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Preparation of (*R*)- and (*S*)-(*E*)-4-Hydroxy-2-unsaturated Acids by Asymmetric Hydrolysis of their Racemic Esters

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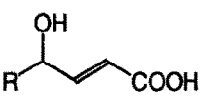
Abstract: Several optically active (*R*)- and (*S*)-(*E*)-4-hydroxyalk-2-enoic acids, metabolites of bioactive compounds of lipid peroxidation, were prepared from the corresponding racemic methyl esters by enantioselective hydrolysis mediated by porcine pancreas lipase and porcine liver esterase.

In connection with some biological problems concerning lipid peroxidation (LPO),[1] we needed to synthesise the unreported (*R*)- and (*S*)-(*E*)-4-hydroxy-2-unsaturated acids **1a-f** which have been shown to be possible metabolites of important cytotoxic (*E*)-4-hydroxyalk-2-enals produced in LPO.[2,3]



(1)

a: R = C_3H_7 **d**: R = C_6H_{13}
b: R = C_4H_9 **e**: R = C_7H_{15}
c: R = C_5H_{11} **f**: R = C_8H_{17}

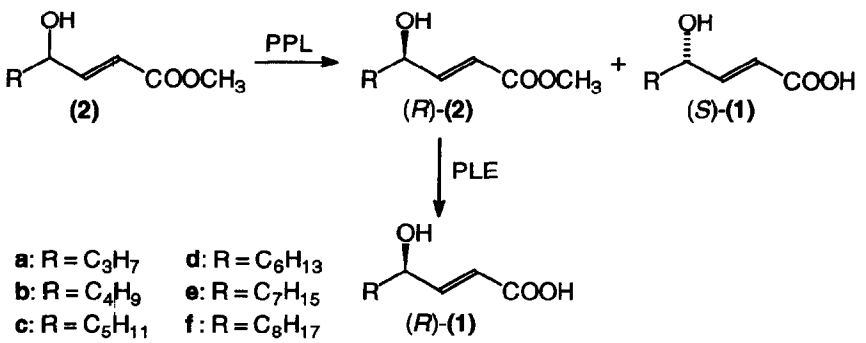
Previous literature has shown that the corresponding racemic (*E*)-4-hydroxy-2-unsaturated esters are versatile synthons in synthesis[4] and are easily prepared in good yields *via* β–keto sulfoxide alkylation[5] or by reacting 2-unsubstituted aldehydes with sulphinyl acetate esters in the presence of piperidine,[6] in a process referred[7] as "SPAC" (Sulfoxide Piperidine and Carbonyl) reaction. They can be obtained also in highly optically active form by lipase mediated irreversible acylation of their 4-hydroxy group[7] or by a combination of asymmetric SPAC reaction and a similar biocatalytic resolution.[4,8-10]

However, in spite of the easy preparation of both (*R*)- and (*S*)-enantiomers of (*E*)-4-hydroxy-2-unsaturated esters, their chemical hydrolysis to the corresponding acids could not be realised by using carbonates or bicarbonates of alkali metals[11] but only by strong basic solutions,[12] in conditions which could cause their racemization or isomerization.[13] Use of other reagents such as $AlCl_3$-alkyl sulphides,[14] sodium benzenselenoate,[15] and iodotrimethylsilane,[16] was restricted by side reactions such as Michael addition of the nucleophile to the α,β-unsaturated system[17] or iodination of the 4-hydroxy group.[16]

In this paper we report the results obtained by performing the biocatalytic resolution of a number of (*E*)-4-hydroxy-2-unsaturated esters by lipase from porcine pancreas (PPL), an enzyme able to realise an enantioselective hydrolysis of the ester function.[18] This enzyme was found to be the best choice after a preliminary screening performed on methyl (*E*)-4-hydroxynon-2-enoate.[19]

The enantioselective hydrolysis of racemic esters **2a-f** with PPL was accomplished in a mixture of 0.1 M phosphate buffer (pH 7) and acetone (2:1, v:v). The use of other cosolvents (iPr$_2$O, tBuOH and DMSO) or of a minor ratio of acetone resulted in a lower enantiospecificity.

Table. Lipase Mediated Hydrolytic Resolution of (E)-4-Hydroxyalk-2-enoic Acids Methyl Esters



			(*R*)-2		(*S*)-1		(*R*)-1	
R	**conv[a] %**	**(time days)**	**ee %[b]**	**yield %[c]**	**ee %[d]**	**yield %[c]**	**ee %[d]**	**(time h)[e]**
a	47	(6)	75	47	84	39	75	(0.5)
b	48	(6)	77	46	82	40	77	(0.5)
c	56	(3)	95	40	74	50	94	(2)
d	53	(2)	93	43	82	48	93	(3)
e	50	(1)	85	45	85	43	85	(5)
f	52	(0.3)	88	45	83	48	87	(8)

[a]Determined by HPLC. All the reactions were performed on a 1-1.5 mmol scale using equal masses of substrates and PPL[18] at 0.1 M concentration of substrates in pH 7, 0.1 M phosphate buffer containing acetone (2:1; v:v), at 25 °C. The pH was kept constant by periodic addition of a 0.1 M NaOH solution. Enantiomeric ratio (E) was always > 20. [b]Enantiomeric excesses were determined by ^{1}H-NMR analysis of the Mosher's esters. [c]Isolated yields after flash chromatography. [d]Enantiomeric excesses were determined by ^{1}H-NMR analysis of the Mosher's esters after treatment of **1a-f** with CH_2N_2. [e]Referred to the hydrolysis of (*R*)-**2a-f** performed on a 0.5-0.7 mmol scale, using equal mmol equivalent of PLE[21] in pH 8 phosphate buffer (0.1 M, 10 ml), at 25 °C, keeping the pH constant by periodic addition of a 0.1 M NaOH solution. Yields were quantitative from the (*R*)-**2a-f**.

The results reported in Table show that, independent of the length of the alkyl substituent bonded at the hydroxymethinic group, the enantiomeric ratio[20] of the kinetic resolution is good (E > 20) and occurs with the same sense of enantioselection, the (*S*)-enantiomers being hydrolysed in preference. The length of the alkyl chain influences only the rate of the hydrolysis, the more polar substrates being hydrolysed more slowly.

Enantiomer (*R*)-(*E*)-hydroxyacids **1a-f**, in turn, were obtained by hydrolysis of the unreacted (*R*)-esters **2a-f** by means of porcine liver esterase (PLE)[21] which, in preliminary screening experiments, had exhibited a high hydrolysis rate, associated to a low enantiospecificity. The hydrolysis occurred in a short time and afforded the (*R*)-hydroxyacids quantitatively, with the same enantiomeric excess observed for the starting methyl esters.

The enantiomeric excess of the unreacted esters (*R*)-**2a-f** was determined by ^{1}H-NMR analysis of their Mosher's esters[22] [(*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetates; (*R*)-MTPA esters]; that of the (*R*)- and (*S*)-acids **1a-f** was determined in the same way, after esterification with diazomethane.

The absolute configuration of all obtained (*R*)- and (*S*)-acids **1a-f** was determined, after treatment with diazomethane, by analysis of the ^{1}H-NMR data of their (*R*)- and (*S*)-MTPA esters, according to the Mosher's modified method.[23] Diagnostic for the assignment in (*S*)-enantiomers were the chemical shifts of the protons at position 2, 3 and 5. In fact, the chemical shifts of the protons at 2 and 3 of the (*R*)-MTPA esters appear significatively shielded with respect to those of the (*S*)-MTPA diastereomer; the chemical shifts of the protons at position 5 appear, on the contrary, deshielded in (*R*)-MTPA esters relative to (*S*)-MTPA ones. Specular results were obtained for the acids (*R*)-**1a-f**.[24]

The stereochemical assignments for the acids **1a-c** were also confirmed by comparison of the optical rotation of their methyl esters with those reported for known compounds.[4,8]

In conclusion, the ready access to racemic (*E*)-4-hydroxy-2-unsaturated esters, by SPAC reaction[4-6] coupled to the ability of PPL to catalyse their asymmetric hydrolysis, affords a profitable method for obtaining some unreported (*E*)-4-hydroxy-2-unsaturated acids of biological interest with good optical purity.

In addition, since in our experiments the unreacted esters were the (*R*)-isomers, the result complements the reported efficient enzymatic resolution of SPAC reaction products in which the (*S*)-esters are directly obtained.[4,25]

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13. Attempted saponification of the hydroxyesters by means of Na_2CO_3 at reflux caused the isomerization of the hydroxyacids to the corresponding β-ketoacid as observed for the Z-isomer by means of NaOH, see ref. 12.
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18. Lipase from porcine pancreas was purchased from Sigma [type II, crude (Steapsina) cat. no. L 3126].
19. Screening experiments were conducted with commercially available enzymes, all showing low enantioselectively and/or hydrolysis rate: porcine liver esterase (Sigma,cat. no. E 3128); lipase from *Candida cylindracea* (type VII, Sigma, Cat. no. L 1754), Subtilopeptidase A (Sigma, cat. no. P 5380), α-chymotripsin (type II from Bovine Pancreas, Sigma, cat. no. C 4129); acylase I (Aldrich, cat. no. 37,302-8).
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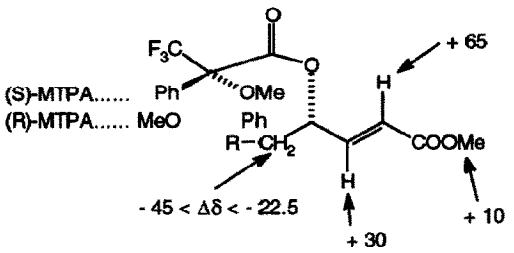
24. All new compounds gave satisfactory elemental analyses and were fully characterised by ^{1}H-NMR, IR and chemical transformations. The assignments of the absolute configuration of 4-hydroxyacids (*S*)-**1a-f** was based on the ^{1}H-NMR Δδ values obtained for their (*S*)- and (*R*)-MTPA esters, after esterification with CH_2N_2. Δδ values (δ_S - δ_R) are expressed in hertz (500 MHz):



The obtained compounds (*S*)-**1a-f**, with the ee reported in Table, showed the following $[\alpha]_D^{23}$ values for $CHCl_3$ 1% solutions: + 22.8 for (*S*)-**1a**; + 22.5 for (*S*)-**1b**; + 22.0 for (*S*)-**1c**; + 19.6 for (*S*)-**1d**; + 18.2 for (*S*)-**1e**; + 19.2 for (*S*)-**1f**. (*R*)-Enantiomers showed $[\alpha]_D^{23}$ values, in the same sequence: -20.3; -21.1; -27.9; -22.2; -18.2; -20.1 The esters (*R*)-**2a-f** showed $[\alpha]_D^{23}$: -16.5 for (*R*)-**2a**; -18.3 for (*R*)-**2b**; -22.8 for (*R*)-**2c**; -20.2 for (*R*)-**2d**; -20.3 for (*R*)-**2e**; -18.0 for (*R*)-**2f**. ^{1}H-NMR ($CDCl_3$, 500 MHz) diagnostic signal for acids **1a-f** were at: δ 7.03 (1 H, dd, J 15.9 and 5.0 Hz, H- 3), 6.03 (1 H, dd, J 15.9 and 2.0 Hz, H-2), 4.33 (1 H, ddt, J 6.5, 5.0 and 2.0 Hz, H-4).

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