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APPLICATION OF LIPASE-CATALYZED TRANSFORMATION FOR SYNTHESES OF INSECT PHEROMONES AND RELATED COMPOUNDS

1992

Eiichiro Fukusaki

APPLICATION OF LIPASE-CATALYZED TRANSFORMATION FOR SYNTHESES OF INSECT PHEROMONES AND RELATED COMPOUNDS

（リパーゼを利用した昆虫フェロモンおよび関連物質の合成）

A thesis submitted to Department of Biotechnology, Faculty of Engineering, Osaka University for doctoral degree

1992

Eiichiro Fukusaki

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INTRODUCTION

Enzymes have been widely used as catalysts in organic synthesis. The immense potential of enzymes as catalysts in organic synthesis is well documented. The major advantage of enzymes for organic synthesis is their selectivity. Enzymes are selective not only in the type of reaction they catalyze, but also in their site of attack on a molecule. Well documented phenomena include chemoselective reaction of one of different functional groups in a molecule, regioselective reaction of one of identical groups in a molecule, enantioselective reaction of one of the enantiomers in a racemic pair, and enantiotopic reaction of a prochiral molecule.[1)-4)]

Among the enzymes, lipases have been widely used for the resolution of various racemic alcohols, either *via* hydrolysis of the corresponding esters in aqueous media or *via* esterification and transesterification in organic solvent,[4)-8)] because they have a remarkable ability to assuming a variety of conformations to accommodate substrates of varying sizes and shapes. Lipases usually do not require any cofactor regeneration and they are stable. These are very strong points for their application. In addition to this catalytic versatility, these enzymes are readily available commercially in sizable quantities and at relatively low cost. For example, one can purchase some 20 lipases isolated from different origins; even though all of them are called lipases and catalyze the same reactions, each one's specificities are highly specialized.[9)] One kilogram of porcine pancreatic lipase can be obtained from Amano Pharmaceutical Co., Japan for less than ¥5,000; although this enzyme preparation is very crude, it has

proved to be quite suitable as a practical catalyst. A number of other lipases can be bought in amounts of 100g for ¥10,000. Additionary, lipase is uniquely stable in nonpolar organic solvents.[5)-8)] This trait of lipases is very important for their application because water is not the ideal reaction medium for most organic processes. For preparative organic syntheses, it would be better to carry out enzymatic conversions in organic solvents rather than water for several reasons[9)]: 1) many organic substrates dissolve better in organic solvents than they do in water; 2) enzymes can be easily recovered and reused without immobilization because enzymes are insoluble in organic solvents; 3) compounds that are unstable in water, such as anhydrides, halogenates could be used as substrates; 4) thermodynamic equilibria of many processes are unfavorable in water -- examples include syntheses of esters from carboxylic acids and alcohols; 5) product recovery from aqueous solutions is often difficult and expensive. For these reasons, lipase-catalyzed transformation is becoming increasingly important in organic synthesis. A recent study has compared the utility of the lipase-catalyzed resolution of racemic alcohol, as a way of obtaining chiral alcohol, with asymmetic reduction of the corresponding ketone by a chemical catalyst.[10)] Although the chemical method is efficient at the laboratory scale, it has some disadvantages and is thereby not practical on a large scale. The disadvantages of the chemical method include: 1) the method requires expensive asymmetric reducing agents; 2) in many cases, the reaction temperature should be kept at very low temperatures -- below -50°C; and; 3) it requires dangerous reagents such as lithium aluminum hydride. For the above reasons, in a standpoint of industrial application,

lipase-catalyzed transformation is expected to become a new powerful procedure that is safe and inexpensive.

The aim of this study is to develop the potential of lipase-catalyzed transformations for preparing of valuable compounds on a comparatively large scale. The target compounds are sex pheromones of pests (including the Japanese beetle (*Popillia japonica* Newman),[11] the cupreous chafer beetle (*Anomala cupurea* Hope),[12] and the gypsy moth (*Lymantria dispar* L.)[13]), that are devastating a variety of trees and crops. Also investigated is carboxyalkyl acrylate, which is a new material for the polymer industry.

Insect sex pheromones are expected to become a new type of pesticides that work by disrupting mating or mass trapping.[14] All these target pheromones are optically active molecules whose chiral centers are critical to their activities. Unnatural enantiomers that correspond to natural ones strongly inhibit a pheromone's male response. Therefore extremely high optical purity is essential for practical use of pheromone. These pheromones' utility is obvious, but efficient routes for large scale preparation of these compound have not been developed. The author successfully applied the lipase-catalyzed enantioselective transformations for their preparation.

Another target compound, carboxyalkyl acrylates, are used as special monomers in the synthesis of the new polymers. Polymers containing acryl esters bearing carboxyalkylside chains are expected to be useful for preparing such products as drug carriers, biomaterials, and pressure sensitive adhesive polymers. Commonly used acrylate, such as methyl acrylate, ethyl acrylate, butyl acrylate, and 2-ethylhexyl acrylate, can be produced by a direct chemically-catalyzed transesterification method.[15] However,

this method cannot serve as an efficient route for the preparation of carboxyalkyl acrylates that have two functional groups, i.e., the ester and carboxyl groups, since it would yield a complex mixture of unreacted hydroxy carboxylic acid and partially and fully esterified compounds. The author applied a lipase-catalyzed regioselective transformation.

In this thesis, the author employs the E value (enantiomeric ratio) developed by Chen *et al.* [16] to evaluate the enantioselectivity of enzymatic reactions.

In chapter 1, the author describes two lipase-catalyzed reactions. One is enantioselective acylation of methyl 4-hydroxy-5-tetradecynoate, and the other is enantioselective lactonization of methyl 4-succinoyloxy-5-tetradecynoate. Consequently, A facile synthesis of the Japanese beetle's (*Popillia japonica* Newman) sex pheromone, (*R*,*Z*)-(-)-5-(1-decenyl)oxacyclopentan-2-one, was established by a combination of two lipase-catalyzed reactions.

Chapter 2 describes, a synthesis of both enantiomers of the cupreous chafer beetle's (*Anomala cuprea* Hope) sex pheromone, (*Z*)-5-(1-octenyl)oxacyclopentan-2-one. The lipase-catalyzed enantioselective acylation of methyl 4-hydroxy-5-dodecynoate was repeated twice as a key reaction.

In the third chapter, the author describes a lipase-catalyzed optical resolution of 8-methyl-2,3-epoxy-1-nonanol, the key intermediate of the synthesis of the gypsy moth's (*Lymantria dispar* L.) sex pheromone. Enantioselective acylation and alcoholysis are discussed.

Chapter 4 outlines a large scale synthesis of (+)-disparlure, the sex

pheromone of the gypsy moth, by a combination of Sharpless asymmetric epoxidation and lipase-catalyzed enantioselective acylation.

Finally, a facile synthesis of carboxyalkyl acrylate by two lipase-catalyzed regioselective reactions is described in chapter 5. One of these reactions is a regioselective transesterification of methyl hydroxyalkcanoate with vinyl acrylate to yield methoxycarbonylalkyl acrylates. The other is a regioselective hydrolysis of methoxycarbonylalkyl acrylates to yield carboxyalkyl acrylates.

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CHAPTER 1

LIPASE-CATALYZED KINETIC RESOLUTION OF METHYL 4-HYDROXY-5-TETRADECYNOATE AND ITS APPLICATION TO A FACILE SYNTHESIS OF JAPANESE BEETLE PHEROMONE

INTRODUCTION

The Japanese beetle, *Popillia japonica* Newman, is a devastating pest of a variety of trees and crops in the United States. Tumlinson et al. have isolated its pheromone from virgin females and identified it as (*R*,*Z*)-(-)-5-(1-decenyl)oxacyclopentan-2-one ((*R*,*Z*)-**1**).

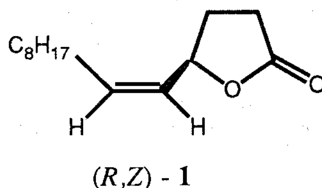
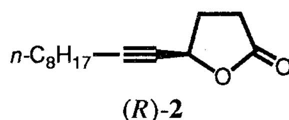


Fig. 1-1 The Japanes beetle sex pheromone

This pheromone has a unique feature whereby a small amount of unnatural (*S*,*Z*)-isomer strongly inhibits the male response to it. Indeed, 2% contamination of (*S*,*Z*)-isomer causes the mixture to be three times less active than optically pure pheromone.[1] Therefore, extremely high optical

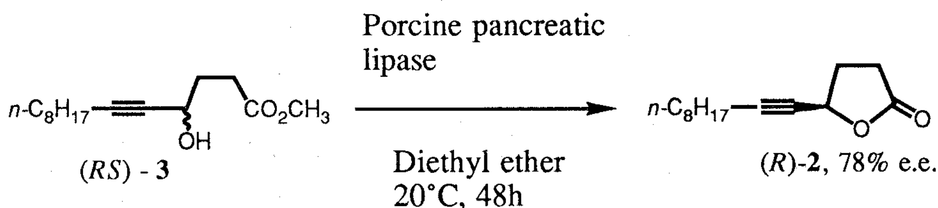
purity is essential for the practical use of this pheromone.

In the original synthesis of Doolittle et al. (*R*)-(-)-glutamic acid was used as its starting material.[2] Since then, a number of other syntheses have been reported.[3)-13] In several of these studies, the acetylenic lactone (*R*)-**2** has been established as the key-intermediate.[4)-9]



(*R*)-**2**

Recently, a synthesis involving the lipase-catalyze enantioselective lactonization of (*RS*)-**3** to yield (*R*)-**2** as the key-reaction (Scheme 1-1) was reported by Sugai et al.[12]



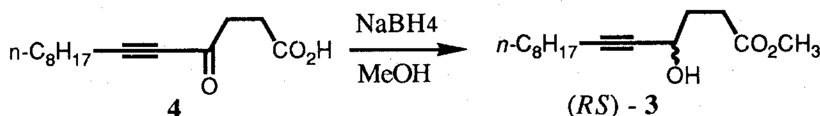
Scheme 1-1 Lipase-catalyzed enantioselective lactonization of (*RS*)-3 in an organic solvent

In Sugai's synthesis, optically active lactone (*R*)-**2** of 97% ee was prepared by repetitions of the above reaction . Although this method was very

efficient for the optical enrichment of (*R*)-**2**, it could not raise the optical purity sufficiently high for practical use because the enantioselectivity of the above reaction was not so high (E=20).[14] This chapter described a practical chemicoenzymatic synthesis of Japanese beetle pheromone (*R*,*Z*)-**1** of over 99% e.e. which involves two lipase-catalyzed crucial steps : enantioselective acylation of γ-hydroxy ester **3** and enantioselective lactonization of γ-acyloxy ester **5** . In addition, it was described that the repetition of the first process, lipase-catalyzed enantioselective acylation, was also successful to obtain (*R*)-**2** over 99% e.e..

RESULTS AND DISCUSSIONS

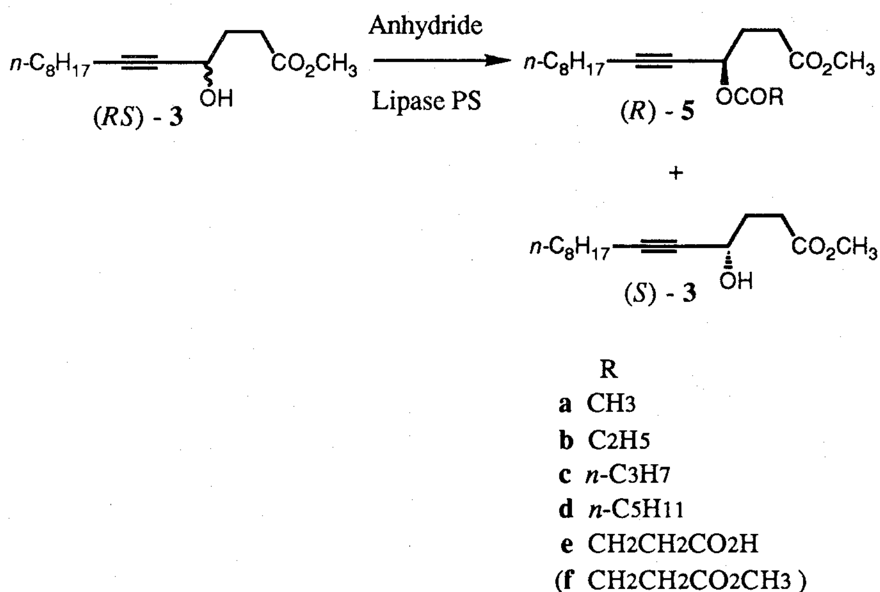
The starting material, methyl 4-hydroxy-5-tetradecynoate ((*RS*)-**3**), was easily prepared by the reduction of methyl 4-oxo-5-tetradecynoate[9] with sodium borohydride.[12] (Scheme 1-2)



Scheme 1-2 Preparation of (*RS*)-3

First, several commercially available lipases were surveyed for the acylation of (*RS*)-**3** in organic solvent. Lipase PS from *Pseudomonas* sp. (Amano Pharm. Co., Japan) was found to catalyze the enantioselective

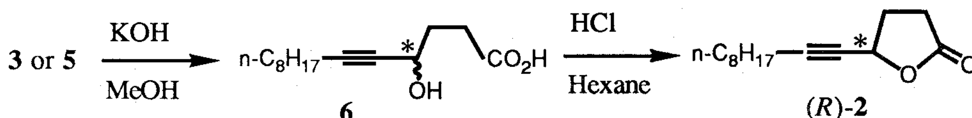
acylation of (*RS*)-**3** with several anhydrides[15] as acylating reagents in diisopropyl ether (Scheme 1-3). Acyclic acid anhydrides such as acetic, propionic, n-butyric, and n-caproic anhydride were examined for acylating activity. Cyclic acid anhydrides such as succinic anhydride, glutaric anhydride, and phthalic anhydride were also examined.



Scheme 1-3 Lipase-catalyzed enatioselective aclation of (*RS*)-3

The reactions were carried out at 30°C and the conversion was monitored by HPLC. When the conversion reached ca. 50%, the reaction was stopped by the removal of enzyme by filtration. Then, acyloxy esters **5** and unreacted substrate **3** were separated by silica gel column chromatography. To determine the ee of **3** and **5**, they were hydrolyzed

with KOH in methanol to yield hydroxy acid **6**. Afte acidification with HCl, **6** was converted to the corresponding lactone **2** by heating in hexane at 60°C (Scheme 1-4).



Scheme 1-4

The ee of **2** was determined by HPLC equipped with a column with a chiral stationary phase, Chiralcel OD (Daicel Chemical Co., Japan). The absolute configuration was determined by comparing the HPLC data of **2** with that of (*R*)-**2** prepared by a previously reported procedure.[9)]

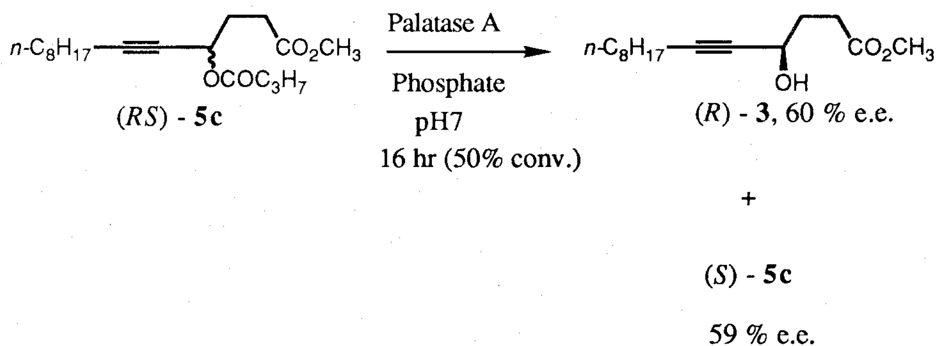
Results are summarized in Table 1-1. The best result was obtained, when succinic anhydride was used as the acylating reagent (E=115). On the contrary, glutaric anhydride and phthalic anhydride provided very poor yields (data not shown). Among acyclic anhydrides, n-butyric anhydride was proved to be the best acylating reagent (E=94). As for solvents, toluene and isooctane, which are recognized as suitable for enzymatic reactions in non-aqueous media[16)], were also examined as reaction solvents. Diisopropyl ether was selected as the best solvent because the reaction was faster than toluene, and lactone **2** was formed as a by-product in isooctane. Acylation with enol esters[17)] such as vinyl acetate were examined, but the reaction rates were too slow for practical application.

Table 1-1 Acylation of (*RS*)-2 by Lipase PS

entry	anhydride	conv.(%)	time (h)	product , ee (%)		E[a]
1	acetic	51	18	(*R*)-**5a** , 85	(*S*)-**2** , 90	36
2	propionic	51	18	(*R*)-**5b** , 86	(*S*)-**2** , 91	40
3	n-butyric	50	18	(*R*)-**5c** , 93	(*S*)-**2** , 93	94
4	n-caproic	50	18	(*R*)-**5d** , 92	(*S*)-**2** , 92	79
5	succinic	50	24	(*R*)-**5e** , 94	(*S*)-**2** , 94	115

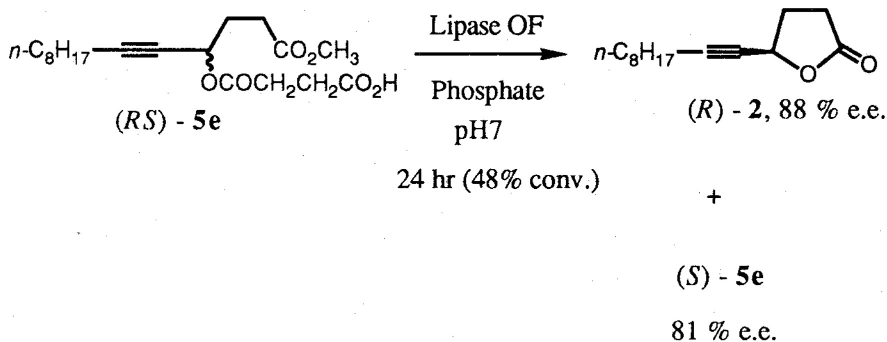
a) E value [E=ln[1-c(1+ee(P))]/ln[1-c(1-ee(P))]] was calculated on the basis of the ee of **5**.

To enrich further the ee of desired (*R*)-enantiomer, asymetric hydrolysis of ester **5** was attempted. First, hydrolysis of butanoate **5c** was examined. After surveying of several commercially available lipases, Palatase A, a lipase from *Aspergillus niger* (Novo Ind. Co., Denmark), was found to catalyze the hydrolysis of **5c** (Scheme 1-5). It hydrolyzed (*RS*)-**5c** in 0.1M phosphate buffer (pH 7) to give (*R*)-**3** (60%ee) and (*S*)-**5c** (59%ee). The reaction was moderately stereoselective (E=7). These results, however, were unsatisfactory for preparing the extremely pure target lactone (*R*)-**2**.



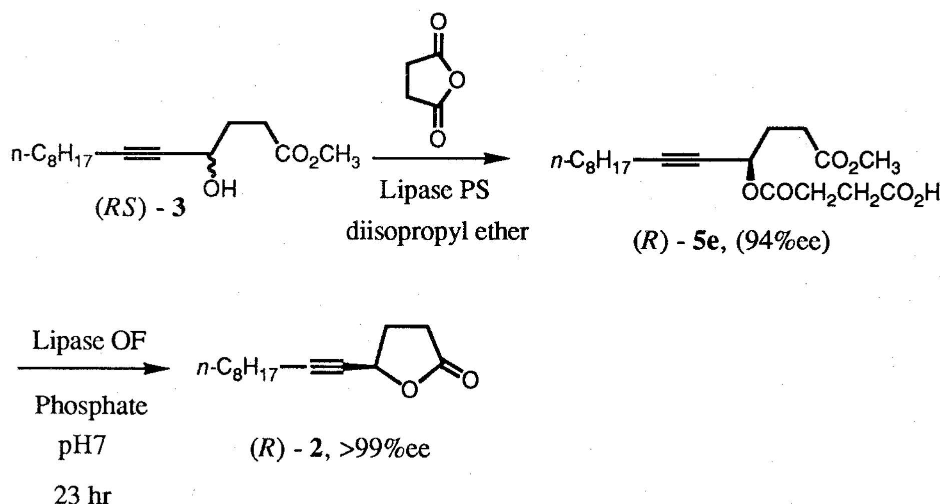
Scheme 1-5 Lipase-catalyzed enantioselective hydrolysis of (*RS*)-5c

Higher stereoselectivity was then expected by using succinoate **5e** as the substrate. Although the enzymatic hydrolysis of **5e** was unsuccessful, we found that lipase OF from *Candida cylindracea* (Meito Sangyo Co., Japan) catalyzed the lactonization of **5e** to yield (*R*)-**2** (88% e.e.) (Scheme 1-6). The E value in this case was 39.



Scheme 1-6 Lipase-catalyzed enantioselective lactonization of (*RS*)-5e

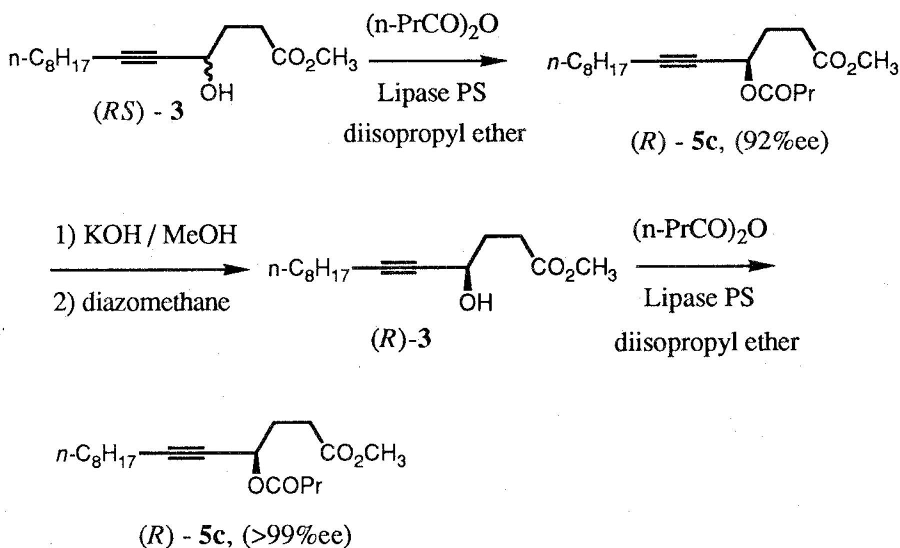
Lipase catalyzed lactonization of hydroxy esters in organic solvents have been previously reported.[18), 19)] Following those procedures, lactonization of (*RS*)-**5c** or (*RS*)-**5e** was tried in organic solvents such as toluene, isooctane, diethyl ether and diisopropyl ether. Those attempts, however, were not successful. In order to refine the methodology to obtain highly optically pure lactone (*R*)-**2**, this lipase OF-catalyzed reaction was applied to (*R*)-**5e**, which was prepared in 94% ee with Lipase PS (Scheme 1-7). The reaction was stopped after 23 hr and (*R*)-**2** was isolated with over 99% e.e. (83% yield).



Scheme 1-7 Preparation of optically pure lactone (*R*)-2 by the conbination of lipase-catalyzed acylation and lanconization

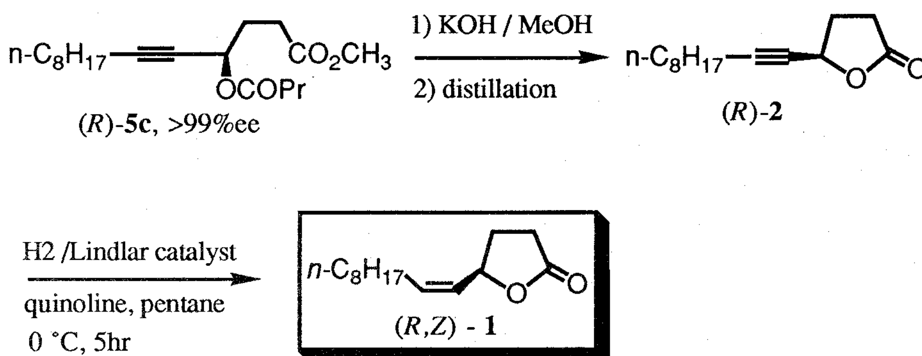
Another successful way to increase the ee of **5** was the repetition of

lipase PS-catalyzed esterification (Scheme 1-3). These reactions with n-butyric anhydride or succinic anhydride as acyl donor are so highly enantioselective (E values is 94 and 115 respectively) that one repetition of these reactions was enough to obtain the optically active (*R*)-**5** with over 99% e.e.. For example, (*R*)-**5c** (92% e.e.) obtained by the first enzymatic reaction was converted to (*R*)-**3** by hydrolysis with KOH in methanol followed by methylation with diazomethane. That (*R*)-**3** was then subjected to the second enzymatic esterification. The second reaction was stopped after a shorter period (10 hr) and (*R*)-**5c** was isolated with over 99%ee (80% yield). (Scheme 1-8)



Scheme 1-8 Preparation of optically pure (*R*)-5c by the repetition of lipase-catalyzed aclation

(R)-**5c** was hydrolyzed with KOH to yield hydroxy acid (R)-**6**,which was distilled to give optically pure lactone (R)-**2**. Semihydrogenation of (R)-**2** by a previously reported procedure[9] (Scheme 1-9) gave the Japanese beetle pheromone (R,Z)-**1**, $[\alpha]_D^{25}$ -73.9°(chloroform). Among the reported values of the specific rotations in a chloroform solution (-70.82°,[11] -70.4°,[9),10)] -70.0°,[2),3)] -69.93°,[7)] -69.7°,[8)] -68.1°,[12)] -63.1°,[13)]), this result (-73.9°) provided the highest value.



Scheme 1-9 Preparation of Japanese beetle pheromone

In conclusion, a new effective synthesis of the Japanese beetle pheromone (R,Z)-**1** was established by combining enzymatic and chemical methods without using any chiral auxiliaries.

SUMMARY

A kinetic resolution of methyl 4-hydroxy-5-tetradecynoate is accomplished by a lipase-catalyzed enantioselective acylation in organic solvent. Acylation of methyl (*RS*)-4-hydroxy-5-tetradecynoate with succinic anhydride in an organic solvent yields methyl (*R*)-4-succinoyloxy-5-tetradecynoate with over 90% e.e.. Furthermore, this optically active diester was converted to (*R*)-5-(1-decynyl)oxacyclopentan-2-one by lipase-catalyzed enantioselective lactonization which enhanced its e.e. over 99%. The Japanese beetle pheromone (*R*,*Z*)-(-)-5-(1-decenyl)oxacyclopentan-2-one is synthesized in one step from this optically active lactone.

EXPERIMENTAL SECTION

Boiling points are uncorrected. IR spectra were recorded in neat on JASCO A-810 spectrophotometer. 1H-MNR spectra were measured at 400MHz on a JEOL GX-400. 13C-NMR spectra were measured at 100MHz on a JEOL GX-400. Optical rotations were measured on JASCO DIP-181. Column chromatography was effected using Merck Kieselgel 60 (70-230 mesh). Lipase PS was purchased from Amano Pharm. Co. Palatase A was purchased from Novo Ind. Co. Lipase OF was purchased from Meito Sangyo Co. Solvent for enzymatic reaction was distilled before use.

Methyl (RS)-4-butanoyloxy-5-tetradecynoate (RS)-5c

(*RS*)-**3**[12)] (10.0 g, 39 mmol) was acylated with butyryl chloride (5.0

g, 47 mmol) in pyridine (50 ml) in the usual manner and purified by silica gel column chromatography [Elution with hexane/EtOAc (9/1)] to give (*RS*)-**5c** (11.1 g, 88 % yield). νmax 2940, 2860, 2230, 1740, 1180 cm-1; δ (^{1}H, 400 MHz, $CDCl_3$) 0.87(3H, t, J=6.6 Hz), 0.94(3H, t, J=7.3Hz), 1.25-1.35(10H, bm), 1.47(2H, m), 1.65(2H, m), 2.06(2H, m), 2.17(2H, m), 2.28(2H, m), 2.47 (2H, t, J=7.0Hz), 3.67(3H, s), 5.42(1H, deformed t, J=6.1Hz) ; δ(^{13}C, 100 MHz, $CDCl_3$) 13.45, 14.13, 18.43, 18.70, 22.68, 28.48, 28.86, 29.08, 29.19, 29.72, 30.27, 31.85, 36.21, 51.73, 63.22, 76.78, 86.99, 172.51, 173.19 ; (Found: C, 70.15 ; H, 9.95 %. Calc for $C_{19}H_{32}O_4$: C, 70.33 ; H, 9.94 %)

Methyl (RS)-4-succinoyloxy-5-tetradecynoate (RS)-5e

(*RS*)-**3** (10.0 g, 39 mmol) was acylated with succinic anhydride (4.7 g, 47 mmol) in pyridine (50 ml) in the usual manner and purified by silica gel column chromatography [Elution with hexane/EtOAc (5/1)] to give (*RS*)-**5e** (11.5 g, 83 % yield). νmax 3300, 2940, 2850, 2240, 1740, 1710,1160 cm-1 ; δ(^{1}H, 400 MHz, $CDCl_3$) 0.85 (3H, t, J=7.0 Hz), 1.24 (8H, m), 1.32 (2H, m), 1.46 (2H, m), 2.05 (2H, td, J=7.3, 6.2 Hz), 2.46 (2H, t, J=7.6Hz), 2.61 (2H, m), 2.66 (2H, m), 3.66 (3H, s), 5.42 (1H, t, J=6.2Hz); δ(^{13}C, 100 MHz, $CDCl_3$) 14.07, 18.65, 22.64, 28.40, 28.84, 28.91, 29.02, 29.15, 29.57, 30.15, 31.81, 51.74, 63.97, 76.36, 87.38, 170.98, 173.20, 177.86 ; (Found: C, 64.22 ; H, 8.51%. Calc for $C_{19}H_{30}O_6$: C, 64.39 ; H, 8.53 %)

General procedure for acylation of (RS)-2 using lipase PS

(*RS*)-**3** (30 mg, 0.118 mmol) and acid anhydride (0.12 mmol) were dissolved in diisopropyl ether (3 ml). Lipase PS (30 mg) was added to the

solution and the suspension was stirred at 30 °C for indicated time in Table 1. The conversion of the reactions were determined by HPLC analysis on the system consisting of pump [JASCO 880-PU] and UV detector [JASCO 875-UV] equipped with ODS column [NUCLEOSIL 7C-18, 4.6 x 250mm; acetonitrile/distilled water (70/30), 2ml/min, 215nm]. The retention times of **2**, **3**, and **5** were: **2**, 3.7min; **3**, 3.1min; **5a**, 4.9min; **5b**, 6.1min; **5c**, 7.6min; **5d**, 12.4min; **5f**, 4.8min. In monitoring of acylation of **3** with succinic anhydride, product **5e** was methylated to yield **5f**, which was analyzed by HPLC analysis. After the lipase powder had been removed by filtration, the filtrate was evaporated and chromatographed on a silica gel column [hexane/EtOAc (9/1)] to give **5** and **3**. To determine the enantiomeric excess(ee) of **3**, and **5**, they were hydrolyzed with 5 % KOH in methanol to yield hydoroxy acid **6**, and **6** was converted to **2** by heating in hexane/2N-HCl (1/2) at 60°C for 30min. And then **2** derived from **4** and **5** was subjected to HPLC analysis on the system consisting of pump [JASCO 880-PU] and UV detector [JASCO 875-UV] equipped with a column with a chiral stationary phase [Chiralcel OD, 4.6 x 250mm; hexane/2-propanol(99/1), 1 ml/min, 215nm]. The retention times of (*R*)-**2** and (*S*)-**2** were 17.1 min and 19.6 min respectively. Results were summarized in Table 1.

Lipase catalyzed hydrolysis of (RS)-5c

(*RS*)-**5c** (500 mg, 1.54 mmol) was suspended in 50 ml of phosphate buffer (pH 7). Palatase A (500mg) was added to the solution and the suspension was stirred at 25 °C for 16 hr. The reaction mixture was extracted with ether. The extract was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purifed by

silica gel column chromatography (10 g). Elution with hexane/EtOAc (9/1) gave (*R*)-**3** (165 mg, 43% yield, 60% e.e.). νmax 3480, 2940, 2860, 2230, 1740, 1440, 1250, 1165, 1160 cm-1; δ(^{1}H, 400 MHz, $CDCl_3$) 0.86(3H, t, J=6.2 Hz), 1.25-1.33(10H, bm), 1.45(2H, m), 1.98(2H, m), 2.17(2H, m), 2.25(1H, bs), 2.51(2H, m), 3.66(3H, s), 4.44(1H, t, J=6.2 Hz) Further elution gave (*S*)-**5c** (219 mg, 45% yield, 59% e.e.). Its IR and NMR spectra were identical with those of (*RS*)-**5c** obtained above respectively.

Lipase catalyzed lactonization of (RS)-5e to yield (R)-5-(1-decynyl)oxacyclopentan-2-one (R)-2

(*RS*)-**5e** (500 mg, 1.41 mmol) was suspended in 50ml of phosphate buffer (pH 7). Lipase OF (500mg) was added to the solution and the suspension was stirred at 25 °C for 24 hr. The reaction mixture was extracted with ether. The extract was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 g). Elution with hexane/EtOAc (9/1) gave (*R*)-**2** (128 mg, 41% yield, 88% e.e.). bp 130-134 °C at 0.3 mmHg; νmax 2940, 2860, 2240, 1790, 1185, 1150, 1020 cm-1 ; δ(^{1}H, 400 MHz, $CDCl_3$) 0.89(3H, t, J=7.1 Hz), 1.26-1.39 (10H, m), 1.49 (2H, tt, J=7.1 Hz), 2.19-2.27 (1H, m), 2.21 (2H, td, J=7.1, 2.0 Hz), 2.43-2.53 (2H, m), 2.59-2.69 (1H, m), 5.2 (1H, m); δ(^{13}C, 100 MHz, $CDCl_3$) 14.00, 18.60, 22.56, 27.90, 28.20, 28.74, 28.96, 29.07, 30.07, 31.74, 69.69, 76.46, 88.78, 176.23. Further elution with hexane/EtOAc(5/1) gave (*S*)-**5e** (195mg, 39% yield, 81% e.e.). Its IR and NMR spectra were identical with those of racemic one obtained above.

Large scale preparation of methyl (R)-4-succinoyloxy-5-tetradecynoate (R)-5e

(*RS*)-**3** (30.0 g, 118.1 mmol) and succinic anhydride (5.9 g, 59.1 mmol) were dissolved in diisopropyl ether (1000 ml). Lipase PS(10 g) was added and the suspension was stirred at room temp for 48hr. After lipase powder had been removed by fiftration, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography(800 g). Elution with hexane/EtOAc(9:1) gave (*S*)-**3** (12.7 g, 43% yield, 88% e.e.). Its IR and NMR spectra was identical with those of racemic one obtained above. Further elution with hexane/EtOAc(5/1) gave (*R*)-**5e** (17.1 g, 41% yield, 94% e.e.). Its IR and NMR spectra were identical with those of racemic one obtained above.

Preparation of (R)-5-(1-decynyl)oxacyclopentan-2-one (R)-3 by lactonization of (R)-4e

(*R*)-**5e** (94 % e.e., 500 mg, 1.41 mmol) was suspended in 50ml of 0.1M phosphate buffer (pH 7). Lipase OF (500 mg) was added to the solution and the suspension was stirred at 25 °C for 23 hr. The reaction mixture was extracted with ether. The extract was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purifed by silica gel column chromatography (10 g). Elution with hexane/EtOAc(9/1) gave (*R*)-**2** (260 mg, 83% yield, >99%ee). bp 135-140 °C at 0.6 mmHg; Its IR and NMR spectra were identical with those of racemic one obtained above.

Large scale preparation of methyl (R)-4-butanoyloxy-5-tetradecynoate (R)-5c

(*RS*)-**3** (20.0 g, 78.7mmol) and n-butyric anhydride (6.2 g, 39.4mmol) were dissolved in diisopropyl ether (200 ml). Lipase PS (5 g) was added and the suspension was stirred at room temp for 30 hr. After lipase powder had been removed by fiftration, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (300 g). Elution with hexane/EtOAc(9:1) gave (*R*)-**5c** (11.0 g, 43 % yield, 92 %ee). Its IR and NMR spectra were identical with those of racemic one obtained above. Further elution gave (*S*)-**3** (9.2 g, 46% yield, 94 %ee). Its IR and NMR spectra were identical with those of racemic one obtained above.

Methyl (R)-4-hydroxy-5-tetradecynoate (R)-3

(*R*)-**5c** (92 % ee, 10.0 g, 30.9 mmol) in 5% KOH in MeOH(100 ml) was stirred and heated under reflux for 15 min. The reaction mixture was concetrated *in vacuo*, diluted with water, adjusted to pH 4 with ice-cooled 1N-HCl, and extrated with ether. The aqueous layer was again extracted with ether. The organic layers were combined, dried over sodium sulfate, and treated with diazomethane. The solution was concetrated *in vacuo*. The residue was purified by silica gel column chromatography (200 g). Elution with hexane/EtOAc (9:1) gave (*R*)-**3** (7.1 g, 91% yield). Its IR and NMR spectra was identical with those of racemic one obtained above.

Second acylation of (R)-2 to yield (R)-4c

(*R*)-**3** (92%ee, 7.10 g, 28.0 mmol) and butyric anhydride (4.15 g, 26.2 mmol) were dissolved in diisopropyl ether (100 ml). Lipase PS (2 g) was added to the solution. The suspension was stirred at room temp for 10 hr. Work up and purification were performed in the same manner

descrived above to give (*R*)-**5c** (7.30 g, 80% yield, >99%ee). Its IR and NMR spectra were identical with those of the primary acylation product.

Preparation of (R)-5-(1-decynyl)oxacyclopentan-2-one (R)-3 from (R)-5c

(*R*)-**5c** (>99 %ee, 7.30 g, 22.5 mmol) was hydrolyzed in 5% KOH in MeOH(80 ml) was stirred and heated under reflux for 15 min. The reaction mixture was concetrated *in vacuo*, diluted with water, adjusted to pH 4 with ice-cooled 1N-HCl, and extracted with ether. The aqueous layer was again extracted with ether. The organic layers were combined, dried over sodium sulfate and concentrated *in vacuo*. The residue was heated at 110-120 °C for 30 min to effect lactonization. Distillation gave 4.03g of (*R*)-**2** (81% yield). bp 135-140 °C (0.6 mmHg). Its IR and NMR spectra were identical with those of (*R*)-**2** obtained above. Its ee was determined to be more than 99%.

(R,Z)-(-)-5-(1-Decenyl)oxacyclopentan-2-one (R,Z)-1

Following the reported prodedure,[9)] semihydrogenation of (*R*)-**2** (>99 %ee, 4.03 g, 18.2 mmol) was accomplished. The crude material was purifed by silica gel column chromatography [hexane/ether(9/1)]. The organic solvent was removed under reduced pressure. The residue was distilled to give (*R*,*Z*)-**1** (3.71 g, 91% yield). bp 100-105 °C (0.5 mmHg) ; $[\alpha]_D^{25}$=-73.9° (c=1.004, $CHCl_3$); νmax 2940, 2860, 1780, 1660, 1460, 1420, 1380, 1330, 1295, 1220, 1180, 1125, 1015, 980, 910, 870, 810, 720 cm-1; δ(^1H, 400 MHz, $CDCl_3$) 0.87 (3H, t, J=6.8 Hz), 1.26 (10H, m), 1.36-1.48 (2H, m), 1.88-1.98 (1H, m), 2.02-2.18 (2H, m), 2.32-2.41 (1H, m), 2.52-2.57

(2H, m), 5.24 (1H, dddd, J=11.7, 11.7, 9.8, 1.2 Hz), 5.44 (1H, ddt, J=9.8, 9.8, 1.7 Hz), 5.65 (1H, dtd, J=10.7, 7.8, 1.2 Hz); δ(^{13}C, 100 MHz, $CDCl_3$) 14.08, 22.65, 27.83, 28.99, 29.19, 29.22, 29.32, 29.40, 29.43, 31.85, 76.42, 127.27, 135.83, 177.07 ; (Found: C, 74.70 ; H, 10.74 %. Calc for $C_{14}H_{24}O_2$: C, 74.95 ; H, 10.78 %)

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CHAPTER 2

SYNTHESIS OF THE ENANTIOMERS OF (Z)-5-(1-OCTENYL)OXACYCLOPENTAN-2-ONE, A SEX PHEROMONE OF THE CUPREOUS CHAFER BEETLE, *ANOMALA CUPREA* HOPE

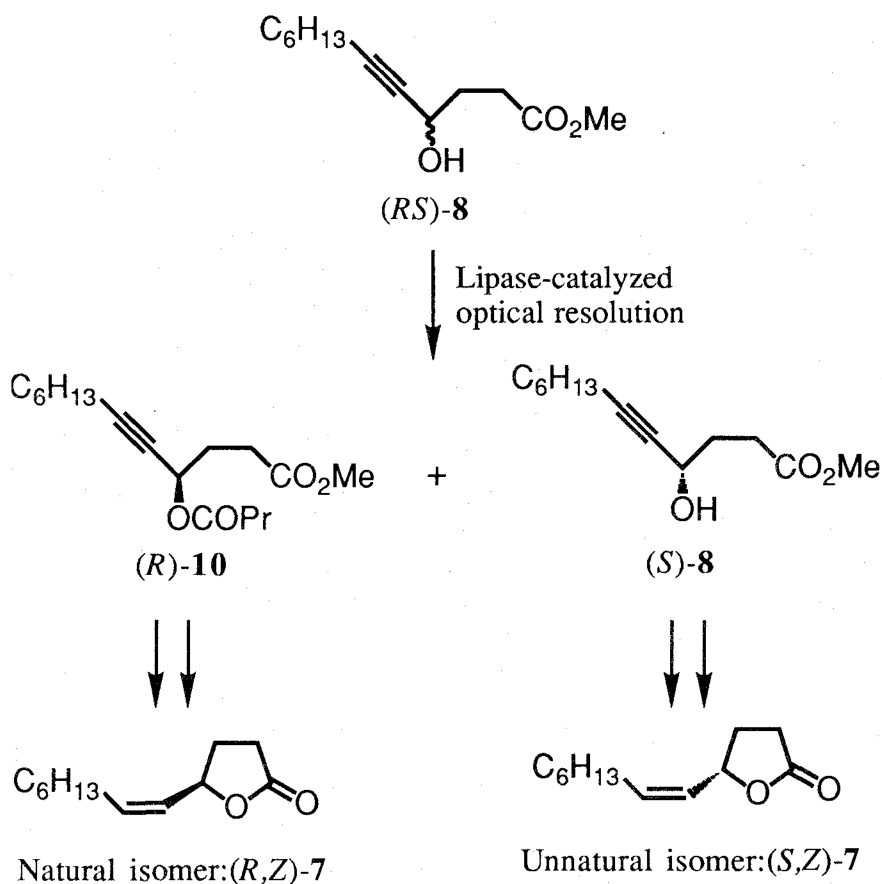
INTRODUCTION

The cupreous chafer beetle, *Anomala cuprea* Hope, is a devastating pest to a variety of crops in Japan. Leal has isolated its pheromone from field-captured female beetles and identified it as (*R*,*Z*)-(-)-5-(1-octenyl)oxacyclopentan-2-one (*R*,*Z*)-**7**.[1] Its structure closely resembles

Fig.2-1 Sex pheromones of Cupreous chafer beetle ((*R*,*Z*)-7) and Japanese beetle ((*R*,*Z*)-1)

sex pheromone (*R*,*Z*)-**1** of the Japanese beetle, *Popillia japonica* Newman.[2] The sex pheromone of the Japanese beetle has the unique feature whereby a small amount of the unnatural (*S*,*Z*)-isomer strongly inhibits the male response to it. The author synthesized both optically pure

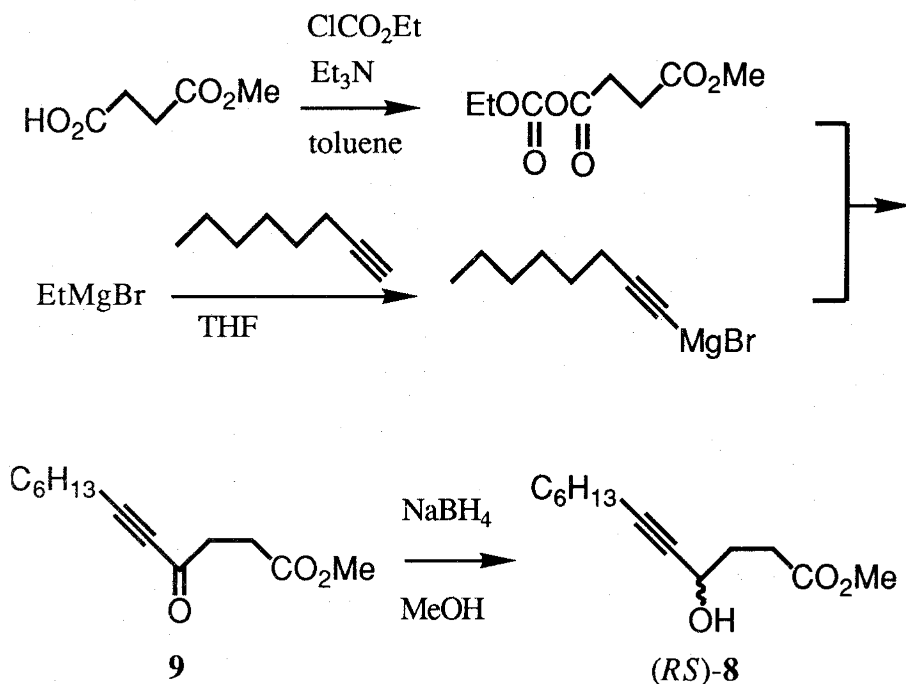
enantiomers of **1** according to the same strategy for the preparation of Japanese beetle sex pheromone (*R*,*Z*)-**1**[3], which was discussed in chapter 1, in order to evaluate the male response to them. The synthetic plan was outlined in Scheme 2-1. The key reaction of this synthesis was an lipase-catalyzed enantioselective acylation of (*RS*) methyl-4-hydroxy-5-dodecynoate ((*RS*)-**8**).



Scheme 2-1 Synthetic plan of both isomers of Cupreous chafer beetle

RESULTS AND DISCUSSIONS

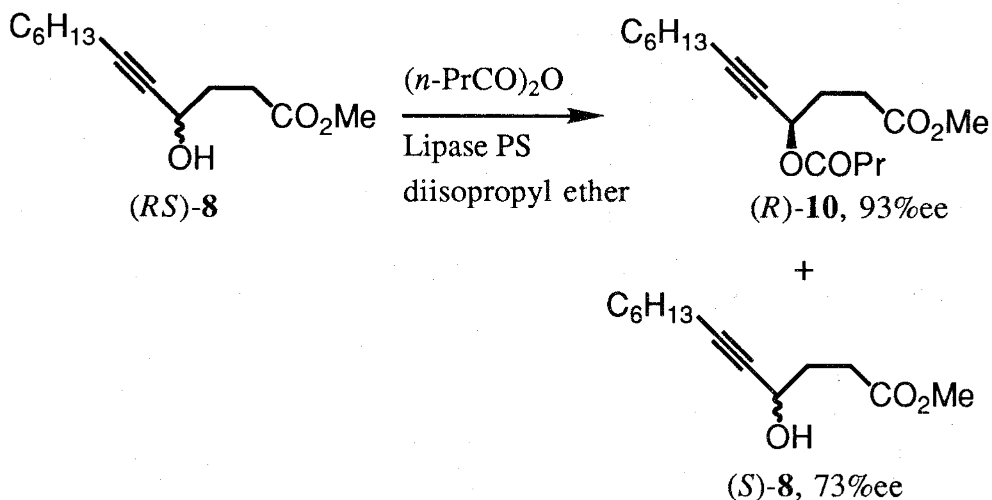
The starting material hydroxy ester (*RS*)-**8**, was obtained by the reduction of keto ester **9** prepared from 1-octyne and succinic acid monomethyl ester according to the reported procedure.[4] (Scheme 2-2)



Scheme 2-2 Preparation of (*RS*)-8

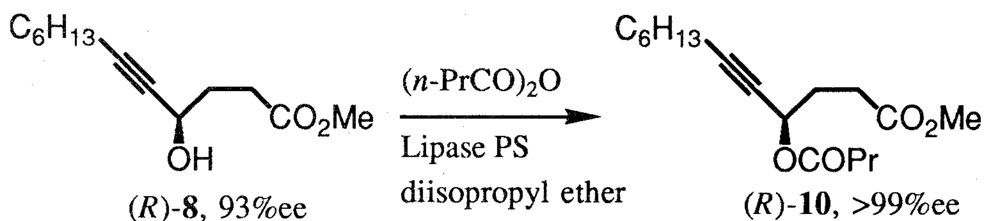
Hydroxy ester **8** was subjected to a lipase-catalyzed enatioselective acylation with n-butyric anhydride as a acylating agent in diisopropyl ether to give (*R*)-butanoyloxy ester (*R*)-**10** in 93% ee. (Scheme 2-3) The absolute configuration of **10** was determined by comparing the specific rotation value of final product (Z)-**7** prepared from **10** with that of (*R*,*Z*)-

7 previously reported by Leal.[1)]



Scheme 2-3 Lipase-catalyzed enantioselective acylation of (*RS*)-8

Further enantiomeric purification was carried out by repetition of the enzymatic reaction. Primary product (*R*)-**10** (93% ee) was converted to (*R*)-**8** by hydrolysis with potassium hydroxide and subsequent methylation with diazomethane. Then (*R*)-**8** was subjected to the second enzymatic reaction under the same conditions to yield (*R*)-**10** with over 99% ee. (Scheme 2-4) The optically pure (*R*)-**10** was converted to optically pure lactone (*R*)-**11**, by hydrolysis and subsequent distillation. The product, (*R*)-**11** was semihydrogenated to give natural pheromone (*R*,*Z*)-**7** according to the reported procedure.[5)]



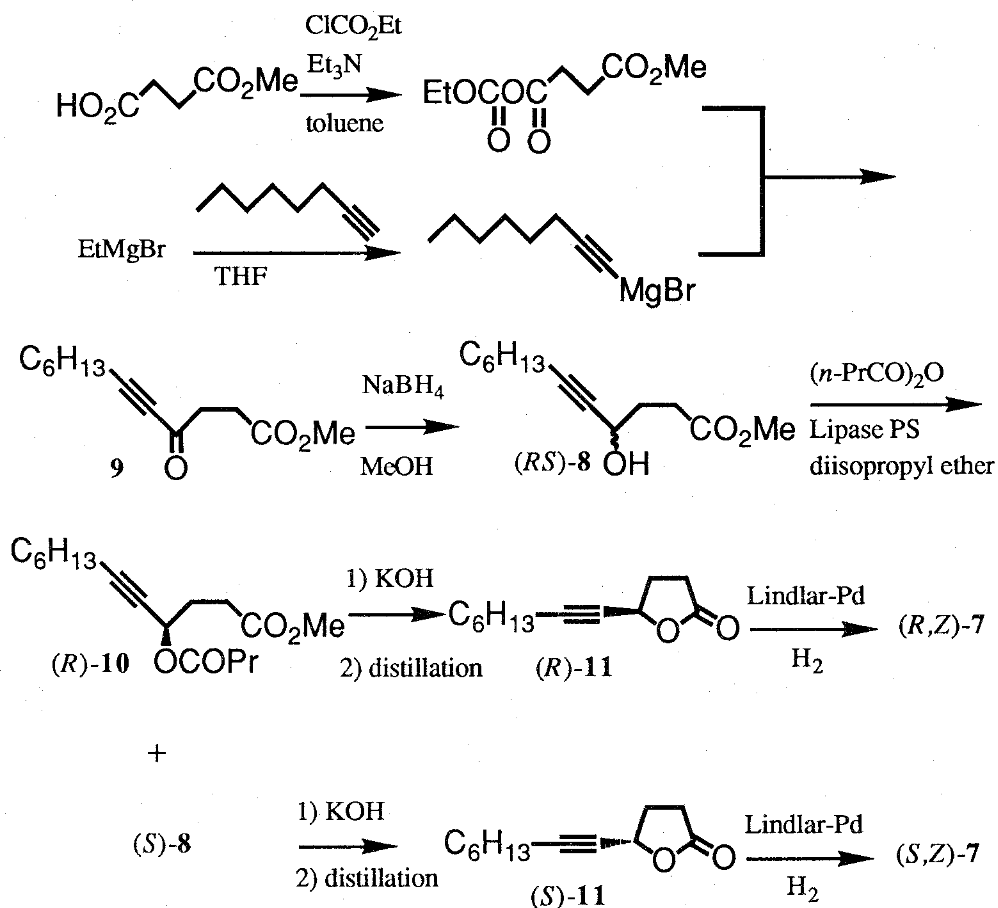
Scheme 2-4 The second enzymatic reaction to yield the optically pure (*R*)-10

In the primary enzymatic reaction, (*S*)-**8** was obtained as the remaining substrate in 73%ee, which was subjected to the same enzymatic reaction to give more optically pure (*S*)-**8** as a remaining substrate with over 99%ee. The product was converted to the optically pure unnatural pheromone (*S*,*Z*)-**7** in 2 steps according to the foregoing procedure.

SUMMARY

Acylation of methyl (*RS*)-4-hydroxy-5-dodecynoate with n-butyric anhydride in an organic solvent yields methyl (*R*)-4-butanoyloxy-5-dodecynoate (93%ee) and methyl (*S*)-4-hydroxy-5-dodecynoate (73%ee). The product obtained by primary enzymatic reaction was subjected to the second enzymatic reactions independently to yield optically pure product, which were converted to (*R*)- and (*S*)-(-)-5-(1-octynyl)oxacyclopentan-2-one respectively. Both enantiomeres of the cupreous chafer beetle

pheromone, (*R*,*Z*)- and (*S*,*Z*)-(-)-5-(1-octenyl)oxacyclopentan-2-one were synthesized in one step from these optically active lactones.



Scheme 2-5 Synthesis of both enantiomers of the cupreous chafer beetle pheromone

EXPERIMENTAL SECTION

Boiling points (bp) are uncorrected. IR spectra refer to films, and NMR spectra were recorded in deuteriochloroform. Lipase PS (from *Pseudomonas* sp.) was purchased from Amano Pharmaceutical Co., Japan.

Methyl 4-oxo-5-dodecynoate (9)

By following the reported procedure,[4)] succinic acid monomethyl ester (69 g, 523 mmol) and triethylamine (82 ml) were dissolved in toluene (600 ml) and cooled to -30°C. Ethyl chloroformate (57 g, 523 mmol) was added dropwise at below -20°C, and the mixture was stirred at that temperature for 1hr. (1-Octynyl)magnesium bromide[4)] (1.74 mol/l in THF) was added dropwise at that temperature and the reaction mixture was stirred at the same temperature for 1 hr. A saturated aqueous solution of ammonium chloride (15 ml) was added to stop the reaction. 6N-HCl (130 ml) was then added, and the mixture was left at room temperature. The organic phase was separated, and the remaining aqueous phase was extracted with ether. The combined organic layer was washed with brine, dried over magnesium sulfate and concentrated *in vacuo* . The residue was distilled to give **9** (68.2 g, 58 % yield) , bp 118-120 °C at 0.5 mm Hg; IRνmax (cm^{-1}): 2950, 2840, 2190, 1740, 1680, 1460, 1440, 1420, 1360, 1320, 1220, 1160, 1060, 1030, 990, 930, 900, 840, 800, 720; ^{1}H-NMR δ (90 MHz): 0.90(3H, deformed t, J=5.4Hz), 1.10-1.75(8H,br), 2.37(2H, t, J=7.1Hz), 2.55-2.71(2H, m), 2.83-2.98(2H, m), 3.69(3H, s). *Anal.* Found: C, 69.33; H, 8.90. Calcd. for $C_{13}H_{20}O_3$: C, 69.61; H, 8.99%.

Methyl (RS)-4-hydroxy-5-dodecynoate ((RS)-8)

Methyl 4-oxo-5-dodecynoate **3**; (50 g, 223 mmol) was reduced with

$NaBH_4$ in MeOH according to the reported procedure[3)] to give (*RS*)-**8** (40 g, 80% yield), IRνmax (cm^{-1}): 3420, 2910, 2840, 2210, 1740, 1440, 1370, 1340, 1260, 1210, 1170, 1140, 1070, 1030, 920. This was employed in the next step without further purification.

Lipase-catalyzed acylation of (RS)-8.

(*RS*)-**8** (35 g, 155 mmol), *n*-butyric anhydride (12.2 g, 77 mmol) and lipase PS (10 g) in diisopropyl ether (500 ml) were stirred at room temperature for 40 hr. After filtering the enzyme powder, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography. The eluate with hexane/EtOAc (10/1) gave (*R*)-**10** (19.2 g, 42 % yield, 93 % ee); IRνmax (cm^{-1}): 2910, 2840, 2210, 1740, 1460, 1440, 1380, 1350, 1260, 1180, 1080, 1040, 1010, 980, 880; ^{1}H-NMR δ (90MHz): 0.87-1.02(6H, m), 1.54-1.86(10H, m), 1.93-2.59(8H,m), 3.69(3H,s), 5.36-5.50(1H,m). *Anal.* Found: C, 68.77; H, 9.39. Calcd. for $C_{17}H_{28}O_4$: C, 68.89, H, 9.52%. Further elution gave (*S*)-**8** (16.1 g, 46 %, 73 % ee). Its IR spectrum was identical with that of (*RS*)-**8**. To determine their enantiomeric excess (ee), they were converted to lactone **11** according to the reported procedure.[3)] This lactone was subjected to a GC analysis[1)] with a chiral capillary column of Chiraldex G-TA (ASTEC).

Second enzymatic reaction to yield optically pure (R)-10.

(*R*)-**10** (19.2 g, 65 mmol, 93 % ee) was hydrolyzed with KOH in MeOH, and subsequent methylation with diazomethane gave (*R*)-**8** (13.7 g, 93 %). (*R*)-**8** (13.7 g, 93 %) was subjected to the same enzymatic reaction with *n*-butyric anhydride (8.7 g, 55 mmol) and lipase PS (5 g) to

give (*R*)-**10** (15.1 g, 84 % yield, >99 % ee). Its IR and NMR spectra were identical with those of the primary product.

(R)-5-(1-Octynyl)oxacyclopentan-2-one ((R)-11).

(*R*)-**10** (14.8 g, 50 mmol) was hydrolyzed with KOH in MeOH, and subsequent distillation gave (*R*)-**11** (8.6 g, 89 %, >99 % ee). IRνmax (cm^{-1}): 2900, 2840, 2210, 1790, 1460, 1420, 1360, 1330, 1300, 1270, 1210, 1190, 1150, 1040, 1020, 980, 920, 880, 810; ^{1}H-NMR δ (90MHz): 0.89(3H, deformed t, J=5.7Hz), 1.0-1.7(8H, br), 2.0-2.7(6H,m), 5.0-5.2(1H, m). *Anal.* Found: C, 74.05; H,9.21. Calcd. for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34%.

(R,Z)-(-)-5-(1-Octenyl)oxacyclopentan-2-one ((R,Z)-7).

By following the reported procedure,[5] (*R*)-**11** was semihydrogenated to give (*R*,*Z*)-**7** (7.1 g, 88 %, >99 % ee). This ee value was determined by a GC analysis under the same conditions as those described above,[1] bp 115° at 0.2mm Hg, $[\alpha]_D^{24}$ -80.9° (c=0.986, chloroform), -61.8°(c=1.000, hexane) [Lit.[1] $[\alpha]_D^{25}$ -61.2°(c=0.85, hexane)]. IRνmax (cm^{-1}): 2900, 2840, 1780, 1660, 1460, 1430, 1380, 1330, 1300, 1220, 1180, 1130, 1020, 980, 910, 870, 810, 720; ^{1}H-NMR δ (400MHz): 0.87(3H, t, J=6.8Hz), 1.26-1.38(8H, br), 1.93(1H, dddd, J=13.2, 9.8, 9.8, 8.8Hz), 2.09(2H, m), 2.36(1H, dddd, J=13.2, 8.3, 6.8, 5.9Hz), 2.52(1H, ddd, J=17.6, 9.8, 8.3Hz), 2.57(1H, ddd, J=17.6, 9.3, 5.4Hz), 5.24(1H, dddd, J=8.8, 8.8, 6.8, 0.9Hz), 5.44(1H, ddt, J=10.8, 8.8, 1.5Hz), 5.65(1H, dtd, J=10.8, 7.8, 0.9Hz); ^{13}C-NMR δ (100MHz): 14.0, 22.5, 27.8, 28.8, 29.0, 29.2, 29.3, 31.6, 76.4, 127.2, 135.8, 177.1. *Anal.* Found: C, 73.33; H, 10.24. Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27%.

Methyl (S)-4-hydroxy-5-dodecynoate ((S)-8).

(*S*)-**8** (15.5 g, 68.6 mmol, 73 % ee) obtained as the remaining substrate from the primary enzymatic reaction, *n*-butyric anhydride (3.2 g, 20 mmol) and lipase PS (5 g) was stirred in diisopropyl ether at room temperature for 30hr. The same work up was performed to give (*S*)-**8** (9.8 g, 63 %, >99 % ee). Its IR was identical with that of (*RS*)-**8**.

(S,Z)-(-)-5-(1-Octenyl)oxacyclopentan-2-one ((S,Z)-7).

By following same procedure as that described above, (*S*)-**8** (4.5 g, 20 mmol) was hydrolyzed, and subsequent distillation gave (*S*)-**11** (2.5 g, 64 %, >99 % ee), which was semihydrogenated to give (*S,Z*)-**7** (2.1 g, 85 %, >99 % ee), $[\alpha]_D^{25}$ +81.4°(c=1.010, chloroform), +61.9°(c=1.110, hexane). The other spectra were identical with those of (*R,Z*)-**7**.

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CHAPTER 3

LIPASE-CATALYZED KINETIC RESOLUTION OF 2,3-EPOXY-8-METHYL-1-NONANOL, THE KEY INTERMEDIATE IN THE SYNTHESIS OF THE GYPSY MOTH PHEROMONE

INTRODUCTION

(+)-Disparlure, *cis*-(7*R*,8*S*)-7,8-epoxy-2-methyloctadecane (**12**) was identified as the sex pheromone for gypsy moth, *Lymantria dispar* L. , which is a harmful forest pest.[1), 2)]

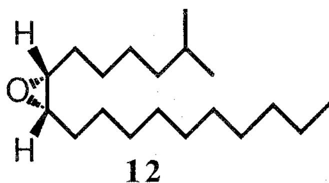
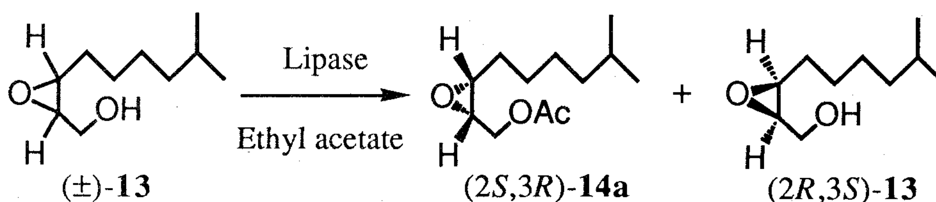


Fig. 3-1 (+)-Disparlure

Mori *et al* reported an interesting synthesis of (+)-disparlure which was derived from the optically active epoxy alcohol, (2*S*,3*R*)-2,3-epoxy-8-methyl-1-nonanol ((2*S*,3*R*)-**13**) prepared by Sharpless asymmetric epoxidation .[3)] Recently, an enzymatic enantioselective transesterification of (**13**) with ethyl acetate as acyl donor and as reaction medium was

reported.[4] (Scheme 3-1)

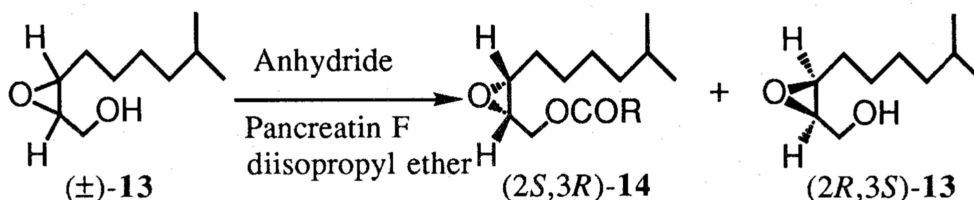


Scheme 3-1 Lipase-catalyzed enantioselective transesterification of (±)-13

By above method, it's very difficult to control the conversion of the reaction around 50% that theoretically would give high optically yield of the target enantiomer. Under the condition of large excess amount of acyl donor, the conversion readily proceeded over 50% resulting in relatively lower optical purity of desired enantiomer. To overcome this drawback, acid anhydride[5] was selected as an acyl donor in lipase-catalyzed enantioselective acylation of (**13**) because conversion of the reaction could be controlled by amount of anhydride. On the other hand, a lipase catalyzed enantioselective alcoholysis of the epoxy ester, corresponding to (**13**), in organic solvent was also attempted. In this chapter, a practical synthesis of (2*S*,3*R*)-2,3-epoxy-2-methyl-1-nonanol ((2*S*,3*R*)-**13**) involving two lipase-catalyzed steps, enantioselective acylation of (±)-**13** with acid anhydride as acylating agent and enantioselective alcoholysis of epoxy ester corresponding (±)-**13** in organic solvent was described.

RESULTS AND DISCUSSIONS

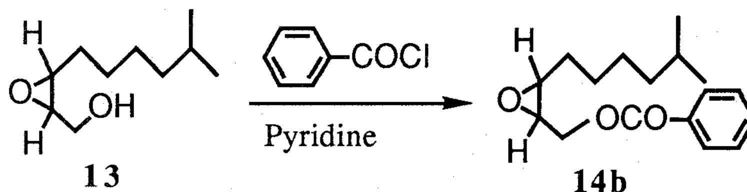
The starting material, (±)-*cis*-2,3-epoxy-8-methyl-1-nonanol (±)-**13** was easily prepared by *cis*-epoxidation of 8-methyl-2-nonen-1-ol[3] with *m*-chloroperoxybenzoic acid. Initially, several commercially available lipases were surveyed for the acylation of (±)-**13** to yield corresponding epoxy esters (**14**) with several anhydrides, such as acetic, propionic, *n*-butyric, *n*-caproic and *i*-butyric anhydride. Pancreatin F(Amano Pharm. Co., Japan), Lipase from porcine pancreas, was found to catalyze the enantioselective acylation of (±)-**13** with several anhydrides as acylating agent. (Scheme 3-2)



Scheme 3-2 Lipase-catalyzed enantioselective acylation of (±)-13 with acid anhydride in diisopropyl ether

In all cases, a half equivalent of anhydrides were used and the reactions were carried out at 30°C in diisopropyl ether. After indicated time in Table1, the enzyme powder was removed by filtration to terminate the reaction. After filtration, the epoxy ester (**14**) and the unreacted epoxy alcohol (**13**) were separated by silica gel column chromatography. To determine the optical purities of the epoxy ester (**14**), it was hydrolyzed

with KOH in MeOH to yield epoxy alcohol (**13**) ,which was converted to epoxy benzoate (**14b**) (Scheme 3-3).



Scheme 3-3

The enantiomeric excess(ee) of **14b** was determined by HPLC analysis by chiral column, Chiralcel OJ(Daicel Chem. Co.,Japan). The absolute configuration of **14** was determined by a comparing the HPLC data of **14b** to that of (2*S*,3*R*)-**14b** derived from (2*S*,3*R*)-**13** prepared by reported procedure[3]. Enantioselectivities of reactions were evaluated by E value[6]. Results are summarized in Table 3-1.

The highest enantioselectivities were obtained when acetic anhydride was used as acylating agent (E=13). Other acyclic anhydrides, such as propionic, *n*-butyric and *n*-caproic anhydride, had almost same property as well as acetic anhydride, however, the enantioselectivities of the reactions with them were slightly lower than that of the reaction with acetic anhydride. The anhydrides which had branch at α-position, such as *i*-butyric anhydride and benzoic anhydride showed very low reactivities.

Cyclic anhydride, such as succinic and phthalic anhydride were also examined. However both didn't give good results, especially phthalic anhydride provided very poor yield (data not shown).

Table 3-1. Lipase-catalyzed enantioselective acylation of epoxy alcohol ((±)-13) with anhydride

Anhydride	Time (h)	Conv.[a] (%)	ee(%), Recov.(%) ester	ee(%), Recov.(%) alcohol	E[b]
Acetic	7	57	66, 49	86, 44	13
Propionic	7	56	59, 48	76, 44	9
n-Butyric	7	56	61, 46	78, 48	10
n-Caproic	7	55	65, 47	80, 45	11
i-Butyric	50	33	72, 34	35, 64	8
Benzoic	200	28	83, 25	33, 70	11
Succinic	24	33	66, 11	33, 56	7
Phthalic	200	-	-	-	-[c]

a Calculated on the basis of ee(S) and ee(P).
[conv.= ee(S)/{ee(S)+ee(P)}]

b E value=ln[1-c(1+ee(P))] / ln[1-c{1-ee(P)}]

c Product was not detected.

Above results showed the acetic anhydride was the best acylating agent, however, in this case, the optical purity of the formed ester (66%ee) was lower than that of remaining alcohol(86%ee). It suggested that the reaction proceeded over 50% conversion, although only half equivalent of acetic anhydride was added as acylating agent. As the reason, it is speculated that the free acetic acid, that formed as by-product derived from acetic anhydride,

might be involved in further acetylation. To solve above problem, removal of free acetic acid was attempted by addition of weak bases[7]. The addition of potassium bicarbonate and 2,6-lutidine were examined. The amount of base added was two equivalent to acetic anhydride and other condition were kept same to those of above reaction. Results were summarized in Table 3-2.

Table 3-2. Effects of base addition and enzyme immobilization on lipase-catalyzed enatioselective acylation of (±)-13

Entry[a]	Base[b]	Enzyme	Temp (°C)	Time (h)	Conv.[c] (%)	ee(%), Recov.(%) ester	alcohol	E[d]
1	KHCO3	free	30	6	51	70, 50	73, 46	12
2	2,6-Lutidine	free	30	6	40	74, 41	62, 46	12
3	None	free	30	6	57	66, 49	86, 44	13
4	None	on celite	30	6	53	73, 49	81, 47	16
5	None	on celite	5	14	50	79, 47	79, 46	23

a In all reactions, acetic anhydride was used as the acylating agent.

b Added in equal equivalent to substrate.

c Calculated on the basis of ee(S) and ee(P).

[conv.= ee(S)/{ee(S)+ee(P)}]

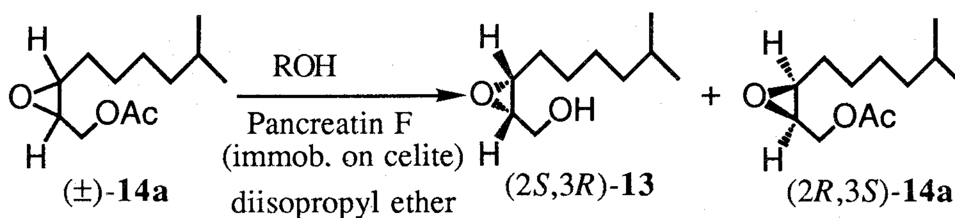
d E value=ln[1-c{1+ee(P)}] / ln[1-c{1-ee(P)}]

Addition of bases was effective to repress the excess of the reaction over

50% conversion. (Table 3-2; entry 1 and 2) As another way to solve above problem, enzyme adsorbed on celite[5),7)] was examined.

Use of Pancreatin F which was adsorbed to celite was also effective (Table 3-2; entry 4), and in this case, the enantioselectivity was higher (E=16) than that of control (Table 3-2; entry 3 (E=12)) To obtain higher enantioselectivity, the reaction at lower temperature[8)] was examined. The reaction by Panceatin F adsorbed on celite at 5°C gave higher enantioselectivity (Table 3-2; entry 5, (E=23)).

For further enrichment of the optical purity of desired (2*S*,3*R*)-enantiomer, enantioselective alcoholysis of epoxy acetate (**14a**) in organic solvent was attempted. After surveying of several commercially available lipases, Pancreatin F(Amano Pharm. Co.,Japan), the same enzyme in above reaction, was found to catalyze the alcoholysis of (±)-**14a** with several alcohol (Scheme 3-4).



Scheme 3-4 Lipase-catalyzed enantioselective alcoholysis of (±)-14a in diisopropyl ether

Results were summarized in Table 3-3. The reactions were carried out in diisopropyl ether with several alcohol, such as methanol, ethanol, *n*-propanol

and *i*-propanol at 30°C for indicated times. After indicated times in Table 3-3 the reactions were stopped by enzyme filtration. After filtration, epoxy alcohol (**13**) and unreacted epoxy acetate (**14a**) were separated by silica gel column chromatography and optical purities of those were determined by same procedure described above.

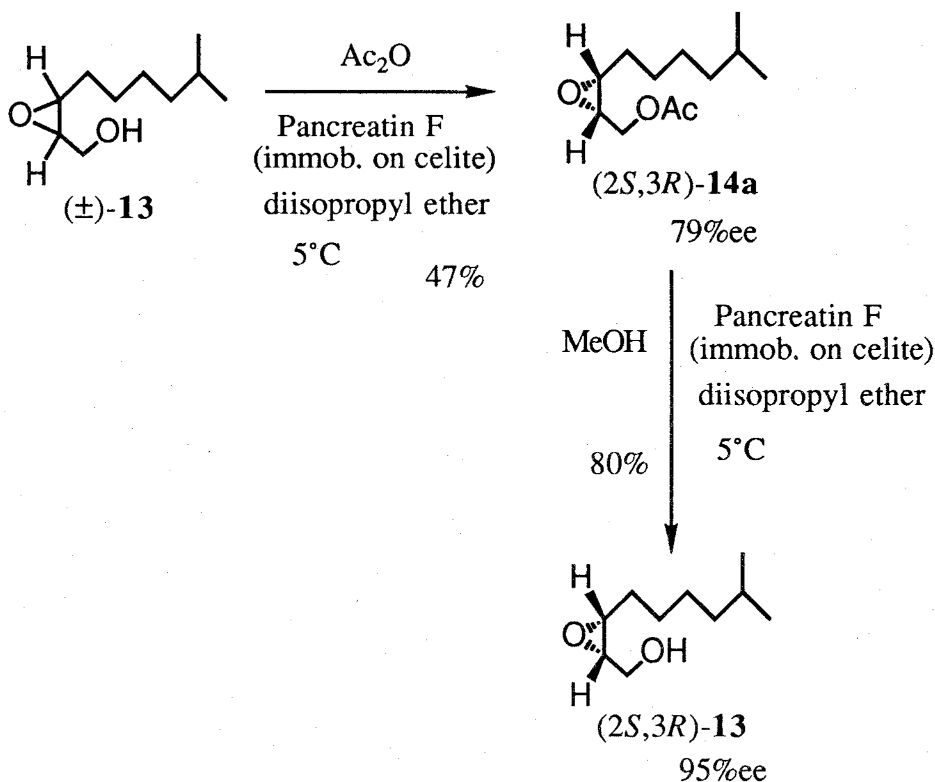
Table 3-3. Lipase-catalyzed enantioselective alcoholysis of (±)-14a in diisopropyl ether

R	Temp (°C)	Time (h)	Conv.[a] (%)	ee(%), Recov.(%) alcohol	ester	E[b]
Methyl	30	64	49	72, 49	69, 48	13
Ethyl	30	85	41	75, 40	51, 57	12
n-Propyl	30	90	41	78, 38	55, 58	14
i-Propyl	30	140	19	47, 19	11, 72	1
Methyl	5	110	35	85, 34	45, 65	20

a) Calculated on the basis of ee(S) and ee(P).
[conv.= ee(S)/{ee(S)+ee(P)}]
b) E value=ln[1-c{1+ee(P)}] / ln[1-c{1-ee(P)}]

When linear alcohol were used as the acceptors, increasing the chain length of the alcohol made the reactivities lower, however didn't affect to the enantioselectivities of the reaction. *i*-Propanol which had branch at α-position gave very low enantioselectivitiy. (E=1) This reaction at lower temperature (5°C) gave higher enantioselectivity (E=20).

In order to refine the methodology to obtain highly optically pure epoxy alcohol (2*S*,3*R*)-**13**, the second reaction, lipase-catalyzed enantioselective alcoholysis, was applied to (2*S*,3*R*)-**14a**, which was prepared in 79%ee by the first reaction, lipase-catalyzed enantioselective acylation. The reaction was stopped after 90hr and (2*S*,3*R*)-**13** was isolated with 95%ee (Scheme 3-5).



Scheme 3-5 Kinetic resolution of (±)-13 by the combination of lipase-catalyzed enantioselective acylation and alcoholysis

In conclusion, epoxy alcohol (2*S*,3*R*)-**13** was prepared with 95%ee from its racemate by lipase-catalyzed acylation followed alcoholysis in

organic solvent.(Scheme 3-5) The gypsy moth pheromone (**12**) could be easily prepared from this epoxy alcohol (2*S*,3*R*)-**13** in two steps.

SUMMARY

Lipase-catalyzed enantnioselective acylation of (±)-2,3-epoxy-8-methyl-1-nonanol with acetic anhydride in diisopropyl ether yielded (2*S*,3*R*)-1-acetoxy-2,3-epoxy-8-methylnonane in 79% enantiomeric excess. Furthermore, that optically active epoxyester was converted to (2*S*,3*R*)-2,3-epoxy-8-methyl-1-nonanol by lipase-catalyzed enantioselective alcoholysis in diisopropyl ether in 95% enantiomeric excess.

EXPERIMENTAL SECTION

IR spectra were measured in neat on JASCO A-810 spectrometer. ^{1}H-NMR spectra were measured at 400MHz on a JEOL GX-400. ^{13}C-NMR spectra were measured at 22.5 MHz on a JOEL FX-90Q. Column chromatography was effected using Merck Kieselgel 60 (70-230 mesh). Pancreatin F was purchased from Amano Pharm.Co. Solvent for enzymatic reaction was distilled before use.

Preparation of (±)-8-methyl-2,3-epoxy-1-nonanol ((±)-13)

(Z)-8-methyl-2-nonen-1-ol[3)] (10.0 g, 64.1 mmol) was oxidized by

m-chroloperoxybenzoic acid (17.4 g, 70.5 mmol) in methylene chloride (500 ml) in the usual manner and purified by silica gel column chromatography [elution with hexane/ethyl acetate (3/1)] to give (±)-**13** (11.0 g , quantitative). νmax 3420, 2950, 2930, 2860, 1460, 1380, 1360, 1040, 1000, 910, 840, 790. δ(^{1}H, 400MHz, $CDCl_3$) 0.85(6H, d, J=6.6Hz), 1.10-1.60(9H, m), 1.91(1H, brs), 3.01(1H, m), 3.14(1H, m), 3.64(1H, m), 3.82(1H, m). Its IR and NMR spectra were consistent with those of (2*S*,3*R*)-**13** (ref.3).

Preparation of (±)-1-acetoxy-2,3-epoxy-8-methyl-1-nonanol (±)-14a

(±)-**13** (4 g, 23.3 mmol) was acylated with acetyl chloride (2.5 g, 31.8 mmol) in pyridine (50 ml) in the usual manner and purified by silica gel column chromatography [elution with hexane/ethyl acetate (20/1)] to give (±)-**14a** (4.43 g, 89 %) νmax 2950, 2930, 2860, 1740, 1460, 1360, 1230, 1030. δ(^{1}H, 400MHz, $CDCl_3$) 0.85(6H, d, J=6.9Hz), 1.13-1.54(9H, m), 2.08(3H, s), 2.99(1H, m), 3.15(1H, m), 4.01(1H, m), 4.30(1H, m). δ(^{13}C, 22.5MHz, $CDCl_3$) 20.7, 22.6, 26.8, 27.1, 27.9, 28.0, 38.8, 53.7, 56.5, 62.9, 170.7. (Found: C,67.21; H,10.38%. Calc. for $C_{19}H_{32}O_4$: C,67.26; H,10.35%)

Adsorption of enzyme to celite[5)]

Celite 544 (4 g) was washed with distilled water and 0.1M K-phosphate buffer(pH 7) and then added to a solution of 1g of Pancreatin F(Amano Pharm. Co.,Japan) in 20 ml of 0.1M K-phosphate buffer(pH 7). The mixture was lyophilized to give the enzyme adsorbed on celite (5 g).

General procedure of lipase-catalyzed acylation of (±)-13 to yield (2S,3R)-14 and (2R,3S)-13

(±)-**13** (100 mg, 0.58 mmol) and acid anhydride (0.29 mmol) were dissolved in diisopropyl ether (5 ml). Pancreatin F (100 mg) was added to the solution and the suspension was stirred at 30 °C for indicated time. In the experiment of entry 4 and 5 in Table 2, 500 mg of Adsorbed enzyme which was corresponding to 100mg of enzyme was used. After stirring for indicated time, the lipase powder was removed by filtration. The filtrate was evaporated and chromatographed on a silica gel column (hexane/ethyl acetate (20/1)) to give (2*S*,3*R*)-**14**. Further elution with hexane/ethyl acetate(3/1) gave (2*R*,3*S*)-**13**. Results are summarized in Table 1 and 2.

Determination of enantiomeric excess(ee) of 13 and 14

14 was hydrolyzed with 5% KOH in MeOH to yield **13**. **13** was converted to the corresponding benzoate **14b** by acylation with benzoyl chloride in pyridine in the usual manner. And then **14b** derived from **13** and **14** was subjected to HPLC analysis on the system consisting of pump [JASCO 880-PU] and UV detector [JASCO 875-UV] equipped with a chiral column [Chiralcel OJ , Daicel Chem. Co., 4.6 x 250 mm; hexane/*i*-propanol(98/2); 1 ml/min; 230 nm at room temp.] The retention time of (2*S*,3*R*)-**14b** and (2*R*,3*S*)-**14b** were 9.1 min and 12.2 min respectively.

General procedure of lipase-catalyzed alcoholysis of (±)-14a to yield (2S,3R)-13 and (2R,3S)-14a

(±)-**14a** (100mg, 0.47mmol) and alcohol(4.7mmol) were dissolved in diisopropyl ether(5 ml). Pancreatin F adsorbed on celite (500 mg)

(corresponding to 100 mg of enzyme) was added to the solution and the suspension was stirred at 30 °C for indicated time in Table 3. After the lipase powder was removed by filtration the filtrate was evaporated and chromatographed on a silica gel column (hexane/ethyl acetate (20/1)) to give (2*R*,3*S*)-**14a**. Further elution with hexane/ethyl acetate(3/1) gave (2*S*,3*R*)-**13**. Results are summarized in Table 3.

Preparation of (2S,3R)-1-acetoxy-2,3-epoxy-8-methyl-1-nonane (2S,3R)-14a

(±)-**13** (500 mg, 2.9 mmol) and acetic anhydride (150 mg, 1.45 mmol) were dissolved in diisopropyl ether(25 ml). Pancreatin adsorbed on celite (2500 mg) was added to the solution and the suspension was stirred for 14 hr at 5 °C. After the lipase powder was removed by filtration, the filtrate was evaporated. The residue was purified by silica gel column chromatography(10 g). Elution with hexane/ethyl acetate (20/1) gave (2*S*,3*R*)-**14a** (291 mg, 47 % yield, 79 %ee). Its IR and NMR spectra were identical with those of (±)-**14a** obtained above. Further elution with hexane/ethyl acetate (3/1) gave (2*R*,3*S*)-**13** (227 mg, 46 % yield, 79 %ee). Its IR and NMR spectra were identical with those of (±)-**13** obtained above.

Preparation of (2S,3R)-2,3-epoxy-8-methyl-1-nonanol (2S,3R)-13

(2*S*,3*R*)-**14a** (79 %ee, 200 mg, 0.94 mmol) and methanol(0.4 ml) were dissolved in diisopropyl ether (10 ml) . Pancreatin adsorbed on celite (1000 mg) was added to the solution and the suspension was stirred for 90hr at 5°C. After the lipase powder was removed by filtration, the

filtrate was evaporated. The residue was purified by silica gel column chromatography(4g). Elution with hexane/ethyl acetate (3/1) gave (2*S*,3*R*)-**13** (130 mg, 80 % yield, 95 %ee). Its IR and NMR spectra were identical with those of (±)-**13** obtained above.

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CHAPTER 4

LARGE-SCALE PREPARATION OF (+)-DISPARLURE, THE GYPSY MOTH PHEROMONE, BY A PRACTICAL CHEMICO-ENZYMATIC PROCEDURE

INTRODUCTION

(+)-Disparlure *cis*-(7*R*,8*S*)-7.8-epoxy-2-methyloctadecane (**12**) was identified as the sex pheromone of the gypsy moth, *Lymantria dispar* L. , which is a harmful forest pest.[1),2)]

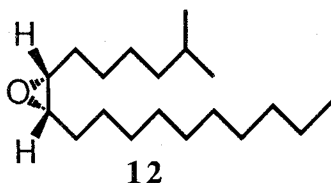
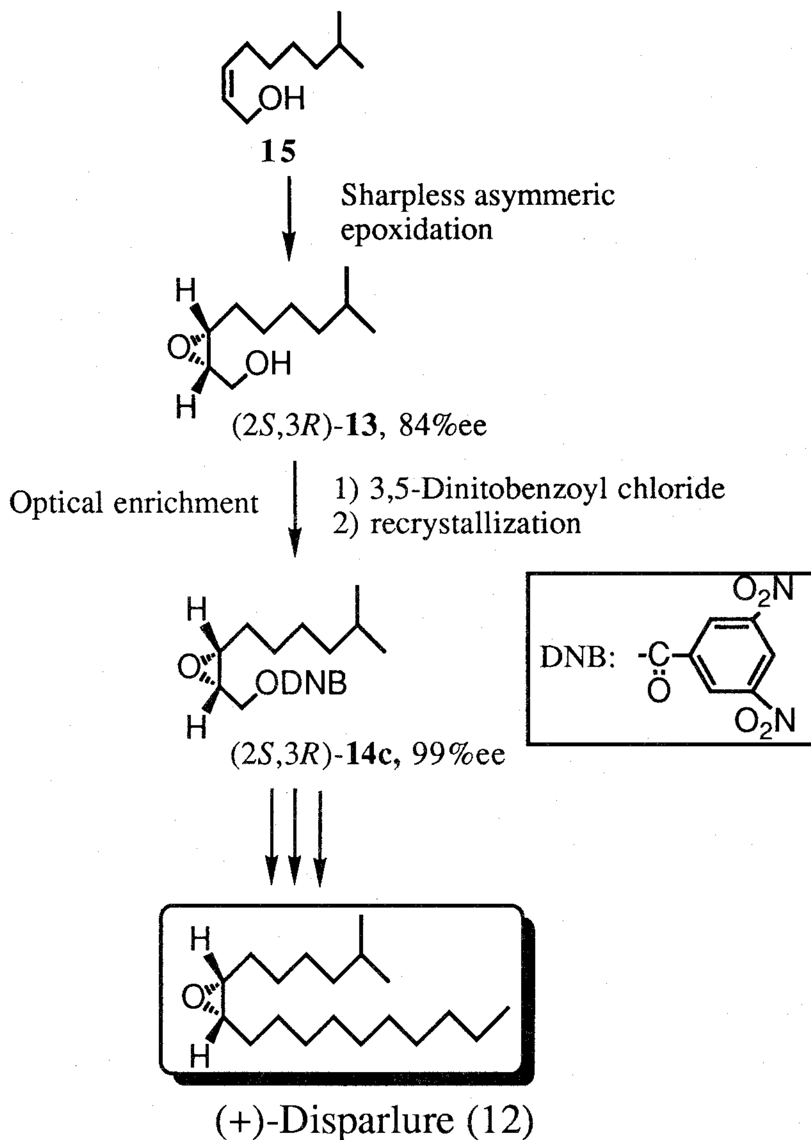


Fig. 4-1 (+)-Disparlure

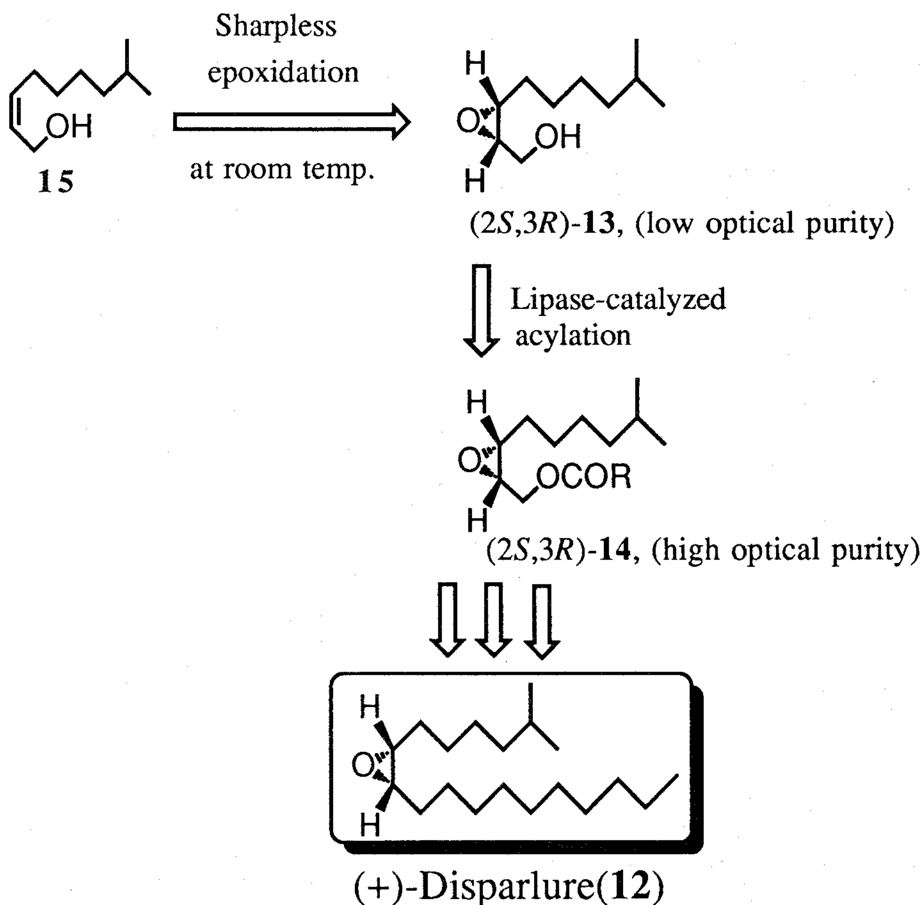
Later, Mori *et al* reported an interesting synthesis of (+)-disparlure starting from the optically active epoxy alcohol, (2*S*,3*R*)-2,3-epoxy-8-methyl-1-nonanol ((2*S*,3*R*)-**13**) prepared by Sharpless asymmetric epoxidation.[3)] In this synthesis, Sharpless asymmetric epoxidation[4)] of 8-methyl-2-nonen-1-ol (**15**) was performed to yield optically active epoxyalcohol ((2*S*,3*R*)-**13**)

in 84%ee. Followed optical enrichment of (2*S*,3*R*)-enantiomer by recrystallization of corresponding 3,5-dinitobenzoate gave optically pure (2*S*,3*R*)-**13**. (Scheme4-1)



Scheme 4-1 Mori's synthesis of (+)-disparlure

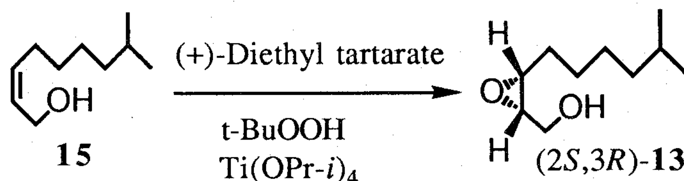
Above synthesis is very efficient in laboratory scale, but it is very costly in industry scale because of its necessity of very low temperature (below -30°C) and long reaction period (over 3days).In this chapter, the author described a practical large scale preparation of (2*S*,3*R*)-**13** by the combination of the Sharpless asymmetric epoxidation and lipase-catalyzed enantioselective acylation. The synthetic plan was summarized in Scheme 4-2



Scheme 4-2 Synthetic plan of (+)-disparlue

RESULTS AND DISCUSSIONS

As a preliminary experiment, we attempted above Sharpless epoxidation (Scheme 4-3) in large scale.



Scheme 4-3 Sharpless asymmetric epoxidation of 15

It was successful to obtain (2*S*,3*R*)-**13** over 80%ee of optical purity, though long reaction time over 7 days and low temperature below -20°C were essentially required. (Table 4-1) Above reaction was very efficient in laboratory scale but it is very costly in large scale, especially the necessity of long reaction time over 7 days had been a problem for industrial use. The reaction at higher temperature could reduce the required time but it would cause a remarkable drop of steleoselectivity.

The reaction at 5°C required 40hr to be completed and the optical purity of obtained (2*S*,3*R*)-**13** was 65%ee. And the reaction at 15°C required only 15hr to be completed and the optical purity of obtained (2*S*,3*R*)-**13** was 52%ee. These optical purities were too low to perform the optical enrichment by recrystallization of corresponding 3,5-dinitrobenzoate in satisfied recovery yield (over 60%).

Table 4-1. Effects of reaction temperature for Sharpless asymmetric epoxidation[a] of 15

Temp. (°C)	Reaction time (h)	ee of **13**[b]
-20	170	82
5	40	65
15	15	52

a Reaction conditions : **15** (42 g, 0.27 mol), (+)-diethyltartarate (38 g, 0.19 mol), $Ti(OPr\text{-}i)_4$ (46 g, 0.16 mol), *t*-BuOOH (3.0 M, 134 ml, 0.40 mol), Molecular sieves 3A (55 g), dichloromethane (5 *l*)
b Determined by the procedure described in Chapter 3.

In a preliminary experiment, we found that over 80 %ee of optical purity was necessary for good recovery over 60 %. To obtain higher optical purity over 80 %ee, application of lipase-catalyzed kinetic resolution which was discussed in Chapter 3 to (2*S*,3*R*)-**13** with low optical purity (<60%ee) prepared by Sharpless epoxidation at room temperature was examined.
The starting material, 8-methyl-2-nonen-1-ol **15** was prepared by the reported procedure.[3)] Initially the Sharpless asymmetric epoxidation of olefinic alcohol **15** was performed at room temperature in mole scale. **15** was submitted to the modified Sharpless epoxidation which used molecular

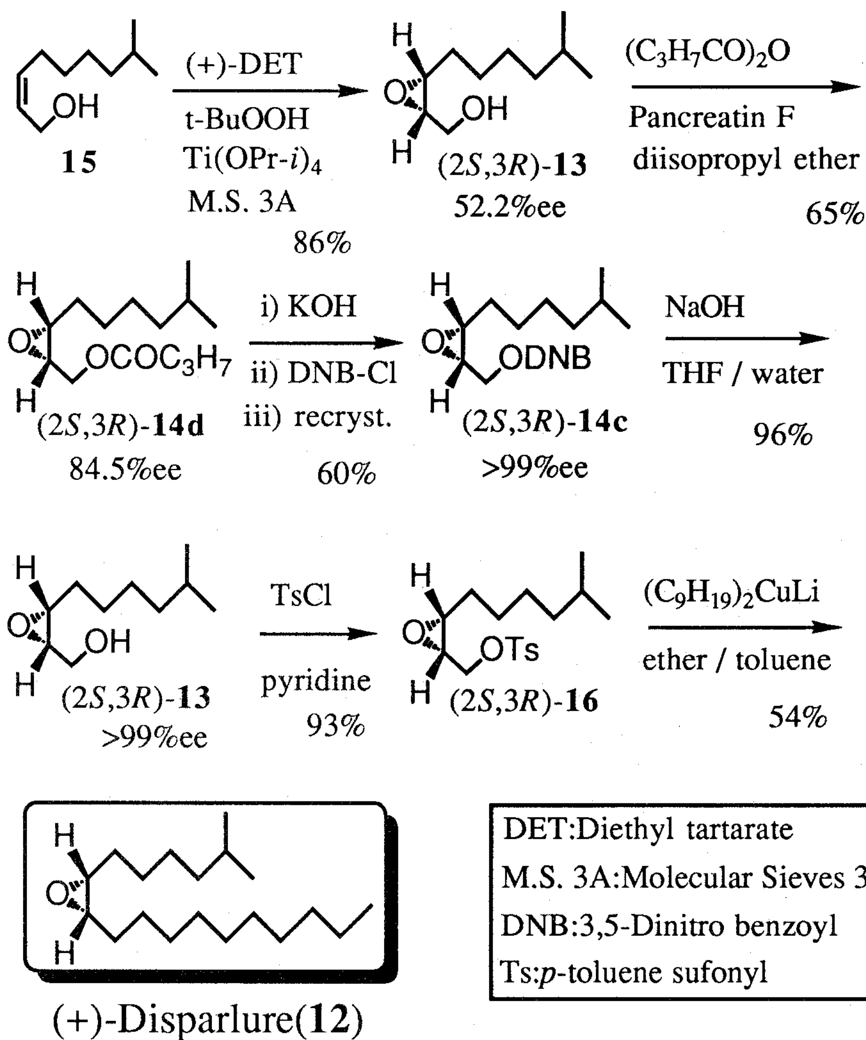
sieves 3A[5]. L-(+)-Diethyl tartrate was employed as chiral auxiliary. The reaction took 18hr to be completed and gave optically active epoxyalcohol (2*S*,3*R*)-**13**, whose optical purity was determined to be 52.2 % by the procedure presented in Chapter 3.

Next, obtained epoxyalcohol with low optical purity(52.2 %ee) was submitted to the lipase-catalyzed enantioselective acylation to obtain more optically pure one. The enzymatic reaction was carried out in diisopropyl ether with *n*-butyric anhydride as acylating agent at room temperature for 5hr to give epoxyester **14d** in 84.5 %ee.

To further enrich the optical purity of the desired (2*S*,3*R*)-enantiomer, epoxyester obtained by above reaction **14d** was converted to the corresponding 3,5-dinitorobenzoate **14c** and recrystallization of **14c** from MeOH was carried out to yield the pure **14c**. Its optical purity was determined to be over 99%ee by HPLC analysis equipped with a chiral column, Chiralcel OJ (Daicel Chem. Co.).The 3,5-dinitro benzoate **14d** was hydrolyzed with NaOH in THF/water (1/1) to yield epoxyalcohol **13**. Its optical purity was confirmed to be over 99%ee.

Finally, the tosylate **16** derived from (2*S*,3*R*)-**13** was treated with $(n\text{-}C_9H_{19})_2CuLi$ in ether and toluene according to the reported procedure[3] to give (+)-disparlure **12**, $[\alpha]_D^{23}$ +0.56±0.28° (c=0.71, chloroform). Its IR and NMR spectra coincided with those of an authentic sample[3].

In conclusion, (+)-disparlure was synthesized from optically pure (2*S*,3*R*)-**13** prepared by the combination of Sharpless asymmetric epoxidation and lipase-catalyzed enantioselective acylation.(Scheme 4-4)



Scheme 4-4 Large scale preparation of (+)-disparlure by the combination of Sharpless asymmetric epoxidation and lipase-catalyzed enantioselective acylation

SUMMARY

Sharpless asymmetric epoxidation of 8-methyl-2-nonen-1-ol performed in large scale (over 5 mole) at room temperature to yield (2*S*,3*R*)-2,3-epoxy-8-methyl-1-nonanol in 52%ee of optical purity. This epoxy alcohol with low optical purity was subjected to lipase-catalyzed enantioselective acylation to enrich its optical purity to 85%ee from 52%ee. Followed recrystallization of corresponding 3,5-dinitrobenzoate gave optically pure epoxy alcohol. (+)-Disparlure, the gypsy moth pheromone, was synthesized in two steps from this optically pure epoxy alcohol.

EXPERIMENTAL SECTION

Boiling points were uncorrected. IR spectra were measured in neat on JASCO A-810 spectrometer. ^{1}H-NMR spectra were measured at 400MHz on a JEOL GX-400. ^{13}C-NMR spectra were measured at 22.5 MHz on a JOEL FX-90Q. Optical rotations were measured on JASCO DIP-181. Column chromatography was effected using Merck Kieselgel 60 (70-230 mesh). Pancreatin F was purchased from Amano Pharm.Co. Solvent for enzymatic reaction was distilled before use.

Large scale Sharpless asymmetric epoxidation of (Z)-8-methyl-2-nonen-1-ol (15) to yield (2S,3R)-2,3-epoxy-8-methyl-1-nonanol

((2S,3R)-13)

According to the reported procedure[3), 6)] , Sharpsess asymmetric epoxidation was performed. Ground molecular sieves 3A (1030 g) was dried and suspended in dried CH_2Cl_2(60 l) under N_2. L-(+)-Diethyl tartrate (377 g, 1.88 mol) was added to the suspension. Ti(OPr-*i*)$_4$ (459 g, 1.61 mol) was added to the suspension dropwise under 5°C. *t*-BuOOH (3.02 M, 2.67 l, 8.07 mol) was added to the suspension. A solution of **15** (840 g, 5.38 mol) in dry CH_2Cl_2 (8 l) was added dropwise. The reaction mixture was stirred and allowed at room temperature for 15 hr. To quench the reaction, the reaction mixture was cooled to -5°C, then poured gradually into the ice-cooled aqueous solution (10 l) containing Fe(II)SO_4 (789 g, 2.84 mol) and (+)-tartaric acid (1150 g, 7.67 mol). The mixture was left to stand for 15 hr at room temperature. The organic phase was separated and the aqueous phase was extracted with ether. The organic phase was combined and cooled to 5 °C. The basic brine (7 l) containing NaOH(282 g) was gradually added to the solution below 5 °C , then the mixture was stirred at around 5 °C for 1 hr. The organic phase was separated and washed with brine, dried over sodium sulfate and concentrated *in vacuo* . The residue was purified by silica gel column chromatography (15 kg). Elution with *n*-hexane/ethyl acetate (4/1) gave (2*S*,3*R*)-**13** (800 g, 86 % yield). νmax 3420, 2950, 2930, 2860, 1460, 1380, 1360, 1040. Its ee was determined to be 52.2 % by the procedure described in Chapter 3.

Large scale lipase-catalyzed eantioselective acylation of (2S,3R)-13 to yield 14d

(2*S*,3*R*)-**13** (500 g, 2.90 mol, 52.2 %ee) and *n*-butyric anhydride (315 g, 2.00 mol) were dissolved in diisopropyl ether (5 l). Pancreatin F

(500 g) was added to the solution , then the mixture was stirred at room temperature for 5 hr. After lipase powder had been removed by filtration, the filtrate was washed with sat. $NaHCO_3$ aq., dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 kg). Elution with hexane/ethyl acetate(20/1) gave **14d** (460 g, 65.4 % yield). νmax 2950, 2930, 2860, 1740, 1460, 1360, 1180, 1080. δ (^1H, 400MHz, $CDCl_3$) 0.85(6H, d, J=6.6Hz), 0.94(3H, t, J=2.7Hz), 1.14-1.69(11H, m), 2.33(3H, d, J=7.3Hz), 2.99(1H, m), 3.16(1H, m), 4.03(1H, m), 4.31(1H, m). δ(^{13}C, 22.5MHz, $CDCl_3$) 13.6, 18.3, 22.6, 26.8, 27.1, 27.8, 28.0, 35.9, 38.8, 53.7, 56.4, 62.5, 173.3. (Found: C,69.38; H,10.86%. Calc. for $C_{14}H_{26}O_3$: C, 69.38; H,10.81%) Its ee was determined to be 84.5 %.

(2S,3R)-1-(3',5'-Dinitrobenzoyloxy)-2,3-epoxy-8-methylnonane (14c)

14d (442 g, 1.83 mol, 84.5 %ee) was hydrolyzed with KOH(169 g) in MeOH(2l) in the usual manner to give (2*S*,3*R*)-**13** (308 g, 98.4 %). Obtained (2*S*,3*R*)-**13** was acylated with 3,5-dinitorobenzoyl chloride (500 g, 2.17mol) in ether(3l)/pyridine(300ml) in the usual manner to give **14c**. This was recrystallized from MeOH to give pure **14c** as colorless leaflet (398 g, 61% yield). mp. 80-81°C. $[\alpha]_D^{28}$ -9.65° (c=1.0, ether) ([Lit.][3], $[\alpha]_D^{22}$ -10.5° (c=0.74, ether))
Its ee was determined to be over 99% by HPLC analysis described below.

Determination of enantiomeric excess(ee) of 14c

14c was subjected to HPLC analysis on the system consisting of pump [JASCO 880-PU] and UV detector [JASCO 875-UV] equipped with a

chiral column [Chiralcel OJ , Daicel Chem. Co., 4.6 x 250mm; hexane/*i*-propanol(95/5); 1ml/min; 256nm at room temp.] The retention time of (2*S*,3*R*)-**14c** and (2*R*,3*S*)-**14c** were 51.2 min and 45.5 min respectively.

(2S,3R)-2,3-epoxy-8-methyl-1-nonanol ((2S,3R)-13)

14c (398 g, 1.09 mol, >99 %ee) was dissolved in THF(1.6 l) and cooled to 5°C. NaOH(56.3 g, 1.31mol) in 1.6l of water was added dropwise below 20 °C. The mixture was stirred for 30min at room temperature. Ether (2.5 l) was added to the reaction mixture and stirred for 5min. The organic phase was separated, washed with brine, dried over sodium sulfate and concentrated *in vacuo* to give (2*S*,3*R*)-**13** (179 g, 95.5% yield). $[\alpha]_D^{23}$ -7.60° (c=1.00, chloroform)[3], $[\alpha]_D^{21}$ -7.83° (c=0.78, chloroform)). νmax 3420, 2950, 2930, 2860, 1460, 1380, 1360, 1040. δ (^{1}H, 400MHz, $CDCl_3$) 0.85(6H, d, J=6.6Hz), 1.10-1.60(9H, m), 1.91(1H, brs), 3.01(1H, m), 3.14(1H, m), 3.64(1H, m), 3.82(1H, m). Its IR and NMR spectra were consistent with those of an authentic sample[3].

(2S,3R)-2,3-epoxy-8-methylnoyl tosylate (16)

13 (179 g, 1.04 mol) was tosylated with *p*-TsCl(300 g, 1.57 mol) in dry pyridine(400 ml) in the usual manner and purified by silica gel column chromatography (3 kg). Elution with hexane/ethyl acetate (10/1) gave **16** (315 g, 93.3 % yield). νmax 2950, 2925, 2860, 1595, 1460, 1365, 1190, 1175, 970, 810, 780. Its IR spectrum was consistent with that of an authentic sample[3].

(+)-Disparlure (12)

According to the reported procedure[3], the coupling reaction of tosylate (**16**) (240 g, 0.74 mol) and $(n\text{-}C_9H_{19})_2CuLi$ (0.32 M, 4.4 l, 1.41mol) were carried out to yield (**12**). Obtained crude product was purified by silica gel column chromatography (30 kg). Elution with hexane/ether(100/1-20/1) gave (**12**). The residue was distilled to give pure (+)-disparlure 224 g(54.0 %). bp. 135-145 °C at 0.5 mmHg. $[\alpha]_D^{23}$ +0.56±0.25° (c=0.71, chloroform) (ref.3, $[\alpha]_D^{20}$ +0.6±0.2°(c=5.6, carbon tetrachloride). νmax 2960, 2935, 2860, 1465, 1385, 1370. δ (^{1}H, 400MHz, $CDCl_3$) 0.86(6H, d, J=6.6Hz), 0.88(3H, t, J=6.6Hz), 1.10-1.60(27H, m), 2.89(2H, m). (Found: C,80.75; H,13.79%. Calc. for $C_{19}H_{38}O$: C, 80.78; H,13.56%). Its IR and NMR spectra were consistent with those of an authentic sample[3].

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CHAPTER 5

PREPARATION OF CARBOXYALKYL ACRYLATE BY LIPASE-CATALYZED REGIOSELECTIVE HYDROLYSIS OF CORRESPONDING METHYL ESTER

INTRODUCTION

Polymers resuted in polymerization of acrylate (**17**) have been widely used in a large number of important industrial applications[1].

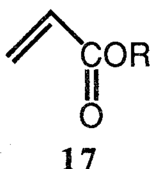
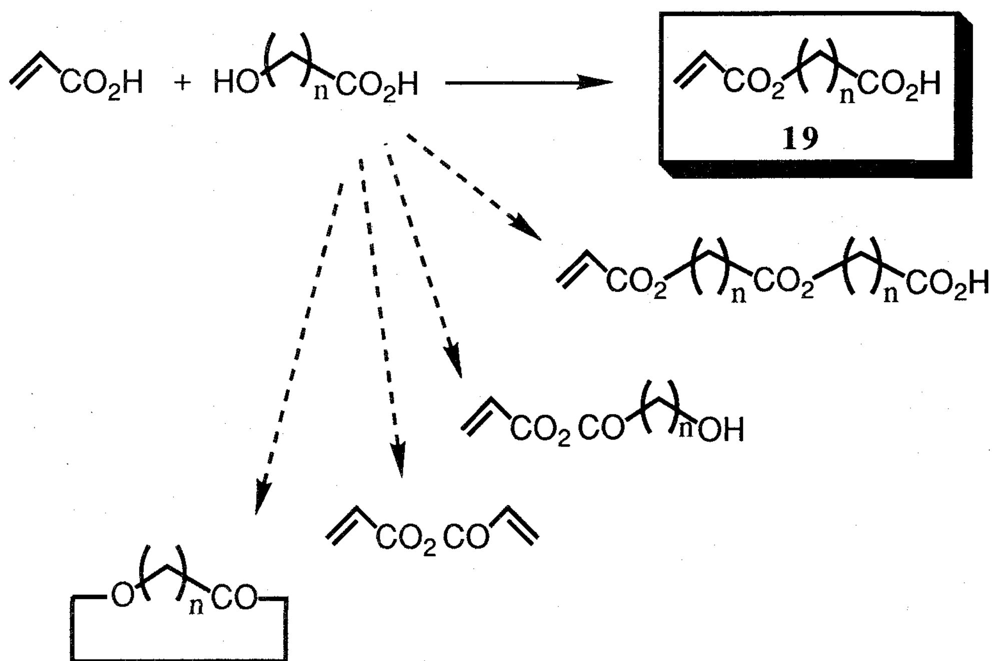


Fig. 5-1 Structure of acrylate

These polymers are normally produced from the lower acrylates such as methyl, ethyl or butyl acrylate. Pendant hydroxyalkyl groups or carboxyalkyl groups, providing sites for further chemical modification or ionic effect, may also be incorporated into the polymers by copolymerization with special acrylates bearing hydroxyalkyl or carboxyalkyl side chaines, namely hydroxyalkyl acrylate (**18**) and carboxyalkyl acrylate (**19**).

A direct chemically catalyzed transesterification method[2] cannot, however, serve as an efficient route for the preparation of these acrylates, since it would yield a complex mixture of unreacted alcohol and partially and fully substituted ester products. (Scheme 5-1)

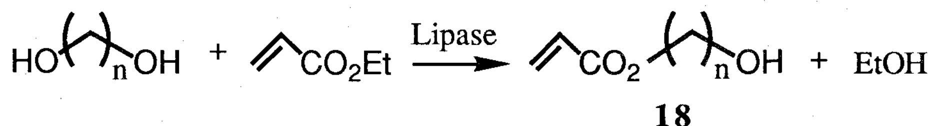
Fig. 5-2 Hydroxyalkyl acrylate (18) and carboxyalkyl acrylate(19)



Scheme 5-1 Problem of chemical synthesis for preparation of carboxyalkyl acrylate (19)

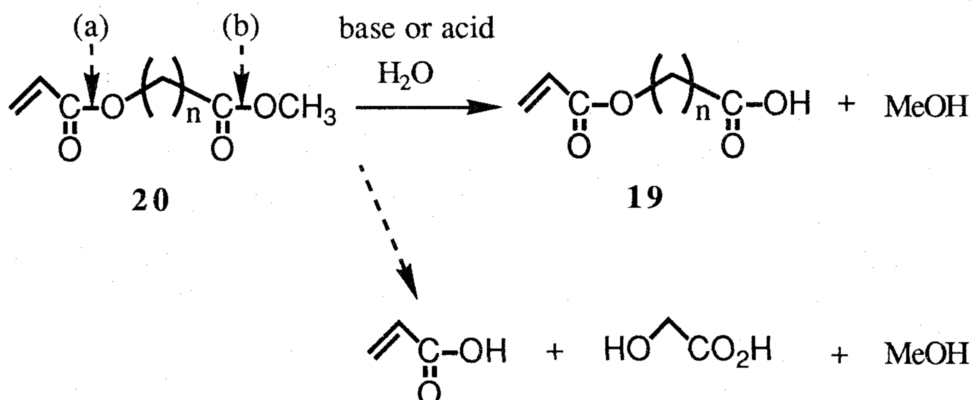
Although interesting syntheses of hydroxyalkyl acrylates (**18**) by enzymatic transesterification have been reported[3] (Scheme 5-2), there are

no convenient routes to acrylates bearing carboxyalkyl side chains.



Scheme 5-2 Preparation of hydroxyalkyl acrylate by lipase

Chemical hydrolysis of methoxycarbonylalkyl acrylate (**20**), which includes two ester bonds, is inefficent for preparation of carboxyalkylacrylate (**19**) in good yield because of low regioselectivity. In this procedure, a mixture of **19** and acrylic acid would be obtained. (Scheme 5-3)



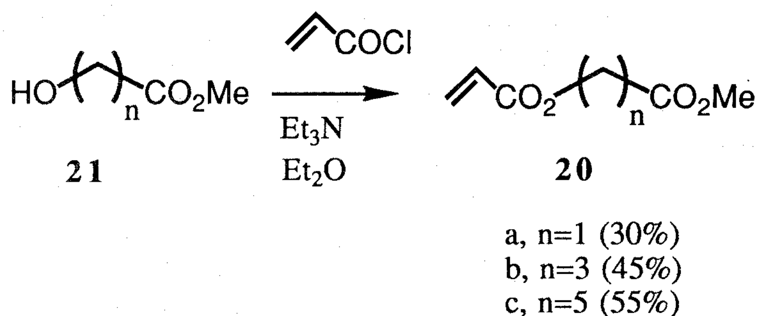
Scheme 5-3 Problem of chemical hydrolysis of methoxycarbonyl alkyl acrylate (20)

In this Chapter, the author present a regioselective hydrolysis of methoxycarbonylacrylate **20** by lipase to yield carboxyalkyl acrylate **19**. In addition, following reported procedures[4)], a convenient synthesis of **20**

by lipase-catalyzed transesterification of hydroxy ester and vinyl acrylate in an organic solvent is described.

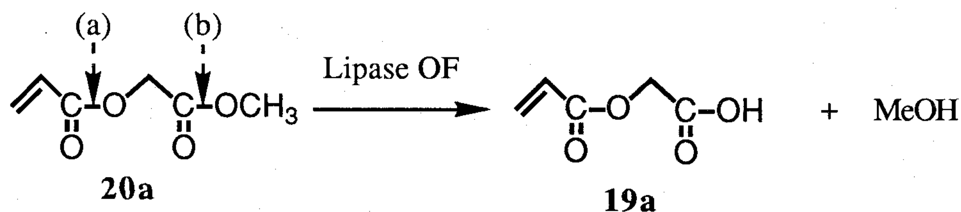
RESULTS AND DISCUSSIONS

Starting materials, methoxycarbonylalkyl acrylates (**20**) were readily prepared from hydroxycarboxylic acids methyl ester (**21**) and acryloyl chloride in the usual manners. (Scheme 5-4)



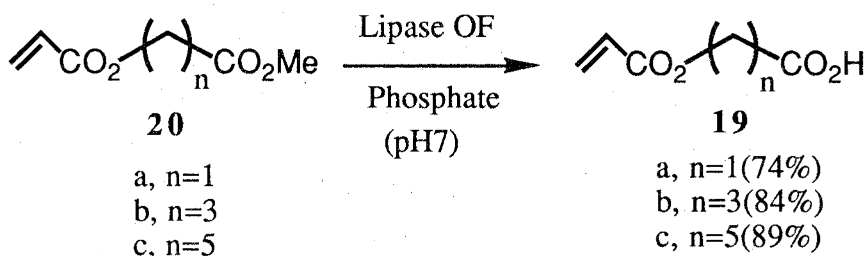
Scheme 5-4 Preparation of methoxycarbonylalkyl acrylate (20)

First, several commercially available lipases were surveyed for the hydrolysis of methoxycarbonylmethyl acrylate (**20a**) in phosphate buffer. Lipase OF, a lipase from *Candida cylindracea*, Meito Sangyo Co., Japan, was found to recognized site(b). **20a** was hydrolyzed by lipase OF to yield carboxymethyl acrylate (**19a**) without any by-product.(Scheme 5-5)



Scheme 5-5

Other substrates, **20b** and **20c**, were also subjected to the same reaction, Lipase OF-catalyzed regioselective hydrolysis to yield **19b** (84% yield) and **19c** (89% yield) respectively (Scheme 5-6)

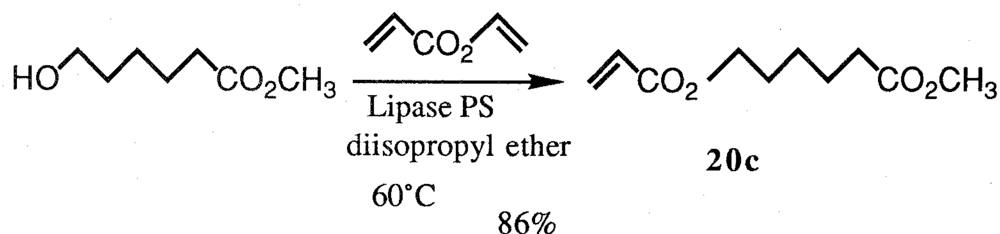


Scheme 5-6 Lipase-catalyzed regioselective hydrolysis of methoxycarbonylalkyl acrylate (20)

Among these acrylates, **19c** was especially demanded as a monomer

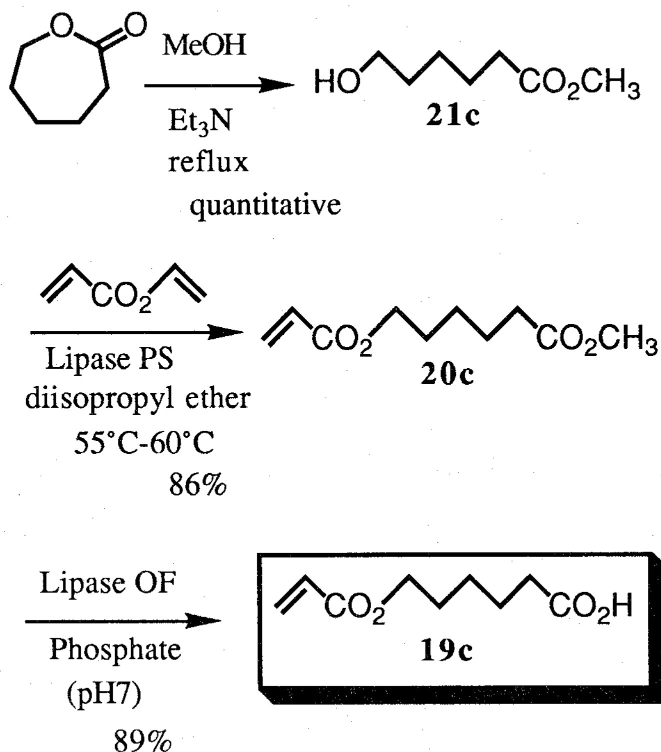
for synthesis of a pressure sensitive adhesive polymer. If a large scale preparation of **19c** is required to be performed by this new method (Scheme 5-6), the current method for preparation of the substrate(**20c**) (shown in Scheme 5-4) also needs to be improved because of its low yield (55%).

Lipase-catalyzed transesterification was applied to preparation of **20c**. After surveying several commercially available lipases, Lipase PS, a lipase from *Pseudomonas* sp., Amano Pharm.Co., Japan, was found to catalyze the transesterification of vinyl acrylate and methyl 6-hydroxyhexanoate in isopropyl ether to give **2c** (86% yield).(Scheme 5-7)



Scheme 5-7 Lipase-catalyzed regioselective transesterification to yield methoxycarbonylpentyl acrylate

In conclution, a convenient route for preparation of carboxyalkyl acrylate was established by enzymatic procedure. (Scheme 5-8)



Scheme 5-8 New route for preparation of carboxypentyl acrylate (19c) by lipase-catalyzed regioselective transesterification and hydrolysis

SUMMARY

Carboxyalkyl acrylate was synthesized by lipase-catalyzed regioselective hydrolysis of corresponding methyl ester, methoxycarbonylalkyl acrylate, which was conveniently prepared from vinyl acrylate and hydroxyalkanoic acid methyl ester by lipase-catalyzed transesterification in an organic solvent.

EXPERIMENTAL SECTION

IR spectra were measured in neat on JASCO A-810 spectrometer. ^{1}H-NMR spectra were measured at 90MHz on a JEOL FX-90Q or 400MHz on a JEOL GX-400. ^{13}C-NMR spectra were measured at 22.5 MHz on a JOEL FX-90Q or 100MHz on a JOEL GX-400. Column chromatography was effected using Merck Kieselgel 60 (70-230 mesh). Pancreatin F was purchased from Amano Pharm.Co. Solvent for enzymatic reaction was distilled before use.

Preparation of methyl hydroxy carboxylates (21a-c)

methyl glycolate (**21a**) was purchased from Aldrich Chemical Co., USA. 4-butyrolactone was methanolyzed[5] in methanol with triethylamine as catalyst at reflux temperature for 1hr. Methanol and triethylamine were removed *in vacuo* and 4-hydroxybutanoate (**21b**) was obtained. (quantitative yield) νmax 3400, 2920, 2850, 1740, 1440, 1370, 1330, 1260, 1210, 1180, 1080, 1060, 1030, 970. 6-hexanolactone was subjected to same procedure to give 6-hydroxyhexanoate (**21c**). (quantitative yield) νmax 3400, 2920, 2850, 1740, 1440, 1370, 1330, 1260, 1210, 1180, 1080, 1060, 1030, 970. These were employed in the next steps without further purification.

Preparation of methoxycarbonylalkyl acrylates (20a-c)

Methyl glycolate (**21a**) (5.0 g, 56 mmol) was acylated with acryloyl

chloride (6.0 g) in diethyl ether with triethylamine in the usual manner and purified by silica gel column chromatography [Elution hexane/ethyl acetate (10/1)] to give **20a** (2.9 g, 30 %yield), νmax 3000, 2940, 1740, 1640, 1620, 1520, 1450, 1430, 1410, 1390, 1300, 1220, 1180, 1100, 1060, 1010, 980, 900, 850, 810. $\delta(^{1}H, 90MHz, CDCl_3)$ 3.78(3H, s), 4.71(2H, s), 5.92(1H, dd, J=9.8, 2.7Hz), 6.18(1H, dd, J=16.9, 9.8Hz), 6.54(1H, dd, J=16.9, 2.7Hz) $\delta(^{13}C, 22.5MHz, CDCl_3)$ 52.2, 60.6, 127.3, 132.1, 165.2, 168.1. Mass spectra *m/e* 145 $(M+H)^+$

Methyl 4-hydroxybutanoate(**21b**) (8.3 g, 70 mmol) was subjected above procedure to give **20b** (5.5 g, 45 %yield), νmax 2930, 1740, 1640, 1620, 1460, 1410, 1370, 1320, 1300, 1280, 1200, 1100, 1060, 1040, 990, 940, 890, 810. $\delta(^{1}H, 90MHz, CDCl_3)$ 1.83-2.17(2H, m), 2.43(2H, t, J=7.0Hz), 3.69(3H, s), 4.20(2H, t, J=7.0Hz), 5.82(1H, dd, J=9.8, 2.7Hz), 6.10(1H, dd, 16.8, 9.8Hz), 6.43(1H, dd, 16.8, 2.7Hz).. $\delta(^{13}C, 22.5MHz, CDCl_3)$ 23.9, 30.4, 51.4, 63.3, 128.2, 130.6, 165.8, 173.0. Mass spectra *m/e* 173$(M+H)^+$.

Methyl 6-hydroxyhexanoate(**21c**) (5.0 g, 34 mmol) was subjected to above procedure to give **20c** (4.1 g, 55 %yield), νmax 2930, 2850, 1740, 1640, 1620, 1460, 1440, 1410, 1370, 1300, 1280, 1200, 1100, 1060, 980, 810, 740. $\delta(^{1}H, 90MHz, CDCl_3)$ 1.38-1.42(2H, m), 1.62-1.71(4H, m), 2.31(2H, t, J=7.8Hz), 3.63(3H, s), 4.14(2H, t, J=6.6Hz), 5.80(1H, dd, J=10.1, 1.4Hz), 6.09(1H, dd, J=17.1, 10.7Hz), 6.38(1H, dd, J=17.1, 1.4Hz). $\delta(^{13}C, 22.5MHz, CDCl_3)$ 25.5, 27.5, 28.3, 33.8, 51.4, 64.2, 128.5, 130.4, 166.2, 173.8. Mass spectra *m/e* 201$(M+H)^+$.

Lipase-catalyzed regioselective hydrolysis of methoxycarbonyl acrylate (20) to yield carboxyalkyl acrylates (19)

20a (500 mg, 3.5 mmol) was suspended in phosphate buffer (50 ml,

0.1 M, pH 7). Lipase OF (250 mg) was added to the solution and the mixture was stirred at room temperature for 16hr. The reaction mixture was extracted with ether. The extract was washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to give **19a** (234 mg, 74 %yield) νmax 3180, 2940, 1740, 1640, 1620, 1430, 1420, 1300, 1280, 1180, 1100, 1060, 900, 860, 820. δ(^{1}H, 400MHz, deuterio acetone) 4.72(2H, s), 5.92(1H, dd, J=10.3, 1.1Hz), 6.19(1H, dd, J=17.6, 10.6Hz), 6.50(1H, dd, J=17.4, 1.3Hz). δ(^{13}C, 100MHz, deuterio acetone) 61.0, 128.6, 132.2, 165.8, 169.1. Mass spectra *m/e* 131(M+H)$^+$.

20b (500 mg, 2.9 mmol) was subjected above procedure to give **19b** (387 mg, 84 %yield). νmax 3200, 2940, 2850, 1730, 1640, 1620, 1420, 1300, 1280, 1200, 1080, 1030, 990, 940, 890, 820. δ(^{1}H, 400MHz, deuterio acetone) 1.93-2.00(2H, m), 2.42(2H, t, J=7.3Hz), 4.19(2H, t, J=6.2Hz), 5.88(1H, dd, J=10.4, 1.6Hz), 6.15(1H, dd, J=17.6, 10.6Hz), 6.35(1H, dd, J=17.2, 1.5Hz). δ(^{13}C, 100MHz, deuterio acetone) 24.8, 30.6, 64.1, 129.4, 131.0, 166.3, 174.2. Mass spectra *m/e* 159(M+H)$^+$.

20c (500 mg, 2.9 mmol) was subjected above procedure to give **19c** (415 mg, 89 %yield). νmax 3400, 3200, 2930, 2850, 1720, 1710, 1640, 1620, 1460, 1420, 1300, 1280, 1200, 1100, 1060, 980, 820. δ(^{1}H, 400MHz, deuterio acetone) 1.39-1.48(2H, m), 1.60-1.73(4H, m), 2.31(2H, t, J=7.3Hz), 4.14(2H, t, J=6.6Hz), 5.86(1H, dd, J=10.8, 1.6Hz), 6.14(1H, dd, J=17.2, 10.3Hz), 6.34(1H, dd, J=17.2, 1.6Hz). δ(^{13}C, 100MHz, deuterio acetone) 25.2, 26.1, 29.0, 34.0, 64.8, 129.5, 130.8, 166.3, 174.9. Mass spectra *m/e* 187(M+H)$^+$.

Lipase-catalyzed transesterification of methyl 6-hydroxyhexanoate and vinyacrylate to yield methoxycarbonyl

pentyl acrylate (20c)

Methyl 6-hydroxyhexanoate (5 g) and vinyl acrylate (4 g) were dissolved in isopropyl ether (250 ml). Lipase PS (1 g) was added to the solution and the mixture was stirred at 60°C for 6 hr. After filtration and concentration, the residue was purified by silica gel column chromatography(hexane/diethyl ether(3/1)) to give **20c** (5.9 g, 86 %). IR and NMR spectra were idntical with those of **20c** obtained above respectively.

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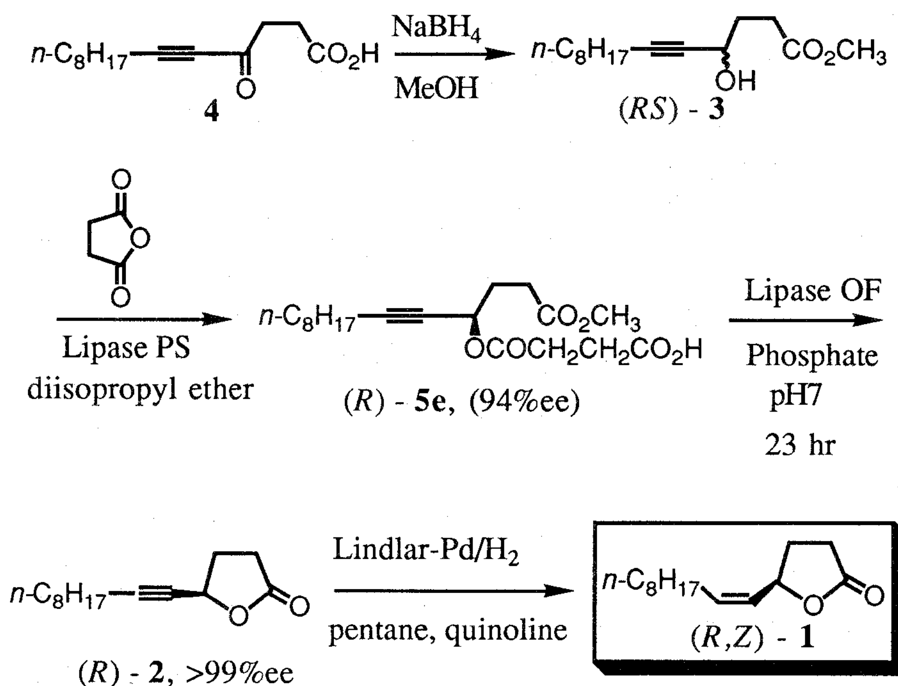
5 Sugai, T.; Ohsawa, S.; Yamada, H.; Ohta, H.: Preparation of enantiomerically enriched compounds using enzymes; VII. A synthesis of Japanese beetle pheromone utilizing lipase-catalyzed enatioselective lactonization. *Synthesis* , 1112-1114 (1990)

GENERAL CONCLUSION

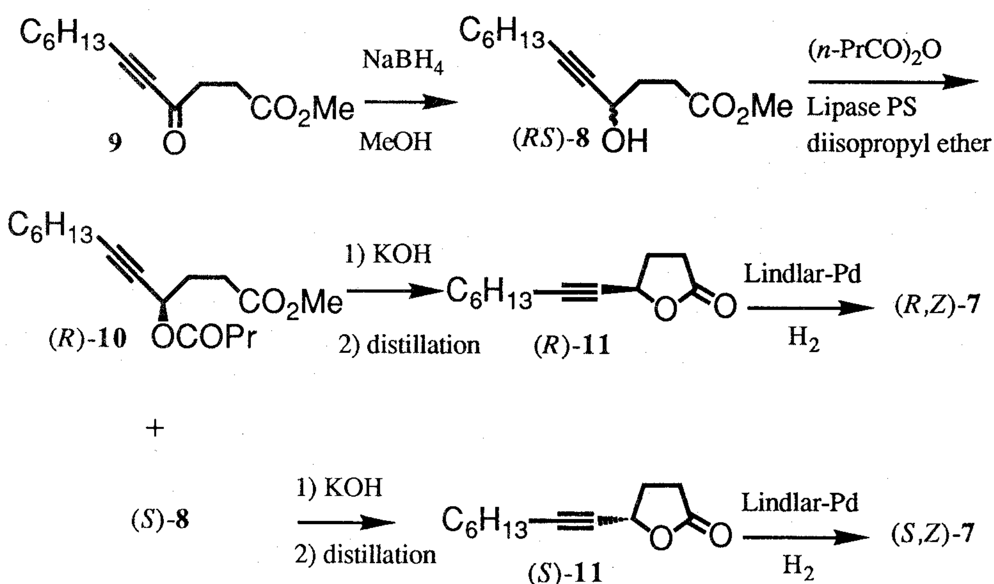
In this thesis, the author described utility of lipase-catalyzed transformation for preparation of valuable compounds. In chapter 1, a new synthesis of the Japanese beetle pheromone, (*R*,*Z*)-(-)-5-(1-decenyl)oxacyclopentan-2-one ((*R*,*Z*)-**1**), established by combining enzymatic and chemical methods without using any chiral auxiliaries was described. (Scheme 1) The key reaction is a combination of two lipase-catalyzed enantioselective transformation.



Scheme 1

One is acylation of methyl 4-hydroxy-5-tetradecynoate with succinic anhydride in an organic solvent to yield methyl (*R*)-4-succinoyloxy-5-tetradecynoate. The other is lactonization of (*R*)-4-succinoyloxy-5-tetradecynoate to yield (*R*)-5-(1-decynyl)oxacyclopentan-2-one

In chapter 2, a chemico-enzymatic procedure for the synthesis of both enantiomers of cupreous chafer beetle pheromone, (*R*,*Z*)- and (*S*,*Z*)-5-(1-octenyl)oxacyclopentan-2-one ((*R*,*Z*)-**7** and (*S*,*Z*)-**7**) was described. (Scheme 2)

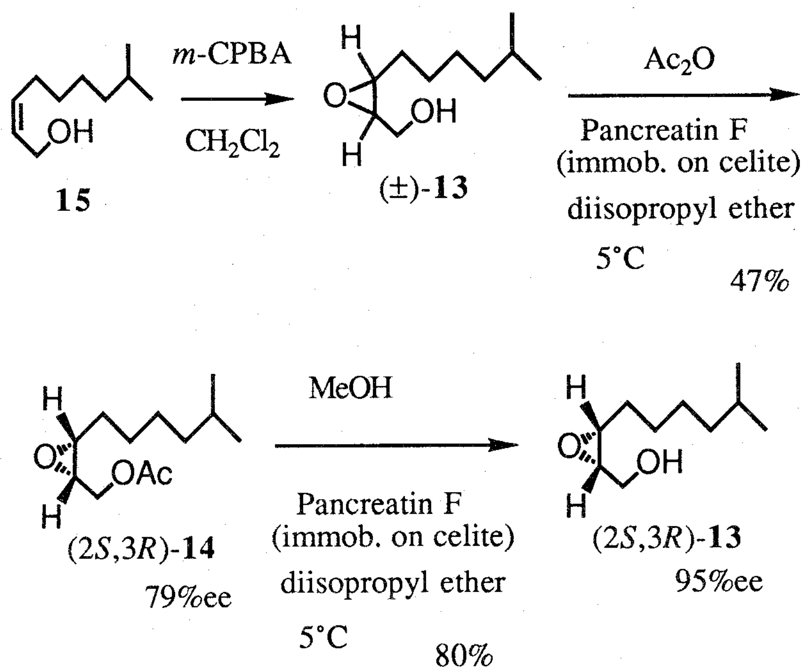


Scheme 2

The key reaction is a repetition of lipase-catalyzed enantioselective acylation

of methyl 4-hydroxy-5-dodecynoate ((*RS*)-**8**) with n-butyric anhydride in an organic solvent.

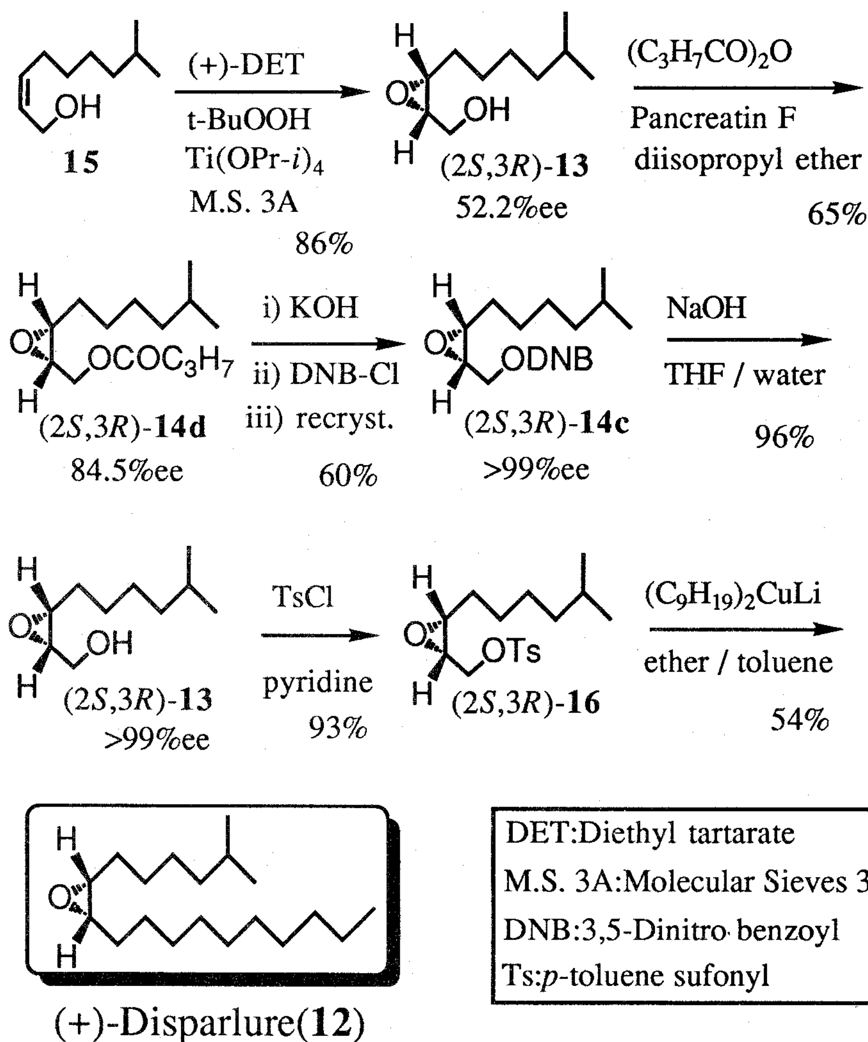
In chapter 3, a optical resolution of (±)-2,3-epoxy-8-methyl-1-nonanol, (**13**) the key intermediate of the synthesis of gypsy moth pheromone, was described. The key reactions are the combination of lipase-catalyzed enantioselective acylation of epoxy alcohol (**13**) and enantioselective alcoholysis of corresponding ester (**14**) in an organic solvent. (Scheme 3)



Scheme 3

In chapter 4, a practical chemico-enzymatic synthesis of (+)-disparlure,

(**12**) in large scale was described.(Scheme 4)

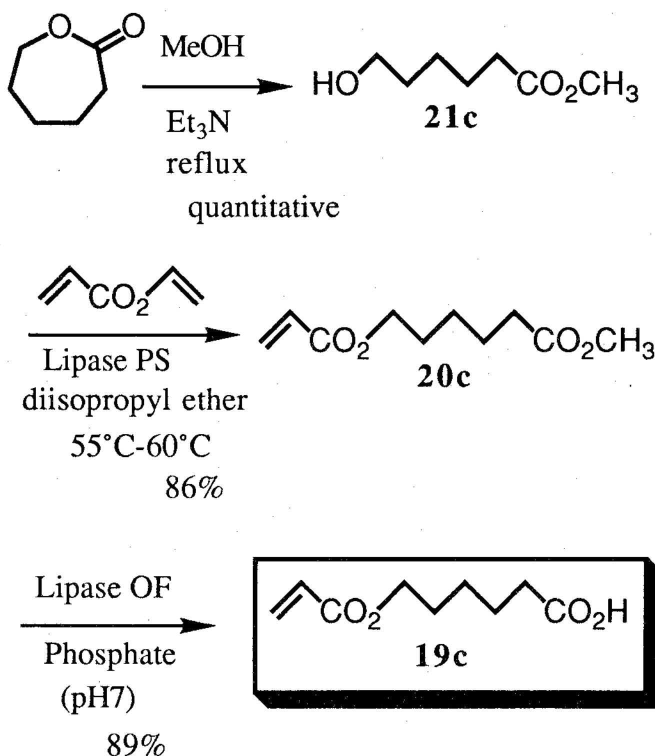


Scheme 4

In this synthesis, modified Shapless asymmetric epoxidation was carried

out at room temperature to yield epoxy alcohol ((2*S*,3*R*)-**13**) with low optical purity. Obtained epoxy alcohol ((2*S*,3*R*)-**13**) was subjected lipase-catalyzed enantioselective acylation in an organic solvent to enrich its optical purity. Optically pure (+)-disparlure (**12**), the gypsy moth pheromone, was synthesized from this epoxy alcohol.

In chapter 5, the establishment of a convenient route for preparation of carboxyalkyl acrylate (**19c**) by enzymatic procedure was described. (Scheme 5)



Scheme 5

In this synthesis carboxyalkyl acrylate (**19c**) was synthesized by lipase-catalyzed regioselective hydrolysis of corresponding methyl esters, methoxycarbonylalkyl acrylates (**20c**), which were conveniently prepared from vinyl acrylate and hydroxyalkanoic acid methyl esters by lipase-catalyzed transesterification in an organic solvent.

The potential of lipase-catalyzed transformation as a tool for preparative synthesis is proved through this study.

LIST OF PUBLICATION

1. Lipase-catalyzed kinetic resolution of methyl 4-hydroxy-5-tetradecynoate and its application to a facile synthesis of Japanese beetle pheromone
   Eiichiro Fukusaki, Shuji Senda, Yutaka Nakazono, and Tetsuo Omata
   Tetrahedron, **47**, 6223-6230 (1991)

2. Synthesis of the enantiomers of (Z)-5-(1-octenyl)oxacyclopentan-2-one, a sex pheromone of the cupreous chafer beetle, *Anomala cuprea* Hope
   Eiichiro Fukusaki, Shuji Senda, Yutaka Nakazono, and Tetsuo Omata
   Biosci. Biotech. Biochem., **56**, 1160-1162 (1992)

3. Lipase-catalyzed kinetic resolution fo 2,3-epoxy-8-methyl-1-nonanol, the key intermediate in the synthesis of the gypsy moth pheromone
   Eiichiro Fukusaki, Shuji Senda, Yutaka Nakazono, Hiroyuki Yuasa, and Tetsuo Omata
   J. Ferment. Biotech., **73**, 280-283 (1992)

4. Large-scale preparation of (+)-disparlure, the gypsy moth pheromone, by a practical chmico-enzymatic procedure
   Eiichiro Fukusaki, Shuji Senda, Yutaka Nakazono, Hiroyuki Yuasa, and Tetsuo Omata
   J. Ferment. Biotech., **73**, 284-286 (1992)

5. Preparation of carboxyalkyl acrylate by lipase-catalyzed regioselective hydrolysis of corresonding methyl ester
Eiichiro Fukusaki, Shuji Senda, Yutaka Nakazono, Hiroyuki Yuasa, and Tetsuo Omata
Bioorganic & Medicinal Chemistry Letters, **2**, 411-414 (1992)

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