

Enzymatic Enantioselective Synthesis of (R)-2-Trimethylsilyl-2-hydroxyl-propionitrile by Defatted Apple Seed Meal

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The enantioselective synthesis of (R)-2-trimethylsilyl-2-hydroxyl-propionitrile by (R)-oxynitrilase contained in defatted apple seed meal in a biphasic system was successfully performed. The influences of some factors on the reaction were investigated systematically. Diisopropyl ether was found to be the best for this reaction among all the organic solvents examined. The optimal concentration of defatted apple seed meal, aqueous phase content, concentrations of acetyltrimethylsilane and acetone cyanohydrin, buffer pH, reaction temperature were 4% (W/V), 23% (V/V), 20 mmol·L⁻¹, 40 mmol·L⁻¹, pH = 5.0 and 40 °C, respectively, under which the initial reaction rate, substrate conversion and product enantiomeric excess were 9.4 mmol·L⁻¹·h⁻¹, 99% and >99%, respectively.

Keywords asymmetric synthesis, ketone-cyanohydrin, (R)-oxynitrilase, (R)-2-trimethylsilyl-2-hydroxyl-propionitrile

Introduction

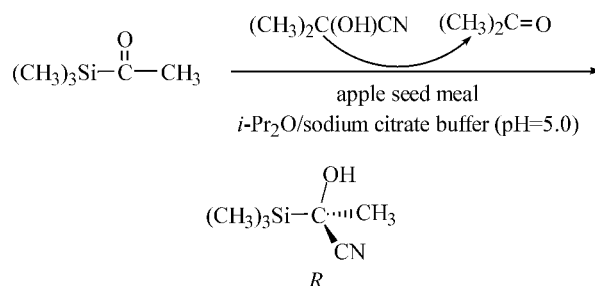
Over the past years, the synthesis of chiral aldehyde-cyanohydrins was well studied.¹ In contrast to chiral aldehyde-cyanohydrins, there were only few reports about the preparation of optically active ketone-cyanohydrins,² which are useful starting materials and intermediates for the synthesis of many chiral natural products.³ We therefore focused on the preparation of optically active silicon containing (R)-ketone-cyanohydrin ((R)-2-trimethylsilyl-2-hydroxyl-propionitrile) using acetone cyanohydrin as transcyanation agent and powdered, defatted apple seed meal as biocatalyst (Scheme 1), which was studied for the first time in this field.

Organosilicon compounds show unique chemical and physical properties compared to their carbon analogues due to the specific characteristics of silicon atom, and therefore they not only play an important role in asymmetric synthesis and functional materials, but also are bioactive and could be used for the synthesis of drugs with better pharmacological effect, higher selectivity and lower toxicity than their carbon counterparts. Recently, many investiga-

tions have been carried out to transform organosilicon compounds because of their importance in fundamental study of enzymology and the production of useful organosilicon compounds.⁴ We have already reported the bioconversion of organosilicon compounds with alcohol dehydrogenase and lipase.⁵ In these cases, the silicon atom served as a more effective atom than the carbon atom to enhance the activity and enantioselectivity of the enzymes.

In addition to almond meal, apple seed meal is another rich source of (R)-oxynitrilase. As previously shown, (R)-oxynitrilase from apple seed meal is a better biocatalyst than that from almond meal for reactions with sterically hindered aldehydes (e.g., pivalaldehyde) as substrates.⁶

Scheme 1 Synthesis of (R)-2-trimethylsilyl-2-hydroxyl-propionitrile



Experimental

Materials

Apple seed meal was collected and ground, then defatted with ethyl acetate and stored at 4 °C for use. *n*-Nonane, acetyltrimethylsilane were purchased from Sigma and Aldrich (USA). Acetone cyanohydrin was from Tokyo Kasei Kogyo Co., Ltd (Japan). All other chemicals were also from commercial sources and of analytical grade.

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Received March 24, 2003; revised May 22, 2003; accepted June 23, 2003.

Project supported by the National Natural Science Foundation of China (No. 20076019) and the Natural Science Foundation of Guangdong Province (No. 000444).

Enzymatic reaction

The reaction was carried out in a biphasic system. Diisopropyl ether (10 mL) containing $20 \text{ mmol} \cdot \text{L}^{-1}$ acetyltrimethylsilane, $40 \text{ mmol} \cdot \text{L}^{-1}$ acetone cyanohydrin and $10 \text{ mmol} \cdot \text{L}^{-1}$ *n*-nonane (used as internal standard) was mixed with citrate buffer (1.5 mL, $100 \text{ mmol} \cdot \text{L}^{-1}$, pH 5.0) containing powdered, defatted apple seed meal (unless specified). The mixture was incubated within a flask (50 mL) capped with a septum and shaken in a water bath shaker.

Assay of reaction mixture

At predetermined time intervals, 0.4 mL of sample was taken from the organic phase with a syringe (more than 99% of the substrate was dissolved in the organic phase and no side product was detectable by GC equipped with different columns) and assayed by HP 4890 Gas Chromatography with a flame ionization detector and a chiral column (20% permethylated β -cyclodextrin 30 m \times 0.32 mm, HP, USA). The column temperature was programmed as being upgraded from 80 °C to 139 °C at the rate of 7 °C/min and further increased to 142 °C at the rate of 1 °C/min. The retention-time of acetyltrimethylsilane, *n*-nonane, acetone cyanohydrin, (*S*)-product and (*R*)-product was 2.851, 3.651, 6.323, 9.715 and 9.780 min, respectively.

NMR spectral analysis

Kugelrohr distillation gave 2-trimethylsilyl-2-hydroxyl-propionitrile (65–70 °C, 8 mm). NMR spectra were obtained using CDCl_3 solution (CHCl_3 taken as δ 7.24) and a Bruker AVANCE Digital 400 MHz spectrometer. ^1H NMR δ : 0.18 (s, 9H), 1.54 (s, 3H), 2.85 (br s, 1H). ^{13}C NMR δ : -5.0, 23.3, 59.9, 123.1. Anal. calcd for $\text{C}_6\text{H}_{13}\text{NOSi}$: C 50.30, H 9.15, N 9.78; found C 50.40, H 9.27, N 9.89.

Results and discussion

Effect of apple seed meal concentration

As could be seen in Fig. 1, both initial reaction rate and product *ee* increased with the increase of apple seed

meal concentration when it was below 4% (W/V). This was due to the higher enzymatic reaction rate at higher apple seed meal concentration. Further increase in apple seed meal concentration, however, resulted in a decrease in initial reaction rate, which is in accordance with the previous report.^{1c} The reason for this was that obvious aggregation of apple seed meal at high concentration (> 4%, W/V) led to a low enzymatic activity due to mass transfer limitation. Clearly, there existed a mass transfer rate limiting enzyme concentration ($c_{E,MTL}$), and under the conditions used, the $c_{E,MTL}$ was 4% (W/V) for acetyltrimethylsilane.

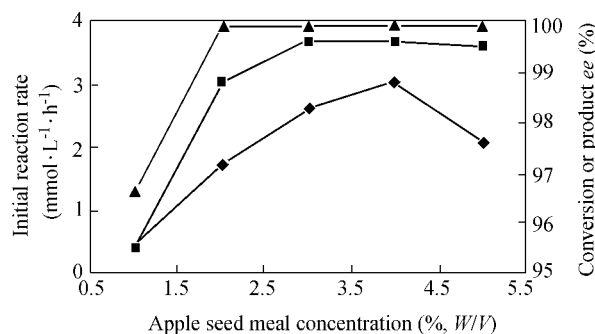


Fig. 1 Effect of apple seed meal concentration on the reaction. The reaction was performed in the biphasic system of 13% (V/V) of citrate buffer ($100 \text{ mmol} \cdot \text{L}^{-1}$, pH = 5.0) and diisopropyl ether at 30 °C and 150 r/min. The reaction mixture contained $20 \text{ mmol} \cdot \text{L}^{-1}$ acetyltrimethylsilane and $40 \text{ mmol} \cdot \text{L}^{-1}$ acetone cyanohydrin. —◆— initial reaction rate; —■— conversion; —▲— product *ee*.

Effect of organic solvent

As shown in Table 1, the more hydrophobic the organic solvent, the higher the initial reaction rate. This could be explained by the higher concentration of HCN owing to the presence of a greater amount of acetone cyanohydrin in the aqueous phase when more hydrophobic solvent was used as the organic phase. Two competitive reactions (enantioselective enzymatic reaction and non-enantioselective chemical one) took place simultaneously. Too much HCN in the aqueous phase might inhibit the enzymatic but enhance the chemical reaction, thus leading to a low product *ee*. This could account for the low product *ee* in the

Table 1 Effect of organic solvent on the reaction^a

Organic solvent	log <i>P</i>	v_0 ($\text{mmol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	<i>t</i> (h)	Conversion (%)	<i>ee</i> (%)
Ethyl acetate	0.68	0.4	30	30	> 99
Diisopropyl ether	1.9	2.2	23	99	> 99
Cyclohexane	3.0	3.6	19	98	90
<i>n</i> -Hexane	3.5	3.2	19	98	87

^aThe reaction was initiated by addition of 4% (W/V) apple seed meal in the biphasic system of 13% (V/V) of citrate buffer ($100 \text{ mmol} \cdot \text{L}^{-1}$, pH = 5.0) and organic solvent at 30 °C, 150 r/min. The reaction mixture contained $20 \text{ mmol} \cdot \text{L}^{-1}$ acetyltrimethylsilane and $40 \text{ mmol} \cdot \text{L}^{-1}$ acetone cyanohydrin.

cases with *n*-hexane and cyclohexane as the organic phase. Among the four organic solvents, diisopropyl ether was the most suitable for the reaction.

Effect of aqueous phase content

It was reported that the influence of the aqueous phase content varies widely.⁷ In this reaction, acetone cyanohydrin was used as transcyanation agent. Relatively high water content was necessary for the decomposition of acetone cyanohydrin at a reasonable rate.⁸ Fig. 2 showed the variation of the product *ee* and the initial reaction rate with the aqueous phase content. At low water content, acetone cyanohydrin decomposed slowly, and therefore, HCN concentration in aqueous phase was rather low, resulting in the low initial reaction rate and high product *ee*. On the other hand, an excess of water resulted in the dilution of HCN, leading to the low initial reaction rate and the low product *ee*.

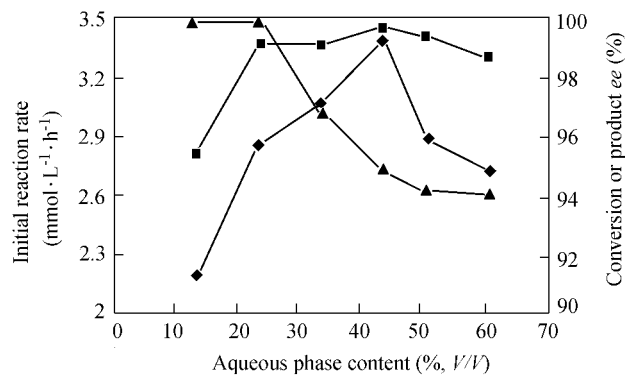


Fig. 2 Effect of the aqueous phase content on the reaction. This was investigated in the biphasic system of citrate buffer (100 mmol · L⁻¹, pH = 5.0) and diisopropyl ether at 30 °C and 150 r/min. The reaction mixture contained 20 mmol · L⁻¹ acetyltrimethylsilane, 40 mmol · L⁻¹ acetone cyanohydrin, and 4% (W/V) apple seed meal. —◆— initial reaction rate; —■— conversion; —▲— product *ee*.

Effect of substrate concentration

It is well known that, thermodynamically, high sub-

strate concentration pushes the reaction towards the synthesis of the product. Substrate inhibition, however, might occur at excessively high substrate concentration. Therefore, it is important to investigate the influence of substrate concentration on the reaction. It has been found that the optimal acetyltrimethylsilane concentration was 20 mmol · L⁻¹ and the product *ee* dropped at higher substrate concentration (Table 2). Substrate inhibition might be the main reason for this.

Effect of buffer pH

The buffer pH has a significant influence on enzymatic enantioselectivity and activity.^{2a, 8} At the same time, the effect of pH on the formation of CN⁻ is apparent.⁸ At low pH (≤ 6.0), the formation of HCN proceeded slowly, which contributed to a high product *ee*. As could be seen in Fig. 3, the maximal initial reaction rate was achieved at pH = 5.4, which is identical with Griengl's result.⁹ High product *ee* and low initial reaction rate at low pH (≤ 5.0) demonstrated the depression of nonenzymatic reaction at low pH.

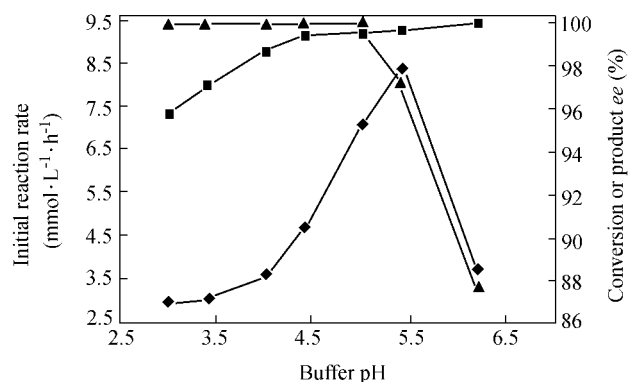


Fig. 3 Effect of buffer pH on the reaction. The reaction was initiated by addition of 4% (W/V) apple seed meal in the biphasic system of 23% (V/V) of citrate buffer (100 mmol · L⁻¹) and diisopropyl ether at 30 °C and 150 r/min. The reaction mixture contained 20 mmol · L⁻¹ acetyltrimethylsilane and 40 mmol · L⁻¹ acetone cyanohydrin. —◆— initial reaction rate; —■— conversion; —▲— product *ee*.

Table 2 Effect of substrate concentration on the reaction^a

Acetyltrimethylsilane concentration (mmol · L ⁻¹)	Acetone cyanohydrin concentration (mmol · L ⁻¹)	<i>t</i> (h)	Conversion (%)	<i>ee</i> (%)
10	20	23	99	> 99
20	40	23	> 99	> 99
30	60	25	99	98
40	80	30	99	97
50	100	27	99	96
80	160	23	> 99	95
160	320	30	96	92

^a The reaction was initiated by addition of 4% (W/V) apple seed meal in the biphasic system of 23% (V/V) of citrate buffer (100 mmol · L⁻¹, pH = 5.0) and diisopropyl ether at 30 °C and 150 r/min.

Effect of reaction temperature

Temperature influences the activity, selectivity and stability of a biocatalyst and the equilibrium of a reaction as well significantly. The initial reaction rate, the maximum conversion and the product *ee* were explored at different temperatures (Table 3). Within the range from 15 °C to 45 °C, higher temperature enhanced the initial reaction rate and shortened the reaction time greatly. When the temperature was below 40 °C, it had no effect on the product *ee*, which was against the previous study,¹⁰ and further increase of the temperature resulted in a lower product *ee*.

Table 3 Effect of reaction temperature on the reaction^a

<i>T</i> (°C)	<i>v</i> ₀ (mmol·L ⁻¹ ·h ⁻¹)	<i>t</i> (h)	Conversion (%)	<i>ee</i> (%)
45	10.8	4	99	96
40	9.4	8	99	> 99
35	7.6	10	> 99	> 99
30	5.4	18	99	> 99
25	4.7	30	99	> 99
20	3.6	32	99	> 99
15	2.5	47	98	> 99

^a The reaction was initiated by addition of 4% (W/V) apple seed meal in the biphasic system of 23% (V/V) of citrate buffer (100 mmol·L⁻¹, pH = 5.0) and diisopropyl ether at 150 r/min. The reaction mixture contained 20 mmol·L⁻¹ acetyltrimethylsilane and 40 mmol·L⁻¹ acetone cyanohydrin.

Conclusion

Optically active silicon containing aliphatic (*R*)-ketone-cyanohydrin ((*R*)-2-trimethylsilyl-2-hydroxy-propionitrile) could be efficiently synthesized with defatted apple seed meal in a water/organic solvent biphasic system. Apple seed meal seems to be a more effective (*R*)-oxynitrilase source for the reaction than almond meal, in terms of the initial reaction rate, substrate conversion and product *ee*.¹¹

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(E0303242 ZHAO, X. J.; LING, J.)