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Almond oxynitrilase-catalyzed transformation of substituted aldehydes. Part 2

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Abstract

Different α- and β-alkyl substituted aldehydes **(1–5)** have been submitted to the catalytic action of almond oxynitrilase, in order to explore the influence of a stereocenter already present in the substrate molecule on the selectivity of this enzyme. The results indicate that substituents in α-position to the aldehyde group significantly influence the stereochemical outcome of the AON-catalyzed transformation, especially when they are of different size. On the other hand, the presence of a β-substituent seems to have little effect on AON selectivity.

Keywords: Almond oxynitrilase; Cyanohydrin; Substituted aldehydes; Diastereoselectivity

1. Introduction

Oxynitrilase-catalyzed addition of hydrogen cyanide to aldehydes and ketones is the most important methodology for the obtainment of optically active cyanohydrins in terms of efficiency and enantiomeric purity of the products [1,2]. Besides the well-known and abundant oxynitrilase isolated from almond that catalyzes the formation of (*R*)-cyanohydrines, two additional enzymes with opposite stereospecificity have been recently cloned and overexpressed, thus expanding the exploitation of these biocatalysts even more [3,4].

In the frame of our interest in biocatalytic transformations performed in organic solvents (Refs. [5,6] and references therein), we have recently included oxynitrilases among our research topics, with the aim to explore the influence of a stereocenter already present in the substrate molecule on the selectivity displayed by these enzymes.

In a preliminary communication [7], we have described the results obtained with the model compound (±)-2-phenyl-propanal (**1**). Addition of HCN was performed using the enzyme isolated from grounded almonds [8], following the methodology described by Kyler [9] (Scheme 1). Four different diastereoisomers were obtained and the relative composition as well as the absolute configurations of their stereogenic

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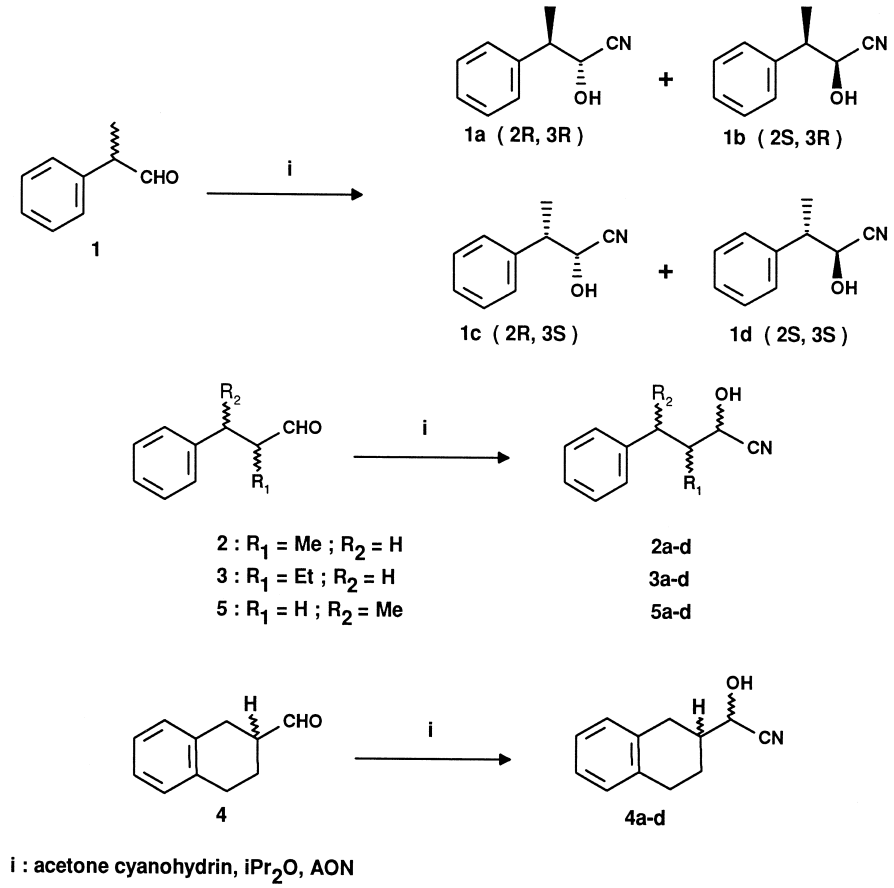


Scheme 1.

centers [7] are reported in the first line of Table 1.

These data were quite intriguing, showing that the stereocenter in α to the aldehyde deeply influenced the stereochemical outcome of the reaction. Specifically, when the stereocenter had an (*S*)-configuration, almond oxynitrilase (AON) showed the expected preference for the synthesis of (*R*)-cyanohydrins and the (2*R*,3*S*)-isomer was formed with a diastereomer ratio of 17.3. On the other hand, when the stereocenter had an (*R*)-configuration, the selectivity of the enzyme was almost completely lost and the two cyanohydrins were obtained in nearly equivalent amounts (diastereoisomer ratio 1.6). Notably, in this case the selectivity of AON was inverted, as the slightly more abundant diastereoisomer had the (2*S*)-configuration.

To gain more information on this topic, we synthesized other α-alkyl substituted aldehydes that were submitted to the catalytic action of AON, and in the following we describe and comment on the results obtained.

Table 1
Almond oxynitrilase-catalyzed synthesis of α- (or β-) substituted cyanohydrins

Substrate	Products (isol. yields)	Diastereoisomeric composition %			
		2*R*,3*R*	2*S*,3*R*	2*R*,3*S*	2*S*,3*S*
1[a]	**1a–d** (60)	17.6	27.6	51.8	3.0
2	**2a–d** (55)	26.5	13.8	57.1	2.6
3	**3a–d** (80)	42.8	0	57.2	0
4	**4a–d** (70)	46.7	4.2	44.5	4.6
5[a]	**5a–d** (80)	44.5	3.9	48.5	3.1

[a] See Ref. [7].

2. Experimental

2.1. Materials and methods

Oxynitrilase was isolated from grounded almonds [8]. Lipase P from *Pseudomonas cepacia* was purchased from Amano. Acetone cyanohydrin and other reagents were from Aldrich.

HPLC analyses were performed using a Chiralcel OD column (from DIACEL) and a Jasco 880/PU instrument equipped with a Jasco 875 UV/VIS detector (reading was done at 254 nm). **3a–d**, eluent: hexane-*i*-PrOH, 98:2; flow rate: 0.5 ml/min; ret. time: **3a**, 53.7 min; **3b**, 56.9 min; **3c**, 64.3 min; **3d**, 101.6 min; **4a–d**, eluent: hexane-*i*-PrOH, 98: 2; flow rate: 0.8 ml/min; ret. time: **4a**, 80.5 min; **4b**, 71.6 min; **4c**, 87.2 min; **4d**, 75.4 min; **2a–d** were previously acetylated; **2a–d** acetates, eluent: hexane-*i*-PrOH, 100: 0.5; flow rate: 0.5 ml/min; ret. time: **2a**, 36.6 min; **2b**, 38.6 min; **2c**, 41.1 min; **2d**, 35.1 min. For **1a–d** and **5a–d**, see [7].

2.2. Synthesis of aldehydes *1–5*

Racemic 2-phenyl propanal (**1**) and 3-phenyl butanal (**5**) were commercially available (Aldrich).

Racemic 3-phenyl-2-methyl propanal (**2**) was synthesized by catalytic (Pd/C) hydrogenation of the commercially available α-methylcinnamaldehyde. B.p. 50°C (1 mmHg). ^{1}H-NMR data ($CDCl_3$) δ: 9.7 (1H, br s, H-1); 7.4 − 7.1 (5H, m, ArH); 3.08 (1H, dd, $J_1 = 13.0$ Hz, $J_2 = 7.8$ Hz, H-3a); 2.67 (1H, m, H-2); 2.60 (1H, dd, $J_1 = 13.0$ Hz, $J_2 = 7.8$ Hz, H-3b); 1.10 (3H, d, $J = 6.5$ Hz, CH_3).

Racemic 2-benzyl butanal (**3**) was prepared by exhaustive catalytic hydrogenation (Pd/C) of α-ethylcinnamaldehyde (obtained by aldolic condensation of butanal and benzaldehyde) and subsequent oxidation to the corresponding aldehyde with the Swern procedure [10]. B.p. 100°C (1 mmHg). ^{1}H-NMR data ($CDCl_3$) δ: 9.7 (1H, d, $J = 4$ Hz, H-1); 7.4 − 7.0 (5H, m, ArH); 2.97 (1H, dd, $J_1 = 13.5$ Hz, $J_2 = 8.2$ Hz, H-3a); 2.65 (1H, m, H-2); 2.0 (1H, dd, $J_1 = 13.5$ Hz, $J_2 = 6.8$ Hz, H-3b); 1.65 (2H, m, CH_2); 0.95 (3H, t, $J = 10$ Hz, CH_3).

Racemic 1,2,3,4-tetrahydro-2-naphtaldehyde (**4**) was prepared from 1,2,3,4-tetrahydro-2-naphtoic acid, which was esterified with MeOH, reduced to the corresponding alcohol with $LiAlH_4$ and then oxidized with NaOCl and a catalytic amount of TEMPO [11]. B.p. 150°C (1 mmHg). ^{1}H-NMR data ($CDCl_3$) δ: 9.8 (1H, br s, H-1); 7.2 − 7.0 (4H, m, ArH); 3.05 − 2.60 (5H, m, H_2-1, H_2-4, H-2); 2.30 − 1.25 (2H, m, H_2-3).

2.3. General procedure for the chemical synthesis of cyanohydrins

To a solution of 20 mg of aldehyde in 1 ml of CH_3COOH 80%, NaCN (3 eq) dissolved in 1 ml of water was added dropwise at 0°C. At the end of the reaction water was added and the mixture was extracted with diethyl ether. The organic phases were washed with $NaHCO_3$, dried with Na_2SO_4 and evaporated. The cyanohydrines were purified by flash-chromatography.

^{1}H-NMR data ($CDCl_3$) **2a–d** δ: 7.40 − 7.10 (5H, m, ArH); 4.33 and 4.37 (each 0.5H, d, $J = 6.2$ Hz, H-1); 2.92 and 2.80 (each 0.5H, dd, $J_1 = 7.5$ Hz, $J_2 = 13.7$ Hz, H-3a); 2.64 and 2.60 (each 0.5H, dd, $J_1 = 7.5$ Hz, $J_2 = 13.5$ Hz, H-3b); 2.22 (1H, m, H-2); 1.12 and 1.10 (each 1.5H, d, $J = 6$ Hz, CH_3).

3a–d δ: 7.4 − 7.1 (5H, m, ArH); 4.45 and 4.38 (each 0.5H, d, $J = 4.5$ Hz, H-1); 2.90 and 2.73 (each 0.5 H, dd, $J_1 = 13.4$ Hz, $J_2 = 6.0$ Hz, H-3a); 2.75 and 2.68 (each 0.5 H, dd, $J_1 = 13.4$ Hz, $J_2 = 7.5$ Hz, H-3b); 2.0 (1H, m, H-2); 1.55 (2H, m, CH_2); 1.12 and 1.10 (each 1.5H, t, $J = 7$ Hz, CH_3).

4a–d δ: 7.2 − 7.0 (4H, m, ArH); 4.43 and 4.40 (each 0.5H, d, $J = 6$ Hz, CH–CN); 3.1 − 2.7 (5H, m, CH_2-1, CH_2-4, H-2); 2.3 − 1.5 (2H, m, CH_2-3).

2.4. General procedure for the enzymatic synthesis of the cyanohydrins

To a solution of 500 mg of aldehyde in 23 ml of diisopropyl ether containing 1.3 eq of acetone cyanohydrin, AON ($\approx$ 1500 units), dissolved in 500 μl of 0.1 M citrate buffer pH 5.5, was added and the biphasic system shaken at room temperature for 3–6 days. At the end of the reaction the two phases were separated, the aqueous phase was extracted with diisopropyl ether, the organic phases were dried with Na_2SO_4 and evaporated. The cyanohydrins were purified by flash-chromatography.

2.5. General procedure for the acylation with lipase P

About 30 mg/ml of substrate (alcohol or cyanohydrin) were dissolved in methyltert-butylether containing 10% v/v vinyl acetate and lipase P immobilized on celite (20 mg/ml) was added and the suspension was shaken at room temperature until about 50% of conversion was reached. The enzyme was filtered, the solvent evaporated and the products purified by flash-chromatography.

2.6. Stereochemical correlations

(*R*)-3-Phenyl-2-methylpropanal (**2**) was prepared from the corresponding (*R*)-alcohol (Scheme 3) obtained as described by Delink and Margolin [12]. The corresponding (3*R*)-cyanohydrins were obtained chemically as previously described.

(*S*)-2-Benzyl butanal (**3**) was prepared from the corresponding (*S*)-alcohol (Scheme 3) obtained as described by Sih and Gu [13]. The corresponding (3*S*)-cyanohydrins were obtained chemically as previously described.

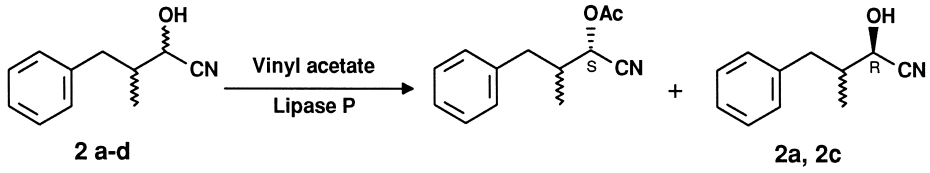
(*S*)-1,2,3,4-Tetrahydro-2-naphtaldehyde (**4**) was obtained from the corresponding (*S*)-1,2,3,4-tetrahydro-2-naphtoic acid methyl ester (Scheme 3) as described by Cohen et al. [14]. The corresponding (2*S*)-cyanohydrins were obtained chemically as previously described.

(*R*)-3-Phenyl-3-methylpropanal (**5**) was prepared from the corresponding (*R*)-acid (Scheme 3) obtained by selective crystallization [15]. The corresponding (3*R*)-cyanohydrins were obtained chemically as previously described.

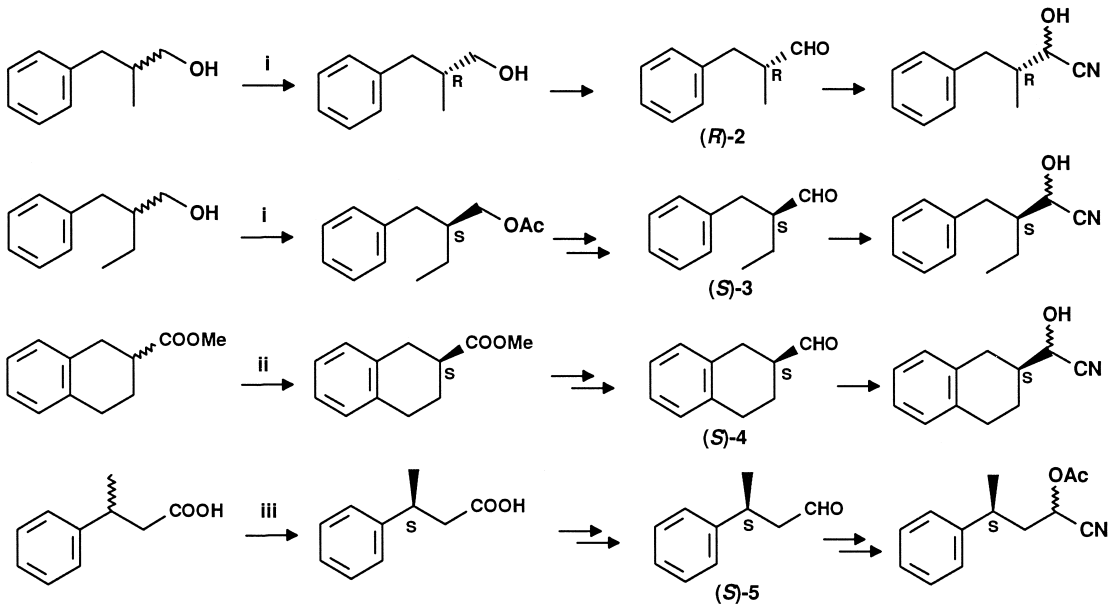
3. Results and discussion

Aldehydes **2**–**4** were prepared following standard chemical procedures (see Section 2), and Kyler's protocol [9] was used for the AON-catalyzed synthesis of the corresponding cyanohydrins. The diastereomeric composition in each product mixture (**2a–d**, **3a–d**, **4a–d**) was obtained by chiral HPLC, which allowed a base-line separation of the peaks corresponding to the four different isomers. The absolute configuration of the stereogenic centers of cyanohydrins was determined by first exploiting the selectivity of lipase P for the acylation of (*S*)-cyanohydrins, as exemplified in Scheme 2 [16].

Then, to complete the correlation between the absolute configurations of the products and their own chiral HPLC peaks, preliminary resolution



Scheme 2.



Scheme 3.

of the synthetic precursors of the aldehydes **2–4** was done, as shown in Scheme 3 (for details see Section 2).

The combination of the whole of this information gave the results reported in Table 1.

As expected, (±)-3-phenyl-2-methylpropanal (**2**) behaved quite similarly to **1**, even though the (2*R*)-enantiomer of **2** did not show an inversion of the HCN facial addition to the carbonyl. On the other hand, a dramatic increase of selectivity was observed with the ethyl homologue of **2**, (±)-3-phenyl-2-ethylpropanal (**3**): only two diastereoisomeric cyanohydrins were detected, both of them with the 'natural' (2*R*) configuration. Similarly, a very high (*R*)-selectivity was observed with the two enantiomers of **4**, the cyclic analogue of **3**.

The results indicate that substituents in α-position to the aldehyde group significantly influence the stereochemical outcome of the AON-catalyzed transformation, especially when they are of different size (compare substrates **1** and **2**, and substrates **2** and **3**). On the other hand, the presence of a β-substituent, as in 3-phenyl butanal (**5**) [7], seems to have little effect on the natural AON stereospecificity.

These data are not easy to rationalize in the absence of information on the structure of the active site of AON; further work will be dedicated to study the behaviour of other compounds, for instance (±)-2-naphtyl-propanal. The influence of oxygenated substituents on the selectivity of AON and of other oxynitrilases will be also reported soon.

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