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Synthesis of (*R*)-cyanohydrins by crude (*R*)-oxynitrilase-catalyzed reactions in micro-aqueous medium

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Abstract

In diisopropyl ether or ethyl acetate under micro-aqueous conditions, the enantioselective synthesis of (*R*)-cyanohydrins from aldehydes and methyl ketones was studied using crude (*R*)-oxynitrilase prepared from almonds. This reaction system performed well over the temperature range of 4°C to 30°C.

Asymmetric cyanohydrination is an important process in organic synthesis because cyanohydrins can be easily converted into a variety of valuable synthetic intermediates such as α-hydroxyketones, α-hydroxyacids, β-aminoalcohols, amino-nitriles and aziridines. Due to the availability and the excellent stereoselectivities, the biocatalytic formation of (*R*)-cyanohydrins using almond enzymes has been thoroughly investigated and is considered to be a reliable method.[1,2]

The (*R*)-oxynitrilase [EC 4.1.2.10] catalytic reaction has been reported so far to be successful with both crude enzyme preparations[3–8] and purified enzyme.[1,9,10] In the case of isolated oxynitrilase, it is necessary to establish certain conditions (essential water, pH, temperature, solvent, etc.) to retain the catalytic activity of the enzyme. In general, aqueous buffer media was chosen for its favorable reaction conditions,[3,11] however, due to a competing nonenzymatic reaction of the substrate with hydrogen cyanide in aqueous media, it is most likely to lead to a decrease in the enantiomeric purity of the products. Moreover, the enantiomeric purity of the enzymatic products could be further compromised by their racemization in the aqueous buffer during the course of the reaction. Many substrates have been investigated by Effenberger et al. in detail with the purified oxynitrilase both in water/ethanol and in water/water-immiscible organic solvent systems.[1,9,10] They found that higher yields and enantiomeric excesses could be achieved in water/water-immiscible organic biphases. Recently it was reported that 2–10% (v/v) aqueous buffers were employed in the reaction with the crude enzyme.[4–8]

The crude enzyme is swollen with aqueous buffer to congeal to a pulp (System I in Scheme 1), so efficient stirring is essential for ensuring the contact of enzymes with substrates. Nonetheless, the

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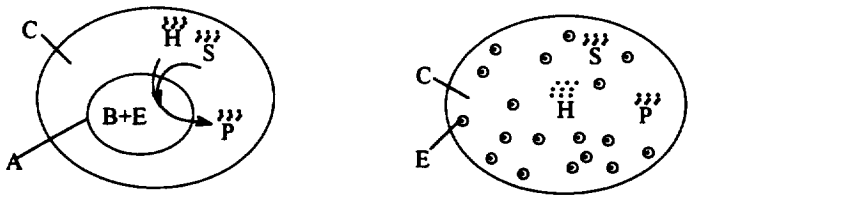
nonenzymatic addition still exists in these aqueous phases. Therefore, on most occasions in System I it is necessary to carry out the enzymatic reaction in a lower range of pH values (3.5–5.5) and lower temperatures (0–4°C) to supress the competitive chemical addition.[4,6–8]



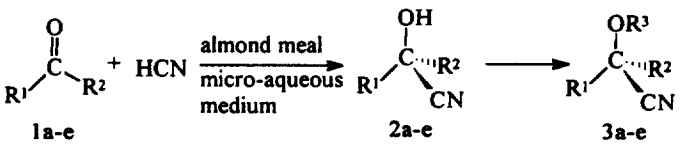
a). System I : biphase system b). System II : micro-aqueous phase crude enzyme system

A: Aqueous phase; B: Buffer solution; C: Organic phase;

E: Enzyme; H: Hydrogen cyanide; S: Substrate; P: Product.

Scheme 1. Comparison of a water–organic solvent biphase system with a micro-aqueous organic phase system

Herein, we wish to report our results where almond meal is used as an (*R*)-oxynitrilase source for the enantioselective synthesis of (*R*)-cyanohydrins (Scheme 2) in a micro-aqueous organic phase (System II in Scheme 1). In this case the organic phase serves as a big reservoir of substrates and products, where the catalytic enzyme powder can mix well and act as a highly effective mode of transfer, therefore it is easier for the substrates to interact with the enzymes to give the products. Furthermore, it is very easy to recover the enzyme for re-use from System II.



1a-e 2a-e 3a-e

a. R^1 = Ph, R^2 = H, R^3 = Ac; b. R^1 = 2-Furyl, R^2 = H, R^3 = Ac; c. R^1 = Ph-CH=CH, R^2 = H, R^3 =TBDMS;

d. R^1 = Ph, R^2 = Me, R^3 = Ac; e. R^1 = n-Butyl, R^2 = Me, R^3 = p-ClC_6H_4CO

Scheme 2.

The results of the enzymatic cyanohydrinations are presented in Table 1 (System I) and Table 2 (System II) for comparison. At a lower temperature (4°C), the synthesis of (*R*)-mandelonitrile **2a** was accomplished in quantitative yield and excellent enantiomeric purity (>99% ee) both in System I (Entry 1 and 2) and in System II (Entry 8). With an increase in temperature in System I, Table 1 shows that at 12°C the yields and enantiomeric excesses of **2a** decreased to 92% and 97.5% respectively in IPE/buffer (Entry 4), and at 30°C decreased to 65% and 92% in EA/buffer (Entry 5) as well as to 77% and 93.9% in IPE/buffer (Entry 6), respectively. This means that a lower temperature was necessary to have better results for the reaction in a water–organic biphase (System I). At higher temperatures (30°C) the rates of chemical addition and decomposition were enhanced, resulting in a decrease of the chemical yields and ees. While in System II (see Table 2) higher yields and enantioselectivities were achieved, ie, at 12°C 100% yield and >99% ee of **2a** (Entry 9 and 10), at 30°C 98% yield and 98.2% ee in EA (Entry 11) as well as 98% yield and 97% ee in IPE (Entry 12) of **2a**, respectively. As shown in Fig. 1, the nonenzymatic addition and decomposition that occurred in the aqueous phase (A) were suppressed deeply in micro-aqueous media (B and C).

Furthermore, in System II (*S*)-(+)-2-(2-furyl)-2-hydroxyacetonitrile **2b** was obtained not only with 100% yield and 99% ee at 4°C (Entry 13) but also with 90% yield and 95% ee at 30°C (Entry 14), while in System I it was only obtained with 70% yield and 73% ee at 30°C (Entry 7). In contrast to the reported result that (*E*)-cinnamaldehyde **1c** was not accepted by the enzyme in aqueous medium,[3] it could be

Table 1
Investigations for the enantioselective syntheses of (*R*)-mandelonitrile in System I

Entry	Substrate	Solvent	Time/h	Temp./°C	Yield/%[a]	ee/%[b]
1	1a	EA+Buffer	48	4	100	>99
2	1a	IPE+Buffer	48	4	100	>99
3	1a	EA+Buffer	48	12	94	>99
4	1a	IPE+Buffer	48	12	92	97.5
5	1a	EA+Buffer	24	30	65	92
6	1a	IPE+Buffer	24	30	77	93.9
7	1b	IPE+Buffer	12	30	70	73

EA: Ethyl acetate; IPE: Diisopropyl ether; Buffer: 5%(v/v) 0.02M citric buffer (pH5.5).

a) Isolation yield; b) Determined by HPLC analysis on CHIRALPAK AD column as *O*-protected derivatives.

Table 2
Investigations for the enantioselective syntheses of (*R*)-cyanohydrins in System II

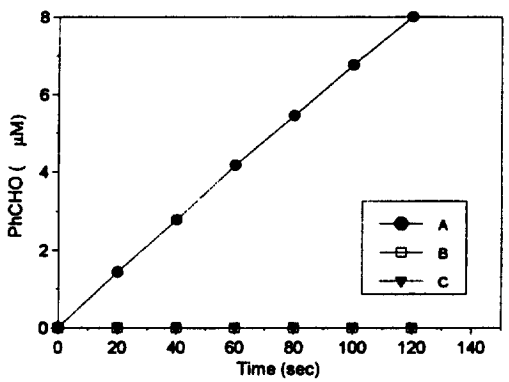
Entry	Substrate	Solvent	Time/h	Temp./°C	Yield/%[a]	ee/%[b]
8	1a	IPE	48	4	100	>99
9	1a	EA	48	12	100	>99
10	1a	IPE	48	12	100	>99
11	1a	EA	24	30	98	98.2
12	1a	IPE	24	30	98	97
13	1b	IPE	24	4	100	99
14	1b	IPE	12	30	90	95
15	1c	IPE	48	4	9.6	57
16	1c	IPE	24	30	36.5	51.5
17	1c	IPE	48	30	58	49
18	1d	IPE	120	25	33	78.1
19	1e	IPE	48	25	68	98.6

EA(water content 0.20-1.52% v/v), IPE(water content 0.14-0.32% v/v).

a) Isolation yield; b) Determined by HPLC analysis on CHIRALPAK AD column as *O*-protected derivatives.

converted into the corresponding (*R*)-cyanohydrin with moderate enantiomeric purity by using System II (Entry 15, 16 and 17), the yield of **2c** which was only 9.6% at 4°C (Entry 15) reached 58% at 30°C (Entry 17). The reactions of methyl ketones under the conditions of System II gave the results of 78% ee of **2d** (Entry 18) and 98.6% ee of 2e (Entry 19).

In conclusion, (*R*)-cyanohydrins can be synthesized with high enantiomeric excess by the application of almond meal to a micro-aqueous organic phase in the temperature range of 4°C to 30°C. The procedure for System I: In a typical experiment, the enzyme preparation (500 mg)[12] was swollen with 0.53 ml of a 0.02 M citrate buffer pH 5.5 for 10 min and then mixed with freshly distilled aldehyde (2.5 mmol) and 1.5 equiv. HCN in 10 ml diisopropyl ether.[13] The resulting aqueous pulp of almond meal in organic solvent was stirred at 30°C for the times indicated in Table 1. The procedure for System II: In a typical experiment, the enzyme preparation (500 mg), freshly distilled aldehyde (2.5 mmol) and 1.5 equiv. HCN in 10 ml diisopropyl ether (the solution was dried over Na_2SO_4 before use, water content 0.32% v/v)



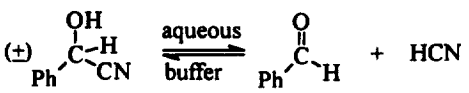


Fig. 1. Comparison of the rate of decomposition of (±)-mandelonitrile (420 μM) to that of benzaldehyde in pH=5.5 citrate buffer (A) and in micro-aqueous medium (EA: B; IPE: C) at 25°C, by determining the intensity of the absorbance of PhCHO at 249.6 nm on a Beckman DU-70 spectrometer

were mixed. The fine enzyme powder mixed homogeneously in the organic solvent when the mixture was stirred at 30°C for the times indicated in Table 2. Upon removal of the crude enzyme by filtration, the filtrate was concentrated under reduced pressure, and the crude cyanohydrin was purified by column chromatography on silica gel. The enantiomeric purity of the cyanohydrins were determined with the chiral HPLC resolution on CHIRALPAK AD column as the corresponding *O*-protected derivatives.

Acknowledgements

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12. Almond was collected from mature garden fruits, after being granulated in a homogenizer, the preparation was defatted four times by treatment with ethyl acetate, then sealed and stored in a refrigerator at 4°C (water content in the meal was 8% w/w).
13. Hydrogen cyanide in diisopropyl ether or ethyl acetate solution was prepared according to the literature[4] method.