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Primary allenic alcohols of high optical purity via lipase catalyzed resolution

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Abstract

The lipase from *Candida rugosa* lipase (CRL) was used for the preparation of optically active primary allenic alcohols with axial chirality. The biocatalytic material used was the commercial CRL (C-CRL) and propan-2-ol-treated CRL (PT-CRL). The kinetic resolution of (±)-**1** and (±)-**3a–c** in water using C-CRL resulted in either the absence of or low enantiomeric ratio, whereas PT-CRL increased the *E*-value. Under optimized conditions (temperature and medium used) (+)-**S-3a** and (−)-**R-4** were isolated in excellent yields and high optical purity (o.p.). The resolution was carried out on multigram scale in water/*n*-hexane at 4 °C. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Lipases; *Candida rugosa*; Allenic alcohols; Enantioselectivity

1. Introduction

The importance of allenes is well established and considerable progress has been made in the area of naturally occurring allenes, most of which are chiral [1]. Different methods have been proposed to construct cumulenic-bond systems, bearing different types of substituents, to have chiral allenes in optically active forms or at least, enantio-enriched forms [2,3].

To date, the methods for resolving allenic racemate have been based almost exclusively on classical methodology. Even though enzymes have been shown to discriminate enantiomers of different classes of compounds, their use in the allene field has been limited to a few cases [4].

To our knowledge, the only enzymatic resolution of allenic alcohols concerns the esterification of these compounds with crude *Candida rugosa* lipase (CRL) in organic solvents [5].

However, none of the reactions were optimized and the procedure is unsuitable for preparing sufficient amounts of optically active material that would allow further transformations.

In an attempt to provide a general, easy route to the required molecules, based on our previous experience in this area [6,7], we decided to explore the possibility of obtaining the title compounds by lipase-catalyzed hydrolysis of the corresponding esters.

2. Experimental

2.1. General

CRL (E.C.3.1.1.13 type VII) was purchased from Sigma Chemicals Co. It was purified and its activity was determined as previously described [6,7].

All the organic solvents were of reagent grade. All other chemicals were obtained from Aldrich Chemical Co. (±)-**2** and (±)-**4** were synthesized according to [8,9].

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2.2. Preparation of racemic esters (±)-*1* and (±)-*3a–c*

The corresponding alcohol (13.5 mmol) and dry pyridine (10 ml) were mixed at 0 °C under N_2 atmosphere. To this, the corresponding acetyl, benzoyl or lauryl chloride (20 mmol) was then added dropwise over a 5–10 min period. The mixture was left at room temperature for 1–5 h and then quenched with water and extracted with ethyl ether. The organic layer was washed with HCl 4% solution (3 × 20 ml), then with $NaHCO_3$ and finally with water. The organic layer was dried over Na_2SO_4 and evaporated at reduced pressure. The residue was purified by column chromatography on silica gel eluted with petroleum ether/ethyl ether (95/5).

The (±)-**1**: yield 70%, oil, MS (EI): *m/z* (relative intensity) 84 (100), 83 (44), 67 (14), 66 (26), 65 (22), 55 (14), 43 (74); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.68 (dd, 3H, CH_3C=C, J = 6.9, 3.4 Hz) 2.05 (s, 3H, $COCH_3$) 4.64 (dd, 2H, CH_2O, J = 6.2, 4.5 Hz) 4.84–4.94 (m, 1H, C_4H) 5.11–5.18 (m, 1H, C_2H).

The (±)-**3a**: yield 89%, oil, MS (EI): *m/z* (relative intensity) 168 (M^+,1), 153 (78), 111 (41), 95 (44), 93 (42), 91 (20), 79 (17), 77 (24), 67 (55), 55 (39), 53 (17), 43 (100); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.04 (s, 6H, $C(CH_3)_2$) 1.66 (dd, 3H, CH_3C=C, J = 6.9, 3.2 Hz) 2.07 (s, 3H, $COCH_3$) 3.85 (s, 2H, CH_2O) 5.04 (dq, 1H, C_3H, J = 6.5, 3.2 Hz) 5.16 (dq, 1H, C_5H, J = 6.9, 6.5 Hz).

The (±)-**3b**: yield 80%, oil, MS (EI): *m/z* (relative intensity) 230 (M^+,1),215 (41), 106 (19), 105 (100), 93 (41), 77 (67), 67 (25), 51 (21); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (s, 6H, $C(CH_3)_2$) 1.63 (dd, 3H, CH_3C=C, J = 6.7 Hz, 3.5 Hz) 4.11 (s, 2H, CH_2O) 5.13 (dq, 1H, C_3H, J = 6.5, 3.5 Hz) 5.15 (dq, 1H, C_5H, J = 6.7, 6.5 Hz) 7.44–7.56 (m, 3H, H arom) 8.04–8.09 (m, 2H, H arom).

The (±)-**3c**: yield 86%, oil, MS (EI): *m/z* (relative intensity) 308 (M^+,2), 183 (39), 111 (34), 109 (44), 108 (25), 95 (78), 93 (100), 85 (25), 71 (35), 67 (55), 57 (73), 55 (56), 43 (69); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, 3H, CH_2CH_3, J = 6.7 Hz) 1.04 (s, 6H, $C(CH_3)_2$) 1.20—1.30 (m, 16H $(CH_2)_8$) 1.63–1.65 (m, 2H, CH_2) 1.66 (dd, 3H, CH_3C=C, J = 6.9 Hz, 3.2 Hz) 2.33 (t, 2H, $COCH_2$, J = 7.3 Hz) 3.85 (s, 2H, CH_2O) 5.03 (dq, 1H, C_3H, J = 6.4, 3.2 Hz) 5.15 (dq, 1H, C_5H, J = 6.9, 6.4 Hz).

2.3. Determination of optical purities

The optical purities (o.p.) of alcohols (−)-**R-2** and (−)-**R-4** obtained in the enzymatic hydrolysis was determined by comparing the specific optical rotation of sample with those of optically pure compounds [10,11]. The esters (+)-**S-1** and (+)-**S-3** were first hydrolyzed to the corresponding alcohols (+)-**S-2** and (+)-**S-4** by treatment with NaOH 0.5 M/ethanol (4/1, v/v) for 3–15 h at reflux.

2.4. Enzymatic hydrolysis in the presence of commercial CRL in aqueous medium

In a standard experiment (Table 1, Fig. 1 entry 3), crude CRL (120 mg) was suspended in water (12 ml) and phosphate buffer (NaH_2PO_4–Na_2PO_4 0.1 M, pH 7.2, 2 ml), stirred at room temperature and the pH adjusted to 7.2. Racemic ester **3a** (0.168 g, 1 mmol) was added and the mixture was maintained at pH 7.2 by automatic titration with NaOH 0.2 M using a Mettler DK pH-stat under vigorous stirring. When the hydrolysis reached 53% conversion, the reaction was terminated by adding a saturated solution of NaCl (10 ml). The solution was then filtered through celite and the mixture was extracted with ethyl ether (3 × 20 ml). The organic layer was dried with Na_2SO_4 and concentrated in vacuo. Alcohol (−)-**R-4** and ester (+)-**S-3a** were separated by column chromatography on silica gel using petroleum ether/ethyl ether (95/5) as eluent.

The mixture worked up afforded (−)-**R-4** (60 mg) yield 89%, $[\alpha]_D^{20}$ − 4.2 (c 1.0, MeOH), o.p. 45% and (+)-**S-4** (27 mg) yield 46%, $[\alpha]_D^{20}$ +4.6 (c 1.0, MeOH), o.p. 50%.

2.5. Enzymatic hydrolysis in the presence of propan-2-ol-treated CRL in aqueous medium

In a standard experiment (Table 1, entry 4), 12 ml of solution of propan-2-ol-treated CRL (PT-CRL) (225 units with *p*-NPA assay) and phosphate buffer (NaH_2PO_4–Na_2PO_4 0.1 M, pH 7.2, 2 ml) were stirred at room temperature and the pH adjusted to 7.2. Racemic ester **3a** (0.168 g, 1 mmol) was added and the mixture was maintained at pH 7.2 by automatic titration with NaOH 0.2 M under vigorous stirring. When the hydrolysis reached 38% conversion, the

Table 1
C-CRL- and PT-CRL-catalyzed hydrolysis of racemic allenic esters (±)-**1** and (±)-**3a**–**c** in water at pH 7.2 and at room temperature

Entry	Enzyme	Substrate	*t* (h)	Conversion (%)[a]	Products	o.p. (%)[b]	*E*[c]
1	C-CRL	(±)-**1**	25	55	(−)-**R-2**	–	–
					(+)-**S-1**	–	
2	PT-CRL	(±)-**1**	23	62	(−)-**R-2**	2	–
					(+)-**S-1**	–	
3	C-CRL	(±)-**3a**	23	53	(−)-**R-4**	45	4
					(+)-**S-3a**	50	
4	PT-CRL	(±)-**3a**	5	38	(−)-**R-4**	65	7
					(+)-**S-3a**	39	
5	C-CRL	(±)-**3b**	31	27	(−)-**R-4**	19	2
					(+)-**S-3b**	7	
6	PT-CRL	(±)-**3b**	31	25	(−)-**R-4**	12	1
					(+)-**S-3b**	4	
7	C-CRL	(±)-**3c**	29	30	(−)-**R-4**	25	2
					(+)-**S-3c**	11	
8	PT-CRL	(±)-**3c**	28	29	(−)-R-**4**	22	2
					(+)-S-**3c**	9	

[a] Conversion of reaction determined by GC after the required amount of NaOH 0.2 N was consumed.
[b] Isolated products (experimental errors ± 4% in all cases).
[c] Enantioselectivity factor [20].

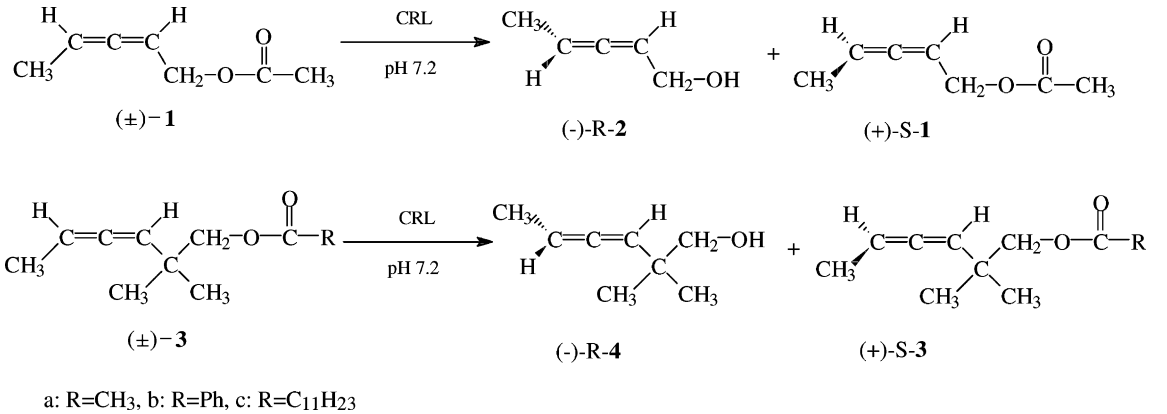

Fig. 1.

reaction mixture was worked up as described above and afforded (−)-**R-4** (45 mg) yield 94%, $[\alpha]_D^{20}$ − 6.1 (c 1.0, MeOH), o.p. 65% and (+)-**S-4** (40 mg) yield 51%, $[\alpha]_D^{20}$ + 3.7 (c 1.0, MeOH), o.p. 39%.

2.6. Enzymatic hydrolysis in the presence of commercial CRL in water/n-hexane medium

In a standard experiment (Table 2, entry 1), crude CRL (120 mg) was suspended in water (12 ml) and phosphate buffer (NaH_2PO_4–Na_2PO_4 0.1 M, pH 7.2, 2 ml), stirred at room temperature and the pH adjusted to 7.2. The resulting enzymatic solution was added to a solution of racemic ester **3a** (0.168 g, 1 mmol) in *n*-hexane (2 ml) and the mixture was maintained at pH 7.2 by automatic titration with NaOH 0.2 M under vigorous stirring. When the hydrolysis reached 36% conversion, the reaction mixture was worked up as described earlier and afforded (−)-**R-4** (40 mg) yield 89%, $[\alpha]_D^{20}$ − 4.7 (c 1.0, MeOH), o.p. 51% and (+)-**S-4** (26 mg) yield 33%, $[\alpha]_D^{20}$ + 2.7 (c 1.0, MeOH), o.p. 29%.

Table 2
C-CRL- and PT-CRL-catalyzed hydrolysis of racemic allenic esters (±)-**3a–c** in water/*n*-hexane at pH 7.2 and at room temperature

Entry	Enzyme	Substrate	*t* (h)	Conversion (%)[a]	Products	o.p. (%)[b]	*E*[c]
1	C-CRL	(±)-**3a**	24	36	(−)-**R-4**	51	4
					(+)-**S-3a**	29	
2	PT-CRL	(±)-**3a**	16	42	(−)-**R-4**	76	13
					(+)-**S-3a**	55	
3	C-CRL	(±)-**3b**	47	30	(−)-**R-4**	23	2
					(+)-**S-3b**	10	
4	PT-CRL	(±)-**3b**	7	35	(−)-**R-4**	40	3
					(+)-**S-3b**	22	
5	C-CRL	(±)-**3c**	50	4	(−)-**R-4**	–	–
					(+)-**S-3c**	–	
6	PT-CRL	(±)-**3c**	52	5	(−)-**R-4**	–	–
					(+)-**S-3c**	–	

[a] Conversion of reaction determined by GC after the required amount of NaOH 0.2 N was consumed.
[b] Isolated products (experimental errors ± 4% in all cases).
[c] Enantioselectivity factor [20].

2.7. Enzymatic hydrolysis in the presence of propan-2-ol-treated CRL in water/n-hexane medium

In a standard experiment (Tables 2 and 3, entry 2), 12 ml of solution of PT-CRL (225 units with *p*-NPA assay) and phosphate buffer (NaH_2PO_4–Na_2PO_4 0.1 M, pH 7.2, 2 ml) were stirred at room temperature and the pH adjusted to 7.2. The resulting enzymatic solution was added to a solution of racemic ester **3a** (0.170 g, 1 mmol) in *n*-hexane (2 ml) and the mixture was maintained at pH 7.2 by automatic titration with NaOH 0.2 M under vigorous stirring. When the hydrolysis reached 42% conversion, the reaction mixture was worked up as described earlier and afforded (−)-**R-4** (50 mg) yield 94%, $[\alpha]_D^{20}$ − 7.1 (c 1.0, MeOH), o.p. 76% and (+)-**S-4** (42 mg) yield 58%, $[\alpha]_D^{20}$ + 5.1 (c 1.0, MeOH), o.p. 55%.

Table 3
PT-CRL-catalyzed hydrolysis of substrate (±)-**3a** in water/*n*-hexane at pH 7.2 and at different temperatures

Entry	*T* (°C)	*t* (h)	Conversion (%)[a]	Products	o.p. (%)[b]	*E*[c]
1	40	20	36	(−)-**R-4**	18	2
				(+)-**S-3a**	10	
2	22	16	42	(−)-**R-4**	76	13
				(+)-**S-3a**	55	
3	10	18	47	(−)-**R-4**	78	18
				(+)-**S-3a**	70	
4	4	20	36	(−)-**R-4**	100	100
				(+)-**S-3a**	57	

[a] Conversion of reaction determined by GC after the required amount of NaOH 0.2 N was consumed.
[b] Isolated products (experimental errors ± 4% in all cases).
[c] Enantioselectivity factor [20].

*2.8. Enzymatic hydrolysis of (±)-**3a** in the presence of propan-2-ol-treated CRL in water/n-hexane medium on a multigram scale*

Twenty milliliter of phosphate buffer (20 mM, pH 7.2) solution of PT-CRL (450 U, with *p*-NPA assay) were stirred at 4 °C for 15 min in a closed vessel. The resulting enzymatic preparation was added to a solution of **3a** (1.36 g) in *n*-hexane (7 ml). The mixture was maintained at pH 7.2 under stirring by automatic titration with NaOH 0.2 M using a Mettler DK pH-Stat. When the hydrolysis reached 44% conversion, a saturated solution of NaCl (15 ml) was added to the reaction mixture. The mixture, worked up as described earlier, afforded 0.4 g of (−)-**R-4** yield 91%, $[\alpha]_D^{20}$ − 9.1 (c 1.0, MeOH), o.p. 98% and 0.44 g of (+)-**S-4** yield 59%, $[\alpha]_D^{20}$ + 8.4 (c 1.0, MeOH), o.p. 90%.

3. Results and discussion

First, we studied the hydrolysis of esters **1** and **3a–c** in water in the presence of C-CRL and PT-CRL.

The results of the reactions carried out in aqueous medium at pH 7.2 and at room temperature are illustrated in Table 1. All the esters were hydrolyzed by C-CRL and PT-CRL but only the allene **3a**, the acetyl derivative of the most hindered alcohol, was hydrolyzed with low enantiomeric selectivity. By using the benzoate and laurate of allenic alcohol, instead of acetate, the hydrolysis of esters under the same experimental conditions were slow and proceeded with very low enantio-recognition.

The alcohol (−)-**R-4** and the ester (+)-**S-3** were obtained in all the experiments. The absolute configurations and the optical rotations of the alcohols are well-known [10–12].

These results cannot be considered useful from a preparative point of view so we decided to optimize the bioconversion and scale-up the preparation of substrate **3a**.

*3.1. Optimization of enantioselectivity of Candida rugosa lipase in the resolution of racemic allenic ester **3a***

Organic solvents are commonly added to water to produce a positive effect on the enantioselectivity of lipases [13]. In water/*n*-hexane medium, CRL often increases its enantioselectivity in the resolution of racemic esters [14]. Therefore, the influence of *n*-hexane as a cosolvent was investigated. As can be seen from Table 2, the activity and enantioselectivity of C-CRL towards the substrates **3a** and **3b** were not enhanced due to the presence of *n*-hexane, while PT-CRL increased the enantioselectivity.

The enhanced enantioselectivity of PT-CRL can be justified on the basis of a favorable conformational change of the enzyme as a consequence of the displacement of its polypeptide lid [15]. The favorable effect of the organic solvent on enantioselectivity of PT-CRL is probably caused by the more flexible conformation induced by propan-2-ol [6]. Although PT-CRL enantioselectivity towards **3a** was good, we decided to examine the effect of temperature in an effort to improve the results.

As shown in Table 3, the enantioselctivity of PT-CRL increased as the reaction temperature decreased from 40 to 4 °C with excellent enantioselectivity ($E = 100$) at low temperatures. In contrast, the hydrolysis rate of **3a** was independent of temperature in the 4–40 °C range. This fact is important because the alcohol (−)-**R-4** can be obtained in optically pure form under experimental conditions where the reaction rate is acceptable.

This is the first reported preparation of allenic alcohol of high optical purity by enzymatic hydrolysis of the corresponding ester. Under the reaction conditions described in Table 3 entry 4, an excellent resolution of 1.36 g of **3a** was obtained, which rendered 0.4 g of alcohol (−)-**R-4** (98% o.p., 91% yield) and 0.64 g of ester (+)-**S-3a** (90% o.p., 85% yield).

Temperature has been shown to be a useful tool for improving the enantioselectivity of enzyme-catalyzed reactions [16,17]. It has been established that the effect of temperature is specific so it cannot be used to make predictions. In fact, both increases and decreases of selectivity have been observed with the lowering of temperature [18] and, in some cases, temperature had no effect on the *E*-value [19].

Generally, the decrease of enzyme selectivity with increasing temperature could be explained with a more flexible protein conformation, that can accept and transform enantiomers without selectivity. Another possible explanation for lipases is a significant change in the protein conformation that is probably associated with the lid movement. This modification could positively or negatively influence the enantioselectivity due to the contemporary capacity to change the active site structure according to the substrate properties (induced fit enzyme).

4. Conclusions

In summary, we have demonstrated that PT-CRL, used in water/*n*-hexane medium and at 4 °C, is an excellent catalyst for preparing (+)-**S-3a** and (−)-**R-4** in optically pure form and on a multigram scale. Temperature plays a significant role in the optimization of the final result.

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