CHEMISTRY LETTERS, pp. 379-380, 1989.

Microbial Synthesis of Optically Pure α-Methoxy-α-trifluoromethyl-α-phenylacetic Acid

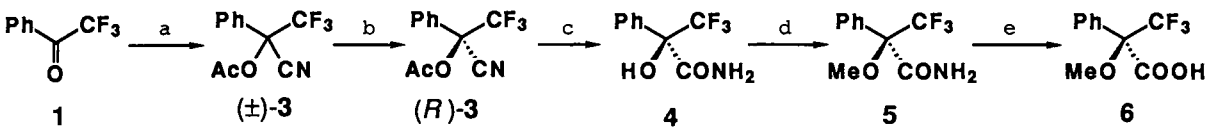
Hiromichi OHTA, Yoshitaka MIYAMAE, and Yoichi KIMURA
Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223

Preparatin of optically pure α-methoxy-α-trifluoromethyl-α-phenylacetic acid via microbial asymmetric hydrolysis of cyanohydrin acetate as the key step is described.

We have already reported that microbial hydrolysis is effective for the preparation of esters of chiral cyanohydrins of aldehydes[1] and ketones.[2] As cyanohydrins can be easily converted to α-hydroxycarboxylic acids, the present method is applied to the preparation of optically active α-methoxy-α-trifluoromethyl-α-phenylacetic acid (MTPA), which is widely used for the determination of optical purities of alcohols and amines.[3] In addition, the interaction of fluorine containing groups with enzyme systems is attracting increasing attentions.[4]

Among microorganisms tested, *Bacillus coagulans*, which was isolated by us (FERM P-9237),[5] was found to hydrolyze 1-cyano-1-phenyl-2,2,2-trifluoroethyl acetate (**3**)[6] in an enantioselective manner. The substrate **3** was prepared starting from 2,2,2-trifluoro-1-phenylethanone (**1**), which in turn was derived to **2** by the reaction with KCN in H_2O-EtOH[3] followed by acetylation. Incubation of (±)-**3** with grown cells of *B. coagulans* in a nutrient medium[7] at 30 °C resulted in the recovery of optically active **3** and the ketone **1** in nearly quantitative yield. As shown in Table 1, optically pure acetate **3** was obtained when the hydrolysis exceeded far more 50%. Two possibilities were supposed for the relatively loose enantioselectivity; essential character of the enzyme and the undesirable effect of cyanide anion. As summarized in Table 2, the addition of potasium cyanide to the incubation medium accelerated the rate of hydrolysis, but lowered the stereoselectivity. Thus, to reduce the effect of cyanide, relatively large amount of liophilyzed dry cells to the substrate were used. As shown in the column 5 of Table 1, incubation of **3** with ten fold weight of dry cells in phosphate buffer (pH 7.2) afforded optically pure **3**[8] in 30% yield, $[\alpha]_D^{25}$ -27° (c 1.1, $CHCl_3$).

Optically active **3** was hydrolyzed by conc H_2SO_4 to afford α-hydroxy-α-tri-



1 (±)-**3** (*R*)-**3** **4** **5** **6**

a. 1) KCN/H_2SO_4 2) Ac_2O/Py b. *B. coagulans* c. H_2SO_4 d. Me_2SO_4/KOH e. NaOH/H_2O

Table 1. Hydrolysis of 1-Cyano-1-phenyl-2,2,2-trifluoroethyl Acetate (3)

Run	Method[a)]	Dry cell/mg	Cult/h	Concn/%	Recovery/%	e.e./%
1	A	-	12	0.2	32	80
2	A	-	24	0.2	9	100
3	A	-	72	0.4	35	36
4	B	500	24	0.4	18	90
5	B	1000	3	0.2	30	100

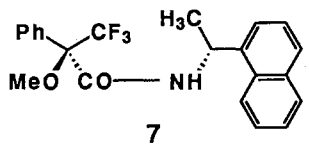
a) Method A: The substrate was added to the suspension of grown cells of *B. coagulans*. Method B: Dry cells of *B. coagulans* and the substrate were incubated in phosphate buffer of pH 7.2.

fluoromethyl-α-phenylacetamide (**4**): Yield 89%; $[\alpha]_D^{25}$ +45° (c 1.9, MeOH). Treatment of **4** with excess Me_2SO_4 and KOH gave methyl ether **5** in a yield of 77%: $[\alpha]_D^{26}$ +41° (c 1.0, MeOH). The final product MTPA (**6**) was obtained by heating **5** in an aqueous solution of KOH under reflux for 5 h, followed by distillation in vacuo: Bp 95-110 °C/ 1.5-2.0 mmHg; Yield, 60%, $[\alpha]_D^{26}$ +69° (c 1.6, MeOH). The specific rotation indicates that **6** has R configuration[9)] of very high optical purity. The enantio excess of **6** was further determined by derivation to (R)-1-(α-naphthyl)ethyl amide **7**. While the amide prepared from (±)-**6** separated into two peaks on HPLC analysis,[10)] the one from chiral **6** showed only one peak, indicating that MTPA obtained through the sequence of reactions is 100% e.e. within the experimental error.

Table 2. Effect of CN^- on the Hydrolysis of 3[a)]

Cult/h	KCN/mg	Recovery of 3/%	e.e./%
12	no	47	56
	100	26	28
24	no	25	80
	100	4	50

a) Medium, 50 ml; substrate 3, 100 mg.



7

References

1) H. Ohta, Y. Miyamae, and G. Tsuchihashi, Agric. Biol. Chem., 50, 3181 (1986); H. Ohta, S. Hiraga, K. Miyamoto, and G. Tsuchihashi, ibid., 52, No. 12 (1988).
2) H. Ohta, Y. Kimura, and Y. Sugano, Tetrahedron Lett., in press.
3) J. A. Dale, D. L. Dull, and H. S. Mosher, J. Org. Chem., 34, 2543 (1969).
4) T. Kitazume and T. Yamazaki, Yuki Gosei Kagaku Kyokai Shi 45, 888 (1987).
5) H. Ohta, Y. Miyamae, and G. Tsuchihashi, Agric. Biol. Chem., 53, No. 1 (1989).
6) All the compounds gave satisfactory IR, ^{1}H-NMR, and mass spectra.
7) The medium consists of glucose (1%), pepton (0.7%), yeast extract (0.5%) and K_2HPO_4 (0.5%) in distilled water, pH 7.2.
8) Determined by HPLC using a chiral column: DAICEL CHIRALCEL, OC (4.6 D x 250 mm); hexane-isopropanol (9:1), 0.3 ml/min; retention time 16.1 and 17.3 min.
9) D. L. Dull, H. S. Mosher, J. Am. Chem. Soc., 89, 4230 (1967); W. H. Pirkle, S. D. Beare, Tetrahedron Lett., 1968, 2579.
10) Zorbax-SIL (4.6 D x 250 mm); hexane-ethyl acetate (9:1), 0.5 ml/min; retention time 21.3 and 23.3 min.

(Received October 20, 1988)