Enzymatic Resolution of (*R*)- and (*S*)-(*E*)-4-Hydroxyalk-2-enals Related to Lipid Peroxidation

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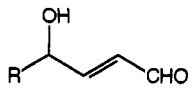
Introduction

Among the many different unsaturated aldehydes which can be formed during lipid peroxidation, (*E*)-4-hydroxyhex-2-enal (**1a**), (*E*)-4-hydroxynon-2-enal (**1b**), and (*E*)-4-hydroxyundec-2-enal (**1c**), produced in the peroxidation



1a: R = C_2H_5
1b: R = C_5H_{11}
1c: R = C_7H_{15}

of (*n*–3)-, (*n*–6)-, and (*n*–9)-polyunsaturated fatty acids, are of interest since they could mediate many pathophysiological and possibly also physiological effects occurring in cells or organs in response to oxidative stress and lipid peroxidation.[1] Although the hydroxy aldehydes **1a–c** possess a chiral carbon at the 4-position, no papers have until now been reported concerning the stereochemistry at this carbon of the compounds formed in lipid peroxidation, and no study has been reported on a possible different biological behavior of each enantiomer, even if it was shown that racemic (*E*)-4-hydroxynon-2-enal could mimic many biological effects observed with the corresponding biogenic aldehyde, isolated from suspensions of peroxidized microsomes.[2]

Our interest in studying the mechanism by which racemic (*E*)-4-hydroxynon-2-enal effects heat shock gene expression[3] and the most general consideration about the utility of these compounds for studies on their biological interactions prompted us to obtain both *R*- and *S*-enantiomers of aldehydes **1a–c**.

Previous literature analysis showed several syntheses of racemic (*E*)-4-hydroxyalk-2-enals[4] but only one method for their optical resolution by a route which uses pure chiral iron tricarbonyl complex of sorbic acid.[5] The method was verified for the preparation of both optically active forms of (*E*)-4-hydroxynon-2-enal (**1b**).

In order to set up a simpler method for a general preparation of pure (*R*)- and (*S*)-(*E*)-4-hydroxyalk-2-enals, we decided to explore the possibility of effecting the enzymatic resolution of racemic **1a–c** or that of one of their suitable precursors by means of hydrolytic enzymes able to enantioselectively acylate the hydroxy group in organic solvents.[6]

In this paper we report how we obtained the (*R*)- and (*S*)-hydroxy aldehydes **1a–c** by enantioselective esterification of the 4-hydroxy group of their racemic dimethyl acetals **2a–c** by using *Pseudomonas fluorescens* lipase (PFL)[7] in organic solvent[8] as catalyst.

Results and Discussion

Preliminary experiments were aimed at resolving the racemic hydroxy aldehyde **1b** by means of biological acylation catalyzed by commercially available lipases working in organic solvents. However, porcine pancreatic lipase displayed extremely low activity and very poor selectivity (enantiomeric ratio E[9] < 1), in various solvents (diethyl ether,[10] cyclohexane and nitromethane;[11] in the last solvent the reaction does not occur) and in the presence of trifluoroethyl laurate or vinyl acetate as acylating agents. Similarly, PFL exhibited low enantiospecificity (E < 6).

Better results were obtained in the separation of the dimethyl acetal **2b**, from which the parent aldehyde **1b** is regenerated by simple treatment with acids[12a] or by column chromatography on silica gel.[12b] In fact, in this case, screening experiments showed an improved stereoselectivity for PFL (E > 70) when vinyl acetate in *tert*-butyl methyl ether (*t*-BuOMe) was used. Then this lipase was selected for the separation of the hydroxy acetals **2a–c**, which were efficiently resolved into nearly pure unreacted alcohols (*S*)-**2a–c** and acetates (*R*)-**3a–c**, letting the reaction run to nearly 50% conversion extent. These reaction products can be separated in pure form by TLC, while attempted separation by column chromatography (on silica gel or alumina) resulted, in all cases, in a partial hydrolysis of the acetal groups.[12b] Since the aim of our work was to obtain *S*- and *R*-enantiomers of hydroxy aldehydes **1a–c**, we decided to regenerate the aldehyde groups by acidification of the reaction produts, after filtration of the enzyme. In this way, successive column chromatography allowed an efficient recovery of the hydroxy aldehydes (*S*)-**1a–c** and of the acetates (*R*)-**4a–c**.

The results reported in Table I show that, independent of the length of the alkyl substituent bonded at the carbinol group, the enantiomeric ratio of the kinetic resolution is excellent (E > 70) and occurs with the same sense of enantioselection, the (*R*)-enantiomers being acetylated in preference. In fact, the hydroxy aldehydes (*S*)-**1a–c** are

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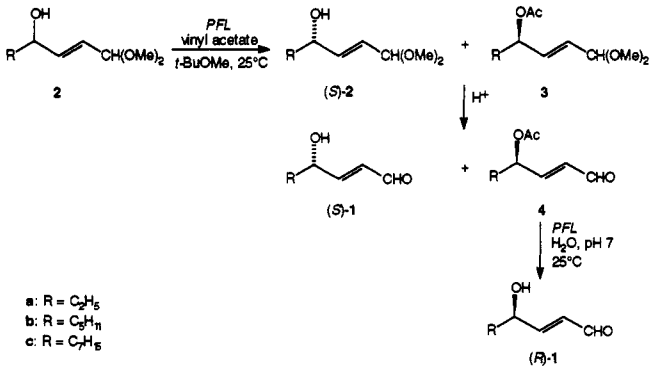
Table I. Chemoenzymatic Obtention of (*R*)- and (*S*)-1a–c



R	convn[a] (%) (time, days)[b]	(*S*)-1 ee (%)[c] (yield, %)[d]	4 ee (%)[e] (yield, %)[d]	(*R*)-1 ee (%)[c] (yield, %)[d]
a	51 (1)	>95 (44)	90 (48)	>95 (78)
b	52 (5)	>95 (43)	89 (46)	>95 (77)
c	52 (5)	>95 (43)	88 (46)	>95 (75)

[a] Determined by HPLC after acidification of the reaction mixture. [b] Referred to enzymatic esterification. [c] Enantiomeric excesses were determined by ^{1}H NMR analysis of the Mosher esters. [d] After flash chromatography. [e] Enantiomeric excesses were determined by ^{1}H NMR using 0.5–0.8 equiv of Eu(hfc)$_3$.

obtained in over 95% enantiomeric excess while the acetylated isomers (*R*)-**4a–c** are obtained in 88–90% enantiomeric excess. However, the aldehydes (*R*)-**1a–c** can also be obtained in an improved enantiomeric excess (over 95%) by hydrolysis of their acetates (*R*)-**4a–c**, performed in aqueous medium under catalysis of the same enzyme which showed the same *R* stereospecificity. The enzymatic hydrolysis is remarkable since the chemical hydrolysis of acetylated 4-hydroxyalk-2-enals, both in the presence of mild bases[2] or acids,[13] causes the rapid decomposition of the regenerated aldehydes.

The enantiomeric excess of the hydroxy aldehydes **1a–c** was determined by ^{1}H NMR analysis of their (*R*)- and (*S*)-Mosher esters [(*R*)- and (*S*)-2-methoxy-2-phenyl-2-trifluoromethyl acetates; (*R*)- and (*S*)-MTPA esters][14] while those of the acetates (*R*)-**4a–c** were evaluated by ^{1}H NMR, using Eu(hfc)$_3$.[15] The configurational assignment in the case of the (*R*)- and (*S*)-hydroxy aldehydes **1b** could be done by comparison of their optical rotations with those of compounds previously obtained.[5] However we confirmed their absolute stereochemistry and established that of the other unreported hydroxy aldehydes (*S*)-**1a** and (*S*)-**1c** by analysis of the ^{1}H NMR data of their Mosher esters, according to the Mosher's modified method.[16] Diagnostic for the assignment were the chemical shifts of the protons at positions 1–3 and 5. In fact, the chemical shifts of the protons at 1–3 of the (*R*)-MTPA esters appear significantly shielded with respect to those of the (*S*)-MPTA diastereomer; the chemical shifts of the protons at position 5 appear, on the contrary, deshielded in (*R*)-MTPA esters relative to (*S*)-MPTA ones (Figure 1). Specular results were obtained for the Mosher esters of the hydroxy aldehydes (*R*)-**1a–c**.

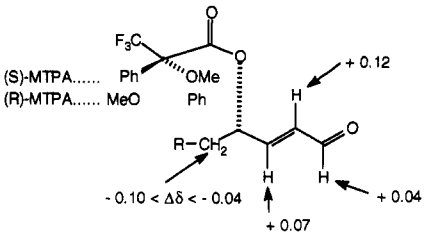


Figure 1. Configurational assignment of the alcohols (*S*)-**1a–c** based on the ^{1}H NMR $\Delta\delta$ values obtained for their (*S*)- and (*R*)-MTPA esters. $\Delta\delta$ values ($\delta_S - \delta_R$) are expressed in ppm (500 MHz).

Some rules[17,18] have been reported for predicting the fast-reacting enantiomer in the resolution of secondary alcohols mediated by various lipase from *Pseudomonas*, on the basis of the sizes of the substituents at the stereocenter. In our experiments no change in the sense of the enantioselection and in the enantiomeric ratio was observed regardless of the steric size of the alkyl substituent at the chiral center, and so it appears difficult to apply the reported rules.

In conclusion, the (E)-4-hydroxyalk-2-enals **1a–c**, important products of lipid peroxidation, have been efficiently resolved by enantioselective acetylation of their acetals mediated by PFL in an organic solvent. The (*S*)-aldehydes were directly obtained by successive acidification, while the (*R*)-enantiomers were obtained after acidification and enzymatic hydrolysis of the regenerated 4-acetoxy aldehydes, catalyzed by the same enzyme in aqueous medium. A uniform sense of enantioselection and high enantiselectivities were observed in substrates bearing a short (C_2) and a long (C_7) chain bonded to the carbinol group.

The ready access[4] to racemic (*E*)-4-hydroxy aldehydes coupled with their efficient and simple enzymatic resolution here reported make these compounds now easy available for biological studies.

Experimental Section

The ^{1}H NMR spectra (500.13 MHz) were recorded in $CDCl_3$ at 303 K and were referenced to $CHCl_3$ at 7.24 ppm. Mass spectra (MS) were recorded at 70 eV. Mass spectra were reported as *m/z* (relative abundance). HPLC analyses were carried out on a Merck superspher 100 RP-18 column, 4 mm × 25 cm, the flow rate was 1 mL/min, and the detection was performed at 220 nm. Optical rotations were measured for 1% $CHCl_3$ solutions. TLC was carried out on silica gel HF_{254} microplates. Column chromatography refers to flash chromatography.[19] (*S*)- and (*R*)-MTPA ester derivatives were prepared from commercially available (*R*)- and (*S*)-MTPA.[14,16] Porcine pancreatic lipase (type II) was purchased from Sigma; *Pseudomonas fluorescens* lipase (SAM-2) was purchased from Fluka (cat. no. 62312). All organic solvents were dried before use.

Lipase-Mediated Acylation of Racemic Acetals (2a–c) in Organic Solvents. General Procedure. Vinyl acetate (2.2 mL, 24 mmol) and *Pseudomonas fluorescens* lipase (500 mg) were added to a solution of the racemic hydroxy acetals **2a–c** (3 mmol) in *t*-BuOMe (10 mL). The resulting suspension was shaken at 25 °C and monitored by HPLC (performed on samples acidified with ion-exchange resin). When the desired conversion was reached, the reaction was stopped by filtering away the enzyme. The solvent was then removed in vacuo to give a mixture of the

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unreacted starting (*S*)-alcohol and of the (*R*)-acetate which are separable on TLC (CH_2Cl_2–Et_2O (80:20 v/v)).

The residue (formed by a mixture of the acetals (*S*)-**2** and **3**) was dissolved in moist acetone (10 mL), treated with ion-exchange resin (Dowex 50 × 8-200; 600 mg), and stirred for different times (0.5, 1.0, and 2.5 h for the series **a**, **b**, and **c**, respectively). After filtration of the resin and drying and evaporation of the solvent, the (*S*)-hydroxy aldehyde and the (*R*)-acetoxy aldehyde were separated by flash chromatography (hexane–AcOEt (75:25 v/v)).

The obtained (*S*)-hydroxy aldehydes –**1a–c** (purity >97% by HPLC, eluent MeOH–H_2O (70:30 v/v)) showed correct physicochemical properties identical with those reported for their racemates.[4,20] Their optical rotations, $[\alpha]^{25}_D$, were +51.6° for (*S*)-**1a**, +46.3° for (*S*)-**1b** (lit.[5] +48°, *c* 0.69), and +42.0° for (*S*)-**1c**.

All (*R*)-acetoxy aldehydes **4a–c** (purity > 97%) showed at ^1H NMR (500 MHz) identical proton signals diagnostic for the assigned α,β unsaturated system: δ 9.54 (1 H, d, J = 7.7 Hz, H-1), 6.70 (1 H, dd, J = 15.4 and 4.9 Hz, H-3), 6.17 (1 H, ddd, J = 15.4, 7.7, and 1.4 Hz, H-2), 5.47 (1 H, ddt, J = 6.5, 4.9, and 1.4 Hz, H-4), 2.09 (3 H, s, $OCOCH_3$).

(*E*)-4-Acetoxyhex-2-enal (4a): an oil; TLC R_f = 0.45 (CH_2Cl_2–Et_2O (95:5 v/v)); HPLC rt_R (relative retention time) = 1.26 (eluent MeOH–H_2O (75:25 v/v); rt_R of **1a** = 1); $[\alpha]^{25}_D$ +34.5°; MS 127 (M^+ – CHO, 2), 55 (100).

(*E*)-4-Acetoxynon-2-enal (4b): an oil; TLC R_f = 0.52 (CH_2Cl_2–Et_2O (95:5 v/v)); HPLC rt_R = 1.31 (eluent MeOH–H_2O (80:20 v/v)); rt_R of **1b** = 1); $[\alpha]^{25}_D$ +12.7°; MS 169 (M^+ – CHO, 2), 55 (100).

(*E*)-4-Acetoxyundec-2-enal (4c): an oil; TLC R_f = 0.53 (CH_2Cl_2–Et_2O (95:5 v/v)); HPLC rt_R = 1.55 (eluent MeOH–H_2O (80:20 v/v)); rt_R of **1c** = 1); $[\alpha]^{25}_D$ +8.9°; MS 197 (M^+ – CHO, 2), 55 (100).

Lipase-Mediated Hydrolysis of the (*R*)-Acetoxy Aldehydes 4a–c. General Procedure. *Pseudomonas fluorescens* lipase (90 mg) was added to a suspension of the acetates **4a–c** (1 mmol) in 0.1 M phosphate buffer (30 mL, pH 7). The pH value of the mixture was kept constant at the starting value by controlled addition of 0.1 N sodium hydroxide. The hydrolysis was followed in HPLC, and after 80% conversion extent (1 day) the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), filtered, and evaporated to afford a residue which was purified by flash chromatography (hexane–ethyl acetate (75:25 v/v)) to give the aldehydes (*R*)-**1a–c**, which showed appropriate physicochemical properties identical to those reported above for (*S*)-enantiomers, apart from the optical rotations, $[\alpha]^{25}_D$, which were –52.3° for (*R*)-**1a**, –48.2° for (*R*)-**1b** (lit.[5] –46°, *c* 0.45), and –43.5 for (*R*)-**1c**.

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