ORGANIC LETTERS

2002
Vol. 4, No. 3
371−373

Microbial Deracemization of α-Substituted Carboxylic Acids

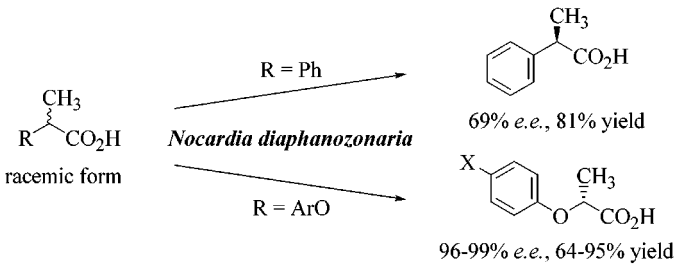
Dai-ichiro Kato,[†] Satoshi Mitsuda,[‡] and Hiromichi Ohta*,[†]

Center for Life Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan, and Sumitomo Chemical Co. Ltd., 4-2-1 Takatsukasa, Takarazuka 665-8555, Japan

hohta@chem.keio.ac.jp

Received November 15, 2001

ABSTRACT



An enzyme system of *Nocardia diaphanozonaria* JCM 3208 catalyzes the inversion of the chirality of various α-substituted carboxylic acids, such as 2-phenylpropanoic acid and 2-phenoxypropanoic acid derivatives, via a novel deracemization reaction.

The methods of preparation of optically active compounds are classified into two broad categories: optical resolution of racemic compounds and asymmetrization of prochiral compounds. Biocatalysts are widely utilized in both cases.[1] When the starting material is a racemic mixture, the most popular enzymatic approach to obtain the optically active compounds is kinetic resolution. However, the maximum theoretical yield is limited to 50% and the tedious procedures for the separation of the recovered starting material and the product are inevitable.

To overcome this drawback, there is a fascinating method known as dynamic resolution.[2] In this case, it is essential that the starting material racemizes under the reaction conditions, while the product does not. Accordingly, a combination of sophisticated design of the substrate and incubation conditions is the key to the successful procedure. The most straightforward method is the synthesis of the target molecule in racemic form and its conversion to the optically active form.[2c]

In this Letter, we report a preparation of optically active α-substituted carboxylic acids via a novel enzymatic deracemization reaction using whole cells. This deracemization reaction inverts the chirality of one enantiomer of a racemate to the other antipode, resulting in an optically active compound starting from a racemic mixture. Thus this process is entirely different from the above-mentioned dynamic resolution,[2] because in this reaction the starting material and the product are the same compound except for their chirality. The most common enzymatic deracemization processes previously reported are oxidation of *sec*-alcohol followed by enantioselective reduction to the antipode.[2c] Here we present a different option.

The (*S*)-enantiomers of 2-arylpropanoic acid derivatives constitute a subgroup of the widely used nonsteroidal antiinflammatory drugs,[3] while the (*R*)-enantiomer is either inactive or only weakly active in vivo. The preparation of an optically pure enaniomer is extremely important for these

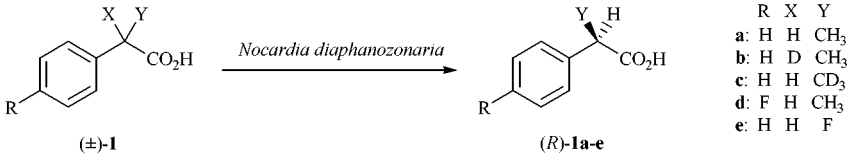
[†] Keio University
[‡] Sumitomo Chemical Co. Ltd.

(1) (a) Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Spriger-Verlag: Berlin, 2000. (b) Drauz, K., Waldmann, H., Ed. *Enzyme Catalysis in Organic Synthesis*; VCH: Weinheim, 1995. (c) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.

(2) (a) Stecher, H.; Faber, K. *Synthesis* **1996**, 1−16. (b) Caddick, S.; Jenkins, K. *Chem. Soc. Rev.* **1996**, *25*, 447−456. (c) Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107−117. (d) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475−1490. (e) Huerta, F. F.; Minidis, A. B. E.; Backvall, J.-E. *Chem. Soc. Rev.* **2001**, *30*, 321−331.

10.1021/ol0170556 CCC: $22.00
Published on Web 01/09/2002

Table 1. Deracemization of α-Substituted Phenylacetic Acids by the Aid of the Enzyme System of *N. diaphanozonaria*



entry	starting compd	reactn time (h)	product[a]	yield[b] (%)	$[\alpha]_D$[c]	ee[d] (%)
1	(±)-**1a**	48	(*R*)-**1a**	81	−71.3	69
2	(*R*)-**1a** (88% ee)	48	(*R*)-**1a**	58		72
3	(*S*)-**1a** (93% ee)	48	(*R*)-**1a**	48		52
4	(±)-**1b**[e] (D content: 98%)	48	(*R*)-**1a**[e] (D content: 20%)	66		78
5	(±)-**1c**[e] (D content: 100%)	48	(*R*)-**1a**[e] (D content: 100%)	61		71
6	(±)-**1d**	16	(*R*)-**1c**	58	−5.6	6
7	(±)-**1e**	48	(*R*)-**1d**	74		55

[a] The corresponding starting compounds were incubated with grown cells of *N. diaphanozonaria* at 30 °C. [b] Isolated yield after conversion to the corresponding methyl ester by treatment with diazomethane. [c] Optical rotation was measured in EtOH, $c = 1.00-1.03$ after conversion to the corresponding methyl ester. [d] ee (%) of the product was determined by HPLC analysis after conversion to the corresponding methyl ester. [e] D content was calculated from ^{1}H NMR analysis.

compounds, and some of these approaches have been previously reported.[4,5]

Only a few reports are known for the enzymatic deracemization of 2-arylpropanoic acid, i.e., the use of rat liver[6,7] and two fungi, *Cordyceps militaris*[3,8] and *Verticillium lecanii*,[9] which are capable of inverting the chirality of 2-arylpropanoic acid derivatives from the (*R*)-enantiomer to its (*S*)-antipode. In this case, the mechanism of the biotransformation system was proposed based on various studies using enantiomerically pure compounds and deuterated derivatives.[3,6,8,9] These studies, however, have been carried out from the perspective of the pharmacological effect of racemic compounds and were intended to clarify the destiny of two enantiomers in vivo. As described above, this type of reaction is also interesting from the standpoint of synthetic chemistry. Thus, we tried to examine the applicability of such a novel type of reaction as well as the substrate specificities and mechanism of the reaction.

After some screening, we found a deracemization activity in a type of actinomycetes, *Nocardia diaphanozonaria* JCM 3208.[10] The enzyme system of *N. diaphanozonaria* catalyzes the inversion of the chirality of 2-phenylpropanoic acid from (*S*)- to the (*R*)-configuration. To the best of our knowledge, this is the first example of inverting the chirality of 2-arylpropanoic acid derivatives from *S* to *R*.

The best incubation conditions so far obtained for 2-phenylpropanoic acid are as follows. To 90 mL of a nutrient medium was added a suspension of 48 h incubated cells of *N. diaphanozonaria* in 10 mL of broth, and the incubation was carried out at 30 °C for 24 h (first incubation). Then, 0.1% w/v of (±)-2-phenylpropanoic acid was added to the suspension of the grown cells, and the mixture was shaken for another 48 h (second incubation). Extraction of the broth, followed by treatment with diazomethane and subsequent purification of the product, gave the methyl ester of the starting acid. The yield and enantiomeric excess were determined to be 81% and 69% (*R*), respectively. Elongation of the time of the second incubation resulted in the remarkable decrease of the yield and enantiomeric excess of the product.

There are two possible paths for the asymmetrization of the substrates. One is deracemization of the substrate via some mechanism and the other is the enantioselective degradation of the (*S*)-enantiomer. The latter is supposed to be a minor path, if at all, based on the yield and the ee mentioned above. To clarify, if the enantioselective degradation path was actually working, optically active 2-phenylpropanoic acid was subjected to the reaction (Table 1). Regardless of the configuration of the starting material (88% *R* or 93% *S*), the recovery of the product was around 70% and the configuration was *R* (72% ee from *R* and 52% ee from *S*-starting acid). These results indicate that the enantioselective degradation process is very unlikely in this system. In addition, when (±)-2-deuterio-2-phenylpropanoic acid (D content 98%) was incubated with the whole cells of

(3) Rhys-Williams, W.; McCarthy, F.; Backer, J.; Hung, Y.-F.; Thomason, M. J.; Lloyd, A. W.; Hanlon, G. W. *Enzyme Microb. Technol.* **1998**, *22*, 281−287 and reference cited therein.

(4) (a) Wu, S.-H.; Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 2, 1990−1995. (b) Cambou, B.; Klibanov, A. M. *Biotechnol. Bioeng.* **1984**, *XXVI*, 1449−1454. (c) Liu, Y.-Y.; Xu, J.-H.; Hu, Y. *J. Mol. Catal. B: Enzymol.* **2000**, *10*, 523−529.

(5) Colton, I. J.; Ahmed, S. N.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 212−217.

(6) (a) Knihinicki, R. D.; Day, R. O.; Williams, K. M. *Biochem. Pharmacol.* **1991**, *42*, 1905−1911. (b) Knights, K.; Talbot, U. M.; Baillie, T. A. *Biochem. Pharmacol.* **1992**, *44*, 2415−2417. (c) Menzel, S.; Waibel, R.; Brune, K.; Geisslinger, G. *Biochem. Pharmacol.* **1994**, *48*, 1056−1058.

(7) (a) Shieh, W.-R.; Chen, C.-S. *J. Biol. Chem.* **1993**, *268*, 3487−3493. (b) Brugger, R.; Alía, B. G.; Reichel, C.; Waibel, R.; Menzel, S.; Brune, K.; Geisslinger, G. *Biochem. Pharmacol.* **1996**, *52*, 1007−1013. (c) Reichel, C.; Brugger, R.; Bang, H.; Geisslinger, G.; Brune, K. *Mol. Pharmacol.* **1997**, *51*, 576−582.

(8) Rhys-Williams, W.; Thomason, M. J.; Hung, Y.-F.; Hanolon, G. W.; Lloyd, A. W. *Chirality* **1998**, *10*, 528−534.

(9) Rhys-Williams, W.; Thomason, M. J.; Lloyd, A. W.; Hanlon, G. W. *Pharm. Sci.* **1996**, *2*, 537−540.

(10) The strain used in this experiment is available from the Japan Collection of Microorganism: The Institute of Physical and Chemical Research (Riken), 2-1 Hirosawa, Wako 351-0106, Japan.

N. diaphanozonaria, the D content of the product decreased to 20%. Clearly the reaction path includes C−D bond fission. On the other hand, (±)-3,3,3-trideuteriophenylpropanoic acid was recovered as its optically active form without losing any deuterium atom. This fact indicates that no C−D bond fission occurred on the methyl group, such as formation of methylene followed by reduction to the methyl group. Thus, the most probable mechanism is the formation of enolate-type intermediate as proposed by W-.R. Shieh et al.[7a] The direct binding of the phenyl group to the α-position will facilitate the formation of such an intermediate.

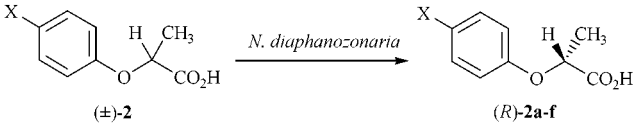
Introduction of a fluorine atom on the *p*-position is expected to stabilize the enolate-type intermediate by its electronegative effect and accelerate the reaction. However, with 2-(4-fluorophenyl)propanoic acid, the apparent chiral inversion process did not occur to any significant extent (entry 6, Table 1). This fact shows that this enzyme system is affected dramatically by the electronic effect of the substituent on the aromatic ring. Unfortunately, other *p*-substituted derivatives that have a chlorine atom or a methoxy group did not appear to undergo the deracemization reaction.

The enzyme system accepted a compound that has a fluorine atom on the α-carbon instead of a methyl group. α-Fluorophenylacetic acid underwent the deracemization reaction to give the corresponding optically active form (entry 7, Table 1). On the other hand, the enzyme system was shown to be inactive to 2-phenylbutanoic acid, probably because of the steric bulkiness of the α-substituent.

In the second group of substrates, we used 2-phenoxypropanoic acid derivatives.[11] (*R*)-2-Phenoxypropanoic acid derivatives are utilized as herbicides, while the (*S*)-antipodes are inactive. In addition, (*R*)-2-(4-chlorophenoxy)propanoic acid lowers the level of serum cholesterol and prevents platelet aggregation.[5] As the (*S*)-antipode inhibits the chloride channel in muscles, it causes side effects, such as muscle irritability and spasms.

As these type of compounds have an oxygen atom between the aromatic ring and the asymmetric center, we presumed that it was unfavorable for the reaction. To our surprise, however, the deracemization reaction of 2-phenoxypropanoic acid (entry 1 to 4, Table 2) cleanly proceeded, and enantiomeric excess of the product reached up to 97% after a 72 h incubation. Moreover, judging from the sign of optical rotation, the absolute configuration of the product was revealed to be *R*.[12] This means that the spacial arrangement of the ligands around the asymmetric center was opposite to that of 2-phenylpropanoic acid. The mode of the interaction between the enzyme and the substrates is not clear at present, but it is very interesting that insertion of only one atom brought about a dramatic change in enantioselectivity.

Table 2. Deracemization of 2-Phenoxypropanoic Acids by the Aid of the Enzyme System of *N. diaphanozonaria*



(±)-**2** (*R*)-**2a-f**

a: X= H; b: X= F; c: X= Cl
d: X= Br; e: X= I; f: X= CF_3

entry	product[a]	X	reactn time (h)	yield[b] (%)	$[\alpha]_D$[c]	ee[d] (%)
1	**2a**	H	36	72		68
2	**2a**	H	48	75	+41.0	75
3	**2a**	H	60	66		91
4	**2a**	H	72	71	+41.2	97
5	**2b**	F	48	64	+52.0	96
6	**2c**	Cl	12	75		92
7	**2c**	Cl	48	95	+44.5	97
8	**2d**	Br	48	83	+47.5	99
9	**2e**	I	48	76	+43.4	97
10	**2f**	CF_3	48	64	+43.3	99

[a] The corresponding racemic substrates were incubated with grown cells of *N. diaphanozonaria* at 30 °C. [b] Isolated yield after conversion to the corresponding methyl ester by treatment with diazomethane. [c] Optical rotation was measured in EtOH, $c = 0.71-1.05$, except for entry 4 in which $CHCl_3$ was used: $c = 0.58$, after conversion to the corresponding methyl ester. [d] ee (%) of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.

In this case, introduction of an electronegative substituent on the aromatic ring had no negative effect on the reaction, in contrast to the case of 2-phenylpropanoic acid (entries 5−10, Table 2). As seen in entry 7, 2-(4-chlorophenoxy)-propanoic acid deracemized smoothly, with the yield and ee of the product being as high as 95% and 97% (*R*), respectively.

In conclusion, we found an enzyme system that gives (*R*)-2-phenoxypropanoic acid, which is the pharmacologically active enantiomer, via a novel deracemization reaction. This process can be performed under mild reaction conditions using an extremely simple procedure and gives good to high yield and enantiomeric excess. Further investigation on the scope and limitation of the reaction, as well as the mechanistic study, is now underway.

Supporting Information Available: Synthetic details and spectroscopic data for a representative product. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0170556

(11) (a) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836−6841. (b) Bewick, D. W. Eur. Pat. Appl. EP 133,033 (*Chem. Abstr.* **1985**, *102*, 165249a). (c) Bewick, D. W. Eur. Pat. Appl. EP 133,034 (*Chem. Abstr.* **1985**, *102*, 165251v).

(12) Chordia, M. D.; Harman, W. D. *J. Am. Chem. Soc* **2000**, *122*, 2725−2736.