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# Hydrolysis of allylglycidyl ether by marine fungus *Trichoderma* sp. Gc1 and the enzymatic resolution of allylchlorohydrin by *Candida antarctica* lipase type B

**Mariana P. Martins, Ana M. Mouad, and André L. M. Porto***

Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, CEP 13560-970, CP 780, São Carlos-SP, Brazil

## ABSTRACT

Enzymatic hydrolysis of (±)-2-((allyloxy)methyl) oxirane (**1**) using whole cells of the marine fungus *Trichoderma* sp. Gc1 that had been grown in artificial seawater produced (*S*)-(+)-2-(allyloxy-methyl)oxirane (**1**) (34% *ee*) in 23% yield, together with (*R*)-(-)-3-(allyloxy)propane-1,2-diol (**2**) in 60% yield (10% *ee*). The fungal hydrolases exhibited selectivity with preference for oxirane (*R*)-**1,** while the concomitant formation of (*R*)-diol **2** indicated that the mechanism involved retention of configuration. The esterification of (±)-1-(allyloxy)-3-chloropropan-2-ol (**4**) by lipase type B from *Candida antarctica* resulted in (*R*)-(+)-**4** (72% *ee*) and 45% yield, and (*S*)-(+)-1-(allyloxy)-3-chloropropan-2-yl acetate (**5**) (77% *ee*) and 41% yield. The enzyme showed acceptable selectivity with an enantiomeric ratio (*E*) of 16 for the resolution of the chlorohydrin *rac*-**4**.

**KEYWORDS:** marine fungus, oxirane, chlorohydrin, lipase, *Trichoderma* sp.

## INTRODUCTION

Chiral glycidyl ethers are valuable substrates in the asymmetric synthesis of a variety of bioactive compounds [1-4]. For example, the synthesis of (*R*)-bicalutamide, a potent non-steroidal anti-androgen that has been used in the treatment of prostate cancer, was prepared from enantiopure (*R*)-2-(benzyloxy)propane-1,2-diol as start material [1]. In this context, the use of enzymes to produce enantiopure compounds has become an important strategy [5, 6], and a number of biocatalytic studies using published enzymatic methodologies have been carried out for aryl glycidyl ether derivatives. Thus, whole cells of *Rhodococcus ruber* catalyzed the resolution of (±)-2-methyl-glycidyl benzyl ether with an enantioselectivity (expressed as the enantiomeric ratio *E*) of more than 200 [7], while the hydrolysis of (±)-glycidyl ether catalyzed by *Bacillus megaterium* produced (*S*)-enantiomer glycidyl ether and (*R*)-diol with an *E* value of 47.8 [8]. Additionally, *Trichosporum loubierii* catalyzed the formation of (*R*)-oxiranes and (*S*)-diols from aryl glycidyl ether derivatives with good enantioselectivity [9-10]. In contrast, the biocatalyzed hydrolytic kinetic resolution of benzyl glycidyl ether proceeded with low selectivity ($E<10$), [11].

Chlorohydrins are important intermediates for the synthesis of a range of biologically active natural and synthetic products. Synthesis of chlorohydrins includes cleavage of epoxide with enzymes, as epoxide hydrolase from microorganisms. Highly efficient methodologies for the enantioselective preparation of aryl chlorohydrins by chemoenzymatic reactions have been described in the literature [2, 3, 12-17]. It has been shown, for example, that

*Corresponding author
almporto@iqsc.usp.br

*Candida antarctica* type B lipase (CALB) is able to catalyze the kinetic resolution of racemic secondary alcohols with vinyl acetate as acylating agent to yield enantiopure compounds [18-20]. Recently, we have used Brazilian marine fungi *Aspergillus sydowii* and *Trichoderma* sp. to catalyze the hydrolysis of benzyl glycidyl ether [21].

In the present study, the application of whole cells of the marine fungus *Trichoderma* sp. Gc1 in the enzymatic hydrolysis of a (±)-allyl glycidyl ether, namely, 2-((allyloxy)methyl)oxirane (**1**), is addressed. Additionally, the chemoenzymatic resolution of (±)-1-(allyloxy)-3-chloropropan-2-ol (**4**) by CALB is described for the first time. The objective of these studies was attained by biocatalytic methods, using whole cells of marine fungus *Trichoderma* sp. Gc5 and lipase CALB, yielding enantiomerically enriched compounds.

## MATERIALS AND METHODS

### Preparation of diol **2**, diacetate **3** and chlorohydrin acetate **5**

Racemic 3-(allyloxy)propane-1,2-diol (**2**) was synthesized by hydrolysis of (±)-2-((allyloxy) methyl)oxirane (**1**; Sigma-Aldrich) in acidic medium. 3-(Allyloxy)propane -1,2-diyl diacetate (**3**) and 1-(allyloxy)-3-chloropropan-2-yl acetate (**5**) were synthesized by classical methods using acetic anhydride and pyridine, (Scheme 1) [21].

3-(Allyloxy)propane-1,2-diol (**2**):
Yield: 68% (160 mg, 1.20 mmol); yellow oil; $^1$H NMR (200 MHz) $\delta$ (ppm): 2.20 (2H, sl), 3.39-4.05 (7H, m), 5.17-5.33 (2H, m), 5.81-6.00 (1H, m); $^{13}$C NMR (50 MHz) $\delta$ (ppm): 134.2, 117.6, 72.5, 71.8, 70.6, 64.1; IR (film) $\nu$ (cm$^{-1}$): 3390, 2900, 2350, 1730, 1650, 1415, 1070; MS [ESI$^+$] *m/z* (relative intensity): 132 (absent), 101 (16), 83 (34), 75 (37), 71 (63), 61 (100), 58 (89), 57 (84), 56 (28), 55 (35).

3-(Allyloxy)propane-1,2-diyl diacetate (**3**):
Yield: 95% (76 mg, 0.35 mmol); yellow oil; $^1$H NMR ($CDCl_3$, 200 MHz,) $\delta$ (ppm): 2.09 (3H, s), 2.10 (3H, s), 3.40-3.60 (2H, m), 3.90-4,40 (5H, m), 5.10-5.40 (2H, m), 5.70-5.90 (1H, m); $^{13}$C NMR (50 MHz) $\delta$ (ppm):170.7, 170.5, 134.2, 117.5, 72.3, 70.3, 68.8, 62.9, 21.0, 20.8; IR (film) $\nu$ (cm$^{-1}$): 3085, 1743, 1635, 1373, 1230.

1-(Allyloxy)-3-chloropropan-2-yl acetate (**5**):
Yield: 96% (110 mg, 0.64 mmol); yellow oil; $^1$H NMR (200 MHz) $\delta$ (ppm): 2.11 (3H, s), 3.60-3.85 (4H, m), 4.20-3.90 (3H, m), 5.40-5.01 (2H, m), 6.00-5.90 (1H, m); $^{13}$H NMR (50 MHz) $\delta$ (ppm): 170.2, 134.1, 117.6, 72.4, 71.7, 68.0, 42.7, 21.0; IR (film) $\nu$ (cm$^{-1}$): 3082, 2866, 1745, 1647, 1431, 1373, 1234, 1101, 1051.

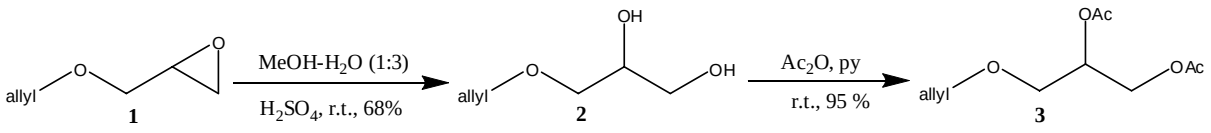
### Preparation of chlorohydrin **4**

Oxirane **1** (1.14 g, 10 mmol), dissolved in DMSO (2 mL), was added to a stirred solution of NaCl (15.2 g, 0.38 mol) in water (100 mL) and maintained at room temperature for 72 h with constant stirring. The reaction products were extracted with ethyl acetate (3 x 20 mL), the combined organic phases were dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (CC) over silica gel (230-400 mesh) using hexane/ethyl acetate (1:1) as eluent, and racemic chlorohydrin **4** was obtained in 90% yield.

1-(Allyloxy)-3-chloropropan-2-ol (**4**):
Yield: 90% (1.35 g, 9 mmol); yellow oil; $^1$H NMR (200 MHz) $\delta$ (ppm): 2.20 (1H, sl), 3.51-3.72 (4H, m), 4.01-4.14 (3H, m), 5.18-5.34 (2H, m), 5.60-5.80 (1H, m); $^{13}$C NMR (50 MHz) $\delta$ (ppm): 134.2, 117.7, 72.4, 70.6, 70.3, 46.0; IR (film) $\nu$ (cm$^{-1}$): 3390, 2900, 2350, 1730, 1650, 1415, 1070.

### Analytical methods

Column chromatographic separations of the products of synthesis and of enzymatic resolutions were




**Scheme 1.** Preparation of diol **2** and diacetate **3**.

monitored by TLC using aluminum-backed pre-coated silica gel 60 F254 layers (Sorbent) eluted with hexane/ethyl acetate and visualized by anysaldehyde. Reaction products derived from racemic substrates were analyzed on a Hewlett Packard model HP-5890 gas chromatograph equipped with flame ionization detector (FID) and a Varian CP-Chiralsil-DEX CB (β-cyclodextrin) chiral column (25 m x 0.25 mm i.d.; 0.39 μm). The GC conditions employed for the analysis of **1**-**3** were: oven temperature initially 60°C then increased to 180°C at 2°C/min; run time 60 min; injector temperature 200°C; detector temperature 200°C; injector split ratio 1: 20; hydrogen carrier gas at a pressure of 60 kPa. For the GC analysis of **4** and **5**, similar conditions were employed except that the oven temperature was initially 90°C then increased to 150°C at 1°C/min. The concentrations and enantiomeric excesses (*ee*) of analytes were determined from peak areas at retention times corresponding to: (*S*)-**1** (8.2 min) and (*R*)-**1** (8.4 min); (*R*)-**2** (30.1 min); (*S*)-**2** (30.1 min); (*R*)-**3** (37.4 min) and (*S*)-**3** (38.0 min); (*R*)-**4** (11.2 min) and (*S*)-**4** (11.4 min); and (*R*)-**5** (12.4 min) and (*S*)-**5** (13.7 min).

## Culture of *Trichoderma* sp. Gc1

The marine fungus *Trichoderma* sp. Gc1 was isolated from a specimen of the sponge *Geodia corticostylifera* that had been collected off the north coast of the State of São Paulo, Brazil, adjacent to the city of São Sebastião, by Professor Roberto G. S. Berlinck (Instituto de Química de São Carlos, Universidade de São Paulo). The fungal strain was identified at the Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, and a voucher specimen was deposited in the Coleção Brasileira de Microrganismos de Ambiente e Indústria (CBMAI) located in the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) at Universidade de Campinas (UNICAMP), [22].

All manipulations involving *Trichoderma* sp. Gc1 were carried out under sterile conditions in a Veco laminar flow cabinet. Artificial sea water (ASW) used for preparing the culture media contained (g/L): $CaCl_2.2H_2O$ (1.36), $MgCl_2.6H_2O$ (9.68), KCl (0.61), NaCl (30), $Na_2HPO_4$ (1.4 x $10^{-5}$), $Na_2SO_4$ (3.47), $NaHCO_3$ (0.17), KBr (0.1), $SrCl_2.6H_2O$ (0.04) and $H_3BO_3$ (0.03). Stock cultures of the fungal strain were maintained at 4°C (refrigerator) in Petri dishes containing solid medium comprising ASW supplemented with malt extract (30 g/L), soy peptone (3 g/L) and agar (15 g/L) and adjusted to pH 8 with 3 M KOH solution. Liquid cultures were prepared as required using the same medium but without agar.

## Hydrolysis of oxirane **1** catalyzed by *Trichoderma* sp. Gc1

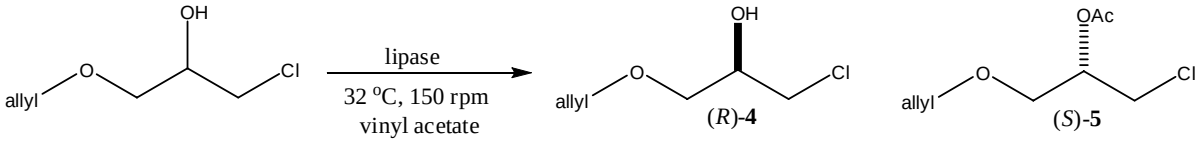
Small slices of solid medium (0.5 x 0.5 cm) bearing fungal mycelia were cut from the stock culture and inoculated into 1 L of liquid culture medium contained in a 2 L Erlenmeyer flask and incubated for 5 days at 32°C on a Technal TE-421 or Superohm G-25 orbital shaker (150 rpm). Fresh mycelia were harvested by Buchner filtration and 5.0 g (wet weight) portions were suspended in 100 mL aliquots of 0.1 M $Na_2HPO_4$/ $KH_2PO_4$ buffer (pH 7.0) contained in 250 mL Erlenmeyer flasks. Biocatalysed reactions were initiated by the addition of 100 μL of oxirane **1** (dissolved in 300 μL of DMSO) to the fungal suspensions, and the mixtures were incubated at 32°C on an orbital shaker (150 rpm). The reaction progress was monitored by collecting samples (0.1 mL) regularly every to 2 h and analyzed by CG-FID. Reaction progress was assessed after 24 h by filtering the fungal mycelia and extracting the aqueous phase with ethyl acetate (3 x 30 mL).

The organic phase was dried over $Na_2SO_4$, the solvent evaporated under vacuum, and the products purified by CC over silica gel (230-400 mesh) using hexane/ethyl acetate as eluent to yield (*S*)-oxirane **1** (23%) and (*R*)-diol **2** (60%).

## Reactions of chlorohydrin **4** catalyzed by CALB

Chlorohydrin **4** (40 mg, 0.26 mmol) was transferred to a 50 mL Erlenmeyer flask containing 5.0 mL of HPLC-grade hexane, 0.5 mL (5.4 mmol) of vinyl acetate and 40 mg of CALB (Novozym 435; Novo Nordisk, PR, Brazil) and the mixture was incubated at 32°C on an orbital shaker (150 rpm). In order to monitor the progress of the reaction, samples (0.1 mL) were collected at the times shown in Table 1 and aliquots (1 μL) were analyzed by GC/FID using a chiral capillary column. The chromatographic

**Table 1.** Chemoenzymatic esterification of chlorohydrin *rac*-**4** by lipase CALB.




| Entry | Time (h) | c(%)[c] **4** | *ee*[c,d] **4** (ac)[c] | c (%)[c] **5** | *ee*[c] **5** (ac)[c] | *E* |
|---|---|---|---|---|---|---|
| 1[a] | 1 | 95 | - | 5 | >99 | - |
| 2[a] | 5 | 80 | - | 20 | >99 | - |
| 3[a] | 10 | 56 | - | 44 | 86 | - |
| 4[a] | 18 | 55 | - | 45 | 85 | - |
| 5[a] | 24 | 53 | 72 (*R*) | 47 | 81 (*S*) | 20 |
| 6[b] | 24 | 52 | 72 (*R*) | 48 | 77 (*S*) | 16 |

[a]Reaction conditions: chlorohydrin **4** (40 mg, 0.26 mmol), 5.0 mL HPLC-grade hexane, 0.5 mL vinyl acetate, 40 mg CALB. The reaction mixture was incubated at 32°C on an orbital shaker (150 rpm).
[b]Reaction conditions: chlorohydrin **4** (400 mg, 2.6 mmol), 20 mL HPLC-grade hexane, 5.0 mL vinyl acetate, 400 mg CALB. The isolated yields were chlorohydrin **4** (45%) and chlorohydrin acetate **5** (41%).
[c]Symbols: c (%) - concentration; *ee* (%) - enantiomeric excess determined by GC; ac - absolute configuration; *E*: enantioselectivity expressed as the enantiomeric ratio [22].
[d]Enantiomeric excess determined after derivatization of (*R*)-chlorohydrin **4** to (*R*)-chlorohydrin acetate **5**.

profiles of the reaction products were compared with those of racemic standards that had been analyzed previously. The reaction was repeated on a larger scale by incubating 0.4 g (2.6 mmol) of *rac*-**4,** 20 mL of hexane, 5.0 mL of vinyl acetate and 400 mg of CALB, contained in a 100 mL Erlenmeyer flask, under the conditions indicated above for 24 h. The products were purified by CC over silica gel (230-400 mesh) using hexane/ethyl acetate as eluent to produce (*R*)-chlorohydrin **4** (45% yield) and (*S*)-chlorohydrin acetate **5** (41% yield).

### Hydrolysis of (*S*)-chlorohydrin acetate **5** to (*R*)-diol **2**

LiOH (40 mg, 1.66 mmol) was added to a stirred solution of (*S*)-(+)-chlorohydrin acetate (**5**; 96 mg, 0.5 mol) in ethanol 95% (0.5 mL) and maintained at room temperature for 24 h with constant stirring. The reaction mixture was then diluted with 5 mL of water, the ethanol evaporated under vacuum and the products was extracted with diethyl ether (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and the organic solvent evaporated under reduced pressure. The product was purified by CC over silica gel (230-400 mesh) using hexane/ethyl acetate as eluent to produce (*R*)-(-)-diol **2** in 84% yield (56 mg, 0.42 mmol) with $[\alpha]_D^{25}$ = -2.4° (c 0.5, $CHCl_3$). The absolute configuration of (*R*)-(-)-diol **2** was determined by comparison of optical rotation value described in the literature [24].

### Assignment of absolute configuration

The optical rotations of the products of the biocatalytic reactions were measured on a Perkin-Elmer model 241 polarimeter using a 1 dm cuvette and were referenced to the Na-D line. The absolute configurations of (*S*)-(+)-2-((allyloxy) methyl)oxirane (**1**) and (*R*)-(-)-3-(allyloxy)propane-1,2-diol (**2**) were established by comparing the specific signs of rotation determined for the products with those reported in the literature (Table 2) [23, 24]. The absolute configurations of (*R*)-(+)-1-(allyloxy)-3-chloropropan-2-ol (**4**) and (*S*)-(+)-1-(allyloxy)-3-chloropropan-2-yl acetate (**5**) were suggested through application of Kazlauskas' empirical rule [25] and, for the latter, verified on the basis of the specific optical rotation of the (*R*)-(-)-diol **2** prepared from the chlorohydrin acetate.

## RESULTS AND DISCUSSION

Enzymatic hydrolysis of *rac*-**1** in spent ASW culture medium, from which the mycelia of

**Table 2.** Specific optical rotations and absolute configurations of oxirane **1**, diol **2**, chlorohydrin **4** and chlorohydrin acetate **5**.

| Reaction products | Specific optical rotation $[\alpha]_D^{25}$ | |
|---|---|---|
| | Experimental value | Literature value |
| (*S*)-(+)-2-((Allyloxy)methyl)oxirane (**1**) | +22.1° (*c* 2.0, $CHCl_3$) [34% *ee*] | +9.6° (*c* 0.94, EtOH) [26] |
| (*R*)-(-)-2-((Allyloxy)methyl)oxirane (**1**) | | -10.19° (*c* 2.59, EtOH) [26] |
| (*R*)-(-)-3-(Allyloxy)propane-1,2-diol (**2**) | -8.82° (*c* 2.0, $CHCl_3$) | -1.576° (*c* 1.60, $CHCl_3$)[a] [24] |
| (*S*)-(-)-3-(Allyloxy)propane-1,2-diol (**2**) | | -1.887° (*c* 1.70, $CHCl_3$)[b] [24] |
| (*R*)-(+)-1-(Allyloxy)-3-chloropropan-2-ol (**4**) | +2.3° (*c* 0.5, $CHCl_3$) [72% *ee*] | |
| (*S*)-(+)-1-(Allyloxy)-3-chloropropan-2-yl acetate (**5**) | +0.84° (*c* 0.5, $CHCl_3$) [77% *ee*] | |
| (*R*)-(-)-1-(Allyloxy)propane-1,2-diol (**2**)[c] | -2.4° (*c* 0.5, $CHCl_3$) | |

[a]Value shown is $[\alpha]_D^{28}$
[b]Value shown is $[\alpha]_D^{26}$
[c]Produced synthetically from **5**.

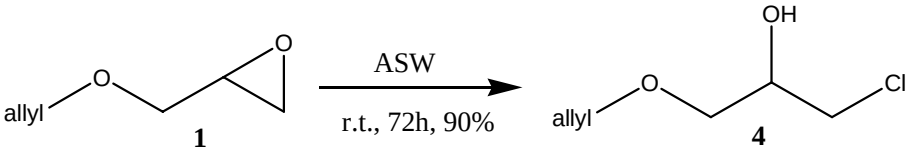
*Trichoderma* sp. Gc1 had been removed by filtration, produced racemic chlorohydrin **4** in quantitative yield. Additionally, incubation of *rac*-**1** in fresh ASW produced chlorohydrin **4** in 90% yield (Scheme 2). Since ASW, which contains a high concentration of chloride ions (1.20 M), induced spontaneous ring opening of oxirane **1**, it was not possible to evaluate the enzymatic resolution of this substrate using filtered ASW culture medium in which *Trichoderma* sp. Gc1 had been grown.

In order to evaluate the presence of the hydrolytic enzymes present in ASW-grown *Trichoderma* sp. Gc1 mycelia, biocatalytic reactions were carried out with resting fungal cells. For this purpose, mycelia were filtered from the spent medium, suspended in 0.1 M phosphate buffer solution (pH 7), and substrate was subsequently added. Enzymatic hydrolysis produced (*S*)-(+)-2-(allyloxymethyl)oxirane (**1**) (34% *ee*) in 23% yield together with (*R*)-(-)-3-(allyloxy)propane-1,2-diol (**2**) in 60% yield after purification by CC over silica gel (Scheme 3). The purified diol **2** was derivatized with acetic anhydride and pyridine, and the diacetate **3** so produced showed low optical purity (10% *ee*) following GC/FID analysis using a chiral column.

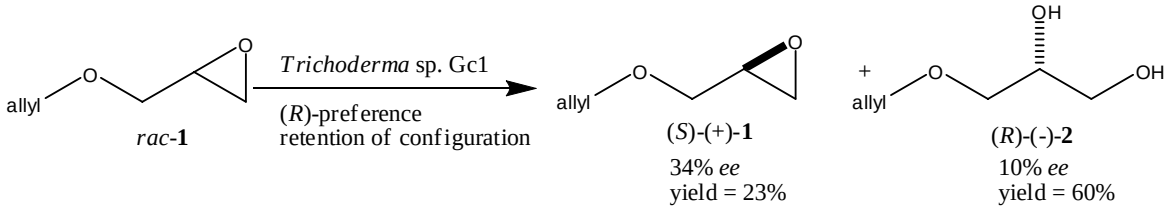
It is concluded, therefore, that hydrolases from *Trichoderma* sp. Gc1 exhibit selectivity with preference for (*R*)-**1** and, since diol (*R*)-**2** was concomitantly formed, the mechanism appeared to involve retention of configuration. Whole cells of the fungal strain exhibited a poor enantioselective performance in the hydrolysis of glycidyl oxirane **1**, a finding similar to that previously reported for the hydrolysis of **1** by whole microbial cells [26]. It would seem that enantioselective enzymatic hydrolysis of oxiranes in aqueous solution is difficult because spontaneous hydrolysis frequently occurs.

By virtue of the ease of formation of **4**, a study of the lipase-catalyzed esterification of racemic chlorohydrin with vinyl acetate as acylating agent was carried out since this type of methodology has proven to be effective in synthesizing chiral alcohols with high enantioselectivity [18-20]. Using type B lipase from *Candida antarctica* as catalyst, (*R*)-chlorohydrin **4** and (*S*)-chlorohydrin acetate **5** were obtained in reasonable optical purities and the enzyme showed acceptable selectivity ($E$ = 16) for the resolution of chlorohydrin *rac*-**4** (Table 1).

The results obtained were in accord with Kazlauskas' empirical rule, which predicts that $E$ values should be high for *sec*-alcohols when groups linked at the stereocenter differ significantly in size [25]. Interestingly, $E$ values




**Scheme 2.** Spontaneous opening of oxirane **1** in artificial sea water.




**Scheme 3.** Enzymatic resolution of oxirane *rac*-**1** by whole cells of *Trichoderma* sp.

obtained using CALB are good for large ($CH_2CHCH_2OCH_2$-) and medium ($ClCH_2$-) groups, but smaller groups, i.e. $CH_3$-, typically promote high enantioselectivity ($E$ >30) probably due to the difference in size of the substituents at the stereocenter resulting in perfect isomer interactions with an active lipase site [5]. The influence of substituents on enantiomeric ratio in transesterification of halohydrin derivatives using CALB was studied. Enantiomeric ratio ($E$) depended on the hydroxyl protecting groups in 1-position and the halogens in 3-positions, and some substrates the $E$ was dependent on the acylating group. In general, the resolution of the chlorohydrins by CALB showed moderate enantiomeric ratio [27].

Basic hydrolysis of product **5** from lipase-catalyzed reaction, using LiOH in ethanol produced (*R*)-(-)-diol **2**. The measured specific optical rotation of (*R*)-(-)-diol **2** was compared with the published value [24]. In addition, this result confirmed the absolute configuration of the (*S*)-(+)-chlorohydrin acetate **5** previously suggested by Kazlauskas' empirical rule. A number of enzymatic resolutions of racemic chlorohydrins by lipases have been reported to provide enantiopure compounds in agreement with Kazlauskas' empirical rule [1, 12].

In the literature, the absolute stereochemistry of various compounds was predicted by their interactions with chiral entities, e.g., a lipase or a chiral column [28]. On the basis of these studies, is possible predict the absolute configuration to structurally related and analogs compounds. In study investigated by us, the lipase CALB showed enantiopreference for enzymatic resolution of *rac*-**5** and faster-reacted with (*S*)-enantiomer and producing (*S*)-**5** acetate. In addition, the elution order of enantiomers *rac*-**5** on CP-chirasil-Dex CB column were in accordance with the studies describes by Hoff, the (*R*)-acetate first eluted [28]. In conclusion, the known stereopreference of CALB on ezymatic resolution of secondary alcohols and the elution order of *rac*-**5** on CP-chirasil-Dex CB permitted assign the absolute stereochemistry of (*S*)-**5**.

Preparation of enantiopure synthons with low selectivities from epoxide hidrolases of native microorganisms is a serious problem. To obtain an increase of enantioselectivity and activity of chemical compounds has applied the protein engineering with epoxide hydrolase from *Aspergillus niger*. This study showed that amino acid exchange at protein enhanced the activity of epoxide hydrolase from *A. niger* overexpression in *Escherichia coli* [29].

## CONCLUSIONS

Whole cells of the marine fungus *Trichoderma* sp. Gc1 that had been grown in medium containing artificial sea water were able to catalyze the enzymatic hydrolysis of oxirane **1**. The fungal

hydrolases exhibited selectivity with preference for oxirane (*R*)-**1** forming diol (*R*)-**2**, which suggests that the mechanism involves retention of configuration. Spent culture medium from which fungal mycelia had been removed hydrolyzed *rac*-**1** into racemic chlorohydrin **4** in quantitative yield. CALB-catalyzed transesterification of **4** with vinyl acetate as acylating agent has been shown to be an efficient method for preparing the halohydrins with high enantioselectivity and good yields.

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