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Journal of Molecular Catalysis B: Enzymatic 19–20 (2002) 223–230

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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(*R*)-Oxynitrilase-catalyzed transformation of ω-hydroxyalkanals

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Received 19 October 2001; received in revised form 29 April 2002; accepted 13 May 2002

Abstract

Optically active aliphatic ω-hydroxycyanohydrins are valued materials in organic synthesis. We describe herein the effect of the different reaction parameters as organic solvent, temperature, concentration of enzyme or concentration of buffer in the (*R*)-oxynitrilase-catalyzed synthesis of optically enriched aliphatic ω-hydroxycyanohydrins from 4-hydroxybutanal and 5-hydroxypentanal.

Keywords: Transcyanation; (*R*)-Oxynitrilase; Aliphatic ω-hydroxycyanohydrins; 4-Hydroxybutanal; 5-Hydroxypentanal

1. Introduction

(*R*)-Oxynitrilase from almonds [EC 4.1.2.10] catalyzes the enantioselective preparation of cyanohydrins from aldehydes or ketones [1]. As α-substituted carboxylic acid derivatives, these cyanohydrins have a great synthetic potential in organic synthesis; simple racemization-free transformations of the CN function or the OH group yield a great variety of important compounds [2]. As a part of our research on the enzymatic preparation of optically active cyanohydrins and their application into the synthesis of nitrogen heterocycles, we are very interested in the preparation of ω-functionalized cyanohydrins. In a previous report, we have prepared, in high yield and enantiomeric excesses ω-bromocyanohydrins through an enzymatic transcyanation process catalyzed by (*R*)-oxynitrilase [3]. We showed that these cyanohydrins are suitable starting materials for the preparation of (*S*)-pipecolic acid, 2-substituted piperidine alkaloids [4] or (*R*)-azacycloalkan-3-ols [5].

In the present paper, we study the (*R*)-oxynitrilase catalyzed addition of HCN to 4-hydroxybutanal and 5-hydroxypentanal, using acetone cyanohydrin as a source of HCN and the powdered defatted almond meal as a rich source of (*R*)-oxynitrilase.

Hydroxyalkanals have not much been studied as substrates for (*R*)-oxynitrilase. Effenberger et al. [6,7] have reported the enzymatic hydrocyanation of hydroxypivalaldehyde and *O*-protected α-hydroxyalkanals. In both cases, the enantioselectivity of the reaction strongly depends on the *O*-protecting group. Recently, Gerrits et al. [8] achieved good results in the hydrocyanation reaction of several aromatic hydroxylated aldehydes using an aqueous-organic two-phase system. Also, the hydrocyanation of α- and β-oxygenated aldehydes catalyzed by (*R*)-oxynitrilase has been studied [9]. Until now, the reaction of ω-hydroxyalkanals which are mainly as hemiacetalic form in the reaction media, had not been investigated. These aldehydes can be considered difficult substrates for (*R*)-oxynitrilase because of their relative high water solubility [10].

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1381-1177/02/$ – see front matter
PII: S1381-1177(02)00171-6

2. Experimental

2.1. Preparation of almond meal

Almonds were soaked in distilled water for 1 h, peeled, air dried and powdered. The powder was defatted by 10 washes with diethyl ether, filtered and stored at 4 °C.

2.2. Materials and methods

The commercial defatted almond meal was purchased from Sigma. MeHNL (4333 U/ml, 42.4 mg/ml), LuHNL (60.9 U/ml, 3.1 mg/ml) and PaHNL (70.4 U/ml, 7.6 mg/ml) are available from Jülich Fine Chemicals as purified enzymes.

Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of $10^{-1}\,^{\circ}\,cm^2\,g^{-1}$. ^1H , ^{13}C NMR and DEPT spectra were performed using AC 200 (^1H, 200.13 MHz and ^{13}C, 50.3 MHz), AC 300 (^1H, 300.13 MHz and ^{13}C, 75.5 MHz) and DPX-300 (^1H, 300.13 MHz and ^{13}C, 75.5 MHz) Bruker spectrometers. Mass spectra were recorded on Hewlett-Packard 1100 Series and Perkin-Elmer 5987A spectrometers. Microanalyses were performed on a Perkin-Elmer 240B elemental analyzer. IR spectra were recorded on a Perkin-Elmer 1720-X FT Infrared spectrophotometer. All reagents were purchased from Aldrich. Solvents were distilled over an adequate desiccant and stored under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh).

2.3. Synthesis of racemic 2-hydroxytetrahydropyran (*1*)

A solution of DIBAL-H 1.0 M in toluene (55 ml) was added dropwise to a solution of δ-valerolactone (5.0 g, 50 mmol) in THF (40 ml). The mixture was stirred at −70 °C for 5 h and then quenched by slow addition of H_2O (20 ml). The reaction mixture was warmed up to room temperature and stirred for 15 min. Then, HCl 0.5 M (175 ml) was added and the reaction was extracted with CH_2Cl_2 (5 × 200 ml). The organic phase was dried over anhydrous Na_2SO_4. Solvents were evaporated and the product was purified by distillation (62 °C, 4 mm Hg). Colorless oil. Yield: 70%. IR (KBr): ν 3400 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.47–1.60 (m, 4H), 1.78–1.89 (m, 2H), 3.52–3.61 (m, 1H), 4.01–4.08 (m, 1H), 4.21 (s, 1H) and 4.90 (m, 1H). ^{13}C NMR ($CDCl_3$, 50.4 MHz): δ 19.8 (CH_2), 24.7 (CH_2), 31.4 (CH_2), 63.4 (CH_2) and 93.9 (CH). MS (EI, *m/z*): 101 (M-H, 9%), 85 (100), 67 (14) and 56 (33). Anal. Calcd. (%) for $C_5H_{10}O_2$: C, 58.80; H, 9.87. Found: C, 58.6; H, 10.1.

2.4. Synthesis of racemic 2-hydroxytetrahydrofuran (*2*)

2-Ethoxytetrahydrofuran (2.5 g, 21.5 mmol) was dissolved in 40 ml of H_2SO_4 0.1N at room temperature and the mixture was stirred for 1 week. Then, acetic acid (2.5 ml) was added and the mixture was neutralized with NaOH 1.0N. The product was extracted with CH_2Cl_2 (5 × 20 ml). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated to give the hydroxyaldehyde **2** as colorless oil. This compound was used without further purification. Yield: 60%. IR (KBr): ν 3408 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.72–2.05 (m, 4H), 3.72–3.81 (m, 1H), 3.96–4.08 (m, 1H), 5.16 (s, 1H) and 5.50 (m, 1 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 23.3 (CH_2), 32.9 (CH_2), 67.2 (CH_2) and 98.2 (CH). MS (EI, *m/z*) 87 (M-H, 11%), 71 (100) and 53 (32). Anal. Calcd. (%) for $C_4H_8O_2$: C, 54.53; H, 9.15. Found: C, 54.8; H, 9.3.

2.5. General procedure for the enzymatic synthesis of aliphatic (R)-hydroxycyanohydrins using a crude enzyme

2.5.1. Synthesis of (R)-2,6-dihydroxyhexanenitrile ((*R*)-*3*) and (R)-2,5-dihydroxypentanenitrile ((*R*)-*4*)

The defatted almond meal was activated for 15 min with 500 μl of 0.1 M citrate buffer, pH 5.4. Then, a solution of 1.14 mmol of **1** or **2** in 5 ml of diisopropyl ether or ethyl acetate containing 1.5 eq. of acetone cyanohydrin was added. The mixture was shaken at 250 rpm and monitored by TLC ($CHCl_3$:methanol, 8:1). When the reaction was finished, the catalyst was filtered off and washed with ethyl acetate (20 ml). The solvent was evaporated and the chiral cyanohydrins (***R***)-**3** or (***R***)-**4** were purified by flash chromatography ($CHCl_3$:methanol, 8:1).

*2.5.1.1. (R)-2,6-Dihydroxyhexanenitrile ((**R**)-**3**).*

Colorless oil. Yield: 72%. $[\alpha]_D^{25}$ +3.23 (*c* 1.2, acetone), ee 50%. IR (KBr): ν 3313 and 2270 cm^{-1}. ^1H NMR (MeOD-d_4, 300 MHz): δ 1.70–1.82 (m, 4H), 1.96–2.03 (m, 2H), 3.77 (t, 2H, $^3J_{HH}$ 5.9 Hz) and 4.67 (t, 1H, $^3J_{HH}$ 6.8 Hz). ^{13}C NMR (MeOD-d_4, 75.5 MHz): δ 22.3 (CH_2), 32.9 (CH_2), 36.3 (CH_2), 61.6 (CH), 62.5 (CH_2) and 121.8 (CN). MS (EI, *m/z*): 129 (M^+, 40%), 111 (30), 85 (100) and 71 (22). Anal. Calcd. (%) for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 56.0; H, 8.4; N, 10.6.

*2.5.1.2. (R)-2,5-Dihydroxypentanenitrile ((**R**)-**4**).*

Colorless oil. Yield: 78%. $[\alpha]_D^{25}$ +2.70 (*c* 1.0, acetone), ee 62%. IR (KBr): ν 3320 and 2255 cm^{-1}. ^1H NMR (MeOD-d_4, 200 MHz): δ 1.84–1.95 (m, 2H), 1.99–2.10 (m, 2H), 3.79 (t, 2H, $^3J_{HH}$ 6.0 Hz) and 4.71 (t, 1H, $^3J_{HH}$ 6.3 Hz). ^{13}C NMR (MeOD-d_4, 75.5 MHz): δ 29.3 (CH_2), 33.7 (CH_2), 62.0 (CH), 62.6 (CH_2) and 122.2 (CN). MS (EI, *m/z*): 115 (M^+, 56%), 97 (50), 71 (70) and 57 (100). Anal. Calcd. (%) for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.16. Found: C, 52.0; H, 8.1; N, 12.4.

*2.6. General procedure for the enzymatic synthesis of (R)-2,6-dihydroxyhexanenitrile ((**R**)-**3**) using purified enzymes*

To a solution of 200 mg (1.96 mmol) of **1** in 8 ml of diisopropyl ether containing 1.5 eq. of acetone cyanohydrin, HNL (~250 units) dissolved in 500 μl of citrate buffer 0.1 M, pH 5.4, was added and the biphasic system shaken at 250 rpm. At the end of the reaction, the mixture was washed with a solution of $NaHSO_3$ 10% (10 ml) and extracted with diethyl ether (3 × 15 ml). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The cyanohydrin (***R***)-**3** was purified by flash chromatography ($CHCl_3$:methanol, 8:1).

*2.7. General procedure for the non-enzymatic synthesis of racemic 2,6-dihydroxyhexanenitrile (**3**) and racemic 2,5-dihydroxypentanenitrile (**4**)*

To a solution of 5.9 mmol of **1** or **2** in 1 ml of water containing 1.1 eq. of KCN, 2 ml of an aqueous solution of $NaHSO_3$ 40% were added dropwise at 0 °C. The reaction was stirred for 20 h. Then, the mixture was extracted with AcOEt (3 × 10 ml). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The cyanohydrins were purified by flash chromatography ($CHCl_3$:methanol, 8:1). Yield **3**: 25% and **4**: 21%.

2.8. Determination of the enantiomeric excesses

A total of 115 μl of acetic anhydride and 95 μl pyridine were added to a solution of 0.5 mmol of (***R***)-**3** or (***R***)-**4** in 3 ml of CH_2Cl_2. After stirring at room temperature for 6 h, 5 ml of CH_2Cl_2 were added and the mixture was washed with HCl 1.0N (4 × 10 ml). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated to give (*R*)-2,6-diacetoxyhexanenitrile (***R***)-**5** or (*R*)-2,5-diacetoxypentanenitrile (***R***)-**6**. The enantiomeric excesses were determined by ^1H NMR using a chiral shift reagent, Europium tris[3-(heptafluropropylhydroxymethylene)-(+)-camphorate].

*2.8.1. (R)-2,6-Diacetoxyhexanenitrile ((**R**)-**5**)*

Pale yellow oil. Yield: 84%. $[\alpha]_D^{25}$ +3.31 (*c* 1.0, $CHCl_3$), ee 50%. IR (KBr): ν 2311 and 1747 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.55–1.70 (m, 4H), 1.87–1.95 (m, 2H), 2.03 (s, 3H), 2.12 (s, 3H), 4.06 (t, 2H, $^3J_{HH}$ 6.1 Hz) and 5.30 (t, 1H, $^3J_{HH}$ 6.6 Hz). ^{13}C NMR ($CDCl_3$, 50.4 MHz): δ 20.2 (CH_3), 20.8 (CH_3), 21.7 (CH_2), 27.7 (CH_2), 31.7 (CH_2), 60.7 (CH), 63.6 (CH_2), 116.6 (CN), 169.0 (C=O) and 171.2 (C=O). MS (E.S.I.$^+$, *m/z*): 252 [$(M + K)^+$, 32%], 236 [$(M + Na)^+$, 100] and 214 [$(M + H)^+$, 6]. Anal. Calcd. (%) for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.2; H, 7.2; N, 6.4.

*2.8.2. (R)-2,5-Diacetoxypentanenitrile ((**R**)-**6**)*

Pale yellow oil. Yield: 82%. $[\alpha]_D^{25}$ +2.81 (c = 1.14, $CHCl_3$), ee 62%. IR (KBr): ν 2300 and 1740 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.82–1.91 (m, 2H), 1.95–2.04 (m, 2H), 2.07 (s, 3H), 2.15 (s, 3H), 4.12 (t, 2H, $^3J_{HH}$ 6.2 Hz) and 5.37 (t, 1H, $^3J_{HH}$ 6.4 Hz). ^{13}C NMR ($CDCl_3$, 50.4 MHz): δ 20.3 (CH_3), 20.8 (CH_3), 23.8 (CH_2), 29.1 (CH_2), 60.6 (CH), 62.9 (CH_2), 116.5 (CN), 169.0 (C=O) and 170.8 (C=O). MS (E.S.I.$^+$, *m/z*): 238 [$(M + K)^+$, 49%], 222 [$(M+Na)^+$, 100] and 200 [$(M + H)^+$, 4]. Anal. Calcd. (%) for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.5; H, 6.4; N, 6.8.

3. Results and discussion

5-Hydroxypentanal (**1**) and 4-hydroxybutanal (**2**) were isolated for the most part as its hemiacetalic form (Scheme 1) (determined by ^{1}H and ^{13}C NMR analysis). The hemiacetal **1** was obtained by reduction of the δ-lactone with DIBAL-H and the hemiacetal **2**, from 2-ethoxytetrahydrofuran by hydrolysis with diluted sulfuric acid and aftermost treatment with acetic acid and neutralization with sodium hydroxide, (see Section 2).

Firstly, we carried out the enzymatic reaction under usual conditions, acetone cyanohydrin as a source of HCN, 30 °C, 250 μl mmol^{-1} of buffer and diisopropyl ether or ethyl acetate as organic solvent (Scheme 2).

As one can see from the results shown in Table 1 (entries 4, 7, 11 and 13), both aldehydes are substrates for the enzyme but the enantiomeric excesses of the products are only low or moderate. The main reason for these low enantiomeric excesses is the significant amount of product formed through the undesirable parallel chemical reaction. In order to minimize this chemical addition, we studied the enzymatic process under different reaction conditions. Diisopropyl ether and ethyl acetate are the more common solvents in (*R*)-oxynitrilase-catalyzed reactions. In general, the reaction rate is faster in diisopropyl ether, but the optical yields of the products can be better in diisopropyl ether or in ethyl acetate depending on other reaction parameters. Then, we study the effect of these parameters testing both solvents.

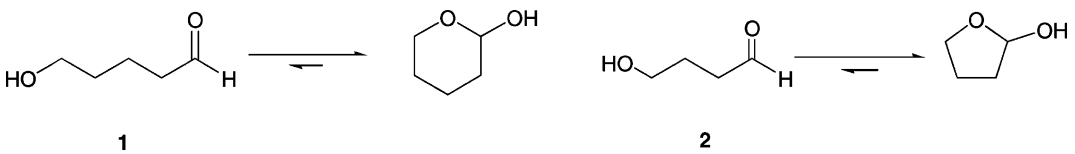
3.1. Temperature effect

First, we study the effect of temperature in the transformation of substrate **1** (entries 1–7, Table 1). As it can be expected, the conversion of both processes, the enzymatic and the competing spontaneous reaction, increase with temperature. The slower rate of the chemical process at lower temperatures enables an improvement of the optical yields of the cyanohydrins, even though higher reaction times are necessary for the total conversion of the substrate. The best optical yields are obtained at 15 °C. At lower temperature the activity of the enzyme dramatically decrease. These results are clearly shown in Fig. 1.

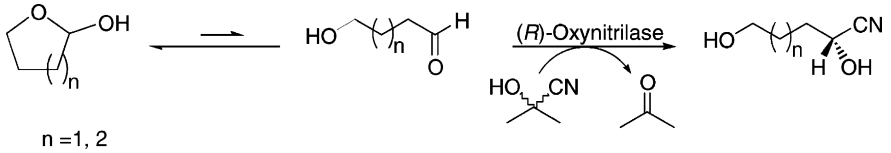
Similar behavior is observed for substrate **2**, at the same reaction conditions (Table 1, entries 10–13). In both solvents, ethyl acetate and diisopropyl ether, the optical purity of the cyanohydrins is higher when the reactions are carried out at 15 °C.

3.2. Enzyme concentration

As can be expected, increasing the amount of enzyme increases the rate of the enzymatic reaction. For substrate **1**, a 100% conversion can be achieved in 16 h at 30 °C when double amount of enzyme was employed and the enantiomeric excess slightly increase from 17 (entry 4) to 28%. Under the same conditions, similar results are obtained for substrate **2**: a total conversion is achieved in 60 h at 30 °C and the enantiomeric excess increase from 4 (entry 11) to 10%.



Scheme 1.



Scheme 2.

Table 1
Enzymatic transcyanation over substrates **1** and **2** using a buffer–organic solvent system

Entry	Substrate	Solvent	Temperature (°C)	t (h)	c[a] (%)	ee[b] (%)	c[c] (%)
1	1	$^{i}Pr_2O$	10	278	90	26	17
2	1	$^{i}Pr_2O$	15	168	100	46	21
3	1	$^{i}Pr_2O$	20	96	100	32	24
4	1	$^{i}Pr_2O$	30	25	100	17	32
5	1	$^{i}Pr_2O$	40	15	100	13	41
6	1	AcOEt	15	168	90	30	20
7	1	AcOEt	30	35	100	23	24
8[d]	1	$^{i}Pr_2O$	15	168	75	33	21
9[d]	1	$^{i}Pr_2O$	30	48	92	20	32
10	2	$^{i}Pr_2O$	15	240	97	25	38
11	2	$^{i}Pr_2O$	30	96	100	4	48
12	2	AcOEt	15	240	85	27	40
13	2	AcOEt	30	140	94	15	45

Buffer = 250 μl mmol^{-1}.
[a] Conversion of the enzymatic process, crude enzyme = 200 mg mmol^{-1}.
[b] Values determined after acetylation by ^{1}H NMR in presence of Eu(hfc)$_3$.
[c] Conversion of the process carried out in absence of enzyme.
[d] These reactions were carried out using (*R*)-oxynitrilase from *Prunus amygdalus* commercialized by Sigma.

3.3. Micro-aqueous medium

The use of a small amount of buffer (2–10%) in an immiscible organic solvent is the usual procedure for (*R*)-oxynitrilase-catalyzed reactions. Lin and co-workers [11] claimed that, as the competing non-enzymatic reaction occurs in the aqueous phase, this undesirable process can be suppressed using a micro-aqueous medium. In order to compare the results, we repeat the biotransformations at 15 and 30 °C without buffer. The data in Table 2 clearly show a significant improvement of enantiomeric excess for substrate **1** in both solvents (entries 14–17), even though the reaction conversion considerably

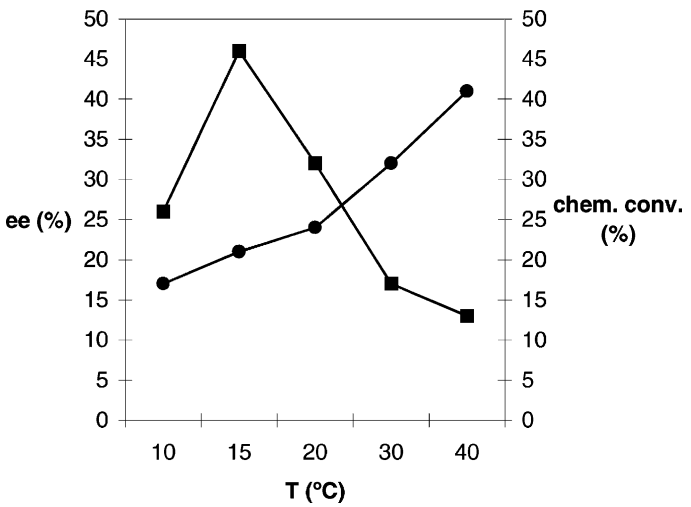


Fig. 1. Effect of the temperature in the enantioselectivity of the enzymatic transcyanation of **1** catalyzed by PaHNL (■) (Table 1, entries 1–5) and percent of the non-enzymatic reaction at each temperature (●).

Table 2
Enzymatic transcyanation over substrates **1** and **2** in micro-aqueous medium

Entry	Substrate	Solvent	Temperature (°C)	t (h)	c^a (%)	ee^b (%)	c^c (%)
14	1	^{i}Pr$_2$O	15	200	84	50	14
15	1	^{i}Pr$_2$O	30	72	80	21	17
16	1	AcOEt	15	200	78	46	12
17	1	AcOEt	30	76	74	24	15
18	2	^{i}Pr$_2$O	15	280	64	62	13
19	2	^{i}Pr$_2$O	30	140	96	43	21
20	2	AcOEt	15	300	55	60	15
21	2	AcOEt	30	190	90	45	22

[a] Conversion of the enzymatic process, crude enzyme = 200 mg mmol^{-1}.
[b] Values determined after acetylation by ^{1}H NMR in presence of Eu(hfc)$_3$.
[c] Conversion of the process carried out in absence of enzyme.

decreases. More dramatic is the effect of the suppression of water in the reaction media for substrate **2** (entries 18–21), the enantiomeric excess increases from 25 to 62% in diisopropyl ether at 15 °C. Similar behavior is observed in ethyl acetate at 15 °C and in both solvents at 30 °C. Whereby, as for substrate **1**, the reaction rate decrease in these conditions.

3.4. Effect of the conversion

In order to minimize the effect of the chemical reaction, we decided to study the enzymatic process at lower conversions. We carried out a typical enzymatic reaction over substrate **2** (Table 3). As we expected, there was a little increase in the enantiomeric purity of the (*R*)-cyanohydrin (***R*)-4** when we stopped the reactions at shorter times of reaction. We can only obtain (***R*)-4** with an enantiomeric excess of 63% at 15% of conversion in 17 h (entry 22), although we had reduced the chemical reaction to only a 4% of conversion.

Table 3
Effect of the conversion vs. the enantiomeric excess over substrate **2**[a]

Entry	t (h)	c^b (%)	ee^c (%)	c^d (%)
22	15	15	63	4
23	30	34	56	9
24	70	63	49	16
19	140	96	43	21

[a] Reactions were carried at 30 °C, in micro-aqueous medium and using diisopropyl ether as the solvent.
[b] Conversion of the enzymatic process, crude enzyme = 200 mg mmol^{-1}.
[c] Values determined after acetylation by ^{1}H NMR in presence of Eu(hfc)$_3$.
[d] Conversion of the process carried out in absence of enzyme.

3.5. Acetone cyanohydrin content

We also studied the effect of the acetone cyanohydrin content in the enzymatic synthesis of (***R*)-4** (Table 4). The enzymatic transcyanation of aldehydes is a two-step reversible process, where in a first step catalyzed by the (*R*)-oxynitrilase, hydrogen cyanide is liberated from the acetone cyanohydrin. The concentration of acetone cyanohydrin and hydrogen cyanide in the reaction medium can affect the activity of the enzyme (Fig. 2). We carried out all the enzymatic reactions using 1.5 eq. of acetone cyanohydrin, a content of cyanohydrin which had been optimized in our previous works [3]. When we used 2 eq. of

Table 4
Effect of the concentration of acetone cyanohydrin in the enzymatic transcyanation of **2**[a]

Entry	n^b	c^c (%)	ee^d (%)	c^e (%)
25	1.0	10	61	0
23	1.5	34	56	9
26	2.0	38	53	12
27	4.0	43	5	35

[a] Reactions were carried at 30 °C, in micro-aqueous medium, using diisopropyl ether as the solvent and were stopped at 2 h.
[b] n = number of equivalents.
[c] Conversion of the enzymatic process, crude enzyme = 200 mg mmol^{-1}.
[d] Values determined after acetylation by ^{1}H NMR in presence of Eu(hfc)$_3$.
[e] Conversion of the process carried out in absence of enzyme.

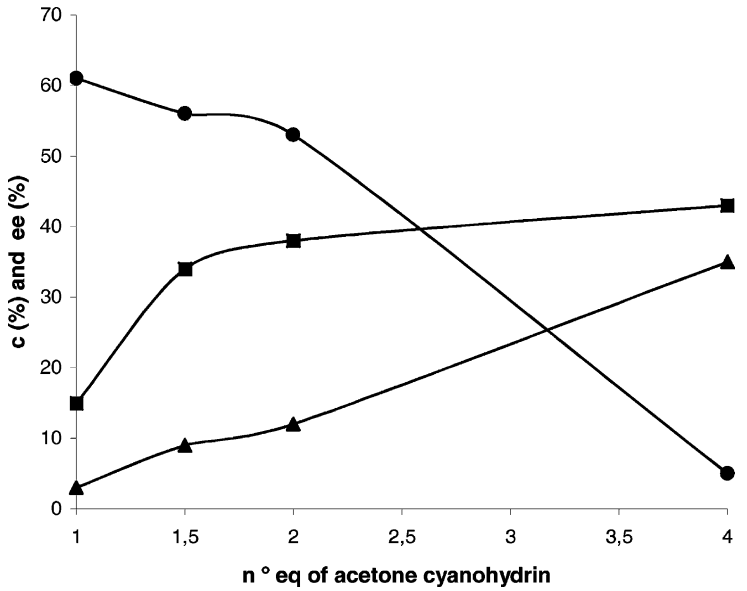


Fig. 2. Effect of the content of acetone cyanohydrin in the enantioselectivity of the enzymatic transcyanation of **2** catalyzed by PaHNL (●) (Table 4, entries 23 and 25–27), conversion of the enzymatic process (■) and percent of the non-enzymatic reaction at each condition (▲).

cyanohydrin (entry 26), we obtained similar values of the conversion and the enantiomeric excess. Then, we made the same process using 1 eq. of the cyanide source (entry 25). In these conditions the reaction was very slow, with only a 15% of conversion at 30 h, but we almost suppressed the chemical reaction and the cyanohydrin was obtain with a slightly bigger optical purity. We decided to increase the concentration of acetone cyanohydrin by using 4 eq. (entry 27), but in this case we found that the cyanohydrin obtained was almost racemic. The conversion of this reaction is very similar to the process carried out with 2 eq. and mainly this conversion is debt to the chemical reaction, so there must be some effect in the activity of the (*R*)-oxynitrilase when we work with contents of acetone cyanohydrin higher than 2 eq.

3.6. Other sources of enzyme

Finally, we carried out the reaction with substrate **1** using several commercial (*S*)- and (*R*)-oxynitrilases. Processes carried out with (*S*)-oxynitrilase from *Manihot esculenta* and with (*R*)-oxynitrilases from *Linux usiatissimum* and *Prunus amygdalus* commercialized by Jülich achieved racemic cyanohydrins. (*R*)-Oxynitrilase from *P. amygdalus*, commercialized as a crude by Sigma, yields similar enantiomeric excess but lower conversion than the crude enzyme prepared in our laboratory in the process carried out in diisopropyl ether at 30 °C (entry 9). At 15 °C in the same solvent, we observe poorer conversion and enantiomeric excess (entry 8).

4. Conclusions

We have studied the (*R*)-oxynitrilase-catalyzed transcyanation of ω-hydroxyalkanals. These aldehydes are isolated mainly in its hemiacetal form and can be considered difficult substrates because of its relative high water solubility. The predictable low enantiomeric excesses of the obtained cyanohydrins are mainly due to the non-enzymatic reaction that occurs in competition with the enzymatic hydrocyanation. By varying the different reaction parameters we can reduce the extension of the undesirable competing reaction and optimize the optical purity of the obtained cyanohydrins. The effect of temperature in the hydrocyanation of both substrates is noteworthy; the best results are obtained a 15 °C. The effect of concentration of water in the reaction media is also remarkable. For 4-hydroxybutanal (**2**), the suppression

of buffer in the enzymatic transcyanation significantly increases the enantiomeric excess of the product. This effect is less important for 5-hydroxypentanal (**1**). Nevertheless, the enantiomeric excesses can be slightly improved in the micro-aqueous medium and by carrying out the reactions at lower conversions.

Acknowledgements

Financial support of this work by CICYT (Project BIO-98-0770) is gratefully acknowledged.

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