Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1129–1133

TETRAHEDRON: *ASYMMETRY*

PLE and HLE catalyzed reverse enantiomeric separation of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-1-carboxylate derivatives

Cihangir Tanyeli,[a,*] Bengu Sezen,[a] Ayhan S. Demir,[a] Rosemeire Brondi Alves [b] and Simeon Arseniyadis [b]

[a] *Department of Chemistry, Middle East Technical University, 06531 Ankara, Turkey*
[b] *Institut de Chimie des Substances Naturelles, CNRS, F-91198 Gif-sur-Yvette, France*

Received 12 February 1999; accepted 4 March 1999

Abstract

We describe the diversities of hydrolase-type enzymes PLE and HLE on the hydrolysis of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-1-carboxylate and (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate to afford both enantiomers with 92–96% ee.

1. Introduction

2,5-Cyclohexadiene-1-carboxylate derivatives are appealing synthons for the asymmetric synthesis of natural products. Among this class of substances, 1-methyl-2,5-cyclohexadiene-1-carboxyaldehyde, specifically, is used as a starting material in the synthesis of taxoids, the promising anticancer drugs.[1–3]

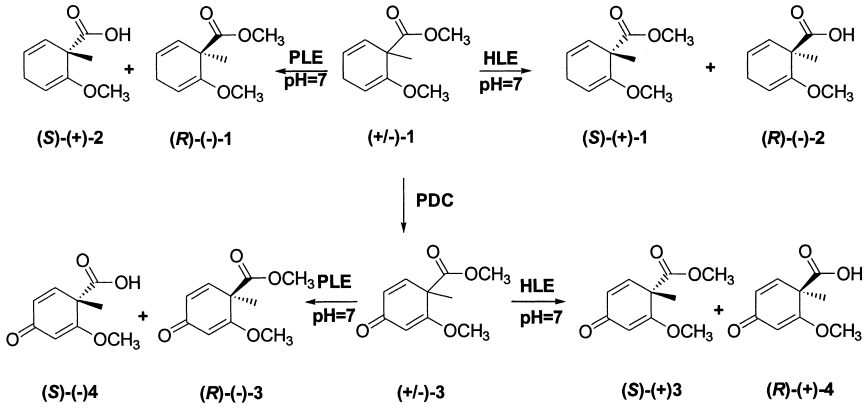
Recently, we have reported on the esterase catalyzed enantiomeric separation of (±)-α′-acetoxy-α,β-unsaturated cyclic ketones,[4] α-acetoxy aryl alkyl ketones[5] and 2-furyl carbinols.[6] During the course of our studies on the biotransformations of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-1-carboxylate (±)-**1** and (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate (±)-**3**, the screening reactions were first examined with various lipases (i.e. CCL, PLE, HLE, PPL and CAL) by the substrate:enzyme ratio which varied from 1:1 to 1:0.5. Among the lipases studied, PLE and HLE proved suitable for the enantioselective hydrolysis of these substrates showing an interesting enzyme-depending reversal of enantioselectivity. The observed promising preliminary results directed us towards catalytic studies on this subject. Thus, PLE catalyzed reactions afforded (*S*)-configured carboxylic acids.

* Corresponding author. E-mail: cihangir.tanyeli@oc.rwth-aachen.de

0957-4166/99/$ - see front matter
PII: S0957-4166(99)00080-4

In contrast to this, HLE yielded (*R*)-configured carboxylic acids. Both enzymes exhibited high ee values and manifested unusual versatility and diversity of the enantioselective hydrolysis of these substrates.

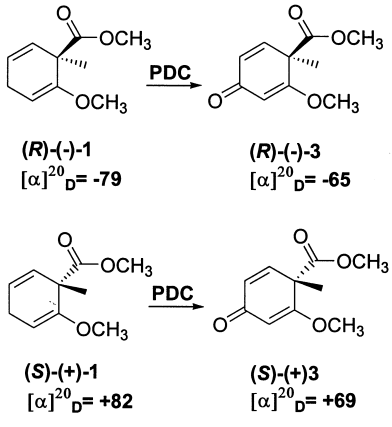
This report describes the highly efficient diverse enantioselective resolution of the racemic substrates (±)-**1** and (±)-**3** with PLE and HLE to afford (*R*)-(−)-**1** and (*R*)-(−)-**3** esters with the former, while (*S*)-(+)-**1**, and (*S*)-(+)-**3** esters were obtained with the latter enzyme (Scheme 1).



Scheme 1.

2. Results and discussions

Racemic **1** was obtained using slightly modified literature procedures.[3,7] The first bioconversion was performed by PLE according to the following general procedure. To a stirred solution of 500 mg (±)-**1** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE was added in one portion and the reaction mixture was stirred at 15°C in a pH stat unit. The conversion was monitored by TLC. After 43 h, 45% conversion was observed. The products were separated using flash column chromatography and compound (−)-**1** was isolated in 36% yield and in 93% ee. Absolute configuration of compound (−)-**1** was assigned as (*R*) by transforming it into the corresponding (*R*)-(−)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate via oxidation with pyridinium dichromate (PDC)[8] (Scheme 2).



Scheme 2.

Table 1
Results of the PLE and HLE catalyzed hydrolysis of (±)-**1** and (±)-**3**

Substrate	Enzyme	Time (h)	Esters[b]	Yield (%)	$[\alpha]^{20}_D$	ee (%)[a]	Acids	Yield (%)	$[\alpha]^{20}_D$	E[c]
(±)-1	PLE	43	(*R*)-(-)-1	36	-79	93	(*S*)-(+)-2	32	+24	42
(±)-1	HLE	48	(*S*)-(+)-1	42	+82	96	(*R*)-(-)-2	34	-20	80
(±)-3	PLE	52	(*R*)-(-)-3	36	-67	94	(*S*)-(-)-4	30	-12	96
(±)-3	HLE	40	(*S*)-(+)-3	38	+65	92	(*R*)-(+)-4	34	+12	65

a) Enantiomeric excess values are determined by the Chiralcel OC chiral column HPLC analysis. b) The absolute configurations are determined by the comparison with the α values of the esters in ref. 8. c) E, enantiomeric ratio, values are calculated according to ref.9.

The second attempt was done on the same substrate using HLE under the same conditions as above, in which 10 mg of enzyme was used. When the hydrolysis of the above substrate catalyzed by HLE (10 mg) was allowed to proceed to 47% conversion (48 h at 15°C), the unreacted ester of (*R*) absolute configuration, (+)-**1**, was obtained in 42% yield and 96% ee. Absolute configuration of the isolated product (+)-**1** was determined by transforming it into the corresponding (*S*)-(+)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate with PDC to correlate with the result given above (Scheme 2).

Related to this study, hydrolyses of (±)-**3** with PLE and HLE under the same reaction conditions were examined. Absolute configurations of the products were assessed by comparison of their specific rotations with the literature data.[8] The results proved again that PLE and HLE have different enantioselectivities on the substrate. The results are given in Table 1 and show that the enantiomeric excess (ee) varied from 92% to 96%.

In conclusion, an enzyme-dependent reversal of enantioselectivity is demonstrated. Commercially available and inexpensive enzymes, PLE and HLE, have shown specificity for the (*S*)- and (*R*)-enantiomer, respectively, of the two esters tested thus providing access to optically active esters of high enantiomeric purity and with opposite configurations. The availability and very low cost of the lipases, used in catalytic levels, renders the process very attractive for large-scale preparations.

3. Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometers. Chemical shifts are given in ppm downfield from tetramethylsilane. IR spectra were obtained from a Perkin–Elmer Model 1600 series FT-IR spectrometer and are reported in cm^{-1}. Optical rotations were measured in $CHCl_3$ solution in a 1 dm cell using a Bellingham & Stanley P20 polarimeter at 20°C. Elemental analyses were performed on a LECO 932. PLE and HLE were purchased from Boehringer Manheim GmbH as a suspension in ammonium sulfate solution (3.2 mol/L) and as liver acetonic powder, respectively.

*3.1. PLE hydrolysis of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-1-carboxylate (±)-**1***

To a stirred solution of 500 mg (±)-**1** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE was added in one portion and the reaction mixture was stirred at 15°C (TLC monitoring). The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The crude

product was purified by flash column chromatography (EtOAc:hexane, 1:3 as eluent) to yield (−)-**1** (0.18 g, 36% yield). $[\alpha]_D^{20}$=−79 (*c*, 0.52). ^{1}H NMR: δ 1.58 (s, 3H, CH_3), 2.68 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, $COOCH_3$), 5.68 (s, 1H, CH), 6.28 (d, 1H, CH, J=10 Hz), 6.53 (d, 1H, CH, J=10 Hz). ^{13}C NMR: 22.3, 30.1, 40.5, 53.4, 56.5, 102.9, 128.8, 144.8, 171.2, 175.5. IR (neat): 2954, 1700 cm^{-1}. Anal. calcd for $C_{10}H_{14}O_3$ (182.22): C, 65.91; H, 7.74. Found: C, 65.97; H, 7.76.

The acidified water layer was extracted with ethyl acetate to isolate (+)-**2** (0.15 g, 32% yield). $[\alpha]_D^{20}$=+24 (*c*, 0.30). ^{1}H NMR: δ 1.55 (s, 3H, CH_3), 2.75 (s, 2H, CH_2), 3.65 (s, 3H, OCH_3), 5.65 (s, 1H, CH), 6.25 (d, 1H, CH, J=10 Hz), 6.53 (d, 1H, CH, J=10 Hz), 10.25 (s, 1H, OH). IR (neat): 3453, 2977, 1704 cm^{-1}.

*3.2. HLE hydrolysis of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-1-carboxylate (±)-**1***

To a stirred solution of 500 mg (±)-**1** in 50 mL pH 7.00 phosphate buffer, 10 mg HLE was added in one portion and the reaction mixture was stirred at 15°C (TLC monitoring). The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc:hexane, 1:3) to yield (+)-**1** (0.21 g, 42% yield). $[\alpha]_D^{20}$=+82 (*c*, 0.57).

The acidified water layer was extracted with ethyl acetate to give (−)-**2** (0.15 g, 34% yield). $[\alpha]_D^{20}$=−20 (*c*, 0.30).

*3.3. (±)-Methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate (±)-**3***

Compound (±)-**1** (2.0 g, 11 mmol) and pyridinium dichromate (PDC) (12.0 g, 32 mmol) in 100 mL $CHCl_3$ was refluxed for 24 h with continuous removal of water using a Dean–Stark trap. The reaction mixture was extracted with 1 N HCl, H_2O and brine. The organic phase was dried and purified by flash column chromatography (EtOAc:hexane, 1:3) (1.98 g, 92% yield). ^{1}H NMR: δ 1.58 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.75 (s, 3H, $COOCH_3$), 5.75 (s, 1H, CH), 6.25 (d, 1H, CH, J=10 Hz), 6.52 (d, 1H, CH, J=10 Hz). ^{13}C NMR: 22.3, 30.1, 50.3, 56.4, 126.2, 129.1, 144.4, 170.4, 173.4, 207.7. IR (neat): 2953, 1702, 1667 cm^{-1}. Anal. calcd for $C_{10}H_{12}O_4$ (196.20): C, 61.22; H, 6.16. Found: C, 61.25; H, 6.19.

*3.4. PLE hydrolysis of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate (±)-**3***

To a stirred solution of 500 mg (±)-**3** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE was added in one portion and the reaction mixture was stirred at 15°C (TLC monitoring). The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc:hexane, 1:3) to yield (−)-**3** (0.18 g, 36% yield). $[\alpha]_D^{20}$=−67 (*c*, 0.45)

The acidified water layer was extracted with ethyl acetate to give (−)-**4** (0.14 g, 30% yield). $[\alpha]_D^{20}$=−12 (*c*, 0.30). ^{1}H NMR: δ 1.60 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.67 (s, 1H, CH), 6.26 (d, 1H, CH, J=9 Hz), 6.54 (d, 1H, CH, J=9 Hz), 10.22 (s, 1H, OH). IR (neat): 3419, 2999, 1707, 1655 cm^{-1}.

*3.5. HLE hydrolysis of (±)-methyl-2-methoxy-1-methyl-2,5-cyclohexadiene-4-one-1-carboxylate (±)-**3***

To a stirred solution of 500 mg (±)-**3** in 50 mL pH 7.00 phosphate buffer, 10 mg HLE was added in one portion and the reaction mixture was stirred at 15°C (TLC monitoring). The reaction mixture was

extracted with ethyl acetate, dried over $MgSO_4$ and the product (+)-**3** was separated by flash column chromatography (EtOAc:hexane, 1:3) (0.19 g, 38% yield). $[\alpha]_D^{20}$=+65 (*c*, 0.48).

The acidified water layer was extracted with ethyl acetate to afford (+)-**4** (0.16 g, 34% yield). $[\alpha]_D^{20}$=+12 (*c*, 0.30).

Acknowledgements

We thank the Alexander von Humboldt Foundation, the Middle East Technical University for a grant (AFP-1997) and the Turkish Scientific and Technical Research Council for a grant (TBAG-1461).

References

1. Arseniyadis, S.; Yashunsky, D. V.; Freitas, R. P.; Dorado, M. M.; Potier, P. *Tetrahedron* **1996**, *52*, 12443–12458.
2. Arseniyadis, S.; Alves, R. B.; Freitas, R. P.; Dorado, M. M.; Yashunsky, D. V.; Potier, P. *Heterocycles* **1997**, *46*, 727–764.
3. Arseniyadis, S.; Yashunsky, D. V.; Dorado, M. M.; Alves, R. B.; Wang, Q.; Potier, P.; Toupet, L. *Tetrahedron* **1996**, *52*, 6215–6232.
4. Tanyeli, C.; Demir, A. S.; Dikici, E. *Tetrahedron: Asymmetry* **1996**, *7*, 2399–2402.
5. Demir, A. S.; Hamamci, H.; Tanyeli, C.; Akhmedov, I. M.; Doganel, F. *Tetrahedron: Asymmetry* **1998**, *9*, 1673–1677.
6. Tanyeli, C.; Demir, A. S.; Arkin, A. H.; Akhmedov, I. M. *Enantiomer* **1997**, *2*, 433–439.
7. Schultz, A. G.; Lavieri, F. P.; Snead, T. E. *J. Org. Chem*. **1985**, *50*, 3086–3091.
8. Schultz, A. G.; Lavieri, F. P.; Macielag, M.; Plummer, M. *J. Am. Chem. Soc*. **1987**, *109*, 3991–4000.
9. Chen, C. S.; Fujimoto, Y.; Girdakus, G.; Sih, C. J. *J. Am. Chem. Soc*. **1982**, *104*, 7294–7299.