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Enzymatic separation of hardly separable mixtures of structural isomers

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Abstract

Lipase-meditated separation of six hardly separable mixtures of structural isomers was carried out. It has become apparent that the hydrolysis rate of the C(5)-acetoxyl group of methyl (±)-5-acetoxy-4-aryl-(2*E*)-pentenoate possessing the *ortho*-substituents in the aromatic ring was slower than that of the corresponding compound with no substituents at the *ortho*-position. The hydrolysis rate of the C(5)-acetoxyl group of methyl (±)-5-acetoxy-2-aryl-(3*E*)-pentenoate possessing a remote reaction site from the *ortho*-substituents in the aromatic ring was proved to be faster than that of methyl (±)-5-acetoxy-4-aryl-(2*E*)-pentenoate.

Keywords: Lipase; Enzymatic separation; Regio-selective hydrolysis; Structural isomer.

1. Introduction

In recent years, the advantages of using enzymatic catalysis in preparative organic synthesis have become apparent, as observed in comprehensive review books. [1] Organic chemists seeking a stereoselective transformation, which will eventually lead to variety of optically active intermediates in the synthesis of enantiomerically pure compounds, prefer lipases among enzymes. The reason for this choice is that hydrolytic enzymes do not require cofactor regeneration and are easy to handle. Lipase-catalyzed acyl transfer has been applied to solve a number of different synthetic problems. The majority of applications have dealt with the asymmetrization of prochiral and *meso*-diols or with the kinetic resolution of racemic primary and secondary alcohols, while there are no reports concerning enzymatic separation of structural isomers except for some examples [2–4] so far. A stereoisomeric mixture of the allylic terpene alcohols (*E*)-geraniol (**1**) and (*Z*)-nerol (**1**) was separated by selective acylation with an acid anhydride using porcine pancreatic lipase (PPL) as catalyst [2]. Depending on the acyl donor employed, the slightly less hindered (*E*)-geraniol (**1**) was acylated faster to (*E*)-geranyl acylate (**2**), leaving the (*Z*)-nerol (**1**) behind. (Scheme 1)

The lipase Amano PS from *Pseudomonas* spp. catalyzed separation of a mixture of *trans*- and *cis*-4-*tert*-butylcyclohexane methanols (**3**) with an excess of vinyl acetate in *tert*-butylmethyl ether at room temperature was reported to give the acetate (*trans*-**4**) and the unreacted alcohol (*cis*-**3**) in yields of 60% and 35%, respectively. [3] (Scheme 1).

On the other hand, we reported the boron trifluoride-mediated reaction of methyl (±)-4,5-epoxy-(2*E*)-pentenoate (**5**) with benzene derivatives to give 4-aryl-5-hydroxy-(2*E*)-pentenoate (A) and 2-aryl-5-hydroxy-(3*E*)-pentenoate (B) in good yields. [4,5] An application of the pentenoate (A) to the synthesis of bisabolane type sesquiterpenes is also reported. [6] In this process, separation of some structural isomers due to the substitution pattern in the aromatic ring of the pentenoates (A) and (B) was found to be difficult by means of conventional column chromatography (Scheme 2). In this paper, we describe the enzymatic separation of the hardly separable mixtures of the 4-aryl-5-hydroxy-(2*E*)-pentenoate (A) and 2-aryl-5-hydroxy-(3*E*)-pentenoate (B).

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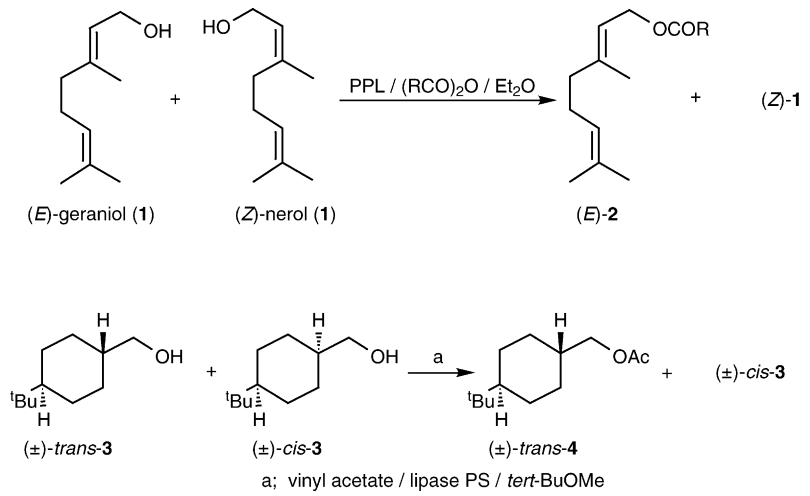


Scheme 1.

The following acetates ((±)-**8**, (±)-**9**, (±)-**12**, (±)-**13**, (±)-**16**, (±)-**17**, (±)-**20**, (±)-**21**, (±)-**24**, (±)-**25**, (±)-**29**, (±)-**30**, and (±)-**31**) were selected as the substrates for the competitive hydrolysis by using lipases. The syntheses of the substrates are shown in Scheme 3.

2. Material and methods

2.1. Analytical methods

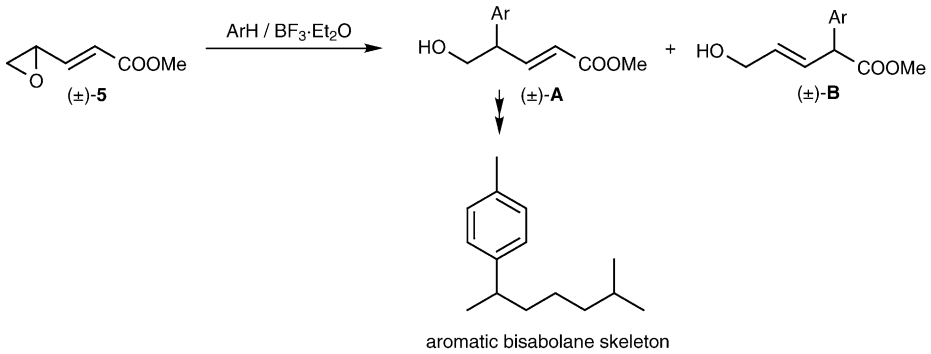
^{1}H-NMR spectra were recorded by a JEOL EX 400 spectrometer (Tokyo, Japan). Spectra were taken with 5–10% (w/v) solution in $CDCl_3$ with Me_4Si as an internal reference. The FAB mass spectra were obtained with a JEOL JMS-DX 303 (matrix; glycerol, *m*-nitrobenzyl alcohol) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. The HPLC system was composed of a detector (UV detector SSC-5200, Senshu), pump (SSC-3210, Senshu) and integrator (chromatocorder SIC 21). All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

2.2. Materials

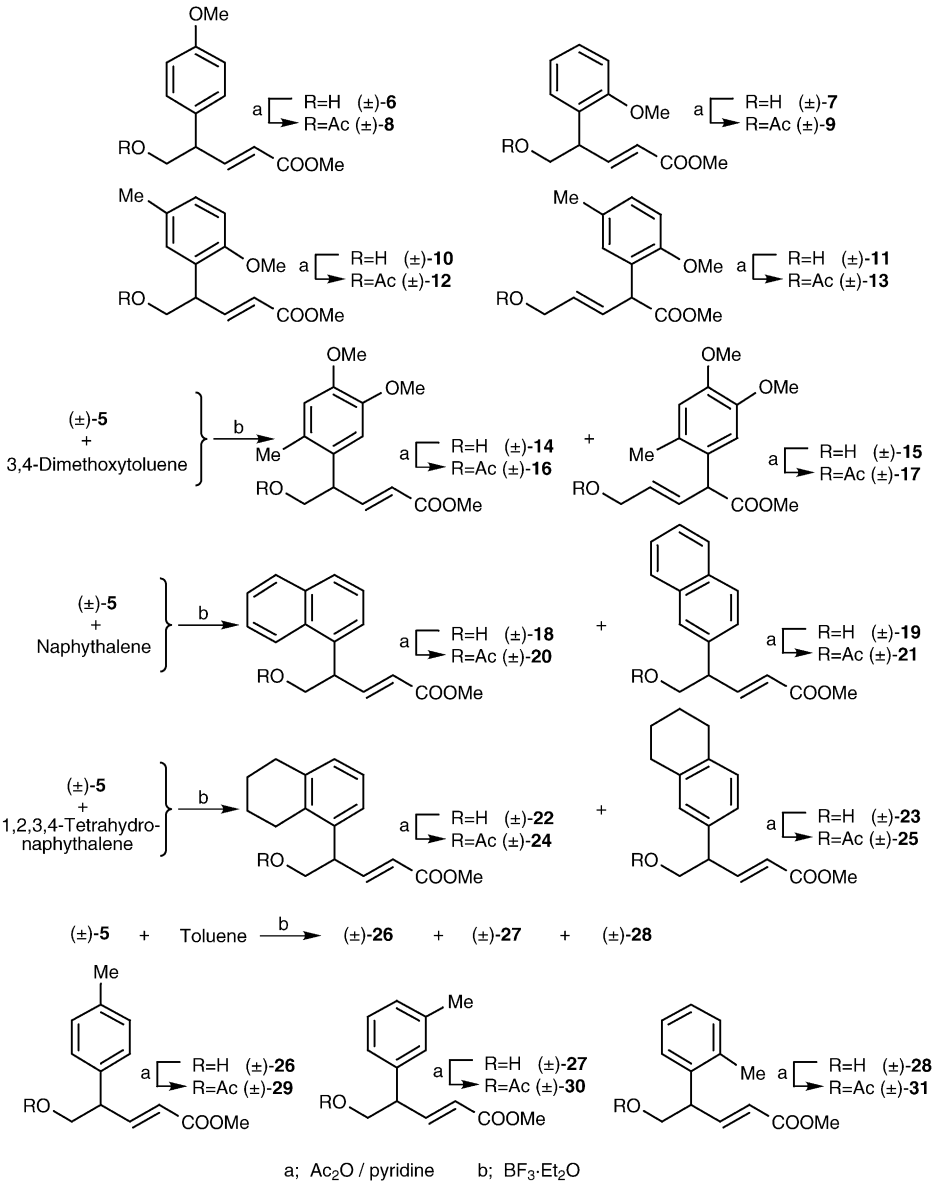
The substrates ((±)-**8**, (±)-**9**, (±)-**12**, (±)-**13**, (±)-**16**, (±)-**17**, (±)-**20**, (±)-**21**, (±)-**24**, (±)-**25**, (±)-**29**, (±)-**30**, and (±)-**31**) for competitive hydrolysis by lipase were prepared by the following method.

2.2.1. Methyl (±)-5-acetoxy-4-(4′-methoxyphenyl)-2(E)-pentenoate *8* and methyl (±)-5-acetoxy-4-(2′-methoxyphenyl)-2(E)-pentenoate *9*

A solution of (±)-**6** (2.15 g, 9.1 mmol)[5] and Ac_2O (2.02 g, 19.7 mmol) in pyridine (3.0 mL) was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with 2 M aqueous HCl, 7% aqueous $NaHCO_3$, and saturated brine. After drying over $MgSO_4$, the organic layer was evaporated to give a residue, which was separated by column chromatography on silica gel (30 g, *n*-hexane:AcOEt = 6:1) to give (±)-**8** (2.19 g, 86% yield) as an oil. (±)-**8**: IR (neat): 1740 cm^{-1}, ^{1}H-NMR: δ 2.02 (3H, s), 3.73 (3H, s), 3.78 (1H, br,q, *J* = 7.0 Hz), 3.79 (3H, s), 4.28–4.35 (2H, m), 5.86 (1H, dd, *J* = 16.0, 2.0 Hz), 6.88 (2H, d, *J* = 9.0 Hz), 7.09 (1H, dd,



Scheme 2.



Scheme 3.

J = 16.0, 7.0 Hz), 7.12 (2H, d, J = 9.0 Hz); FAB MS m/z: 279 $(M+1)^+$; *Anal*. Found: C, 64.34; H, 6.49. Calculated for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52%.

A solution of (±)-**7** (0.331 g, 1.4 mmol)[5] and Ac_2O (0.28 g, 2.74 mmol) in pyridine (1.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford (±)-**9** (0.323 g, 88% yield) as oil. (±)-**9**: IR (neat): 1740 cm^{-1}, ^{1}H-NMR: δ 2.02 (3H, s), 3.72 (3H, s), 3.82 (3H, s), 4.23 (1H, br,q, J = 7.0 Hz), 4.33 (1H, dd, J = 11.0, 5.0 Hz), 4.40 (1H, dd, J = 11.0, 9.0 Hz), 5.87 (1H, dd, J = 16.0, 2.0 Hz), 6.88 (1H, dd, J = 8.0, 2.0 Hz), 6.92 (1H, dt, J = 8.0, 2.0 Hz), 7.11 (1H, dd, J = 8.0, 2.0 Hz), 7.16 (1H, dd, J = 16.0, 7.0 Hz), 7.25 (1H, dt, J = 8.0, 2.0 Hz); FAB MS m/z: 279 $(M+1)^+$; *Anal*. Found: C, 64.60; H, 6.50. Calculated for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52%.

2.2.2. *Methyl (±)-5-acetoxy-4-(2′-methoxy-5′-methylphenyl)-2(E)-pentenoate* ***12*** *and methyl (±)-5-acetoxy-2-(2′-methoxy-5′-methylphenyl)-3(E)-pentenoate* ***13***

A solution of (±)-**10** (0.99 g, 3.91 mmol)[5] and Ac_2O (0.93 g, 9.11 mmol) in pyridine (2.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up by the same way as for (±)-**8** to afford (±)-**12** (0.93 g, 81% yield) as oil. (±)-**12**: IR (neat): 1740 cm^{-1}, ^{1}H-NMR: δ 2.03 (3H, s), 2.26 (3H, s), 3.72 (3H, s), 3.80 (3H, s), 4.19 (1H, br,q, J = 7.5 Hz), 4.30 (1H, dd, J = 10.0, 6.0 Hz), 4.39 (1H, dd, J = 10.0, 9.0 Hz), 5.87 (1H, dd, J = 16.0, 2.0 Hz), 6.78 (1H, d, J = 9.0 Hz), 6.90 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 9.0, 2.0 Hz), 7.15 (1H, dd, J = 16.0, 7.5 Hz); FAB MS m/z: 293 $(M+1)^+$; *Anal*. Found: C, 65.41; H, 7.29. Calculated for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90%.

A solution of (±)-**11** (0.74 g, 2.96 mmol) [5] and Ac_2O (0.64 g, 6.27 mmol) in pyridine (3.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford (±)-**13** (0.55 g, 81% yield) as oil. (±)-**13**: IR (neat): 1738 cm^{-1}, ^{1}H-NMR: δ 2.04 (3H, s), 2.28 (3H, s), 3.68 (3H, s), 3.78 (3H, s), 4.55 (2H, d, *J* = 6.0 Hz), 4.60 (1H, d, *J* = 8.0 Hz), 5.63 (1H, dt, *J* = 16.0, 6.0 Hz), 6.15 (1H, dd, *J* = 16.0, 8.0 Hz), 6.78 (1H, d, *J* = 9.0Hz), 7.00 (1H, d, *J* = 2.0 Hz), 7.04 (1H, dd, *J* = 9.0, 2.0 Hz); FAB MS *m/z*: 292 (M^+); *Anal*. Found: C, 65.52; H, 7.30. Calculated for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90%.

2.2.3. Methyl (±)-5-acetoxy-4-(3′,4′-dimethoxy-6′-methylphenyl)-2(E)-pentenoate **16** and methyl (±)-5-acetoxy-2-(3′,4′-dimethoxy-6′-methylphenyl)-3(E)-pentenoate **17**

Under dry ice/acetone cooling, $BF_3{\cdot}Et_2O$ (0.71 g, 5.0 mmol) was added to a solution of (±)-**5** (1.92 g, 15.0 mmol) and 3,4-dimethoxytoluene (1.521 g, 10 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred for 1 h at −78 °C, then gradually warmed to −20 °C. After addition of saturated brine at −20 °C, the whole was extracted with CH_2Cl_2. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of CH_2Cl_2 gave an oily product, which was separated by column chromatography on silica gel (60 g, *n*-hexane:AcOEt = 2:1) to give a mixture (2.202 g, 64% yield) of (±)-**14** and (±)-**15** as an oil. A solution of the above-mentioned mixture (1.00 g, 3.57 mmol) and Ac_2O (0.73 g, 7.1 mmol) in pyridine (5.0 mL) was stirred for 3.5 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford a mixture of (±)-**16** and (±)-**17** (0.88 g, 76% yield) as oil. This mixture was shown to be a single spot by thin-layer chromatography using several kinds of solvent systems. Characterization of the products was carried out after enzymatic separation followed by chromatographic purification as described later in the text.

2.2.4. Methyl (±)-5-acetoxy-4-(1'-naphtyl)-2(E)-pentenoate **20** and methyl (±)-5-acetoxy-4-(2'-naphtyl)-2(E)-pentenoate **21**

Under dry ice/acetone cooling, $BF_3{\cdot}Et_2O$ (2.16 g, 15.2 mmol) was added to a solution of (±)-**5** (2.0 g, 15.6 mmol) and naphythalene (4.0 g, 31.2 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 1 h at −78 °C, then gradually warmed to −20 °C. After addition of saturated brine at −20 °C, the whole was extracted with CH_2Cl_2. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of CH_2Cl_2 gave an oily product, which was separated by column chromatography on silica gel (60 g, *n*-hexane:AcOEt = 6:1) to give a mixture (2.82 g, 71% yield) of (±)-**18** and (±)-**19** as an oil. A solution of the above-mentioned mixture (1.70 g, 6.63 mmol) and Ac_2O (0.7 g, 6.8 mmol) in pyridine (3.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford a mixture of (±)-**20** and (±)-**21** (1.61 g, 81% yield) as oil. This mixture was shown to be a single spot by thin-layer chromatography using several kinds of solvent systems. Characterization of the products was carried out after enzymatic separation followed by chromatographic purification as described later in the text.

2.2.5. Methyl (±)-5-acetoxy-4-(1′-6′,7′,8′,9′-tetrahydronaphtyl)-2(E)-pentenoate **24** and methyl (±)-5-acetoxy-4-(2′-6′,7′,8′,9′-tetrahydronaphtyl)-2(E)-pentenoate **25**

Under dry ice/acetone cooling, $BF_3{\cdot}Et_2O$ (2.30 g, 16.2 mmol) was added to a solution of (±)-**5** (2.01 g, 15.7 mmol) and tetralin (4.17 g, 16.1 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 1 h at −78 °C, then gradually warmed to −20 °C. After addition of saturated brine at −20 °C, the whole was extracted with CH_2Cl_2. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of CH_2Cl_2 gave an oily product, which was separated by column chromatography on silica gel (60 g, *n*-hexane:AcOEt = 6:1) to give a mixture (2.43 g, 60% yield) of (±)-**22** and (±)-**23** as an oil. A solution of the above-mentioned mixture (1.521 g, 5.87 mmol) and Ac_2O (1.19 g, 11.7 mmol) in pyridine (3.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford a mixture (1.57 g, 89% yield) of (±)-**24** and (±)-**25** as oil. This mixture was shown to be a single spot by thin-layer chromatography using several kinds of solvent systems. Characterization of the products was carried out after enzymatic separation followed by chromatographic purification as described later in the text.

2.2.6. (±) Methyl (±)-5-acetoxy-4-(3′-methylphenyl)-2(E)-pentenoate **30** and methyl (±)-5-acetoxy-4-(2′-methylphenyl)-2(E)-pentenoate **31**

Under dry ice/acetone cooling, $BF_3{\cdot}Et_2O$ (4.26 g, 30.0 mmol) was added to a solution of (±)-**5** (3.85 g, 30.0 mmol) and toluene (11.06 g, 120.0 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred for 1 h at −78 °C, then gradually warmed to −20 °C. After addition of saturated brine at −20 °C, the whole was extracted with CH_2Cl_2. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of CH_2Cl_2 gave an oily product, which was separated by column chromatography on silica gel (60 g, *n*-hexane:AcOEt = 5:1) to give a mixture (2.845 g, 43% yield) of (±)-26, (±)-27 and (±)-**29** as an oil. A solution of the above-mentioned mixture (1.01 g, 4.59 mmol) and Ac_2O (0.94 g, 9.21 mmol) in pyridine (3.0 mL) was stirred for 24 h at room temperature. The reaction mixture was worked up in the same way as for (±)-**8** to afford a mixture (1.09 g, 90% yield) of (±)-29, (±)-30 and (±)-**31** as oil. This mixture was shown to be a single spot by thin-layer chromatography using several kinds of solvent systems. The ratio ((±)-**29**:(±)-**30**:(±)-**31**) of this mixture was found to be 2.5:1.0:1.2 based on the intensity of the aromatic

protons at the *ortho*-position ((±)-**29** (δ 5.86), (±)-**30** (δ 5.87), (±)-**31** (δ 5.83)), respectively. Characterization of the products was carried out after enzymatic separation followed by chromatographic purification as described later in the text.

3. Results and discussion

As mentioned in introduction, we happened to meet difficulty of the separation of the structural isomers, the pentenoates (A) and (B) by means of conventional column chromatography. Therefore, we focus on the lipase-mediated separation of the structural isomer because this type separation except for some examples [2,3,7] is not reported so far. Then enzymatic separation using six kinds of mixtures was carried out in order to establish the advantage of the lipase-catalyzed separation of structural isomers.

3.1. Screening for suitable enzyme

At first, screening experiments in water-saturated diisopropyl ether using thirty kinds of commercially available lipases possessing hydrolysis activity against the substrates (±)-8 and (±)-9 were carried out. The following lipases such as lipase Amano AY 30 from *Candida rugosa*, Meito lipase MY 30 from *Candida rugosa*, Meito lipase OF 360 from *Candida rugosa*, C.C. Sigma (L-1754) from *Candida rugosa*, Meito lipase PL 679 from *Alcaligenes* spp. were found to exhibit hydrolysis activity against both (±)-**8** and (±)-**9** based on thin-layer chromatographic analysis. When the following lipases such as lipase Amano P from *Pseudomonas* spp., lipase Nagase P from *Pseudomonas* spp., GODO lipase E-4 from *Pseudomonas* spp., Meito lipase PL 266 from *Alcaligenes* spp. and Meito lipase AL from *Alcaligenes* spp. were employed, hydrolysis rate of (±)-**8** was found to be faster than that of (±)-**9**. Among the above-mentioned five lipases, hydrolysis rate using lipase Amano P was found to be the fastest against (±)-**8**, and (±)-**9** was obtained intact. Actually, when a 1:1 mixture of (±)-**8** and (±)-**9** was exposed to the lipase Amano P in phosphate buffer solution (pH 7.4) for 1.5 h at 33 °C, (±)-**8** was perfectly hydrolyzed and (±)-**9** remained intact. Moreover, the hydrolyzed product (±)-**6** from (±)-**8** was found to be racemic by means of HPLC analysis as described in the next section. Examples illustrating the hydrolysis using the lipase Amano P will hereinafter, be described and the results are shown in Table 1.

3.2. Competitive hydrolysis of thirteen kinds of substrates using lipase Amano P in phosphate buffer

3.2.1. Methyl (±)-5-hydroxy-4-(4′-methoxyphenyl)-2(E)-pentenoate **6** and methyl (±)-5-acetoxy-4-(2′-methoxyphenyl)-2(E)-pentenoate **9**

A suspension of a mixture of (±)-**8** (0.10 g) and (±)-**9** (0.10 g), lipase Amano P (0.10 g) in phosphate buffer (pH 7.4; 150 mL) was stirred at 33 °C for 2 h. The reaction mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (10 g) to give (±)-**9** (0.091 g, 91% recovery) as an oil (*n*-hexane:AcOEt = 6:1) and (±)-**6** (0.071 g, 83% conversion) as an oil (*n*-hexane:AcOEt = 2:1). The NMR data of (±)-**6** and (±)-**9** were identical with those of the authentic (±)-**6** and (±)-**9**, respectively [5]. The present samples (±)-**6** and (±)-**9** were found to be racemic by means of HPLC analysis using a chiral column, respectively. The alcohol (±)-**6** was subjected to HPLC (chiral column; CHIRALPAC AS, solvent; *n*-hexane:EtOH = 10:1, flow rate; 1 mL/min) analysis to afford two separated peaks (13.2 min and 16.1 min). The acetate (±)-**9** was subjected to HPLC (chiral column; CHIRALCEL OD, solvent; *n*-hexane:EtOH = 1000:2, flow rate; 1 mL/min) analysis to provide two separated peaks (83.6 min and 97.2 min).

3.2.2. Methyl (±)-5-acetoxy-4-(2′-methoxy-5′-methylphenyl)-2(E)-pentenoate **12** and methyl (±)-5-hydroxy-2-(2′-methoxy-5′-methylphenyl)-3(E)-pentenoate **11**

A suspension of a mixture of (±)-**12** (0.104 g) and (±)-**13** (0.10 g), lipase Amano P (0.10 g) in phosphate buffer (pH 7.4; 200 mL) was stirred at 33 °C for 2 h. The reaction

Table 1
Enzymatic separation of six kinds of mixtures using lipase Amano P in phosphate buffer (pH 7.4)

Entry	Substrate	Time (h)	Products	
1	(±)-**8** + (±)-**9** (1:1 mixture)	2	(±)-**6** (83% conversion)	(±)-**9** (91% recovery)
2	(±)-**12** + (±)-**13** (1:1 mixture)	2	(±)-**11** (>99% conversion)	(±)-**12** (>99% recovery)
3	(±)-**16** + (±)-**17** (1:3 mixture)	24	(±)-**15** (61% conversion)	(±)-**16** (92% recovery)
4	(±)-**20** + (±)-**21** (2:1 mixture)	21	(±)-**19** (83% conversion)	(±)-**20** (95% recovery)
5	(±)-**24** + (±)-**25** (1:2 mixture)	21	(±)-**23** (67% conversion)	(±)-**24** (75% recovery)
6	(±)-**29** + (±)-**30** + (±)-**31** (2.5:1.0:1.2 mixture)	21	(±)-**26** + (±)-**27** (81% conversion)	(±)-**31** (>99% recovery)

mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (10 g) to give (±)-**12** (0.10 g, >99% recovery) as an oil (*n*-hexane:AcOEt = 8:1) and (±)-**11** (0.097 g, >99% conversion) as an oil (*n*-hexane:AcOEt = 3:1). The NMR data of (±)-**11** and (±)-**12** were identical with those of the authentic (±)-**11** and (±)-**12**, respectively [5].

3.2.3. Methyl (±)-5-acetoxy-4-(3′,4′-dimethoxy-6′-methylphenyl)-2(E)-pentenoate *16* and methyl (±)-5-hydroxy-2-(3′,4′-dimethoxy-6′-methylphenyl)-3(E)-pentenoate *15*

A suspension of a 1:3 mixture (0.200 g; (±)-**16**; corresponding to 0.05 g, (±)-**17**; corresponding to 0.15 g) of (±)-**16** and (±)-**17**, lipase Amano P (0.10 g) in phosphate buffer (pH 7.4; 200 mL) was stirred at 33 °C for 24 h. The reaction mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (10 g) to give (±)-**16** (0.046 g, 92% recovery) as an oil (*n*-hexane:AcOEt = 4:1) and (±)-**15** (0.080 g, 61% conversion) as an oil (*n*-hexane:AcOEt = 2:1). The NMR data of (±)-**15** was identical with those of the reported (±)-**15**.[5] (±)-**16**: IR (neat): 1710 cm^{-1}, ^{1}H-NMR: δ 2.00 (3H, s), 2.24 (3H, s), 3.68 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.98 (1H, dddd, *J* = 8.0, 8.0, 7.0, 2.0 Hz), 4.26 (1H, d, *J* = 8.0 Hz), 4.30 (1H, d, *J* = 8.0 Hz), 5.78 (1H, dd, *J* = 16.0, 2.0 Hz), 6.56 (1H, s), 6.64 (1H, s), 7.03 (1H, dd, *J* = 16.0, 7.0 Hz); EI MS (High); *Anal*. Found: 322.1440. Calculated for $C_{17}H_{22}O_6$: 322.1416.

3.2.4. Methyl (±)-5-acetoxy-4-(1′-naphtyl)-2(E)-pentenoate *20* and methyl (±)-5-hydroxy-4-(2′-naphtyl)-2(E)-pentenoate *19*

A suspension of a 2:1 mixture (1.77 g; (±)-**20**; corresponding to 1.18 g, (±)-**21**; corresponding to 0.59 g) of (±)-**20** and (±)-**21**, lipase Amano P (0.50 g) in phosphate buffer (pH 7.4; 100 mL) was stirred at 33 °C for 21 h. The reaction mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (30 g) to give (±)-**20** (1.13 g, 95% recovery) as an oil (*n*-hexane:AcOEt = 10:1) and (±)-**19** (0.421 g, 83% conversion) as an oil (*n*-hexane:AcOEt = 5:1). (±)-**19**: IR (neat): 1721 cm^{-1}, ^{1}H-NMR: δ 2.12 (1H, s), 3.70 (3H, s), 3.81 (1H, br,q, *J* = 7.0 Hz), 3.95 (2H, d, *J* = 7.0 Hz), 5.92 (1H, dd, *J* = 16.0, 2.0 Hz), 7.22 (1H, dd, *J* = 16.0, 7.0 Hz), 7.31 (1H, dd, *J* = 8.0, 2.0 Hz), 7.42-7.50 (2H, m), 7.66 (1H, d, *J* = 2.0 Hz), 7.76-7.83 (3H, m); FAB MS *m/z*: 257 $(M+1)^+$; *Anal*. Found: C, 74.74; H, 6.33. Calculated for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29%. The structure of (±)-**19** was confirmed by the n.O.e., enhancement for the C(4)-proton and aromatic C(1′)-proton (8.4%) and C(3′)-proton (4.2%), respectively, as shown in Fig. 1. (±)-**20**: IR (neat): 1725 cm^{-1}, ^{1}H-NMR: δ 2.03 (3H, s), 3.71 (3H, s), 4.46 (1H, dd, *J* = 10.0, 12.0 Hz), 4.55 (1H, dd, *J* = 6.0, 12.0 Hz), 4.71 (1H, br. q, *J* = 7.0 Hz), 5.94 (1H, dd, *J* = 16.0, 2.0 Hz), 7.26 (1H, dd, *J* = 16.0, 7.0 Hz), 7.34 (1H, br. d, *J* = 8.0 Hz), 7.43 (1H, t, *J* = 8.0 Hz), 7.49 (1H, br.t, *J* = 8.0 Hz), 7.54 (1H, br.t, *J* = 8.0 Hz), 7.78 (1H, br.d, *J* = 8.0 Hz), 7.87 (1H, br.d, *J* = 8.0 Hz), 8.08 (1H, br.d, *J* = 8.0 Hz); FAB MS *m/z*: 298 $(M + 1)^+$; *Anal*. Found: C, 72.33; H, 6.10. Calculated for $C_{18}H_{18}O_4$: C, 72.49; H, 6.08%.

Methyl (±)-5-hydroxy-4-(1′-naphtyl)-2(*E*)-pentenoate **18**
A mixture of (±)-**20** (1.13 g, 3.79 mmol) and K_2CO_3 (0.06 g, 0.43 mmol) in MeOH (5 mL) was stirred for 3 h at room temperature. The reaction mixture was diluted with saturated brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated. The residue was subjected to column chromatography on silica gel (30 g, *n*-hexane:AcOEt = 5:1) to give (±)-**18** (0.73 g, 75% yield) as an oil. (±)-**18**: IR (neat): 3429, 1715 cm^{-1}, ^{1}H-NMR: δ 1.88 (1H, s), 3.70 (3H, s), 4.02–4.10 (2H, m), 4.54 (1H, br, q, *J* = 7.0 Hz), 5.95 (1H, dd, *J* = 16.0, 2.0 Hz), 7.28 (1H, dd, *J* = 16.0, 7.0 Hz), 7.37 (1H, br. d, *J* = 8.0 Hz), 7.45 (1H, t, *J* = 8.0 Hz), 7.47-7.57 (2H, m), 7.78 (1H, br. d, *J* = 8.0 Hz), 7.88 (1H, br. d, *J* = 8.0 Hz); FAB MS *m/z*: 257 $(M + 1)^+$; *Anal*. Found: C, 75.00; H, 6.42. Calculated for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29%. The structure of (±)-18 was confirmed by the n.O.e., enhancement for the C(4)-proton and aromatic C(2′)-proton (19%) as shown in Fig. 1.

3.2.5. Methyl (±)-5-acetoxy-4-(1′-6′,7′,8′,9′-tetrahydronaphtyl)-2(E)-pentenoate *24* and methyl (±)-5-hydroxy-4-(2′-6′,7′,8′,9′-tetrahydronaphtyl)-2(E)-pentenoate *23*

A suspension of a 1:2 mixture (0.78 g; (±)-**24**; corresponding to 0.26 g, (±)-**25**; corresponding to 0.52 g) of (±)-**24** and (±)-**25**, lipase Amano P (0.20 g) in phosphate buffer (pH 7.4; 40 mL) was stirred at 33 °C for 21 h. The reaction mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (30 g) to give (±)-**24** (0.20 g, 76% recovery) as an oil (*n*-hexane:AcOEt = 8:1) and (±)-**23** (0.30 g, 67% conversion) as an oil (*n*-hexane:AcOEt = 5:1). (±)-**23**: IR (neat): 3433, 1720 cm^{-1}, ^{1}H-NMR: δ 1.76–1.82 (4H, m), 1.78 (1H, s), 2.70–2.78 (4H, m), 3.60 (3H, br. q, *J* = 7.0 Hz), 3.71 (3H, s), 3.88 (2H, d, *J* = 7.5 Hz), 5.90 (1H, dd, *J* = 16.0, 2.0 Hz), 6.90 (1H, br. s), 6.92 (1H, dd, *J* = 8.0, 2.0 Hz), 7.03 (1H, d, *J* = 8.0 Hz), 7.12 (1H, dd, *J* = 16.0, 7.5 Hz); FAB MS *m/z*: 261 $(M + 1)^+$; *Anal*. Found: C, 72.34; H, 7.82. Calculated for $C_{16}H_{20}O_3 \cdot 1/5H_2O$: C, 72.81; H, 7.79%. The structure of (±)-**23** was confirmed by the n.O.e., enhancement for the C(4)-proton and aromatic C(1′)-proton (6%) and C(3′)-proton (6.5%), respectively, as shown in Fig. 1. (±)-**24**: IR (neat): 1732 cm^{-1}, ^{1}H-NMR: δ 1.72-1.87 (4H, m), 2.03 (3H, s), 2.64-2.82 (4H, m), 3.72 (3H, s), 3.99 (1H, br,q, *J* = 7.0 Hz), 4.27–4.36 (4H, m), 5.83 (1H, dd, *J* = 16.0, 2.0 Hz), 6.95 (1H, dd, *J* = 8.0, 2.0 Hz), 6.99

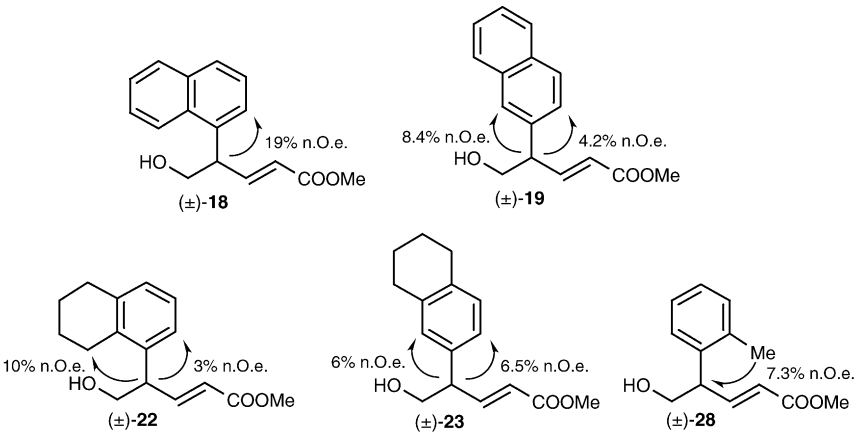


Fig. 1.

(1H, dd, J = 8.0, 2.0 Hz), 7.07 (1H, dd, J = 16.0, 7.0 Hz), 7.08 (1H, t, J = 8.0 Hz); FAB MS m/z: 303 $(M + 1)^+$; *Anal*. Found: C, 71.60; H, 7.40. Calculated for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33%.

Methyl (±)-5-hydroxy-4-(1′-6′,7′,8′,9′-tetrahydronaphtyl)-2(*E*)-pentenoate **22** A mixture of (±)-**24** (0.20 g, 0.65 mmol) and K_2CO_3 (0.06 g, 0.43 mmol) in MeOH (4 mL) was stirred for 2.5 h at room temperature. The reaction mixture was diluted with saturated brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated. The residue was subjected to column chromatography on silica gel (30 g, *n*-hexane:AcOEt = 5:1) to give (±)-**22** (0.15 g, 87% yield) as an oil. (±)-**22**: IR (neat): 3431, 1719 cm^{-1}, ^{1}H-NMR: δ 1.71 (1H, s), 1.72-1.87 (4H, m), 2.64–2.83 (4H, m), 3.72 (3H, s), 3.88–3.92 (2H, m), 3.98 (1H, br, q, J = 7.0 Hz), 5.85 (1H, dd, J = 16.0, 2.0 Hz), 6.98 (1H, br. d, J = 8.0 Hz), 7.00 (1H, br. d, J = 8.0 Hz), 7.08 (1H, dd, J = 16.0, 7.0 Hz), 7.11 (1H, t, J = 8.0 Hz); FAB MS m/z: 261 $(M + 1)^+$; *Anal*. Found: C, 73.61; H, 7.79. Calculated for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74%. The structure of (±)-**22** was confirmed by the n.O.e., enhancement for the C(4)-proton and aromatic C(3′)-proton (3%) and C(9′)-proton (10%), respectively, as shown in Fig. 1.

*3.2.6. Methyl (±)-5-hydroxy-4-(4′-methylphenyl)-2(E)-pentenoate **26**, methyl (±)-5-hydroxy-4-(3′-methylphenyl)-2(E)-pentenoate **27** and methyl (±)-5-acetoxy-4-(2′-methylphenyl)-2(E)-pentenoate **31***

A suspension of a 2.5:1.0:1.2 mixture (2.34 g; (±)-**29**; corresponding to 1.245 g, (±)-30; corresponding to 0.498 g and (±)-**31**; corresponding to 0.597 g) of (±)-**29**, (±)-**30** and (±)-**31**, lipase Amano P (0.60 g) in phosphate buffer (pH 7.4; 40 mL) was stirred at 33 °C for 21 h. The reaction mixture was filtered, and the precipitate was washed with ether. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was separated by column chromatography on silica gel (50 g) to give (±)-31 (0.69 g, >99% recovery) as an oil (*n*-hexane:AcOEt = 10:1) and a mixture (1.19 g, 81% conversion) of (±)-**26** and (±)-**27** as an oil (*n*-hexane:AcOEt = 7:1). (±)-**31**: IR (neat): 1732 cm^{-1}, ^{1}H-NMR: δ 2.04 (3H, s), 2.35 (3H, s), 3.73 (3H, s), 4.08 (1H, br,q, J = 7.0 Hz), 4.32–4.37 (2H, m), 5.83 (1H, dd, J = 16.0, 2.0 Hz), 7.10 (1H, dd, J = 16.0, 7.0 Hz), 7.12–7.22 (4H, m); FAB MS m/z: 263 $(M+1)^+$; *Anal*. Found: C, 68.42; H, 7.04. Calculated for $C_{15}H_{18}O_4$: C, 68.68; H, 6.91%.

Methyl (±)-5-hydroxy-4-(2'-methylphenyl)-2(*E*)-pentenoate **28** A mixture of (±)-**31** (0.52 g, 1.98 mmol) and K_2CO_3 (0.06 g, 0.43 mmol) in MeOH (3 mL) was stirred for 1 h at room temperature. The reaction mixture was diluted with saturated brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated. The residue was subjected to column chromatography on silica gel (20 g, *n*-hexane:AcOEt = 5:1) to give (±)-**28** (0.38 g, 87% yield) as an oil. (±)-28: IR (neat): 3432, 1716 cm^{-1}, ^{1}H-NMR: δ 2.10 (1H, s), 2.33 (3H, s), 3.70 (3H, s), 3.86–3.91 (2H, m), 3.94 (1H, br, q, J = 7.0 Hz), 5.84 (1H, dd, J = 16.0, 2.0 Hz), 7.11 (1H, dd, J = 16.0, 7.0 Hz), 7.13–7.21 (4H, m); FAB MS m/z: 221 $(M+1)^+$; *Anal*. Found: C, 70.61; H, 7.36. Calculated for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32%. The structure of (±)-**28** was confirmed by the n.O.e., enhancement for the C(4)-proton and aromatic C(2′)-methyl proton (7.3%) as shown in Fig. 1.

3.3. Discussion

When the reaction site of hydrolysis by lipase was located near the *ortho*-substituents in the aromatic ring of the substrates such as (±)-**9**, (±)-**20**, (±)-**24**, and (±)-**31**, the rate of hydrolysis of the acetoxyl group is found to be particularly slower than that of the substrates, (±)-**8**, (±)-**21**, (±)-**25**, (±)-**29**, and (±)-**30** with no substituent at the *ortho*-position. Moreover, the rate of hydrolysis of the acetoxyl group of the substrates (±)-**13** and (±)-**17** possessing a remote reaction site from the *ortho*-position was ascertained to be particularly faster than that of the substrates (±)-**12** and (±)-**16**. These phenomena indicate that the less hindered acetoxyl group is hydrolyzed faster than the more hindered one.

4. Conclusion

Enzymatic separation of six hardly separable mixtures of structural isomers was carried out. It has become apparent that the hydrolysis rate of the C(5)-acetoxyl group of methyl (±)-5-acetoxy-4-aryl-(2*E*)-pentenoate possessing the *ortho*-substituents in the aromatic ring was slower than that of the substrates with no substituents at the *ortho*-position of the aromatic ring. Moreover, the hydrolysis rate of the C(5)-acetoxyl group of methyl (±)-5-acetoxy-2-aryl-(3*E*)-pentenoate possessing a remote reaction site from the *ortho*-substituents was proved to be faster than that of methyl (±)-5-acetoxy-4-aryl-(2*E*)-pentenoate.

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