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Enantioselective formation of mandelonitrile acetate—investigation of a dynamic kinetic resolution

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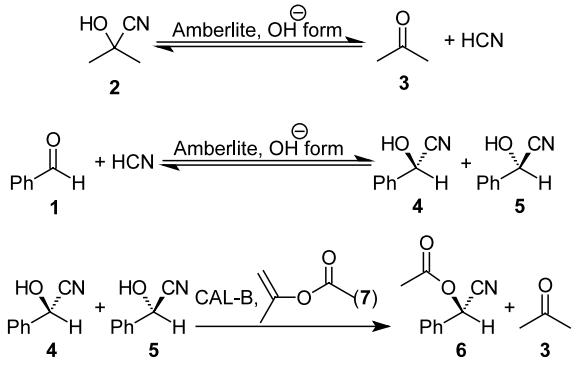
Abstract—Investigations into the separate reactions of a dynamic kinetic resolution and the combined reactions revealed that the overall sequence is highly susceptible to the water content of the reaction mixture. While the racemization and formation of mandelonitrile as well as its kinetic resolution proceeded rapidly when performed independently, the dynamic kinetic resolution was severely hampered by the undesired formation of acetic acid during the reaction. The utilization of drying reagents and neutralizing agents in order to suppress the formation of acetic acid or its consequences were investigated. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Enantiomerically pure cyanohydrins and their esters are important building blocks in organic chemistry.[1–3] Their synthesis has therefore attracted considerable attention.[4,5] One particularly elegant approach is the dynamic kinetic resolution of cyanohydrins formed in situ from an aldehyde **1** and acetone cyanohydrin **2**, the cyanide source. The utilization of an enantioselective lipase for catalyzing the esterification leads to high yield and enantiomeric purity of the formed cyanohydrin ester **6** (Scheme 1). This method was first published in 1991[6] and in more detail in 1992.[7,8] These papers have been quoted frequently; however, only one report has since described the application of the methodology.[9] Since all the chemicals and the enzyme utilized in this synthesis of the cyanohydrin acetates **6** are readily available, this is somewhat surprising. All the more so, since the method has even been included in a book on preparative biotransformations.[10] The main drawback of the published synthesis is the relatively long reaction time of 6–8 days. In order to obtain a better insight into the possible reasons for the long reaction time we investigated this multi component reaction and its separate steps. Few modifications were introduced. As an enzyme the highly selective *Candida antarctica* lipase B (CAL-B, chirazyme L-2, c.-f., C2, Lyo)[11,12] rather than *Pseudomonas cepacia* lipase was utilized.[7,10] The study was performed in dry toluene (instead of diisopropyl ether), a solvent that proved to be particularly suitable for the kinetic resolution of cyanohydrin acetates with CAL-B. Furthermore no zeolite molecular sieve was added for water adsorption,[13] since this might also act as a Lewis or Brønsted acid,[14] thereby disturbing the hydrocyanation which is catalyzed by alkaline Amberlite ($^-$OH form).[7,10] Benzaldehyde **1** was used as a model substrate, since it is known to be a particularly good substrate for this reaction sequence.



Scheme 1.

Three separate reactions constitute the overall synthetic sequence from benzaldehyde **1** to the final product (*S*)-mandelonitrile acetate **6**. The elimination of HCN

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from acetone cyanohydrin **2** as well as the addition of HCN to benzaldehyde **1** are reversible, base catalyzed, reactions. Under acidic conditions **2**, **4** and **5** are stable, and the reactions are extremely slow in either direction. The two reversible reactions are essential for the formation and racemization of mandelonitrile (**4**+**5**), the substrate of the enzyme reaction. They can be driven to completion by the third, irreversible reaction.

This third step is the CAL-B catalyzed acylation of **4** with *iso*-propenyl acetate **7** to form (*S*)-mandelonitrile acetate **6** and acetone **3**. The enantiopurity of the product depends on the enantioselectivity *E* of the enzyme for the specific substrate under the specific conditions. In a kinetic resolution the ee of the product decreases with the degree of conversion, since the availability of the favored substrate enantiomer (here **4**) decreases much more rapidly than that of the less favored substrate enantiomer (here **5**). In order to achieve the greatest possible enantiomeric purity in an enzymatic dynamic kinetic resolution, it is therefore important not only to utilize an enzyme with a high *E* value, but also to ensure that the substrate remains (almost) racemic throughout its conversion. Thus, it is essential that the racemization is faster than the acylation. Therefore we first studied the reactions individually and then in combination. In the overall reaction, the molar ratio of **1** to acetone cyanohydrin **2** to *iso*-propenyl acetate **7** was fixed at 1:2:3.

2. Results and discussion

Utilizing 0.05 mol equiv. of alkaline Amberlite, the equilibrium constants and the equilibration times of the combined first two reactions were determined. The concentrations of **1** and **2** were quantified simultaneously by IR (Fig. 1a). Even at room temperature the equilibrium of the combined reactions was reached within 5 h (Fig. 1b).

The racemization of **5** under reaction conditions (40 or 60°C) proceeded completely within an hour or less (Fig. 2), which is in accordance with data reported earlier.[9] Moreover, the racemization reaction was not influenced by the presence of CAL-B. It was therefore anticipated that the elimination and addition reactions of HCN would not form the bottleneck of the overall procedure.

In the CAL-B catalyzed acylation of the racemic mixture of **4** and **5** with **7**, only **4** is converted. Several conditions for the esterification of the racemate were investigated (Fig. 3). At 25, 40 and 60°C in toluene with different enzyme concentrations CAL-B always showed

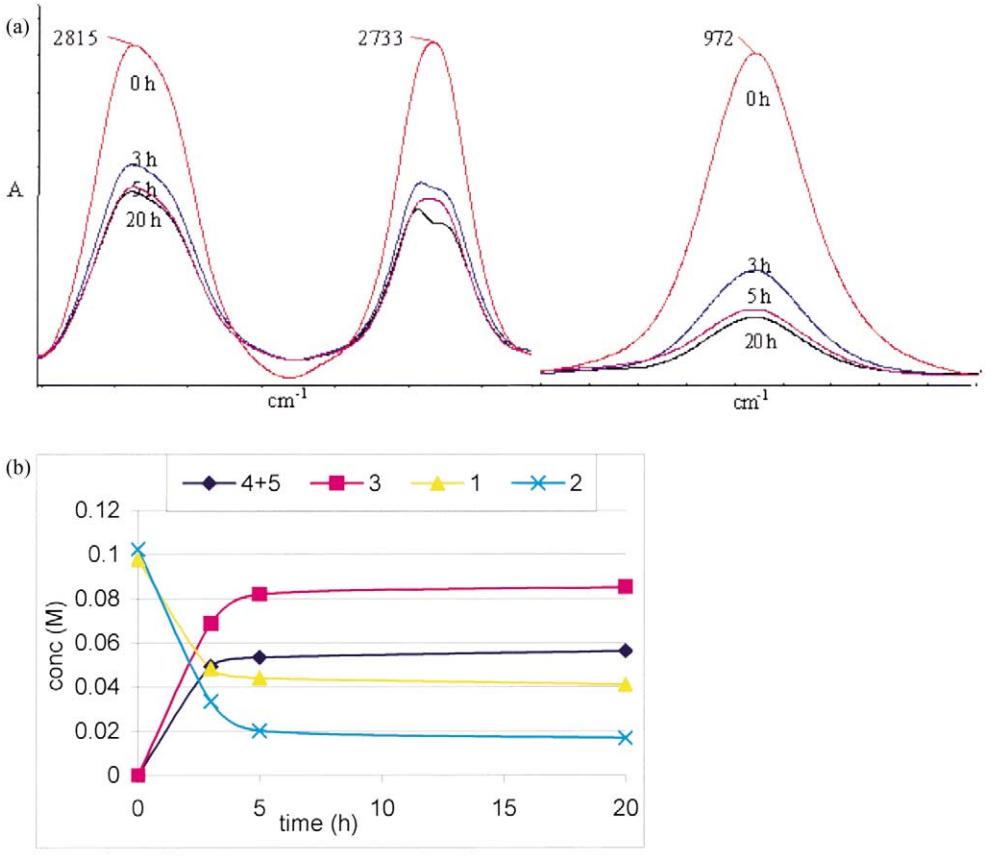


Figure 1. (a) IR spectra of the measurements of the reaction of **1** and **2** in the presence of Amberlite (0.0625 equiv. $^{-}$OH) to form **3**, **4** and **5**. The concentration of **1** was measured at 2733 and 2815 cm^{-1} and the concentration of **2** at 972 cm^{-1}. The equilibrium constant for the racemic reaction was determined as $K_{eq} = 6.53$. (b) Reaction of **1** and **2** in the presence of Amberlite (0.0625 equiv. $^{-}$OH) to form **3**, **4** and **5**. The concentration of HCN is not shown.

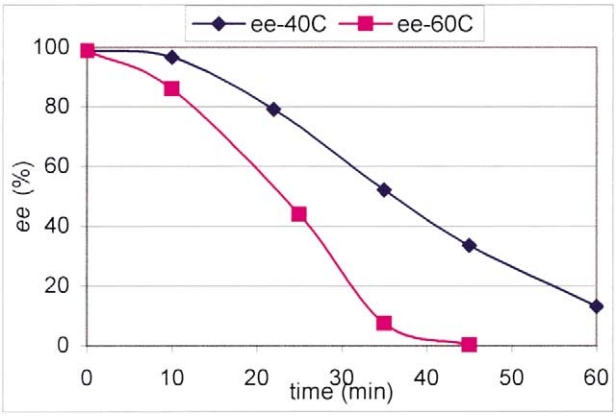


Figure 2. Racemization of **5** at 40 and 60°C in the presence of Amberlite (0.05 equiv. $^{-}$OH).

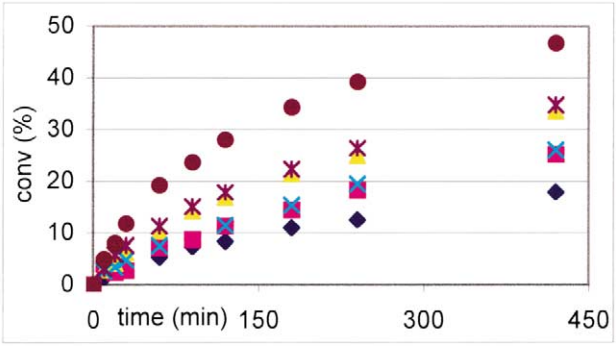


Figure 3. Kinetic resolution of (±)-mandelonitrile (**4**+**5**) with CAL-B and **7** at different temperatures and enzyme concentrations. ◆ 25°C and 37 U/mL; ■ 40°C and 37 U/mL; ▲ 60°C and 37 U/mL; × 25°C and 74 U/mL; * 40°C and 74 U/mL; ● 60°C and 74 U/mL.

excellent selectivity (E[15] always larger than 400). In every case **6** was formed with an ee ≥99%. The reaction rate was proportional to the amount of enzyme.

These results clearly indicate that the separate reactions are fast and proceed with high selectivity. As is required for a successful dynamic kinetic resolution, racemization is significantly faster than acylation if either 370 or 740 units of CAL-B per mmol of substrate are utilized. In order to achieve overall reaction times that are significantly shorter than those described in the literature, the reaction temperature should be maintained at 40°C or above. No side products were detected in any case. Moreover, Amberlite did not catalyze the acylation step, ruling out any interference of the chemical catalyst with the enzyme catalyzed reaction.

These results indicate that the combination of the racemization of mandelonitrile (**4**+**5**) and its kinetic resolution should lead to the fast and highly selective formation of (*S*)-mandelonitrile acetate **6**. This is indeed the case during the initial period of the reaction (Fig. 4). Comparison of Figs. 3 and 4 show that the observed slowing down of the formation of **6** can be attributed to the formation of **1** rather than to any inhibition of the enzyme. In the course of the reaction, however, a change in rates was observed. Instead of rapid racemization and acylation of mandelonitrile, the concentration of **1** seems to almost stabilize on a plateau. Moreover, the ee of **5** increases, a clear indication of a kinetic resolution without racemization of the initially racemic starting material. This strongly suggests that the Amberlite is deactivated during the course of the reaction. Similar results were obtained at 40°C. The same observations were also made when performing the overall reaction of **1** (0.1 M) with **2** and **7** in the presence of CAL-B (37 U/mL) and Amberlite (0.05 equiv. $^{-}$OH). The reaction was extremely slow and even after 47 h the yield of **6** was only 9.5%.

Recently, it has been demonstrated that the presence of even very small quantities of water can lead to significant enzyme-catalyzed hydrolysis of the acylating reagent.[16] The toluene utilized in the reactions described above contained 120 ppm of water. In order to investigate the consequences of this amount of water, the CAL-B catalyzed hydrolysis of (±)-mandelonitrile acetate and **7** in dry toluene (obtained from Aldrich) was examined separately. Both compounds were initially rapidly hydrolyzed. At 25°C and in the presence of 120 ppm of water, 18% of (±)-mandelonitrile acetate and 21% of **7** were hydrolyzed within 5 h. The hydrolysis proceeded even after an amount of water equal to the original amount of water in the dry toluene had been consumed. This indicates that water adsorbed on the enzyme and its carrier must also be utilized in this hydrolysis. During the dynamic kinetic resolution of **4**+**5** some enzymatic hydrolysis of **6** and **7** can also be expected, although **6** and **7** were stable in the presence of Amberlite alone. If this is indeed the case, the acetic acid that is formed will quickly and completely neutralize the alkaline ion exchanger, which is present in only 0.05 mol equiv. The reaction mixture turns from alkaline to acidic. This stabilizes **2**, **4** and **5**. Such neutralization regenerates water that had been consumed by hydrolysis, so the water concentration will remain constant, while the alkaline Amberlite will be depleted (Scheme 2).

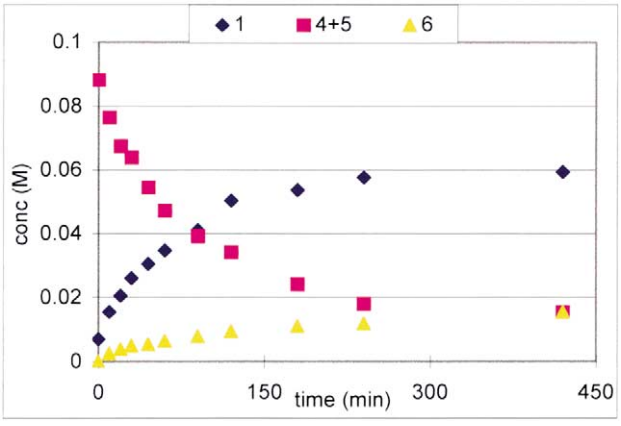
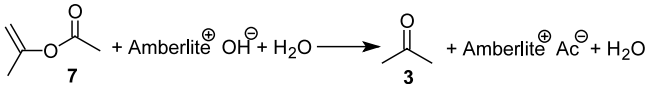


Figure 4. Dynamic kinetic resolution of (±)-mandelonitrile (**4**+**5**) in the presence of **7**, CAL-B and Amberlite (0.05 equiv. $^{-}$OH) at 60°C.



Scheme 2.

Upon deactivation of the racemization catalyst, the dynamic kinetic resolution is turned into a conventional kinetic resolution. In addition, not all of **1** has at this stage been converted into **4** and **5** nor has all of **2** been converted into HCN and acetone **3**. Therefore, the concentration of **4**+**5** is relatively low. Further drying of the solvent with molecular sieve prior to the incubation of (±)-mandelonitrile acetate with CAL-B lowered the water content to 60 ppm. This did reduce the rate of the hydrolysis, but the problem remained. In order to overcome this problem and to ensure that the reaction medium remained alkaline, 0.25 mol equiv. of base was added in the form of Amberlite. This did indeed lead to an improvement in the overall reaction. The conversion of **1** was significantly improved and the yield of **6** after 45 h was higher than before (15.9 instead of 9.5%).

This clearly demonstrates that the Amberlite and CAL-B catalyzed synthesis of cyanohydrin acetate **6** is very sensitive to hydrolysis and the acids released thereby. In order to overcome this problem and to improve the yield of **6** a large amount of Amberlite (1.0 mol $^{-}$OH equiv.) was added. However, instead of improving the yield, only 2.1% of **6** was formed after 45 h. Moreover, this reaction mixture turned brownish, something not observed in the other reactions. This points to a base induced polymerization of the HCN, which is well known to yield brown and even black products.[17] Adding more base, in order to neutralize the released acid does not circumvent the problem of hydrolysis. It seems to be necessary to suppress hydrolysis or its consequences more efficiently.

In summary, low concentrations of alkaline Amberlite efficiently catalyze the formation and racemization of mandelonitrile (**4**+**5**) and do not inhibit the enzyme-catalyzed acylation. Similarly, CAL-B is an excellent catalyst for the enantioselective formation of cyanohydrin acetates **6**. However, the combination of these reactions to a dynamic kinetic resolution is severely hampered by the acid formed by the hydrolysis of **6** and **7**. Utilization of larger quantities of base does not solve this problem, possibly due to the polymerization of HCN.

3. Experimental

3.1. Materials and methods

Benzaldehyde, acetone, acetone cyanohydrin and *iso*-propenyl acetate were distilled prior to use and were stored under nitrogen. Technical grade (±)-mandelonitrile from Acros was purified by silica gel column chromatography and stored under nitrogen. Dry toluene was obtained from Aldrich. Molecular sieves (4 Å) were obtained from Aldrich and used as supplied. CAL-B (Chirazyme L-2, cf., C2, Lyo) was a gift from Roche Diagnostics (Penzberg, Germany). Its activity was determined according to the procedure developed by Roche Diagnostics.[18] The enzyme was stored in a dessicator with silica gel as a drying agent at room temperature. The activity of the stored enzyme (3.7 U/mg) remained unchanged over a period of half a year. Amberlite IRA904 was purchased from Acros and conditioned according to the literature.[7] HPLC analyses were conducted with a Waters 510 pump, a Chiracel OB-H column (0.46×25 cm), a Waters 486 UV detector at 215 nm and a mixture of hexane and *iso*-propanol (92:8) with 0.1% acetic acid as eluent. The flow rate was 1.0 mL/min. 1,3,5-Triisopropylbenzene was used as an internal standard. Retention times: 1,3,5-triisopropylbenzene 3.7 min, **7** 5.7 min, **1** 7.4 min, **5** 12.4 min; **4** 13.2 min; **6** 15.1 min and (*R*)-mandelonitrile acetate 20.3 min. The water concentration in toluene was measured by Karl–Fischer Titration with a Mettler DL35. Hydranal®–Titrant 2 (from Riedel–deHaen) was used as titrant. IR analysis was performed with a Perkin–Elmer FT-IR Spectrometer Spectrum 1000 in a KBr cell with a path length of 0.115 mm. Absorptions were measured at 2733 and 2815 cm^{-1} **1** and 972 cm^{-1} **2**.

3.2. Kinetic resolution of (±)-mandelonitrile (4+5)

In an 8 mL vessel (±)-mandelonitrile (**4**+**5**) (66.5 mg, 0.5 mmol) and *iso*-propenyl acetate **7** (125 mg, 1.25 mmol) were dissolved in 5 mL dry toluene. A baseline sample was taken with a microsyringe. The solution was then transferred to a nitrogen gas flushed vessel with CAL-B (50 mg, 185 U or 100 mg, 370 U) in it. The reaction system was closed with a rubber septum and stirred at the desired temperature (25, 40 or 60°C). Samples (15 μL) were taken with a microsyringe at the time intervals given in Fig. 3 and diluted with 1 mL solvent (same as the mobile phase for HPLC) for chiral HPLC analysis. The enantiomeric excess of **6** was always >99%.

3.3. Dynamic kinetic resolution of (±)-mandelonitrile (4+5)

In an 8 mL vessel (±)-mandelonitrile (**4**+**5**) (66.5 mg, 0.5 mmol) and *iso*-propenyl acetate **7** (125 mg, 1.25 mmol) were dissolved in 5 mL of dry toluene. A baseline sample was taken with a microsyringe. The solution was then transferred to a nitrogen gas flushed vessel with CAL-B (50 mg, 185 U) and Amberlite IRA904 ($^{-}$OH form, 19.2 mg, 0.025 mmol $^{-}$OH) in it. The reaction system was closed with a rubber septum and stirred at 60°C. Samples (15 μL) were taken with a microsyringe at the time intervals given in Fig. 4 and diluted with 1 mL of solvent (the same as the mobile phase for HPLC) for chiral HPLC analysis. The enantiomeric excess of **6** was always >99%.

3.4. Racemization of (*R*)-mandelonitrile 5

Amberlite IRA904 ($^{-}$OH form, 19.1 mg, 0.025 mmol $^{-}$OH) was added to a solution of **5** (66.5 mg, 0.5 mmol) in dry toluene (5 mL). The reaction mixture was stirred at the desired temperature (40 or 60°C). Samples (15

μL) were taken with a microsyringe at the time intervals given in Fig. 2 and analyzed by chiral HPLC to measure the ee values.

3.5. Chemical transcyanation between benzaldehyde 1 and acetone cyanohydrin 2 and vice versa

Amberlite IRA 904 ($^-$OH form, 38.5 mg, 0.05 mmol $^-$OH) was added to a solution of **1** (84.8 mg, 0.8 mmol) and **2** (70.0 mg, 0.82 mmol) in dry toluene (8 mL). The mixture was stirred in a closed system at room temperature (25°C). Samples were taken (at the times given in Fig. 1b) with a syringe for infrared spectroscopic analysis (see Section 3.1). After 5 h the equilibrium situation was reached with 0.35 mmol **1**, 0.16 mmol **2**, 0.66 mmol **3** and 0.45 mmol (±)-mandelonitrile (**4**+**5**).

The reverse reaction was carried out similarly using racemic mandelonitrile (109.3 mg, 0.82 mmol) and **3** (51.0 mg, 0.88 mmol) in dry toluene (8 mL). After 5 h the equilibrium situation was reached with 0.38 mmol **1**, 0.14 mmol **2**, 0.74 mmol **3** and 0.44 mmol (±)-mandelonitrile (**4**+**5**).

3.6. Hydrolysis of (±)-mandelonitrile acetate or *iso*-propenyl acetate 7

(±)-Mandelonitrile acetate (or **7**) (0.5 mmol) and CAL-B (50 mg, 185 U) were added to dry toluene (5 mL) that contained 120 ppm water or toluene (5 mL) that contained 60 ppm water (obtained by drying the dry toluene from Aldrich with molecular sieves). The reaction mixture was stirred at room temperature (25°C). The conversion was measured by chiral HPLC. After 5 h in the presence of 120 ppm H_2O, 21.2% **7** and 18.6% (±)-mandelonitrile acetate were hydrolyzed. After 5 h in the presence of 60 ppm H_2O, 12.9% (±)-mandelonitrile acetate were hydrolyzed.

3.7. One-pot synthesis of (*S*)-mandelonitrile acetate 6 from benzaldehyde 1, acetone cyanohydrin 2 and *iso*-propenyl acetate 7

1 (84.8 mg, 0.8 mmol), **2** (136 mg, 1.6 mmol) and **7** (240 mg, 2.4 mmol) were added to dry toluene (8 mL). To this solution different $^-$OH equivalents of Amberlite IRA904 ($^-$OH form, 30.8 mg/0.05 equiv., 154 mg/0.25 equiv., or 616 mg/1.0 equiv.) and CAL-B (80 mg, 296 U) were added. The reaction mixture was stirred at 40°C and 15 μL samples were taken for the analysis by chiral HPLC. The enantiomeric excess of **6** was always >99%. The results are shown in Table 1.

Acknowledgements

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Table 1. One-pot synthesis of **6** from **1**, **2** and **7** in the presence of different amounts of Amberlite ($^-$OH form)

Amberlite ($^-$OH equiv.)	Time (h)	Yield of **6** (%)	Conv. of **1** (%)	ee of **5** (%)
1.0	21	1.8	49.7	0.6
	45	2.1	47.9	6.2
0.25	21	9.1	60.1	2.4
	45	15.9	67.3	0.8
0.05	20	4.1	40.3	2.9
	47	9.5	59.7	3.0