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CATALYTIC ACTIVITIES OF MODIFIED HYDROLYTIC ENZYMES IN ORGANIC MEDIA : SYNTHESES OF OPTICALLY ACTIVE TRIFLUOROMETHYLATED COMPOUNDS

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SUMMARY

Modifications of hydrolytic enzymes by incorporation of fluorine-containing molecules have been found to improve the catalytic activity of the parent enzymes. Syntheses of optically active compounds possessing a trifluoromethyl group, via enzymatic chiral Michael addition reaction and synthesis of heterocycles, have been undertaken in organic solvents.

INTRODUCTION

The importance of enzymatic immobilization and modification as techniques for modifying the catalytic activities and/or capabilities of enzymes has been recognized [1-3]. In particular, modified enzymes have been shown to function in lipophilic organic media in the presence of a few percent of water [4-7].

Recently, we have reported the incorporation of fluorine compounds to improve the catalytic activity of hydrolytic enzymes [8,9].

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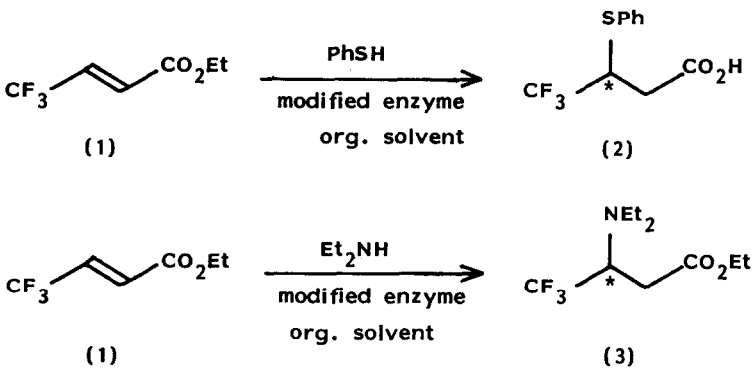
As part of our work in this area [10], we now present some results establishing a new catalytic reaction of modified hydrolytic enzymes with fluorine-containing molecules. This is an enzyme-assisted Michael addition reaction to introduce a center of chirality into fluorocompounds in organic media.

RESULTS AND DISCUSSION

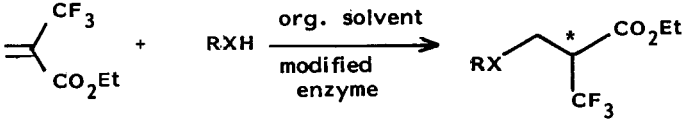
Enzyme-assisted Michael addition in organic media

Reports concerning enzymes active in an organic medium have appeared [11-16]. However, enzymes have not been widely used in organic synthesis because organic reactions are usually run in organic solvents, and most enzymatic reactions are run in water. In our previous research work, enzyme-assisted asymmetric Michael addition reactions of ethyl 3-(trifluoromethyl)propenate (1) with nucleophiles, did not proceed in an aqueous medium.
Therefore, we have attempted to use the catalytic activity of modified enzymes to introduce a center of chirality into the fluorocompounds in organic media.

The results shown in Table 1 show that chiral Michael addition was achieved using a thiol and a secondary amine as nucleophiles. However, water and phenol did not react even in this system. In the case of thiophenol as a nucleophile, both chiral Michael addition and hydrolysis of the ester group proceeded, to give the optically active (-)-3-phenylthio-4,4,4-trifluorobutanoic acid (2).



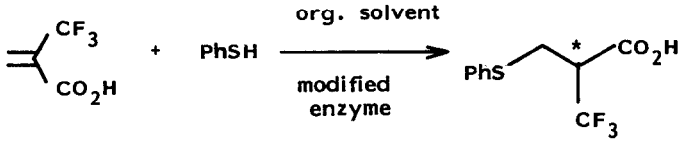
Furthermore, it is also possible to use ethyl 2-trifluoropropenate (4) as a Michael acceptor in the above system.



(4a)

(5) RX : PhS

(6) RX : Et_2N



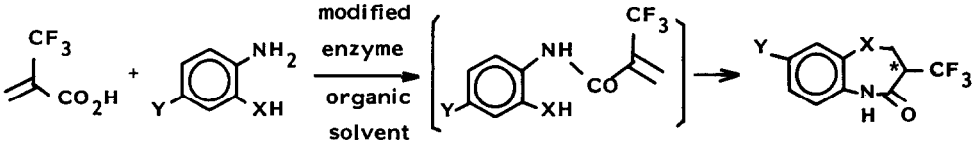
(4b)

(7)

Preparation of optically active heterocycles

Numerous stereocontrolled syntheses of heterocycles have been reported by several workers. However, the introduction of a center of chirality into fluorinated heterocycles expected to be bioactive materials has not been reported in the literature.

In our biosynthetic applications based on the catalytic capability of modified hydrolases, we have also found a facile route to optically active trifluoromethylated heterocycles. Various kinds of compounds with bifunctional groups were examined to prepare optically active heterocycles. The results shown in Table 3 indicate that modified hydrolases transform 2-trifluoromethylpropenoic acid into optically active heterocycles with higher optical purities, but in lower yields than do native hydrolases.



(8) : X= O, Y= H

(9) : X= O, Y= Me

(10): X= S, Y= H

TABLE 1

Asymmetric Michael addition reaction using the compound, $CF_3CH=CHCO_2Et$ (1)

Origin of enzyme or modified enzyme	Reactant	Solvent	Yield (%)	$[\alpha]_D$/MeOH	Optical purity %e.e.
Candida cylindracea [a]	PhSH[d]	H_2O	46	+0.80(c 1.27)	47[g]
Candida cylindracea - C(O)(CF_3)C=CH_2 [c]	PhSH[d]	hexane	47	-0.31(c 0.94)	18[f]
Candida cylindracea - C(O)(CF_3)C=CH_2	PhSH[d]	benzene	54	-0.56(c 0.89)	33
α-*Chymotrypsin* [b] - C(O)(CF_3)C=CH_2	PhSH[d]	benzene	53	-0.26(c 0.97)	15
Pig liver esterase [b] - C(O)(CF_3)C=CH_2	PhSH[d]	benzene	43	-0.19(c 1.07)	11
Candida cylindracea - C(O)(CF_3)C=CH_2	Et_2NH[e]	hexane	38	-1.26(c 0.98)	26[g]
Candida cylindracea - C(O)(CF_3)C=CH_2	Et_2NH[e]	benzene	47	-1.80(c 1.28)	37
α-*Chymotrypsin* - C(O)(CF_3)C=CH_2	Et_2NH[e]	benzene	36	-0.92(c 1.43)	19
Pig liver esterase -C(O)(CF_3)C=CH_2	Et_2NH[e]	benzene	41	-0.39(c 1.56)	8
Candida cylindracea - C(O)(CF_3)C=CH_2	PhOH	benzene	no reaction		

[a] lipase-MY: Meito Sangyo Co. Ltd. [b] Sigma Co. Ltd. [c] modified enzyme
[d] product: $PhS(CF_3)CHCH_2CO_2H$(2) [e] product: $Et_2N(CF_3)CHCH_2CO_2Et$(3) [f] The optical purity was determined by g.l.c. and/or ^{19}F nmr after conversion of optically active acid into its diastereomeric amide by optically active α-methylbenzylamine. [g] The optical purity was determined by ^{19}F nmr signal intensities by commercially available (+)-tris[di(perfluoro-2-propoxypropionyl)metanate]europium(III).

TABLE 2

Asymmetric Michael addition reaction using compounds, $CH_2=C(CF_3)CO_2R$ (4a and 4b)

R	Origin of enzyme or modified enzyme	Reactant	Solvent	Yield (%)	$[\alpha]_D$/MeOH	Optical purity %e.e.[d]
H	*Candida cylindracea*[a]	PhSH[e]	H_2O	64	+1.13(c 2.06)	50
	Candida cylindracea - $C(O)(CF_3)C=CH_2$[c]	PhSH[e]	hexane	52	-0.34(c 1.08)	15
	Candida cylindracea - $C(O)(CF_3)C=CH_2$	PhSH[e]	benzene	70	-0.47(c 1.09)	21
	α-*Chymotripsin*[b] - $C(O)(CF_3)C=CH_2$	PhSH[e]	benzene	61	-0.62(c 0.76)	28
	Pig liver esterase[b] - $C(O)(CF_3)C=CH_2$	PhSH[e]	benzene	63	-0.74(c 1.45)	33
Et	*Candida cylindracea* - $C(O)(CF_3)C=CH_2$	PhSH[f]	H_2O	no reaction		
	Candida cylindracea - $C(O)(CF_3)C=CH_2$	PhSH[f]	benzene	86	-1.91(c 1.89)	44
	Candida cylindracea - $C(O)(CF_3)C=CH_2$	Et_2NH[g]	benzene	82	-3.44(c 1.44)	35

[a] lipase-MY: Meito Sangyo Co. Ltd. [b] Sigma Co. Ltd. [c] modified enzyme
[d] The optical purity was determined by ^{19}F nmr signal intensities by commercially available (+)-tris[di(perfluoro-2-propoxypropionyl)metanate]europium(III).
[e] product (7) [f] product (5) [g] product (6)

TABLE 3

^{19}F and ^{1}H NMR spectrum

Compound No	^{19}F nmr[a] (J_{CF_3-CH} ; Hz)	^{1}H nmr chemical shift
(2)	-6.5 (d, J= 8.5 Hz)	3.35-3.45(3xH, m), 7.2(Ar-H), 10.6(CO_2H)
(3)	-8.0 (d, J= 8.5 Hz)	1.14(CH_3, t, $J_{CH_3-CH_2}$= 6.5 Hz), 1.33(CH_3, t, $J_{CH_3-CH_2}$= 7.0 Hz), 2.50-2.83(CH, m), 2.60(CH_2, q), 3.83(CH_2, m), 4.10(CH_2, q)
(5)	-11.7(d, J= 7.5 Hz)	1.35(CH_3, t, $J_{CH_3-CH_2}$= 7.1 Hz), 3.41-3.71(CH, m), 4.04 (CH_2), 4.28(CH_2, q), 7.20-7.51(Ar-H)
(6)	-12.5(d, J= 8.5 Hz)	1.13(CH_3, t, $J_{CH_3-CH_2}$= 6.7 Hz), 1.33(CH_3, t, $J_{CH_3-CH_2}$= 7.1 Hz), 2.61(CH_2, q), 3.29-3.64(CH, m), 4.05(CH_2, m), 4.26(CH_2, q)
(7)	-11.5(d, J= 7.0 Hz)	3.26-3.57(CH, m), 4.09(CH_2), 7.26-7.40(Ar-H), 10.7(CO_2H)

[a] δ ppm from external CF_3CO_2H in CCl_4.

TABLE 4

Preparation of optically active heterocycles

Substrate (product[e] No)	origin of enzyme or modified enzyme	solvent	Yield (%)	O.P.[d] %e.e.
2-aminophenol	*Trichoderma viride*[a]	H_2O	94	56
(8)	*Trichoderma viride*-C(O)(CF_3)C=CH_2[c]	hexane	57	65
	Trichoderma viride-C(O)(CF_3)C=CH_2	benzene	61	79
	Aspergillus niger[b]	H_2O	90	67
	Aspergillus niger -C(O)(CF_3)C=CH_2	hexane	48	73
	Aspergillus niger -C(O)(CF_3)C=CH_2	benzene	64	78
4-methyl-2-aminophenol	*Aspergillus niger*	H_2O	64	71
(9)	*Aspergillus niger* -C(O)(CF_3)C=CH_2	hexane	32	71
	Aspergillus niger -C(O)(CF_3)C=CH_2	benzene	54	77
2-aminothiophenol	*Trichoderma viride*	H_2O	72	36
(10)	*Trichoderma viride*-C(O)(CF_3)C=CH_2	hexane	33	61
	Trichoderma viride-C(O)(CF_3)C=CH_2	benzene	48	68

[a] Yakult Pharmaceutical Industry Co. Ltd. [b] Amano Seiyaku Co. Ltd.
[c] modified enzyme [d] The optical purities were determined by ^{19}F nmr signal intensities by commercially available (+)-tris[di(perfluoro-2-propoxypropionyl)metanate]europium(III).
[e] T. Kitazume, T. Ikeya and K. Murata, J. Chem. Soc. Chem. Commun., (1986) 1331.

TABLE 5

NMR spectral data and elemental analysis of heterocycles

Compound No	^{19}F nmr[a] δ ppm	^{1}H nmr δ ppm	Analysis: Found (Calcd) C	H	N
(8)	-12.3 (d, J_{CF_3-CH}= 8.5 Hz)	3.50-3.70 (3xH), 6.30-8.33 (5xH)	51.76 (51.96)	3.64 (3.49)	6.31 (6.06)
(9)	-11.0 (d, J_{CF_3-CH}= 8.0 Hz)	2.36 (s, Me), 2.90-3.40 (3xH), 6.40-7.50 (4xH)	54.05 (53.88)	3.85 (4.11)	6.10 (5.71)
(10)	-10.8 (d, J_{CF_3-CH}= 7.5 Hz)	2.83-3.33 (3xH), 6.33-7.67 (5xH)	48.79 (48.58)	3.46 (3.26)	5.47 (5.67)

[a] δ ppm from external CF_3CO_2H in $CDCl_3$

EXPERIMENTAL

(-)-3-Phenylthio-4,4,4-trifluorobutanoic acid (2)(nc)

A suspension of modified lipase-MY (*Candida cylindracea* - $C(O)(CF_3)=CH_2$, 10 g), ethyl 3-(trifluoromethyl)propenate (1) (1.8 g, 10 mmol) and thiophenol (2.2 g, 20 mmol) in benzene (50 ml) was stirred at 40-41°C. After 2 days of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel (5:1, hexane/ethyl acetate) to give optically active compound in a 54 % yield.

$[\alpha]_D$/MeOH -0.56 (c 0.89); 33 %e.e.

Analysis: Found : C, 48.24 ; H, 3.85 %

Calcd for $C_{10}H_9SO_2F_3$: C, 48.00 ; H, 3.63 %

(-)-Ethyl 3-diethylamino-4,4,4-trifluorobutanate (3)(nc)

A suspension of modified lipase-MY (10 g), ethyl 3-(trifluoromethyl)propenate (1.8 g, 10 mmol) and diethylamine (1.5 g, 20 mmol) in benzene (50 ml) was stirred at 40-41°C. After 2 days of stirring, workup gave the corresponding product in a yield of 47 %.

$[\alpha]_D$/MeOH -1.80 (c 1.28); 37 %e.e.

Analysis: Found : C, 49.57 ; H, 7.69 %

Calcd for $C_{10}H_{18}NO_2F_3$: C, 49.79 ; H, 7.52 %

(-)-Ethyl 3-phenylthio-2-(trifluoromethyl)propanate (5)(nc)

A suspension of modified lipase-MY (10 g) in benzene (50 ml) was stirred for 15 min at 40-41 C. Into the mixture, ethyl 2-(trifluoromethyl)propenate (2)(1.8 g, 10 mmol) and thiophenol (2.2 g, 20 mmol) was stirred for 40-41°C. After 24 h of stirring, the solvent was removed. the resulting crude products were chromatographed on silica gel (5:1, hexane/ethyl acetate) to give optically active compound in a yield of 86 %.

$[\alpha]_D$/MeOH -1.91 (c 1.89); 44 %e.e.

Analysis: Found : C, 51.86 ; H, 4.58 %

Calcd for $C_{12}H_{13}SO_2F_3$: C, 51.79 ; H, 4.71 %

(-)-Ethyl 3-diethylamino-2-(trifluoromethyl)propanate (6)(nc)

In the above system, modified lipase-MY (10 g), diethylamine (1.5 g, 20 mmol) and ethyl 2-(trifluoromethyl)-propenate (1.8 g, 10 mmol) in benzene (50 ml) were used, and then worked up similarly. the product was obtained in a yield of 82 %. $[\alpha]_D$/MeOH 3.44 (c 1.44); 35 %e.e.

Analysis: Found : C, 49.63 ; H, 7.85 %
Calcd for $C_{10}H_{18}NO_2F_3$: C, 49.79 ; H, 7.52 %

(-)-3-Phenylthio-2-(trifluoromethyl)propenoic acid (7)(nc)

A suspension of modified lipase-MY (10 g), 2-(trifluoromethyl)propenoic acid (1.4 g, 10 mmol) and thiophenol (2.2 g, 20 mmol) in benzene (50 ml) was stirred at 40-41°C. After 2 days of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel (5:1, hexane/ethyl acetate) to give optically active compound in 70 % yield. $[\alpha]_D$/MeOH -0.47 (c 1.09); 21 %e.e.

Analysis: Found : C, 48.16 ; H, 3.47 %
Calcd for $C_{10}H_9SO_2F_3$: C, 48.00 ; H, 3.63 %

Preparation of optically active heterocycles

In a typical procedure, a suspension of modified cellulase (*Trichoderma viride* - $C(O)(CF_3)C=CH_2$,10 g), 2-(trifluoromethyl)propenoic acid (1.5 g, 10 mmol) and o-aminophenol (1.3 g, 12 mmol) in benzene (50 ml) was stirred at 40-41°C. After 24 h of stirring, the solvent was removed. The resulting crude products were chromatographed over silica gel (1:1, hexane/diethyl ether) to give optically active heterocycles in the yields shown in Table 4.

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