Pergamon

TETRAHEDRON

Tetrahedron 58 (2002) 2979–2983

Selectivity of the (*S*)-oxynitrilase from *Hevea brasiliensis* towards α- and β-substituted aldehydes☆

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Received 9 November 2001; accepted 21 February 2002

Abstract—The performance of (*S*)-oxynitrilase from *Hevea brasiliensis* (HbHNL) has been investigated with several α- and β-substituted alkyl or alkoxy aldehydes and the results have been compared to the data previously obtained with the (*R*)-specific enzyme from almonds (PaHNL). With both enzymes there was no chiral discrimination between the enantiomers of the racemic substrates, therefore this reaction cannot be used as a preparative method to achieve both the kinetic resolution of the starting racemic aldehyde and the production of diastereomerically pure (or enriched) cyanohydrins. Additionally, in comparison with the (*R*)-PaHNL the (*S*)-selective enzyme from *Hevea* gave products with higher de, but was more negatively effected by oxygenated substituents. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Within the last few years the enzymatic synthesis of cyanohydrins has become a valuable enantioselective access to these compounds.[2] However, less is known about the influence of substituents in the starting aldehyde or ketone on the outcome of the reaction. In particular, it is important to know if and to what extent a stereocentre, situated in the starting carbonyl compound close to the aldehyde functionality, influences the stereochemical outcome of the reaction with respect to diastereoselectivity.

Recently, protected α-hydroxy aldehydes were investigated using the (*R*)-selective oxynitrilase from almonds (*Prunus amygdalus*, PaHNL)[1] and the (*S*)-selective enzymes from manihot (*Manihot esculenta*, MeHNL)[3] and *Hevea brasiliensis* (HbHNL).[1] Despite the fact that good selectivity with respect to the newly formed stereocentre was observed, there was no directing effect of the stereogenic centre already present in the substrate.

In this publication these investigations have been extended to the HbHNL-catalyzed cyanuration of alkyl substituted aldehydes and of some α-alkoxy aldehydes with related structures. The results have been compared to the data previously obtained with PaHNL,[4] allowing us to draw some general conclusions on the selectivity of these two enzymes.

2. Results and discussion

The performances of HbHNL were investigated with the substrates **1–13** (Scheme 1), which were synthesised as previously reported.[4a–c] The reaction products from compounds **1** and **7** are given as an example in Scheme 2. The enzymatic transformations were carried out in a biphasic system buffer/diisopropyl ether; substrates were quantitatively converted into the corresponding cyanohydrins which were isolated by flash chromatography (recovered yields 75–90%). Analyses of the products were performed either by chiral HPLC or, after acetylation, by chiral GC.[4a–c] The assignment of the absolute configuration of the products corresponding to the baseline-separated chromatographic peaks was performed by means of stereochemical correlations, as previously described for the reaction catalysed by the (*R*)-selective enzyme from almonds.[4a–c]

The results obtained with HbHNL are reported in Tables 1 and 2, the data previously collected with the oxynitrilase from almonds also being included for the sake of comparison.

☆ Oxynitrilases in organic synthesis. Part 5. For Part 4 see Ref. 1.

Keywords: oxynitrilases; cyanohydrins; *Hevea brasiliensis*; *Prunus amygdalus*; substituted aldehydes.

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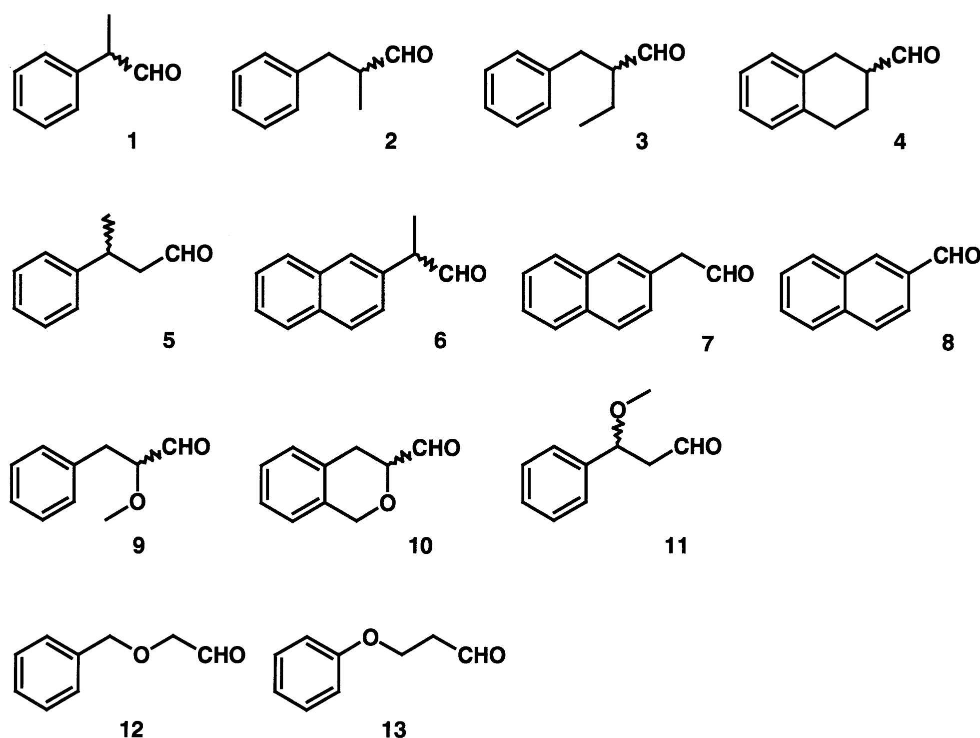
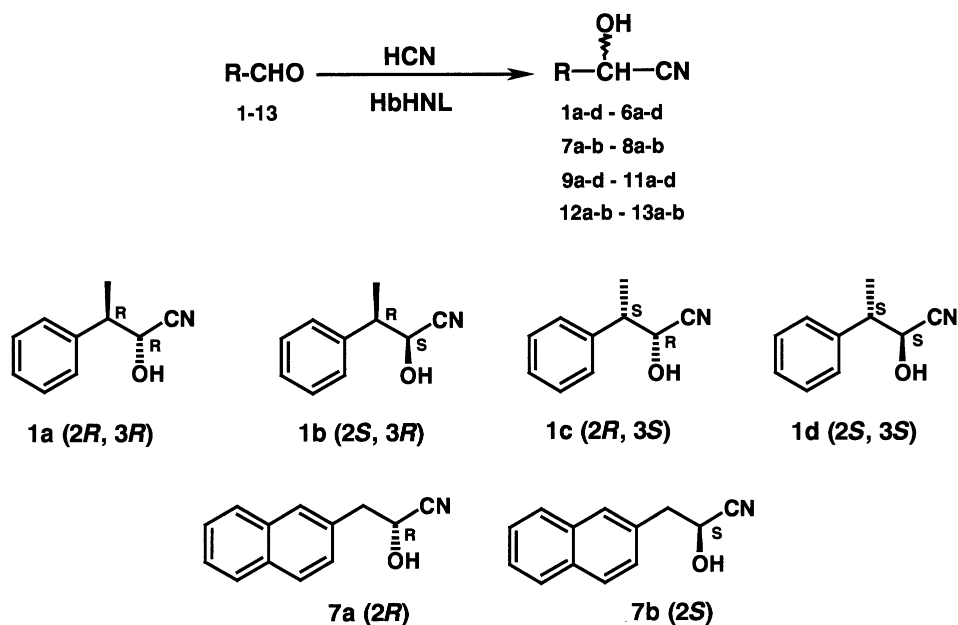
PII: S0040-4020(02)00187-4



Scheme 1.



Scheme 2.

Both enantiomers of the racemic aldehydes **1–6** and **9–11** were cyanurated with comparable rates, therefore this reaction cannot be used as a preparative method to achieve both the kinetic resolution of the starting racemic aldehyde and the production of diastereomerically pure (or enriched) cyanohydrins. However, the de of the products **1a–d—6a–d** and **9a–d—11a–d** were strongly dependent on the configuration of the stereocentre already existing in the starting molecule.

Specifically, applying HbHNL the de was, as a rule, 89–98% (although the result with substrate **4** was anomalous) with the (*R*)-enantiomers of the aldehydes **1–6**, whereas the de was lower, sometimes significantly, with the (*S*)-enantiomers. A similar effect had been found

Table 1. Diastereomeric composition of reaction products **1a–d—6a–d** and **9a–d—11a–d**

Substrate	HNL	Cyanide source	% Diastereomeric composition					
			2*R*,3*R*	2*S*,3*R*	de 3*R*	2*R*,3*S*	2*S*,3*S*	de 3*S*
1	(*S*)-HbHNL	HCN	0.4	49.5	98.3	3.3	46.8	86.8
	(*S*)-HbHNL	$(CH_3)_2C(OH)CN$	2.5	48.5	90.2	10.3	38.6	57.8
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[a]	17.6	27.6	22.1	51.8	3.0	89.1
	(*R*)-PaHNL	HCN	21.9	28.2	12.6	37.9	11.9	52.2
2	(*S*)-HbHNL	HCN	2.7	47.7	89.3	7.4	42.2	70.2
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[a]	26.5	13.8	31.5	57.1	2.6	91.3
3	(*S*)-HbHNL	HCN	0.7	50.2	97.2	0.9	48.2	96.3
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[a]	42.8	0	100	57.2	0	100
4	(*S*)-HbHNL	HCN	11.2	38.2	54.7	11.8	38.8	53.4
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[a]	46.7	4.2	83.5	44.5	4.6	81.3
5[b]	(*S*)-HbHNL	HCN	0.5	49.2	98.0	6.0	44.3	76.1
	(*S*)-HbHNL	$(CH_3)_2C(OH)CN$	7.0	43.7	72.4	37.3	12.0	51.3
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[a]	44.5	3.9	83.9	48.5	3.1	88.0
	(*R*)-PaHNL	HCN	40.9	7.9	67.6	42.2	9.1	64.5
6	(*S*)-HbHNL	HCN	4.2	45.9	83,2	21.2	28.7	15.0
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[c]	25.6	20.0	12.3	24.0	30.4	11.8
9	(*S*)-HbHNL	HCN	27.6	20.6	14.5	27.6	24.2	6.4
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[c]	8.4	40.1	65.4	8.4	43.1	67.4
10	(*S*)-HbHNL	HCN	39.3	14.8	45.3	31.3	14.5	36.7
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[c]	21.1[d]	28.7[e]	15.3	21.5[d]	28.6[e]	14.2
11[b]	(*S*)-HbHNL	HCN	16.6	33.9	34.3	17.6	31.9	28.9
	(*R*)-PaHNL	$(CH_3)_2C(OH)CN$[c]	34.3	14.0	42.0	45.2	6.5	74.9

[a] Data from Ref. 4b for comparison.
[b] Diastereoisomers are 2*R*,4*R*; 2*S*,4*R*; 2*R*,4*S*; 2*S*,4*S*.
[c] Data from Ref. 4a for comparison.
[d] Values might be exchanged.
[e] Values might be exchanged.

Table 2. Enantiomeric composition of the reaction products **7a–b**, **8a–b**, **12a–b**, **13a–b**

Substrate	HNL	% Enantiomeric composition		
		2*R*	2*S*	ee
7	(*S*)-HbHNL	15.6	84.3	68.8
	(*R*)-PaHNL[a]	33.2	66.8	33.6
8	(*S*)-HbHNL	17.0	83.0	66.0
	(*R*)-PaHNL[a]	97.6	2.4	45.2
12	(*S*)-HbHNL	50.0	50.0	0
	(*R*)-PaHNL[a]	43.9	56.1	12.2
13	(*S*)-HbHNL	4.2	95.8	91.6
	(*R*)-PaHNL[a]	74.3	25.7	48.6

[a] Data from Ref. 4a for comparison.

with the (*S*)-selective enzyme from *M. esculenta* by Bühler with compounds **1** and **5**.[5] As might be expected, the (*R*)-selective oxynitrilase from almonds showed a similar but opposite effect: the (*S*)-enantiomers were much better substrates than the (*R*)-aldehydes.[4] The substrate that gave the lowest de with HbHNL was the (*S*)-enantiomer of **6** (de 15.0%), a compound that was also non-selectively transformed by PaHNL.

Different reaction protocols were compared with **1** and **5** as substrates, using either HCN or acetone cyanohydrin as cyanide source. Longer reaction times were always needed using acetone cyanohydrin and the transhydrocyanation protocol, while the de's obtained with this approach (compared with the direct cyanuration with HCN) were lower with HbHNL and higher with PaHNL.

The negative influence of an oxygenated substituent can be clearly evidenced by comparing the de obtained with the aldehydes **3–5** and with their oxygenated analogues **9–11**. In the case of molecules **3** and **9**, for instance, the de values moved from 97.2 and 96.3% (with HbHNL acting on **3**) to 14.5 and 6.4% (with HbHNL acting on **9**). A similar, but less pronounced effect, had been previously observed with PaHNL:[4] considering the same two aldehydes the de values decreased from 100 to 65.4 and 67.4%.

The data in Table 2 show the performance of HbHNL towards other substrates that had been previously investigated with PaHNL.[4] The α-oxygenated aldehyde **12** was a substrate which was transformed with poor selectivity by both enzymes, while the other three aldehydes yielded products with higher ee with HbHNL. It has to be pointed out that HbHNL always produced cyanohydrins enriched in the expected (*S*)-isomer, while PaHNL sometimes acted in an 'unnatural' way (see data obtained with **7** and also with (*R*)-**1** and (*S*)-**6**, giving products enriched in the (*S*)-enantiomer).

In conclusion, this systematic investigation allows a comparison of the performances of two oxynitrilases with opposite stereoselectivity. As a general trend, in comparison with the (*R*)-PaHNL the (*S*)-selective enzyme from *Hevea* gave products with higher de, but this was diminished by oxygenated substituents. Neither of the enzymes are able to catalyse the kinetic resolution of α- or β-substituted racemic aldehydes, and from the limited data available this is also the case for the enzyme isolated from *Manihot*.[5] In future work we will try to rationalize these experimental results by molecular modelling.[6]

3. Experimental

3.1. Materials and methods

The oxynitrilase from *H. brasiliensis* (HbHNL) was obtained from Roche Diagnostics. Acetone cyanohydrin and other reagents were from Aldrich. HPLC analyses were performed using a Chiralcel OD column (from DIACEL) and a Jasco 880/PU instrument equipped with a Jasco 875 UV/Vis detector (reading was done at 254 nm). GC analyses were performed using a Chrompack capillary column fused silica gel coated with CP-cyclodex B236M and a Hewlett–Packard 5890 series II instrument.

3.2. Synthesis of aldehydes

Aldehydes **1**, **5**, **8**, and **12** were commercially available (Aldrich). Aldehydes **2–4**, **6**, **7**, **9**, **10**, **11**, **13**, and **14** were prepared as previously described.[4a,b]

3.3. General procedures for the enzymatic synthesis of the cyanohydrins

3.3.1. Protocol using HCN. HbHNL (~6360 units in 1.2 ml of citrate buffer pH 4.5) was added to a solution of 250 mg of aldehyde in 1.5 ml of isopropyl ether. Neat HCN (5 equiv.) was added at 0°C, and the reaction was stirred at 15°C until the complete conversion of the substrate was reached. Celite was added to absorb the enzyme and the aqueous phase; the organic phase was dried with Na_2SO_4 and evaporated. The cyanohydrins were purified by flash chromatography (eluents were the same described in previous papers of this series[4a,b]).

3.3.2. Protocol using acetone cyanohydrin. To a solution of 250 mg of aldehyde in 10 ml of isopropyl ether containing 1.3 equiv. of acetone cyanohydrin, HbHNL (~1500 units) dissolved in 500 μl of 0.1 M citrate buffer pH 5.5, was added and the biphasic system shaken at room temperature for 5 days. At the end of the reaction the two phases were separated, the aqueous phase was extracted with isopropyl ether, the organic phases were dried with Na_2SO_4 and evaporated. The cyanohydrins were purified by flash chromatography (eluents were the same described in previous papers of these series[4a,b]).

^{1}H NMR data ($CDCl_3$) **1a–d** δ: 7.30 (m, 5H, ArH); 4.49 and 4.48 (d, 1H, J=7.1 Hz, H-2); 3.15 (m, 1H, H-3); 1.46 and 1.44 (d, 3H, J=7.3 Hz CH_3). **2a–d** δ: 7.40–7.10 (m, 5H, ArH); 4.33 and 4.37 (d, each 0.5H, J=6.2 Hz, H-2); 2.92 and 2.80 (dd, each 0.5H, J_1=7.5 Hz, J_2=13.7 Hz, H-4a); 2.64 and 2.60 (dd, each 0.5H, J_1=7.5 Hz, J_2=13.5 Hz, H-4b); 2.22 (m, 1H, H-3); 1.12 and 1.10 (d, each 1.5H, J=6 Hz, CH_3). **3a–d** δ: 7.4–7.1 (m, 5H, ArH); 4.45 and 4.38 (d, each 0.5H, J=4.5 Hz, H-2); 2.90 and 2.73 (dd, each 0.5H, J_1=13.4 Hz, J_2=6.0 Hz, H-4a); 2.75 and 2.68 (dd, each 0.5H, J_1=13.4 Hz, J_2=7.5 Hz, H-4b); 2.0 (m, 1H, H-3); 1.55 (m, 2H, CH_2); 1.12 and 1.10 (t, each 1.5H, J=7 Hz, CH_3). **4a–d** δ: 7.2–7.0 (4H, m, ArH); 4.43 and 4.40 (each 0.5H, d, J=6 Hz, *CH*–CN); 3.1–2.7 (5H, m, CH_2-1, CH_2-4, H-2); 2.3–1.5 (2H, m, CH_2-3). **5a–d** δ: 7.30 (m, 5H); 4.17 (t, 1H, J=7.2 Hz, H-2); 3.12–2.92 (m, 1H, H-4); 2.20–2.02 (m, 2H, H-3); 1.31 and 1.33 (d, 3H, J=7.3 Hz, CH_3). **6a–d**: δ=8.00–7.30 (m, 7H, ArH); 4.60 (dd, 1H, J_1=8.6 Hz, J_2=6.8 Hz, H-2); 3.35 (quint, 1H, J=6.8 Hz, H-3); 1.60 (d, 3H, J=6.8 Hz, CH_3). **7a–b**: ($CDCl_3$+D_2O) δ=8.00–7.20 (m, 7H, ArH); 4.75 (t, 1H, J=8 Hz, H-2); 3.30 (d, 2H, J=8 Hz, CH_2-3). **8a–b**: δ=8.02 (s, 1H, ArH); 7.95–7.82 (m, 3H, ArH); 7.62–7.52 (m, 3H, ArH); 5.72 (d, 1H, J=6.8 Hz, H-2); 2.25 (d, 1H, J=6.8 Hz, OH). **9a–d** δ: 7.25 (m, 5H, ArH); 4.25 (d, 1H, J=3 Hz, H-2); 3.63 (m, 1H, H-3); 3.51 and 3.48 (s, each 1.5H, OCH_3); 3.15 and 2.98 (dd, each 0.5H, J_1=14.2 Hz, J_2=6 Hz, H-4a); 2.88 and 2.82 (dd, each 0.5H, J_1=14.2 Hz, J_2=7.5 Hz, H-4b). **10a–d** δ: 7.30–7.00 (m, 4H, ArH); 4.95 (dd, 2H, J_1=5 Hz, J_2=14 Hz, H-1a); 4.88 (dd, J_1=8 Hz, J_2=14 Hz, 2H, H-1b); 4.58 (d, 0.5H, J=4 Hz, *CH*–CN); 4.52 (d, 0.5H, J=5 Hz, *CH*–CN); 3.98 (m, 1H, H-3); 3.05 (m, 2H, H-4a); 2.8 (m, 2H, H-4b). **11a–d** δ: 7.35 (m, 5H, ArH); 4.75 (dd, 1H, J_1=6 Hz, J_2=3 Hz, H-4); 4.42 (dd, 1H, J_1=6 Hz, J_2=3 Hz, H-2); 3.30 and 3.22 (s, each 1.5H, OCH_3); 2.35 and 2.08 (m, each 1H, H-3a, H-.3b). **12a**,**b**: δ: 7.40 (m, 5H, ArH); 4.65 (s, 2H, benzylic CH_2); 4.55 (t, 1H, J=7 Hz, H-2); 3.75 (d, 2H, J=7 Hz, CH_2-3). **13a**,**b**: δ: 7.40–6.80 (m, 5H, ArH); 4.83 (m, 1H, H-2); 4.28 (m, 2H, CH_2-4); 2.90 (d, 1H, J=6 Hz, OH); 2.33 (m, 2H, CH_2-3).

3.4. Stereochemical correlation

GC- and HPLC-analyses and stereochemical correlations were performed as previously described.[4a,b] In this way it was possible to evaluate the diastereomeric or the enantiomeric compositions of the enzymatically produced cyanohydrins, obtaining the data reported in Tables 1 and 2.

Acknowledgements

Grants obtained within the framework of the Italian–Austrian treaty on scientific and technological cooperation are gratefully acknowledged. Work in Graz was also financed from the special research program 'Biocatalysis' of the Austrian Research Fund.

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