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Enzymatic Resolution of 2-Acyl-3-hydroxymethyl-4-butanolide and Preparation of Optically Active IM-2, the Autoregulator from *Streptomyces* sp. FRI-5

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Abstract : Racemic 2-acyl-3-hydroxymethyl-4-butanolides were resolved through a lipase-catalyzed acylation with acetic anhydride. Optically active forms of 2-butyryl-3-hydroxymethyl-4-butanolide **14** obtained were used to prepare enantiomers of IM-2 **10**, the autoregulator from *Streptomyces* sp. FRI-5. The absolute configuration of IM-2 was deduced to be (2R,3R,6R).

In *Streptomyces*, a number of butyrolactone autoregulators which control cytodifferentiation or secondary metabolites production are known, such as A-factor **1**[1], factor-1 **2**[2], Gräfe's factors **3**, **4** and **5**[3], virginiae butanolides (VB) A~E **3**, **6**, **7**, **8** and **9**[4], and IM-2 **10**[5]. These molecules have a common 2,3-disubstituted butanolide skeleton but differ in the C-2 side chain. All of autoregulators which have a C-6 hydroxyl group possess a 2,3-*trans* configuration, but stereochemistry of a C-6 hydroxyl group is different among them as shown in Fig. 1[6]. The absolute configurations of A-factor **1** and VBs (A, B and C **3**, **6** and **7**) have been assigned to (3R) and (2R, 3R, 6S), respectively, with their chiral synthesis from (S)-paraconic acid being carried out by Mori *et al.*[7], but those of other molecules are not determined as yet. Since autoregulators of *Streptomyces* are usually produced in trace amounts in culture broths, synthetic samples are very important to investigate their functions. 2-Acyl-3-hydroxymethyl-4-butanolides, one of which is A-factor **1**, are synthetic intermediates of autoregulators with a C-6 hydroxyl group[7] and also biosynthetic precursors of them[8]. Recently, in order to study the substrate stereospecificity of the enzyme concerning the reduction from 3-hydroxymethyl-2-(5-methylhexanoyl)-4-butanolide **11** to VB-A **3**, we have attempted to prepare optically active forms of **11** by resolution of its racemate. As a result, enantiomers of **11** each having high optical purity have been obtained using a lipase-mediated acylation. In this paper, we describe the enzymatic resolution of **11** and its congeners, and its application to a preparation of enantiomers of IM-2 **10**, which led to determine the absolute configuration of **10** as (2R, 3R, 6R).

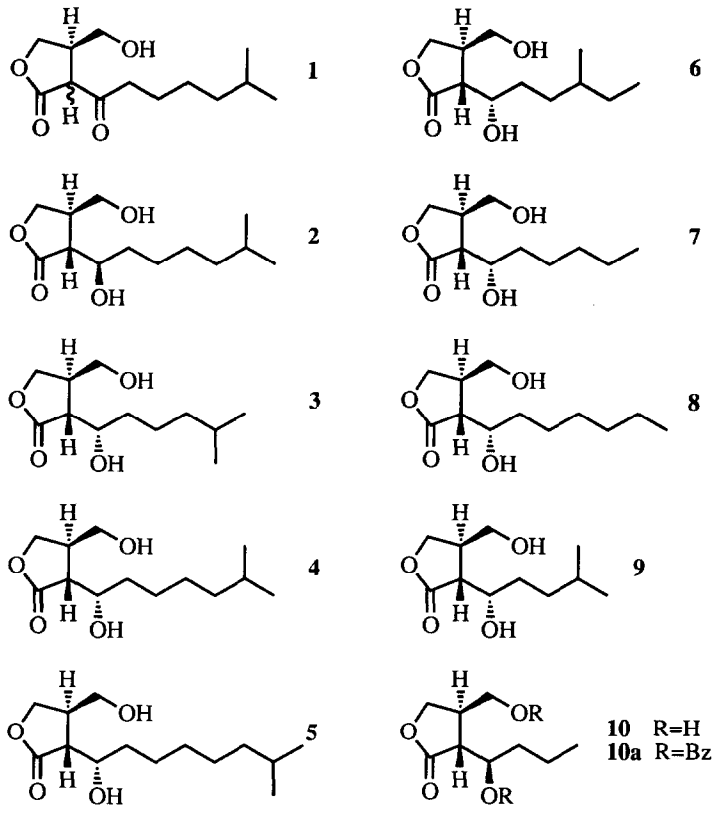


Fig.1 Structures of Autoregulators from *Streptomyces*.

Enzymatic Resolution of 2-Acyl-3-hydroxymethyl-4-butanolides.

Resolution of racemic **11** was attempted by an enzyme-catalyzed acetylation in organic solvent. We screened lipases using acetic anhydride as the acylating agent and benzene as the organic solvent[9]. When the conversion from **11** to **11a** reached ca. 50%, which was monitored by TLC, the reaction was stopped, and the acetate **11a** along with the unreacted **11** were isolated from the reaction mixture by preparative TLC. The optical rotation value of each compound was measured to evaluate the optical purity. By the screening against 21 commercially available lipases, lipase L-10 and olipase 4SD were found to afford (-)-**11** and (+)-**11a** which have high optical rotation values (Table 1). Lipase L-10 gave (-)-**11** in higher optical purity than olipase 4SD, but the optical rotation value of (+)-**11a** obtained by olipase 4SD was higher than that obtained by lipase L-10 (Table 1).

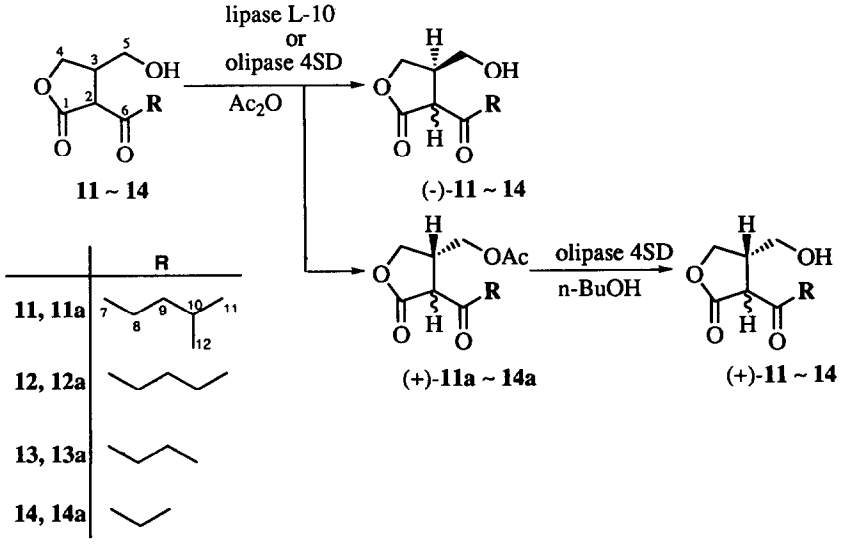
Table 1. Enzymatic Resolution of **11** ~ **14**

Substrate	Enzyme	Time (hr)	(-)-**11** ~ **14**			(+)-**11a** ~ **14a**	
			Yield (%)	$[\alpha]_D^{22}$	ee% [a]	Yield (%)	$[\alpha]_D^{22}$
11	lipase L-10	2	22	-14°	>97	52	+11°
11	olipase 4SD	3.5	44	-11°	_ [b]	34	+21°
12	lipase L-10	2	25	-12°	88	49	+10°
12	olipase 4SD	5	38	-12°	-	31	+22°
13	lipase L-10	2	36	-11°	-	49	+11°
13	olipase 4SD	5	34	-15°	>95	46	+16°
14	lipase L-10	2	38	-12°	73	50	+12°
14	olipase 4SD	5	51	-11°	-	39	+19°

a) Enantiomeric excess determined as described in the text.

b) not determined.

To determine the optical purity of resulting (-)-**11**, it was successively converted to the Mosher ester **15**[10] and the enol acetate **19**, as shown in scheme 2. Because of the keto-enol tautomerization, **15** couldn't afford sharp peaks on HPLC or clear signals on the ^{1}H-NMR spectrum. But, the diastereomer ratios could be deduced from the methyl signals of the acetyl group observed in the ^{1}H-NMR spectrum of **19**, and it was revealed that (-)-**11** obtained by lipase L-10 has a high optical purity (>97% ee).



Scheme 1

Next, in order to prepare (+)-**11**, (+)-**11a** obtained above was butanolyzed using olipase 4SD in isopropyl ether. Three samples which differed in optical purity were subjected to this reaction. As a result, all materials afforded (+)-**11** having almost the same optical rotation values as shown in Table 2. The optical purity of (+)-**11** (run 3) was determined by the same method as mentioned above to be high (94% ee). Totally, we have succeeded to prepare (-)-**11** and (+)-**11** in high optical purities from racemic **11** using two kinds of lipases as shown in scheme 1.

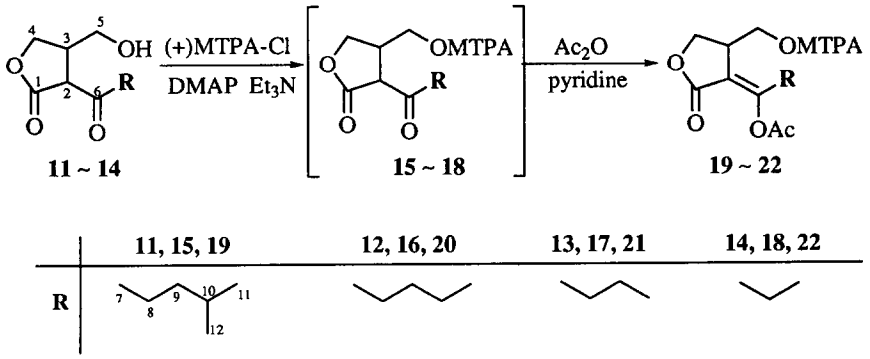
Table 2. Butanolysis of (+)-**11a** ~ **14a** by olipase 4SD

Run	Substrate		Time (hr)	Produced (+)-**11** ~ **14**		
	Compound	$[\alpha]_D^{22}$		Yield (%)	$[\alpha]_D^{22}$	ee% [a]
1	**11a**	+12°	2.5	36	+16°	- [b]
2	**11a**	+14°	3.1	46	+17°	-
3	**11a**	+21°	2.8	44	+16°	94
4	**12a**	+22°	3.0	72	+16°	>97
5	**13a**	+11°	3.0	55	+14°	90
6	**14a**	+19°	3.0	58	+18°	92

a) Enantiomeric excess determined as described in the text.

b) not determined.

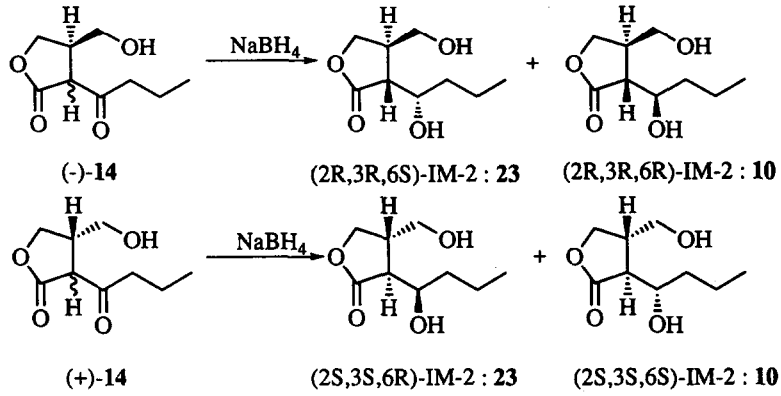
The resolution of other 2-acyl-3-hydroxymethyl-4-butanolides **12**, **13** and **14**, which have a different length of the C-2 acyl chain, was next carried out according to scheme 1. The acetylation and butanolysis reactions to afford their enantiomers are summarized in Table 1 and 2, respectively. Optical purities were determined by NMR analysis of the enol acetates **20**, **21** and **22** which were obtained from optically active **12**, **13** and **14**, respectively, like the case of **11**. All (+)-forms were obtained in high optical purity by the two-step enzymatic reactions, but the enantiomeric excess of (-)-forms varied from 73% to 95%. The absolute configurations of (-)-**11** and (-)-**12** have been determined as (3R) by Mori *et al.*[7]. Close structural similarities of **13** or **14** to **12** indicated that (3R) and (3S) configurations could be assigned to (-)-forms of **13** and **14**, and (+)-forms of them, respectively.



Scheme 2

Determination of the Absolute Configuration of IM-2 **10**.

Reduction of optically active **14** with sodium borohydride was carried out to prepare IM-2 **10**. This reduction afforded only two 2,3-*trans* products, **10** and **23**, which are stereoisomers at C-6[6]. Thus, enantiomers of **10** and **23** were obtained by reduction of (-)-**14** (73% ee) and (+)-**14** (92% ee) as shown in scheme 3. Since there had been no chiroptical data about IM-2 to determine its absolute configuration, the synthetic (2R, 3R, 6R)-(-)-**10** and (2S, 3S, 6S)-(+)-**10** as well as natural **10** were converted to their dibenzoate **10a**, and their CD spectra were measured. As shown in Fig.2, both of the CD spectra of **10a** derivatized from natural **10** and synthetic (-)-**10** showed a negative extremum at 236nm, but that from (+)-**10** showed a positive one, indicating that IM-2 has (2R, 3R, 6R) configuration.



(-)-**14** (2R,3R,6S)-IM-2 : **23** (2R,3R,6R)-IM-2 : **10**

(+)-**14** (2S,3S,6R)-IM-2 : **23** (2S,3S,6S)-IM-2 : **10**

Scheme 3

The blue pigment-inducing activities of synthetic **10** and **23** were measured against *Streptomyces* sp. FRI-5, and the minimum effective concentrations c [ng/ml] were determined as follows : (2R, 3R, 6R)-**10**, $0.3<c<0.6$; (2S, 3S, 6S)-**10**, $1.5<c<2.0$; (2R, 3R, 6S)-**23**, $2.0<c<<2.5$; (2S, 3S, 6R)-**23**, $2.0<c<<2.5$. (2R, 3R, 6R)-**10** showed an activity similar to that of natural IM-2 (ca. 0.6ng/ml)[5].

Hitherto known autoregulators from *Streptomyces* are classified into three groups according to the functional groups and stereochemistry at C-6. A-factor **1**, virginiae butanolides **3**, **6~9**, and IM-2 **10** belong to different groups each other, but (3R) configuration is common to them.

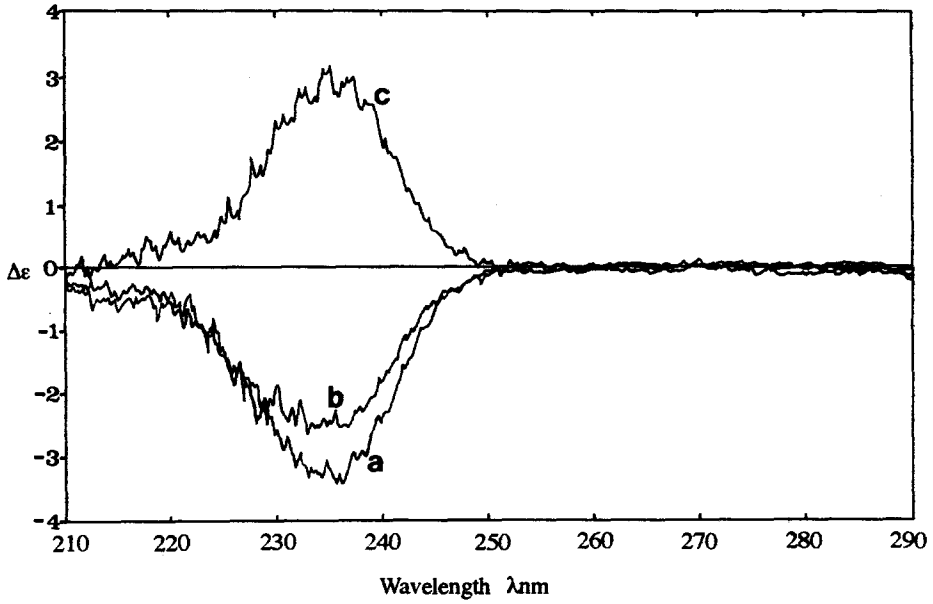


Fig. 2 CD Spectra of **10a** from natural **10** (a), synthetic (2R, 3R, 6R)-**10** (b) and (2S, 3S, 6S)-**10** (c) (in CH_3CN). In a, ext.(nm) : 236 ($\Delta\varepsilon$ = -3.3). In b, ext.(nm) : 236 ($\Delta\varepsilon$ = -2.4). In c, ext.(nm) : 236 ($\Delta\varepsilon$ = +3.0).

EXPERIMENTAL

General Methods.

^{1}H NMR spectra were recorded at 600MHz on a Bruker AM 600, or at 400MHz on a JEOL JNM-GSX-400 spectrometer, using TMS δ_H 0.0 as an internal reference. All *J* values are given in Hz. Mass spectra were obtained on a JEOL JMS-DX-303 spectrometer. CD spectra were recorded on a JASCO J-600 spectropolarimeter and optical rotation values were measured on a JASCO DIP-181 polarimeter. IR spectra were recorded on a HORIBA FT-210 spectrometer. Lipase L-10 and olipase 4SD were gift from Amano Pharm. Co. and Nagase Sangyo. Co., respectively.

Acetylation of 2-Acyl-3-hydroxymethyl-4-butanolides with Lipases.

Preparation of 2-acyl-3-hydroxymethyl-4-butanolides (**11**, **12**, **13**, and **14**) have been described previously[11]. The following procedure is representative. Lipase L-10 (10mg) was added to a solution of 3-hydroxymethyl-2-(5-methylhexanoyl)-4-butanolide **11** (50mg) and acetic anhydride (30μl) in dry benzene (1ml), and the reaction mixture was stirred at room temperature. Reaction was monitored by TLC until ca. 50% conversion was reached, and the reaction was stopped. After the solid enzyme was filtered off, the filtrate was purified by preparative thin-lalyer chromatography with hexane-ethyl acetate 2:1 as a solvent to give (-)-**11** (11mg) and (+)-**11a** (31mg). In the case of acetylation of **11** (50mg) with olipase 4SD, 20mg of the enzyme was used for the reaction. In the same manner, acetylation of **12**, **13**, and **14** was performed

with lipase L-10 or olipase 4SD. The yields and optical rotation values of the products and unacetylated substrates are summarized in Table 1.

11a : EI-MS m/z 270 $(M)^+$, 228 $(M\text{-}Ac)^+$; HREI-MS m/z 228.1357 $(M\text{-}Ac)^+$ (calcd for $C_{12}H_{20}O_4$ 228.1362); IR (film) 1774, 1743, 1720, 1645cm^{-1}; ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.45 (1H, dd, *J*=9 and 8Hz, H-4a), 4.14 (1H, dd, *J*=12 and 5Hz, H-5a), 4.11 (1H, dd, *J*=12 and 5Hz, H-5b), 4.09 (1H, dd, *J*=9 and 6Hz, H-4b), 3.55 (1H, d, *J*=7Hz, H-2), 3.39 (1H, m, H-3), 2.96 (1H, dt, *J*=18 and 7Hz, H-7a), 2.59 (1H, dt, *J*=18 and 7Hz, H-7b), 2.06 (3H, s, Ac), 1.15-1.65 (5H, m, H-8, 9 and 10), 0.87 (6H, d, *J*=7Hz, H-11 and 12).

12a : EI-MS m/z 256 $(M)^+$, 214 $(M\text{-}Ac)^+$; HREI-MS m/z 256.1307 $(M)^+$ (calcd for $C_{13}H_{20}O_5$ 256.1311); IR (film) 1774, 1743, 1716, 1645cm^{-1}; ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.45 (1H, dd, *J*=9 and 8Hz, H-4a), 4.13 (1H, dd, *J*=11 and 5Hz, H-5a), 4.10 (1H, dd, *J*=11 and 5Hz, H-5b), 4.08 (1H, dd, *J*=9 and 6Hz, H-4b), 3.55 (1H, d, *J*=7Hz, H-2), 3.39 (1H, m, H-3), 2.96 (1H, dt, *J*=18 and 7Hz, H-7a), 2.60 (1H, dt, *J*=18 and 7Hz, H-7b), 2.06 (3H, s, Ac), 1.25-1.65 (6H, m, H-8, 9 and 10), 0.88 (3H, t, *J*=7Hz, H-11).

13a : EI-MS m/z 242 $(M)^+$, 200 $(M\text{-}Ac)^+$; IR (film) 1774, 1743, 1720, 1645cm^{-1}; ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.45 (1H, dd, *J*=9 and 8Hz, H-4a), 4.13 (1H, dd, *J*=12 and 5Hz, H-5a), 4.10 (1H, dd, *J*=12 and 5Hz, H-5b), 4.08 (1H, dd, *J*=9 and 6Hz, H-4b), 3.55 (1H, d, *J*=7Hz, H-2), 3.39 (1H, m, H-3), 2.97 (1H, dt, *J*=18 and 7Hz, H-7a), 2.59 (1H, dt, *J*=18 and 7Hz, H-7b), 2.06 (3H, s, Ac), 1.3-1.65 (4H, m, H-8 and 9), 0.91 (3H, t, *J*=7Hz, H-10).

14a : EI-MS m/z 228 $(M)^+$, 186 $(M\text{-}Ac)^+$; IR (film) 1774, 1743, 1716, 1645cm^{-1}; ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.44 (1H, dd, *J*=9 and 8Hz, H-4a), 4.12 (1H, dd, *J*=11 and 5Hz, H-5a), 4.10 (1H, dd, *J*=11 and 5Hz, H-5b), 4.07 (1H, dd, *J*=9 and 6Hz, H-4b), 3.55 (1H, d, *J*=7Hz, H-2), 3.38 (1H, m, H-3), 2.94 (1H, dt, *J*=18 and 7Hz, H-7a), 2.58 (1H, dt, *J*=18 and 7Hz, H-7b), 2.05 (3H, s, Ac), 1.68 (2H, m, H-8), 0.93 (3H, t, *J*=7Hz, H-9).

*Butanolysis of (+)-**11a** - **14a** with Olipase 4SD.*

The following procedure is representative. Olipase 4SD (20mg) was added to a solution of (+)-**11a** (20mg) and n-BuOH (30μl) in dry isopropylether (1ml), and the reaction mixture was stirred at room temperature for 2.8 hours. After the lipase was filtered off, the filtrate was purified by preparative thin-layer chromatography with hexane-ethyl acetate 2:1 as a solvent to afford (+)-**11** (7.4mg). In the same way, butanolysis of **12a**, **13a**, and **14a** was carried out (Table 2).

*Determination of Enantiomeric Excess of Optically Active **11** ~ **14**.*

The following procedure is representative. Dimethylaminopyridine (6.1mg), triethylamine (2.9μl) and (R)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 4.8μl) was added to a solution of (-)-**12** (2.8mg) in dry dichloromethane (0.5ml), and the solution was stirred at room temperature for 4 hours. After adding 3-dimethylaminopropylamine (3.2μl), the solution was further stirred for 10 min. The reaction mixture was purified by preparative thin-layer chromatography to give the MTPA ester (3R)-**16** (4.1mg), which was then acetylated with pyridine (0.17ml) and acetic anhydride (0.33ml) for 1 hour at room temperature. The reaction solution was poured into ice-cooled water (10ml), which was extracted with

dichloromethane (50ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. Purification of the residue with a reverse-phase HPLC (column : Wakosil-II $5C_{18}$-HG, 4.6x250mm, Wako Pure Chemical Ind. ; mobile phase: gradient elution of 60-100% CH_3CN in water in 20min ; flow rate : 1ml/min) afforded the enol acetate (3R)-**20** (2.7mg). In the same manner, (3R)-**19**, (3S)-**19**, (3S)-**20**, (3R)-**21**, (3S)-**21**, (3R)-**22**, and (3S)-**22** were prepared from (-)-**11**, (+)-**11**, (+)-**12**, (-)-**13**, (+)-**13**, (-)-**14**, and (+)-**14**, respectively, in about the same yield as the case of (3R)-**20**. Diastereomers of each enol acetate had the same retention time on HPLC under the conditions above mentioned. Racemic **11** ~ **14** were also converted to the enol acetates in the same way and each diastereomer ratios of **19** ~ **22** produced was confirmed as 1 : 1 by the NMR analysis. Stereochemistry of the double bond of the enol acetates was not determined.

(3R)-**19** : EI-MS m/z 486 $(M)^+$, 444 $(M\text{-}Ac)^+$; HREI-MS m/z 486.1863 $(M)^+$ (calcd for $C_{24}F_3H_{29}O_7$ 486.1865); IR (film) 1753, 1680cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 600MHz) 7.3-7.5 (5H, m, Ph). 4.62 (1H, dd, *J*=11 and 4Hz, H-5a), 4.13 (1H, dd, *J*=9 and 8Hz, H-4a), 4.03 (1H, dd, *J*=9 and 2Hz, H-4b), 3.97 (1H, dd, *J*=11 and 10Hz, H-5b), 3.51 (3H, s, OCH_3), 3.43 (1H, m, H-3), 2.95 (1H, dt, *J*=15 and 8Hz, H-7a), 2.87 (1H, dt, *J*=15 and 8Hz, H-7b), 2.31 (3H, s, OAc), 1.2-1.6 (5H, m, H-8, 9 and 10), 0.86 (6H, d, *J*=7Hz, H-11 and 12).

(3S)-**19** : EI-MS m/z 486 $(M)^+$, 444 $(M\text{-}Ac)^+$; HREI-MS m/z 486.1872 $(M)^+$ (calcd for $C_{24}F_3H_{29}O_7$ 486.1865); IR (film) 1755, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 600MHz) 7.3-7.5 (5H, m, Ph). 4.54 (1H, dd, *J*=11 and 4Hz, H-5a), 4.21 (1H, dd, *J*=9 and 8Hz, H-4a), 4.11 (1H, dd, *J*=9 and 2Hz, H-4b), 4.06 (1H, dd, *J*=11 and 9Hz, H-5b), 3.48 (3H, s, OCH_3), 3.45 (1H, m, H-3), 2.97 (1H, dt, *J*=15 and 8Hz, H-7a), 2.83 (1H, dt, *J*=15 and 8Hz, H-7b), 2.28 (3H, s, OAc), 1.15-1.6 (5H, m, H-8, 9 and 10), 0.85 (6H, d, *J*=7Hz, H-11 and 12).

(3R)-**20** : EI-MS m/z 473 $(M+H)^+$, 430 $(M\text{-}Ac)^+$; HREI-MS m/z 472.1707 $(M)^+$ (calcd for $C_{23}F_3H_{27}O_7$ 472.1708); IR (film) 1755, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.62 (1H, dd, *J*=11 and 4Hz, H-5a), 4.13 (1H, dd, *J*=9 and 8Hz, H-4a), 4.03 (1H, dd, *J*=9 and 2Hz, H-4b), 3.97 (1H, dd, *J*=11 and 10Hz, H-5b), 3.51 (3H, s, OCH_3), 3.43 (1H, m, H-3), 2.97 (1H, dt, *J*=15 and 8Hz, H-7a), 2.88 (1H, dt, *J*=15 and 8Hz, H-7b), 2.31 (3H, s, OAc), 1.2-1.5 (6H, m, H-8, 9 and 10), 0.87 (3H, t, *J*=7Hz, H-11).

(3S)-**20** : EI-MS m/z 473 $(M+H)^+$, 430 $(M\text{-}Ac)^+$; HREI-MS m/z 472.1721 $(M)^+$ (calcd for $C_{23}F_3H_{27}O_7$ 472.1708); IR (film) 1755, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.53 (1H, dd, *J*=11 and 4Hz, H-5a), 4.22 (1H, dd, *J*=9 and 8Hz, H-4a), 4.11 (1H, dd, *J*=9 and 2Hz, H-4b), 4.06 (1H, dd, *J*=11 and 9Hz, H-5b), 3.48 (3H, s, OCH_3), 3.45 (1H, m, H-3), 2.99 (1H, dt, *J*=15 and 8Hz, H-7a), 2.84 (1H, dt, *J*=15 and 8Hz, H-7b), 2.28 (3H, s, OAc), 1.2-1.5 (6H, m, H-8, 9 and 10), 0.87 (3H, t, *J*=7Hz, H-11).

(3R)-**21** : EI-MS m/z 459 $(M+H)^+$, 416 $(M\text{-}Ac)^+$; IR (film) 1755, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.62 (1H, dd, *J*=10 and 4Hz, H-5a), 4.13 (1H, dd, *J*=9 and 7Hz, H-4a), 4.03 (1H, dd, *J*=9 and 2Hz, H-4b), 3.97 (1H, dd, *J*=11 and 10Hz, H-5b), 3.51 (3H, s, OCH_3), 3.42 (1H, m, H-3), 2.98 (1H, dt, *J*=15 and 8Hz, H-7a), 2.88 (1H, dt, *J*=15 and 8Hz, H-7b), 2.31 (3H, s, OAc), 1.2-1.5 (4H, m, H-8 and 9), 0.90 (3H, t, *J*=7Hz, H-10).

(3S)-**21** : EI-MS m/z 459 $(M+H)^+$, 416 $(M-Ac)^+$; IR (film) 1753, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.53 (1H, dd, *J*=11 and 4Hz, H-5a), 4.22 (1H, dd, *J*=9 and 8Hz, H-4a), 4.11 (1H, dd, *J*=9 and 2Hz, H-4b), 4.06 (1H, dd, *J*=11 and 9Hz, H-5b), 3.49 (3H, s, OCH_3), 3.45 (1H, m, H-3), 3.00 (1H, dt, *J*=15 and 8Hz, H-7a), 2.84 (1H, dt, *J*=15 and 8Hz, H-7b), 2.28 (3H, s, OAc), 1.2-1.5 (4H, m, H-8 and 9), 0.89 (3H, t, *J*=7Hz, H-10).

(3R)-**22** : EI-MS m/z 445 $(M+H)^+$, 402 $(M-Ac)^+$; IR (film) 1751, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.62 (1H, dd, *J*=11 and 4Hz, H-5a), 4.13 (1H, dd, *J*=9 and 7Hz, H-4a), 4.03 (1H, dd, *J*=9 and 2Hz, H-4b), 3.97 (1H, dd, *J*=11 and 9Hz, H-5b), 3.51 (3H, s, OCH_3), 3.42 (1H, m, H-3), 2.96 (1H, dt, *J*=15 and 8Hz, H-7a), 2.86 (1H, dt, *J*=15 and 8Hz, H-7b), 2.30 (3H, s, OAc), 1.51 (2H, m, H-8), 0.93 (3H, t, *J*=7Hz, H-9).

(3S)-**22** : EI-MS m/z 445 $(M+H)^+$, 402 $(M-Ac)^+$; IR (film) 1753, 1682cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 400MHz) 7.3-7.5 (5H, m, Ph). 4.53 (1H, dd, *J*=11 and 4Hz, H-5a), 4.22 (1H, dd, *J*=9 and 7Hz, H-4a), 4.11 (1H, dd, *J*=9 and 2Hz, H-4b), 4.07 (1H, dd, *J*=11 and 9Hz, H-5b), 3.49 (3H, s, OCH_3), 3.44 (1H, m, H-3), 2.98 (1H, dt, *J*=15 and 8Hz, H-7a), 2.82 (1H, dt, *J*=15 and 8Hz, H-7b), 2.27 (3H, s, OAc), 1.49 (2H, m, H-8), 0.92 (3H, t, *J*=7Hz, H-9).

Preparation of Enantiomers of IM-2 ***10***.

Sodium borohydride (6.8mg) was added to a solution of (-)-**14** (33.4mg) in methanol (7ml) at 0 °C, and the solution was stirred for 2.5 hours at room temperature. After adjusting the pH to 3-4 with 3N acetic acid, the reaction solution was concentrated *in vacuo*. The residue was purified on reverse-phase HPLC (column : Cosmosil $5C_{18}$, 10x250mm, Jasco ; mobile phase: isocratic elution of 20% CH_3CN in H_2O ; flow rate : 2.0ml/min) to afford (2R, 3R, 6S)-**23** (7.7mg) and (2R, 3R, 6R)-(-)-**10** (13.4mg).

(2R,3R,6S)-**23** : $[\alpha]_D^{19}$ = -24.8° (*c* 0.38, $CHCl_3$); EI-MS m/z 189 $(M+H)^+$; HREI-MS m/z 170.0952 $(M-H_2O)^+$ (calcd for $C_9H_{14}O_3$ 170.0943); IR (film) : 1747cm^{-1}. ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.41 (1H, t, *J*=9Hz, H-4a), 4.14 (1H, m, H-6), 4.09 (1H, dd, *J*=9 and 7Hz, H-4b), 3.75 (1H, dd, *J*=10 and 6Hz, H-5a), 3.71 (1H, dd, *J*=10 and 6Hz, H-5b), 2.86 (1H, m, H-3), 2.56 (1H, dd, *J*=7 and 4Hz, H-2), 1.3-1.6 (4H, m, H-7 and 8), 0.96 (3H, t, *J*=7Hz, H-9).

(2R,3R,6R)-(-)-**10** : $[\alpha]_D^{19}$ = -1° (*c* 0.66, $CHCl_3$); EI-MS m/z 189 $(M+H)^+$; HREI-MS m/z 170.0955 $(M-H_2O)^+$ (calcd for $C_9H_{14}O_3$ 170.0943); IR (film) : 1755cm^{-1}. ^{1}H-NMR δ ($CDCl_3$, 600MHz) 4.41 (1H, t, *J*=9Hz, H-4a), 4.02 (1H, m, H-6), 3.98 (1H, t, *J*=9Hz, H-4b), 3.75 (1H, dd, *J*=11 and 5Hz, H-5a), 3.69 (1H, dd, *J*=11 and 7Hz, H-5b), 2.76 (1H, m, H-3), 2.64 (1H, dd, *J*=9 and 5Hz, H-2), 1.35-1.65 (4H, m, H-7 and 8), 0.95 (3H, t, *J*=7Hz, H-9).

In the same manner, (+)-**14** (18.2mg) was reduced by sodium borohydride (5.0mg) to give (2S, 3S, 6R)-**23** (4.7mg) and (2S, 3S, 6S)-(+)-**10** (8.3mg).

(2S,3S,6R)-**23** : $[\alpha]_D^{19}$ = +24.2° (*c* 0.23, $CHCl_3$); EI-MS m/z 189 $(M+H)^+$.

(2S,3S,6S)-(+)-**10** : $[\alpha]_D^{19}$ = +1° (*c* 0.41, $CHCl_3$); EI-MS m/z 189 $(M+H)^+$.

Preparation of IM-2 Dibenzoate ***10a***.

Synthetic (2R, 3R, 6R)-**10** (1.0mg) was benzoylated with benzoyl cyanide (ca. 3mg) and tributylamine (3μl) in dry CH_3CN (100μl) for 10min. The reaction solution was directly purified on reverse-phase HPLC (column: Daisopak SP-120-5-ODS-B, Daiso ; mobile phase : gradient elution of 50-100% CH_3CN in H_2O in 15min ; flow rate : 1ml/min) to afford (2R, 3R, 6R)-**10a** (1.1mg). In the same way, benzoylation of (2S, 3S, 6S)-**10** (1.0mg) and natural **10** (ca. 100μg) gave (2S, 3S, 6S)-**10a** (0.9mg) and natural IM-2 dibenzoate (125μg), respectively.

10a : EI-MS m/z 396 $(M)^+$; HREI-MS m/z 396.1575 $(M-H_2O)^+$ (calcd for $C_{23}H_{24}O_6$ 396.1573); IR (film) 1776, 1720cm^{-1}; ^{1}H-NMR d ($CDCl_3$, 600MHz) 7.4-8.1 (10H, m, Ph), 5.56 (1H, m, H-6), 4.46 (1H, dd, *J*=11 and 5Hz, H-5a), 4.40 (1H, dd, *J*=9 and 7Hz, H-4a), 4.38 (1H, dd, *J*=11 and 5Hz, H-5b), 4.13 (1H, dd, *J*=9 and 6Hz, H-4b), 2.97 (2H, m, H-2 and 3), 1.35-2.15 (4H, m, H-7 and 8), 0.95 (3H, t, *J*=7Hz, H-9).

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