Tetrahedron: Asymmetry Vol. 4, No. 6, pp. 1081-1084, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

Asymmetric Hydrolysis of a Disubstituted Malononitrile by the Aid of a Microorganism

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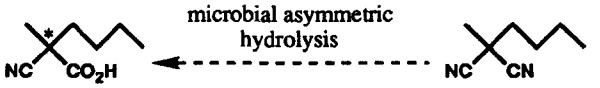
(*Received* 11 *February* 1993)

Abstract: *Rhodococcus rhodochrous* ATCC 21197 hydrolyzed prochiral butylmethylmalononitrile to afford the corresponding amide-carboxylic acid with high enantiomeric excess. The reaction proceeds *via* the hydration of the starting dinitrile by a nitrile hydratase and the subsequent enantioselective hydrolysis of the intermediate diamide by an amidase.

Enzymatic hydrolysis of nitriles to the corresponding amides and carboxylic acids has found increasing interest in recent years, some examples demonstrating potential utility of the reaction in organic synthesis being reported.[1] The mild reaction conditions permit selective functional group transformation even in the presence of an acid- or base-labile moieties. Moreover, the reaction is often characterized by remarkable stereoselectivity which can not be realized by usual chemical hydrolysis.

Enzyme-catalyzed enantioselective conversion of racemic and prochiral nitriles would be an efficient method for preparing optically active carboxylic acid derivatives, which may serve as versatile intermediates in asymmetric synthesis. This idea has been exemplified by several groups: we have recently shown that an enzyme system of *Rhodococcus rhodochrous* ATCC 21197[2] is effective for the kinetic resolution of racemic α-arylpropionitriles[3] and asymmetric hydrolysis of prochiral β-substituted glutaronitriles.[4] In the latter case, pro-(*S*) cyano group of the dinitriles is preferentially hydrolyzed, resulting in the formation of the corresponding cyano-carboxylic acids. Independently Turner *et al.* have reported the enantioselective hydrolysis of nitriles by using an immobilized whole cell system derived from a *Rhodococcus* sp.[5] Enzymatic production of optically active α-hydroxy acids[6] and α-amino acids[7] from the corresponding racemic nitriles have also been reported. For a further investigation of the microbial transformation of prochiral dinitriles, our attention was then directed toward the hydrolysis of disubstituted malononitriles.

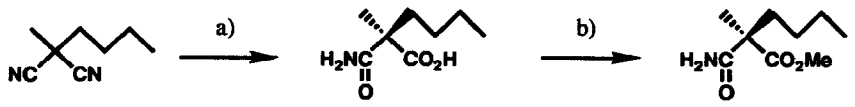
Optically active 2-cyano-2-methylhexanoic acid **1** is a starting material used in the synthesis of a class of ferroelectic liquid crystals, and has been obtained *via* an optical resolution.[8] Based on the results of our previous study,[4] we expected that *R. rhodochrous* would hydrolyze butylmethylmalononitrile **2** to afford the cyano-carboxylic acid **1** directly. Thus the malononitrile **2** was employed as the substrate of this microbial reaction.



1 **2**

When the malononitrile **2** was incubated with the grown cells of *R. rhodochrous*[3] (0.5%w/v, pH 6.0, 30°C, 1 day), the substrate was smoothly consumed. To our surprise, the formation of the expected cyano-carboxylic acid **1** was not detected, and optically active 2-carbamoyl-2-methylhexanoic acid **3**[9] was exclusively obtained.

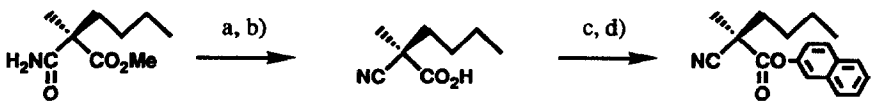
The crude acid **3** was esterified with diazomethane to give methyl ester **4** ($[\alpha]^{20}_D$ −15.6 (c 1.0, $CHCl_3$)) in 92% yield from the dinitrile **2** (**Scheme 1**). The enantiomeric excess of the hydrolysis product **3** was determined to be 96% as follows. The amide-ester **4** was converted chemically into the cyano-carboxylic acid **1** ($[\alpha]^{19}_D$ +8.6 (c 2.0, MeOH)) without changing the optical purity. Subsequently, its *e.e.* was estimated by chiral HPLC analysis of the corresponding β-naphthyl ester **5**[10] (**Scheme 2**).



2 (*R*)–**3** (*R*)–**4**

a) *Rhodococcus rhodochrous*, pH 6, 30°C, 1 day; b) CH_2N_2 / Et_2O (92%, 2 steps)

Scheme 1

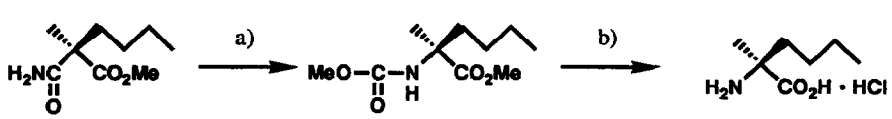


(*R*)–**4** (*R*)–**1** **5**

a) P_2O_5 / toluene; b) KOH / H_2O–EtOH (80%, 2 steps); c) $SOCl_2$ / toluene; d) β–naphthol, pyridine / Et_2O (81%, 2 steps)

Scheme 2

The next task was the determination of absolute configuration. Thus amide-ester **4** was subjected to the Hofmann rearrangement in methanolic sodium hypobromite solution to afford optically active carbamate **6** ($[\alpha]^{21}_D$ +15.5 (c 1.1, $CHCl_3$)) in good yield (**Scheme 3**). It has already been established that the Hofmann rearrangement of amides with a quaternary asymmetric carbon atom at α position proceeds with nearly complete retention of configuration.[11] Hydrolysis of the carbamate **6** then furnished optically active α-methylnorleucine hydrochloride **7**.[12] Comparison of its (+) plain ORD curve[13] with the reported one[14] showed that the amino acid **7** had (*S*)-configuration, which revealed the absolute configuration of the amide-carboxylic acid **3** to be (*R*). The efficient conversion of **4** to **7** (78%) indicates that the present microbial hydrolysis would add a new entry for preparation of optically active α,α-disubstituted α-amino acids, some of which are important for their biological and pharmaceutical properties.



(*R*)–**4** (*S*)–**6** (*S*)–**7**

a) Br_2, MeONa / MeOH (93%); b) conc. HCl (84%)

Scheme 3

Nitrile-hydrolyzing microorganisms generally have three enzymes concerned in the conversion of nitriles, *i.e.*, nitrile hydratase (RCN to $RCONH_2$), amidase ($RCONH_2$ to RCO_2H) and nitrilase (RCN to RCO_2H).[15] The fact that *R. rhodochrous* hydrolyzed both cyano groups of the malononitrile **2** to give two different functionalities was very interesting, since it indicated that plural enzymes should be participated in the total reaction. To clarify the conversion pathway of nitrile **2**, we next studied the time course of the hydrolysis.

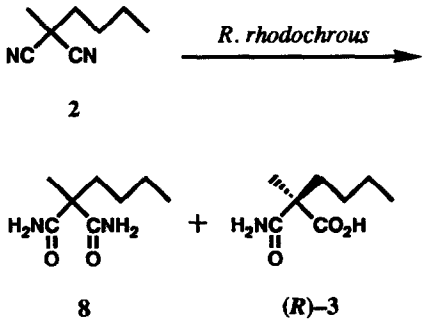
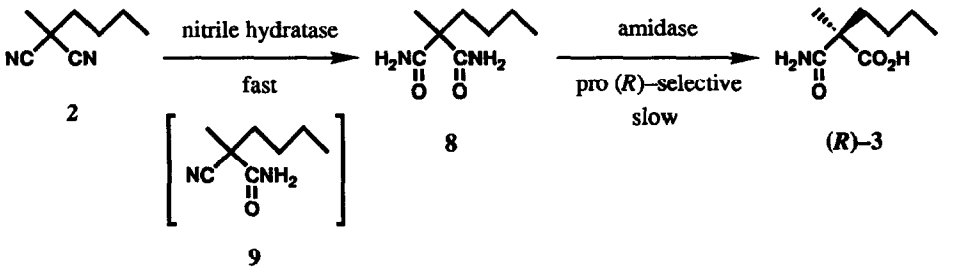


Table 1. Time course of the hydrolysis of **2**[a]

	Yield / %	
Time / hr	**8**	**3**[b]
0.5	64	26
1.0	51	43
2.0	25	71
4.0	5	94

a) substrate concentration: 0.1%w/v
b) determined as methyl ester **4**

The observed time course of the reaction is given in **Table 1**. The dinitrile **2** was completely consumed within half an hour to afford the corresponding diamide **8** as well as amide-carboxylic acid **3**, and the proportion of **3** gradually increased. This result suggests the conversion pathway as shown in **Scheme 4**. In the initial step, malononitrile **2** is rapidly converted to prochiral diamide **8** by the nitrile hydratase. This conversion must proceed *via* formation of cyano-amide **9**, although we failed to detect it. The intermediate diamide **8** is subsequently hydrolyzed slowly to the monoacid **3** by the pro-(*R*) enantiotopic group selective amidase. When the diamide **8** was subjected to the reaction, the nearly same result was obtained ((*R*)-**3** with 97% *e.e.*). This result strongly supports that the observed high enantioselectivity is solely due to the action of the amidase.



Scheme 4

The non-enantioselective hydration in the initial step was further confirmed by the following experiment. We chose racemic cyano-amide **9** as the substrate to know whether the nitrile hydratase would discriminate between the cyano group in (*R*)-**9** and that in (*S*)-**9**. Actually, when **9** was subjected to the microbial reaction

(0.5%w/v, 10 min), both (*R*)-**9** and (*S*)-**9** were converted to diamide **8** (82%) without any recovery of the substrate. This result supported lack of enantioselectivity in the initial step.

In summary, *R. rhodochrous* ATCC 21197 hydrolyzed prochiral malononitrile **2** to the corresponding amide-carboxylic acid (*R*)-**3** with 96% *e.e.* The whole reaction consists of sequential conversion of the dinitrile by an enzyme system; the initial non-selective conversion to the diamide and the subsequent enantioselective hydrolysis to the optically active product. Further study on this microbial transformation, including its application to the synthesis of optically active α-methyl-α-amino acids, will be published in due course.

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9. An analytical sample of **3** was obtained by recrystallization from hexane–diethyl ether. M.p. 90°C, $[\alpha]^{26}_{D}$ –9.8° (c 1.0, $CHCl_3$).
10. Conditions for HPLC analysis; column, CHIRALCEL OJ (Daicel Chemical Industries, Ltd.); mobile phase, hexane/2–propanol=9/1; flow rate, 0.7 mL/min; retention time, 25 min (*S*) and 32 min (*R*).
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12. The *e.e.* of **7** was determined to be >99% by chiral HPLC analysis of *N*-benzyloxycarbonyl-*O*-methyl derivative (CHIRALCEL OJ, hexane/2–propanol=180/1, 0.5 mL/min).
13. Selected values of $[\alpha]^{23}$ (c 2.0, 5*N* HCl); $[\alpha]_{300}$ +65 , $[\alpha]_{365}$ +35 , $[\alpha]_{400}$ +27 $[\alpha]_{435}$ +22 , $[\alpha]_{500}$ +15 , $[\alpha]_{D}$ +10 .
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