Tetrahedron: Asymmetry Vol. 3, No. 10, pp. 1223-1226, 1992
Printed in Great Britain

0957-4166/92 $5.00+.00

Enzyme-Catalysed Synthesis of Optically Active Aliphatic Cyanohydrins

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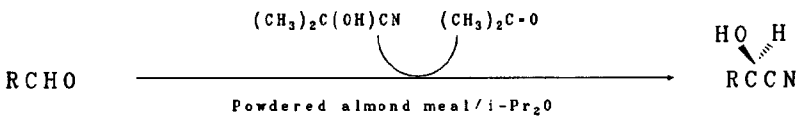
(*Received* 14 *August* 1992)

Abstract: The enantioselective preparation of aliphatic (*R*)-cyanohydrins was studied using powdered almond meal, a rich source of mandelonitrile lyase, as a catalyst in organic solvent. The feasibility of powdered almond meal as a catalyst was compared to that of a purified enzyme preparation.

Optically active cyanohydrins are versatile intermediates in organic synthesis[1]. During the last decade the preparation of these compounds has received a considerable amount of attention[2]. One of the most succesful ways of preparing aromatic and aliphatic (*R*)-cyanohydrins has been the use of mandelonitrile lyase (EC 4.2.1.0) purified from sweet almonds[3]. A crude extract from ground almonds in an aqueous buffer or ground almond meal itself have also been used as catalysts[4,5].

In this work, we describe the first systematic study on the synthesis of optically active aliphatic cyanohydrins using acetone cyanohydrin as a transcyanation agent[3b,6] and powdered, defatted almond meal as a catalyst (Scheme 1). Powdered almond meal provides an inexpensive catalyst, the use of which eliminates the need to purify and immobilize the enzyme.



1 **2**

R = a: propyl
b: i-propyl
c: butyl
d: i-butyl
e: pentyl
f: heptyl
g: nonyl

Scheme 1. Aliphatic (*R*)-cyanohydrins from aliphatic aldehydes.

The reactions were carried out in a biphasic mixture of 10 % (v/v) of diisopropyl ether and citrate buffer (0.02 M, pH 5.5)[7]. The enzyme is known to require some water to retain it's catalytic activity[8] and the amount of the buffer used was found to be optimal in terms of enzyme activity and enantioselectivity. Samples from the reaction mixture were analysed with a gas chromatograph equipped with a chiral capillary column[9]. This method allows us to obtain both the extent of conversion and the enantiomeric purity of the product simultaneously. The results of the present work are shown in Table 1.

Table 1. The Preparation of Optically Active Aliphatic Cyanohydrins.

Substrate	Time (h)	Conv. (%)	Product	e.e. (%)
1 a	19	100	(*R*)-2 a	95
1 a	41	100	(*R*)-2 a	89[a]
1 b	15	99	(*R*)-2 b	83
1 c	13	100	(*R*)-2 c	97
1 c	15	36	(*R*)-2 c	97[b]
1 d	15	100	(*R*)-2 d	94
1 e	18	100	(*R*)-2 e	94
1 f	26	98	(*R*)-2 f	87
1 f	24	24	(*R*)-2 f	87[b]
1 g	70	94	(*R*)-2 g	63

[a] Zandbergen *et al.*[3] The reaction was conducted in ethyl acetate at 4 °C, using HCN as a cyanogen source.

[b] The reactions were catalyzed by immobilized enzyme.

The results in Table 1 indicate that the use of defatted almond meal to catalyse the reactions of the substrates **1a-f** allows the preparation of both straight and branched chain aliphatic (*R*)-cyanohydrins in extremely high yield (corresponding to more than 95 % conversion) and enantiomeric purity (e.e. 83-97 %). According to our results and contrary to the proposal made by Brussee *et al.*[4] mandelonitrile lyase accepts as substrates aliphatic aldehydes with at least ten carbon atoms. However, longer reaction times are needed and the enantiomeric purity of the product gets worse with increasing chain length (Table 1, entries 8-10). This is at least partly due to the slow enzymatic reactions which allow the proportion of the corresponding non-stereoselective chemical reaction to become significant. The retarded enzymatic reactions, in turn, are caused by the partitioning of the more hydrophobic substrates mainly to the organic phase which diminishes their concentration in the aqueous phase in the vicinity of the enzyme[8,10].

In order to compare the feasibility of powdered almond meal with that of a purified enzyme preparate, the reactions with the substrates **1c** and **1f** were also carried out with commercially available mandelonitrile lyase immobilized on Celite[11] (Table 1, entries 5 and 9). The results of Table 1 do not indicate any loss of enantioselectivity when the reactions of the two substrates are catalysed by almond meal instead of the purified enzyme. On the other hand, the reactions carried out with the commercial enzyme were found to be much slower. This is most propably caused by the partial denaturation of the enzyme during it's purification and immobilization.

By comparing our results with the only result previously reported for the preparation of an aliphatic cyanohydrin with almond powder (Table 1, entry 2)[5], it can be noticed that our method affords somewhat greater enantioselectivity. Although the two results for the substrate **1a** are not obtained exactly in the same conditions, different enzyme activities in different organic solvents can be used to explain this. As a support, Wehtje[8] has found, using benzaldehyde as a substrate, that mandelonitrile lyase is almost 7 times more active in diisopropyl ether than in ethyl acetate. Based on this, it can be expected that the chemical reaction, leading to the formation of racemates, plays a greater role in the reactions conducted in ethyl acetate than in the reactions in diisopropylether.

In summary the advances in our methodology are the extremely low cost of almond meal, the simplicity of the experimental procedure and the safety of acetone cyanohydrin as a cyanogen source.

Acknowledgement. Thanks are due to the Technology Development Centre (TEKES) for financial support.

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7. The general procedure for preparing optically active aliphatic cyanohydrins was as follows: 0.2 g of defatted, ground almond meal was incubated with 500 μl of 0.02 M citrate buffer (pH 5.5) in a 10 ml reaction vessel. After 15 minutes diisopropyl ether, enough to bring the final volume to 5 ml, was added. The freshly distilled substrate aldehyde (1,0 mmol) was added and the reaction was started by the addition of 1.5 eq of acetone cyanohydrin. The mixture was shaken at 420 rpm on a rotary shaker at room temperature. When commercial mandelonitrile lyase was used as a catalyst, 100 mg of celite containing 1.6 mg of the enzyme was employed in the place of almond meal.
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11. It is estimated that not less than 0.4 % by weight of sweet almonds is mandelonitrile lyase[12]. The amount of immobilized enzyme used in the reactions was chosen to correspond to the estimated enzyme content in the reactions catalyzed by almond powder. The mandelonitrile lyase was purchased from Sigma Chemical Co. (St Louis, USA) and it was immobilized following the procedure by Wehtje et al.[13].
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