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ENANTIOSELECTIVE SYNTHESIS OF MONOFLUORINATED MALONIC ACID MONOESTERS WITH ENZYMES OF MICROBIAL ORIGIN

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The optically active (+)- and (-)-2-fluoro-2-substituted malonic acid monoesters useful as the practical chiral synthon were obtained by the microbial hydrolysis of the corresponding malonic acid diesters with esterases or cellulases.

The current study in fluorine chemistry reflects the increasing interest in the molecular design concerning the biological activities.[1-4] However, no stereocontrolled synthesis of fluorinated compounds with high optical purity, considered to be a key process to achieve the above purpose, has been reported except for few approaches to suicide inactivator[5-8] and asymmetric synthesis.[9-14] We have found that microbial transformation can provide with a useful synthetic technique to give a potential solution for designing the optically active fluorinated compounds.[15-17] The microbial approach to the new chiral synthons of monofluorinated compounds developed here may open up a new avenue for the biologically active compounds containing fluorine atom.

$$RCF(CO_2R')_2 \longrightarrow RCF(CO_2H)CO_2R'$$

Attempted microbial hydrolysis of 2-fluoro-2-substituted malonic acid diesters with several kinds of esterases or cellulases gave the optically active (+)- or (-)-2-fluoro-2-substituted malonic acid monoesters. Such asymmetric hydrolysis of prochiral compounds with enzymes of microbial or animal origin has been extensively studied recently.[18-23]

Furthermore, it is of particular interest to compare fluorine with other halogens or alkyl groups, which is possible to confirm the mimic effect of

fluorine atom.[24)]

The results shown in Table 1 clearly suggest the mimic effect of fluorine atom in the hydrolysis by microorganisms and a great advantage of fluorine atom for the no racemization under these conditions.

The asymmetric hydrolysis by esterase (*Candida Cylindraceae*) proceeded smoothly to afford the (-)-2-fluoro-2-methyl[or (-)-2-fluoro-2-ethyl] malonic acid monoesters, and that by cellulase (*Trichoderma Viride*) afforded the enantiomer, (+)-2-fluoro-2-methyl[or (+)-2-fluoro-2-ethyl] malonic acid monoesters. However, in the case of 2-fluoro malonic acid diesters, both esterase and cellulase gave only the (+)-2-fluoro malonic acid monoesters. These results clearly demonstrate that the new synthetic approaches are useful for the design of the desired monofluorinated chiral molecules, which represent extension of the syntheses of a wide variety of fluorinated and biologically active compounds.

Furthermore, no explanation for the dependence of the chiral half-esters on the enzymes of microbial origin is available at the present time and the detailed study will be necessary in the future.

Present microbial hydrolysis based on the utility of the mimic effect of fluorine atom is considered to be the most convenient one-step process for preparing the practical monofluorinated chiral synthons.

In a typical procedure, a suspension of lipase-MY (*Candida Cylindraceae*, Meito Sangyo Co. Ltd., 30 g) in buffer solution (600 ml, PH 7.3), which are prepared from 1/15 M aq. Na_2HPO_4 solution (460.8 ml) and 1/15 M aq. KH_2PO_4 solution (139.2 ml), was stirred for 15 min at 40-41 °C in Jarfermentor (M-100, Tokyo Rikakikai Co. Ltd.). Into the mixture, ethyl 2-fluoro-2-methyl malonate (20 g, 104 mmol) was added, and then the whole mixture was stirred at 40-41 °C. After 6 h with stirring, the flocculant (200 ppm solution prepared from p-713, Dai-ichi Kogyo Seiyaku, 100 ml) was added into the stirring mixture for a few minute. After 1 h with standing, the mixture acidified with 3% HCl and then the precipitates were 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 the corresponding malonic acid monoester in a 87% (14.8 g) yield, bp 88-89 °C/0.6 mmHg.

Table 1. Asymmetric Hydrolysis

Substrate	Origin of enzyme	Method	Yield %	Bp θ_m/°C(mmHg)	α_D/MeOH	Optical purity %ee[i]
$MeCF(CO_2Et)_2$	*Candida Cylindraceae*[b]	A[g]	87	88-89(0.6)	-20.9(c 2.81)	91
	Candida Cylindraceae	B[h]	60		-20.6(c 1.95)	91
	Porcine Pancreas[c]	B	23		-12.0(c 1.20)	61
	Aspergillus sp.[d]	B	80		-6.46(c 1.11)	25
	Trichoderma Viride[e]	A	60		+13.1(c 2.24)	56
$MeCF(CO_2Me)_2$	*Candida Cylindraceae*	A	74	98-101(2)	-23.2(c 2.84)	95
	Trichoderma Viride[e]	A	83		+12.5(c 2.70)	46
$CHF(CO_2Et)_2$	*Candida Cylindraceae*	A	70	100-102(1)	+8.67(c 2.20)	62
	Trichoderma Viride[e]	A	74		+5.33	38
	Trichoderma Viride[f]	A	51		+8.12	58
$EtCF(CO_2Et)_2$	*Candida Cylindraceae*	A	87	94-95(0.7)	-14.0(c 1.86)	93
	Candida Cylindraceae	B	62		-13.8(c 2.08)	93
	Trichoderma Viride[e]	A	no reaction			
	Trichoderma Viride[f]	A	no reaction			
$EtCF(CO_2Me)_2$	*Candida Cylindraceae*	A	87	105-108(4)	-18.1(c 1.97)	99
	Trichoderma Viride[e]	A	no reaction			
$n\text{-}PrCF(CO_2Et)_2$	*Candida Cylindraceae*	B	30	108-111(1)	-2.90(c 1.51)	33
$n\text{-}BuCF(CO_2Et)_2$	*Candida Cylindraceae*	A	78	93-97(0.7)	-1.53(c 2.26)	11
$MeCCl(CO_2Et)_2$	*Candida Cylindraceae*	B	no reaction			
$MeCBr(CO_2Et)_2$	*Candida Cylindraceae*	B	no reaction			
$CF_3CH(CO_2Me)_2$	*Candida Cylindraceae*	B	no reaction			

a) Each structure was determined by means of IR, NMR and mass spectral data.
b) Meito Sangyo Co.Ltd. c) Sigma Co.Ltd. d) Amano Seiyaku Co.Ltd. e) Yakult Pharmaceutical Industry Co.Ltd. f) Meijiseika Co.Ltd. g) Method A : Substrate (10 mmol)/Lipase or Cellulase(3 g)/Buffer solution(100 ml)/6 h. h) Method B : Substrate(10 mmol)/Lipase or Cellulase(2 g)/Buffer solution(50 ml)/3 h.
i) The optical purities were determined by glc and/or ^{19}F NMR after conversion of the malonic acid monoesters to their diastereomeric amides by optically active α-methylbenzylamine.

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