Communications

Highly Enantioselective Microbial Hydrolysis of *cis*-2-Arylcyclopropanecarbonitriles†

FENG, Guo-Qiang(冯国强) **WANG, Mei-Xiang***(王梅祥)

Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

Hydrolysis of racemic *cis*-2-arylcyclopropanecarbonitriles catalyzed by *Rhodococcus sp*. AJ270 whole cells proceeded enantioselectively to afford the corresponding amide and acid with enantiomeric excess higher than 99%.

Keywords Enantioselective hydrolysis, *cis*-2-arylcyclopropanecarbonitriles, 1*R*, 2*S*-2-arylcyclopropanecarboxamides, 1*S*, 2*R*-2-arylcyclopropanecarboxylic acids, *Rhodococcus sp*. AJ270

Much effort has been devoted in recent years to the asymmetric synthesis of enantiopure cyclopropyl compounds, because such compounds occur widely in natural products, in synthetic pharmaceuticals and agrochemicals; and their enantiomers often show different biological activities. While asymmetric cyclopropanation reaction is effective for the preparation of optically active *trans*-cyclopropane compounds, the method generally shows deficiency in the synthesis of the *cis*-isomers.[1] Only in a very recent publication, has the cyclopropanation of styrene with α-diazoacetate using chiral (ON^+) Ru-salen complex as a catalyst been illustrated to give *tert*-butyl *cis*-2-phenylcyclopropane-1-carboxylate with high enantiomeric excess, but in a very low chemical yield.[2] Kinetic resolution of the esters of 2-substituted cyclopropane-1-carboxylic acids using ester-hydrolyzing enzymes has been used successfully to prepare optically active *trans*-2-substituted cyclopropane-1-carboxylic acids and their esters.[3,4] In contrast, however, no enzymatic preparation of *cis*-2-substituted cyclopropane-1-carboxylic acids and their derivatives has been reported so far.[3]

Biotransformation of nitriles using microbial cells and nitrile-hydrolyzing enzymes is a convenient and efficient method for the preparation of amides and acids.[5] It has been demonstrated recently that *Rhodococcus sp*. AJ270 is a powerful and robust nitrile hydratase/amidase-containing microorganism.[6] Compared with other microorganisms obtained, it has a very broad activity against almost all types of nitriles[7] and shows excellent selectivity in hydrolyzing aromatic dinitriles and a variety of aliphatic dinitriles.[8] It also shows excellent enantioselectivity when catalyzing the hydrolysis of some racemic α-substituted phenylacetonitriles[9] and prochiral dinitriles.[10] In our previous paper, we disclosed enantioselective biotransformation of racemic *trans*-2-arylcyclopropanecarbonitriles using *Rhodococcus sp*. AJ270, an efficient synthesis of optically active *trans*-2-arylcyclopropanecarboxylic acids and amides.[11] To explore further its potential in organic synthesis, and also to go deep insight into the effect of the structure of substrates on the reaction efficiency and stereoselectivity, we undertook the study of *Rhodococcus sp*. AJ270 whole-cell-catalyzed hydrolysis of *cis*-2-arylcyclopropanecarbonitriles.

In contrast to *trans*-2-arylcyclopropanecarbonitriles that were readily hydrolyzed[11] within hours by *Rhodococcus sp*. AJ270, all of the *cis*-2-arylcyclopropanecarbonitriles (**1**) tested underwent hydrolysis slowly under the identical conditions . As indicated in

* E-mali: mxwang@infoc3.icas.ac.cn

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Scheme 1

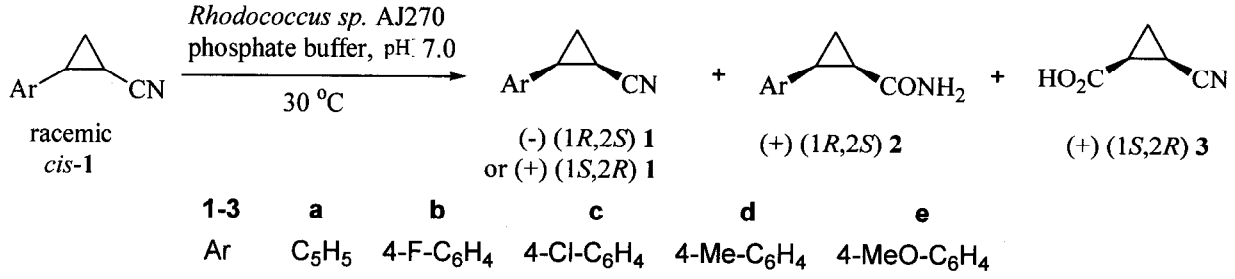

Table 1 Hydrolysis of *cis*-2-arylcyclopropanecarbonitriles[12]

Entry	1	Conc. (mmol/L)	Time (d)	Recovered **1**			1*R*,2*S*-**2**		1*S*,2*R*-**3**	
				yield	*ee*	conf.	yield	*ee*	yield	*ee*
1	**1a**	20	7	15	100	1*R*, 2*S*	32	> 99	49	> 99
2	**1a**	20 [a]	7	27	> 99	1*R*, 2*S*	24	> 99	43	> 99
3	**1a**	20 [b]	7	90	9	1*R*, 2*S*	0	-	9	90
4	**1a**	10	2	49	> 99	1*R*, 2*S*	6	66	43	> 99
5	**1a**	4	7	0	-		41	> 99	48	> 99
6	**1b**	12	4	24	84	1*R*, 2*S*	25	> 99	46	> 99
7	**1b**	12	7	11	> 99	1*R*, 2*S*	38	> 99	46	> 99
8	**1c**	20	7	84	2	nd [e]	8	> 99	8	> 99
9	**1c**	12	7	81	5	nd [e]	12	> 99	6	> 99
10	**1c**	5	7	67	6	nd [e]	18	> 99	8	> 99
11	**1d**	5	7	80	8	nd [e]	11	> 99	0	-
12	**1e**	5	7	77	17	1*S*, 2*R*	18	> 99	0	-
13	**1e**	5 [c]	7	71	22	1*S*, 2*R*	17	> 99	0	-
14	**1e**	5 [d]	7	62	27	1*S*, 2*R*	15	> 99	0	-

[a] A biphasic system of buffer (25 mL) and *n*-hexane (25 mL) was used. [b] Acetone (3 mL) was added as a co-solvent. [c] 4 grams of wet weight cell were used. [d] β-CD (100 mg) was added. [e] Configuration was not determined.

Table 1, the biotransformation of **1** was strongly dependent upon the nature of the substituent on the phenyl ring. The hydrolysis of parent *cis*-2-phenylcyclopropanecarbonitrile (**1a**) after one week gave a mixture of the corresponding amide **2a**, acid **3a** and nitrile **1a** (Entry 1). The complete conversion of **1a** was effected when the concentration of the substrate went down to 4 mmol/L, yielding 1*R*, 2*S*-2-phenylcyclopropanecarboxamide **2a** and 1*S*, 2*R*-2-phenylcyclopropanecarboxylic acid **3a** in excellent yield with high enantiomeric excess (Entry 5). Similar results were obtained when 2-(4-fluorophenyl)-cyclopropanecarbonitrile (**1b** was employed as the substrate (Entry 7). While the hydrolysis of chloro-substituted analogue **1c** led to the formation of amide **2c** and acid **3c** in low yields, the methyl-(**1d**) and methoxy-(**1e**) substituted analogs only gave the hydrated product **2d** and **2e**, respectively. In all later three cases, a large amount of nitrile was recovered even when low concentration (5 mmol/L) of substrate was applied. The use of organic additive such as acetone and β-cyclodextrin, and of bisphasic system of phosphate buffer and hexane did not improve the conversion. The results suggest that both nitrile hydratase and amidase involved in *Rhodococcus sp*. AJ270 are limited to the steric hindrance of the substrate, with the amidase being more sensitive. It is noteworthy that 1*R*, 2*S*-amide **2** and 1*S*, 2*R*-acid **2** obtained from biotransformation always show enantiomeric excesses higher than 99% after a long period of incubation, indicating a remarkable 1*S*, 2*R*-enantioselectivity of amidase. It should also be noted that the nitrile **1a** recovered from the reaction was optically active with high enantiomeric excess after 50% conversion. This is probably the first example that nitrile hydratase is of excellent enantioselectivity. More surprisingly, the 1*S*, 2*R*-enantioselectivity of nitrile hydratase against **1a** changed into 1*R*, 2*S*-enantioselectivity when nitrile **1e** was used. Though the precise reason awaits further study, the nature of the substituent on

the phenyl ring apparently played a role in determining the enantioselectivity of the nitrile hydratase in *Rhodococcus sp*. AJ270.

In conclusion, *Rhodococcus sp*. AJ270 can hydrolyze racemic *cis*-2-arylcyclopropanecarbonitriles to produce the corresponding optically active *cis*-2-arylcyclopropanecarboxamides and carboxylic acids. Both nitrile hydratase and amidase are highly enantioselective, and both the reaction rate and stereochemistry were strongly dependent upon the nature of the substituent on the phenyl ring of the substrates.

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12 For a general procedure of biotransformation, see ref. 10. A suspension of cells (2 g wet weight) in phosphate buffer (0.1 mol/L, pH 7.0) was used.

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