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Microbial Hydrolysis of 3-Substituted Glutaronitriles

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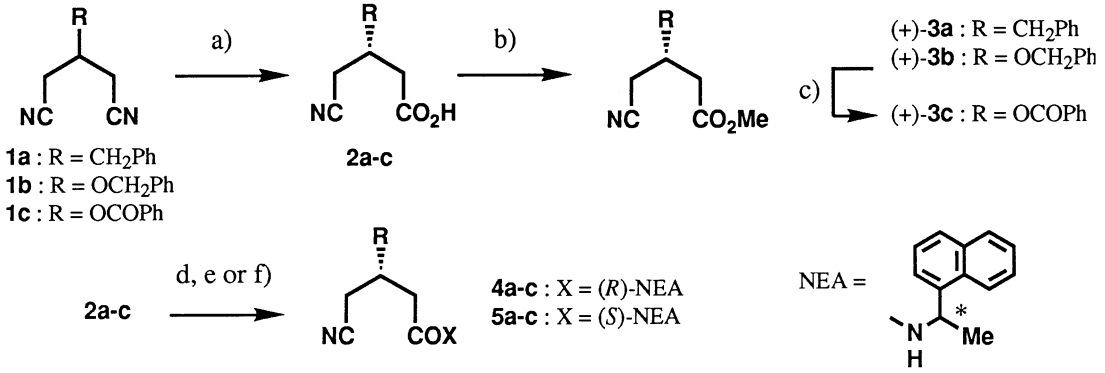
Rhodococcus butanica ATCC 21197 preferentially hydrolyzed pro-S cyano group of 3-substituted glutaronitriles with an aromatic ring. A product with high *e.e.* (>99%) was obtained from 3-benzoyloxy derivative.

Recognition of enantiotopic groups in prochiral substrates is one of the characteristic features of enzymatic reactions. For example, stereoselective hydrolysis of 3-substituted glutarates has been investigated extensively.[1] On the other hand, there have been few reports so far on the transformation of nitrogen-containing functional groups such as amides and nitriles. Very recently, we found an enzyme system of *Rhodococcus butanica* ATCC 21197 to be effective for the enantioselective hydrolysis of racemic 2-arylpropionitrile.[2a] Here we report its extention to 3-substituted glutaronitriles.



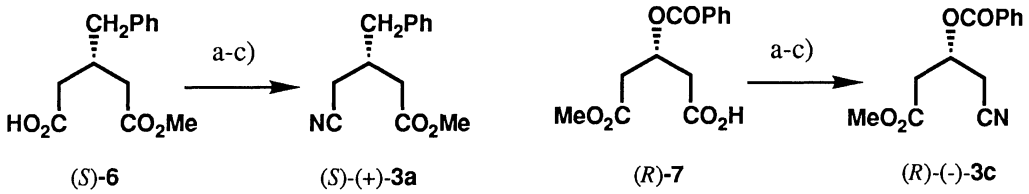
a) *Rhodococcus butanica* ATCC 21197; b) CH_2N_2; c) RuO_4, $NaIO_4/H_2O$-MeCN-CCl_4; d) $SOCl_2$/toluene; e) (*R*)-NEA/Et_2O; f) (*S*)-NEA/Et_2O.

Substrate	Yd. of **2** from **1** /%	Abs. config.	*e.e.* /%
1a	59	*S*	29
1b	68	*S*	90
1c	71	*S*	>99

When 3-benzyl derivative **1a** was incubated with grown cells of *R. butanica*,[2b] smooth hydrolysis (30°C, 6 h) took place to afford monocarboxylic acid **2a** (59%). Its enantiomeric excess (*e.e.*) was determined to be 29% by the NMR analysis[3] of naphthylethylamide derivative[4] **4a** and **5a**. The absolute configuration

was determined to be *S*, by comparison of the optical rotation of methyl ester (+)-**3a**[5] with an authentic sample derived from a halfester (*S*)-**6** whose absolute configuration has been unambiguously determined.[1d]

Since the *e.e.* of the product was rather low, we designed other substrates, expecting more matching with the enzyme. Insertion of an oxygen atom between benzyl and carbon chain (**1b**) brought about an enormous enhancement of the *e.e.* of the product (**2b**) to 90%.[6] It was suggested that the polar functional group such as an ether or an ester moiety affects the selectivity of chiral recognition. The benzoate **1c** whose oxidation level is further enhanced, was therefore subjected to this microorganism. As expected, almost enantiomerically pure product was obtained.[7] The resulting (+)-**3c** was proved to be (*S*)-isomer[8] by the comparison with (*R*)-(-)-**3c** derived from (*R*)-**7** with known absolute configuration.[1e] Furthermore, (+)-**3b** prepared from hydrolysis product **2b** was correlated to (*S*)-(+)-**3c**[9] *via* ruthenium tetraoxide oxidation.[10]



(*S*)-**6** (*S*)-(+)-**3a** (*R*)-**7** (*R*)-(-)-**3c**

a) $SOCl_2$/toluene; b) *aq* NH_3; c) P_2O_5/benzene.

From these results, it is concluded that the microbial hydrolysis prefers pro-*S* cyano group of 3-substituted glutaronitriles with an aromatic ring. Thus, a suitably protected nitrile (*S*)-**2c** of much synthetic utility was efficiently obtained in a high *e.e.*

References

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2) a) H. Kakeya, N. Sakai, T. Sugai, and H. Ohta, *Tetrahedron Lett.*, **32**, 1343 (1991); b) H. Kakeya, N. Sakai, T. Sugai, and H. Ohta, *Agric. Biol. Chem.*, **55**, 1877 (1991).

3) **4a**: δ ($CDCl_3$) 1.66 (1.94H, d, *J*=6.8 Hz), 1.69 (1.06H, d, *J*=6.8 Hz); **5a**: 1.66 (1.06H), 1.69 (1.94H).

4) B. J. Bergot, R. J. Anderson, D. A. Schooley, and C. A. Henrick, *J. Chromatogr.*, **155**, 97 (1978).

5) **3a** from **2a**: $[\alpha]_D^{22}$ +4.7° (MeOH). Authentic (*S*)-(+)-**3a** from (*S*)-**6**,[1d] $[\alpha]_D^{21}$ +7.2° (MeOH).

6) **4b**: δ 4.32 (0.05H, d, *J*=11.2 Hz), 4.49 (0.05H, d, *J*=11.2 Hz), 4.60 (0.95H, d, *J*=11.2 Hz), 4.67 (0.95H, d, *J*=11.2 Hz); **5b**: δ 4.32 (0.95H), 4.49 (0.95H), 4.60 (0.05H), 4.67 (0.05H).

7) **4c**: δ 2.69 (1H, dd, *J*=5.9, 15.1 Hz), 2.82 (1H, dd, *J*=7.1, 15.1 Hz), 2.95 (1H, dd, *J*=4.9, 17.3 Hz), 3.07 (1H, dd, *J*=4.9, 17.3 Hz); **5c**: 2.73 (1H, dd, J=6.4, 14.8 Hz), 2.82 (1H, dd, *J*=6.6, 14.8 Hz), 3.01 (1H, dd, *J*=4.4, 17.1 Hz), 3.08 (1H, dd, *J*=4.4, 17.1 Hz).

8) **3c** from **2c**: $[\alpha]_D^{22}$ +46.6° ($CHCl_3$). Authentic (*R*)-(-)-**3** from (*R*)-**7**,[1e] $[\alpha]_D^{21}$ -18.6° ($CHCl_3$). *R* configuration of **7** was further confirmed by the correlation to (*R*)-3-hydroxyglutaric acid monomethylester.[1c]

9) **3b** from **2b**: $[\alpha]_D^{21}$ +12.0° ($CHCl_3$); **3c** from **3b** (51%): $[\alpha]_D^{19}$ +39.5° ($CHCl_3$).

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