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Analyzing the hydrocyanation reaction: chiral HPLC and the synthesis of racemic cyanohydrins

Pieter Jan Gerrits,* Friedhelm Zumbrägel and Jan Marcus

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

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Abstract—A method to directly analyze unprotected cyanohydrins (with regard to enantiomeric purity and conversion) via chiral HPLC is described. The influence of the solvent composition on the stability of the unprotected cyanohydrins is investigated. By acidifying the solvent during HPLC analysis, hydrocyanation reactions can be directly followed in time. For many chiral cyanohydrins it is possible to determine both conversion and enantiomeric purity without prior protection of the cyanohydrin. Using a special aqueous-organic two-phase system racemic cyanohydrins can be synthesized in excellent yields.

1. Introduction

Cyanohydrins are versatile chiral building blocks for pharmaceutical chemistry.[1] The synthesis of chiral cyanohydrins catalyzed by *R*-hydroxynitrile lyase (*R*-PaHNL) (EC 4.1.2.10) from (*Prunus amygdalus*) in a two-phase system using mass-transfer-limitation has been described before.[2] The reaction is depicted in Scheme 1. The synthesis of chiral cyanohydrins is not restricted to catalysis by *R*-HNL. Catalysis is also possible by using chiral catalysts, like cyclic dipeptides or chiral Lewis acids.[3]

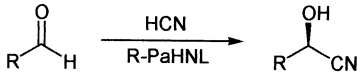
Determination of the enantiomeric purity of organic compounds is increasingly important, mainly for those with a biological activity. It is well known that this activity depends on the structure of the substrates, in such a way that the biological response due to each enantiomer may be different. Because of this, it is necessary to have a method to accurately determine the enantiomeric excess.

In earlier investigations, the optical purity of cyanohydrins was solely determined from the optical rotation.[4,5] Over the years several methods have been developed to determine the enantiomeric excess of cyanohydrins. These include the following:

1. derivatization with (*R*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride followed by ^{1}H NMR[6a] or ^{19}F NMR[6b] analysis, gas chromatography[7] or HPLC analysis;[8]
2. by ^{1}H NMR using tris-[3-(heptafluoropropylhydroxymethylene)-D-camphorate] europium(III) ($Eu(hfc)_3$) as a chiral shift reagent;[9]
3. derivatization as silyl-ethers, to prevent decomposition of the cyanohydrin, followed by chiral-HPLC (Chiralcel OD);[10]
4. derivatization as acetates using trifluoroaceticanhydride (TFAA), to prevent decomposition of the cyanohydrin, followed by gas chromatography analysis using a β-cyclodextrin column;[11]
5. ^{1}H NMR of mixtures of mandelonitrile and cyclodextrin in an aqueous solution;[12]
6. (*S*)-Naproxen® as a derivatization agent followed by HPLC analysis.[13]

One of the drawbacks of the above mentioned methods is the requirement for derivatization or the addition of a shift reagent. Derivatization of the product may be complicated and time consuming, and excludes the possibility of a direct analysis and therefore, quantitative determinations like conversions.

It was our aim to develop an HPLC-method to analyze the hydrocyanation reaction, with regard to the aldehyde and the corresponding unprotected cyanohydrin (conversion and enantiomeric excess). In this way, it may be possible to directly follow the course of the reaction and the enantiomeric excess over time. Such an analysis can be used to investigate the possible racemization of chiral cyanohydrins. To understand the catalytic reactions we



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

Keywords: chiral-HPLC; cyanohydrins; hydrogen cyanide; hydroxynitrile lyase; two-phase system.

* Corresponding author. Present address: Elandstraat 1E, 2513 GL Den Haag, The Netherlands. Tel.: +31-70-3607570; fax: +31-84-8717567; e-mail: PJGerrits@Yahoo.com

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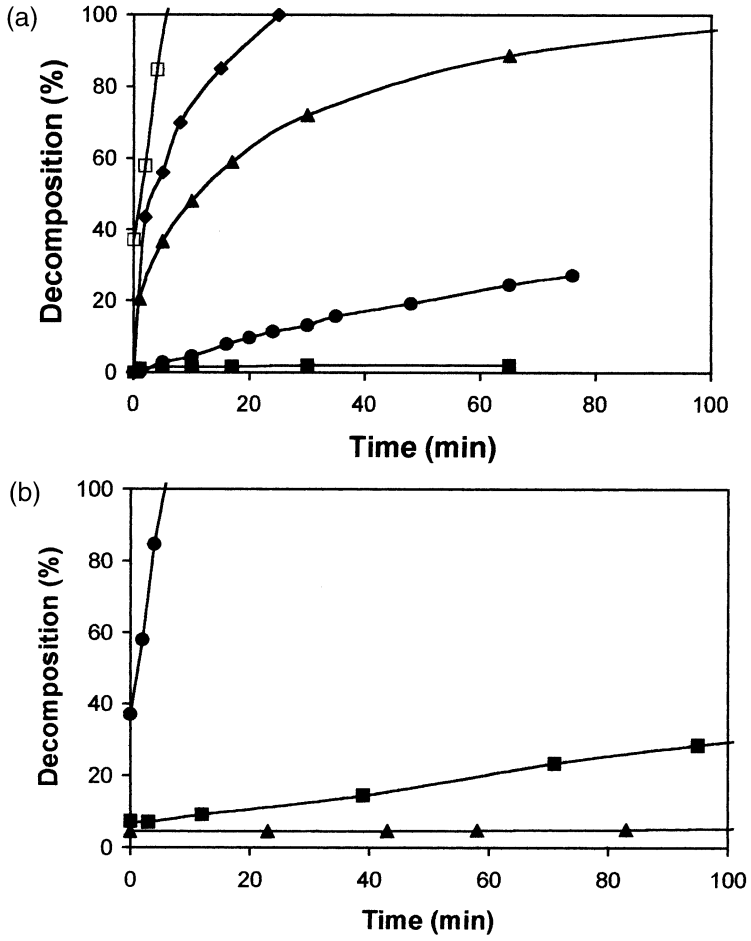


Figure 1. (a) The effect of water (pH 7) and alcohols on the stability of 3*E*-2-hydroxy-4-phenylbutenenitrile dissolved in a mixture of *n*-hexane and: water (□), methanol (◆), ethanol (▲), *iso*-propanol (●), and *tert*-butanol (■); (b) The effect of the pH on the stability of 3*E*-2-hydroxy-4-phenylbutenenitrile dissolved in water of pH 3 (▲), pH 5 (■), and pH 7 (●).

will investigate the racemization reaction and include a method to improve the reaction.

2. Results and discussion

The enantiomeric excess of cyanohydrins, produced in the enzyme-catalyzed hydrocyanation reaction, has normally been determined by chiral-HPLC analysis of the corresponding *tert*-butyldimethylsilyl (TBDMS) or *tert*-butyldiphenylsilyl (TBDPS) protected cyanohydrins.[10] This protection is necessary because unprotected cyanohydrins are prone to degradation.[14] The disadvantages of protecting cyanohydrins are the additional reaction and thereby purifications. An additional reaction is time consuming and may introduce errors, and excludes the direct analysis of the progress of the hydrocyanation reaction. To the best of our knowledge no method to analyze unprotected cyanohydrins has been reported before.

Table 1. The stability of 3*E*-2-hydroxy-4-phenylbutenenitrile in the presence of H_2O and alcohols

Co-solvent	Effect in time
Water (pH 7)	Total decomposition after 7 min
MeOH	Total decomposition after 30 min
EtOH	90% decomposition after 1 h
iso-PrOH	Partial decomposition after 1 h
tert-BuOH	No significant decomposition

2.1. UV intensities

In order to establish a method to analyze the progress of the hydrocyanation reaction, the effects of the conditions during chiral-HPLC analysis on the unprotected cyanohydrins was examined more closely.

The separation of the substrate (aldehyde) and both enantiomers can be realized on a chiral Chiralcel column[15] using an eluent combination. The separated substrate and enantiomers are then detected by UV.

The UV-intensity measured by the UV-detector is directly dependent upon the concentration, the wavelength and the extinction coefficient of the compounds. By taking a UV-spectrum of a corresponding aldehyde and cyanohydrin pair, the wavelength at which the UV-intensities of the aldehyde and cyanohydrins are comparable with respect to the concentration can be found. With this knowledge it is possible to adjust the wavelength of the UV-detector correspondingly.

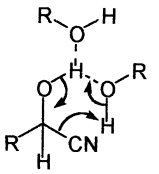


Figure 2. Proposed mechanism showing the influence of different alcohols.

When side reactions are minimal, it is possible to calculate the conversion of the substrate into the product. The conversion can be calculated using the ratio between the produced peak areas of the aldehyde (A_{ald}) and both cyanohydrin enantiomers (A_R and A_S). This ratio is shown in Eq. (1). For some substrates the use of a correction factor (cf) is necessary.[16]

$$\text{conv} = 100\% - \left(\frac{A_{ald}}{\text{cf}\ (A_{ald} + A_R + A_S)} \times 100 \right) \quad (1)$$

2.2. Stability of cyanohydrins in different eluents

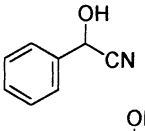
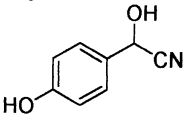
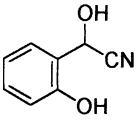
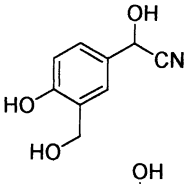
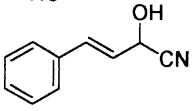
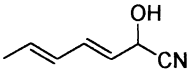
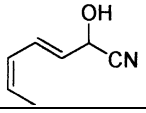
To solve the problem of the stability of unprotected cyanohydrins during HPLC analysis, the influence of the eluent was examined. For the analysis of protected cyanohydrins mixtures of *n*-hexane and *iso*-propanol are used. Variation of the eluent combination revealed that decomposition of the unprotected cyanohydrins only occurred in the presence of small alcohols and water. In pure *n*-hexane the cyanohydrin remained stable.

The decomposition of racemic 3*E*-2-hydroxy-4-phenylbutenenitrile (Entry 5 in Table 2), dissolved in mixtures of *n*-hexane and different alcohols and water, was followed by UV-spectrometry. Progress curves were recorded for the decomposition of the cyanohydrin. The results are presented in Fig. 1a and b and Table 1.

The kind of alcohol used has an important effect on the speed of the decomposition. Water and primary alcohols lead to fast decomposition, whereas tertiary alcohols do not show this effect. *iso*-Propanol shows an intermediate effect.

The extent of decomposition differs with the kind of cyanohydrin used. Mandelonitrile, the cyanohydrin from benzaldehyde (Entry 1 in Table 2) is less stable than 3*E*-2-hydroxy-4-phenylbutenenitrile, the cyanohydrin of cinnamaldehyde, which has an additional double bond between the benzyl ring and the cyanohydrin group. Generally it can be said that the decomposition of unprotected cyanohydrins is

Table 2. Analyzed samples containing the aldehyde and the corresponding unprotected racemic cyanohydrin.

Entry	Racemic cyanohydrin	Column			λ[a] (nm)	cf[b]	Retention time (min)		
		Type	T[c] (°C)	Eluent H/I[d] (%)			$R_{t,ald}$	$R_{t,R}$	$R_{t,S}$
1		OD	40	97/3	220	2.1	5.74	18.5	17.4
2		OJ	40	87/13	230	0.32	6.65	25.3	28.3
3		OJ	20	80/20	220	1	4.8	8.2	9.0
4		OJ	20	87/13	230	1	10.3	36.6	30.2
5		OD	20	87/13	261	1	7.78	14.42	17.40
6		OD	5	97/3	231	1	5.9	12.6	11.3
7		OD	5	97/3	231	1	6.2	10.5	10.1

[a] This λ-value is not necessarily λ_{max}.
[b] Correction factor related to a typical wavelength.[16]
[c] Changing the temperature of the column often results in better base-line separations.
[d] H=*n*-Hexane; I=*i*-propanol. To all eluents 0.1% acetic acid was added.

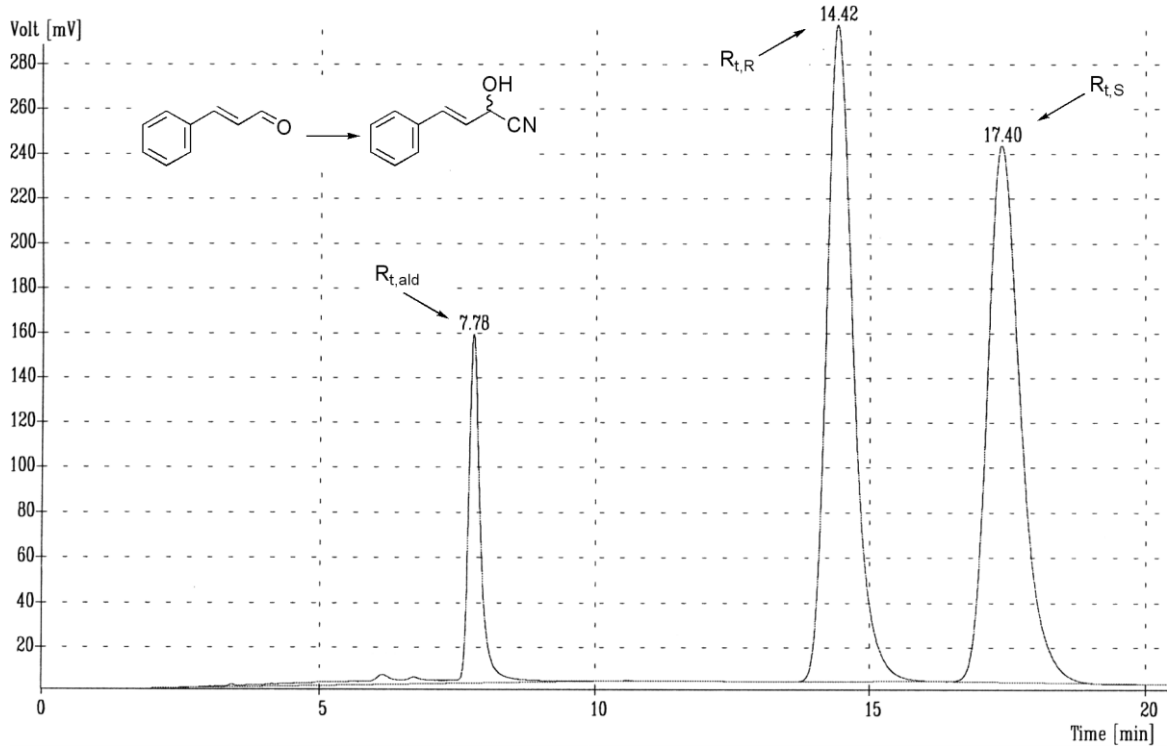
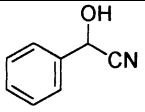
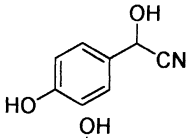
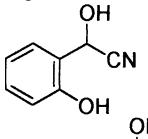
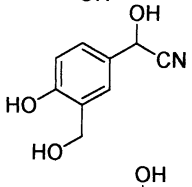
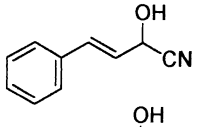
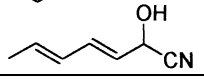


Figure 3. HPLC chromatogram of a reaction mixture containing cinnamaldehyde and the corresponding *R*- and *S*-enantiomers. Shown are three peaks: (1) the aldehyde ($R_{t,ald}$=7.78 min); (2) the *R*-enantiomer ($R_{t,R}$=14.42 min) and (3) the *S*-enantiomer ($R_{t,S}$=17.40 min). The order of the *R*- and *S*-enantiomers depends on the substrate used. Notice the baseline separation between the three peaks. This is essential for a reliable result.

Table 3. The synthesis of racemic cyanohydrins using a two-phase system

Entry	Product	Conv. (%)[a]	Rt (h)[b]
1		99.8	5
2		91.0	24
3		99.8	24
4		90.1	72
5		98.0	48
6		97.0	24

The reaction is monitored and analyzed using HPLC.
[a] Conversion.
[b] Reaction time.

accelerated by the use of water and small alcohols (in a steric way) as co-solvents.

A possible explanation for the above described phenomenon is shown in Fig. 2. Small alcohols form hydrogen bonds with the hydroxy groups of the cyanohydrins and weaken the oxygen–hydrogen bond. The strength of the hydrogen bonds depends on the pH and the kind of alcohol used.[17]

To prevent the decomposition of cyanohydrins based upon the proposed mechanism, the use of bigger alcohols[18] and the possibility of acidifying the eluent was investigated. An unprotected cyanohydrin in an eluent with a small amount of added pure acetic acid shows no decomposition on HPLC. By using this composition of eluent, racemic unprotected cyanohydrins can be analyzed. The results are presented in Table 2 and Fig. 3.

Fig. 3 shows an example of an HPLC chromatogram of a reaction mixture. The peaks of both enantiomers and the aldehyde can be clearly seen.

Based upon the effect of alcohols on the stability of cyanohydrin, it is unfavorable to use MeOD as a solvent in NMR analyses and MeOH or EtOH as solvents in the determination of the optical rotation.[19]

2.3. The synthesis of racemic cyanohydrins

The production of racemic cyanohydrins can sometimes be troublesome. Normally, a solution of NaCN in water is added to a solution of the aldehyde in acetic acid.[20,21] As

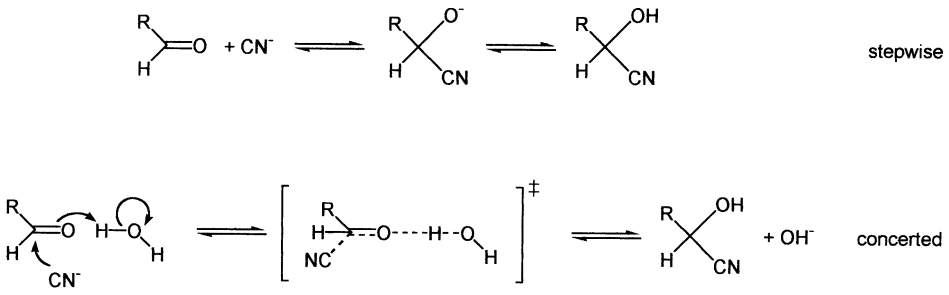


Figure 4. Two possible mechanisms[22] for cyanohydrin formation and decomposition. Top: a stepwise pathway involving an anionic cyanohydrin. Bottom: a concerted pathway involving a water molecule.

an example, the synthesis of racemic α-hydroxy-(4-hydroxy-3-hydroxymethylphenyl)-acetonitrile (Entry 4, Table 2) results in a low conversion of 30%.

To optimize the results of the synthesis of racemic cyanohydrins, a modified procedure was developed based upon the successful enzymatic hydrocyanation reaction.[2] In this procedure the pH of a solution of NaCN in water was adjusted to 5.5 by addition of citric acid followed by extraction with MTBE. The MTBE-layer, an aqueous buffer layer of pH 6.8 and the aldehyde were then added to the reaction flask. After stirring for some time the racemate was isolated by extraction.

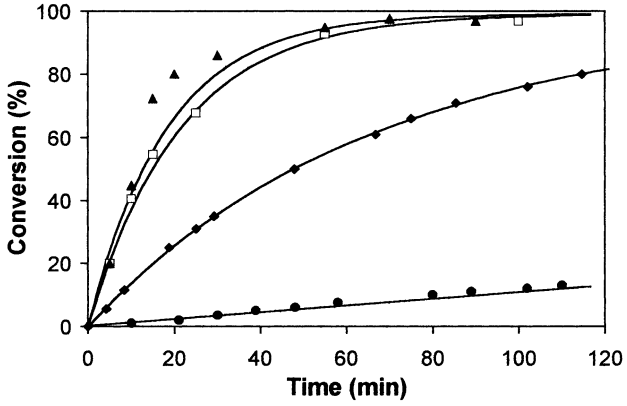


Figure 5. Progress curves for the synthesis of racemic mandelonitrile at pH 5.5 (●), 7.0 (◆), 8 (□) and 9 (▲) at 5°C in an aqueous-organic biphasic system. The line is the simulated curve, markers are the experimental values. The experimental conditions used were described before.[2]

For racemic α-hydroxy-(4-hydroxy-3-hydroxymethylphenyl)-acetonitrile (entry 4) a conversion and yield of 90% could be achieved. In Table 3 the results of the synthesis of other racemic cyanohydrins using this method are presented.

The formation and decomposition of cyanohydrins involve both neutral reactants (HCN and aldehyde) and products (cyanohydrins). For the racemic cyanohydrin formation two distinct mechanisms are possible, differing in whether the proton addition to the carbonyl oxygen atom is stepwise or concerted (Fig. 4). Ching and Kallen concluded, based upon elaborate kinetic studies, that a stepwise pathway is the most likely.[22]

To investigate the rate of the formation of racemic cyanohydrins under different conditions, progress curves were recorded for the synthesis of racemic mandelonitrile in a two-phase system at pH 5.5, 7, 8 and 9. The results are shown in Fig. 5.

Fig. 5 clearly shows that the rate increases with an increasing concentration of hydroxide-ions. Performing the reaction at very high pH values however does not seem to be the best choice. The hydroxide-ion has a dual role in the

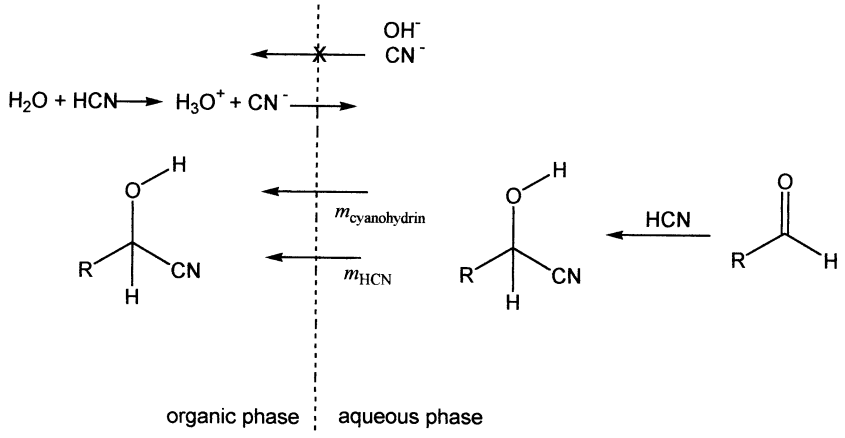


Figure 6. The hydrocyanation reaction can go to completion because the concentration of cyanohydrin is kept low in the aqueous phase. Consequently, the decomposition of cyanohydrins by hydroxide ions hardly occurs. Remarkable is the distribution of HCN (partition coefficient[2] HCN: 2.6; CN^- will stay in the aqueous layer) to the organic phase layer. After dissociation of HCN, the hydroxide and cyanide ions will leave the organic phase and become therefore unavailable for the degradation of the cyanohydrin in the organic layer. pH=($pK_a \times c_{HCN}$) ≈3.1; m_{HCN}=mass transport of HCN; $m_{cyanohydrin}$=mass transport of the cyanohydrin.

mechanism of the racemic hydrocyanation reaction. On the one hand it deprotonates the hydroxyl moiety of the cyanohydrin. On the other hand it deprotonates HCN forming a cyanide-ion in a rate-determining step. Increasing the concentration of hydroxide-ions favors the decomposition of the cyanohydrins. After all, the concentration of HCN (and CN^-) is not significantly increased. The effect of the increased decomposition at higher pH values may be suppressed by performing the reaction in a two-phase system.

The progress curves in Fig. 5 were also simulated (curve) using the reaction rate constants for the formation of racemic mandelonitrile at pH 5.5, 7.0, 8.0 and 9. These values are 0.0044, 0.0702, 0.43 and 2.6 $l_{aq}\,mol^{-1}\,s^{-1}$, respectively, as determined by extrapolation of the values reported by Niedermeyer.[23] There is a remarkable consistency between the experimental values (markers) and the simulated data (curves). This may imply that in a two phase-system the formation of cyanohydrins has the upper hand (compared to the decomposition reaction) and causes the equilibrium to go to completion.

The racemic hydrocyanation reaction, which is an equilibrium, can go to completion if the concentration of the cyanohydrin formed is kept as low as possible and the concentration of cyanide as high as necessary. This can be accomplished by excluding the racemic cyanohydrin from the equilibrium that occurs in the aqueous layer (see Fig. 6). The organic layer is acidified by the dissociation of HCN in dissolved water. The pH will be roughly 3 in accordance with the formula which states that the pH is equal to the square root of the multiplication of the pK_a and the concentration of HCN. The partition coefficient of the cyanohydrin will be the driving force of the racemic hydrocyanation reaction. This phenomenon is also observed by the enantiomeric excess progression analysis whereby the enantiomeric excess is constant throughout the reaction.

The same exclusion-principle can be seen in the enzyme catalyzed hydrocyanation reaction producing chiral cyanohydrins. The chiral reaction will go to completion and the chirality of the cyanohydrin may be 'stabilized' in the organic phase. Racemization of chiral cyanohydrins may only be possible using one-phase systems (especially by using co-solvents such as alcohols).[5,7,24]

3. Conclusion

By acidifying the eluent and adjusting the detection wavelength, column material and column temperature a new chiral HPLC analysis method was developed for unprotected cyanohydrins that can be used to follow the progress of the hydrocyanation reaction and directly determine the enantiomeric excess and conversion.

The negative effect of alcohols on the stability of cyanohydrin make it unfavorable to use MeOD as a solvent in NMR analyses and MeOH or EtOH as solvents in the determination of the optical rotation.

Using a special aqueous-organic two-phase system racemic cyanohydrins can be synthesized in excellent yields. Racemization of chiral cyanohydrins may only be possible using one-phase systems.

4. Experimental

4.1. General methods and materials

^{1}H NMR spectra were recorded on a JEOL FX-200 instrument. Samples were measured in acetone-d_6 or $CDCl_3$ with Me_4Si as an internal standard for ^{1}H NMR and $CDCl_3$ for ^{13}C NMR; δ in ppm, J in Hz. The HRMS spectrum was obtained with a Finnigan MAT ITD 700 electron impact (70 eV). The enantiomeric excess and conversions were determined by HPLC using Chiralcel OD and OJ columns[15] (250×4.6 mm^2) followed by UV-detection (Applied Biosystems 759A Absorbane detector). As eluent, mixtures of hexane (H), isopropyl alcohol (I) and acetic acid (HAc), which are specified in each case, were applied. Flow rate=1 ml min^{-1}. All cyanohydrins were also prepared in racemic form to optimize the conditions for peak separation. Melting points were measured on a Büchi melting-point apparatus and are uncorrected. Spectrophotometer: Varian DMS 200 UV–visible. Acetic acid (99–100%) and isopropyl alcohol (99.5%) were purchased from Baker, hexane (HPLC-grade) from Biosolve, MTBE (99%) from Acros, benzaldehyde (99%) from Merck, *p*-hydroxyaldehyde (99%) and salicylaldehyde (98%) from Aldrich, 4-hydroxy-2-hydroxymethyl-benzaldehyde (99%) from Maybridge Chemical Company, cinnamaldehyde (99%) from Aldrich, sorbic aldehyde (95%) from Aldrich, and NaCN (p.a.) from Baker.

4.2. Calculation of conversion and enantiomeric excess

In the described experiments, the conversion and the *ee* are based on the analysis of the organic phase. Since the aqueous phase volume is small compared to the organic phase volume and most of the aldehyde and cyanohydrin resides in the organic phase, the amounts of aldehyde and cyanohydrin in the aqueous phase were considered negligible for the calculation of the conversion. The definitions for the extent of conversion and the enantiomeric excess are integrated in a computer program. The computer program[25] was written to integrate the UV-activities in time, and process the data provided by the HPLC and the spectrophotometer. Formulas (1) and (2) including the correction factor were used in this program

$$ee = \frac{A_R - A_S}{A_R + A_S} 100\% \qquad (2)$$

4.3. Stability measurements

Rates of cyanohydrin decomposition were measured spectrophotometrically by monitoring the aldehyde using a UV/VIS spectrophotometer. All measurements involving benzaldehyde were made at 280 nm. For cinnamaldehyde a wavelength of 284 nm was used. The stability experiments were started by adding 0.5 ml of aqueous buffers or alcohols (buffers of pH 3, 5 and 7, MeOH, EtOH, *i*-PrOH and *t*-BuOH) to a 4 ml cuvet (length 1 cm) at room temperature,

containing 3 ml of a 3.42×10^{-7} M mixture of the cyanohydrin in hexane.

4.3.1. 2-Hydroxybenzeneacetonitrile (entry 1, Table 3).[8] To a thermostatically cooled (5°C) double-walled reaction vessel, 60 ml of a 0.1 M phosphate/citrate buffers (pH 5.5, 7.0, 8.0, 9.0) were added. Meanwhile 10 g of NaCN was dissolved in 100 ml of cold water. The pH of this solution was adjusted to 5.5 by addition of citric acid. *CAUTION: Formation of toxic hydrogen cyanide!* The hydrogen cyanide solution was extracted with MTBE (3×40 ml). The combined MTBE layers were added to the reaction vessel. Freshly distilled benzaldehyde (26 mmol) was added to the solution to start the reaction. After 5 h the reaction was converted for 99.8%. After separation of the two layers, the organic layer was dried ($MgSO_4$) and the solvent evaporated. Pure racemic mandelonitrile was obtained as a clear oil which solidified at 5°C. HPLC, eluent H/I/HAc=97:3:0.1, λ 220 nm. λ_{max}=220 nm (λ_{max} aldehyde=250 nm). ^{1}H NMR ($CDCl_3$): δ=3.15 (br, 1H, OH), 5.52 (s, 1H, C*H*OH), 7.49 (m, 5H, H-arom); ^{13}C NMR ($CDCl_3$):=63.22 (COH), 119.16 (CN), 126.70, 129.12 (C-arom), 129.71 (C_{para}), 135.14 (C_{ipso}).

4.3.2. 2-Hydroxy-2-(4-hydroxyphenyl)-acetonitrile (entry 2, Table 3).[26,27] Prepared as described above for 2-*hydroxybenzeneacetonitrile* using 24.6 mmol of 4-hydroxybenzaldehyde. After 24 h the reaction was 91% converted into cyanohydrin. Cyanohydrin was obtained as a light pink solid and could be separated from the aldehyde by crystallization in dichloromethane/petroleum-ether 40–60 to give pure cyanohydrin as light pink colored crystals. HPLC: OJ-column, eluent H/I/HAc=87:13:0.1, λ 230 nm. λ_{max} 230 nm (λ_{max} aldehyde 218 and 270 nm). ^{1}H NMR (acetone-d_6): δ=5.66 (s, 1H, CHCN), 6.91 (d, 2H, *J*=8.78 Hz, H-arom), 7.41 (d, 2H, *J*=8.78 Hz, H-arom); ^{13}C NMR (acetone-d_6):=62.28 (CHOH), 115.43 (C-arom), 119.20 (CN), 128.10 (C-arom), 157.97 (C_{ipso}), 166.58 (C_{ipso}).

4.3.3. 2-Hydroxy-2-(2-hydroxyphenyl)-acetonitrile (entry 3, Table 3).[26] Prepared as described above for 2-*hydroxybenzeneacetonitrile* using 47.5 mmol of salicylaldehyde and 60 ml phosphate/citrate buffer (pH 7.5). After 24 h the reaction was 99.8% converted into the cyanohydrin. Pure cyanohydrin was obtained as a yellow oil. HPLC: OJ-column, eluent H/I/HAc=80:20:0.1, λ 220 nm. λ_{max} 215 and 255 nm (λ_{max} aldehyde 220 nm). ^{1}H NMR (acetone-d_6): δ=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):=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}).

4.3.4. α-Hydroxy-(4-hydroxy-3-hydroxymethylphenyl)-acetonitrile (entry 4, Table 3).[26] To a round bottom flask, 5 ml of a 0.1 M phosphate/citrate buffer (pH 6.8) were added. Meanwhile 1 g of NaCN was dissolved in 17 ml of cold water. The pH of this solution was adjusted to 5.5 by addition of citric acid. *CAUTION: Formation of toxic hydrogen cyanide!* The hydrogen cyanide solution was extracted with MTBE (1×40 ml). 23 ml hydrogen cyanation extract and 0.66 mmol of 4-hydroxy-3-hydroxymethylbenzaldehyde were added to the flask. After stirring for three days a conversion of 90% into the cyanohydrin was reached. Crude cyanohydrin was obtained as oil, 0.12 g yield. HPLC: OJ-column, eluent H/I/HAc=87:13:0.1, λ 230 nm, λ_{max} 210 and 231 (λ_{max} aldehyde 278 nm). ^{1}H NMR (acetone-d_6): δ=4.76 (s, 2H, CH_2OH); 5.65 (s, 1H, CHCN); 6.87 (d, 1H, *J*=8.2 Hz, H-arom); 7.26 (dd, 1H, *J*=2.1, 8.2 Hz, H-arom). HRMS (EI): M^+, found 179.1720. $C_9H_9NO_3$ requires 179.1727.

4.3.5. 3*E*-2-Hydroxy-4-phenylbutenenitrile (entry 5, Table 3).[7,26] Prepared as described above for 2-*hydroxybenzeneacetonitrile* using 40 mmol of cinnamaldehyde. After two days a conversion of 98% was reached. Crude cyanohydrin was obtained as a clear yellow solid, yield 97% cyanohydrin. The crude cyanohydrin was separated from the aldehyde by crystallizing in CH_2Cl_2/*n*-hexane, to give pure cyanohydrin as light yellow colored crystals. Mp 78°C. HPLC: eluent H/I/HAc=87:13:0.1, λ 261.5 nm, λ_{max}=253 nm (λ_{max} aldehyde=284 nm). ^{1}H NMR ($CDCl_3$): δ=3.96 (br, 1H, OH), 5.16 (d, 1H, *J*=6.2 Hz, CHCN), 6.27 (dd, 1H, *J*=6.2, 15.9 Hz, =C*H*CHCN), 6.89 (d, 1H, *J*=15.9 Hz, C*H*=CH), 7.39 (m, 5H H-arom); ^{13}C NMR ($CDCl_3$):=61.60 (CHOH) 118.30 (CN), 122.00 (C-arom), 127.00 (CH=CH), 128.70 (C-arom), 128.90 (CH=CH), 134.60 (C_{ipso}), 135.00 (C-arom).

4.3.6. 2-Hydroxy-3*E*,5*E*-heptadienenitrile and 2-hydroxy-3*E*,5*Z*-heptadienenitrile (entry 6, Table 3).[9b,26] Prepared as described above for 2-*hydroxybenzeneacetonitrile* using 57.2 mmol of 2,4-hexadienal, 15 g of NaCN in 120 ml of cold water and 60 ml of a phosphate/citrate buffer (pH 7). After 24 h, a conversion of 97% was observed. The cyanohydrin was obtained as a yellow solid. The reaction was followed and no additional isomerization was observed during the reaction. HPLC: eluent H/I/HAc=97:3:0.1, λ 231 nm, λ_{max} 232 nm, (λ_{max} aldehyde 260 nm). ^{1}H NMR ($CDCl_3$): δ=1.80 (d, 3H, CH_3, *J*=5.6 Hz), 3.05 (br, 1H, OH), 5.01 (d, 1H, CH–CN, *J*=5.9 Hz), 5.63 (dd, 1H, =C*H*CHCN, *J*=15.2, 6.2 Hz), 5.91 (dd, 1H, MeC*H*=CH, *J*=15, 7 Hz), 6.10 (dd, 1H, MeCH=C*H*, *J*=15, 0.5 Hz); ^{13}C NMR ($CDCl_3$):=18.10 (CH_3), 61.55 (CHOH), 118.35 (CN), 122.61 (CH_3C=C), 129.14 (*C*=CHCHCN), 134.66 (CH_3C=*C*) 135.63 (=*C*HCHCN).

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References

1. (a) Marcus, J. Enantioselective Bio-Organic Synthesis and Application of Chiral Cyanohydrins, PhD Thesis, University of Leiden, 2001. (b) Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1555–1564. (c) Gregory, R. J. H. Biocatalytic Preparation and Chemistry of Some Novel Cyanohydrin Systems, PhD Thesis, University of Liverpool, 1998.

(d) Schmidt, M.; Griengl, H. *Biocatalysis—Discovery Applic.* **1999**, *200*, 193. (e) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649. (f) Effenberger, F. *Chimia* **1999**, *53*, 3.

2. Gerrits, P. J.; Willeman, W. F.; Straathof, A. J. J.; Heijnen, J. J.; Brussee, J.; van der Gen, A. *J. Mol. Cat.: B, Enz.* **2001** in press.
3. (a) Oku, J.-I.; Ito, N.; Inoue, S. *Makromol. Chem.* **1979**, 1089. (b) Reetz, M. T.; Kunisch, F.; Heitmann, P. *Tetrahedron Lett.* **1986**, *27*, 4721. (c) North, M. *Synlett* **1993**, 807. (d) Shvo, Y.; Gal, M.; Becker, Y.; Elgavi, A. *Tetrahedron: Asymmetry* **1996**, *7*, 911. (e) Iovel, I.; Popelis, Y.; Fleisher, M.; Lukevics, E. *Tetrahedron: Asymmetry* **1997**, *8*, 1279. (f) Noe, C. R.; Weigand, A.; Pirker, S.; Liepert, P. *Monatshefte Chem.* **1997**, *128*, 301. (g) Kogut, E. F.; Thoen, J. C.; Lipton, M. A. *J. Org. Chem.* **1998**, *63*, 4604. (h) Thoen, J. C.; Lipton, M. A. *Tetrahedron: Asymmetry* **1998**, *9*, 3947. (i) Hwang, C. D.; Hwang, D. R.; Uang, B. J. *J. Org. Chem.* **1998**, *63*, 6762. (j) Brunel, J. M.; Legrand, O.; Buono, G. *Tetrahedron: Asymmetry* **1999**, *10*, 1979. (k) Belokon, Y. N.; North, M.; Parsons, T. *Org. Lett.* **2000**, *2*, 1617.
4. Rosenthaler, L. *Biochem. Z.* **1908**, *14*, 238.
5. Becker, W.; Freund, H.; Pfeil, E. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 1079.
6. (a) Dale, J.; Dull, D.; Mosher, H. *J. Org. Chem.* **1969**, *34*, 2543. (b) Mendez, E.; Brieva, R.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Chem. Commun.* **1995**, 989.
7. Effenberger, F.; Ziegler, T.; Förster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458.
8. (a) Brussee, J.; Roos, E. C.; van der Gen, A. *Tetrahedron Lett.* **1988**, *29*, 4485. (b) Brussee, J.; Loos, W. T.; Kruse, C. G.; van der Gen, A. *Tetrahedron* **1990**, *46*, 979.
9. (a) Effenberger, F.; Ziegler, T.; Förster, S.; Ziegler, T. *Tetrahedron Lett.* **1990**, *31*, 1249. (b) Ognyanov, V. I.; Datcheva, V. K.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6992.
10. (a) Zandbergen, P.; van den Nieuwendijk, M. C. H.; Brussee, J.; van der Gen, A.; Kruse, C. G. *Tetrahedron* **1992**, *48*, 3977. (b) Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. Preparative Biotransformations. In *Whole Cell and Isolated Enzymes in Organic Synthesis*, Roberts, S. M., Ed.; *Update 5,4:5.1*; Wiley: New York, 1995.
11. (a) Huuhtanen, T. T.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1992**, *3*, 1223. (b) Kiljunen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1994**, *5*, 311.
12. Hickel, A.; Gradnig, G.; Scall, M.; Zangger, K.; Griengl, H. *Spectr. Chim. Acta, Part A* **1997**, *53*, 451.
13. Solis, A.; Luna, H.; Pérez, H. I.; Manjarrez, N.; Sánchez, R. *Tetrahedron Lett.* **1998**, *39*, 8759.
14. Brussee, J.; Loos, W. T.; Kruse, C. G.; van der Gen, A. *Tetrahedron* **1990**, *46*, 979.
15. For more information see http://www.daicel.co.jp/chiral.
16. Because the aldehyde and the cyanohydrin have different maximum wavelengths and extinction coefficients, it is not always possible to find the wavelength where the peak intensities are equal. It is therefore necessary to apply a correction factor (cf). To establish the correction factor, equal amounts of aldehyde and cyanohydrin are weighed and dissolved in the eluent. This mixture is then injected onto the chiral column and analyzed. The peak intensities of the aldehyde and the cyanohydrin are then set to match by applying the correction factor.
17. March, J. *Advanced Organic Chemistry*; 4th ed; Wiley: New York, 1992; pp. 75–79.
18. Marcus, J.; Vandermeulen, G. W. M.; Brussee, J.; van der Gen, A. *Tetrahedron: Asymmetry* **1999**, *10*, 1617.
19. (a) Johnson, H.; Griengl, H. *Tetrahedron* **1997**, *53*, 617. (b) Ognyanov, V. I.; Datcheva, V. K.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6992.
20. Danieli, B.; Barra, C.; Carrea, B.; Riva, S. *Tetrahedron: Asymmetry* **1996**, *7*, 1682.
21. 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.
22. Ching, W. M.; Kallen, R. G. *J. Am. Chem. Soc.* **1978**, *19*, 6119.
23. Niedermeyer, U. Untersuchungen zur enzymatischen C-C Verknupfung am Beispiel der chiralen Cyanhydrinbildung, PhD Thesis, Forschungszentrum Julich, Germany, 1990.
24. Wehtje, E.; Adlercreutz, P.; Mattiasson, B. *Appl. Microbiol. Biotechnol.* **1988**, *29*, 419.
25. Peter Schakel. Computer program for chromatography. AD prog. version 3.0, department CEA, Gorlaeus Laboratoria, Leiden Institute of Chemistry, Leiden University.
26. Gerrits, P. J.; Birikaki, L.; Marcus, J.; van der Gen, A. *Tetrahedron: Asymmetry* **2001**, *12*, 971.
27. Kiljunen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 1225.