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Sorghum bicolor Shoots in the Synthesis of (S)-Mandelonitrile

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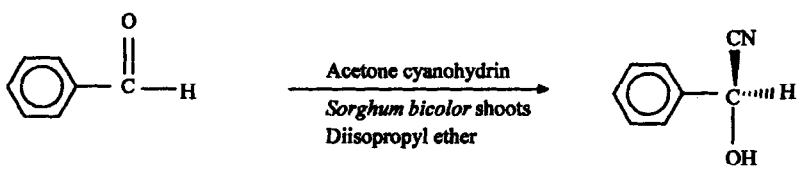
Abstract: An inexpensive method for the preparation of (*S*)-mandelonitrile from benzaldehyde and HCN in diisopropyl ether with the chemical yield and enantiomeric excess of *ca.* 90 % is described. In this method, (*S*)-oxynitrilase in etiolated shoots of *Sorghum bicolor* is used as an asymmetric catalyst.

The potential of optically active cyanohydrins as intermediates for the synthesis of different types of chiral compounds, such as α-hydroxy carboxylic acids and β-aminoalcohols is well documented[1]. Biocatalytic approaches to optically active cyanohydrins include the lipase-catalysed resolution of a cyanohydrin or its acylated counterpart[2] as well as the (*R*)- and (*S*)-oxynitrilase (E.C. 4.1.2.10 and 4.1.2.11, respectively)-catalysed condensation of hydrogen cyanide with a corresponding aldehyde[3]. An advantage of oxynitrilase catalysis is the almost quantitative chemical yield of an optically active cyanohydrin; the theoretical yield of kinetic resolution for one enantiomer is 50 % at its best. On the other hand, purified oxynitrilases are rather expensive enzymes which must be immobilized on solid supports before use. Due to the instability of optically active cyanohydrins in aqueous solutions, biocatalytic reactions in organic solvents or in solvents of minor water contents are usually preferred.

Sweet almonds and *Sorghum* seedlings are relatively rich sources of (*R*)- and (*S*)-oxynitrilases, respectively. A crude extract from ground almonds in an aqueous buffer or ground almond meal itself in organic media has been used as an inexpensive catalyst for the preparation of aromatic and aliphatic (*R*)-cyanohydrins[3c-e]. In this work, we describe a simple and inexpensive method for the preparation of (*S*)-mandelonitrile from benzaldehyde and hydrogen cyanide in diisopropyl ether, using acetone cyanohydrin as a transcyanation agent (Scheme 1). In this method lyophilized, powdered and washed *Sorghum* shoots[4] are used as a source of (*S*)-oxynitrilase. This method eliminates the need for the purification and immobilization of the enzyme.



Scheme 1

Etiolated *Sorghum bicolor* shoots were obtained by first soaking the seeds into water for one day followed by etiolation in a dark room for four days at 30 °C. The shoots obtained were then harvested and

frozen[5]. After grinding in a mortar the enzyme preparate was lyophilized for at least 17 h. The crude material thus obtained was used for the preparation of (*S*)-mandelonitrile with relatively high optical purity (e.e. 81 % at 75 % conversion, Table 1) in diisopropyl ether which contained 17.5 % of citrate buffer (0.1 M, pH 3.25)[6]. When unlyophilized enzyme material was used under the same conditions the formation of mandelonitrile was fast (85 % conversion within 16 hours) but the optical purity of the product low (e.e. 56 %). The nonstereospecific chemical formation of mandelonitrile becomes more important in this case (Table 1) because it is more difficult to control the water concentration of the enzyme material compared to the case of the lyophilized preparate.

The enzyme requires some water to retain its catalytic activity. The results for the synthesis of (*S*)-mandelonitrile using different amounts of citrate buffer (0.1 M, pH 3.25) in diisopropyl ether and lyophilized enzyme preparate as a catalysts are shown in Table 1. It is clear according to these results that the rate for the formation of (*S*)-mandelonitrile increases with increasing water content. Enantioselectivity, on the other hand, goes through a shallow maximum at the water content of *ca.* 20 % (v/v). The water content of 17.5 % (v/v) was then used throughout this work.

Table 1. The effect of water for the preparation of (*S*)-mandelonitrile from acetone cyanohydrin (11 mmol) and benzaldehyde (1.0 mmol) in aqueous diisopropyl ether at 25 °C

Water[a]/ % (v/v)	Time/h	Conversion/ % (enzymatic)	e.e.$_{(S)}$/ %	Conversion[b]/ % (chemical)	e.e./ %
0	330	-	-		
10	284	35	75		
17.5	284	75	81	8	-
23	233	88	77		
27	232	89	78	12	-
32.5	260	93	75		
40	260	95	68	30	-

[a]0.1 M citrate buffer (pH 3.25); [b]In the absence of the enzyme.

To improve the optical purity of (*S*)-mandelonitrile obtained in the *Sorghum bicolor* shoot-catalysed addition of hydrogen cyanide to benzaldehyde (Table 1) the enzyme preparate obtained after grinding or lyophilization was washed (dechlorophylled) with different solvents until the filtrate was uncoloured. The lyophilized material was incubated in aqueous diisopropyl ether before the washing. The best result in terms of the optical purity of the product was obtained when the crude enzyme material was washed with a water soluble organic solvent such as acetone or 1,4-dioxane (Table 2). The results also show that in this case it is not necessary to dry the enzyme preparate by lyophilization before the washing.

Table 2. The effect of solvents used to wash the *Sorghum bicolor* shoots on the formation of (*S*)-mandelonitrile in aqueous diisopropyl ether[a] at 25 °C

Solvent[b]	Time/h	Conversion/%	e.e.$_{(S)}$/%
Diethyl ether	355	89	86
Diisopropyl ether	360	89	64
Ethyl acetate	355	69	84
1,4-Dioxane[c]	232	85	89
1,4-Dioxane	210	88	90
Water	118	24	87
Acetone	257	83	91

[a]17.5 % water (v/v, 0.1 M citrate buffer, pH 3.25); 0.74 mmol of benzaldehyde and 11 mmol of acetone cyanohydrin; [b]Lyophilized shoots incubated in aqueous diisopropyl ether; [c]Ground shoots.

Owing to the base-catalysed nonenzymatic addition of hydrogen cyanide to aldehydes the higher the pH of an aqueous reaction medium the lower is the optical purity of a cyanohydrin product obtained in oxynitrilase catalysis[3f]. In water (*S*)-oxynitrilase purified from *Sorghum bicolor* exhibits high stability over a wide pH range from 2 to 10 enabling the work in solutions of low pH[7]. In the biphasic systems of diisopropyl ether and 0.1 M citrate buffer (17.5 % (v/v)), the pH over the range from 3.25 to 3.75 had not an effect on the optical purity or yield of the product. Thus, the enantiomeric excess of (*S*)-mandelonitrile obtained was 87-88 % at 50-55 % conversion within 160 hours when lyophilized shoots washed with diethyl ether were used as a catalyst.

In aqueous solutions of pH < 4.0 acetone cyanohydrin is a stable compound[8]. Lyases, *e.g.*, α-hydroxynitrile lyase from *Hevea*, are known to catalyse the dissociation of acetone cyanohydrin at pH > 3[8]. To test the availability of hydrogen cyanide for the *Sorghum bicolor* shoot-catalysed synthesis of (*S*)-mandelonitrile in aqueous diisopropyl ether different amounts of acetone cyanohydrin was used. The conversion of 0.06 M benzaldehyde to mandelonitrile was shown to increase from 53 to 70 % within 210 hours when 0.60 to 1.35 M acetone cyanohydrin was used. This result strongly supports increasing availability of hydrogen cyanide with the increasing concentration of acetone cyanohydrin. Enantiomeric purity (e.e. 86-89 %) of (*S*)-mandelonitrile was not affected by the amount of acetone cyanohydrin.

For the synthesis of (*S*)-mandelonitrile under optimized conditions, 0.74 mmol of benzaldehyde and 11 mmol of acetone cyanohydrin were added to a suspension of 1.4 g of the lyophilized and dechlorophylled (washed with acetone) enzyme material in 11 ml of aqueous diisopropyl ether. The reaction mixture was shaken at 25 °C. After 10 days the reaction was stopped by filtering of the enzyme at 84 % conversion according to benzaldehyde and with the e.e. of 90 % for (*S*)-mandelonitrile. After evaporation (8 mmHg; 70 °C) of the solvent and other reaction components 0.56 mmol (91 % of the theory) of pure (*S*)-mandelonitrile

resulted. The value of $[\alpha]_D^{25}$ = -42 (*c* 0.85, $CHCl_3$) obtained is in good agreement with the literature value of -46 (*c* 2.1, $CHCl_3$) for (*S*)-mandelonitrile[9].

In summary, the present method for the synthesis of (*S*)-mandelonitrile utilizes unpurified (*S*)-oxynitrilase in *Sorghum bicolor* shoots. The chemical and optical yields of the product are comparable with the previous results which exploit highly expensive purified and immobilized (*S*)-oxynitrilase for the condensation of hydrogen cyanide with benzaldehyde in citrate buffer[3f] (chemical yield 80 % and e.e. 96 %) or organic solvents saturated with water[3g] (chemical yield 91 % and e.e. 97 %).

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4. The *Sorghum bicolor* seeds were the generous gift from Pioneer, Hi-bred International, Inc., Johnston (U.S.A.).
5. We purified *ca.* 2000 Units of the enzyme from 1 kg of the frozen shoots using $(NH_4)_2SO_4$ precipitation (see Ref. 10).
6. As a general procedure, 950 mg (corresponds 10 g of frozen shoots) of the enzyme preparate was incubated in aqueous [17.5 % (v/v) 0.1 M citrate buffer, pH 3.25] diisopropyl ether (8.1 ml). Benzaldehyde (1.0 mmol) and acetone cyanohydrin (11.0 mmol) were added and the reaction mixture was shaken at room temperature. The reactions were followed by taking samples at intervals. The cyanohydrin in the samples was derivatized as an acetate (Ac_2O, Pyridiini, DMAP, room temperature) and then analyzed using the chiral GLC (J&M Scientific cyclodex-β, 30 m) method (see Refs. 2f and 3e).
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