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ASYMMETRY

Rhodococcus rhodochrous IFO 15564-mediated hydrolysis of alicyclic nitriles and amides: stereoselectivity and use for kinetic resolution and asymmetrization

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Abstract

The stereocourse and the selectivity of the hydrolysis of alicyclic mono- and dinitriles and amides mediated by *Rhodococcus rhodochrous* IFO 15564 has been examined. The stereochemistry of the substrates, as well as the nature of substituents and presence of double bonds in alicyclic rings greatly affected the rate of hydrolysis by nitrile hydratase and amidase. The rate difference between enantiomers or enantiotopic groups, in some cases, enabled kinetic resolution or asymmetrization. The highest enantioselectivity of amidase was observed in the hydrolysis of 5-benzoyloxy-3-cyclohexene-1-carboxamide (E>200), and both enantiomers of methyl 5-hydroxy-3-cyclohexene-1-carboxylate thus became readily available. © 1998 Elsevier Science Ltd. All rights reserved.

The importance of nitriles in synthetic organic chemistry prompted the elaboration of functional group transformation under mild conditions, *i.e.*, biocatalytic hydrolysis.[1] Many attempts have been devoted to clarify the regio- and stereoselectivity for aromatic substrates, however, few reports of alicyclic substrates have appeared so far.[2] We became interested in the hydrolysis of alicyclic mono- and dinitriles and amides mediated by *Rhodococcus rhodochrous* IFO 15564,[3] and the stereocourse and the selectivity on the substrates was examined.

Table 1 summarizes our results and some comments on them are described below. Firstly, the detection of amides clearly supports that the hydrolysis by this microorganism proceeds in two steps; the hydration by nitrile hydratase and the subsequent hydrolysis of the intermediate by amidase. With regard to the first step, the hydration of cyano groups in the substrates **1** (entry 1), **9** (entry 5), and **14** (entry 7) with a C=C double bond was faster than the corresponding saturated ones **3** (entry 2), **12** (entry 6), **17** (entry 9), respectively, and these results suggested a certain interaction between double bonds and nitrile hydratase. This effect, however, became smaller, between **5** (entry 3) and **7** (entry 4), and between **19** (entry 10) and **24** (entry 15).

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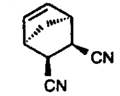
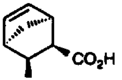
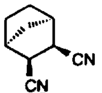
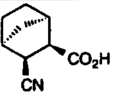
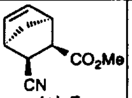
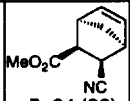
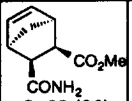
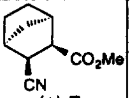
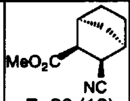
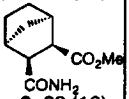
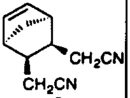
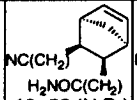
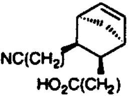
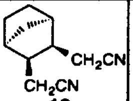
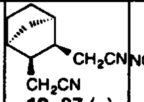
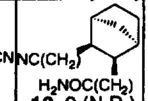
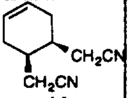
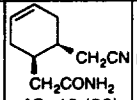
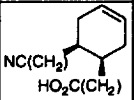
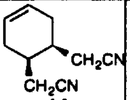
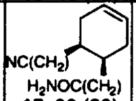
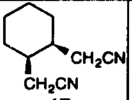
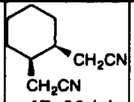
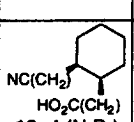
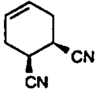
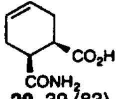
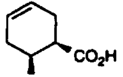
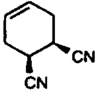
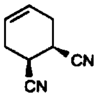
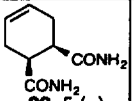
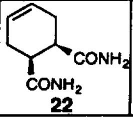
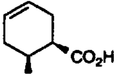
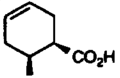
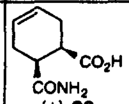
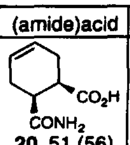
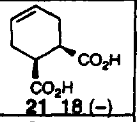
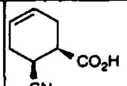
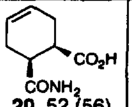
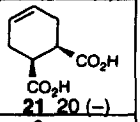
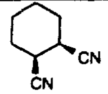
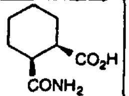
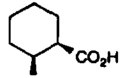
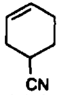
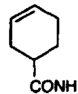
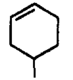
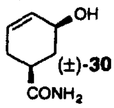
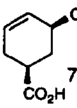
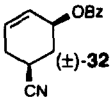
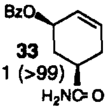
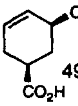
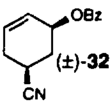
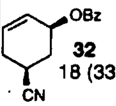
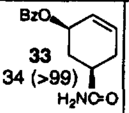
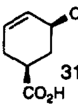
Table 1.

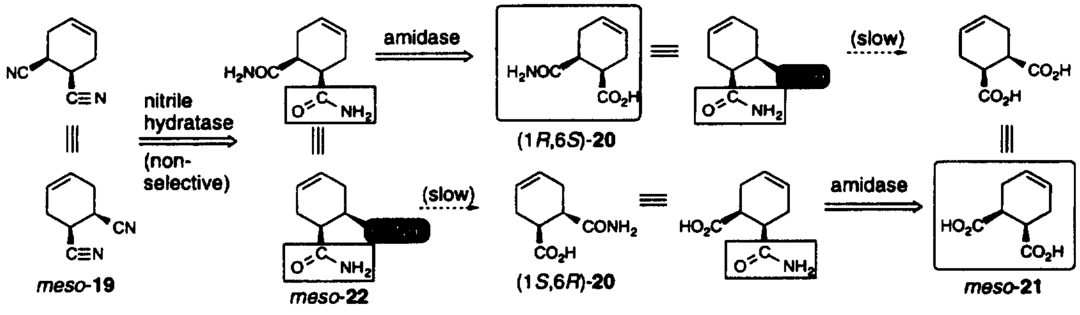
entry	substrate	condi-tion	product: yield / % (*e.e.* / %)					
			recovery	(cyano)amide	(cyano)acid	diamide	(amide)acid	diacid
1	 **1**	a	—	—	—	—	—	 **2** 87 (–)
2	 **3**	a	—	—	 **4** 76 (74)	—	—	—
3	 (±)-**5**	b	 **5** 24 (36)	 **6** 28 (36)	—	—	—	—
4	 (±)-**7**	b	 **7** 36 (18)	 **8** 28 (16)	—	—	—	—
5	 **9**	a	—	 **10** 55 (N.D.)	 **11** 4 (N.D.)	—	—	—
6	 **12**	a	 **12** 87 (–)	 **13** 9 (N.D.)	—	—	—	—
7	 **14**	a	—	 **15** 46 (36)	 **16** 35 (95)	—	—	—
8	 **14**	a(d)	—	 **15** 99 (30)	—	—	—	—
9	 **17**	a	 **17** 66 (–)	—	 **18** 4 (N.D.)	—	—	—
10	 **19**	a	—	—	—	—	 **20** 39 (83)	 **21** 33 (–)
11	 **19**	a(d)	 **19** 82 (–)	—	—	 **22** 5 (–)	—	—
12	 **22**	a	—	—	—	—	 **20** 39 (97)	 **21** 39 (–)

entry	substrate	condi-tion	product: yield / % (*e.e.* / %)					
			recovery	(cyano)amide	(cyano)acid	diamide	(amide)acid	diacid
13	 (±)-**20**	a	—	—	—	—	 **20** 51 (56)	 **21** 18 (–)
14	 (±)-**23**	a	—	—	—	—	 **20** 52 (56)	 **21** 20 (–)
15	 **24**	a	—	—	—	—	 **25** 22 (95)	 **26** 24 (–)
16	 (±)-**27**	c	—	 **28** 20 (N.D.)	 **29** 72 (2)	—	—	—
17	 (±)-**30**	c	—	—	 **31** 74 (0)	—	—	—
18	 (±)-**32**	b(e)	—	 **33** 41 (>99)	 **34** 49 (72)	—	—	—
19	 (±)-**32**	c(e)	 **32** 18 (33)	 **33** 34 (>99)	 **34** 31 (97)	—	—	—

For incubation condition, see ref. 3. **a**: sub. conc. 0.1%, incub. time 4.5-24h; **b**: pre-incubated with $FeSO_4$ (0.1%), sub. conc. 0.1%, incub. time 2-8h; **c**: pre-incubated with $FeSO_4$ (0.1%), sub. conc. 0.5%, incub. time 1.5-8h; **d**: incub. time 10-30 min; **e**: EtOH (10%) was added as co-solvent. For spectral data, determination of the absolute configuration, and *e.e.* of the products, see ref 5–7.

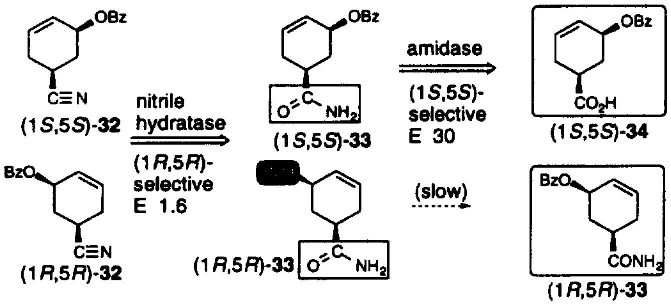
Incubation of *meso*-dinitrile **19** (entry 10) afforded enantiomerically enriched amide-carboxylate **20** and *meso*-dicarboxylate **21**. Starting from *meso*-diamide **22** (entry 12), which was revealed as the intermediate by a shorter-period incubation (entry 11), the same result was obtained. From these results, the hydrolysis of the amide worked in an enantioselective manner. This was further supported by an independent experiment of the kinetic resolution of (±)-**20** (entry 13). In contrast, as the incubation of (±)-**23** (entry 14), a potential precursor of (±)-**20** afforded almost the same result compared with that from (±)-**20**, nitrile hydratase had nearly no enantioselectivity. Indeed, nitrile hydratase of this microorganism showed rather low enantioselectivity to the series of the substrates in this study (E=1.0 to 6.7).[4]

Both an unsaturated (**19**, entry 10) and a saturated (**24**, entry 15) dinitrile were hydrolyzed in a similar manner. Thus, in contrast to nitrile hydratase, the rate of hydrolysis by amidase was greatly affected by the nature and position of substituents on alicyclic rings, rather than the double bonds. The carbamoyl and carboxyl group placed next to (α) the amide group, which is susceptible to hydrolysis, in a counter-clockwise orientation in *meso*-**22** and (1*R*,6*S*)-**20**, retards the hydrolysis of the amide group by amidase (Scheme 1). The result brought about the accumulation of (1*R*,6*S*)-**20** and *meso*-**21**. By protecting carboxylic acids as methoxycarbonyl groups, such an effect was more strongly expressed and the hydrolysis with amidase did not proceed (**5** and **7**, in entry 3 and 4).



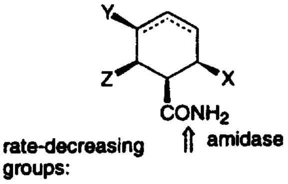
Scheme 1.

We then became interested in the effect of the substituent at the β-position. Smaller substituents such as hydroxyl groups (**30**, entry 17) showed no effect. To our surprise, an esterification with a benzoyl group (**32**, entry 18) dramatically changed the situation. The introduction of a bulky and lipophilic group[8] at the β-position in a clockwise orientation was found to have a retarding effect as in Scheme 2. In this case, nitrile hydratase showed a slight preference for the (1*R*,5*R*)-enantiomer (E 1.6), while amidase had a reverse selectivity:[9] (1*S*,5*S*)-**33** was hydrolyzed with a high selectivity (E 30). Figure 1 summarizes the rate-decreasing substituents on the cyclohex(a/e)ne ring to amidase, which should elucidate the stereostructure of the active site of amidase (*cf.* Deigner et al.).[10]



Scheme 2.

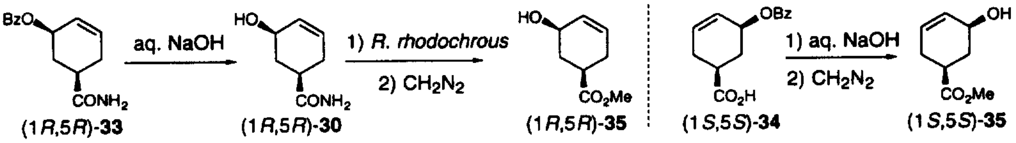
When the substrate concentration of **32** was raised to 0.5% (entry 18 versus 19), the reaction became slow and the recovery of the starting material (1*S*,5*S*)-**32** (33% *e.e.*) as well as (1*S*,5*S*)-acid **34** with an enhanced *e.e.* (97%) was achieved. This result is explained if an enantioselective inhibition caused by the final products brought about an enhancement of the apparent E value (>200) of the amidase. By taking advantage of the high enantioselectivity, a preparative method for the both enantiomers of methyl



$X = CONH_2, CO_2H, CO_2Me$
$Y = OBz$
$Z = CO_2Me$

Fig. 1.

5-hydroxy-3-cyclohexene-1-carboxylate **35**[11] was developed as in Scheme 3.[12,13] Alkaline treatment of (1*R*,5*R*)-**33** and (1*S*,5*S*)-**34** led to deprotection of the benzoyloxy group, and (1*R*,5*R*)-**30** and (1*S*,5*S*)-**35** (as free carboxylic acid) were produced respectively. Due to a beneficial loss of enantioselectivity by substituting the benzoyloxy group for the hydroxyl group, the hydrolysis of amide in (1*R*,5*R*)-**30** proceeded very smoothly under mild conditions (entry 17). In this manner, the enantiomer (1*R*,5*R*)-**35** could be obtained in a highly enantiomerically enriched state (97% *e.e.*).



Scheme 3.

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4. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299. In the case of *meso*-substrates, the *e.e.* of the products is considered to reflect the relative reaction rate between enantiotopic groups.
5. A methyl ester of (1*R*,6*S*)-**20**: m.p. 115–116°C; $[\alpha]_D^{20}$ −6.0 (c=2.22, $CHCl_3$); IR (nujol) vmax 3350, 1720, 1660, 1610, 1200, 1030 cm^{-1}; ^1H NMR δ 2.1–3.3 (m, 6H), 3.70 (s, 3H), 5.6–5.9 (m, 2H), 6.0 (br., 2H); found: C, 58.60; H, 6.77; N, 7.54. Calc. for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.65%. HPLC analysis of the corresponding benzyl ester: Chiralcel OJ, hexane:*i*-PrOH (9:1); 0.3 mL/min, t_R (min): 74.4 (1*S*,6*R*), 81.0 (1*R*,6*S*). The absolute configuration was confirmed by preparing an authentic sample from a known half ester (1*R*,6*S*)-**36**: see Kobayashi, S.; Kamiyama, K.; Iimori, T.; Ohno, M. *Tetrahedron Lett.* **1984**, *25*, 2557–2560.
6. Entry 2: HPLC of **37**, Chiralcel OJ, hexane–*i*-PrOH (50:1); 0.5 mL/min, t_R (min): 43.4 (1*S*,2*R*,3*S*,4*R*), 48.2 (1*R*,2*S*,3*R*,4*S*). See Murata, M.; Uchida, H.; Achiwa, K. *Chem. Pharm. Bull.* **1992**, *40*, 2610–2613. Entry 3: the absolute configuration and *e.e.* was related to that in entry 2 by hydrogenation of the product. Entry 4: the absolute configuration and *e.e.* was related to that in entry 2. Entry 7: HPLC of **38**, Develosil, $CHCl_3$:EtOAc (25:1); 0.55 mL/min, t_R (min): 12.1 (1*S*,6*R*), 14.0 (1*R*,6*S*). See Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. *J. Org. Chem.* **1986**, *51*, 2391–2393. Nagao, Y.; Kume, M.; Wakabayashi, R. C.; Nakamura, T.; Ochiai, M. *Chem. Lett.* **1989**, 239–242. Entry 9: HPLC of **39**, Develosil, $CHCl_3$:EtOAc (25:1); 0.55 mL/min, t_R (min): 12.8 (1*S*,2*R*), 14.0 (1*R*,2*S*). Entry 15: HPLC analysis of the corresponding benzyl ester, Chiralcel OJ, hexane:*i*-PrOH (50:1); 1.0 mL/min, t_R (min): 15.3 (1*S*,2*R*), 17.4 (1*R*,2*S*). The absolute configuration was related to that in entries 10–14 by hydrogenation of the product (Fig. 2).
7. (1*S*,5*S*)-**32**: $[\alpha]_D^{22}$ −30.5 (c=0.93, $CHCl_3$), Chiralcel OJ, hexane:*i*-PrOH (9:1); 0.5 mL/min, t_R (min): 48.3 (1*R*,5*R*), 64.4 (1*S*,5*S*). An authentic (1*R*,5*R*)-**32** from (1*R*,5*R*)-**33**: $[\alpha]_D^{22}$ +112.8 (c=0.90, $CHCl_3$). (1*R*,5*R*)-**33**: m.p. 159.0–159.5°C; $[\alpha]_D^{22}$ +73.3 (c=0.94, $CHCl_3$); IR (KBr) ν_{max} 3440, 1710, 1660, 1620, 1600, 1450, 1320, 1285, 1275, 1250, 715 cm^{-1}; ^1H NMR δ 1.94 (dt, J=9.5, 12.5 Hz, 1H), 2.28–2.47 (m, 3H), 2.65–2.73 (m, 1H), 5.66–5.79 (m, 3H), 5.76–5.79 (m, 1H), 5.91–5.96 (m, 1H), 7.41–7.45 (m, 2H), 7.53–7.57 (m, 1H), 8.02–8.05 (m, 2H); found: C, 68.75; H, 6.28; N, 5.79. Calc. for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71%. HPLC: Chiralcel OJ, hexane:*i*-PrOH (5:1); 0.5 mL/min, t_R (min): 19.1 (1*R*,5*R*), 22.4 (1*S*,5*S*). A methyl ester of (1*S*,5*S*)-**34**: $[\alpha]_D^{22}$ −86.4 (c=1.25, $CHCl_3$); IR (film) ν_{max} 3030, 2950, 1720, 1595, 1580, 1450, 1270, 715 cm^{-1}; ^1H NMR δ 1.96 (ddd, J=8.9, 11.9, 12.5 Hz, 1H), 2.34–2.53 (m, 3H), 2.76–2.87 (m, 1H), 3.67 (s, 3H), 5.61–5.68 (m, 1H), 5.75–5.81 (m, 1H), 5.91–5.98 (m, 1H), 7.40–7.56 (m, 3H), 8.02–8.06 (m, 2H); found: C, 69.00;

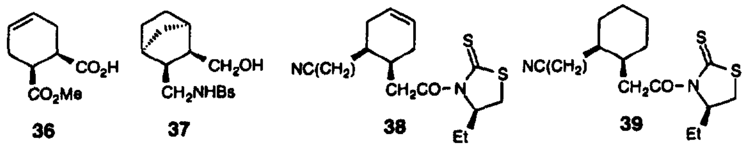

Fig. 2.

H, 6.31. Calc. for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20%. HPLC: Chiralcel OJ, hexane:*i*-PrOH (50:1); 0.5 mL/min, t_R (min): 25.8 (1*R*,5*R*), 31.7 (1*S*,5*S*). The absolute configurations of **33** and **34** were determined at the stage of **35**, respectively.

8. The hydrolysis had worked well with the substrates bearing benzoyloxy groups: Kakeya, H.; Sakai, N.; Sano, A.; Yokoyama, M.; Sugai, T.; Ohta, H. *Chem. Lett.* **1991**, 1823–1824.; Yokoyama, M.; Imai, N.; Sugai, T.; Ohta, H. *J. Mol. Catal. Sect. B enzymatic* **1996**, *1*, 135–141. In a certain case, however, the hydrolysis became very slow by introducing a too bulky protecting group such as TBDMS: Sugai, T.; Katoh, O.; Ohta, H. *Tetrahedron* **1995**, *51*, 11987–11988.
9. The preference of same enantiomer and/or enantiotopic group by nitrile hydratase and amidase was observed in entry 2, 7 and 9. Related example: Beard, T.; Cohen, M. A.; Parratt, J. S.; Turner, N. J.; Crosby, J.; Moilliet, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1085–1104.
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12. Marshall, J. A.; Xie, S. *J. Org. Chem.* **1995**, *60*, 7230–7237.
13. (1*R*,5*R*)-**35**: $[\alpha]_D^{22}$ −5.9 (*c*=0.39, $CHCl_3$) [Ref.[12] $[\alpha]_D^{24}$ −4.6 (*c*=2.25, $CHCl_3$)]; IR (film) ν_{max} 3430, 3040, 2960, 1735, 1440, 1380, 1205, 1175, 1050, 1005, 920 cm^{-1}; ^1H NMR δ 1.69–1.80 (ddd, J=7.9, 10.6, 12.9 Hz, 1H), 2.25–2.33 (m, 3H), 2.67–2.78 (m, 1H), 3.70 (s, 3H), 4.26–4.32 (m, 1H), 5.71–5.81 (m, 2H); ^{13}C NMR δ 175.6, 130.8, 126.8, 65.9, 51.9, 37.7, 34.1, 27.3. The NMR spectra were identical with those reported previously.[12] Found: C, 61.58; H, 7.92. Calc. for $C_8H_{12}O_3$: C, 61.52; H, 7.74%. (1*S*,5*S*)-**35**: $[\alpha]_D^{24}$ +5.5 (*c*=0.45, $CHCl_3$); IR and NMR spectra were identical with those of (1*R*,5*R*)-**35**.