Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 3047–3052

TETRAHEDRON: *ASYMMETRY*

Resolution of *cis*-2-fluorocyclopropanecarboxylic acid by a microbial enantioselective hydrolysis

Akihiro Imura,* Motohiro Itoh and Akihiko Miyadera

Chemical Technology Research Laboratories, Daiichi Pharmaceutical Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

Received 29 June 1998; accepted 3 August 1998

Abstract

The important key intermediate of quinolone analogue synthesis, (1*S*,2*S*)-2-fluorocyclopropanecarboxylic acid, was prepared enantioselectively by a microbial resolution. One of the strains with the highest enzymatic specificity was selected from soil and when lyophilized cells were treated with corresponding ester, the remaining (1*S*,2*S*)-ester was obtained with high enantiomeric purity (98% *e.e.*).

1. Introduction

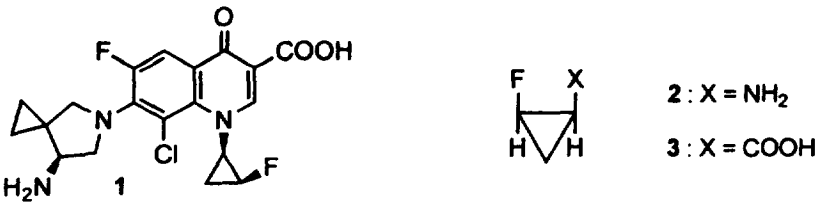
Utilization of optically active fluoroorganic compounds in biological and medicinal chemistry[1] continues to expand and requires the development of a method of synthesis with high stereoselectivity, because of the unique physical and biological features resulting from fluorine. Hayakawa et al. discovered 7-[7(*S*)-7-amino-5-azaspiro[2.4]heptan-5-yl]-8-chloro-6-fluoro-1-quinolinecarboxylic acid **1**,[2] a new-generation antibacterial quinolone carboxylic acid with broad-spectrum antibacterial activity. A number of syntheses of the important key intermediate, *cis*-2-fluorocyclopropylamine **2** and *cis*-2-fluorocyclopropanecarboxylic acid **3**, have been reported (Scheme 1).[3] Although a few methods of resolution of **3** have also been reported, an expensive chiral reagent is used[4] and chromatography is required for the separation of diastereomers.[5] Therefore, practical access to (1*S*,2*S*)-**3** is limited in the above two syntheses.

On the other hand, an enzymatic approach to the synthesis of monofluorinated compounds has been reported.[6] Furthermore, it is particularly interesting to compare fluorine with other halogens or alkyl groups, to confirm the mimic effect of the fluorine atom.[7]

In this paper, we describe, as shown in Scheme 2, the first synthesis of the key chiral compound **7** using a microbial resolution.

* Corresponding author. E-mail: imuraycm@daiichipharm.co.jp

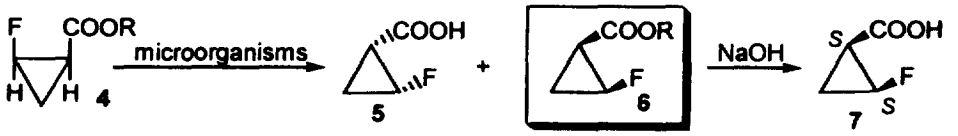
0957-4166/98/$ - see front matter
PII: S0957-4166(98)00321-8



Scheme 1.

2. Results and discussion

The synthesis of *cis*-ethyl-2-fluorocyclopropanecarboxylate **4a** was achieved by Kusuyama's route,[8] derived from the cyclopropanation of butadiene with bromofluorocarbene as the intermediate. We selected this *cis*-ethyl ester **4a** as the substrate for attempted resolution by a microbial enantioselective hydrolysis.



a : R = -CH_2CH_3, b : R = -$CH_2CH_2CH_3$
c : R = -$CH_2(CH_2)_2CH_3$, d : R = -$CH_2(CH_2)_3CH_3$
e : R = -$CH_2(CH_2)_4CH_3$, f : R = -$CH_2(CH_2)_6CH_3$

Scheme 2.

In our first trial of an enantioselective hydrolysis reaction, about 100 commercially available enzymes such as esterases, lipases and proteases were screened for their ability to hydrolyze the ester **4a**. Although many enzymes especially esterases and lipases hydrolyzed **4a**, they had poor specificity, and the acid **5** was formed as a racemate. We therefore attempted to hydrolyze **4a** by use of microorganisms. A few strains from cultures were found to afford good enantiomeric excess of acetate **6a**. However, the configuration of acetate was *R*, and the *e.e.* value of the (*S*)-acid, which is the desirable configuration, was only 27–68% (runs 1–4, Table 1). We directed our efforts to obtain the highest *e.e.* of the (*S*)-acetate. One of the strains, which was selected from soil, exhibited better enantiomeric discrimination, and hydrolyzed the (*R*)-enantiomer. We found the DSC 4011 strain yielded good to excellent *e.e.* of the (*S*)-acetate in a reasonable time (run 5).

The microbial reaction was applied to other aliphatic esters **4b–4f** to study the effect of chain length on reaction rate and enantioselectivity. The reaction was monitored by GC analysis and stopped when *ca.* 55% conversion was achieved. As shown in Table 2, **4b** was the most reactive substrate for the DSC 4011 among the six substrates examined, and longer reaction times were required for substrates with longer chains. The reaction time required to achieve 54% conversion was 144 h for **4e**, and 10 times longer than that for **4b**. For **4f**, the conversion ratio was only 24% at 144 h and never reached 50%, even after a prolonged reaction time. For **4a–4e**, excellent *e.e.* values of the (*S*)-ester were obtained. Therefore the chain length of the alkyl group was found to have little effect on enantioselectivity.

Table 1
Screening of enzymes to catalyze hydrolysis of racemate **4a**

run[a]	strain[b]	conv.[c] (%)	acid **5**		acetate **6a**		E[e] value
			e.e.(%)	config.	*e.e.*(%)	config.	
1	*Brevibacterium* sp. (IAM 1637)	90[d]	-	-	-	-	-
2	*Bacillus* sp. (IFO 3330)	58	28	(1*S*,2*S*)	52	(1*R*,2*R*)	2.8
3	*Pseudomonas* sp. (IFO 3454)	63	27	(1*S*,2*S*)	81	(1*R*,2*R*)	3.9
4	*Bacillus* sp. (IAM 11064)	52	68	(1*S*,2*S*)	73	(1*R*,2*R*)	11
5	*Micrococcus* sp. (DSC 4011)[f]	56	76	(1*R*,2*R*)	98	(1*S*,2*S*)	36

[a] 10mg **4a**, 2mg lyophilized cells, 0.1M phosphate buffer pH7.0 (1ml) at 40℃-24hr.
[b] About 500 strains of type cultures were tested in runs 1-4.
[c] Conversion ratio was determined from %*e.e.* of the acids and the esters.
[d] Conversion ratio was determined by GC.
[e] This enantiomeric ratio E was calculated from the equation : $E = \ln\{(1-c)[1-e.e.(s)]\}/\ln\{(1-c)[1+e.e.(s)]\}$.[9]
[f] DSC 4011 strain was isolated from soil, and was identified as *Micrococcus* sp.

Table 2
Effect of the chain length of alkyl group **4a–4f**

substrate[a]	reaction time (h)	conv.[b] (%)	acid **5**		esters **6a-f**		E[d] value
			e.e.(%)	config.	*e.e.*(%)	config.	
4a	42	55	77	(1*R*,2*R*)	98	(1*S*,2*S*)	38
4b	14	56	78	(1*R*,2*R*)	98	(1*S*,2*S*)	37
4c	20	55	79	(1*R*,2*R*)	96	(1*S*,2*S*)	33
4d	48	57	74	(1*R*,2*R*)	97	(1*S*,2*S*)	27
4e	144	54	80	(1*R*,2*R*)	95	(1*S*,2*S*)	33
4f	144	24[c]	-	-	-	-	-

[a] 100mM **4a-f**, 15mg lyophilized cells (DCS 4011), 0.1M phosphate buffer pH 7.0 (10ml) at 40℃.
[b] Conversion ratio was determined from %*e.e.* of acids and esters.
[c] Conversion ratio was determined by GC.
[d] This enantiomeric ratio E was calculated from the equation : $E = \ln\{(1-c)[1-e.e.(s)]\}/\ln\{(1-c)[1+e.e.(s)]\}$.[9]

3. Conclusion

We have demonstrated a highly enantioselective preparation of the optically active 2-fluorocyclopropanecarboxylic acid **7** using a microbial hydrolysis with the corresponding esters. We are interested in the chiral discrimination of the fluorine atom, and the substrate specificity of other cyclopropyl compounds by the purified enzyme from the DSC 4011. These studies are in progress.

4. Experimental section

4.1. General procedures

Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared spectra were recorded on an FT-720 spectrometer (Horiba). ^{1}H-NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) instrument. Coupling constants are reported in hertz (Hz) and chemical shifts in ppm downfield from internal TMS. ^{19}F-NMR spectra were recorded on a JEOL JNM-GSX500 (500 MHz) instrument and the values are reported on the δ scale relative to hexafluorobenzene as an internal standard. Mass spectra were recorded on a JEOL JMS-HX110 or JMS-AX505W mass spectrometer. Optical rotations were measured with a SEPA-300 polarimeter (Horiba). All chemicals were obtained from commercial sources and were used without further purification.

4.2. Microorganisms, media and culture conditions

Bacteria were obtained from culture collections, except for the *Micrococcus* sp. (DSC 4011). This was selected from soil, cultivating with *cis*-ethyl-2-fluorocyclopropanecarboxylate **4a** as the sole carbon source at 30°C for 7 days. The identification of bacteria (DSC 4011) was based on Bergey's Manual of Determinative Bacteriology. Cultures were performed with a reciprocating shaker (150 rpm/min) on the following medium; pepton (10 g/L), meat extract (3 g/L), NaCl (5 g/L) at pH 7.0 after sterilization (120°C, 15 min). At the late exponential growth phase, the cells were harvested by centrifugation (3000×*g*), resuspended in phosphate buffer (50 mM, pH 7.0), centrifuged again and lyophilized. The cells were stored for several months at 5°C without significant loss of activity.

4.3. Measurement of enantiomeric purity

Enantiomeric excesses were analyzed by GLC (Shimadzu GC-14A) on a CP cyclodextrin-β-236M capillary column (0.25 mm×25 m, Chrompack Co., Ltd.) with flame ionization detection, after derivatization of the carboxylic acid **5** to the methyl ester by diazomethane, at an oven temperature of 70°C. A typical separation is illustrated in Fig. 1.

4.3.1. cis-*Ethyl-2-fluorocyclopropanecarboxylate* ***4a***

cis-2-Fluorocyclopropanecarboxylic acid (12.0 g, 115 mmol) was dissolved in ethanol (150 mL), and sulfuric acid (0.5 g) was added to the solution. The mixture was refluxed for 8 h, and evaporated *in vacuo*. The residue was extracted with dichloromethane (150 mL), and washed with water (3×100 mL). The organic layer was dried and distilled *in vacuo* to obtain **4a** as a colorless liquid (13.7 g, 90.3%). Bp 49–51°C (24 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 1.04–1.18 (m, 1H), 1.29 (t, J=7.3 Hz, 3H), 1.74–1.89 (m, 2H), 4.18–4.26 (q, J=7.3 Hz, 2H), 4.73 (dm, J=70 Hz); ^{19}F-NMR ($CDCl_3$) δ: −59.4 (m, CH*F*); m/z 133 (M^++1).

4.3.2. cis-*Propyl-2-fluorocyclopropanecarboxylate* ***4b***

Yield 92.9%; bp 78–80°C (24 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 0.96 (t, J=7.6 Hz, 3H) 1.01–1.20 (m, 1H), 1.29 (q, J=7.6 Hz, 3H), 1.74–1.89 (m, 2H), 4.18–4.26 (q, J=5.9 Hz, 2H), 4.73 (dm, J=70 Hz); ^{19}F-NMR ($CDCl_3$) δ: −59.3 (m, CH*F*); m/z 147 (M^++1)

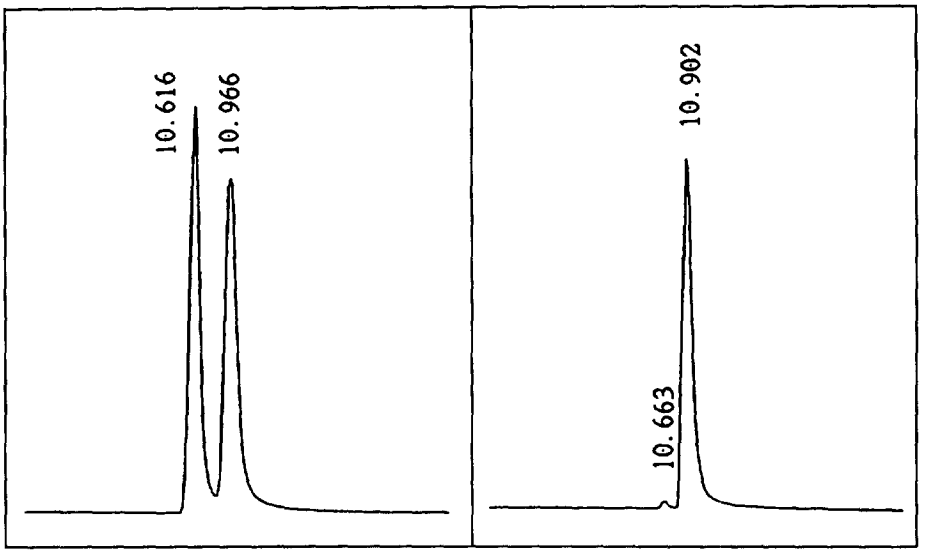


Fig. 1. GC of methyl ester of **7**, (±)-form (left) or (+)-form from microbial resolution (right)

4.3.3. cis-*Butyl-2-fluorocyclopropanecarboxylate* ***4c***

Yield 90.8%; bp 68–70°C (15 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 0.94 (t, J=7.6 Hz, 3H), 1.04–1.23 (m, 1H), 1.30–1.69 (m, 4H), 1.73–1.92 (m, 2H), 4.15 (t, J=6.6 Hz, 2H), 4.74 (dm, J=70 Hz); ^{19}F-NMR ($CDCl_3$) δ: −59.2 (m, CH*F*).

4.3.4. cis-*Pentyl-2-fluorocyclopropanecarboxylate* ***4d***

Yield 89.8% (purified by silica); ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, J=7.6 Hz, 3H), 1.02–1.19 (m, 1H), 1.29–1.72 (m, 6H), 1.74–1.93 (m, 2H), 4.13 (t, J=6.9 Hz, 2H), 4.74 (dm, J=69 Hz).

4.3.5. cis-*Hexyl-2-fluorocyclopropanecarboxylate* ***4e***

Yield 87.6% (purified by silica); ^{1}H-NMR ($CDCl_3$) δ: 0.90 (t, J=7.4 Hz, 3H), 1.01–1.18 (m, 1H), 1.30–1.89 (m, 10H), 4.14 (t, J=6.8 Hz, 2H), 4.74 (dm, J=70 Hz)

4.3.6. cis-*Octyl-2-fluorocyclopropanecarboxylate* ***4f***

Yield 83.6% (purified by silica); ^{1}H-NMR ($CDCl_3$) δ: 0.88 (t, J=7.4 Hz, 3H), 1.03–1.17 (m, 1H), 1.31–1.94 (m, 14H), 4.13 (t, J=6.8 Hz, 2H), 4.75 (dm, J=69 Hz).

4.4. Microbial hydrolysis of ***4b***

Lyophilized microbial cells (DSC 4011, 2.0 g) were suspended in phosphate buffer (500 mL, 0.1 M, pH 7.0) and stirred for 15 min at 40°C. *cis*-Propyl-2-fluorocyclopropanecarboxylate **4b** (20.0 g, 136 mmol) was then added and the whole mixture was stirred at 40°C, while the reaction was monitored by GC. As the pH value of the mixture was decreased by formed acid, it was maintained at pH 7.0 by the addition of 10% aqueous sodium hydroxide with an autotitrator. After an appropriate degree of conversion (0.55) was reached (14 h), ethyl acetate was added (400 mL) and the cells were filtered through Celite. The aqueous layer of the filtrate was extracted with ethyl acetate. The combined organic layers were washed with water, dried and evaporated *in vacuo*. The residue was dissolved in 50% methanol (100 mL) and sodium hydroxide (3.2 g, 80 mmol) was added at 0°C. The mixture was left at room temperature (2

h) then evaporated to half volume, and adjusted to pH 2 with 10% hydrochloric acid, and extracted with ethyl acetate. The organic layer was evaporated *in vacuo* to obtain a colorless solid of (1*S*,2*S*)-2-fluorocyclopropanecarboxylic acid **7** (5.95 g, 38.0%, 98% *e.e.*). $[\alpha]_D^{25}$ +23.0 (*c*=0.50, $CHCl_3$). The solid was crystallized from toluene to obtain colorless crystals (>99% *e.e.*). Mp 62–63°C; ^{1}H-NMR ($CDCl_3$) δ: 1.15–1.32 (m, 3H), 1.56–1.98 (m, 2H), 4.76 (dm, J=69 Hz, 1H), 8.76 (brs, 1H); ^{19}F-NMR ($CDCl_3$) δ: −58.6 (m, CH*F*); m/z 104 (M^+); IR (KBr) 3210, 1727, 1439 and 1190 cm^{-1}; anal. calcd for $C_4H_5FO_2$: C, 46.16; H, 4.84; F, 18.26. Found: C, 46.17; H, 4.80; F, 18.23. The aqueous layer of the filtrate was adjusted to pH 2 and extracted with ethyl acetate. The organic layer was evaporated *in vacuo* to obtain a colorless solid of (1*R*,2*R*)-2-fluorocyclopropanecarboxylic acid **5** (8.45 g, 54.1%, 78% *e.e.*). $[\alpha]_D^{25}$ −18.9 (*c*=0.50, $CHCl_3$). The solid was crystallized from toluene to obtain colorless crystals (84% *e.e.*). Mp 59–61°C; ^{1}H-NMR ($CDCl_3$) δ: 1.15–1.32 (m, 3H), 1.56–1.98 (m, 2H), 4.76 (dm, J=69 Hz, 1H), 8.76 (brs, 1H); ^{19}F-NMR ($CDCl_3$) δ: −58.6 (m, CH*F*); m/z 104 (M^+); IR (KBr) 3210, 1724, 1440 and 1192 cm^{-1}; anal. calcd for $C_4H_5FO_2$: $C_4H_5FO_2$: C, 46.16; H, 4.84; F, 18.26. Found: C, 46.27; H, 4.82; F, 18.20.

References

1. (a) Welch, J. T. *Tetrahedron* **1987**, *43*, 3123–3197. (b) Resnati, G. *Tetrahedron* **1993**, *49*, 9385–9445.
2. (a) Hayakawa, I.; Atarashi, S.; Kimura, Y.; Kawakami, K.; Saito, T.; Yafune, T.; Sato, K.; Une, K.; Sato, M. 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 1991; Abstract No. 1504. (b) Atarashi, S.; Imamura, M.; Kimura, Y.; Yoshida, A.; Hayakawa, I. *J. Med. Chem.* **1993**, *36*, 3444–3448.
3. (a) Tamura, O.; Hashimoto, M.; Kobayashi, Y.; Katoh, T.; Nakatani, K.; Kamada, M.; Hayakawa, I.; Akiba, T.; Terashima, S. *Tetrahedron Lett.* **1992**, *33*, 3483–3486. (b) Tamura, O.; Hashimoto, M.; Kobayashi, Y.; Katoh, T.; Nakatani, K.; Kamada, M.; Hayakawa, I.; Akiba, T.; Terashima, S. *Tetrahedron* **1994**, *50*, 3889–3904. (c) Akiba, T.; Tamura, O.; Hashimoto, M.; Kobayashi, Y.; Katoh, T.; Nakatani, K.; Kamada, M.; Hayakawa, I.; Terashima, S. *Tetrahedron* **1994**, *50*, 3905–3914. (d) Toyota, A.; Ono, Y.; Kaneko, C.; Hayakawa, I. *Tetrahedron Lett.* **1996**, *37*, 8507–8510.
4. Yukimoto, Y.; Ebata, T.; Kaneuchi, T.; Kawanishi, H. *Japan Kokai Tokkyo Koho* **1993**, JP5-255180.
5. Kimura, Y.; Atarashi, S.; Kawakami, K.; Sato, K.; Hayakawa, I. *J. Med. Chem.* **1994**, *37*, 3344–3352.
6. (a) Kitazume, T.; Sato, T.; Ishikawa, N. *Chemistry Lett.* **1984**, 1811–1814. (b) Bucciarelli, M.; Forni, A.; Moretti, I.; Prati, F.; Torre, G.; Resnati, G.; Bravo, P. *Tetrahedron* **1989**, *45*, 7505–7514. (c) Goj, O.; Burchardt, A.; Haufe, G. *Tetrahedron: Asymmetry* **1997**, *8*, 399–408.
7. (a) Kobayashi, Y.; Kumadaki, I. *Kagaku* **1977**, *32*, 538–544. (b) Kitazume, T.: Ishikawa, T.: Taguchi, T. *Chemistry of Fluorine*, Kodansha Scientific, Tokyo, 1993.
8. Kusuyama, Y.; Tokami, K.; Nakanishi, W.; Negoro, T.; Ikeda, Y. *Bull. Fac. Edu. Wakayama Univ. Nat. Sci.* **1984**, *33*, 33–38.
9. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7298.