Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 971–974

TETRAHEDRON: *ASYMMETRY*

Difficult substrates in the R-hydroxynitrile lyase catalyzed hydrocyanation reaction: application of the mass transfer limitation principle in a two-phase system

Pieter Jan Gerrits, Jan Marcus,* Lemonia Birikaki and Arne van der Gen

Leiden Institute of Chemistry, Gorlaeus Laboratoria, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

Received 13 March 2001; accepted 24 April 2001

Abstract—The application of a number of new and/or difficult substrates in the catalyzed hydrocyanation reaction by R-hydroxynitrile lyase from almonds is described. By using an aqueous–organic two-phase system and increasing the rate of the enzymatic reaction relative to the mass transfer rate, the enantiomeric purity was improved. By fine tuning the reaction parameters (temperature, pH, and the amount of enzyme) the hydrocyanation reaction was optimized for all substrates. The general principles described here can also be applied to optimize the reaction conditions for other substrates.

1. Introduction

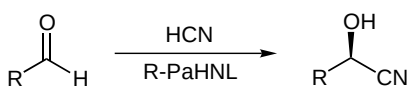
Chiral cyanohydrins can be easily prepared, often in excellent enantiomeric purity, by the addition of HCN to aldehydes and ketones in a reaction catalyzed by the enzyme hydroxynitrile lyase.[1] This enzyme can be found in many plant species including *Prunus amygdalus* (almond),[1,2] *Hevea brasiliensis* (rubber tree)[1,3] and *Sorghum bicolor* (sorghum).[1,2,4]

The enzyme from almonds, R-PaHNL [E.C. 4.1.2.10], is known to only catalyze the formation of cyanohydrins having (*R*)-configuration, provided that the hydroxyl and the nitrile substituents have priority over the alkyl or aryl substituent, R, according to the Cahn–Ingold–Prelog rules[5] (Scheme 1).

Several methods have been developed by which these cyanohydrins can be prepared, often in excellent enantiomeric purity.[1,6] Recently, we published an article that described an optimized aqueous–organic two-phase system using mass-transfer-limitation as a tool to enhance enantioselectivity.[7]

R-PaHNL is characterized by broad substrate acceptance. Aromatic, heteroaromatic, and saturated as well as different types of unsaturated aliphatic aldehydes can be employed in the HNL catalyzed reaction.[1c,1h] Over the years the range of successfully converted substrates has increased considerably. Some substrates however are still considered to be 'difficult' since they can only be synthesized in low enantiomeric purity and/or yield. The enantiomeric purity of the cyanohydrins formed depends on the ratio of the reaction rates of both the enzyme catalyzed reaction and the equivalent non-enzymatic reaction. The latter reaction occurs in the aqueous layer of the two-phase system.

The reason for denoting a substrate as difficult is mainly based upon the sometimes fast non-enzymatic reaction that occurs in competition with the enzyme catalyzed hydrocyanation reaction. These two reactions occur simultaneously during the addition of HCN to the aldehyde. While the enzyme catalyzed reaction produces enantiomerically pure (*R*)-cyanohydrins, the non-enzyme catalyzed reaction produces the racemic product. With difficult substrates, the non-enzymatic reaction competes in such a way that cyanohydrins of poor enantiomeric purity are formed. Therefore, our



Scheme 1. Synthesis of chiral (*R*)-cyanohydrins catalyzed by R-PaHNL.

* Corresponding author. Present address: Goudenregenlaan 21, 2612 VS Delft, The Netherlands. Tel.: +31-70-3607570; fax: +31-84-8820119; e-mail: jmarcus@wanadoo.nl

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00183-5

goal was to reduce the rate of the non-enzymatic reaction, relative to the rate of the enzyme catalyzed reaction. The tools for controlling the rate of the chemical reaction involve changes in the different reaction parameters, based upon the mass-transfer-limitation principle.[7] Herein, we present the results of this investigation.

2. Results and discussion

The aldehyde substrates are presented in Fig. 1. These substrates generally show diminished activity in the enzyme catalyzed hydrocyanation reaction and the outcome of the reactions, both in terms of yield and enantiomeric purity, are often poor. Typical problems with substrates **1a**–**1h** are increased water solubility of the aldehyde and cyanohydrin, and a rapid non-enzymatic reaction.

In a typical hydrocyanation experiment[8] the aldehyde, dissolved in MTBE, is added to a pre-cooled mixture of HCN in MTBE and an aqueous buffer phase containing the enzyme. The reactions were performed in a cooled, double walled, reaction vessel equipped with a mechanical stirrer. In all reactions the type of stirrer, the shape of the reaction vessel and the stirring speed were kept constant. The mass-transfer-limitation principle states that for optimum enantiomeric purity in the enzyme catalyzed hydrocyanation reaction, the concentration of substrate in the aqueous layer must be kept as low as possible. An elaborate explanation for this can be found in our previous publication.[7] Tools for complying with this principle are lowering of the pH and the temperature. It must be remembered that changes in these parameters also influence the course of the enzymatic reaction. Simply lowering the pH does result in suppression of the non-enzymatic reaction. However, the optimum operating pH for R-PaHNL is 5.5–6.0. Deviating from this pH range reduces the activity of the enzyme, resulting in a lower enantiomeric purity of the cyanohydrins formed. In the ideal situation the optimum parameters of the enzyme are retained. Only moderate differentiations of these parameters are allowed to influence the non-enzymatic reaction. Increasing the amount of enzyme has no direct influence on the non-enzymatic reaction but increases the rate of the enzymatic reaction.

By varying the different parameters, good results (both with regard to yield and e.e.) were obtained for the aldehydes shown in Fig. 1. The data for the reactions[9] are presented in Table 1.

2-Propenal **1a** and 3-butenal **1b**, together with previously published[6b] 4-pentenal and 5-hexenal, constitute a new class of substrates: aldehydes with a reactive ω-double bond. The introduced terminal alkene functionality can be exploited in future reaction steps, including intramolecular cycloaddition reactions[6b] and metathesis. To suppress the relatively fast non-enzyme catalyzed reaction both the pH and the temperature of the reaction were lowered compared to the standard protocol[7] (pH 5.5, temp. 5°C). To obtain an optimal result for substrate **1a**, the amount of enzyme was increased. Cyanohydrin **2a** was isolated in 72% yield and with an enantiomeric purity of 86%. Cyanohydrin **2b** was isolated in 90% yield, with an enantiomeric purity of 97%.

The enzymatic hydrocyanation of butyraldehyde **1c** has been described in the literature.[10] By using the almond meal method[6a] cyanohydrin **2c** was isolated in 99% yield with an enantiomeric purity of 95%. By using the purified enzyme and the standard protocol, the enantiomeric purity of cyanohydrin **2c** was increased to 99%, while the yield remained quantitative.

As previously reported by Kyler et al., cyanohydrin **2d** can be obtained from the corresponding aldehyde in 36% yield and 96% enantiomeric purity by a transcyanation procedure.[11] The yield was increased significantly by using the purified enzyme and the standard protocol. Cyanohydrin **2d** was isolated in 91% yield with an enantiomeric purity of 98%.

The published preparation of the cyanohydrin from cinnamaldehyde **1e** are a low yield (45%) and an e.e. of

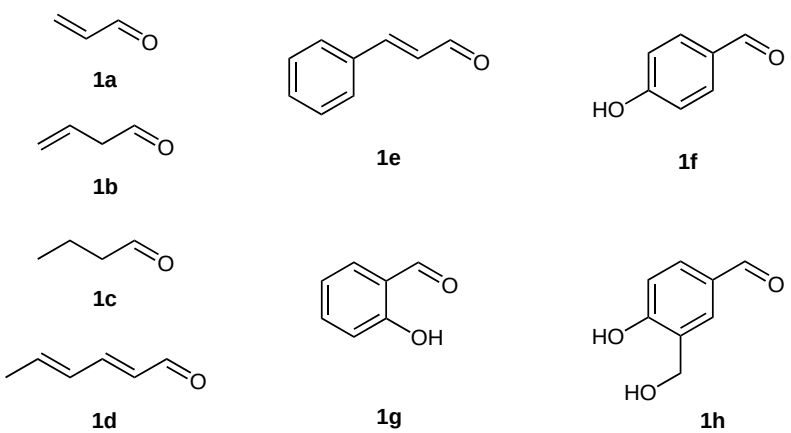
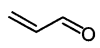
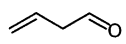
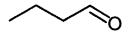
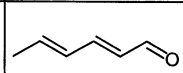
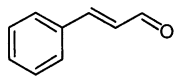
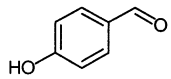
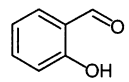
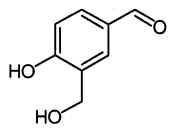


Figure 1. Substrates used in the R-PaHNL catalyzed hydrocyanation reaction.

Table 1. Chiral cyanohydrins by R-PaHNL catalyzed hydrocyanation

Substrate			Pa-HNL	pH	temp	Reaction	(*R*)-Cyanohydrin		
Entry	R	[mmol]	[mg]		[°C]	time [h.]	Entry	Yield [%]	ee [%][a]
1a		50	100	5.0	1	12	**2a**	72	86[b]
1b		25	30	5.0	1	24	**2b**	90	97[b]
1c		82	45	5.5	5	24	**2c**	99	99[b]
1d		50	40	5.5	5	24	**2d**	91	98[b]
1e		60	25	5.5	5	168	**2e**	97	98[c]
1f		50	42	5.5	5	96	**2f**	90	91[c]
1g		50	100	5.5	1	96	**2g**	97	98
1h		3.3	152	5.5	1	340	**2h**	80	58

a) Determined by HPLC; b) E.e. determined after *tert*-butyldiphenylsilyl protection of the hydroxyl group; c) Prior to crystallisation. After crystallisation in both cases: e.e. >99%.

93% (after crystallization).[12] When substrate **1e** was used in the hydrocyanation reaction under standard conditions, the corresponding cyanohydrin **2e** was isolated in 97% yield, with an e.e. of 98%. After crystallization the e.e. was increased to >99%.

Results for (*R*)-cyanohydrins obtained from aromatic aldehydes containing a hydroxyl substituent are normally not very good. Hydroxyl substitution at the *meta*-position is a positive exception,[13] producing the cyanohydrin in 90% yield, with an e.e. of 97%. A hydroxyl group at the *para*-position gives, on a very small scale, the cyanohydrin in 64% yield and an e.e. of 96% (after crystallization).[14] To date it was thought to be impossible to introduce a substituent at the *ortho*-position. For these reasons both substrates **1f** and **1g** were investigated. Cyanohydrin **2f** was formed in 90% yield, with an e.e. of 91%. The enantiomeric purity was determined prior to crystallization. After crystallization an e.e. of 99% was obtained. For the cyanohydrin of salicylaldehyde to be formed in satisfactory yield and enantiomeric purity, twice the standard amount of purified enzyme was needed. Cyanohydrin **2g** was obtained in 97% yield, with an e.e. of 98%.

Effenberger et al. investigated aldehyde **1h** and related compounds for the synthesis of (*R*)-salbutamol.[13] Unfortunately their synthesis was hampered by racemization, which occurred when a protecting group was removed in the final step of the total synthesis. The protecting group that had to be removed was necessary for the preparation of the cyanohydrin. It was reported that the unprotected cyanohydrin could not be formed starting from aldehyde **1h**. By using a very large excess of purified Pa-HNL and performing the hydrocyanation over an extended period of time, cyanohydrin **1h** could be formed in 80% yield, with a moderate e.e. of 58%.

3. Conclusions

By using an aqueous–organic two-phase system and increasing the rate of the enzymatic reaction relative to

the mass transfer rate, the enantiomeric purity of the R-PaHNL catalysed hydrocyanation reaction was optimized. Fine tuning the reaction temperature, pH, and the amount of enzyme allowed the R-PaHNL catalyzed hydrocyanation reaction to predominate over the competing non-enzymatic hydrocyanation reaction. In this way, a range of new and/or difficult substrates could be converted in generally excellent yield and enantiomeric purity. The general principle of mass-transfer-limitation can also be applied to other substrates to optimize the results.

Acknowledgements

This work was carried out as part of the Innovation Oriented Research Program on Catalysis (IOP-katalyse, No. IKA 94026a) funded by The Netherlands Ministry of Economic Affairs.

References

1. For reviews see: (a) Kruse, C. G. In *Chirality in Industry*; Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds.; John Wiley: New York, 1992; pp. 279–299; (b) Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1555–1564; (c) Gregory, R. J. H. In *Biocatalytic Preparation and Chemistry of Some Novel Cyanohydrin Systems*; Thesis, University of Liverpool, 1998; (d) Schmidt, M.; Griengl, H. In *Biocatalysis—From Discovery to Application*; Fessner, W.-D., Ed.; Springer: Berlin, 1999; Vol. 200, p. 193; (e) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649; (f) Effenberger, F. *Chimia* **1999**, *53*, 3; (g) Brussee, J.; van der Gen, A. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; pp. 289–320. (h) Marcus, J. In *Enantioselective Bio-Organic Synthesis and Application of Chiral Cyanohydrins*; Thesis, University of Leiden, 2001.
2. Smitskamp-Wilms, E.; Brussee, J.; van der Gen, A.; van Scharrenburg, G. J. M.; Sloothaak, J. B. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 209.
3. Schall, M. Thesis, Technische Universität Graz, 1996.
4. Wajant, H.; Mundry, K.-W. *Plant Sci.* **1993**, *89*, 127.
5. Notable exceptions are the cyanohydrins derived from 2-furaldehyde and 2-thiophenecarboxaldehyde, in which the furan and thiophene residues have priority over the hydroxyl group.
6. (a) Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. *Synth. Commun.* **1991**, *21*, 1387–1391; (b) Marcus, J.; Brussee, J.; van der Gen, A. *Eur. J. Org. Chem.* **1998**, 2513–2517.
7. (a) Gerrits, P. J.; Willeman, W. F.; Straathof, A. J. J.; Heijnen, J. J.; Brussee, J.; van der Gen, A. *J. Mol. Catal. B, Enzym.* **2001**, accepted for publication; (b) Willeman, W. F.; Gerrits, P. J.; Hanefeld, U.; Brussee, J.; Straathof, A. J. J.; van der Gen, A.; Heijnen, J. J. *Biotechnol. Bioeng.*, accepted for publication.
8. General procedure. (*R*)-2-Hydroxy-3-butenenitrile **2a**: To a cooled double walled reaction vessel at 1°C, R-PaHNL (100 mg) was added to citrate buffer (0.1 M, pH 5.0, 6 mL). Meanwhile NaCN (20 g, 0.41 mol) was dissolved in cold water (150 mL). The pH of this solution was adjusted to 5.4 by addition of citric acid (**CAUTION**: *Formation of toxic hydrogen cyanide!*). The hydrogen cyanide solution thus obtained was extracted with methyl *tert*-butyl ether (3×40 mL). The combined MTBE layers were transferred into the reaction vessel. After stirring for 10 min, 2-propenal (3.3 mL, 50 mmol) was added. The reaction mixture was stirred overnight. The reaction was monitored by taking samples of the organic layer. After drying ($MgSO_4$), the samples were analyzed by HPLC and NMR.
   Upon completion of the reaction, the water layer containing the enzyme was separated from the MTBE layer. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Pure cyanohydrin **2a** was obtained as a yellow oil. The water layer containing the enzyme could be re-used for a new reaction without loss of activity.
9. All analytical data for compounds **2c**–**2f** were in agreement with those previously published. New compounds: (*R*)-2-Hydroxy-3-butenenitrile **2a**: ^{1}H NMR: $\delta = 2.83$ (br s, 1H, OH), 5.02 (br s, 1H, CHOH), 5.48 (dd, 1H, $J = 10.2$, $J = 1.5$, CH_2=CH), 5.67 (dd, 1H, $J = 16.8$, $J = 1.5$, CH_2=CH), 5.98 (m, 1H, CH_2=CH); ^{13}C NMR: $\delta = 61.38$ (CHOH), 117.94 (CN), 119.61 (CH_2=CH), 131.34 (CH_2=CH). (*R*)-2-Hydroxy-4-pentenenitrile **2b**: ^{1}H NMR: $\delta = 2.36$–2.65 (m, 2H, CH_2CHOH), 4.57 (m, 1H, CHOH), 5.07–5.39 (dd, 2H, CH_2=CH), 5.80 (m, 1H, CH_2=CH). (*R*)-Hydroxy-(2-hydroxy-phenyl)-acetonitrile **2g**: ^{1}H NMR (acetone-d_6): $\delta = 5.87$ (s, 1H, CHCN), 6.94 (m, 2H, H-arom), 7.26 (m, 1H, H-arom), 7.55 (m, 1H, H-arom); ^{13}C NMR (acetone-d_6): $\delta = 56.33$ (CHOH), 113.73 (C-arom), 117.83 (CN), 118.19 (C-arom), 121.34 (C_{ipso}), 125.50, 128.56 (C-arom), 152.33 (C_{meta}). (*R*)-α-Hydroxy-(4-hydroxy-3-hydroxymethylphenyl)-acetonitrile **2h**: ^{1}H NMR (acetone-d_6): $\delta = 4.76$ (s, 2H, CH_2OH), 5.66 (s, 1H, CHCN), 6.87 (d, 1H, $J = 8.2$, H-arom), 7.26 (dd, 1H, $J = 2.1$, $J = 8.2$, H-arom), 7.49 (s, 1H, H-arom).
10. Duffield, J. J.; Regan, A. C. *Tetrahedron: Asymmetry* **1996**, *7*, 663–666.
11. Ognyanov, V. I.; Datcheva, V. K.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6992–6996.
12. Effenberger, F.; Ziegler, T.; Förster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458–459.
13. Effenberger, F.; Jäger, J. *J. Org. Chem.* **1997**, *62*, 3867–3873.
14. Kiljunen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 1225–1234.