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SYNTHESIS OF OPTICALLY ACTIVE FLUORINATED MATERIALS BY USE OF IMMOBILIZED ENZYMES FOR ASYMMETRIC HYDROLYSIS

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SUMMARY

A synthetic approach to optically active fluorinated compounds was based on the enantiotopic specificity of asymmetric hydrolysis by an immobilized enzyme.

INTRODUCTION

In recent years the opportunities for asymmetric synthesis, provided by the wide range of catalytic activities of enzymes of microorganisms, have been increased to cover the field of halogen-containing compounds which have unique properties as industrial materials [1-3].

In this paper, we wish to describe a useful technique for the efficient manipulation of enzymes in fluorine chemistry, in which the enzyme is immobilized on the commercially available ceramic 'honeycomb' (NGK Insulators Ltd).

We examined this technique for a number of asymmetric hydrolyses and have found that it is useful to separate optically active materials from the suspension of an enzyme-solvent system on a practical scale.

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RESULTS AND DISCUSSION

Microbial hydrolysis of 2-fluoro-2-methylmalonic acid diethyl ester (1) with immobilized enzymes

We found a simple process to produce (S)-(-)-2-fluoro-2-methylmalonic acid monoethyl ester (2) with high optical purity (>89 %ee) involving the asymmetric hydrolysis of 2-fluoro-2-methylmalonic acid diethyl ester (1)[4] by using an enzyme immobilized with calcium alginate on ceramic "honeycomb".

The results shown in Table 1 clearly demonstrated that immobilized enzymes effected the transformation of the diester into the optically active monoester on a practical scale.

$$\underset{1}{MeCF(CO_2Et)_2} \xrightarrow{\text{immobilized enzyme}} \underset{2}{MeCF(CO_2Et)CO_2H}$$

TABLE 1

Asymmetric hydrolysis of compound(1)

Immobilized enzyme	Reaction time (h)	$[\alpha]_D$/MeOH	Yield (%)	Optical purity % ee	Absolute[c] configuration
lipase-MY[a]	24	-18.2	63	89	S
lipase-MY	108	-17.0	75	86	S
cellulase[b]	24	+6.30	33	29	R
lipase P[b]	24	+6.32	42	30	R

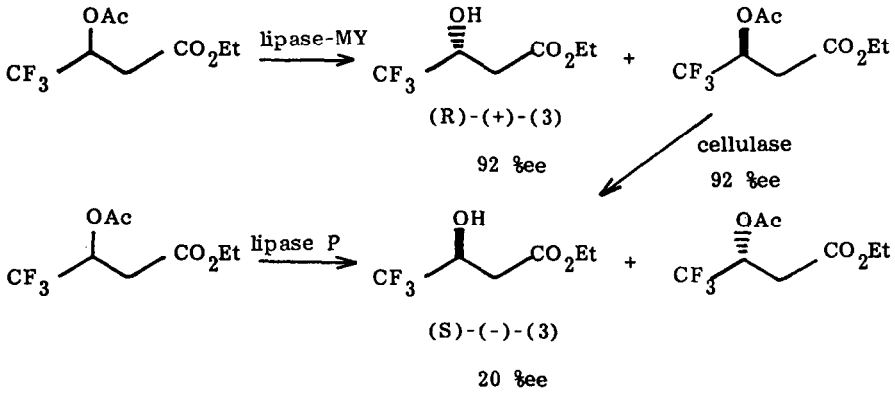
[a] *Candida cylindracea* (Meito Sangyo Co. Ltd.) [b] Amano Seiyaku Co. Ltd.
[c] The optical purities were determined by GLC and/or ^{19}F NMR after conversion of the malonic acid half ester to its diastereomeric amide by optically active α-methylbenzylamine.

(R)-(+)- or (S)-(-)-ethyl 4,4,4-trifluoro-3-hydroxybutanoate (3)

In our previous papers [4-9], we have reported the asymmetric hydrolysis of fluoroalkylated compounds by enzymes of microbial or animal origin. To search for techniques to separate the optically active materials from the suspension of enzyme-solvent system on a practical scale, we examined the asymmetric hydrolysis by a variety of immobilized enzymes on ceramic 'honeycomb', of the acetate derivative of ethyl 4,4,4-trifluoro-3-hydroxybutanoate.

In the hydrolysis of the acetate derivative of ethyl 4,4,4-trifluoro-3-hydroxybutanoate, immobilized lipase-MY gave greater ee than immobilized lipase P. The asymmetric hydrolysis by immobilized lipase-MY proceeded smoothly to afford the (R)-enantiomer, and by immobilized lipase P to afford the (S)-enantiomer. The results shown in Scheme I clearly suggest a great advantage of enzymatic immobilization for the prevention of racemization under hydrolysis conditions. The optical purity of the (R)-enantiomer is sufficiently high to allow its use as a practical chiral intermediate in fluorine chemistry.

A sample of the (S)-enantiomer with higher optical purity, was prepared using the acetate recovered from the hydrolysis by lipase-MY. Hydrolysis of this recovered acetate using cellulase (*Trichoderma viride*), gave (S)-(-)-ethyl 4,4,4-trifluoro-3-hydroxybutanoate (3), having 92 %ee.



Optically active 1-fluoro-2-octanol (4)

We have reported that the asymmetric hydrolysis of 1-fluoro-2-octyl acetate by lipase-MY only produced (R)-(+)-1-fluoro-2-octanol with low optical purity (< 24 %ee). This is one of the important compounds for molecular design, e.g. for ferroelectric liquid crystals.
Therefore, the asymmetric hydrolysis of 1-fluoro-2-octyl acetate by using lipase P immobilized on ceramic hanikamu with calcium alginate was examined. The ee of the product, (S)-(-)-fluoro-2-octanol at 60 % conversion should equal 52 %.

Recently, Sih and his co-workers have reported the relation of three key parameters: the extent of conversion of racemic substrate (c), the optical purity , expressed as enantiomeric excess (ee) of the product or the remaining substrate, and the enantiomeric ratio (E).

For example, the above result shown in Fig. 1 suggest that the ee of remaining substrate at >70 % conversion should equal >85 %. In fact, we obtained the (R)-(+)-1-fluoro-2-octanol with high optical purity (>90 %ee) at 81 % conversion.

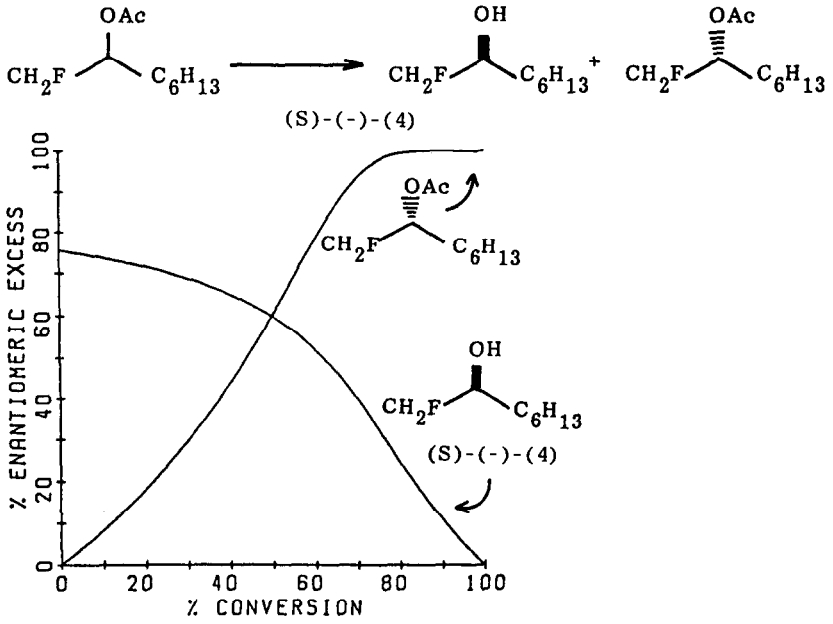


Fig. 1. **Dependence of percent enantiomeric excess(ee) on the percent conversion.**

EXPERIMENTAL

General procedure.

All microbial transformations were carried out in a 'CULSTAR' flask for suspension culture with double arms and jacket (100 ml, Sibata Scientific Technology Ltd.). All commercial available reagents were used without further purification. Infrared spectra were obtained by using a JASCO A-102 spectrometer and KBr pellets. The ^{1}H(internal Me_4Si) and ^{19}F(external CF_3CO_2H) NMR spectra were recorded by using a Varian EM-390(90 MHz) and a Hitachi R-24F(56 MHz) spectrometers. Specific rotations were recorded by using a JASCO DIP-140 digital polarimeter. Yields were those of the products actually isolated.

Immobilized lipase-MY

A suspension of lipase-MY (*Candida cylindracea* , Meito Sangyo Co. Ltd., 7.0 g) and sodium alginate (2.5 g) in water (40 ml) was mixed at room temperature, and then the ceramic 'honeycomb'(35 g)(NGK Insulators Ltd.) was covered with this mixture. Into the aq. 10 % $CaCl_2$ solution, the above ceramic "honeycomb" was dipped for 1 h.

Immobilized lipase P

In the above reaction, lipase P (Amano Seiyaku Co. Ltd., 5 g) and sodium alginate (2.5 g) in water (40 ml) was used, and then worked up as usual.

Immobilized cellulase

In the same mannner, cellulase (*Trichiderma viride* , Amano Seiyaku Co. Ltd., 5 g) and sodium alginate (2.5 g) in water (40 ml) was used, and then the ceramic 'honeycomb' was covered with this mixture. Into the aq. 10 % $CaCl_2$ solution, the above ceramic 'honeycomb' was dipped for 1 h.

(S)-(-)-2-Fluoro-2-methylmalonic acid monoethyl ester (2)

A mixture of the above prepared immobilized lipase-MY(7 g) on ceramic 'honeycomb' and 2-fluoro-2-methylmalonic acid diethyl ester (1)[4](3.9 g, 20 mml) in buffer solution (50 ml, pH 7.3), [which was prepared from 1/15 M aq.Na_2HPO_4 solution (38.4 ml) and 1/15 M aq.KH_2PO_4 solution (11.6 ml)], was stirred at 40-41 °C in the 'CULSTAR' flask. After 108 h of stirring, the mixture was acidified with 1N HCl, and then the immobilized lipase-MY on ceramic 'honeycomb' was separated. The oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate, and then the solvent was removed. Distillation gave (S)-(-)-2-fluoro-2-methylmalonic acid monoethyl ester (2)[4] in a 75 % yield, bp 90-92°C/0.6 mmHg, $[\alpha]_D$ -17.0 (c 2.81, MeOH), 86 %ee.
^{19}F NMR ($CDCl_3$): δ 77.8(q, J_{F-CH_3} = 20.9 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.32(CH_3, t, $^3J_{CH_3-CH_2}$ = 7.1 Hz), 1.77(CH_3, d), 4.27(CH_2, q), 10.90(CO_2H, s).

(R)-(+)-Ethyl 4,4,4-trifluoro-3-hydroxybutanoate (3)

A suspension of the above immobilized lipase-MY(7 g) on ceramic 'honeycomb'(35 g) and acetate derivative of ethyl 4,4,4-trifluoro-3-hydroxybutanoate (15 mmol, 3.0 g) in buffer solution (50 ml, pH 7.3) was stirred at 40-41°C in 'CULSTAR' flask. After 4 h of stirring, the mixture was acidified with 1N HCl and then the oily materials were extracted with diethyl ether. After determining the hydrolysis ratio (45 %) by ^{19}F NMR signal intensities using $C_6H_5CF_3$ as an internal standard, (R)-(+)-ethyl 4,4,4-trifluoro-3-hydroxybutanoate (3)[10]($[\alpha]_D$ (neat) +19.6, >92 %ee) and (S)-acetate (recovered yield 80 %) were separated by column chromatography using the mixture of n-hexane-ethyl acetate (5:1) as an eluent.

Synthesis of (S)-enantiomer

In the above asymmetric hydrolysis, the acetate derivative of ethyl 4,4,4-trifluoro-3-hydroxybutyrate (15 mmol, 3.0 g) was

hydrolyzed for 5 h with immobilized lipase-MY(7 g), and then the mixture was acidified with 1N HCl. The oily materials were extracted with diethyl ether and then the ethereal extract was dried over anhydrous magnesium sulfate. After determining the hydrolysis ratio (58 %) by ^{19}F NMR signal intensities, (R)-(+)-ethyl 4,4,4-trifluoro-3-hydroxybutanoate (>73 %ee) and the corresponding (S)-acetate derivative were separated by by column chromatography using the mixture of n-hexane-ethyl acetate (5:1) as an eluent.

A suspension of cellulase(*Trichoderma viride* , Amano Seiyaku Co. Ltd.) and recovered (S)-acetate derivative of ethyl 4,4,4-trifluoro-3-hydroxybutanoate (5 mmol) in buffer solution (50 ml, pH 7.3) was stirred at 40-41°C. After 6 h of stirring, the mixture was acidified with 1N HCl and then the oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate and then the solvent was removed. The product was separated by column chromatography using the mixture of n-hexane-ethyl acetate (5:1) as an eluent. bp 70°C/15 mmHg, $[\alpha]_D$ (neat) -20.3, >95 %ee.
^{19}F NMR ($CDCl_3$): δ 2.6(d, J_{CF_3-CH}= 6.6 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.25(CH_3, t, $J_{CH_3-CH_2}$= 7.3 Hz), 2.62(2xH), 4.30(4xH, m).

(S)-(-)-1-Fluoro-2-octanol(4)

1-Fluoro-2-octyl acetate (9 mmol, 1.6 g) was hydrolyzed with immobilized lipase P(3.6 g) in buffer solution (50 ml). After 7 h of stirring, the mixture was acidified with 1N HCl and then the oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate and then the solvent removed. (R)-(+)-1-Fluoro-2-octanol (4)[9] (>52 %ee: conversion 60 %) and the corresponding (-)-acetate derivative were separated by column chromatography using the mixture of n-hexane-diethyl ether (5:1).

A mixture solution of recovered (-)-1-fluoro-2-octyl acetate (4 mmol), 2 mole/l aq. NaOH (5 ml)-acetone (5 ml) was stirred at room temperature. After 24 h of stirring, the mixture was acidified with 1N HCl and then oily materials were extracted with diethyl ether. The products were separated by column chromatography using the mixture of n-hexane-diethyl ether.
$[\alpha]_D$ +7.5 (c 0.21, MeOH), >76 %ee.
^{19}F NMR ($CDCl_3$) : δ 145(d.t, J_{F-CH_2}= 46, J_{F-CH}= 17 Hz) ppm.
^{1}H NMR ($CDCl_3$) : δ 0.67-1.67(13xH), 3.23(OH, br), 3.67(CH, m), 4.27(CH_2F, d.d)

Determination of optical purity

A mixture solution of (R)-α-methoxy-α-trifluoromethylphenyl-acetic acid chloride (MTPA-Cl)(1.1 mmol), (S)-(-)-1-fluoro-2-octanol (1 mmol) in pyridine (1 ml) was stirred at room temperature. After 24h of stirring, the whole mixture was poured into water, and then oily materials were extracted with diethyl ether. The ethereal layer was washed with 1N HCl, 5% $NaHCO_3$, sat. $Na_2S_2O_3$ solution and then brine. Removal of the solvent, the diastereomeric ratio was determined by ^{19}F NMR signal intensities.

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