me]; δ_C 20.6, 23.1, 27.0, 27.4, 31.6, 32.0, 54.0, 73.1, 121.5, 123.5, 130.6, 130.7, 133.4, 134.8, 136.4, 150.0 and 164.

(b) Saponification. (6*RS*,9*SR*)-α-Ionol 4-nitrobenzoate ester **6b/c** (6.30 g, 0.018 mol) was saponified with potassium hydroxide (1.21 g, 0.002 mol) in refluxing methanol (50 cm^3). After the usual work-up, ionol (6*RS*,9*SR*)-**2** (2.62 g, 75%) was obtained: de > 99% (GC analysis of the corresponding acetates: second and third peak); δ_H 5.48 (3H, m, olefinic hydrogens), 4.30 (1H, quintet, *J* 6.1, *CH*OH), 2.08 [1H, d, *J* 8.0, C(6)H], 1.99 [2H, m, 2 × C(3)H], 1.59 [3H, m, C(1)Me], 1.42 [1H, dt, *J* 13.5 and 8.0, C(4)H], 1.27 (3H, d, *J* 6.1, CH_3CHOH), 1.17 [1H, dt, *J* 13.5 and 5.2, C(4)H], 0.88 [3H, s, C(5)Me] and 0.80 [3H, s, C(5)Me].

(6*S*,9*S*)- and (6*R*,9*R*)-α-Ionol 2a and 2d. Ionol (6*RS*,9*RS*)-**2** (5.0 g, 0.026 mol) was submitted to enzyme-mediated acetylation in *tert*-butyl methyl ether solution (100 cm^3) in the presence of Lipase PS (5.0 g), using vinyl acetate as acylating agent (20 cm^3). After stirring of the mixture for 72 h at rt, the enzyme was removed by filtration and the solvent was evaporated off under reduced pressure. The residue was chromatographed with gradient elution with hexane ⟶ hexane ethyl acetate (1 : 1). The first eluted fractions gave acetate (6*R*,9*R*)-**3** showing enantiomeric purity (GC, fourth peak) (2.27 g, 37%); $[\alpha]_D^{20}$ 352 (*c* 1, $CHCl_3$); δ_H 5.60–5.20 (4H, m, olefinic hydrogens and *CH*OAc), 2.10–1.90 [6H, m + s, C(6)H, 2 × C(3)H + CH_3CO_2], 1.55 [3H, m, C(1)Me], 1.40 [1H, dt, *J* 8.0 and 13.1, C(4)H], 1.29 (3H, d, *J* 6.1, CH_3CHOAc), 1.15 [1H, dt, *J* 5.2 and 13.1, C(4)H], 0.87 [3H, s, C(5)Me] and 0.79 [3H s, C(5)Me].

Saponification with potassium hydroxide in methanol gave ionol (6*R*,9*R*)-**2** (**2d**) (1.43 g, 77%) showing ee 98%; $[\alpha]_D^{20}$ 338 (*c* 1, $CHCl_3$).

The last eluted fractions gave ionol (6*S*,9*S*)-**2** (**2a**) (1.96 g, 39%) showing ee 87% (GC of the corresponding acetate derivative, first peak); $[\alpha]_D^{20}$ −293 (*c* 1, $CHCl_3$). *E* (Enantiomeric ratio) = 506, *c* (conversion) = 47%.[17]

(6*S*,9*R*)- and (6*R*,9*S*)-α-Ionol 2b and 2c. Ionol (6*RS*,9*SR*)-**2** (**2b/c**) (5.0 g, 0.026 mol) was submitted to enzyme-mediated acetylation in TBME solution (100 cm^3) in the presence of Lipase PS (5.0 g), using vinyl acetate as acylating agent (20 cm^3). After stirring of the mixture for 72 h at rt, the enzyme was removed by filtration and the solvent was evaporated off under reduced pressure. The residue was chromatographed and eluted with (gradient elution) hexane ⟶ hexane–ethyl acetate (1 : 1). The first eluted fractions gave acetate (6*S*,9*R*)-**3** (**3b**) showing ee 97% (GC, second peak) (2.56 g, 42%); $[\alpha]_D^{20}$ −305 (*c* 1, $CHCl_3$); δ_H 5.60–5.20 (4H, m, olefinic hydrogens and *CH*OAc), 2.15–1.95 [6H, m + s, C(6)H, 2 × C(3)H + CH_3CO_2], 1.56 [3H, m, C(1)Me], 1.39 [1H, dt, *J* 13.5 and 8.0, C(4)H], 1.30 (3H, d, *J* 6.1, CH_3CHOAc), 0.88 [3H, s, C(5)Me] and 0.79 [3H, s, C(5)Me].

Saponification with potassium hydroxide in methanol gave ionol (6*S*,9*R*)-**2** (**2b**) (1.59 g, 75%); $[\alpha]_D^{20}$ −290 (*c* 1, $CHCl_3$).

The last eluted fractions gave ionol (6*R*,9*S*)-**2** (**2c**) (1.76 g, 35%) showing ee 93% (GC of the corresponding acetate derivative, third peak); $[\alpha]_D^{20}$ +269 (*c* 1, $CHCl_3$). *E* (enantiomeric ratio) = 222, *c* (conversion) = 48%.[17]

(*R*)- and (*S*)-α-Ionone 1 and 2. Oxidation of the various stereoisomers of α-ionol to enantioenriched ionones was performed in methylene dichloride solution in the presence of manganese(IV) oxide in 69–76% yield. The following results were obtained:

Compound (6*R*,9*R*)-**2** (**2d**) gave (*R*)-ionone showing ee 98% and $[\alpha]_D^{20}$ +420 (*c* 1, $CHCl_3$);

Compound (6*S*,9*S*)-**2** (**2a**) gave (*S*)-ionone showing ee 87% and $[\alpha]_D^{20}$ −364 (*c* 1, $CHCl_3$);

Compound (6*S*,9*R*)-**2** (**2b**) gave (*S*)-ionone showing ee 97% and $[\alpha]_D^{20}$ −418 (*c* 1, $CHCl_3$);

Compound (6*R*,9*S*)-**2** (**2c**) gave (*R*)-ionone showing ee 93% and $[\alpha]_D^{20}$ +389 (*c* 1, $CHCl_3$).

Second procedure to (*R*)-α-ionone *R*-(−)-1

Enzymic hydrolysis of α-ionol acetate 3 and Lipase PS-mediated acetylation of α-ionol recovered from residual acetate. A 1 : 1 mixture of the two diastereoisomers of acetate derivative **3** (10.0 g, 0.042 mol) was suspended in water (150 cm^3): the solution pH was brought to 7.5 by adding NaOH 0.5 M. PPL (10.0 g) was added and the reaction mixture was stirred for 72 h, with the solution pH kept constant by progressive addition of NaOH 0.5 M. After treatment of the reaction mixture with ethyl acetate, the enzyme was filtered off, and the organic phase was separated, and dried on Na_2SO_4. The solvent was removed under reduced pressure to give a residue which was chromatographed (gradient elution) with hexane ⟶ hexane–ethyl acetate (1 : 1). The first eluted fractions gave a 1.6 : 1 mixture (de = 0.21) (5.79 g, 58%) of acetates (6*S*,9*S*)-**3** (ee 11%) and (6*R*,9*S*)-**3** (ee 73%) (GC analysis, see Fig. 1b).

The last eluted fractions gave ionol (6*S*,9*R*)-**2** (**2b**) (1.40 g, 17%) showing ee 98% and de 50% (GC).

The residual acetate was saponified (5.79 g, 0.024 mol) with potassium hydroxide (1.62 g, 0.029 mol) in methanol (40 cm^3) and the corresponding α-ionol was submitted to Lipase PS acetylation according to the usual procedure. After 72 h, the enzyme was filtered off and the solvent was removed under reduced pressure to give a residue, which was chromatographed (gradient elution with hexane ⟶ hexane–ethyl acetate (1 : 1). The first eluted fractions gave acetate (6*R*,9*R*)-**3** (**3d**) (1.30 g, 23%) (ee > 99%, de 66%; GC see Fig. 1c).

(6*R*,9*R*)-α-Ionol 2d. Acetate (6*R*,9*R*)-**3** (**3d**) (ee > 99%, de 66%) (1.30 g, 5.52 mmol) was saponified with potassium hydroxide (0.371 g, 6.62 mmol) in refluxing methanol (15 cm^3) (85% yield) and the alcohol **2d** thus obtained was converted into the corresponding 4-nitrobenzoate ester (76%) according to the usual procedure. Two subsequent crystallisations from hexane (39%), and saponification (84%), allowed us to recover compound (6*R*,9*R*)-**2** (**2d**) (0.225 g, 21%) showing diastereoisomeric purity (GC analysis of the corresponding acetate, see Fig. 1d); $[\alpha]_D^{20}$ 335 (*c* 1, $CHCl_3$).

(*R*)-α-Ionone (*R*)-1. Ionol (6*R*,9*R*)-**2** **2d** (0.220 g, 1.13 mmol)

(ee > 99%, de > 99%) was treated with manganese(IV) oxide in methylene dichloride solution to give (*R*)-α-ionone (0.171 g, 79%) (ee > 99%); $[\alpha]_D^{20}$ +422 (*c* 1, $CHCl_3$).

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