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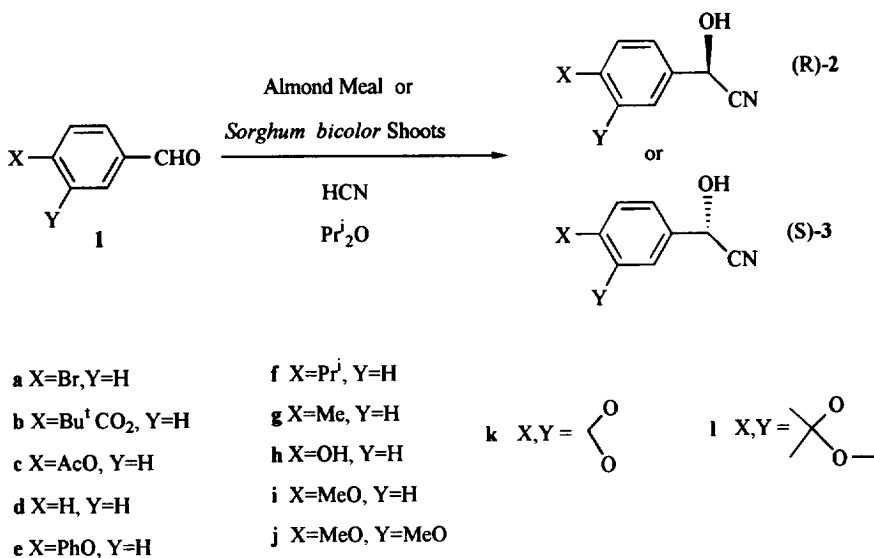
## (*R*)- and (*S*)-Cyanohydrins Using Oxynitrilases in Whole Cells

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**Abstract:** Almond meal and *Sorghum bicolor* shoots were used as the sources of oxynitrilases for the preparation of a number (*R*)- and (*S*)-arylcyanohydrins, respectively, from the corresponding aldehydes in diisopropyl ether. Two different *in situ* methods were used to introduce hydrogen cyanide into the reaction mixture. In method 1, acetone cyanohydrin decomposes enzymatically and/or chemically to hydrogen cyanide. In method 2, hydrogen cyanide freely evaporates from a solution in diisopropyl ether from one compartment of the reaction vessel and ends up to the other where it dissolves into the reaction mixture. Copyright © 1996 Elsevier Science Ltd

The enantiomers of cyanohydrins are fascinating targets for the preparation of different kinds of optically active compounds such as optically active 2-hydroxycarboxylic acids and 2-amino ethanol. In particular, chemical (cyclic dipeptide-catalysed) and enzymatic (oxynitrilase-catalysed) asymmetric syntheses involving carbon-carbon bond formation in the condensation of hydrogen cyanide with an aldehyde have been intensively studied.<sup>1-10</sup> The chemical method using cyclo[(*S*)-phenylalanyl-(*S*)-histidyl] catalysis and the enzymatic process exploiting (*R*)-oxynitrilase (E.C. 4.1.2.10) have been applied for the preparation of aromatic and/or aliphatic aldehyde cyanohydrins with the (*R*) absolute configuration. Due to the need of the corresponding (*R,R*)-dipeptide or expensive (*S*)-oxynitrilase (E.C. 4.1.2.11) the production of the corresponding (*S*)-cyanohydrins has been economically less attractive. In order to avoid the use of isolated enzymes, and accordingly to lower the expense, we previously published a method for the preparation of aliphatic (*R*)-aldehyde cyanohydrins and later for that of (*S*)-mandelonitrile which exploits (*R*)- and (*S*)-oxynitrilases in defatted almond meal and dechlorophyllated *Sorghum bicolor* shoots, respectively.<sup>6,9</sup> In these systems (method 1), acetone cyanohydrin served as the source of hydrogen cyanide. Almond meal catalysis for the preparation of aliphatic (*S*)-ketone and (*R*)-aldehyde cyanohydrins using racemic ketone cyanohydrins in the place of acetone cyanohydrin has been usable as well.<sup>11</sup> In the case of *Sorghum bicolor* shoot-catalysed condensation, however, unreasonably long reaction times (of the order of 2 weeks to reach the conversion  $\geq 90\%$  for mandelonitrile **3d**) were observed.<sup>9</sup> The aim of the present work has been to study the whole cell systems in more detail and to improve the biotransformations, especially those leading to aromatic (*S*)-cyanohydrins. A new whole cell method (method 2) exploiting gaseous hydrogen cyanide which freely diffuses into the reaction mixture is now introduced. The methods have been applied for the synthesis of a number of 4-substituted or 3,4-disubstituted mandelonitriles in diisopropyl ether (Scheme 1). The use of aromatic aldehydes **1** as substrates enabled the preparation of the corresponding (*R*)- and (*S*)-enantiomers. Aliphatic aldehydes or ketones are not the substrates for the *Sorghum* oxynitrilase.



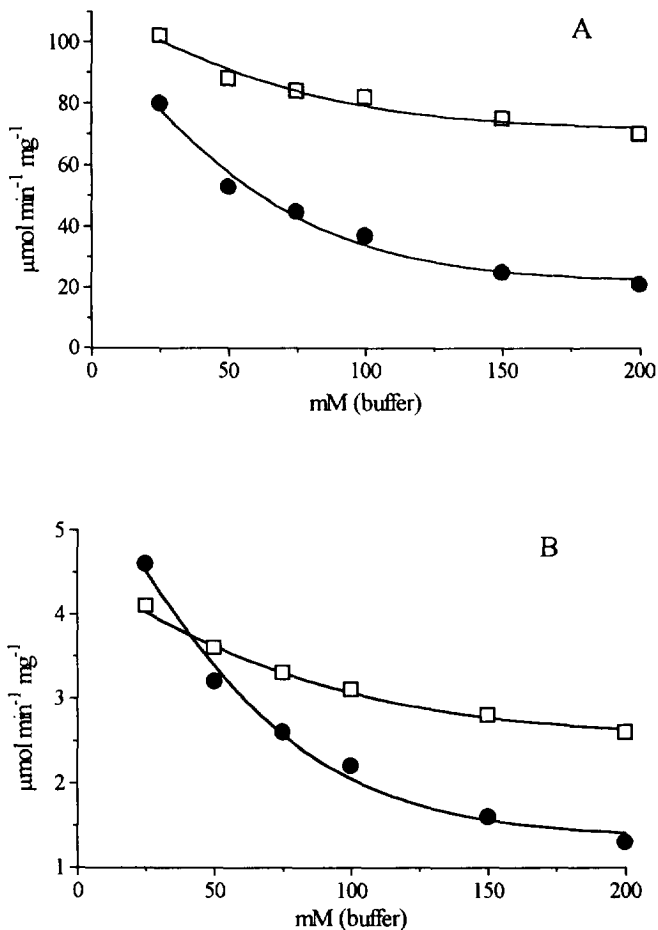
Scheme 1

### The effects of pH and buffer

For oxynitrilase-catalysed condensations, the chemical formation of the corresponding racemic cyanohydrin always competes with the highly enantioselective oxynitrilase-catalysed reaction and may lead to a decrease in the enantiomeric purity of the product. The chemical reaction is most conveniently suppressed by working in an organic solvent or at low pH. On the other hand, the catalytic activity of oxynitrilases always demands the presence of some water, and the rate of an enzymatic reaction is usually pH dependent. Thus, information about the pH behaviour of the enzymes is valuable. Although the two oxynitrilases differ in both structure and catalytic properties<sup>3</sup> the pH profiles for enzymatic activities are almost identical. Thus, for the decompositions of mandelonitrile and 4-hydroxymandelonitrile (the natural substrates of the enzymes) in the presence of commercial oxynitrilases from almond and *Sorghum bicolor*, respectively, the pH optima occur at around 5.5.<sup>12</sup> This pH behaviour is in accordance with the literature data.<sup>14</sup> It is of great importance for (*S*)-oxynitrilase-catalysed condensations that the enzyme still retains some of its catalytic activity at pH < 4 because the values of the product ee rapidly fall off in more basic solutions. Thus, the value of ee was found to drop from 0.92 (conversion 5 %, time 7 h) through 0.83 (conversion 34 %, time 6 h) to 0.74 (conversion 70 %, time 5 h) when pH was changed from 3.75 through 5.0 to 6.0, respectively, for the *Sorghum bicolor* shoot-catalysed synthesis of cyanohydrin **3d** in diisopropyl ether containing acetone cyanohydrin and 0.1 M citrate buffer [17.5 % (v/v)].

Both the quality and quantity of a buffer may affect enzymatic activity. It has been reported that citrate buffers at pH ≥ 5.5 do not cause the inhibition of almond oxynitrilase although monovalent citrate ion prevailing at lower pH may act as an inhibitor.<sup>15</sup> In accordance with this, enzymatic activities for the decompositions of mandelonitrile and 4-hydroxymandelonitrile in the presence of commercial oxynitrilases from almond and *Sorghum bicolor*, respectively, decrease with increasing citrate or tartrate buffer concentrations at pH 3.75 (Figure 1).<sup>12</sup> Similarly, for the *Sorghum bicolor* shoot-catalysed synthesis of mandelonitrile **3d** in aqueous diisopropyl ether lower reactivity is observed in the more concentrated citrate

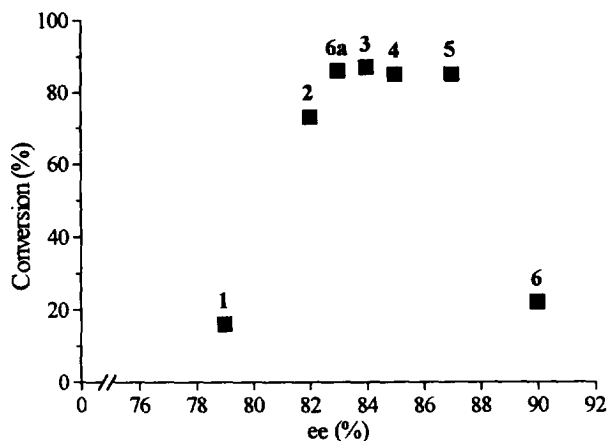
buffer (Figure 2; cases 6 and 6a). Moreover, the quality and quantity of a buffer clearly affect the enantiomeric purity of the synthetic product, the best compromise between reactivity and ee being obtained in the case of 0.1 M tartrate buffer (Figure 2). On this basis, 0.1 M tartrate buffer (pH 5.4 or 3.75 for almond meal or *Sorghum bicolor* shoot catalyses, respectively) is used to maintain the appropriate water level in an organic solvent throughout this work although the buffering effect at pH 5.4 evidently starts to be slight.



**Figure 1.** (R)- and (S)-oxynitrilase activities ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ ) vs. buffer concentration (mM) at pH 3.75 for the decomposition of mandelonitrile (A) and 4-hydroxymandelonitrile (B): (□) tartrate and (●) citrate buffer.

#### Acetone cyanohydrin as the source of hydrogen cyanide; Method 1

For oxynitrilase-catalysed condensations in organic solvents, hydrogen cyanide is introduced into the reaction mixture *in situ* from a suitable donor, added as a neat compound or extracted from an aqueous

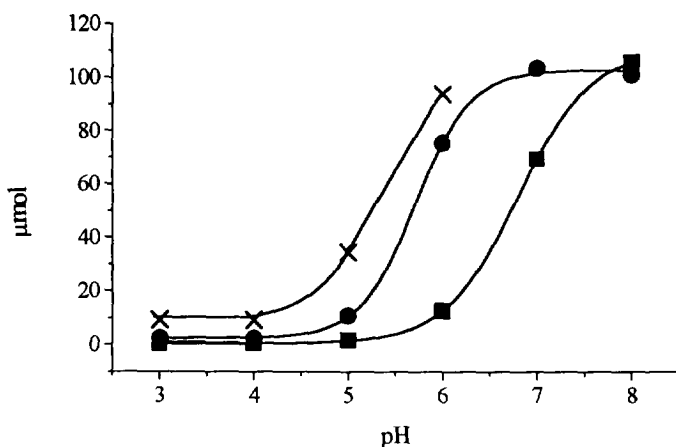


**Figure 2.** The formation of mandelonitrile **3d** (method 1) after 20 h vs. ee in diisopropyl ether, containing 17.5 % (v/v) of 0.1 M buffers, pH 3.75: **1** malate, **2** formate, **3** acetate, **4** phosphate (pH 3.0), **5** tartrate, **6** citrate and **6a** citrate (25 mM).

solution.<sup>3-11</sup> In the case of almond meal catalysis, both the yield and enantiomeric purity of aliphatic aldehyde cyanohydrins and mandelonitrile **2d** (conversion and ee both approaching to 100 %) were practically independent of the source of hydrogen cyanide.<sup>4-6</sup> For the *Sorghum bicolor* shoot-catalysed synthesis of mandelonitrile **3d** in diisopropyl ether in the presence of 0.1 M citrate buffer [17.5 % (v/v), pH 3.25], however, reactivity was low (75 % conversion in 284 h) when hydrogen cyanide was produced *in situ* from acetone cyanohydrin.<sup>9</sup> Moreover, enantioselectivity went through a shallow maximum (ee of the order of 0.90) at the water content of ca. 20 % (v/v).<sup>9</sup> As is reasonable (Figure 2), tartrate buffer [14 % (v/v), pH 3.75] was used in the place of citrate buffer [17.5 % (v/v), pH 3.25] in this work. As a result, mandelonitrile **3d** was obtained at 81 % conversion and high enantiomeric purity in 66 hours (Table 1). Clearly, there are still problems even after changing the buffer.

For the synthesis of cyanohydrins, the concentration of hydrogen cyanide is critical: it must be high enough for reasonable reactivity although too high concentrations may inhibit the enzyme and enhance chemical condensation.<sup>15</sup> Important trends emerge from the data in Figure 3. For the decomposition of acetone cyanohydrin in water at pH  $\leq 6$ , the formation of hydrogen cyanide proceeds slowly. Thus, at pH 6.0 only 11 % of the original acetone cyanohydrin is decomposed after 10 minutes and working at pH 4.0 approximately the same level is obtained in 16 hours.<sup>16</sup> On the other hand, under the synthetic conditions in diisopropyl ether [14 % (v/v) of 0.1 M tartrate buffer, pH 3.75], acetone cyanohydrin (11 mmol) yielded 0.4 mmol of hydrogen cyanide in 24 hours, corresponding to the amount enough to turn ca. 40 % of aldehyde **1** (1 mmol) to the corresponding cyanohydrin. In dry diisopropyl ether or in the solvent saturated with citrate buffers [0.41 % (v/v),<sup>17</sup> pH 4.0 or 6.0], acetone cyanohydrin was practically stable at least for two days. These results show that relatively high water concentrations preferably connected to high pH are necessary for the chemical decomposition of acetone cyanohydrin at a reasonable rate.

The capability of oxynitrilases to catalyse the reaction in both directions will greatly help the decomposition of acetone cyanohydrin.<sup>10</sup> In this respect there is a basic difference between the two



**Figure 3.** The formation of hydrogen cyanide from acetone cyanohydrin in water as a function of pH. Analysis after 10 min (■), 16 h (●) and 64 h (×).<sup>16</sup>

enzymes: (*S*)-oxynitrilase from *Sorghum bicolor* does not accept ketones as substrates whereas the (*R*)-enzyme from almonds does, leading to the increasing availability of hydrogen cyanide in the latter case.<sup>18,19</sup> Accordingly, cyanohydrins **2d**, **g** and **h** were smoothly obtained from the corresponding aldehydes in the presence of almond meal compared to the much slower formation of the corresponding compounds **3** in the presence of *Sorghum bicolor* shoots in diisopropyl ether containing 0.1 M tartrate buffer [7 % (v/v), pH 5.4 and 14 % (v/v), pH 3.75, respectively] (Table 1). As further support, the rate differences become less obvious in the case of method 2 (Table 2, e.g., rows 1 and 2) where the concentration of hydrogen cyanide is identical in the two enzymatic system. Another reason for the rate differences is clearly the difference in pH. Accordingly, considerable rate retardations are evident for the almond meal-catalysed condensations when pH 5.4 is reduced to 3.75 (Table 1).

Acetone cyanohydrin as the donor of hydrogen cyanide may also affect the enantiomeric purity of the product (Table 1). Accordingly, the low ee observed for the formation of compound **2h** is difficult to explain by normal chemical condensation because the almond meal-catalysed condensation using method 2 is successful as is the formation of compound **3h** by method 1 or 2 (Tables 1 and 3). Moreover, the ee values tend to drop with time. This is shown for the *Sorghum bicolor* shoot-catalysed syntheses in Table 1. The drop in this case is at least partially caused by acetone, another decomposition product of acetone cyanohydrin (Table 2).

### Gaseous hydrogen cyanide as the source of hydrogen cyanide; Method 2.

According to the above results, the slow decomposition of acetone cyanohydrin is an important reason for the long reaction times involved in cyanohydrin syntheses by *Sorghum bicolor* shoots. That is why, a new "in situ" method (method 2) was developed. This method exploits a two compartment reaction vessel, one of the compartments (B) containing dry hydrogen cyanide dissolved in diisopropyl ether and the other (A) the reaction mixture. In a closed system, hydrogen cyanide from compartment B evaporates and then freely

**Table 1.** The *Sorghum bicolor* shoot-catalysed synthesis of (*S*)-cyanohydrins at 25 °C.

| Product  | Almond meal; <b>2<sup>a</sup></b> |                 |                    | <i>Sorghum bicolor</i> shoots; <b>3<sup>b</sup></b> |              |                    |
|----------|-----------------------------------|-----------------|--------------------|---|--------------|--------------------|
|          | Time/h                            | Conversion/%    | ee <sup>c</sup> /% | Time/h  | Conversion/% | ee <sup>c</sup> /% |
| <b>d</b> | 20                                | 93              | 98                 | 19  | 30           | 97                 |
|          | 23                                | 22 <sup>d</sup> | 97                 | 66  | 81           | 95                 |
|          |                                   |                 |                    | 162   | 93           | 91                 |
| <b>g</b> | 25                                | 87              | 96                 | 67  | 21           | 93                 |
|          | 23                                | 4 <sup>d</sup>  | 97                 | 162   | 56           | 93                 |
| <b>h</b> | 25                                | 46              | 37                 | 68  | 26           | 98                 |
|          | 23                                | 4 <sup>d</sup>  | 30                 | 165   | 41           | 77                 |

<sup>a</sup>0.1 M tartrate buffer [7 % (v/v), pH 5.4]. <sup>b</sup>0.1 M tartrate buffer [14 % (v/v), pH 3.75]. <sup>c</sup> Determined after acetylation with Ac<sub>2</sub>O. <sup>d</sup> 0.1 M tartrate buffer [7 % (v/v), pH 3.75].

moves along a short glasspipe to A where it dissolves into the reaction mixture. The diffusion rate from B to A is not much affected by the initial amount of hydrogen cyanide in B. Thus, after 24 hours 2.8, 2.4 and 2.1 mmol of hydrogen cyanide was detected in diisopropyl ether (16.1 ml; in A) when the original amounts dissolved in the solvent (10 ml; in B) were 10.0, 7.5 and 5.0 mmol at 5 °C, respectively. As a benefit of this method, neat hydrogen cyanide at an appropriate concentration is generated into the reaction mixture.

Diisopropyl ether is the best reaction medium for the almond meal- and *Sorghum bicolor* shoot-catalysed syntheses of mandelonitriles **2d** and **3d**, respectively (Table 2). Solvent effects on enantioselectivity considerably differ from one whole cell system to another. Under the reaction conditions, the enantiomeric purity of product **2d** is independent of the reaction medium. On the other hand, the enzymatic synthesis of cyanohydrin **3d** in diisopropyl ether results in the highest value of ee. This value decreases when diisopropyl ether is partially or totally replaced by another organic solvent.

**Table 2.** Solvent effects for the syntheses of mandelonitriles **2d** and **3d** at 5 °C.

| Solvent   | Almond meal; <b>2d</b> |                 |                    | <i>Sorghum bicolor</i> shoots; <b>3d<sup>c</sup></b> |                 |                    |
|---|------------------------|-----------------|--------------------|--|-----------------|--------------------|
|   | Time/h                 | Conversion/%    | ee <sup>d</sup> /% | Time/h   | Conversion/%    | ee <sup>d</sup> /% |
| Pr <sup>i</sup> <sub>2</sub> O                        | 23                     | 97 <sup>a</sup> | 98                 | 23   | 87 <sup>e</sup> | 98                 |
| Pr <sup>i</sup> <sub>2</sub> O                        | 14                     | 94 <sup>b</sup> | 98                 | 24   | 75              | 97                 |
| Pr <sup>i</sup> <sub>2</sub> O + acetone [5 % (v/v)]  | 14                     | 89 <sup>b</sup> | 98                 | 24   | 62              | 97                 |
| Pr <sup>i</sup> <sub>2</sub> O + acetone [10 % (v/v)] | 14                     | 92 <sup>b</sup> | 99                 | 24   | 42              | 93                 |
| Pr <sup>i</sup> <sub>2</sub> O + acetone [15 % (v/v)] | 14                     | 74 <sup>b</sup> | 99                 | 24   | 37              | 90                 |
| Pr <sup>i</sup> <sub>2</sub> O + toluene [50 % (v/v)] | 24                     | 96 <sup>a</sup> | 98                 |  |                 |                    |
| Toluene   | 24                     | 88 <sup>a</sup> | 96                 | 25   | 80              | 93                 |
| Pr <sup>i</sup> <sub>2</sub> O + AcOEt [50 % (v/v)]   | 24                     | 90 <sup>a</sup> | >99                |  |                 |                    |
| AcOEt   | 24                     | 59 <sup>a</sup> | 99                 | 25   | 7               | 82                 |

<sup>a</sup>Contains 0.1 M tartrate buffer [2 % (v/v), pH 5.4]. <sup>b</sup>Contains 0.1 M tartrate buffer [7 % (v/v), pH 5.4].

<sup>c</sup>Contains 0.1 M tartrate buffer [14 % (v/v), pH 3.75]. <sup>d</sup>Determined after acetylation with Ac<sub>2</sub>O.

<sup>e</sup>Contains 0.1 M tartrate buffer [2 % (v/v), pH 3.75].

By comparing the results shown in Tables 1-3 it is clear that for the *Sorghum bicolor* shoot-catalysed synthesis of (S)-cyanohydrins the reaction proceeds faster and at higher enantiomeric purity in the case of method 2 than 1. For almond meal catalysis, the difference between the two methods is less obvious. Very importantly, the water content of the system can be reduced to only 2 % (v/v) when method 2 is used. Thus, the possibility for the chemical addition of hydrogen cyanide can be minimized. This is clearly seen by considering the values of ee for the almond meal-catalysed syntheses (Table 3). Thus, in the cases of compounds 2c, h and j the values of ee are lower at the higher water content of 7 % (v/v) (pH 5.4). In accordance with this, the chemical formations of the racemic cyanohydrins under the same conditions were shown to proceed to 15, 37 and 85 % conversions in 76, 77 and 89 hours, respectively. The chemical reaction is negligible at pH 3.75. On the other hand, reactivity considerably decreases with decreasing water content. When the water concentration is low enough such as 2 % (v/v) pH does not play a role any more. In spite of that 0.1 M tartrate buffer (pH 3.75 or 5.4) has been used throughout this work.

**Table 3.** Almond meal- and *Sorghum bicolor* shoot-catalysed syntheses of cyanohydrins 2 and 3 at 5 °C.

| Product | Almond meal: 2 <sup>a</sup> |                 |                    | <i>Sorghum bicolor</i> shoots: 3 <sup>b</sup> |                 |                    |
|---------|-----------------------------|-----------------|--------------------|---|-----------------|--------------------|
|         | Time/h                      | Conversion/%    | ee <sup>c</sup> /% | Time/h  | Conversion/%    | ee <sup>c</sup> /% |
| a       | 48                          | 97              | 98                 | 134   | 87              | 44                 |
| b       | no enzymatic reaction       |                 |                    | no enzymatic reaction                         |                 |                    |
| c       | 64                          | 85              | 96                 | 48  | 60              | 99                 |
|         | 47                          | 90 <sup>e</sup> | 90                 |   |                 |                    |
| d       | 23                          | 97              | 98                 | 42  | 97              | 97                 |
|         |                             |                 |                    | 24  | 75 <sup>d</sup> | 97                 |
| e       | 480                         | 90              | 95                 | 384   | 30 <sup>d</sup> | 93                 |
| f       | 528                         | 61              | 86                 | 174   | 4 <sup>d</sup>  | 37                 |
| g       | 48                          | 94              | 99                 | 99  | 88              | 98                 |
| h       | 68                          | 64              | 96                 | 48  | 60              | 96                 |
|         | 23                          | 45 <sup>e</sup> | 76                 | 49  | 66 <sup>d</sup> | 99                 |
| i       | 91                          | 82              | 98                 | 168   | 23              | 52                 |
|         |                             |                 |                    | 71  | 54 <sup>d</sup> | 71                 |
| j       | 66                          | 82              | 93                 | 123   | 34              | 5                  |
|         | 22                          | 70 <sup>e</sup> | 66                 |   |                 |                    |
| k       | 66                          | 88              | 98                 | 74  | 72              | 89                 |
| l       | no enzymatic reaction       |                 |                    | no enzymatic reaction                         |                 |                    |

<sup>a</sup>Contains 0.1 M tartrate buffer [2 % (v/v), pH 5.4]. <sup>b</sup>Contains 0.1 M tartrate buffer [2 % (v/v), pH 3.75].

<sup>c</sup>Determined after acetylation with Ac<sub>2</sub>O. <sup>d</sup>Contains 0.1 M tartrate buffer [14 % (v/v), pH 3.75].

<sup>e</sup>Contains 0.1 M tartrate buffer [7 % (v/v), pH 5.4].

As is shown by the results in Table 3, the almond meal and *Sorghum bicolor* shoot-catalysed syntheses of cyanohydrins 2 and 3 can be conveniently run in diisopropyl ether containing 2 % (v/v) of 0.1 M tartrate buffer. Usually the reactions smoothly proceed close to 90 % conversion, and it is often reasonable to stop the reaction at that point. Accordingly, e.g., for the *Sorghum bicolor* shoot-catalysed synthesis of mandelonitrile 3d 91 % conversion was reached in 30 hours; 12 more hours were needed in

order to obtain 97 % conversion. Especially steric effects of the substrate seem to be important on reactivity. Thus, aldehydes **1b** and **1** with bulky substituents do not react at all. (*S*)-Oxynitrilase from *Sorghum bicolor* is clearly more sensitive to structural features. Thus, the enzymatic syntheses of cyanohydrins **2i** and **j** conveniently proceeded to *ca.* 80 % conversion although the corresponding reaction to cyanohydrin **3i** was slow. In the latter case, the chemical condensation (5 % in 168 h in the presence of 14 % (v/v) of the buffer) does not explain the low enantiomeric purity observed. Chemical condensation produces the product **3j**. Evidently, the *para*-position at the benzene ring is more restricting because (*S*)-oxynitrilase purified from *Sorghum* was previously shown to catalyse the synthesis of (*S*)-3-methoxymandelonitrile in diisopropyl ether at a reasonable rate and selectivity (93 % yield in 29 hours, ee 0.89).<sup>3</sup> The similar trend was observed for the synthesis of **3e** (Table 3) compared with the formation of (*S*)-3-phenoxymandelonitrile (93 % yield in 144 hours, ee 0.96).<sup>3</sup> The products of Table 3 were identified after separation and the results are described in the experimental section.

### Conclusions

The results of the present work describe the usability of almond meal and *Sorghum bicolor* shoot catalyses for the convenient syntheses of aromatic aldehyde cyanohydrins by two methods which differ in the ways hydrogen cyanide is introduced into the reaction mixture. In method 1, acetone cyanohydrin serves as an *in situ* source of hydrogen cyanide in an one compartment reaction vessel. This method when connected to almond meal catalysis affords (*R*)-cyanohydrins at high chemical yield and enantiomeric purity. Enantiomeric purity obtained in the case of (*S*)-cyanohydrins somewhat suffers from the high water concentrations [14 % (v/v) or even higher] necessary for the decomposition of acetone cyanohydrin as well as from the formation of acetone. Thus, the reactions must be either stopped at an early stage or be contented with somewhat reduced enantiomeric purity. Acetone together with the low pH (3.75) also contribute long reaction times. Method 2 exploits hydrogen cyanide which evaporates from an organic solvent and diffuses into the reaction mixture *in situ* in a two compartment reaction vessel. This method allows the use of lower water contents [2 % (v/v)] and still relatively fast reactions which lead to the yields and enantiomeric purities (in most cases ee  $\geq$  0.95) which are at least comparable to those obtained for (*R*)- and (*S*)-cyanohydrins using isolated enzymes. As a complementary method, the lipase-catalysed resolution of racemic cyanohydrins or their esters enables the preparation of compounds **2** and **3**.<sup>20,21</sup> This possibility is important especially for the preparation of sterically hindered cyanohydrins such as compounds **2b** and **3b**, **i** or **j** which cannot be prepared enantiomerically pure by oxynitrilase catalysis.

### Experimental

Defatted almond meal was purchased from Sigma. Dechlorophylled *Sorghum bicolor* shoots were grown and handled as previously described.<sup>9</sup> The seeds were the generous gift from Pioneer, Hi-bred International, Inc., Johnston (U.S.A.). The solvents were of the best analytical grade, and diisopropyl ether was distilled before use. Aldehydes **a** and **d-k** were the commercial products from Aldrich or Merck and were distilled or recrystallized before use. Aldehydes **b** and **c** were prepared from 4-hydroxybenzaldehyde and acetic acid anhydride and pivaloyl chloride, respectively. 4-Hydroxy-3-hydroxymethylbenzaldehyde was a gift from Leiras Oy. Acetone cyanohydrin was distilled before use. Neat hydrogen cyanide was prepared by adding potassium cyanide solution dropwise into dilute sulphuric



acid<sup>22</sup> and the known amount dissolved in dry diisopropyl ether was stored in a freezer. Racemic cyanohydrins for retention time determinations in GLC were prepared from the corresponding aldehydes and hydrogen cyanide using a known method.<sup>23,24</sup>

<sup>1</sup>H (TMS as an internal standard) and <sup>13</sup>C NMR spectra were measured on a Lambda GX 400 spectrometer in CDCl<sub>3</sub>. The enantiomeric purities of the cyanohydrins were determined after acetylation with the chiral GLC method using Chrompack CP-Cyclodextrin-β-2,3,6-M-9 column.<sup>6,9</sup> Optical rotations were measured using a JASCO DIP-360 polarimeter.

**Method 1.** - In a typical experiment, almond meal (74 mg) was mixed with diisopropyl ether (6.5 ml). 0.1 M Tartrate buffer (0.6 ml, pH 5.4) and freshly distilled benzaldehyde (0.5 mmol) were added. Alternatively, dry *Sorghum bicolor* shoots (0.5 g) were mixed with the solvent (5.9 ml) followed by the addition of 0.1 M tartrate buffer (1.1 ml, pH 3.75) and benzaldehyde (0.5 mmol). The mixtures were stirred half an hour at room temperature and acetone cyanohydrin (1.0 ml) was added. The reactions were followed by taking samples at intervals and using the chiral GLC method.<sup>6,9</sup>

**Method 2.** - In compartment A of the two-compartment reaction vessel, almond meal (150 mg) was mixed with diisopropyl ether (16.1 ml) and 0.1 M tartrate buffer (330 μl, pH 5.4) and freshly distilled benzaldehyde (1.0 mmol) were added. Alternatively, dry *Sorghum bicolor* shoots (1 g) were used in the place of almond meal and the pH of the buffer was 3.75. The reaction mixture was stirred at 5 °C. In compartment B, diisopropyl ether (10 ml) containing hydrogen cyanide (10 mmol) was added. The reaction was followed and the product was handled as in method 1.

The reactions for the formation of cyanohydrins **2** and **3** were stopped by filtering off the enzyme preparates. The residue was washed 5 times with 10 % NaHSO<sub>3</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under reduced pressure. The products were analyzed and the results are shown below. The yields are isolated yields calculated on the basis of conversions.

(R)-2-(4-bromophenyl)-2-hydroxyacetonitrile C<sub>8</sub>H<sub>6</sub>BrNO (**2a**); Yield 78 %; [α]<sub>D</sub><sup>25</sup> = +25.0 (CHCl<sub>3</sub>, c=5.40). (S)-2-(4-bromophenyl)-2-hydroxyacetonitrile C<sub>8</sub>H<sub>6</sub>BrNO (**3a**); Yield 90 %; [α]<sub>D</sub><sup>25</sup> = -11.1 (CHCl<sub>3</sub>, c=5.03). <sup>1</sup>H NMR(CDCl<sub>3</sub>): δ 3.05 (s, 1H, OH); 5.51 (s, 1H, CH); 7.39 (d, 2H, H<sub>arom</sub>, J = 6.6 Hz); 7.56 (d, 2H, H<sub>arom</sub>, J = 6.6 Hz); <sup>13</sup>C NMR(CDCl<sub>3</sub>): δ 63.05 (CH); 118.35 (CN); 124.17, 128.26, 128.26, 128.42, 132.40, 134.15 (C<sub>arom</sub>).

(R)-2-Hydroxy-2-(4-acetylphenyl)acetonitrile C<sub>12</sub>H<sub>11</sub>NO<sub>4</sub> (**2c**); The product corresponding to 85 % conversion; [α]<sub>D</sub><sup>25</sup> = +17.0 (AcOEt, c=9.55). (S)-2-Hydroxy-2-(4-acetylphenyl)acetonitrile C<sub>12</sub>H<sub>11</sub>NO<sub>4</sub> (**3c**); The product corresponding to 60 % conversion; [α]<sub>D</sub><sup>25</sup> = -19.0 (AcOEt, c=4.60). <sup>1</sup>H NMR(CDCl<sub>3</sub>): δ 2.17 (s, 3H, OAc); 2.33 (s, 3H, OAc); 7.19 (d, 2H, H<sub>arom</sub>, J = 6.8 Hz); 7.56 (d, 2H, H<sub>arom</sub>, J = 6.8 Hz); <sup>13</sup>C NMR(CDCl<sub>3</sub>): δ 20.46 (CH<sub>3</sub>); 21.10(CH<sub>3</sub>); 62.26 (CH); 115.94 (CN); 122.56, 129.33, 152.14 (C<sub>arom</sub>); 168.83 (CO); 169.02(CO).

(R)-2-Hydroxy-2-phenylacetonitrile C<sub>8</sub>H<sub>7</sub>NO (**2d**); Yield 91 %; [α]<sub>D</sub><sup>25</sup> = +40.0 (CHCl<sub>3</sub>, c=4.50). (S)-2-Hydroxy-2-phenylacetonitrile (**3d**); Yield 88 %; [α]<sub>D</sub><sup>25</sup> = -34.2 (CHCl<sub>3</sub>, c=5.70). <sup>1</sup>H NMR(CDCl<sub>3</sub>): δ

3.49 (s, 1H, OH); 5.57 (s, 1H, -CH); 7.43-7.53 (m, 5H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  63.84 (CH); 118.82 (CN); 126.64, 129.48, 129.82, 130.15, 130.21, 135.27 ( $C_{\text{arom}}$ ).

**(R)-2-Hydroxy-2-(4-phenoxyphenyl)acetonitrile**  $\text{C}_{14}\text{H}_{11}\text{NO}_2$  (**2e**); The product corresponding to 90 % conversion;  $[\alpha]_{\text{D}}^{25} = +16.5$  ( $\text{CHCl}_3$ ,  $c=5.33$ ). **(S)-2-Hydroxy-2-(4-phenoxyphenyl)acetonitrile**  $\text{C}_{14}\text{H}_{11}\text{NO}_2$  (**3e**); The product corresponding to 30 % conversion;  $[\alpha]_{\text{D}}^{25} = -20.9$  ( $\text{CHCl}_3$ ,  $c=3.40$ ).  $^1\text{H}$  NMR( $\text{CDCl}_3$ )  $\delta$  3.71 (s, 1H, -CH); 7.26-7.68 (m, 9H,  $C_{\text{arom}}$ );  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  63.12 (CH); 117.56 (CN); 118.80, 119.53, 120.47, 124.10, 125.02, 128.45, 129.71, 129.97, 130.17, 132.13, 156.21, 158.90 ( $C_{\text{arom}}$ ).

**(R)-2-Hydroxy-2-(4-isopropylphenyl)acetonitrile**  $\text{C}_{11}\text{H}_{13}\text{NO}$  (**2f**); The product corresponding to 61 % conversion;  $[\alpha]_{\text{D}}^{25} = +17.3$  (AcOEt,  $c=3.50$ ). **(S)-2-Hydroxy-2-(4-isopropylphenyl)acetonitrile**  $\text{C}_{11}\text{H}_{13}\text{NO}$  (**3f**); The product corresponding to 4 % conversion;  $[\alpha]_{\text{D}}^{25} = -10.6$  (AcOEt,  $c=0.35$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.24 (d, 3H,  $\text{CH}_3$ ); 1.26 (d, 3H,  $\text{CH}_3$ ); 2.95 (m, 1H, CH); 3.06 (s, 1H, OH); 5.49 (s, 1H, -CH); 7.29 (d, 2H,  $C_{\text{arom}}$ ); 7.45 (d, 2H,  $C_{\text{arom}}$ );  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  23.81 ( $\text{CH}_3$ ); 23.89 ( $\text{CH}_3$ ); 33.93 (CH); 63.54 (CH); 118.92 (CN); 126.83, 127.32, 132.69, 150.96 ( $C_{\text{arom}}$ ).

**(R)-2-Hydroxy-2-(4-methylphenyl)acetonitrile**  $\text{C}_9\text{H}_9\text{NO}$  (**2g**); Yield 77 %;  $[\alpha]_{\text{D}}^{25} = +48.7$  ( $\text{CHCl}_3$ ,  $c=3.80$ ). **(S)-2-Hydroxy-2-(4-methylphenyl)acetonitrile**  $\text{C}_9\text{H}_9\text{NO}$  (**3g**); Yield 99 %;  $[\alpha]_{\text{D}}^{25} = -35.3$  ( $\text{CHCl}_3$ ,  $c=4.50$ ).  $^1\text{H}$  NMR( $\text{CDCl}_3$ ):  $\delta$  2.38 (s, 3H,  $\text{CH}_3$ ); 3.06 (s, 1H, OH); 5.48 (s, 1H, -CH); 7.25 (d, 2H,  $H_{\text{arom}}$ ,  $J = 8.1$  Hz); 7.41 (d, 2H,  $H_{\text{arom}}$ ,  $J = 8.1$  Hz);  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ): 21.23 ( $\text{CH}_3$ ); 63.51 (CH); 118.89 (CN); 126.68, 129.87, 130.10, 132.45, 140.04 ( $C_{\text{arom}}$ ).

**(R)-2-Hydroxy-2-(4-hydroxyphenyl)acetonitrile**  $\text{C}_8\text{H}_7\text{NO}_2$  (**2h**); The product corresponding to 64 % conversion;  $[\alpha]_{\text{D}}^{25} = +26.0$  (AcOEt,  $c=5.12$ ). **(S)-2-Hydroxy-2-(4-hydroxyphenyl)acetonitrile**  $\text{C}_8\text{H}_7\text{NO}_2$  (**3h**); The product corresponding to 60 % conversion;  $[\alpha]_{\text{D}}^{25} = -17.0$  (AcOEt,  $c=6.40$ ).  $^1\text{H}$  and  $^{13}\text{C}$  NMR product was acylated and the spectra are same as described in **2c** and **3c**.

**(R)-2-Hydroxy-2-(4-methoxyphenyl)acetonitrile**  $\text{C}_9\text{H}_9\text{NO}_2$  (**2i**); Yield 85 %;  $[\alpha]_{\text{D}}^{25} = +36.8$  ( $\text{CHCl}_3$ ,  $c=5.75$ ). **(S)-2-Hydroxy-2-(4-methoxyphenyl)acetonitrile**  $\text{C}_9\text{H}_9\text{NO}_2$  (**3i**); The product corresponding to 47 % conversion;  $[\alpha]_{\text{D}}^{25} = -3.9$  ( $\text{CHCl}_3$ ,  $c=3.84$ ).  $^1\text{H}$  NMR( $\text{CDCl}_3$ ):  $\delta$  3.10 (s, 1H, OH); 3.82 (s, 3H, OMe); 6.94 (d, 2H,  $H_{\text{arom}}$ ,  $J = 8.8$ ); 7.42 (d, 2H,  $H_{\text{arom}}$ ,  $J = 8.8$ );  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  55.43 (OMe); 63.31 (CH); 114.38, 114.55 ( $C_{\text{arom}}$ ); 118.26 (CN); 125.53, 127.56, 128.33, 160.72 ( $C_{\text{arom}}$ ).

**(R)-2-Hydroxy-2-(3,4-dimethoxyphenyl)acetonitrile**  $\text{C}_{10}\text{H}_{11}\text{NO}_3$  (**2j**); Yield 75 %;  $[\alpha]_{\text{D}}^{25} = +33.7$  ( $\text{CHCl}_3$ ,  $c=5.15$ ).  $^1\text{H}$  NMR( $\text{CDCl}_3$ ):  $\delta$  3.10 (s, 1H, OH); 5.40 (s, 1H, CH); 6.89 (d, 1H,  $H_{\text{arom}}$ ,  $J =$

8.3 Hz); 7.02 (d, 1H,  $H_{\text{arom}}$ ,  $J = 2.2$  Hz); 7.08 (d, 1H,  $H_{\text{arom}}$ ,  $J = 8.3$  Hz and  $J = 2.2$  Hz);  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  56.02 (OMe); 56.22 (OMe); 63.66 (CH); 109.65, 111.44 ( $C_{\text{arom}}$ ); 118.89 (CN); 119.67, 128.15, 149.50, 150.22 ( $C_{\text{arom}}$ ).

(R)-2-Hydroxy-2-(3,4-methylenedioxyphenyl)acetonitrile  $\text{C}_9\text{H}_7\text{NO}_3$  (**2k**); Yield 85 %;  $[\alpha]_{\text{D}}^{25} = +45.9$  ( $\text{CHCl}_3$ ,  $c=5.85$ ). (S)-2-Hydroxy-2-(3,4-methylenedioxyphenyl)acetonitrile  $\text{C}_9\text{H}_7\text{NO}_3$  (**3k**); Yield 80 %;  $[\alpha]_{\text{D}}^{25} = -32.7$  ( $\text{CHCl}_3$ ,  $c=4.50$ ).  $^1\text{H}$  NMR( $\text{CDCl}_3$ ):  $\delta$  3.15 (s, 1H, OH); 5.43 (s, 2H,  $\text{OCH}_2\text{O}$ ); 6.83 (d, 1H,  $H_{\text{arom}}$ ,  $J = 6.0$  Hz); 6.98 (s, 1H,  $H_{\text{arom}}$ ); 7.00 (d, 1H,  $H_{\text{arom}}$ ,  $J = 6.0$ );  $^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$  63.42 (CH); 101.67, 107.23, 108.62 ( $C_{\text{arom}}$ ); 118.79(CN); 120.80, 129.13, 148.41, 148.93 ( $C_{\text{arom}}$ ).

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