ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 11 (2000) 37–43

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Optimized methods for the preparation of (*S*)-2-hydroxy-2-phenylpropanenitrile exploiting (*R*)-oxynitrilase in almond meal

Gints Rotčenkovs, Liisa T. Kanerva *

Department of Chemistry, University of Turku, FIN-20014 Turku, Finland

Received 14 April 2000; accepted 6 July 2000

Abstract

Almond meal as the source of (*R*)-oxynitrilase was used for the resolution of *rac*-2-hydroxy-2-phenylpropanenitrile (**1**). Optimization was performed in the presence and absence of an aldehyde paying attention to the nature of the reaction medium (pH, buffer content and organic solvent). Under the optimized conditions, (*S*)-2-hydroxy-2-phenylpropanenitrile as the less reactive enantiomer was obtained at approximately 50% conversion and 98–99% ee, the (*R*)-counterpart decomposing enzymatically to acetophenone (**3**) and hydrogen cyanide. Hydrogen cyanide was conveniently bound by an aldehyde in the formation of the highly enantiopure (*R*)-aldehyde cyanohydrin. At pH > 4.8, the presence of an aldehyde was necessary in order to prevent the reaction of (**3**) with hydrogen cyanide back to (*R*)-2-hydroxy-2-phenylpropanenitrile and the consequent racemization.

Keywords: Oxynitrilase; Almond meal; 2-Hydroxy-2-phenylpropanenitrile; Resolution; Transhydrocyanation

1. Introduction

Optically active cyanohydrins attract synthetic chemists as valuable intermediates for the preparation of several other classes of optically active compounds, such as amino alcohols and hydroxy carboxylic acids [1–5]. In spite of some progress, chemical methods for cyanohydrin enantiomers often suffer from low enantiopurities of products and limited substrate range being accepted. On the other hand, recent discoveries of several (*R*)- and (*S*)-stereoselective oxynitrilase enzymes have opened routes to highly enantiopure cyanohydrins. Oxynitrilases are employed either in catalyzing the addition of hydrogen cyanide to prochiral aldehydes and ketones, or although less commonly, in catalyzing the reverse reaction leading to the less reactive cyanohydrin enantiomer as the result of kinetic resolution (Fig. 1) [6–18]. Ketone cyanohydrins have been used as the in situ source of HCN in oxynitrilase-catalyzed transhydrocyanation [7–9,11].

The enzymatic preparation of (*R*)-cyanohydrins is well established. It is known that the (*R*)-oxinitrilase of various cyanogenic plants can be used as economical defatted meals, eliminating laborious purification of the enzyme. Thus, the use of meals from almond, apple seeds, apricot etc. have been reported [7,10–15]. Previous works on (*S*)-specific oxynitrilase from

* Corresponding author. Tel.: +358-2-3336773; fax: +358-2-3337955.

E-mail address: lkanerva@utu.fi (L.T. Kanerva).

1381-1177/00/$ - see front matter
PII: S1381-1177(00)00196-X

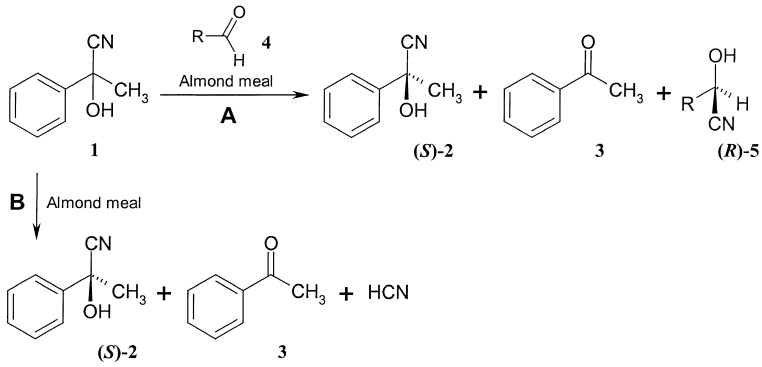

Fig. 1. Almond meal-catalyzed resolution of (**1**).

Hevea brasiliensis (Hb-Hnl) and *Manihot esculenta* have provided excellent tools for the preparation of (*S*)-aldehyde cyanohydrins [6,16–18]. Nevertheless, there is still a lack of good enzymatic methods for (*S*)-ketone cyanohydrins. For example, the condensation of acetophenone (**3**) with HCN in the presence of (*S*)-Hb-Hnl was reported to give (*S*)-(**2**) at only 40% conversion [18]. In our previous work, the almond meal-catalyzed resolution of various ketone cyanohydrins appeared promising for the preparation of (*S*)-ketone cyanohydrins (Fig. 1; route A) [7]. In the present work, the conditions for the almond meal-catalyzed resolution of *rac*-2-hydroxy-2-phenylpropanenitrile (**1**) have been optimized with respect to the reaction medium (pH, buffer content and an organic solvent) in the presence and absence of an aldehyde as an HCN acceptor (Fig. 1; routes A and B, respectively).

Due to the simultaneous enzymatic and chemical reactions and to the possible equilibria involved for the resolution of (**1**) in biphasic systems, enantioselectivity is not discussed in terms of *E* values [19]. Rather, the ee value of the less reactive (*S*)-enantiomer at certain conversion is used throughout the work.

2. Experimental

2.1. Materials

Almond meal (EC 4.1.2.10.) was purchased from Sigma. Diisopropyl ether (DIPE) from Riedel-de-Haën was distilled on a rotary evaporator to remove the stabilizer and thereafter stored in dark under nitrogen. The other solvents were HPLC grade products from Aldrich and used without purification. Aldehydes were from Aldrich or Merck and were distilled before use; (**3**) was from Merck and dodecane from EGA-Chemie.

All reactions were performed on Heidolph Promax 2020 reciprocal shaker at 170 rpm. The course of the reaction was followed by GLC by taking samples (30 μl) at time intervals. The formation of (**3**) against dodecane (an internal standard) was monitored. For ee determination, the cyanohydrins in the sample were acetylated using acetic anhydride (20 μl) and 1% 4-*N*,*N*-dimethylaminopyridine (DMAP) in pyridine (5 μl). GLC analysis was performed on a Perkin Elmer 8500 gas chromatograph (flame ionization detector) using a CP-Chirasil-Dex CB (25 m) column.

^{1}H NMR spectra (internal standard TMS) were recorded on Bruker 200 spectrometer in $CDCl_3$. Perkin Elmer Lambda 10 UV–VIS spectrometer was used for determining HCN contents from the chemical dissociation of 2-phenyl-2-propanenitrile (**1**) to (**3**) and HCN with time.

2.2. Synthesis of racemic 2-hydroxy-2-phenylpropanenitrile

2-Hydroxy-2-phenylpropanenitrile (**1**) was prepared from (**3**) and trimethylsilylcyanide as previously described [20]. The product was used without further purification. ^{1}H NMR ($CDCl_3$): δ 1.85

(s, 3H, CH_3); 3.34 (s, 1H, OH); 7.36–7.46 (m, 3H, H_{arom}); 7.52–7.58 (m, 2H, H_{arom}).

2.3. General procedure for transcyanation reaction

Reactions were performed in 4 ml vials with tightly closed caps. In a typical experiment, an appropriate volume of sodium tartrate buffer (0.1 M, pH between 4.2 and 5.4; 310 μl in the case of 13.5% of buffer by volume) was added on almond meal (20 mg). 2-Hydroxy-2-phenylpropanenitrile (138 mg, 94 mM) was dissolved in the solvent (10 ml) containing dodecane (1% (w/v)) and this solution (2 ml) was added into the vial together with or without one of the aldehydes (0.6–1.0 eq.). If not otherwise stated, propanal (0.67 eq.; 9.1 μl) was used as an aldehyde. The vial was tightly closed and placed on the reciprocal shaker.

The inhibitory effect of propanoic acid was determined using the above procedure except that the added propanal contained propanoic acid (1, 2 or 5% (v/v)).

2.4. Dissociation of 2-hydroxy-2-phenylpropanenitrile

The dissociation of 2-hydroxy-2-phenylpropanenitrile (**1**) in sodium citrate buffers was studied by determining the amount of HCN from the equilibrium [PhC(OH)(CN)Me] = [Ph(C=O)Me] + [HCN] as the function of pH. For that purpose, the previous methods were slightly modified [12,21]. For the calibration curve, the KCN solutions (10 μl) of known amounts were added to the mixture of methanol (0.5 ml) and citrate buffer (0.1 M, 4.5 ml). After 10 min, the solution (10 μl) was added to Reagent 1 (5 ml, see [21]), followed by the addition of Reagent 2 (1 ml). After 10 min, the absorption at 580 nm was measured. Identical absorptions were recorded at pH 3.0, 4.0 and 5.0. The amount of HCN which originated from the dissociation of the cyanohydrin was measured identically, starting with 2-hydroxy-2-phenylpropanenitrile (10 μl; ~ 80 μmol) and citrate buffers (0.1 M) over the pH range 3.0–6.0. Compound (**1**) was rotated for 5 min under reduced pressure before it was used in the experiment in order to remove possible traces of HCN.

3. Results and discussion

3.1. Resolution in the presence of an aldehyde

3.1.1. Optimization of the buffer content and pH

For the enantioselective transhydrocyanation of (**1**), the highest theoretical chemical yield for (*S*)-(**2**) is 50% (Fig. 1; route A). In practice, the chemical yields and ee of the products (*S*)-(**2**) and (*R*)-(**5**) depend on the complex equilibria of simultaneous chemical and enzymatic reactions as well as on the solubility and partition characteristics of the reaction components between the aqueous and organic phases of the medium. Phase volume ratio, pH of the aqueous phase and temperature are the main factors determining the velocity of both chemical and enzymatic reactions and the position of the equilibria. Thus, the proper control of conditions will allow the preparation of highly enantiopure (*S*)-(**2**) while keeping the conversion at around 50%.

Under the previous resolution conditions for (**1**), 98% ee at 58% conversion was reported for (*S*)-(**2**) in DIPE containing tartrate buffer (7% (v/v), 0.1 M, pH 5.4) as a medium and ethanal as an HCN acceptor (Fig. 1; route A) [7]. In the present work, the reaction was repeated with propanal as an HCN acceptor using various buffer contents (2–20% (v/v), pH 5.4) in DIPE (Fig. 2). Propanal was favoured over ethanal due to the high volatility of the latter.

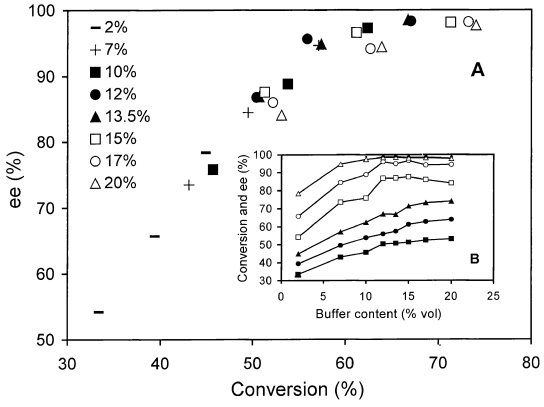

Fig. 2. Resolution of (**1**) in the presence of propanal in DIPE at various (2–20% (v/v)) tartrate buffer (0.1 M, pH 5.4) contents: (A) ee vs. conversion (the symbols for various buffer contents are shown in the figure); (B) conversion (■, ●, and ▲) and ee (□, ○, and △) after 24, 48, and 96 h, respectively vs. the buffer content.

Reactivity (conversion at a certain time; Fig. 2B, closed symbols) increases with increasing buffer content. Thus, after 24 h, the conversions of 33 and 53% were observed at the buffer contents of 2 and 20% (v/v), respectively. At buffer contents of 15% (v/v) or higher, the corresponding ee values start decreasing slightly in spite of increasing conversions. This implies higher possibilities for the chemical decomposition of (**1**) parallel to the enzymatic reaction at high water contents. As the best compromise of ee versus conversion (Fig. 2A), the buffer content of 13.5% (v/v) was chosen for the rest of the work.

The lability of cyanohydrin (**1**) was studied over the pH range 3.0–6.0 in citrate buffers (0.1 M) (Fig. 3). The results clearly indicate that the chemical decomposition of (**1**) in water is significant already at pH > 4. Accordingly, cyanohydrin (**1**) was subjected to enzymatic resolution in DIPE containing tartrate buffers (0.1 M, 13.5% (v/v), pH 4.2–5.4) in the presence of propanal (Fig. 4). When pH is lowered from the optimum pH 5.4 of the almond enzyme, a decrease in reactivity is observed (Fig. 4B, closed symbols). At pH 4.2, enzymatic activity starts becoming negligible (Fig. 4A, (+)). At pH 5.1 or higher, the proportion of the chemical reaction becomes evident (Fig. 4A, (■) and (◆)). On this basis, pH 4.8 was chosen for further experiments concerning solvent effects and the effect of the nature of an aldehyde as an HCN acceptor.

3.1.2. Solvent effects

Several organic solvents containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8) were tested for the

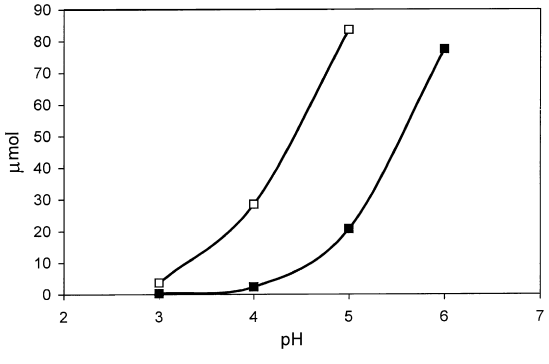

Fig. 3. Dissociation of (**1**) in citrate buffers (0.1 M) at various pHs (3–6): formation of HCN (μmol) after 10 min (■) and 4 h (□) as a function of pH.

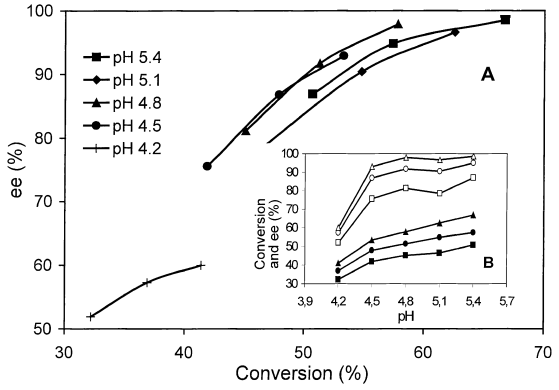

Fig. 4. Resolution of (**1**) in the presence of propanal in DIPE containing tartrate buffers (0.1 M, 13.5% (v/v)) at various pHs (4.2–5.4): (A) ee vs. conversion (the symbols for various pHs are shown in the figure); (B) conversion (■, ●, and ▲) and ee (□, ○, and △) after 24, 48, and 96 h, respectively vs. pH.

almond meal-catalyzed resolution of cyanohydrin (**1**) (Table 1). Clearly, the reactivity of (**1**) was good in DIPE and considerably increased in more hydrophobic toluene and hexane. In hexane, ee dramatically dropped with time and due to the poor solubility of (**1**) a third phase tended to form in the reaction mixture. The course of the resolution in toluene was monitored in more detail with respect to the pH and volume of the buffer (Table 2). The attraction for toluene was somewhat faded by higher conversions needed to achieve good ee as compared to the reac-

Table 1
Enzymatic resolution of (**1**) in organic solvents containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8) in the presence of propanal at 25°C

Solvent	log P[a]	Time/h	Conversion/%	ee (**2**)/%
Ethyl acetate	0.68	24	5	6
		48	10	8
MTBE	1.2	24	7	9
		48	17	14
DIPE	1.9	24	45	90
		48	51	98
Toluene	2.5	24	58	> 99
		48	67	> 99
Hexane	3.5	24	92	80
		48	98	42

[a] P is the partition coefficient of the solvent between water and 1-octanol.

Table 2
Enzymatic resolution of (**1**) in the presence of propanal in toluene containing tartrate buffer (0.1 M, pH 4.8) at 25°C

pH	Buffer/% (v/v)	Time/h	Conversion/%	ee (**2**)/%
4.8	13.5	4	49	82
		9	59	98
4.8[a]	13.5	24	47	89
		48	53	98
4.5	13.5	9	51	92
		12	57	97
4.5	10	9	50	88
		12	55	94

[a] Reaction in toluene/DIPE (1/1).

tion in DIPE (Table 1). The problem lies in significantly higher chemical decomposition of (**1**) in toluene than in DIPE (Table 3). An attempt to use the 1/1 mixture of DIPE and toluene did not yield significant improvements in the time versus conversion-ee relationship (Tables 1 and 2) although the chemical decomposition now followed the pattern typical to the reaction in DIPE (Table 3). In *tert*-butyl methyl ether (MTBE) and ethyl acetate that are less hydrophobic than DIPE, reactivity was negligible (Table 1).

3.1.3. Structural effects of aldehydes as HCN acceptors

The almond meal-catalyzed transcyanation between straight-chain aliphatic aldehydes and acetone cyanohydrin was previously shown to proceed in the formation of highly enantiopure (*R*)-aldehyde cyanohydrins [11]. In the present work, enantioselective transcyanation was exploited for trapping the HCN that liberates when cyanohydrin (**1**) reacts in the presence of almond meal (Fig. 1; route A). Thus, the HCN binding capabilities and enantioselectivity

Table 3
Decomposition[a] of (**1**) in the absence of almond meal: effects of solvent, pH and buffer content at 25°C

pH	Buffer content/%	DIPE	DIPE/toluene[b]	Toluene
4.8	13.5	4	4	18
	10.0	3	3	12
4.5	13.5	3	3	13
	10.0	2	2	9

[a] Formation of acetophenone (% per day).
[b] 1:1 mixture of the solvents.

effects of various straight-chain aldehydes were tested in DIPE containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8). In the process, the aldehyde cyanohydrin (*R*)-(**5**) is obtained at high enantiopurity expecting that one of the aldehydes from butanal to heptanal serves as an HCN acceptor (Table 4). On the other hand, within the limits of the experimental accuracy, the conversion of (**1**) in 24 or 48 h is independent of the nature of the added aldehyde and the molar ratio (**4**)/(**1**). At first sight, propanal and heptanal with an $ee_{(S)\text{-}(2)}$ of 98% at close to 50% conversion seem to be the most favourable acceptors (Table 4; rows 3 and 11–13). An interesting fact arose when the resolution of (**1**) was performed in the presence of decanal (rows 14 and 15 in Table 4). Thus, the $ee_{(S)\text{-}(2)}$ value was high and the resolution smoothly proceeded although the GLC analysis revealed that only a minor part of decanal had reacted. Decanal was known to react slowly on the basis of our previous works [11,13]. This result led to the idea that evidently the removal of the HCN is unnecessary while using a buffer at low pH (see Section 3.2).

Formation and steady increase of a new peak was observed in the GLC analysis of the above reactions.

Table 4
Enzymatic resolution of (**1**) in the presence of an aldehyde in DIPE containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8) at 25°C for reaction times of 24/48 h

Row	Aldehyde (**4**)	Molar ratio of (**4**)/(**1**)	Conversion/%	ee (**2**)/%	ee (**5**)/%
1	Propanal	0.60	47/54	95/ > 99	47/48
2		0.67	48/55	95/ > 99	49/49
3		1.00	46/51	91/98	51/51
4	Butanal	0.67	46/51	83/92	93/92
5		1.00	45/46	79/83	93/91
6	Pentanal	0.67	47/51	91/96	96/95
7		1.00	48/52	86/91	97/96
8	Hexanal	0.60	51/55	94/99	94/94
9		0.67	50/55	95/99	94/94
10		1.00	49/54	88/95	94/94
11	Heptanal	0.60	44/52	93/98	91/98
12		0.67	49/52	94/98	91/98
13		1.00	48/52	92/98	83/96
14	Decanal	0.67	–/58	–/97	–
15		1.00	55/55	94/97	–

It came out that the aldehydes were slowly oxidized to the corresponding carboxylic acids during the reaction. The inhibitory effect is clearly seen from the results in Table 5. Thus, the addition of only 1–2% (v/v) of propanoic acid to propanal affects the reactivity. This emphasizes the importance of distilling the aldehyde before use.

3.1.4. Temperature effects

Propanal and hexanal were used as HCN acceptors for the resolution of (**1**) in DIPE containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8) at 6°C. Highly enantiopure (*S*)-2-hydroxy-2-phenylpropanenitrile (ee 98–99%) was obtained at ca. 50% conversion, indicating good suppression of the chemical decomposition. However, reaction times needed to reach the 50% conversion became hopelessly slow (20–30 days). As a benefit though, the corresponding aldehyde cyanohydrin with high enantiopurity was obtained during the process. Thus, the enantiopurity of (*R*)-butanenitrile ((**5**), R = Et) was increased from 50% at room temperature (Table 4, rows 1–4) to 75% at 6°C. At 6°C in dry DIPE, no chemical reaction was detected between (**1**) and propanal after 2 weeks.

3.2. Resolution in the absence of an aldehyde

For the resolution of (**1**) in biphasic DIPE/tartrate buffer systems, pH 5.4 of the buffer evidently allows the chemical decomposition of the substrate and/or enzymatic reactions of HCN with (**3**) back to cyanohydrin. The latter conclusion is supported by the results from our previous work where the addition of ethanal to bind HCN improved the $ee_{(S)\text{-}(2)}$ to 98% (from 88% without an added aldehyde) at 58% conversion [7]. On the other hand, the lability of (**1**) is evident from the results in Fig. 3.

Table 5
Effect of propanoic acid for the enzymatic resolution of (**1**) in the presence of propanal in DIPE containing tartrate buffer (0.1 M, 13.5% (v/v), pH 4.8) at 25°C

Acid content/% (v/v)	Time/h	Conversion/%	ee (**2**)/%
0	24	48	95
	50	55	> 99
1	24	47	88
	50	52	97
2	24	44	87
	50	49	97
5	24	41	84
	50	48	95

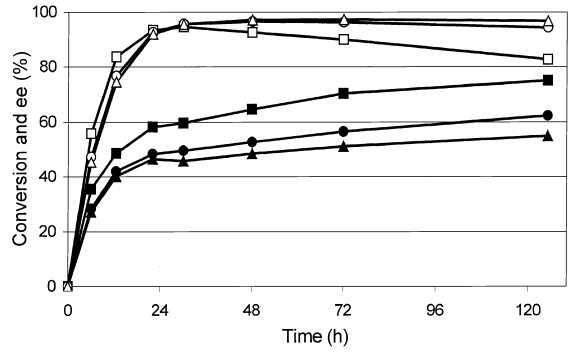

Fig. 5. Resolution of (**1**) in the absence of an aldehyde in DIPE containing tartrate buffers (0.1 M, 13.5% (v/v)) at pH 5.4, 4.8 and 4.5 with conversion (■, ●, and ▲) and ee (□, ○, and △), respectively.

In order to study pH effects in more detail, the resolution of (**1**) in DIPE containing tartrate buffer (0.1 M, 13.5% (v/v), pH 5.4–4.5) was studied (Fig. 1; route B). The results in Fig. 5 show the irrelevance of HCN binding at pH ≤ 4.8. As can be expected, the reaction at the optimum pH value of 5.4 proceeds faster than in more acidic buffers. At every pH, highly enantiopure (*S*)-(**2**) is obtainable. In accordance with the previous work [7], 60% conversion (■) at pH 5.4 is approached when $ee_{(S)\text{-}(2)}$ approaches 97%. Moreover, $ee_{(S)\text{-}(2)}$ starts decreasing with time (□) indicating the reaction of HCN with (**3**) back to (*R*)-(**2**). At pH 4.5 (Δ), (*S*)-(**2**) is virtually stable, leaving ee at 97% at least for 5 days. At pH 4.8 and 4.5, high enantiopurity at 50% conversion is reached already after 24 h.

Clear advantages of this method are simplicity and high enantiopurity of the product at practically optimal conversion of 50% for kinetic resolution. Unfortunately, an ee value larger than 97% was not reached. The presence of free hydrogen cyanide in the reaction is another drawback.

4. Conclusions

Highly enantiopure (*S*)-2-hydroxy-2-phenylpropionitrile as the less reactive enantiomer was ob-

tained by the almond meal-catalyzed resolution in biphasic solvent systems. For that purpose, two methods were studied. In the first method, an aldehyde (from propanal to heptanal and decanal) was used to accept the liberated HCN (Fig. 1; route A). The benefit of this method is that harmful HCN is bound in the form of aldehyde cyanohydrin (*R*)-(**5**) in the reaction mixture. Straight-chain aldehydes up to heptanal are effective enough for that purpose. As another benefit of this method, the production of highly enantiopure (*R*)-aldehyde cyanohydrin becomes possible simultaneously with the resolution product (*S*)-(**2**). In the second method, the resolution of (**1**) was performed without an added aldehyde (Fig. 1; route B). The advantage of this method is that there is no need for the separation of the ketone cyanohydrin from the aldehyde cyanohydrin.

Tartrate buffer (0.1 M, 10–13.5% (v/v)) in DIPE was shown to be the most appropriate medium for the resolution of (**1**) (Fig. 1). In the optimization, pH of the buffer plays a key role when an ee of $\geq 97\%$ at close to 50% conversion is desired. An aldehyde as an HCN acceptor is necessary while working at the optimum pH of 5.4 of the enzyme (Figs. 4 and 5). By lowering the pH of the buffer from 5.4 to 4.8 or lower makes the presence of an aldehyde as an HCN acceptor unnecessary, allowing the preparation of (*S*)-(**2**) at 97% ee at ca. 50% conversion (Fig. 5). This is indicative of the stability of cyanohydrin (**1**) and of the suppression of enzymatic formation (*R*)-(**2**) from (**3**) and HCN.

Acknowledgements

This work was supported by CIMO in Finland (The Centre for International Mobility).

References

[1] F. Effenberger, Angew. Chem. Int. Ed. Engl. 33 (1994) 1555.
[2] C.G. Kruse, in: A.N. Collins, G.N. Sheldrake, J. Crosby (Eds.), Chirality in Industry, 2nd Edition, Wiley, Chichester, 1994, Chapter 14, p. 279.
[3] M. Schmidt, H. Griengl, Top. Curr. Chem. 200 (1999) 193.
[4] F. Effenberger, Chimia 53 (1999) 3.
[5] R.J.H. Gregory, Chem. Rev. 99 (1999) 3649.
[6] F. Effenberger, A. Schwämmle, Biocat. Biotrans. 14 (1997) 167.
[7] E. Kiljunen, L.T. Kanerva, Tetrahedron: Asymmetry 8 (1997) 1551.
[8] V.I. Ognyanov, V.K. Datcheva, K.S. Kyler, J. Am. Chem. Soc. 113 (1991) 6992.
[9] E. Menéndez, R. Brieva, F. Rebolledo, V. Gotor, J. Chem. Soc., Chem. Commun. (1995) 989.
[10] P. Zandbergen, J. van der Linden, J. Brussee, A. van der Gen, Synth. Commun. 21 (1991) 1387.
[11] T.T. Huuhtanen, L.T. Kanerva, Tetrahedron: Asymmetry 3 (1992) 1223.
[12] E. Kiljunen, L.T. Kanerva, Tetrahedron: Asymmetry 7 (1996) 1105.
[13] E. Kiljunen, L.T. Kanerva, Tetrahedron: Asymmetry 8 (1997) 1225.
[14] G. Lin, S. Han, Z. Li, Tetrahedron 55 (1999) 3531.
[15] R.J.H. Gregory, S.M. Roberts, J.V. Barkley, S.J. Coles, M.B. Hursthouse, D.E. Hibbs, Tetrahedron Lett. 40 (1999) 7407.
[16] N. Klempier, H. Griengl, M. Hayn, Tetrahedron Lett. 34 (1993) 4769.
[17] N. Klempier, U. Pichler, H. Griengl, Tetrahedron: Asymmetry 6 (1995) 845.
[18] H. Griengl, N. Klempier, P. Pöchlauer, M. Schmidt, N. Shi, A.A. Zabelinskaja-Mackova, Tetrahedron 54 (1998) 14477.
[19] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.
[20] P.G. Gassman, J.J. Talley, Tetrahedron Lett. 40 (1978) 3773.
[21] D. Selmar, F.J.P. Carvalho, E.E. Conn, Anal. Biochem. 166 (1987) 208.