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MICROBIAL HYDROLYSIS AS A POTENT METHOD FOR THE PREPARATION OF OPTICALLY ACTIVE NITRILES, AMIDES AND CARBOXYLIC ACIDS

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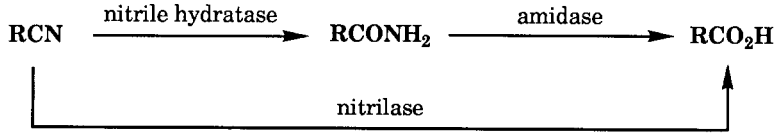
Summary: Many kinds of aromatic nitriles have been hydrolyzed to afford the corresponding amides and carboxylic acids, by the aid of enzyme system of *Rhodococcus butanica* . This enzymatic hydrolysis can be successfully applied to the kinetic resolution of α-arylpropionitriles, resulting in the formation of (R)-amides and (S)-carboxylic acids.

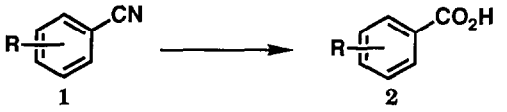
Nitriles are versatile intermediates in organic synthesis, because they can be transformed to amines, amides, carboxylic acids, carbonyl and other compounds. In addition, it is quite advantageous that cyano group can be introduced as " water-stable carbanion ". However, the major drawback of these compounds is that the hydrolysis to amides or carboxylic acids usually requires rather drastic conditions, for example, heating in the presence of a strongly basic or acidic catalyst. Thus enzymatic or microbial transformation of these compounds is a very attractive method because of mild reaction conditions (pH and temperature), and in fact some elegant examples have been demonstrated so far.[1a-c] Very recent report of M. A. Cohen *et al.* [2] prompted us to disclose our own results on microbial hydrolysis of aromatic nitriles as well as its application to the preparation of optically active α-arylpropionic acids.

First, we screened a microorganism which is able to grow utilizing 2-cyanoethanol or benzonitrile as a sole source of nitrogen, and finally selected *Rhodococcus butanica* ATCC 21197 as the best strain, which is available as a type culture. It was found that addition of ε-caprolactam[cf. 3] to the medium is essential to induce three enzymes concerned in the hydrolysis of nitriles, *i.e.*, nitrilase, nitrile hydratase and amidase.



Two typical procedures are as follows. Method A: Sterilized nutrient medium (100 ml, pH 7.2)[4] was inoculated with *R. butanica* and incubated for 2 days at 30 °C. To this suspension, a substrate

(0.1% to the medium, v/v) was added and the incubation was continued for additional 24 hr. The broth was extracted, esterified with diazomethane, and purified by preparative TLC to afford the corresponding amide and carboxylic esters. Method B: The cells of *R. butanica* grown on above medium were harvested by centrifugation and suspended in 50 ml of phosphate buffer (100 mM, pH 8.0) containing 0.1% of the substrate. The flask was shaken for a period specified in Table 2 and 3 (3~24 hr) at 30 °C. The work-up and purification procedures were the same as Method A.



1 2

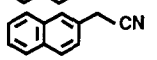
Table 1. Microbial hydrolysis of substituted benzonitriles [a)]

R	Yield of **2** (%)	R	Yield of **2** (%)
2 - OH	59	H	85
3 - OH	88	2 - Cl	29 (32) [c)]
4 - OH	0 [b)]	3 - Cl	96
2 - CH_3	5 (79) [c)]	4 - Cl	86
3 - CH_3	74	3 - CN	77
4 - CH_3	69	4 - OMe	66

a) The reaction was carried out according to Method A.
b) The starting material and/or the product were metabolized.
c) The value in parentheses is the yield of the corresponding amides.

3 4

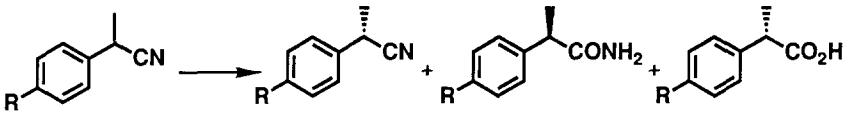
Table 2. Microbial hydrolysis of arylacetonitriles

R	Method	Yield (%)	Substrate	Method	Yield (%)
4 - OH	A	59		B (24 hr)	87
H	A	80			
4 - Cl	A	85		B (24 hr)	85
4 - OMe	A	88			

The broad substrate specificity of this microorganism to catalyze the hydrolysis of aromatic nitriles is shown in Table 1 and 2. A number of monosubstituted (meta and para) benzonitriles and arylacetonitriles are smoothly transformed to the corresponding carboxylic acids. The only exception is 4-hydroxybenzonitrile, which was metabolyzed by this microbe. One characteristic feature of this microorganism is that it also attacks ortho-substituted benzonitrile derivatives, which have been

previously reported to be resistant to enzymatic hydrolysis.[1a] The major products in these cases were found to be amides different from other examples. It is noteworthy that the enzyme system selectively hydrolyzed only one cyano group of isophthalonitrile resulting in the formation of cyano carboxylic acid. The reactivity of the enzyme(s) of *R. butanica* can be said on the whole to largely depend on the steric rather than electronic effects of the substituents. It is suggested that the binding site of this biochemical catalyst may have strong affinity to an aromatic ring.

Then our attention was focused on the problem whether this microorganism distinguishes the chirality of the substrates. Thus, α-arylpropionitriles (**5**) were subjected to the reaction, the results being summarized in Table 3. As the representative substrates, three types of compounds which have sterically bulky (**5a**), electron-withdrawing (**5b**) and donating (**5c**) substituent are selected. In all



(±) - 5 (S) - 5 (R) - 6 (S) - 7

a ; R=iso-Bu, b ; R=Cl, c ; R=OMe

Table 3. Enantioselective microbial hydrolysis of α-arylpropionitriles [a]

Substrate	Reaction time(hr)	Yield (%) / E.e.(%) [b]		
		5	**6**	**7**
5a	3	56 / 27	30 / 95	6 / 75
	6	21 / 73	48 / 99	13 / 87
	24	-	27 / 95	60 / 15
5b	6	-	46 / 76	13 / >99
	24	-	34 / >99	30 / 43
5c	6	-	45 / 99	47 / 99
	24	-	37 / >99	59 / 53

a) The reaction was carried out according to Method B.

b) Enantiomeric exess of **5-7** were determined by HPLC analysis.[5]

cases the corresponding (R)-amides of high enantiomeric excess were obtained in good yields as well as (S)-carboxylic acids in agreeable yields. To the best of our knowledge, present enzyme-mediated kinetic resolution of α-substituted nitriles is the first example utilizing hydrolysis to give optically active 2-arylalkanoic acids, some of which are important as non-steroidal anti-inflammatory drugs. The absolute configuration of **7a** was determined to be S by comparison of the specific rotation of the corresponding alcohol[6] which was readily obtained by the reduction with $LiAlH_4$. The configuration of **7b, c** were confirmed to be also S on the basis of optical rotations of the corresponding methyl esters

(**8**).[7] Acidic hydrolysis of recovered **5a-c** showed their absolute configuration to be S,[8] although partial racemization was observed. Conversion of amides (**6**) to nitriles (with P_2O_5) revealed thier configuration to be R.

The results shown in Table 3 strongly suggest that the reaction proceeds *via* two paths; path A is the stepwise conversion of **5** to **6** catalyzed by nitrile hydratase followed by hydrolysis of **6** to **7** by the aid of amidase, while path B is the direct conversion of **5** to **7** by nitrilase. It is clear that the stepwise path is the major, the activity of nitrile hydratase being the highest because the yield of **6** are relatively high. In a control experiment using racemic amide showed that amidase preferred (S)-amide as the substrate, though the selectivity was not so high as useful in practical resolution. Thus the high optical purities of the obtained amides can be said as the results of double enantiomeric selection in two stepwise reactions, *i.e.*, prefential formation of (R)-enantiomer and faster hydrolysis of (S)-amides. Since optically active amides can be converted to the corresponding carboxylic acids under acidic conditions essentially without any racemization,[9] the above microbial enzyme system is expected to be useful for the preparation of both enantiomers of α-arylpropionic acids.

Reference and Notes

1) a) M. Kobayashi, T. Nagasawa and H. Yamada, *Eur. J. Biochem.*, **182**, 349 (1989); b) P. H. Schmidt and M. P. Schneider, *J. Chem. Soc. Chem. Commun.*, 648 (1990); c) M. Kobayashi, N. Yanaka, T. Nagasawa and H. Yamada, *Tetrahedron*, **46**, 5587 (1990) and the references cited therein.
2) M. A. Cohen, J. Sawden and N. J. Turner, *Tetrahedron Lettt.*, **31**, 7223 (1990).
3) T. Nagasawa, T. Nakamura and H. Yamada, *Abstract of the annual meating of Japan Society of Bioscience, Biothechnology and Agrochemistry*, 2Qp15 (1990)
4) The medium consists of glucose 15 g, KH_2PO_4 0.5 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, ε-caprolactam 5.0 g, and yeast extract 1.0 g in 1000 ml of distilled water.
5) Amides (**6**) were analyzed after dehydration to nitriles (**5**) with P_2O_5 without any racemization and **7a-c** after being converted to the corresponding methyl ester (**8**) with CH_2N_2. Conditions for HPLC analysis; Column CHIRALCEL OJ (Daicel Chemical Industries, Ltd.); Solvent, hexane/2-propanol=180/1 (except for **8c**, hexane/2-propanol=50/1); Retention time (min), **5a**: 20 (R), 23 (S); **5b**: 59 (R), 66 (S); **5c**: 91 (R), 100 (S); **8a**: 23 (R), 20 (S); **8b**: 25 (R), 28 (S); **8c**: 53 (R), 49 (S).
6) T. Sugai and K. Mori, *Agric. Biol. Chem.*, **48**, 2501 (1984).
7) K. Miyamoto and H. Ohta, *J. Am. Chem. Soc.*, **112**, 4077 (1990).
8) 6N H_2SO_4/CH_3CO_2H=1/3, reflux, 48hr.
9) 6N H_2SO_4/CH_3CO_2H=1/2, reflux, 12hr.

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