Tetrahedron: Asymmetry Vol. 4, No. 6, pp. 1287-1294, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00

Stereoselectivity in the Microbial Reduction of (Trifluoroacetyl)ferrocene and 2-Fluoroacetophenones

Yoshimitsu Yamazaki* and Harumi Kobayashi

National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan

(*Received* 21 *December* 1992; *accepted* 12 *February* 1993)

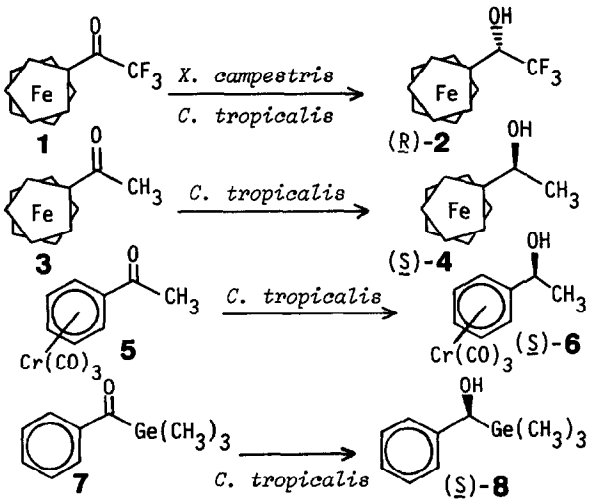
Abstract: Fluorine substitution on the methyl group of acetylferrocene and acetophenone strengthened anti-Prelog-type stereoselectivity in the microbial reduction with 6 strains of yeast (e.g., Rhodotorula rubra IFO 889) and one strain of bacterium. The cell-free extract of R. rubra was fractionated by polyacrylamide gel electrophoresis. Two fractions had prominent activity for NAD(P)H-dependent reduction of (trifluoroacetyl)ferrocene (**1**). One of the two fractions produced preferentially (S)-2,2,2-trifluoro-1-hydroxyethylferrocene (**2**) as following Prelog's rule, while the other produced preferentially (R)-**2** with the anti-Prelog-type stereoselectivity.

INTRODUCTION

The application of enzymes in organic synthesis is expanding into the field of organometallics. Reduction with horse liver alcohol dehydrogenase,[1] baker's yeast,[2] and various species of microorganisms,[3,4] hydrolysis with porcine liver esterase,[5] and hydrolysis or transesterification with lipases[6,7] are mainly used for the enantioselective conversion of functional groups in prochiral or chiral metallocenes[1~3,5,6] and alkyl- or arylsilanes,[7a,c,d] germanes,[4] and stannanes.[7b,e,f] Among the reactions, microbial reduction holds an important position in view of the development of industrial processes.

The previous information about stereoselectivities in microbial reductions of synthetic ketones were summarized as Prelog's rule.[8] Though we recognize the limit of this empirical rule, we are occasionally surprised at the apparently hard-to-understand exceptions.

An organometallic ketone, (trifluoroacetyl)ferrocene (**1**), is more rapidly reduced to the alcohol (**2**) than the non-fluorinated analogue, acetylferrocene (**3**) with baker's yeast.[2b] The fluorine substitution on the methyl group influences not only the increase of reaction rate but also an alteration of the stereoselectivity in the reduction. Xanthomonas campestris reduced **1** to the levorotatory alcohol (**2**) of 98 %e.e.,[3f] whose absolute configuration has been determined to be R by a recent X-ray crystallographic analysis.[9] The reduction of **1** by X. campestris occurred on the si-face of the carbonyl group, and therefore this microbial reduction did not follow Prelog's rule. The same



was found with many species of microorganisms, e.g., Candida tropicalis, Saccharomyces rosei, Saccharomyces sp. H-1, Pseudomonas oleovorans, and Nocardia erythropolis.[3f] These strains reduced, however, acetylferrocene (**3**) preferentially to (S)-1-hydroxyethylferrocene (**4**) and another organometallic ketone, (acetophenone)tricarbonylchromium (**5**), preferentially to (S)-tricarbonyl-(α-methylbenzenemethanol)-chromium (**6**).[3a] In a recent study, C. tropicalis, S. rosei, S. sp. H-1, and Torulopsis magnoliae reduced phenyl trimethylgermyl ketone (**7**) dominantly to (S)-(-)-α-(trimethylgermyl)benzyl alcohol (**8**).[4b] The stereoselectivity in the latter reactions was consistent with Prelog's rule. The abnormal stereoselectivity in the reduction of **1** should be due to the presence of fluorine atom in the substrate molecule.

We had two questions to address how the stereoselectivity changed with changing the number of fluorine atoms and what enzymes in the microorganisms resulted in the anti-Prelog-type reduction for the novel ketone (**1**). This work was started to answer these questions mainly with the yeast R. rubra.

MATERIALS AND METHODS

Chemicals. (Trifluoroacetyl)ferrocene (**1**), 2-fluoro- (**10**), and 2,2-difluoroacetophenone (**11**) were prepared as described previously.[10,11] Acetophenone (**9**), 2,2,2-trifluoro- (**12**) and 2-chloroacetophenone (**13**), and (±)-α-methylbenzyl alcohol (**14**), and (R)-(+)-**14** were purchased from Tokyo Chemical Industries Ltd. (Tokyo). The racemic alcohols (**2**, **15**～**18**) were prepared by the reduction of the corresponding ketone with $NaBH_4$ in MeOH. Yeast alcohol dehydrogenase (YADH) and glucose-6-phosphate dehydrogenase (G6PDH) were obtained from Boehringer. Silica gel F_{254} TLC plates (0.2 mm thick) were obtained from Merck.

Microorganisms. C. tropicalis IFO 199, S. rosei IFO 428, R. rubra IFO 889, S. sp. H-1, X. campestris NIAS 1076, and T. magnoliae IFO 661 were cultured in test tubes containing the modified YM medium[3d] (8 ml/tube) with shaking at 30 °C for 12～18 hr. The cells were collected by centrifugation, and were immediately used for the ketone reduction or else stored in a freezer (-20 °C) until use.

Microbial reduction and product analysis. The fresh cells of each strain (0.1～0.2 g as wet weight) or 50 mg dry baker's yeast were suspended in a mixture of 0.18 ml phosphate buffer (0.1 M, pH 7.0) containing 2 % glucose and 3 % Tween 80 and 20 μl EtOH containing 2 μl of **1**, 3 μl of **9**～**12**, or 3 mg of **13**. The mixtures were shaken at 30 °C for 48 hr and then extracted with ether (1 ml X 2). After concentration by vaporization, the extracts were analyzed by TLC (silica gel F_{254}, benzene or 10 % EtOAc in benzene). The conversion rates of **1** and **13** were determined with a TLC scanner (Shimadzu CS-920, operated at 215 nm) and those of **9**～**12** by GC: column, silica capillary with a

chemically bonded OV-1701 phase (0.25 mm i.d. X 25 m); carrier (He) flow rate, 0.7 ml/min; column temperature, 110 °C; retention time, 4.1, 5.9, 2.7, 1.7, 4.4, 6.3, 6.8, and 5.6 min for **9**, **10**, **11**, **12**, **14**, **15**, **16**, and **17**, respectively. The alcohols were isolated by preparative TLC and their optical purity was determined by chiral HPLC: column (4.6 X 250 mm, Daicel), Chiralcel OB for **14** ~ **16** or OD for **1**, **17**, and **18**; solvent, 7.5 % 2-propanol in hexane; flow rate, 0.5 ml/min ; detection, UV at 215 nm; retention time, 11.9 min for (S)-**14**, 16.8 min for (R)-**14**, 17.1 min for (R)-**15**, 24.2 min for (S)-**15**, 15.5 min for (R)-**16**, 18.0 min for (S)-**16**, 17.9 min for (R)-**2**, 19.1 min for (S)-**2**, 20.6 min for (R)-**17**, 21.7 min for (S)-**17**, 18.8 min for (S)-**18**, and 20.2 min for (R)-**18**.

Reference specimens except for (R)-(-)-**2**[1] and (R)-(+)-**14** were prepared by microbial reduction method[3d] and purified by silica gel column chromatography: (S)-(+)-**15** —— 49 % yield with *T. magnoliae*, oil, $[\alpha]_D^{27}$+42 (c=2.2, CH_2Cl_2) (lit.[12] $[\alpha]_D^{22}$-28.1 (c=3, CH_2Cl_2) for (R)-(-)-**15** of 51.3 %e.e.), MS m/z: 140.0644 (M^+); calcd. C_8H_9FO = 140.0638; (S)-(+)-**16** —— 62 % yield with *T. magnoliae*, oil, $[\alpha]_D^{27}$+19 (c=2.8, CH_2Cl_2) (lit.[12] $[\alpha]_D^{22}$ -7.7 (c=3, CH_2Cl_2) for (R)-(-)-**16** of 43.7 %e.e.), MS m/z: 158.0573 (M^+); calcd. $C_8H_8F_2O$ = 158.0543; (S)-(+)-**17** —— 13 % yield with *X. campestris*, oil, $[\alpha]_D^{23}$+20 (c=0.79, CH_2Cl_2) (lit.[12] $[\alpha]_D^{22}$-1.9 (c=3, CH_2Cl_2) for (R)-(-)-**17** of 6.6 %e.e.), MS m/z: 176.0440 (M^+); calcd. $C_8H_7F_3O$ = 176.0449; and (R)-(-)-**18** —— 13 % yield with *C. tropicalis*, oil, $[\alpha]_D^{23}$ -32 (c=0.59, CH_2Cl_2) (lit.[12] $[\alpha]_D^{22}$-33.7 (c=3, CH_2Cl_2) for (R)-(-)-**18** of 57.3 %e.e.), MS m/z: 156.0328 (M^+); calcd. C_8H_9ClO = 156.0342. The enantiomeric purity of these specimens was 86, 97, 68, and 68 %e.e. for (+)-**15**, (+)-**16**, (+)-**17**, and (-)-**18**, respectively.

Preparation of cell-free extract and its use for reduction of **1**. The frozen cells of *R. rubra* (ca 0.5 g wet weight) were mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.0) containing 2-mercaptoethanol (2 mM) and decanoyl-N-methylglucamide (3.3 mg/ml, as a surface active agent). Each mixture was sonicated with an ultrasonic wave generator (Tomy UR-02) for 10 min under cooling with ice. The remaining cells and cell debris were removed by centrifugation (14000 rpm, 5 min). The supernatant was used as the enzyme extract. Reduction of **1** with the cell-free enzyme was done by adding 20 µl of the extract to a substrate solution made up by mixing 10 µl EtOH containing 0.5 µl of **1** with 0.1 ml of the phosphate buffer containing 0.1 µl Tween 80, 0.1 mg NADH or NADPH, and 10 U YADH (in the case of NADH reaction) or 1 mg glucose 6-phosphate plus 2 U G6PDH (in the case of NADPH reaction). The mixture was incubated at 30 °C for 48 hr, and then extracted with ether. The product was analyzed in the same way as described before.

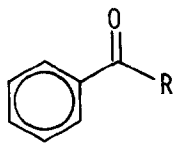
Polyacrylamide gel electrophoresis. A Miniprotean 8[R] apparatus (Bio-Rad) was used. Slab gels (7 %, 7 X 8.13 cm, 0.8 mm thick) and the electrode solutions were prepared according to Laemmli.[13] The enzyme extract was two-fold diluted with 10 % glycerol containing 0.0025 % brom phenol blue (BPB). This sample was charged by 25 µl into the wells. The electrophoresis was run at 5 °C with a constant current of 15 mA until BPB came to the bottom of the gel. The gels were stained actively with a solution containing 1 % alcohol ((±)-**2**, EtOH, 2-propanol, 1-octanol, 3-octanol, or (±)-**14**), 0.5 mg/ml NAD or 0.75 mg/ml NADP, 0.2 mg/ml nitro blue tetrazolium[14] (NBT), and 0.02 mg/ml phenazine methosulfate (PMS) in 0.1 M phosphate buffer (pH 8.5).

Reduction with electrophoretically separated enzymes. The enzyme extract of *R. rubra* was electrophoresed as described above. Two lanes separated by three successive lanes were vertically cut and were stained with the solution containing 1-octanol, (±)-**14**, NAD, NADP, NBT, and PMS at the above-mentioned concentrations. Then the region of the three intermediate lanes was horizontally sectioned with reference to the stains on the both side lanes (see Fig. 2). The cut gels (8 pieces) were separately placed in small vials and were mixed with 0.3 ml of the substrate solution containing 1.4 µl of **1** plus 0.27 mg NADH or NADPH. The gels were crushed with a stainless rod. The mixtures were incubated at 30 °C for 48 hr with being supplemented with 10 µl NADH or NADPH solution (10 mg/ml) in every 12 hr and then were extracted with ether (1 ml X 2) for analysis. In addition, the stained gel itself was inactive for the reduction of **1**.

RESULTS AND DISCUSSION

We attempted to synthesize (fluoroacetyl)- and (difluoroacetyl)ferrocene by the $AlCl_3$-mediated acylation of ferrocene with the corresponding acid chloride, but the synthesis was unsuccessful probably because of the instability of the products. Therefore, a series of α-fluorinated acetophenone derivatives (**9**~**12**) and one chlorine analogue (**13**) were employed as the substrates. The alcohols produced in the microbial reduction of the ketones (**1** and **9**~**13**) with 7 species of microorganisms are summarized in Table 1.

The reduction of the non-fluorinated acetophenone (**9**) usually gave the (S)-alcohol (**14**) of high optical purity, though there was an exception with nearly racemic alcohol being formed by T. magnoliae. On the other hand, 2,2,2-trifluoroacetophenone (**12**) was reduced preferentially to (S)-α-(trifluoromethyl)benzyl alcohol (**17**) by five of the seven strains tested. The product by baker's yeast was (R)-**17** of low optical purity, but Kitazume[15] reported



9 R = CH_3 **14**
10 R = CH_2F **15**
11 R = CHF_2 **16**
12 R = CF_3 **17**
13 R = CH_2Cl **18**

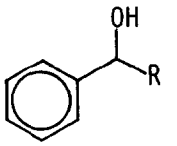


Table 1. Stereoselectivity in the microbial reduction of (trifluoroacetyl)ferrocene (**1**) and acetophenone derivatives (**9**~**13**). Data are given in the order of absolute configuration, optical purity (%e.e.), and conversion rate (in parentheses, %) for the produced alcohol.

Strain	Produced alcohol		
	2	**14**	**15**
R. rubra	R 19 (~100)	S 98 (45)	R 64 (89)
C. tropicalis	R 94 (~100)	S 97 (96)	R 70 (99)
S. rosei	R 20 (~100)	S 89 (9)	R 17 (60)
S. sp. H-1	R 38 (~100)	S 93 (6)	R 76 (97)
T. magnoliae	R 75 (86)	R 2 (94)	S 89 (>99)
X. campestris	R 98 (~100)	S 80 (5)	R <1 (29)
Baker's yeast	R 28 (88)	S 88 (7)	R 88 (43)
	Produced alcohol		
	16	**17**	**18**
R. rubra	R 21 (93)	S 40 (98)	R 94 (65)
C. tropicalis	R 7 (89)	S 51 (99)	R 64 (13)
S. rosei	S 14 (~100)	S 52 (72)	R 64 (27)
S. sp. H-1	R 46 (96)	R 24 (>99)	R 83 (51)
T. magnoliae	S 97 (~100)	S 94 (>99)	S 33 (64)
X. campestris	S 66 (93)	S 86 (93)	S 30 (31)
Baker's yeast	R 72 (92)	R 10 (56)	R 75 (56)

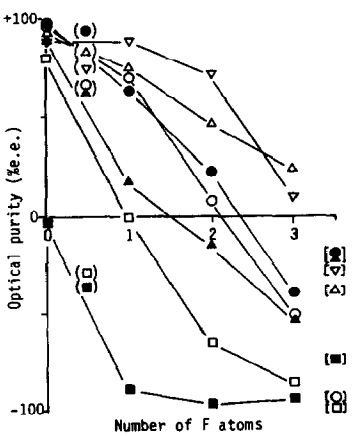


Fig. 1. Plots showing the fluorine substitution favors the (R)-**14**-type enantiomer formation. The precise data are given in Table 1. Details see the text. Marks in parentheses represent plots for **18** and those in brackets plots for **2**. (●) R. rubra, (○) C. tropicalis, (▲) S. rosei, (△) S. Sp. H-1, (■) T. magnoliae, (□) X. campestris, and (▽) baker's yeast.

that (S)-(+)-**17** of >99 %e.e. was produced in his baker's yeast reduction of **12**. Since it cannot be accepted that a trifluoromethyl group is larger than a phenyl group, the stereoselectivity of the five or six strains for **12** was contrary to Prelog's rule. The stereoselectivity in the reduction of 2-fluoro- (**10**), 2,2-difluoro- (**11**), and 2-chloroacetophenone (**13**) seemed to be intermediary between the above two extremes.

The three-dimensional arrangement of carbinol substituents of (R)-monofluoroalcohol **15** is really equivalent to that for (S)-alcohol **14**. The difference in configurational assignment (S to R) is only a matter of the order rule.[16] Therefore, the data in Table 1 are plotted as in Fig. 1, where the abscissa represents the number of fluorine atoms and the ordinate represents the enantiomeric purity with plus sign for (S)-**14** and (R)-**15**~**18** and minus sign for (R)-**14** and (S)-**15**~**18**. Fig. 1 clearly shows that the increased fluorine substitution favors the production of the (R)-**14**-type enantiomer, that is, the stereoselectivity being contrary to Prelog's rule. The plot for the monochlorinated alcohol (**18**) formed by several strains usually comes between the points for the non-fluorinated and monofluorinated alcohols for the corresponding strain. The chlorine atom seems to cause a similar effect on the stereoselectivity as the fluorine atom. The data for the ferrocenyltrifluoroalcohol (**2**) are also given in Fig. 1. The preference to the anti-Prelog-type stereoselectivity for the trifluorinated substrate (**12**) is strengthened by the substitution of the phenyl group for a ferrocenyl group in the case of C. tropicalis, X. campestris, S. sp. H-1, and baker's yeast.

The above-mentioned fluorine effect was found in the reduction with whole cells as catalyst. The next step was to study on the effect with cell-free enzymes.

R. rubra was selected for this study because the actively stained electrophoretograms (see below) was most reproducible for this strain in a preliminary experiment and thus its enzymes were assumed to be relatively stable. The crude extract of R. rubra was subjected to polyacrylamide gel electrophoresis (PAGE) and the gel was processed in the active staining to detect a dehydrogenase for the oxidation of (±)-**2**, i.e., the reverse reaction for the reduction of **1**. Unfortunately, no clear band indicating such a dehydrogenase was found on the gel. The active staining was repeated with simpler alcohols, e.g., ethanol, 1-octanol, and α-methylbenzyl alcohol (**14**), as the substrate. A few violet bands indicative of

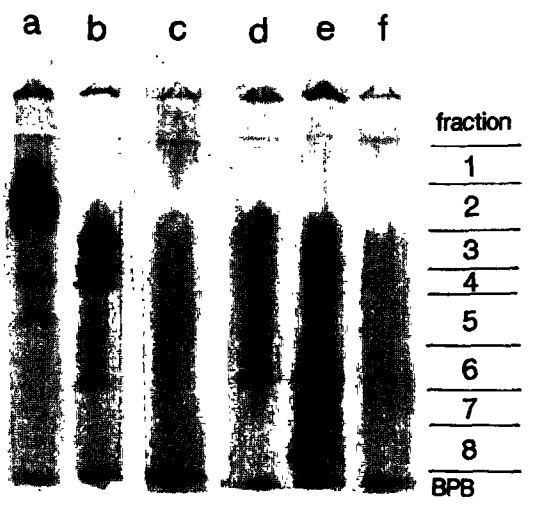


Fig. 2. Electrophoretograms for oxidoreductases in R. rubra actively stained with 1-octanol + NAD (a), 1-octanol + NADP (b), 1-octanol only (c), **14** + NAD (d), **14** + NADP (e), and **14** only (f). Illustration on the right side shows the horizontal sections for the reduction test with **1**.

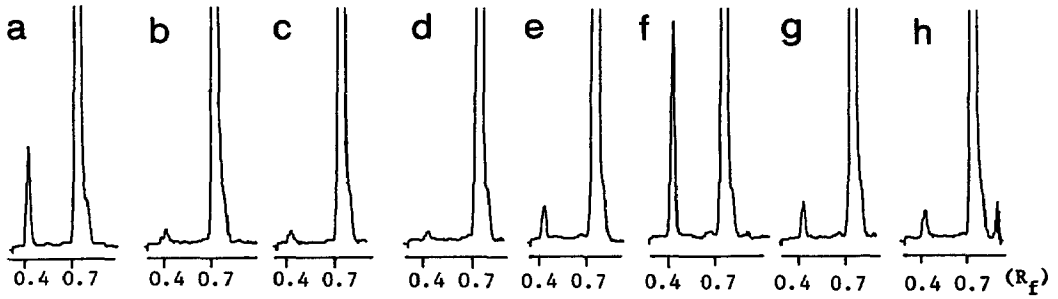


Fig. 3. TLC traces (215 nm) for the reduction of **1** with NADPH and enzymes of R. rubra separated by PAGE in fraction 1 (a), 2 (b), 3 (c), 4 (d), 5 (e), 6 (f), 7 (g), and 8 (h). Peak for R_f 0.42 represents alcohol **2** and peak for R_f 0.78 ketone **1**.

the dehydrogenases appeared on each gel at different sites with different intensities for the combination of substrate and coenzyme (NAD or NADP). The active stains with 1-octanol and (±)-**14** are shown in Fig. 2. The control staining without the coenzyme (Fig. 2 c and f) showed no violet band.

To determine which band corresponded to the reduction of **1**, the gel was cut into 8 fractions with reference to the active stains (see the illustration in Fig. 2) and each fraction was tested for the reduction of **1**. The product was analyzed by TLC (Fig. 3). Clearly, the prominent activity of reduction was present in fractions 1 and 6. Fraction 6 corresponded to the band actively stained with α-methylbenzyl alcohol (**14**), while fraction 1 was a zone actively unstained with any alcohols tested.

The alcohol **2** produced by fraction 1 was rich in the R enantiomer, whereas **2** by fraction 6 was rich in the S enantiomer (Fig. 4). This fact suggests that the reductase (if a single enzyem) for **1** in fraction 1 and another reductase for **1** (if another single

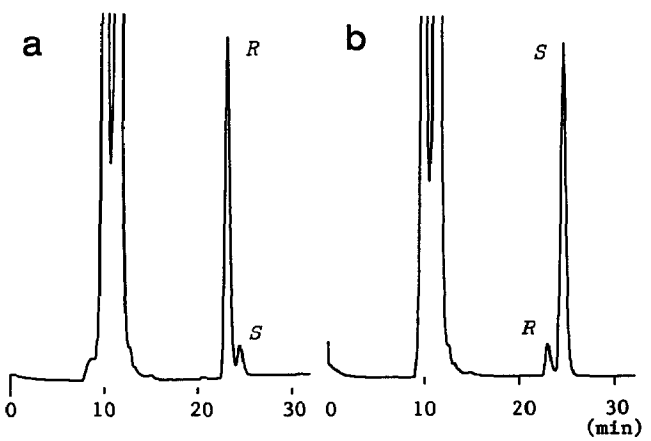


Fig. 4. Chiral HPLC for alcohol **2** formed in the reduction with fraction 1 (a) and fraction 6 (b). Conditions: column, Chiralcel OD (4.6 X 250 mm); solvent, 7.5 % 2-PrOH in hexane; flow rate, 0.3 ml/min. Enantiomers are denoted in the figure.

Table 2. Reduction of (trifluoroacetyl)ferrocene (**1**) with enzymes of R. rubra fractionated by PAGE. Data are given in the same way as in Table 1.
extract = crude enzyme extract

Enzyme	Product and coenzyme	
	2 (NADH)	**2** (NADPH)
fraction 1	R 30 (3)	R 82 (10)
fraction 2	nt (0)	R 44 (2)
fraction 3	R 42 (<1)	R 15 (2)
fraction 4	R 43 (<1)	S 19 (1)
fraction 5	S 54 (<1)	S 63 (4)
fraction 6	S 94 (3)	S 84 (22)
fraction 7	nt (0)	S 26 (4)
fraction 8	R 33 (<1)	R 48 (3)
extract	R 18 (70)	R 50 (91)

Table 3. Stereoselectivity in the reduction of α-halogenated acetophenone derivatives (**9**~**13**) with PAGE fractions 1 and 6 of the R. rubra extract. The coenzyme was NADPH. Data are given in the same way as in Table 1.

Enzyme	Product				
	14	**15**	**16**	**17**	**18**
fraction 1	S 33 (2)	S 66 (2)	S 65 (38)	S 99 (50)	R 58 (<1)
fraction 6	S 79 (5)	R 67 (2)	R 79 (24)	R 98 (36)	R 91 (17)
crude extract	S 79 (4)	R 16 (32)	S 17 (87)	S 90 (49)	R 64 (32)

enzyme) in fraction 6 would have the opposite stereoselectivity with each other. The quantitative results are summarized in Table 2.

The reductase activity for **1** was distributed from fractions 1 to 8, but the major components were contained in fractions 1 and 6. As the coenzyme, NADPH was more efficient than NADH for every fraction. The degree of stereoselectivity by fraction 6 was, however, higher with NADH than with NADPH. On the other hand, fraction 1 became less stereoselective by the use of NADH. The stereoselectivity of the microbial reduction with whole cells should be, therefore, determined by the ratio among the competent enzymes (especially, those in fractions 1 and 6) as well as the ratio of NADPH to NADH present in the cell. These ratios would change depending on the culture conditions (medium, age, aeration, etc.).

Fractions 1 and 6 were further tested for the reduction of acetophenone (**9**) and its halogenated derivatives (**10**~**13**) with NADPH as the coenzyme (Table 3). The reduction by fraction 1 showed the anti-Prelog-type stereoselectivity for all fluorinated ketones (**1**, **10**~**12**) (Table 2 and 3). However, the stereoselectivity in the reduction by fraction 6 followed Prelog's rule for all substrates tested here. This fact shows that the fluorine substitution does not necessarily favor the anti-Prelog-type stereoselectivity in the enzymic level. The competent enzyme in fraction 6 might be a dehydrogenase with a wide substrate specificity for hydrophobic secondary alcohols, because the electrophoretogram was actively stained not only with α-methylbenzyl alcohol (**14**) but also with 2-propanol and 3-octanol (data not shown) at the site of fraction 6.

The zone for fraction 1 was negative in the active staining with any aforementioned alcohols and Na 2-hydroxyisocaproate, Na 3-hydroxybutyrate, and ergosterol. There is no clue to the natural substrate for the oxidoreductase(s) in fraction 1. If the reductive activity of fraction 1 is due to a single (or substantially single) enzyme, its characteristic should be the si-face or pseudo-si-face selectivity striking particularly for the α-fluorinated ketones. This might be reflected in the anti-Prelog-type stereoselectivity in the whole cell reduction of **1**. The fact that the anti-Prelog-type reduction for the trifluoroketones **1** and **12** was found with many species of microorganisms suggests that such a reductase as described above would be widely distributed in microorganisms. Recently, two si-face selective dehydrogenases for synthetic ketones were isolated from bacteria.[17,18] An oxidoreductase for organosilicon and organogermanium ketones was also isolated from a kind of yeast, which showed the re-face selectivity in the reduction.[19]

The enzymic activity for the (trifluoroacetyl)ferrocene reduction in R. rubra was so weak that it was expected to take a long time for clarifying the enzyme systems by the orthodox chromatographic procedure. Alternatively, we did the present approach by electrophoresis to grip the outline. Isolation of the enzymes in fractions 1 and 6 and more strict investigation on their stereoselectivity is to be studied in future.

REFERENCES

1. (a) Y. Yamazaki and K. Hosono, Tetrahedron Lett., 29, 5769 (1988); (b) Y. Yamazaki, M. Uebayasi, and K. Hosono, Eur. J. Biochem., 184, 671 (1989); (c) Y. Yamazaki and K. Hosono, Tetrahedron Lett., 30, 5313 (1989).
2. (a) A. Ratajczak and B. Misterkiewicz, Chem. Ind., 1976, 902; (b) V. I. Sokolov, L. L. Troitskaya, and T. I. Rozhkova, Gazz. Chim. Ital., 117, 525 (1987); (c) S. Top, G. Jaouen, C. Baldoli, P. D. Buttero, and S. Maiorana, J. Organomet. Chem., 413, 125 (1991); (d) J. A. S. Howell, M. G. Palin, H. El Hafa, S. Top, and G. Jaouen, Tetrahedron: Asymmetry, 3, 1355 (1992).
3. (a) Y. Yamazaki and K. Hosono, Agric. Biol. Chem., 52, 3239 (1988); (b) Y. Yamazaki and K. Hosono, Biotechnol. Lett., 11, 627 (1989); (c) Y. Yamazaki and K. Hosono, Biotechnol. Lett., 11, 679 (1989); (d) Y. Yamazaki, M. Uebayasi, J. Someya, and K. Hosono, Agric. Biol. Chem., 54, 1781 (1990); (e) Y. Yamazaki and K. Hosono, Agric. Biol. Chem., 54, 2183 (1990); (f) M. Uebayasi, Y. Yamazaki, and J. Someya, Chem. Express, 6, 221 (1991).
4. (a) C. Syldatk, H. Andree, A. Stoffregen, F. Wagner, B. Stumpf, L. Ernst, H. Zilch, and R. Tacke, Appl. Microbiol. Biotechnol., 27, 152 (1987); (b) Y. Yamazaki and H. Kobayashi, Chem. Express, 8, 97 (1993).
5. (a) Y. Yamazaki and K. Hosono, Ann. N. Y. Acad. Sci., 613, 738 (1990); (b) N. W. Alcock, D. H. G. Crout, C. M. Henderson, S. E. Thomas, J. Chem. Soc., Chem. Commun., 1988, 746; (c) B. Malézieux, G. Jaouen, J. Salaün, J. A. S. Howell, M. G. Palin, P. McArdle, M. O'Gara, and D. Cunningham, Tetrahedron: Asymmetry, 3, 375 (1992).
6. (a) Y. Yamazaki and K. Hosono, Tetrahedron Lett., 31, 3895 (1990); (b) Y. Yamazaki and K. Hosono, Agric. Biol. Chem., 54, 3357 (1990); (c) Y. Yamazaki, N. Morohashi, and K. Hosono, Biotechnol. Lett., 13, 81 (1991); (d) Y. Yamazaki, N. Morohashi, and K. Hosono, J. Chromatogr., 542, 129 (1991); (e) Y. Yamazaki, K. Hosono, H. Matsuda, N. Minami, M. Asai, and H. Nakanishi, Biotechnol. Bioeng., 38, 1218 (1991); (f) N. W. Boaz, Tetrahedron Lett., 30, 2061 (1989); (g) K. Nakamura, K. Ishihara, A. Ohno, M. Uemura, H. Nishimura, and Y. Hayashi, Tetrahedron Lett., 31, 3603 (1990).
7. (a) Y.-F. Wang, J. J. Lalonde, M. Momongan, D. E. Bergbreiter, and C.-H. Wong, J. Am. Chem. Soc., 110, 7200 (1988); (b) M. Therisod, J. Organomet. Chem., 361, C8 (1989); (c) M. A. Sparks and J. S. Panek, Tetrahedron Lett., 32, 4085 (1991); (d) A.-H. Djerourou and L. Blanco, Tetrahedron Lett., 32, 6325 (1991); (e) J. M. Chong and E. K. Mar, Tetrahedron Lett., 32, 5683 (1991); (f) T. Itoh and T. Ohta, Chem. Lett., 1991, 217.
8. C. J. Sih and J. P. Rosazza, "Applications of Biochemical Systems in Organic Chemistry," Part 1, ed. by J. B. Jones, C. J. Sih and D. Perlman, John Wiley & Sons, New York, 1976, pp. 71—78.
9. M. Goto, Y. Yamazaki, and M. Uebayasi, Bull. Chem. Soc. Jpn., 66, 666 (1993).
10. W. E. Truce and B. H. Sack, J. Am. Chem. Soc., 70, 3959 (1948).
11. A. Sykes, J. C. Tatlow, and C. R. Thomas, J. Chem. Soc., 1956, 835.
12. R. Weidmann, A. R. Schoofs, and A. Horeau, C. R. Acad. Sc. Paris, t. 294, Série II, 319 (1982).
13. U. K. Laemmli, Nature, 227, 680 (1970).
14. E. Goldberg, Science, 139, 602 (1963).
15. T. Kitazume, Bio Industry, 5, 733 (1988) [Chemical Abstracts 110: 113136g (1989)].
16. R. S. Cahn, C. Ingold, and V. Prelog, Angew. Chem., 78, 413 (1966).
17. C. W. Bradshaw, H. Fu, G.-J. Shen, and C.-H. Wong, J. Org. Chem., 57, 1526 (1992).
18. C. W. Bradshaw, W. Hummel, and C.-H. Wong, J. Org. Chem., 57, 1532 (1992).
19. L. Fisher, C. Syldatk, and F. Wagner, Ann. N. Y. Acad. Sci., 613, 614 (1990).