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ENZYME-CATALYZED SYNTHESIS OF (S)-CYANOHYDRINS AND SUBSEQUENT HYDROLYSIS TO (S)-α-HYDROXY-CARBOXYLIC ACIDS[1]

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Summary: (*S*)-Cyanohydrins **2** are obtained with high enantioselectivity from aromatic aldehydes and HCN in the presence of (*S*)-oxynitrilase (E.C.4.1.2.11). Acid-catalyzed hydrolysis of the cyanohydrins **2** affords the corresponding (*S*)-α-hydroxy carboxylic acids **3** without racemization.

(*R*)-Cyanohydrins with high optical purity are obtained from the addition of HCN to aldehydes, catalyzed by (*R*)-oxynitrilase (E.C.4.1.2.10) when an appropriate organic solvent is used (e.g. ethyl acetate)[2]. If the reaction is carried out in an aqueous-alcoholic medium, however, the optical yield is reduced significantly[3], especially in the case of substrates which require longer reaction times.

Various procedures for the enantioselective synthesis of cyanohydrins have been published in the last few years. Among these, the use of chiral catalysts[4], the enantioselective hydrolysis of O-acetyl cyanohydrins using esterases as catalysts[5], the lipase-catalyzed enantioselective esterification of racemic cyanohydrins with enol esters[6], and the diastereoselective addition of trimethylsilyl cyanide to optically active aldehydes[7] is reported. A common disadvantage of all catalytic procedures is the poor optical yield at higher conversion rates. At most 50% yield can principally be obtained, relative to the racemic cyanohydrin substrate, for enzyme-catalyzed ester cleavage or esterification.

We now report the enantioselective addition of HCN to aldehydes, yielding (*S*)-cyanohydrins **2** in very high optical purity, with (*S*)-oxynitrilase (E.C.4.1.2.11) as catalyst. This enzyme was first isolated by E.Conn and C.Bové in 1960[8]. High optical yields are obtained, however, only if the non-enzymatic addition, which results in racemic product is successfully suppressed by working in organic solvents (e.g. ethyl acetate or diisopropyl ether DIPE)[9].

The two enzymes, (*S*)- and (*R*)-oxynitrilase, differ significantly both in structure and enzymatic properties[3a,8]. (*S*)-Oxynitrilase exclusively catalyzes addition of HCN to aromatic aldehydes, yielding the (*S*)-cyanohydrins **2**. (*R*)-Oxynitrilase, in contrast, accepts aliphatic aldhehydes as substrates as well[2]. (*S*)-Oxynitrilase-catalyzed reactions as a rule are slower, and the optical purity of the products **2** is sometimes lower than for the obtained products[2] catalyzed by (*R*)-oxynitrilase (see Table 1).

Acid-catalyzed hydrolysis of optically active cyanohydrins affords α-hydroxy carboxylic acids, likewise in optically active form[5d,6,7,10]. This hydrolysis was previously supposed to proceed with partial racemization[4a]. We have now succeeded in directly hydrolyzing the (*S*)-cyanohydrins **2** prepared, without isolation, with conc. hydrochloric acid, and obtained the corresponding (*S*)-α-hydroxy carboxylic acids **3** in high yield, and virtually without racemization[11]. Cyanohydrins **2** with a free hydroxyl group in the aromatic ring, though, are not converted to the respective acids **3** under these conditions (see Table 1).

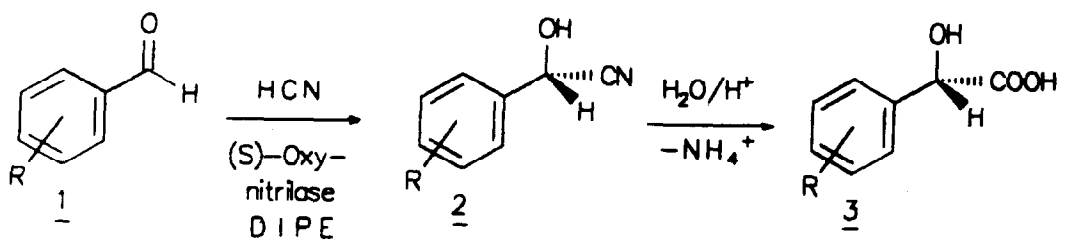

Table 1

Enzyme-Catalyzed Syntheses of (S)-Cyanohydrins 2 and (S)-α-Hydroxy Carboxylic Acids 3

	2		3	
R	Yield (%)	% ee[a]	Yield[b] (%)	% ee[c]
H	91	97	77	98
4-CH_3	61	78	75	78
4-Cl	87	54	63	54
4-OH	84	94	-[d]	-
3-Cl	95	91	69	91
3-Br	94	92	76	90
3-OH	97	91	-[d]	-
3-OCH_3	93	89	67	90
3-OPh	93	96	70	96

[a] as (R)-(+)-MTPA derivatives. - [b] all compounds gave correct elemental analysis. - [c] as (R)-(+)-MTPA derivatives of the isopropylesters. - [d] decomposition.

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9) (*S*)-Cyanohydrins **2**: A suspension of avicel-cellulose (0.5 g) in 0.05 M phosphate buffer (pH 5.4, 10 ml), containing ammonium sulfate (4.72 g), was stirred for 1 h, and a solution of (*S*)-oxynitrilase (E.C.4.1.2.11; 50 µl, 1000 units/ml, specific activity 70 units/mg) added. The mixture was stirred at room temperature for 10 min, filtered, and the immobilized enzyme suspended in diisopropyl ether (DIPE; 10 ml). After addition of al-aldehyde (2 mmol) and HCN (300 µl, 7.5 mmol), the mixture was stirred until all aldehyde has reacted. The filtrate, after removal of the immobilized enzyme, was concentrated to yield **2**. Example: From 3-phenoxy benzaldehyde **1** [R = 3-OPh (0.40 g)] after 6 d, 3-phenoxy mandelonitrile **2** [R = 3-OPh (0.42 g), $[\alpha]_D^{20}$ = -23.4° (c 1.2, chloroform)] was obtained.

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11) (*S*)-α-Hydroxy Carboxylic Acids **3**: A solution of 1.55 mmol of the crude product **2**, prepared according to ref.9, in conc. HCl (5 ml) was stirred 16 h at room temperature and at 60°C for 3-8h. The solution was concentrated in vacuo, and the residue extracted with diethyl ether. Evaporation of the combined extracts yielded **3**. Example: From crude 3-phenoxy mandelonitrile **2**, R = 3-OPh (0.35 g), 3-phenoxymandelic acid **3**, R = 3-OPh (0.26 g), $[\alpha]_D^{20}$ = +21.1° (c 0.6, acetic acid) was obtained.

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