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Lipase behavior in the stereoselective transesterification of zingerol-like derivatives and related biphenyls

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ABSTRACT

Using a highly stereoselective enzymatic procedure based on the irreversible transesterification of alcoholic functions in organic solvent, both enantiomers of zingerol and dehydrozingerol *O*-methyl derivatives **1** and **2** were obtained in high optical purity. The biocatalytic method was then extended to the corresponding biphenyl derivatives **5–8** for which an one-pot resolution/desymmetrization was carried out on the whole racemic/*meso* mixtures to give single stereoisomers with very high enantiomeric and diastereoisomeric excesses. The comparison of kinetic and enantioselective behavior of the lipase AK from *Pseudomonas fluorescens* in the transesterification of monomer (±)-**1** and the related (±)-**5** and meso-**6** biphenyl dimers was also made.

1. Introduction

Many natural phenols are good antioxidants and have a wide spectrum of biological activity related with antibacterial, anticancer, anti-inflammatory and antihypertensive effects. Curcuminoids and their structural analogs show several pharmacological and nutritional properties [1–3] and many of them have been isolated from the rhizome extracts of *Zingiberaceae* as *Curcuma longa* and *zingiber*. Zingerone and dehydrozingerone, key components of ginger, appeared to be useful as antioxidant in the treatment of Parkinson's disease [4], radioprotector against gamma radiation-induced damages in the cancer treatment [5,6], antidiarrheal [7,8], anticancer [9,10] and anti-inflammatory agents [11,12].

Biphenyl analogs of zingerone and dehydrozingerone, **3** and **4** display protective action in the oxidative cells stress (unpublished results) and a marked inhibition in the cell-growth of melanoma and neuroblastoma has been reported for **4** [13].

The related alcohols, zingerol and dehydrozingerol, have been less investigated in their pharmacological properties but therapeutic effects similar to those of the zingerone in the diarrhea treatment have been demonstrated for zingerol [7]. In their racemic form these alcohols are easily obtainable by chemical reduction of the parent keto derivatives [14,15], while only a case exists in the literature in which the optically active (*R*)-zingerol has been isolated from the needless of himalayan *Taxus baccata* [16] and, recently, as phenolic glucoside from leaves of *Staphylea bumalda* [17].

The bioactivity of natural and unnatural compounds is strongly dependent from stereochemistry, thus justifying the interest in the implementation of enantioselective preparation of optically active products, which includes asymmetric synthesis and kinetic resolution of racemates. In this context enzyme catalysis is an useful methodology to achieve enantiopure chiral drugs and lipase-catalyzed resolution of racemic mixtures is a attractive procedure thanks to large substrate acceptance and chemo-, regio- and enantioselectivity of the catalyst at mild conditions of temperature and pressure.

By using anhydrous organic solvents as reaction medium the "natural" activity of lipases can be reversed so allowing their application in the stereoselective transesterification of alcohols or acids and in addition, the modification of the solvent (solvent engineering) influences the enzyme performances [18–21].

Although the most of applications of the lipase catalysis in asymmetric synthesis are found in the kinetic resolution of racemic mixtures, the desymmetrization of *meso*- or prochiral substrates has become very common due to two main factors: (1) the theoretical yield of these reactions is 100% and (2) the enantioselectivity of the process is often high and independent from substrate conversion. A large part of lipase-catalyzed desymmetrizations involves the transesterification of *meso*-diols and effective protocols applied to different families of substrates have been developed for the preparation of optically active building blocks useful in medicinal chemistry or asymmetric synthesis (Fig. 1) [22].

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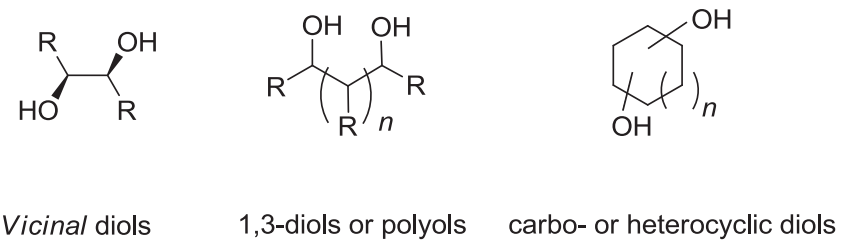


Fig. 1. Families of *meso*-alcohols desymmetrized by lipase-catalyzed reactions.

In the course of our continuous research on the preparation of new chiral alcohols in high enantiomeric purity through biocatalyzed reactions, we have recently reported a one-pot protocol for the simultaneous resolution and desymmetrization of a 1:1 *dl*:*meso* mixture of conformationally flexible 1,1′-bipyridine diol as the first example of lipase-promoted desymmetrization of a biaryl diol [23]. The success of such protocol was strictly related to the high enantioselectivity of the employed lipase that allowed the selective formation of three separable products each one as a single stereoisomer.

Herein we describe the results obtained in the kinetic resolution of racemic zingerol (±)-**1** and dehydrozingerol (±)-**2** *O*-methyl derivatives with lipase AK from *Pseudomonas fluorescens* and, in light of the high stereoselectivity evidenced for the enzyme, the extension of the process to the related racemic and *meso* biphenyl dimers (±)-**5**/*meso*-**6** and (±)-**7**/*meso*-**8** respectively (Fig. 2). Additional experiments have been also performed to compare the behavior of the lipase in the two processes of kinetic resolution of (±)-**1** and (±)-**5** and desymmetrization of *meso*-**6**.

2. Experimental

2.1. General information

Lipase PS-C I (lipase from *Pseudomonas cepacia* immobilized on ceramic), Novozyme 435® (lipase from *Candida antarctica* immobilized on acrylic resin) and Amano Lipase AK (crude lipase from *P. fluorescens*) were purchased from Aldrich. Lipozyme® (lipase from *Mucor miehei* immobilized on a macroporous ion-exchange resin) was obtained from Fluka. Compounds **3** and **4** were prepared according to previously described procedures [13,24].

Thin-layer chromatography (TLC) was carried out on Merck silica gel 60-F254 precoated glass plates and the compounds were detected by 254-nm UV light. Preparative liquid chromatography was performed using LiChroprep® Si 60 (25–40 μm) from Merck.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise specified, on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz respectively. Chemicals shifts (δ) are reported in ppm relative to TMS and coupling constants (J) are given in Hz. Optical rotations were recorded on a DIP 370 JASCO instrument using a ϕ 3.5 mm × 100 mm cell. The enantiomeric excesses were determined by chiral HPLC at 25 °C on Phenomenex Lux® Cellulose-1 or Phenomenex Lux® Cellulose-2 (250 × 4.60 mm) column using *n*-hexane/2-propanol isocratic mixtures as eluting solvent and simultaneous UV-detection at λ 220, 254, 266 and 300 nm. ESI$^+$-MS spectra were acquired on Waters Micromass ZQ2000 instrument (5, 10 or 20 V cone voltage, 150 °C source temperature).

2.2. Synthesis of (±)-*1*

Methylation of commercial zingerone, 4-(4-hydroxy-3-methoxyphenyl)butan-2-one, was performed by addition of K_2CO_3 (0.5 mmol, 69 mg) and CH_3I (0.186 ml, 3 mmol,) to a 5 ml acetone solution of substrate (185 mg, 0.95 mmol). The reaction was refluxed overnight, cooled and taken to dryness under vacuum. The residue was dissolved in CH_2Cl_2 and extracted with water. The organic phase was washed with a saturated solution of NH_4Cl and dried over sodium sulphate. Removal of the solvent gave a white solid (95% yield, 0.94 mmol, 195 mg) that was dissolved in MeOH (5 ml). To this solution $NaBH_4$ (71 mg, 1.9 mmol) was added and the mixture left to react at room temperature for 3 h under stirring.

The reaction was then quenched by addition of NH_4Cl and extracted with CH_2Cl_2. The organic phase was then washed with brine and dried on anhydrous sodium sulfate. The solvent was evaporated under vacuum to give title compound as a transparent oil (0.92 mmol, 193 mg, 97% yield). ^{1}H NMR δ 1.15 (d, J = 6.4, 3H), 1.67 (m, 2H), 2.58 (m, 2H), 2.67 (d, J = 5.6, 1H), 3.74 (m, 7H), 6.68 (m, 3H);

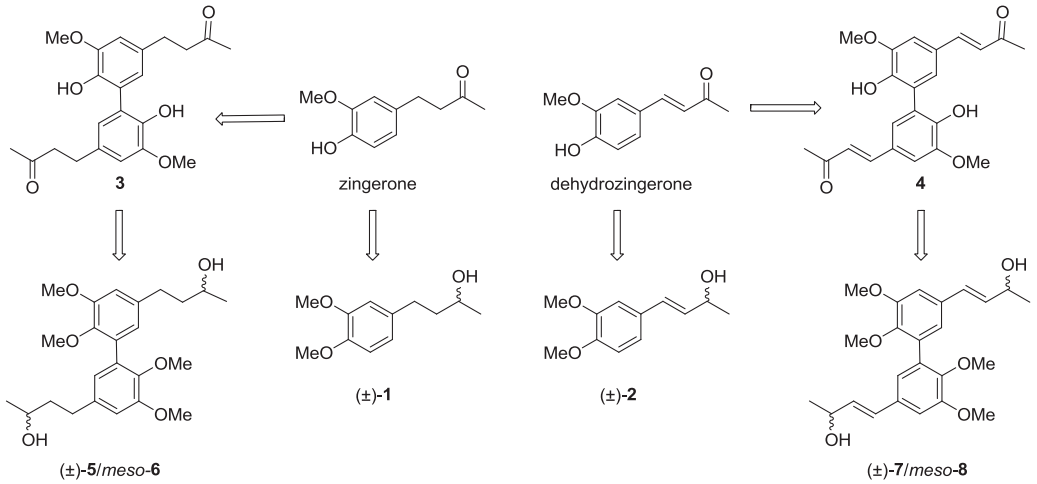


Fig. 2. Zingerol and dehydrozingerol derivatives.

^{13}C NMR δ 23.50, 31.70, 40.99, 55.74, 55.86, 67.21, 111.36, 111.82, 120.16, 134.87, 147.08, 148.79. ESI-MS (+) 20 V: *m/z* 211.7 $[M+H]^+$; 233.6 $[M+Na]^+$ (100); 442.9 $[2M+Na]^+$.

2.3. Synthesis of (±)-**2**

Commercial dehydrozingerone, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, (1.04 mmol, 200 mg) was subjected to methylation and successive reduction using the same procedure above described for (±)-**1** to give (±)-**2** as a colorless oil with a 82% global yield (0.86 mmol, 178 mg). ^{1}H NMR δ 1.34 (d, *J* = 6.4, 3H), 1.97 (bs, 1H), 3.84 (s, 3H), 3.86 (s, 3H), 4.42 (dt, 1H, *J* = 6.5 and 6.4), 6.10 (dd, 1H, *J* = 15.8 and 6.5), 6.46 (d, 1H, *J* = 15.8), 6.78 (d, 1H, *J* = 8.2), 6.88 (m, 2H); ^{13}C NMR δ 23.7, 56.0, 56.1, 68.2, 109.0, 111.4, 119.9, 129.4, 130.0, 131.9, 149.0, 149.2. ESI-MS (+) 20 V: *m/z* 190.7 (100); 230.9 $[M+Na]^+$, 420.7, 454.8.

2.4. General procedure for enzymatic transesterification of (±)-**1** and (±)-**2**

To a solution of the suitable substrate (0.05 mmol) in *tert*-butyl methyl ether (*t*-BME) (2 ml) lipase of choice (20 mg) and vinyl acetate (0.2 mmol) were added. The mixture was incubated in a shaker (250 rpm) at 28 °C and the progress of reaction was monitored by chiral HPLC analysis to check the substrate conversion and enantiomeric purities of both unreacted alcohol and formed acetate.

2.5. Preparative enzymatic resolution of (±)-**1**

Lipase AK (200 mg) and vinyl acetate (0.1 ml, 1.08 mmol) were added to a *t*-BME solution (10 ml) of (±)-**1** (100 mg, 0.48 mmol). Reaction mixture was stirred for 2.5 h at 28 °C and 250 rpm until 50% of substrate conversion was reached, then the enzyme was filtered and the organic solution taken to dryness. The alcohol residue and the acetate were isolated by silica gel chromatography eluting with hexane/ethyl acetate 60:40 vol/vol mixture.

Acetate (+)-**1a** was obtained as a colorless oil in 46% yield (52 mg, 0.22 mmol) and *ee* =98%: $[\alpha]_D^{25}$ = +10.46 (*c* = 1.4, $CHCl_3$). ^{1}H NMR δ 1.21 (d, *J* = 6.3, 3H), 1.74 (m, 1H), 1.89 (m, 1H), 2.01 (s, 3H), 2.56 (m, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 4.90 (dt, *J* = 6.5 and 6.3 1H), 6.67 (m, 2H), 6.76 (d, 1H, *J* = 8.8); ^{13}C NMR δ 20.25, 21.53, 31.61, 37.97, 56.01, 56.12, 70.65, 111.63, 111.92, 120.32, 134.35, 147.48, 149.09, 170.89. ESI-MS (+) 20 V: *m/z* 232.6, 274.8 (100) $[M+Na]^+$, 306.5. Chiral HPLC conditions: Phenomenex Lux® Cellulose-2, 85:15 hexane/2-propanol, flow rate 1 ml/min, *T* 23 °C, 8.30 min (*R* enantiomer) and 9.18 min (*S* enantiomer).

Alcohol (+)-**1** was obtained in 47% yield (46 mg, 0.23 mmol) and *ee* > 98%: $[\alpha]_D^{25}$ = +13.06 (*c* = 1.1, $CHCl_3$). Chiral HPLC conditions: Phenomenex Lux® Cellulose-2, 85:15 hexane/2-propanol, flow rate 1 ml/min, *T* 23 °C, 12.68 min (*R* enantiomer) and 13.52 min (*S* enantiomer).

2.6. Preparative enzymatic resolution of (±)-**2**

To a *t*-BME (10 ml) solution of (±)-**2** (100 mg, 0.48 mmol) lipase AK (200 mg) and vinyl acetate (0.1 ml, 1.08 mmol) were added. The suspension was shaken at 250 rpm and 28 °C temperature for 10 h and then the reaction was stopped at 50% of substrate conversion by filtering off the lipase. After removal of the solvent the residue was purified by chromagraphy on silica gel column eluting with hexane/ethyl acetate 60:40 vol/vol mixture to give acetate (+)-**2a** (55 mg, 0.22 mmol, 45% yield, *ee* = 97%) and alcohol (−)-**2** (44 mg, 0.21 mmol, 45% yield, *ee* > 98%) as colorless oils.

Acetate (+)-**2a**: $[\alpha]_D^{25}$ = +127.65 (*c* = 1.4, $CHCl_3$). ^{1}H NMR δ 1.39 (d, *J* = 6.4, 3H), 2.05 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 5.49 (dq, 1H, *J* = 6.8 and 6.4), 6.04 (dd, 1H, *J* = 16.0 and 6.8), 6.53 (d, 1H, *J* = 16.0), 6.79 (d, 1H, *J* = 8.2), 6.90 (m, 2H); ^{13}C NMR δ 20.70, 21.69, 56.09, 56.17, 71.44, 109.14, 111.36, 120.18, 127.09, 129.64, 131.78, 149.30, 149.36, 170.63. ESI-MS (+) 20 V: *m/z* 190.7 (100), 251.6 $[M+H]^+$, 272.4 $[M+Na]^+$, 312.4, 522.8 $[2M+Na]^+$. Chiral HPLC conditions: Phenomenex Lux® Cellulose-1, 85:15 hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C, 13.19 min (*R* enantiomer) and 15.39 min (*S* enantiomer).

Alcohol (−)-**2**: $[\alpha]_D^{25}$ = −30.53 (*c* = 1.6, $CHCl_3$). Chiral HPLC conditions: Phenomenex Lux® Cellulose-1, 85:15 hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C, 22.41 min (*S* enantiomer) and 24.07 min (*R* enantiomer).

2.7. Synthesis of (±)-**5**/*meso*-**6**

To a solution of biphenyl **3** (400 mg, 1 mmol) in acetone (7 ml) K_2CO_3 (69 mg, 0.5 mmol) and CH_3I (0.186 ml, 3 mmol) were added and the mixture left to react overnight under reflux. After addition of saturated NH_4Cl solution and extraction with CH_2Cl_2 the organic phase was dried on anhydrous sodium sulfate and evaporated under reduced pressure. The residue was dissolved in methanol (5 ml) and to the solution an excess of $NaBH_4$ was added. The reaction was stirred for 3 h at room temperature and then, after addition of saturated solution of NH_4Cl, extracted with CH_2Cl_2 which was washed with brine and dried on anhydrous sodium sulfate. After removal of the solvent, the residue was purified on silica gel column to give a 1:1 mixture of (±)-**5** and *meso*-**6** as pale yellow oil (376 mg, 0.90 mmol, 96% overall yield). ^{1}H NMR δ 1.18 (d, *J* = 6.0, 6H), 1.74 (m, 4H), 2.29 (bs, 2H), 2.64 (m, 4H), 3.58 (s, 6H), 3.79 (m, 2H), 3.84 (s, 6H), 6.67 (d, 2H, *J* = 1.6), 6.73 (d, 2H, *J* = 1.6); ^{13}C NMR δ 23.7, 32.1, 40.9, 55.9, 60.7, 67.5, 112.0, 123.1, 132.6, 137.4, 144.9, 152.6. ESI-MS (+) 20 V: *m/z* 440.0 (100) $[M+Na]^+$, 490.1, 646.9, 859.3 $[2M+Na]^+$. Chiral HPLC (Phenomenex Lux® Cellulose-1, 90:10 hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 41.9 min (*R,R* enantiomer), 43.2 min (*R,S meso*-isomer) and 44.7 min (*S,S* enantiomer).

2.8. Synthesis of (±)-**7**/*meso*-**8**

Biphenyl **4** (340 mg, 0.89 mmol) was methylated and subsequently reduced using the same conditions above described for the synthesis of (±)-**5** and *meso*-**6** from **3**. A 1:1 mixture of (±)-**7** and *meso*-**8** was obtained in 88% overall yield (327 mg, 0.79 mmol). ^{1}H NMR δ 1.31 (d, *J* = 6.4, 6H), 3.61 (s, 6H), 3.87 (s, 6H), 4.43 (dq, 2H, *J* = 6.4 and 6.4), 6.15 (dd, 2H, *J* = 16.0 and 6.4), 6.46 (d, 2H, *J* = 16.0), 6.83 (bs, 2H), 6.92 (bs, 2H); ^{13}C NMR δ 23.3, 55.7, 60.6, 68.7, 109.3, 121.6, 128.8, 132.1, 132.4, 132.9, 146.4, 152.6. ESI-MS (+) 10 V: *m/z* 228.9 268.1, 288.9, 437.5 $[M+Na]^+$. Chiral HPLC (Phenomenex Lux® Cellulose-1, 85:15 *n*-hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 39.4 min (*R,R* enantiomer), 43.0 min (*R,S meso*-isomer) and 49.1 min (*S,S* enantiomer).

2.9. Enzymatic resolution and desymmetryzation of (±)-**5** and *meso*-**6** mixture.

To a solution of (±)-**5** and *meso*-**6** (150 mg, 0.36 mmol) in *t*-BME (15 ml) lipase AK (300 mg) and vinyl acetate (0.15 ml, 1.6 mmol) were added The reaction was kept under stirring at 280 rpm and 28 °C of temperature for 6 h when 76% of substrate conversion was reached. After removal of the enzyme by filtration, the solvent was evaporated and the residue chromatographed on silica gel eluting with 60/40 vol/vol *n*-hexane/ethyl acetate mixture.

Diacetyl derivative (+)-**5b** was the first eluted product and was obtained as pale yellow oil in a 22% yield (40 mg, 0.08 mmol), *ee* > 98% and *de* = 97%: $[\alpha]_D^{25}$ = +13.2 (*c* = 1.5, $CHCl_3$). ^{1}H NMR δ 1.24 (d, *J* = 6.0, 6H), 1.82 (m, 2H), 1.92 (m, 2H), 2.02 (s, 6H), 2.60 (m, 4H),

3.61 (s, 6H), 3.88 (s, 6H), 4.94 (m, 2H), 6.66 (d, 2H, *J* = 2.0), 6.71 (d, 2H, *J*= 2.0); ^{13}C NMR δ 20.3, 21.6, 31.9, 37.8, 56.1, 60.9, 70.9, 112.1, 123.1, 132.8, 136.9, 145.2, 152.8, 171.1. ESI-MS (+) 5 V: *m/z* 525 $[M+Na]^+$, 576 (100). Chiral HPLC (Phenomenex Lux® Cellulose-1, 90:10 hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 16.9 min (*R,R* enantiomer), 18.7 min (*R,S meso*-isomer) and 20.8 min (*S,S* enantiomer).

Monoacetyl derivative (+)-**6a** was obtained as pale yellow oil in 46% yield (74 mg, 0.16 mmol) and *ee* and *de* > 98%. $[\alpha]_D^{25}$ = +10.1 (*c* = 1.1, $CHCl_3$). ^{1}H NMR δ 1.20 (d, *J* = 6.2, 3H), 1.23 (d, *J* = 6.2, 3H,), 1.74 (m, 3H), 1.93 (m, 1H), 2.01 (s, 3H), 2.64 (m, 4H), 3.60 (s, 6H), 3.84 (m, 1H), 3.87 (s, 6H), 4.93 (m, 1H), 6.66 (d, 1H, *J* = 1.8), 6.69 (d, 1H, *J* = 1.8), 6.71 (d, 1H, *J* = 1.8), 6.74 (d, 1H, *J* = 1.8); ^{13}C NMR δ 20.3, 21.6, 23.8, 31.9, 32. 3, 37.8, 41.1, 56.1, 60.7, 60.9, 67.7, 70.9, 112.0, 112.1, 123.1, 123.2, 132.7, 132.9, 136.8, 137.5, 145.1, 145.2, 152.8, 171.1. ESI-MS (+) 5 V: *m/z* 440 (100), 860. Chiral HPLC (Phenomenex Lux® Cellulose-1, 90:10 hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 25.8 min (*R*-acetoxy-*S*-hydroxy enantiomer) and 29.2 min (*S*-acetoxy-*R*-hydroxy enantiomer). To determine the diastereoisomeric excess of monoacetate (+)-**6a**, the isolated product was acetylated by standard procedure using pyridine and acetic anhydride, and the obtained diacetyl derivative was analyzed by chiral HPLC.

Diol (+)-**5**, the last eluted product, was recovered in a 21% yield (32 mg, 0.07 mmol) and *ee* > 98%. $[\alpha]_D^{25}$ = +10.2 (*c* = 1.2, $CHCl_3$).

2.10. Enzymatic resolution and desymmetryzation of (±)-7 and meso-8 mixture

To a solution of (±)-**7** and *meso*-**8** mixture (200 mg, 0.48 mmol) in *t*-BME (20 ml) lipase AK (400 mg) and vinyl acetate (0.2 ml, 2 mmol) were added. The reaction was shaked at 28 °C and 250 rpm until the substrate conversion reached 79% after 34 h. After lipase filtration and evaporation of the solvent, the residue was chromatographed on silica gel, eluting with 60/40 vol/vol *n*-hexane/ethyl acetate mixture. Diacetyl derivative (+)-**7b**, the first eluted product, was recovered as pale yellow oil in 18% yield (43 mg, 0.08 mmol) and 95% *ee*: $[\alpha]_D^{25}$ = +98.4 (*c* = 0.4, $CHCl_3$). ^{1}H NMR δ 1.39 (d, *J* = 6.4, 6H), 2.06 (s, 6H), 3.64 (s, 6H), 3.91 (s, 6H), 5.50 (dq, *J* = 6.8 and 6.4, 2H), 6.10 (dd, *J* = 16.0 and 6.8, 2H), 6.55 (d, *J* = 16.0, 2H), 6.88 (d, *J* = 1.6, 2H), 6.94 (d, *J* = 1.6, 2H); ^{13}C NMR δ 20.7, 21.7, 56.2, 61.1, 71.3, 110.0, 122.1, 128.5, 131.6, 132.1, 132.9, 153.1, 170.7. ESI-MS (+) 10 V: *m/z* 437.3, 479.2, 521.4 $[M+Na]^+$ (100). Chiral HPLC (Phenomenex Lux® Cellulose-1, 85:15 *n*-hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 17.0 min (*R,R* enantiomer), 19.9 min (*R,S meso*-isomer) and 23.9 min (*S,S* enantiomer).

The monoacetyl derivative (+)-**8a** was obtained as yellow oil in 38% yield (83 mg, 0.18 mmol) and 94% *ee*. $[\alpha]_D^{25}$ = +48.6 (*c* = 0.6, $CHCl_3$). ^{1}H NMR δ 1.36 (d, *J* = 6.4, 3H), 1.39 (d, *J* = 6.4, 3H), 2.06 (s, 3H), 3.64 (s, 3H), 3.65 (s, 3H), 3.91 (s, 6H), 4.47 (dq, 1H, *J* = 6.8 and 6.4), 5.50 (dq, 1H, *J* = 6.8 and 6.4), 6.09 (dd, 1H, *J* = 15.8 and *J* = 6.8), 6.18 (dd, 1H, *J* = 15.8 and 6.8), 6.50 (d, 1H, *J* = 15.8), 6.55 (d, 1H, *J* = 15.8), 6.86 (d, 1H, *J* = 1.9), 6.88 (d, 1H, *J* = 1.8), 6.94 (d, 1H, *J* = 1.9), 6.95 (d, 1H, *J* = 1.8); ^{13}C NMR δ 20.7, 21.7, 23.8, 56.2, 61.11, 69.3, 71.37, 109.7, 109.8, 122.0, 122.1, 128.5, 129.4, 131.6, 132.0, 132.4, 132.8, 132.9, 133.2, 146.9, 147.2, 153.0, 170.7. ESI-MS (+) 10 V: *m/z* 229.8, 479.4 $[M+Na]^+$ 935.6 $[2M+Na]^+$ (100). Chiral HPLC (Phenomenex Lux® Cellulose-1, 85:15 *n*-hexane/2-propanol, flow rate 0.5 ml/min, *T* 23 °C): 29.2 min (*R*-acetoxy-*S*-hydroxy enantiomer) and 35.1 min (*S*-acetoxy-*R*-hydroxy enantiomer). To determine the diastereoisomeric excess of monoacetate (+)-**8a**, the isolated product was acetylated by standard procedure using pyridine and acetic anhydride, and the obtained diacetyl derivative was analyzed by chiral HPLC.

The unreacted diol (−)-**7** was recovered in 18% yield (37 mg, 0.09 mmol), *ee* = 93% and *de* = 97%. $[\alpha]_D^{25}$ = −14.6 (*c* = 0.4, $CHCl_3$).

2.11. Kinetic measurements

Three separate solutions of (±)-**1** (10 mg, 0.048 mmol), (±)-**5** (10 mg, 0.024 mmol) and *meso*-**6** (10 mg, 0.024 mmol) in *t*-BME (2 ml) were prepared. To each solution lipase AK (40 mg) and vinyl acetate (0.2 mmol) were added and the mixtures left under stirring at 250 rpm and 28 °C. The progress of reactions was monitored in parallel by chiral HPLC analysis to check the substrate conversion and enantiomeric purity.

3. Results and discussion

3.1. Lipase-catalyzed kinetic resolution of zingerol (±)-1 and dehydrozingerol (±)-2 derivatives

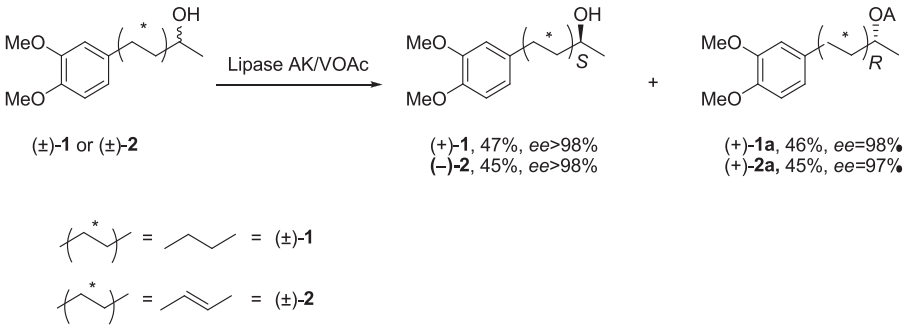
Racemic (±)-**1** and (±)-**2**, respectively zingerol and dehydrozingerol *O*-methyl derivatives, were obtained from carbonyl reduction of commercial zingerone and dehydrozingerone, whose phenolic hydroxyl groups had been methylated to improve the solubility and chemical stability. The reduction with $NaBH_4$ in methanol at room temperature allowed the selective formation of alcohols in quantitative yield, the double bond of dehydrozingerone being unaffected. Structures of (±)-**1** and (±)-**2** were confirmed by ^{1}H-NMR analysis on the basis of methinic resonances at 3.74 and 4.42 ppm, respectively, and a double doublet at 6.10 ppm coupled with a doublet at 6.46 ppm accounting for the ethylenic protons in dehydrozingerol.

In a preliminary phase of the study a screening of some commercially available lipases was performed to compare the degree of selectivity and reactivity of the catalysts. Among the lipases tested for transesterification of (±)-**1** and (±)-**2**, PS-C I from *P. cepacia*, AK from *P. fluorescens*, Lipozyme from *M. miehei* and Novozym 435 from *C. antarctica* were found more selective in the kinetic resolutions. All the reactions were carried out at 28 °C, selected as optimal reaction temperature, in *tert*-butyl methyl ether (*t*-BME) using vinyl acetate (VOAc) as irreversible acyl donor (Scheme 1).

From the obtained data (Table 1) it was evident that the immobilized lipases from *P. cepacia*, *M. miehei* and *C. antarctica* were highly sensitive, in terms of stereoselectivity as well as reaction rate, to the presence of unsaturation in α to the stereogenic center whereas the crude lipase AK maintained excellent levels of stereoselectivity for both substrates.

Taking into the account the good results obtained in the screening, lipase AK was chosen as catalyst for kinetic resolution of (±)-**1** in preparative scale. The reaction was monitored by HPLC and after 2.5 h was stopped when a 50% of substrate conversion was measured. The alcohol (+)-**1** and acetate (+)-**1a** were recovered by silica gel chromatography in high yields and *ee* = 98% and >98% respectively. The acetylation of substrate was confirmed because of the presence, in the ^{1}H-NMR spectrum of (+)-**1a**, of a singlet resonance at 2.01 ppm associated with the acetyl group and a downfield shifted resonance (4.90 ppm) for the methinic proton. Alcohol (+)-**1** was assigned to *S* absolute configuration by comparison of the sign of its optical rotation with literature data previously reported for the related natural zingerol [16] and further support came from the observed reaction outcome in a lipase AK-catalyzed acetylation of (±)-zingerol, that gave unreacted (+)-*S*-zingerol and the corresponding *R*-ester both in optically active form.

Under the same experimental conditions, 50% conversion of (±)-**2** was reached after 11 h when the reaction was stopped by filtering the enzyme and the mixture purified to give alcohol (−)-**2** and acetate (+)-**2a** in high yields and optical purities (*ee* > 98%). Also in



Scheme 1. Enzymatic kinetic resolution of (±)-**1** and (±)-**2**.

this case the ester structure of (+)-**2a** was confirmed by NMR analysis thank the presence of singlet at 2.05 ppm and the downfield shift of the CHOH proton due to esterification. A *S* absolute configuration of (−)-**2** was tentatively assigned on the basis of the sign of its optical rotation that is the same as reported for the structurally similar (*S*)-(−)-4-phenyl-3-buten-2-ol [26].

From such assignments it follows that AK lipase displays *R* stereopreference in the kinetic resolution of both (±)-**1** and (±)-**2**, that is in agreement with the site active model proposed by Burgess et al. for this enzyme by analyzing the substrate-structure/enzyme-activity relationships for different unsaturated alcohols [27].

Taking into the account the high selectivity of AK lipase in the recognition of stereogenic alcoholic centers here evidenced, the kinetic resolution protocol was then extended to compounds (±)-**5**/*meso*-**6** and (±)-**7**/*meso*-**8** that can be viewed as the dimeric forms of (±)-**1** and (±)-**2**, respectively.

3.2. Enzymatic one pot resolution/desymmetryzation of biphenyl mixtures (±)-5/meso-6 and (±)-7/meso-8

The biphenyl zingerone derivative **3** was obtained in 65% yield using a direct C—C coupling reaction [24] of commercial zingerone in the presence of methyl-tri-butylammonium permanganate in CH_2Cl_2 at room temperature. Since this procedure was unsuccessful in the case of dehydrozingerone, the biphenyl **4** was obtained in 50% yield by Claisen-Schmidt condensation of acetone with 5,5′-bivanillin [13]. The obtained biphenyl ketones were *O*-methylated and then reduced with $NaBH_4$ in MeOH to give the corresponding diols, in both cases isolated as a chromatographically inseparable mixture of diastereoisomers. Also in the ^{1}H- and ^{13}C-NMR spectra a single set of resonances was observed for each diol whereas the HPLC chiral analysis clearly revealed the presence of three peaks in 1:2:1 ratio for each diol, accounting for a 1:1 mixture of racemic and *meso* diastereoisomers (±)-**5**/*meso*-**6** and (±)-**7**/*meso*-**8**, respectively.

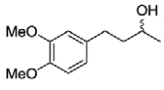
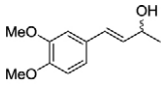
Although the acetylation of a 1:1 mixture of diastereoisomeric diols could be expected to be severely complicated by the presence of up to ten possible stereoisomers in partial or complete acylated form, the enzymatic transesterifications were carried out using the whole mixtures in the hope that the high stereoselectivity of AK lipase could promote a one-pot resolution/*meso*-desymmetrization process giving a reduced number of stereoisomers.

The lipase-catalyzed transesterification of (±)-**5**/*meso*-**6** was then carried out in the same experimental conditions adopted for (±)-**1** and monitored by chiral HPLC at various reaction times. After 3 h the HPLC chromatogram of the reaction mixture displayed 45% of unreacted substrate in the presence of both monoacetate (43%) and diacetate (12%). The reaction was stopped at 6 h when the theoretical composition of 25% of diacetate (+)-**5b**, 50% monoacetate (+)-**6a** and 25% of diol (+)-**5** was reached. Chromatographic purification of the reaction mixture gave diacetate (+)-**5b** with *ee* > 98% and *de* = 97% together with the enantiomerically and diastereoisomerically pure monoacetate (+)-**6a** and diol (+)-**5** (Scheme 2).

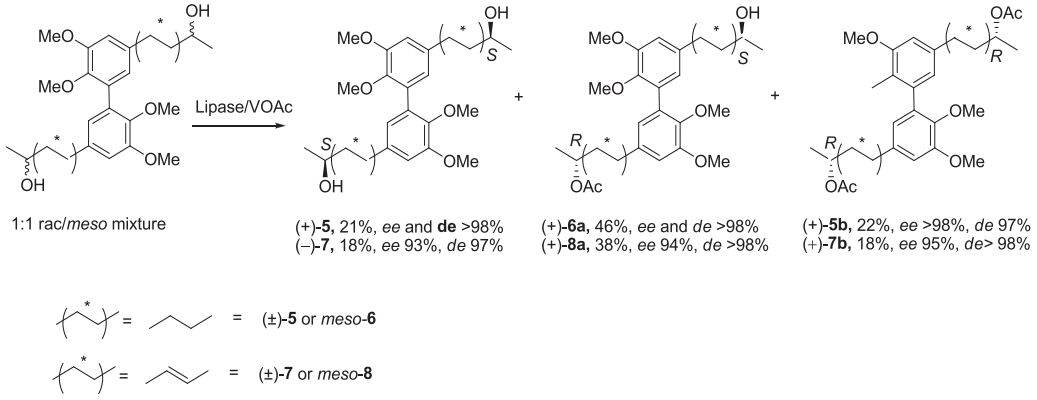
High values of optical purity for all the three compounds confirm that the lipase retained its excellent stereorecognition ability yet displayed in the kinetic resolution of the parent (±)-**1.** The same procedure was also applied to prepare optically active dehydrozingerol biphenyl derivatives. Enzymatic esterification of 1:1 mixture of (±)-**7**/*meso*-**8** was monitored by chiral HPLC analysis until the presence of three main peaks in a ratio about 1:2:1 was observed in the chromatogram (34 h). After quenching the reaction by filtering the enzyme the products were isolated in high yields by column chromatography. Diacetate (+)-**7b** and monoacetate (+)-**8a** were obtained as single diastereoisomers with 95% *ee* and 94% *ee* respectively, while diol (−)-**7** was isolated in 93% *ee* and 97% *de* (Scheme 2).

In agreement with the empirical rules proposed for the enantiorecognition of lipases in the transesterification reactions of secondary alcohols [28] and taking into the account the structural similarity of the alcoholic groups in (±)-**1** and (±)-**2** with

Table 1
Screening of lipases for transesterification[a] of (±)-**1** and (±)-**2**.

	Lipase	Time (h)	*c* (%)[b]	*ee*[b] (*S*)-alcohol	*ee*[b] (*R*)-acetate	*E*[c]
 (±)-1	PS-C	3	38	61	>98	>200
	Lipozyme	11	23	29	98	131
	Novozym	1	40	63	94	62
	AK	3	43	75	>98	>200
 (±)-2	PS-C	24	22	27	96	64
	Lipozyme	24	37	55	94	56
	Novozym	6	45	80	98	>200
	AK	18	46	83	98	>200

[a] Substrate 0.05 mmol, lipase 20 mg, *t*-BME 2 ml, VOAc 0.2 mmol, 28 °C, 250 rpm.
[b] Substrate conversion and *ee* were determined by HPLC using a chiral column.
[c] See Ref. [25].



Scheme 2. Enzymatic resolution/desymmetrization of (±)-**5**/*meso*-**6** and (±)-**7**/*meso*-**8** mixtures.

respect to those in the corresponding dimers **5**–**8** it was assumed that lipase AK maintained the same *R* enantiopreference also in the esterification of the biphenyl diols. Therefore, diester derivatives (+)-**5b** and (+)-**7b** were assigned to *R,R*-absolute configuration and also for monoacetyl derivatives (+)-**6a** and (+)-**8a** it was believed that the acetyl groups are located on the *R*-stereogenic centers.

3.3. Lipase behavior in the kinetic resolution and desymmetrization of biphenyls (±)-5 and meso-6

Despite of a large number of diols has been considered as substrates for lipase-catalyzed transesterification, a direct comparison of the enzyme activity in the recognition of a C_2-symmetric racemic diol and the corresponding *meso*-form has not yet been reported and, in this context, parallel reactions on separate (±)-**5** and *meso*-**6** biphenyls as well as the monomer (±)-**1** as reference compound were carried out for kinetic investigation.

Since the diastereoisomeric diols are obtained as an inseparable mixture from the reduction of the parent ketone **3**, they were prepared in pure form using a different approach. So, *meso*-**6** was easily accessible by chemical hydrolysis of the enzymatic monoacetate (+)-**6a** whereas racemic (±)-**5** was prepared by mixing equal amounts of (+)-**5** and (−)-**5**, the latter obtained from alkaline hydrolysis of enzymatic diacetate (+)-**5b.**

All the enzymatic transesterifications were carried out using a concentration of substrate such that in each reaction an equivalent number of *R* stereogenic centers was present. So, double molar concentration of (±)-**1** with respect to (±)-**5** and *meso*-**6** was required in order to account that two stereogenic centers are present for each molecule of biphenyl diol. The experiments were performed in *t*-BME in the presence of lipase AK and vinyl acetate, monitoring the progress of reactions by chiral HPLC.

Due to the excellent stereoselectivity of lipase AK the measured amount of formed products in the reaction was directly related with the conversion of *R* stereogenic centers, that was plotted against time (Fig. 3). The curves in Fig. 3 display a very similar reaction rate for monomer (±)-**1** and *meso*-**6** indicating that the enzyme takes the same time to select and transform the *R* stereogenic centers discarding the *S*-ones in both cases, either when the *R/S* centers are on separate molecules ((±)-**1**) either when they are located on the same molecule (*meso*-**6**). Although the curve of (±)-**5** was built considering the sum of both monoacetate and diacetate, that contributes for two transformed *R*-centers, the overall speed of esterification appeared about halved. This finding can be explained considering that the exposure of one *R*-center of *R,R*-diol to the active site of the enzyme makes the second one on the same molecule not available for acylation in same time unit.

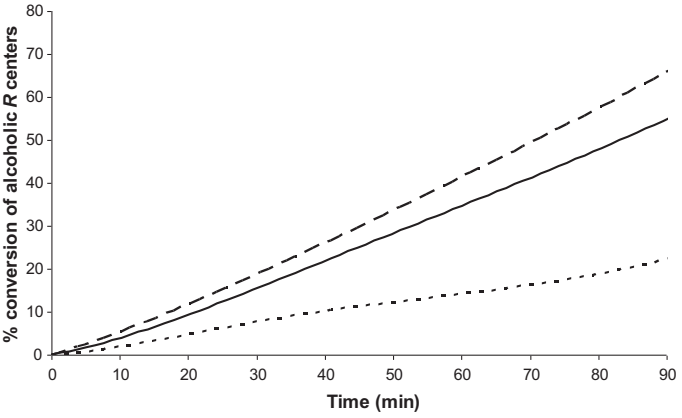


Fig. 3. Enzymatic transesterification rate of alcoholic functions on *R* stereogenic centers of *meso*-**6** (– –), (±)-**1** (—) and (±)-**5** (·····).

4. Conclusions

In summary, a lipase catalyzed kinetic resolution in organic solvent of *O*-methylated zingerol and dehydrozingerol (±)-**1** and (±)-**2** has been successful carried out giving the products in excellent yield and optical purity. The biocatalytic procedure developed for such alcohols was also extended to the preparative resolution and desymmetrization of corresponding rac- and *meso*- biphenyl dimers **5–8**. The high enantioselectivity of lipase AK allowed the complete separation of single diastereoisomers of mixtures, providing the single products in enantiopure form.

Kinetic data of the reactions performed on single diastereoisomeric species evidenced that desymmetrization of *meso*-**6** proceeded with comparable reaction rate with respect to racemic resolution of (±)-**1**, whereas in the case of dimer (±)-**5** the resolution process was slower. This experiment also indicated that the lipase maintained the same stereodifferentiation ability, discriminating independent enantiomeric molecules as in (±)-**1** or stereogenic opposite centers on a same molecule as *meso*-**6** in comparable rate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.01.007.

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