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SYNTHESIS OF THE OPTICALLY PURE MONOETHYL ESTER OF (R)-(+)-2-FLUOROMALONIC ACID BY USE OF IMMOBILIZED LIPASE-MY FOR ASYMMETRIC HYDROLYSIS

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SUMMARY

A synthetic approach to optically pure (+)-2-fluoromalonic acid monoethyl ester was based on the enantiotopic specificity of asymmetric hydrolysis by an immobilized enzyme. The absolute configuration of monoethyl (+)-2-fluoromalonate was determined : it is the (R)-enantiomer.

INTRODUCTION

The control of the absolute stereochemistry of molecules containing a fluorine atom is of fundamental importance for the molecular design of compounds with useful biological activities [1-5]. However, in fluorine chemistry, neither determination of the absolute configuration of chiral materials nor synthetic methods giving high optical purity for a variety of versatile chiral synthetic tools, have been studied in detail.

Recently we have reported examples of microbial hydrolyses which proceeded to optically active monofluorinated materials [6,7]. In our continuing effort to develop the stereocontrolled synthesis of fluorinated compounds, we present here some results leading to a practical route to (+)-2-fluoromalonic acid monoethyl ester(2) with high optical purity (> 99 %ee), and determination of its absolute configuration.

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RESULTS AND DISCUSSION

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

We found a simple process to produce (+)-2-fluoromalonic acid monoethyl ester(2) with high optical purity (> 99 %ee) involving the asymmetric hydrolysis of 2-fluoromalonic acid diethyl ester(1) [6,7] by using an enzyme immobilized with calcium alginate [8,9].

Furthermore, it is of particular interest to compare the immobilized enzyme with native enzymes. The results shown in Table I clearly suggest a great advantage of enzymatic immobilization for the prevention of racemization under these conditions. The optical purity of (+)-2-fluoromalonic acid monoethyl ester(2) was determined by GLC after conversion of the particular malonic acid halfester sample to its diastereomeric amides by optically active (R)-(+)-methylbenzy amine. Results are shown in Fig. 1.

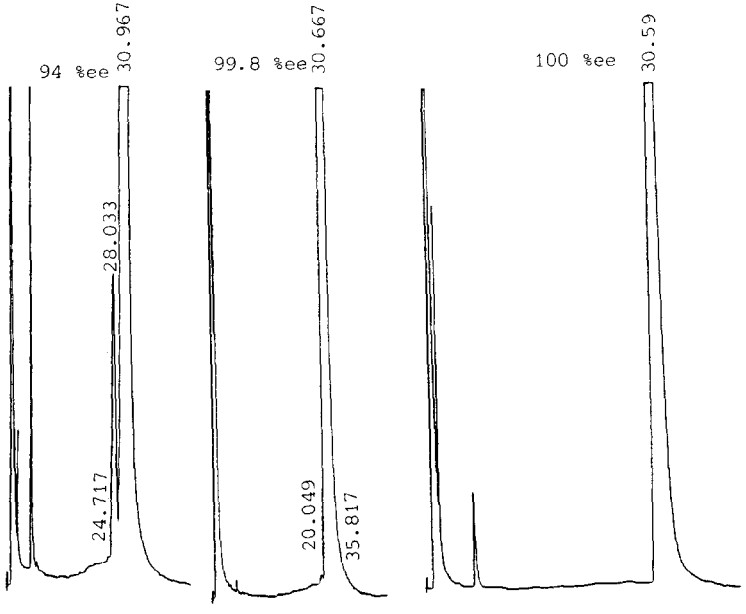


Fig. 1. Optical purity peaks.

TABLE I

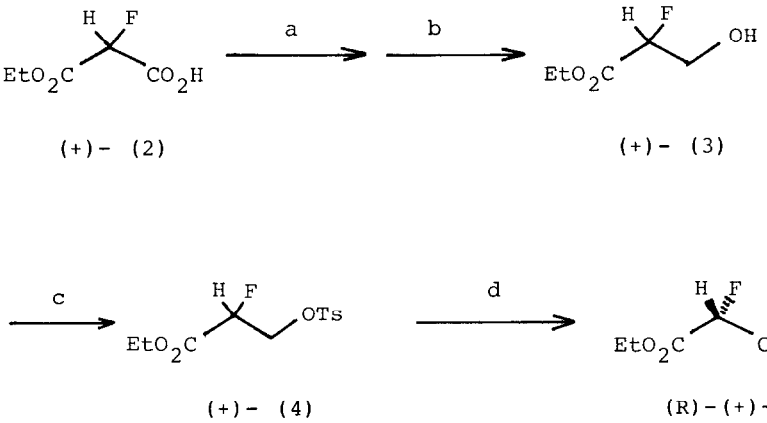
Asymmetric hydrolysis of 2-fluoromalonic acid diethyl ester(1)

Type of lipase-MY[a]	Hydrolysis Time (h)	Method[b]	Yield (%)	$_D$/MeOH	Optical purity of the Mono-ester (2) %ee
native lipase-MY	6	A	73	+8.67 (c 2.20)	62
native lipase-MY	6	B	79	+11.4 (c 1.56)	82
immobilized lipase-MY	24	B	67	+13.9 (c 2.12)	⟩99

[a] lipase-MY : *Candida cylindracea* [b] Method A : 3 g of lipase-MY/10 mmol of substrate/100 ml of buffer solution (pH=7.3) ; Method B : 7 g of lipase-MY/10 mmol of substrate/100 ml of buffer solution (pH=7.3).

Determination of the absolute configuration of (+)-2-fluoromalonic acid monoethyl ester(2)

We believe that the chiral 2-fluoromalonic acid monoethyl ester(2) made using microorganisms has much synthetic potential. To apply this methodology, we first needed to investigate the absolute configuration of the optically active half-acid(2). An outline of the synthetic strategies to achieve the desired structure through which to determine the absolute configuration, is shown in Scheme I.



a Me_2NCHO, $(COCl)_2$, CH_2Cl_2, 0°C: b $NaBH_4$, MeCN-THF, -20°C:

c TsCl, pyridine, r.t.: d $NaBH_3CN$, HMPA.

Scheme I

In the conversions given in Scheme I, the synthetic intermediate was the optically pure hydroxyester(3). The optically active (+)-2-fluoromalonic acid monoethyl ester(2) was selectively reduced with N,N-dimethylchloromethyliminium

chloride [10] and sodium borohydride to give good yields of the optically pure (+)-ethyl 2-fluoro-3-hydroxypropionate(3), $[\alpha]_D$ +4.95 (c 1.31, MeOH). This was then treated with tosyl chloride to give the compound(4) as a potential synthon. Treatment of tosylate(4) with sodium cyanoborohydride [11] gave (+)-ethyl 2-fluoropropionate(5), $[\alpha]_D$ +6.43 (c 3.05, $CHCl_3$), (lit.[12] (R)-(+)-(5) $[\alpha]_D$ +6.49 (c 1.40, $CHCl_3$)). This result shows that (+)-2-fluoromalonic acid monoethyl ester(2) prepared from 2-fluoromalonic acid diethyl ester(1) by mean of the microbial hydrolysis is the (R)-enantiomer.

The general rule based on the study of stereoselective hydrolysis of symmetrical diesters with Pig Liver Esterase by Tamm and co-worker's also supports the allocation of structure as the (R)-enantiomer [13].

The microbial approach to the new monofluorinated chiral synthon developed here may open up a new avenue for making biologically active compounds containing a fluorine atom.

EXPERIMENTAL

General procedure. All microbial transformations were carried out in a Jarfermentor (M-100, Tokyo Rikakikai Co.Ltd.). All commercially available reagents were used without further purification. Infrared spectra were obtained by using a JASCO A-102 spectrometer and KBr pellets. The ^1H(internal Me_4Si) and ^{19}F(external CF_3CO_2H) NMR spectra were recorded by using a Varian EM-390 and a Hitachi R-24F spectrometers. Mass spectra were obtained by using a Hitachi M-52 spectrometer at 20 eV. 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 cholesterol(0.93 g) in 10 % aq. calcium chloride (50 ml) was stirred for 10 min at room temperature. Into the mixture, sodium alginate (7.0 g) in water

(100 ml) was added slowly, and then the whole was stirred for 1h at room temperature. Immobilized lipase-MY was collected by filtration and then washed with water.

(R)-(+)-2-Fluoromalonic acid monoethyl ester(2)(nc)

A mixture of the above prepared immobilized lipase-MY and 2-fluoromalonic acid diethyl ester(1) [6,7] (1.8 g, 10 mmol) in buffer solution(50 ml, PH 7.3), which are 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 Jarfermentor. After 24h of stirring, the mixture was acidified with 1N HCl, and then the immobilized lipase-MY was separated by filtration. 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 (+)-2-fluoromalonic acid monoethyl ester(2) in a 70 % yield, bp 103-106°C/0.4 mmHg, $[\alpha]_D$ +13.9 (c 2.12, MeOH), >99 %ee.
IR (cm^{-1}) : 1770 (C=O).
Anal. Found : C, 40.38 ; H, 4.65 %
Calcd for $C_5H_7O_4F$: C, 40.00 ; H, 4.70 %

Determination of optical purity

A mixture solution of 1-methyl-2-chloropyridinium iodide(0.31 g, 1.2 mmol), (R)-(+)-2-fluoromalonic acid monoethyl ester(0.16 g, 1.0 mmol), $[\alpha]_D$ +13.9 (c 1.56, MeOH), triethylamine(0.24 g, 2.4 mmol) and (R)-(+)-methylbenzylamine, α_D +37.6 (neat), in methylene chloride (10 ml) was heated at 40°C. After heating for 2h, the whole mixture was poured into water, and then oily materials were extracted with diethyl ether. The ethereal layer was washed with 1N HCl solution, 5% aq.$NaHSO_4$, sat.$Na_2S_2O_3$ solution and then brine. After removing the solvent, the diastereomeric ratio was determined by GLC(carrier gas N_2, Flow 20 ml/min, column 3 mm x 3 m packed by Silicone GEXE-60 on Chromosorb W) at 200°C.

(R)-(+)-Ethyl 3-hydroxy-2-fluoropropionate (3)(nc)

After a mixture solution of N,N-dimethylformamide(3.4 ml) and oxalyl chloride(8 ml) in methylene chloride(40 ml) had been stirred for 1h at 0°C, the solvent was removed under dynamic vacuum. Into the reaction vessel, acetonitrile(30 ml) and tetrahydrofuran(100 ml) were added with a syringe under an atmosphere of nitrogen, and then (R)-(+)-2-fluoromalonic acid monoethyl ester(2)(6.5 g, 40 mmol, >99 %ee) was added at-30°C. After 1h of stirring at -30°C, a solution of sodium borohydride (3.5 g, 93 mmol) in N,N-dimethylformamide(20 ml) was added slowly at -78°C. The reaction mixture was stirred for 4h at -20°C and quenched with 3N HCl(50 ml). Oily materials were extracted with ethyl acetate, and the organic layer was washed with 1N HCl, 5% aq.$NaHCO_3$, water and brine. On removal of the solvent, distillation gave (R)-(+)-ethyl 3-hydroxy-2-fluoropropionate (3)(2.8 g, 2.1 mmol) in a yield of 51 %, bp 80-83°C/11 mmHg,
$[\alpha]_D$ +4.95 (c 1.31, MeOH), >99 %ee.
IR (cm^{-1}) : 1750 (C=O)
Anal. Found : C, 44.04 ; H, 6.87 %
Calcd for $C_5H_9O_3F$: C, 44.12 ; H, 6.67 %

(R)-(+)-Tosylate (4)(nc)

A mixture of (+)-ethyl 3-hydroxy-2-fluoropropionate (3) (1.1 g, 8.1 mmol) and tosyl chloride(1.7 g, 9.0 mmol) in pyridine(20 ml) was stirred at room temperature. After 3h of stirring, the whole mixture was poured into water, and then oily materials were extracted with ethyl acetate. Tosylate(4) was purified by column chromatography on silica gel using a mixture of n-hexane-diethyl ether (5:1) as eluent,
in 93 % yield, $[\alpha]_D$ +2.90 (c 1.26, MeOH).
IR (cm^{-1}) : 1765 (C=O)
Anal. Found : C, 49.21 ; H, 5.64 %
Calcd for $C_{12}H_{15}SO_5F$: C, 49.65 ; H, 5.21 %

(R)-(+)-Ethyl 2-fluoropropionate (5)

Into a solution of sodium cyanoborohydride(0.92 g, 14.7 mmol) in freshly dried hexamethylphosphoramide (15 ml), tosylate (4)(0.58 g, 2 mmol) in hexamethylphosphoramide(5 ml) was added slowly at room temperature. After 1 day of stirring at 60-70°C, the reaction mixture was allowed to warm to 130-140°C, and then (R)-(+)-ethyl 2-fluoropropionate was collected by trap to trap system under dynamic vacuum in 25 % yield, $[\alpha]_D$ +6.43 (c 3.05, $CHCl_3$).

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