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A SYNTHESIS OF (-)-DEOXYPODOCARPIC ACID METHYL ESTER VIA AN ENZYMATIC ENANTIOSELECTIVE HYDROLYSIS OF THE KEY INTERMEDIATE ENOL ESTER†

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Abstract -- (1R,4aR,10aS)-(-)-1-Methoxycarbonyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (deoxypodocarpic acid methyl ester **1**), a useful intermediate for the synthesis of various diterpenes, was synthesized from (R)-(+)-6-ethoxycarbonyl-2,6-dimethyl-1-cyclohexenyl acetate **4**. The chiral starting material was prepared by the enantioselective hydrolysis of the corresponding racemate using lipase OF from Candida cylindracea.

Introduction

(1R*,4aR*,10aS*)-1-Methoxycarbonyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (deoxypodocarpic acid methyl ester **1**, Fig. I) is a useful intermediate for the synthesis of various diterpenes. Both enantiomers of **1** are necessary for the natural product syntheses because of the diverse stereochemistry of natural diterpenes. For example, (+)-podocarpic acid requires (1S,4aS,10aR)-(+)-**1** as a synthetic precursor, while (-)-kaurenol, (-)-steviol, and (+)-monogynol were correlated to (1R,4aR,10aS)-(-)-**1**. Chiral syntheses including a derivation from natural product,[1] an optical resolution of itself,[2] and an asymmetric synthe-

†Preparation of Chiral Compound using Enzymes, Part 2. Part 1: T. Sugai and H. Ohta, Agric. Biol. Chem. in press. The chemical experimental part of this work was taken from B. S. thesis of H. K. (March, 1989). X-ray crystallographic work was carried out by M. M and S. O.

(+)-1 (-)-1

Fig. I Both Enantiomers of Deoxypodocarpic Acid Methyl Ester

sis[3] have been reported as well as the racemic[2,4~8] syntheses. Among them, Mori and Matsui's[6] synthesis seems to be the most suitable method for the preparation of racemate of **1** in a large quantity. In their synthesis, 2-ethoxycarbonyl-2,6-dimethylcyclohexanone **2** was the key intermediate (Fig. II). The chiral center at C-2 in **2** was transferred into the one at C-1 in **1**, and chiral centers at C-4a and C-10a in **1** were correctly controlled *via* the diastereoselective cationic cyclization reaction. Therefore, if the quaternary chiral center at C-2 in **2** is constructed in enantiomerically pure state, an efficient chiral synthesis becomes possible. Our continuing efforts in the field of utilization of microorganisms and enzymes in organic syntheses prompted us to develop a method for the preparation of optically active **2** from easily obtainable its racemate.

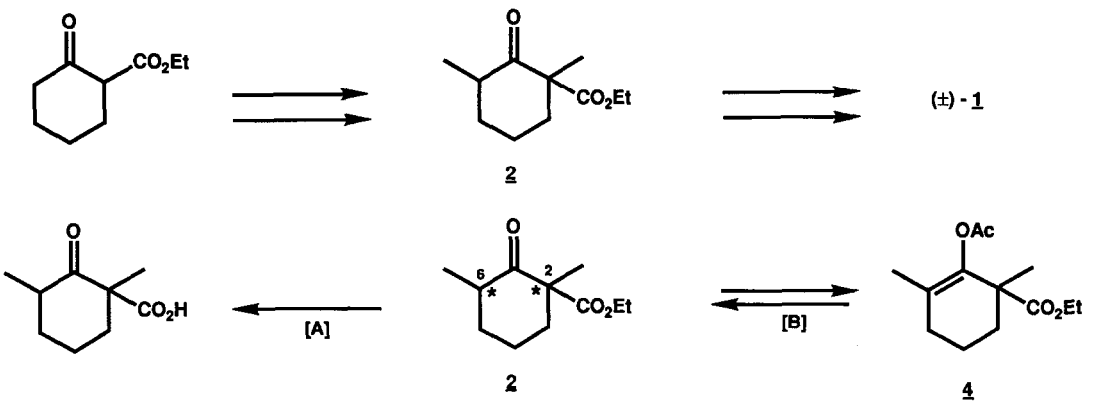
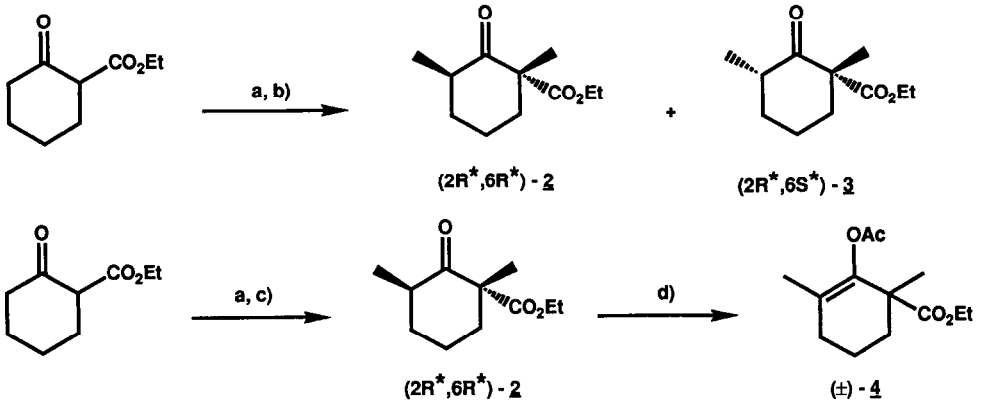


Fig. II Optically Active Starting Materials

Preparation and stereochemistry of the key intermediate (±)- 2

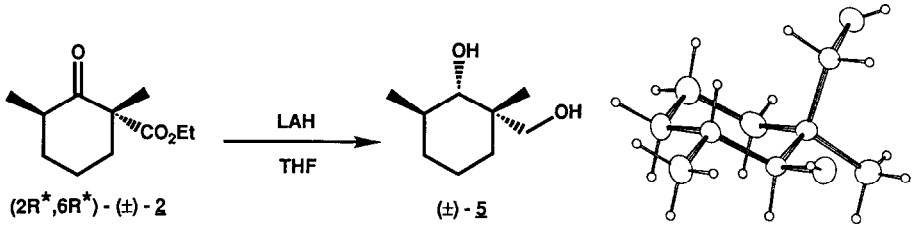
In Mori and Matsui's synthesis,[6] the relative stereochemistry between C-2 and C-6 of **2** was left unknown and the possibility that **2** was a diastereomeric mixture could not be negligible. Therefore, a diastereomeric mixture of the oxoester was prepared *via* another way and chromatographically separated. The relative stereochemistry was determined as (2R*,6R*) for less polar **2** and (2R*,6S*) for more polar

3 respectively (Fig. III), judging from their 400 MHz ^{1}H NMR spectra [2, δ 1.28 (equatorial C-2 Me); 3, δ 1.45 (axial C-2 Me)].[9] Since the proposed stereochemistry was based on the conclusion obtained in the case of homologated compounds (CH_2CO_2R instead of CO_2Et),[10] a diol (±)-5 obtained by the lithium aluminum hydride reduction of (±)-2 was subjected to an X-ray crystallographic analysis for the further proof. Based on the obtained structure of (±)-5 depicted in Fig. IV, the relative configuration of (±)-2 was unambiguously determined to be (2R*,6R*). Now, it became possible for us to correctly determine the stereochemistry and the diastereomeric composition of the product(s) in the process of enzymatic optical resolution. In addition, Mori and Matsui's oxoester was revealed to be a single diastereomer as 2 by comparing its ^{1}H NMR spectrum and GLC analysis.



a) K_2CO_3, MeI/An, 89.0%; b) LDA, MeI, 58.3%; c) i) NaOEt/EtOH; ii) MeI/toluene, 52.7%; d) Ac_2O, cat. $HClO_4/CCl_4$, 92.6%.

Fig. III Preparation of the Substrate



An ORTEP drawing is shown for one of the enantiomeric form in (±)-5.

Fig. IV Determination of the Relative Configuration of (±)-2

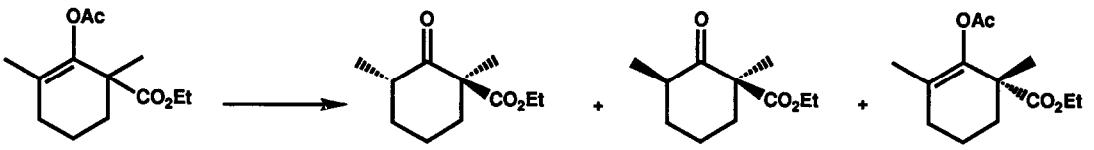
Hydrolysis of enol acetate (±)-4 using Pichia miso

The first attempt was the enantioselective hydrolysis of the carboxylic ester group in 2 by using microorganisms and enzymes (Fig. II, path [A]) only to achieve

non-selective hydrolysis.

Then another substrate **4** was prepared by converting the ketone group of **2** to an enol ester, which was a masked and activated carbonyl compound and could be effected by hydrolytic enzymes (Fig. II, path [B]). Recently, we developed a method for the preparation of optically active ketones by using the enzymatic hydrolysis of corresponding enol esters. The chiral center was brought about by an enantioselective protonation[11] of prochiral enol esters or a kinetic resolution of racemic enol esters.[12] Pichia miso IAM 4682 and Bacillus coagulans FERM P-9237 were found as the potent microorganisms in these cases. Thus, hydrolysis of the enol ester **4** by using those microorganisms was firstly attempted. The substrate (±)-**4** was prepared from **2** in 92.6% yield by the treatment with acetic anhydride in the presence of a catalytic amount of 70% aqueous perchloric acid[13].

Table 1 Hydrolysis of (±)-4 with Pichia miso



(±)-**4** (+)-**2** (-)-**3** (+)-**4**

Run	Sub.conc. (%)	Cult. (h)	Conv. (%)	(+)-2 Yd/ee	(-)-3 Yd/ee	(+)-4 Yd/ee (%)
1	0.2	60	40	11.0/15	15.0/41	44.0/37
2	0.2	90	62	11.0/47	31.0/42	16.0/83
3	0.2	114	100	0/-	3.7/0	0/-
4	0.1	63	53	12.0/37	39.0/55	38.0/72
5	0.5	90	74	11.0/18	45.0/15	19.0/42

Bacillus coagulans[12] hydrolyzed (±)-**4** to give **3** in 67.7% yield. Although the hydrolysis was diastereoselective as revealed from the absence of **2** as the product, recovered **3** was almost racemic. On the other hand, when (±)-**4** was incubated with Pichia miso[11] (see Experimental), (+)-**2** (11.0% yield), (-)-**3** (31.0% yield), and (+)-**4** (16.0% yield) were obtained. The enantiomeric excess of each component was determined as follows: (+)-**2**, 47% e.e. [400 MHz NMR in the presence of chiral NMR shift reagent Tris[3-trifluoromethylhydroxymethylene)-(-)-camphorato]europium [$Eu(tfc)_3$]]; (-)-**3**, 42% e.e. [400 MHz NMR, $Eu(tfc)_3$]; (+)-**4**, 83% e.e. [400 MHz NMR, Tris[3-heptafluoropropylhydroxymethylene)-(+)-camphorato]europium [$Eu(hfc)_3$]].

Then the effect of substrate concentration and period of incubation was examined (Table 1), however, no appreciable enhancement of enantiomeric excess could be obtained. Moreover, since a prolonged incubation period made the total recovery lower, it is supposed that several enzymatic systems were concerned with this conversion and the products were further metabolized. Therefore, commercially available enzymes were tried to find out the more efficient one.

Hydrolysis of enol acetate (±)-**4** using lipase OF

Among 16 kinds of enzymes tested, lipase OF was revealed to show the highest enantioselectivity. When (±)-**4** was incubated with lipase OF (see Experimental), (+)-**2** (5.0% yield; 49% e.e.), (-)-**3** (54.0% yield, 47% e.e.), and (+)-**4** (32.0% yield, >99% e.e.) were obtained. The results obtained by changing the incubation period are listed in Table 2. Although the hydrolyzed product (-)-**3** exhibited only a moderate optical activity, almost optically pure (+)-**4** could be obtained after 72 or 90 h incubation in 32~37% yield.

The scale-up of the enzymatic reaction using several grams of substrate worked well, resulting in the preparative-scale production of (+)-**4** of >99% e.e. in 32.7% yield. Because of the simplicity of the reaction process and the rather low cost of this enzyme, the above enantioselective hydrolysis is expected to be carried out in a several ten gram scale.

In addition, when the reaction was carried out at low temperature[14] (bath temp, -3°C), an enhanced enantioselectivity was achieved although the reaction became slower. After 100 h incubation, (-)-**3** (35.0% yield, 77% e.e.), and (+)-**4** (52.9% yield, 67% e.e.) were obtained. It is supposed that the repetition of the acylation and hydrolysis[14] gives (-)-**3** with a still higher enantiomeric excess, which had been difficult to obtain under a usual hydrolytic condition at 30°C. In this way, the compounds (+)-**4** and (-)-**3** possessing a chiral center correlating to both the enantiomers of **1** were obtained.

Table 2 Hydrolysis of (±)-4 with Lipase OF

Run	Sub.conc. (%)	Cult. (h)	Conv. (%)	(+)-2 Yd/ee	(-)-3 Yd/ee	(+)-4 Yd/ee (%)
1	0.2	48	51	2.0/-	41.0/67	38.0/88
2	0.2	72	57	1.0/-	45.0/55	37.0/>99
3	0.2	90	61	5.0/49	54.0/47	32.0/>99
4	0.2	100 (-3~0°C)	36	0/-	35.0/77	52.9/67

Conversion of (+)-4 into (-)- 2

The next task was the hydrolysis of (+)-4 into the corresponding ketoester. Alkaline hydrolysis under as mild conditions as potassium carbonate in ethanol or non-selective hydrolyses using enzymes resulted in the formation of complex mixture of the products or recovery of the starting material. On the other hand, acidic ethanolysis with a catalytic amount of 70% aqueous perchloric acid (reflux, 13 h) afforded (-)-2 (67.3%, from thermodynamically favorable conformation [A]), (+)-3 (11.5%, from unfavorable conformation [B]) (Fig. V). While the enantiomeric excess of (-)-2 was over 99% e.e. [400 MHz NMR, $Eu(tfc)_3$], (+)-3 was 77% e.e. Epimerization of (-)-2 (less stable axial CO_2Et) into (-)-3 (more stable equatorial CO_2Et) through an intermediate enol [C] under acidic conditions would cause the partial racemization of (+)-3 (Fig. V). Actually, prolonged reaction period caused the further racemization of 3 as low as 48% e.e.

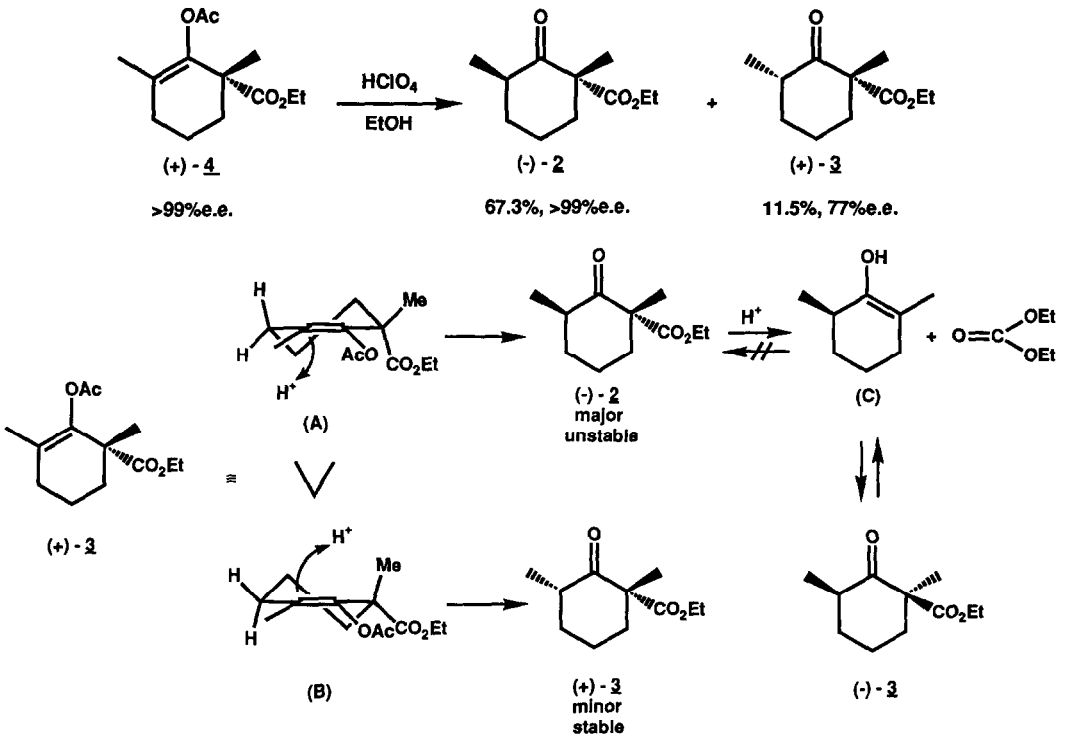


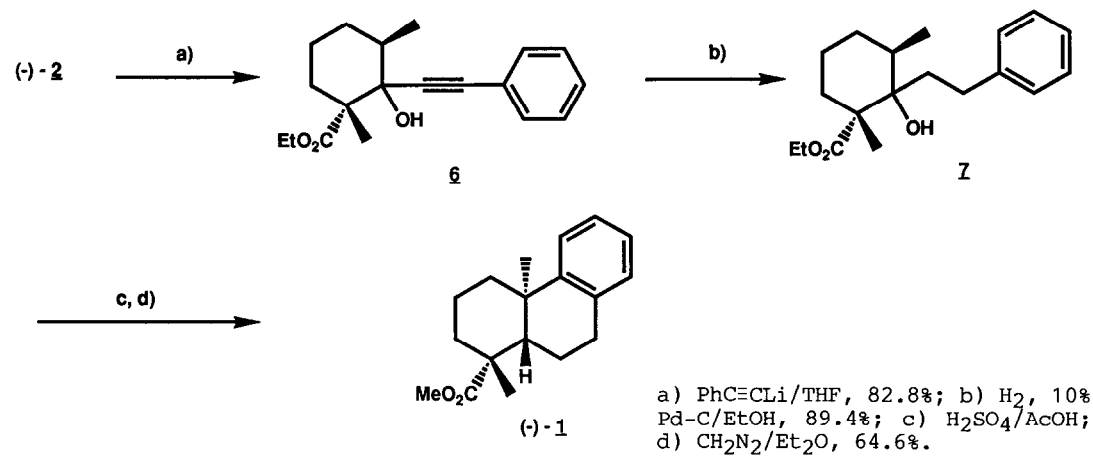
Fig. V Preparation of (-)-2

Interestingly, the major product 3 resulted from enzymatic hydrolysis had the relative configuration contrary to the major product 2 under acidic ethanolysis. This fact suggests that in the case of the enzymatic hydrolysis, the proton comes to the enol double bond in a concerted manner from the catalytic site of enzyme,

not from surrounding water.

Synthesis of (-)-deoxypodocarpic acid methyl ester from (+)-4

The chiral key intermediate (-)-2 was converted to **1** almost according to reported procedures[5,6,15] (Fig. VI). Treatment of (-)-2 with lithium phenylacetylide gave **6** in 82.8% yield, and following catalytic hydrogenation yielded **7** (89.4%). Dehydration and subsequent cyclization of **7** took place smoothly in refluxing acetic acid in the presence of sulfuric acid. The acid was methylated and purified by silica gel column chromatography and recrystallization to give pure (1R,4aR,10aS)-(-)-**1** in 64.6% as prisms; m.p. 141~142°C (lit.[1] m.p. 141~142°C); $[\alpha]_D^{26}$ -149° (c=0.20, EtOH); [lit.[1] $[\alpha]_D$ -149° (EtOH)]. Since our present synthetic sample of **1** was (1R,4aR,10aS) form, the absolute configurations of (-)-2 and (+)-**4** were unambiguously determined to be (2R,6R) and (R), respectively.



a) PhC≡CLi/THF, 82.8%; b) H_2, 10% Pd-C/EtOH, 89.4%; c) H_2SO_4/AcOH; d) CH_2N_2/Et_2O, 64.6%.

Fig. VI Synthesis of (-)-1

In conclusion, it was revealed that lipase OF from Candida cylindracea preferentially hydrolyzed the (S)-enantiomer of enol acetate **4**, leaving (R)-**4** in high enantiomeric excess (>99% e.e.) in 32.7% yield. Starting from (R)-(+)-**4**, (-)-**1** was synthesized in 5 steps and 32.2% overall yield. Present work has established a new synthetic method of optically active diterpenes, utilizing the enzymatic hydrolysis of an unusual substrate, enol ester.

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were measured as films for oils or as KBr discs for solids on a Jasco IRA-202 spectrometer. ^1H NMR spectra were recorded with TMS as an internal standard at 90 MHz on a JEOL JNM FX-90 spectrometer or at 400 MHz on a JEOL JNM GX-400 spectrometer. ^{13}C NMR spectrum was recorded with TMS as an internal standard at 22.5 MHz on a JEOL JNM FX-90 spectrometer. Optical rotations were measured on a Jasco DIP 360 polarimeter. Mass spectra were recorded on a Hitachi M-80 at 70 eV. TLC analyses were performed with Merck

Kieselgel 60 F_{254} (Art 5715). Wako Gel B-5F and silica gel 60 K070-WH (70-230 mesh) of Katayama Chemical Co. were used for preparative TLC and column chromatography, respectively. Peptone and yeast extract were purchased from Kyokuto Seiyaku Co.

(2R*,6R*)-2-Ethoxycarbonyl-2,6-dimethylcyclohexanone (±)-**2** and (2R*,6S*)-2-ethoxycarbonyl-2,6-dimethylcyclohexanone (±)-**3**. An LDA soln was prepared from (i-Pr)$_2$NH (715 mg, 7.0 mmol) and n-BuLi (1.54 M soln in hexane, 4.54 ml, 7.0 mmol) in dry THF (5 ml) at -78°C under Ar. A soln of N,N-dimethylpropyleneurea (2.08 g, 16.2 mmol) in dry THF (5 ml) was added and the mixture was stirred for further 15 min. Then, 2-ethoxycarbonyl-2-methylcyclohexanone (1 g, 5.4 mmol) was added by keeping the reaction temp at -78°C. After 30 min, MeI (0.5 ml, 8.1 mmol) was added with stirring and the reaction temp was gradually raised to room temp. After stirring overnight at room temp, the mixture was poured into sat NH_4Cl soln and extracted with Et_2O. The extract was washed with 5% $Na_2S_2O_3$ soln, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography by the elution with hexane-EtOAc (20:1).

Earlier eluted fractions gave (2R*,6R*)-**2** (241 mg, 22.6%), TLC (hexane-EtOAc 3:1) Rf 0.55; νmax 1730, 1705, 1240, 1205, 1150, 1115, 1080 cm^{-1}; δ (^{1}H, 400 MHz, $CDCl_3$) 1.05 (3H, d, J=6.8 Hz), 1.24 (3H, t, J=7.1 Hz), 1.28 (3H, s), 1.30~1.44 (2H, m), 1.70~1.80 (2H, m), 2.01~2.07 (1H, m), 2.51~2.58 (2H, m), 4.18 (2H, q, J=7.1 Hz); MS: m/z 198 (M^+, 100%); GLC (column, 15% BDS, 100°+5°/min; N_2, 1.0 kg/cm^2) rt 5.4 min.

Later eluted fractions gave (2R*,6S*)-**3** (383 mg, 35.7%), TLC (same condition) Rf 0.45; νmax 1730, 1705, 1240, 1205, 1150, 1115, 1080 cm^{-1}; δ (^{1}H, 400 MHz, $CDCl_3$) 1.04 (3H, d, J=6.4 Hz), 1.28 (3H, t, J=7.1 Hz), 1.45 (3H, s), 1.68~1.85 (3H, m), 2.00~2.08 (1H, m), 2.37~2.70 (3H, m), 4.20 (2H, q, J=7.1 Hz); MS: m/z 198 (M^+, 100%); GLC (same condition) rt 7.9 min.

Further elution gave the starting material (192 mg, 19.2%).

(2R*,6R*)-2-Ethoxycarbonyl-2,6-dimethylcyclohexanone (±)-**2**. According to the reported procedure,[6] (2R*,6R*)-(±)-**2** was prepared from 2-ethoxycarbonyl-2-methylcyclohexanone in 52.7% yield, b.p. 87~97°/2.0 Torr. This was revealed to be a single diastereomer comparing its NMR spectrum, Rf value on TLC and retention time on GLC analysis with those of an authentic sample obtained above.

(1R*,2S*,6S*)-2-hydroxymethyl-2,6-dimethylcyclohexan-1-ol (±)-**5**. In the conventional manner, (±)-**2** was reduced with $LiAlH_4$ to give (±)-**5** in 96.2% yield as prisms, m.p. 62.5~63.0°C; νmax 3345, 2945, 2860, 1460, 1275, 1160, 1040, 1030, 990, 945, 840 cm^{-1}; δ (^{1}H, 400 MHz, $CDCl_3$) 1.02 (3H, d, J=5.9 Hz), 1.19 (3H, s), 1.31~1.40 (2H, m), 1.45~1.50 (1H, m), 1.59~1.62 (2H, m), 1.71~1.79 (2H, m), 2.56~2.63 (2H, m), 3.05 (1H, dd, J=5.4, 10.2 Hz), 3.34 (1H, ddd, J=1.2, 7.3, 10.7 Hz), 4.26 (1H, dd, J=3.2, 11.0 Hz); δ (^{13}C, 22.5 MHz, $CDCl_3$) 19.1, 21.4, 24.8, 34.5, 35.2, 36.9, 38.7, 67.9, 85.0. Single-crystal X-ray diffraction measurement was performed on a Rigaku AFC-5 four-circle diffractometer with Mo Kα radiation. Crystal data are as follows: $C_9H_{18}O_2$, M_r=158.24, monoclinic, C2/c, a=21.592(4), b=6.096(1), c=15.166(3) Å, β=115.00(1)°, V=1809.1(6) Å^3, Z=8, D_m=1.15(1), D_x=1.16 Mg m^{-3}, R=0.044 for 1274 reflections.[16] The crystal structure has a center of symmetry and one independent molecule lies in a general position. The molecular structure is shown in Fig. IV (see text). The molecules are linked into infinite chains by the intermolecular hydrogen bonds between the hydroxy groups.

6-Ethoxycarbonyl-2,6-dimethyl-1-cyclohexenyl acetate (±)-**4**. To a soln of (±)-**2** (4.8 g, 24 mmol) in CCl_4 (20 ml) was added Ac_2O (9.9 g, 97 mmol) and a catalytic amount of 70% $HClO_4$ aq soln at 0°C with stirring. After stirring at room temp overnight, the mixture was poured into sat $NaHCO_3$ soln and extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography. Elution with hexane-EtOAc (20:1) gave (±)-**4** (5.3 g, 92.6%) as an oil, b.p. 118~122°/3.1 Torr (bulb-to-bulb); νmax 1750, 1730, 1700, 1210, 1195 cm^{-1}; δ (^{1}H, 400 MHz, $CDCl_3$) 1.25 (3H, t, J=7.1 Hz), 1.28 (3H, s), 1.51 (3H, s), 1.56~1.73 (4H, m), 2.12~2.20 (2H, m), 2.14 (3H, s); GLC (same condition as for **2**) rt 9.0 min. (Found: C, 64.44; H, 8.27. Calc for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39%). MS: m/z 240.1375 (M^+). Calc for $C_{13}H_{20}O_4$: 240.1361.

Cultivation of Pichia miso IAM 4682. One loop of P. miso IAM 4682 was inoculated into a 100 ml of medium (pH 7.2) containing glucose (10 g), peptone (7 g), yeast extract (5 g), K_2HPO_4 (5 g) in water (1000 ml) placed in a 500 ml-shaking flask. After cultivating at 30°C for 48 h on a reciprocatory shaker (150 cpm), the wet cells (ca. 3.5 g) of P. miso were harvested by centrifugation (2500 rpm for 15 min).

Hydrolysis of (±)-**4** with P. miso. The wet cells of P. miso from 4 flasks were combined (ca. 14 g) and washed twice with 0.2 M phosphate buffer (pH 6.5). This was suspended in 0.2 M phosphate buffer (pH 6.5, 40 ml) and (±)-**4** (80 mg) was added. The mixture was shaken at 30°C for 90 h. The reaction was monitored by GLC analysis. After 60% conversion, Radiolite (a kind of Celite for industrial use) was added to the mixture and was extracted with EtOAc. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed over silica

gel by the elution with hexane-EtOAc (20:1).

Firstly eluted fractions gave (+)-**2** (7.3 mg, 11.0%), $[\alpha]_D^{26}$ +64° (c=0.73, $CHCl_3$). Its IR and NMR spectra were identical with those of racemic **2**. NMR [^{1}H, 400 MHz, C_6D_6, Eu(tfc)$_3$ (1.0 eq)] δ (C-2 Me): 1.76 (0.79H), 1.81 (2.21H). Therefore, the enantiomeric excess was determined to be 47%.

Secondly eluted fractions gave (−)-**3** (20.5 mg, 31.0%), $[\alpha]_D^{27}$ −30° (c=1.1, $CHCl_3$). Its IR and NMR spectra were identical with those of racemic **3**. NMR [^{1}H, 400 MHz, C_6D_6, Eu(tfc)$_3$ (1.0 eq)] δ (C-2 Me): 1.83 (2.13H), 1.85 (0.87H). Therefore, the enantiomeric excess was determined to be 42%.

Thirdly eluted fractions gave (+)-**4** (12.8 mg, 16.0%), $[\alpha]_D^{27}$ +45° (c=1.3, $CHCl_3$). Its IR and NMR spectra were identical with those of racemic **4**. NMR [^{1}H, 400 MHz, C_6D_6, Eu(hfc)$_3$ (1.0 eq)] δ (C-2 Me): 3.23 (0.25H), 3.27 (2.75H). Therefore, the enantiomeric excess was determined to be 83%.

Results obtained under other conditions were listed in Table 1.

Hydrolysis of (±)-**4** with lipase OF at 30°C. A mixture of (±)-**4** (104 mg, 0.434 mmol) and lipase OF (100 mg) in 0.1M phosphate buffer (pH 7.0, 50 ml) was stirred on reciprocatory shaker (150 cpm) at 30°C for 90 h. After the addition of EtOAc (100 ml), the organic layer was separated by the addition of Radiolite. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography.

(+)-**2**: (4.3 mg, 5.0%), $[\alpha]_D^{24}$ +66° (c=0.41, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with authentic spectra. Its enantiomeric excess was determined to be 49% from its ^{1}H NMR spectrum in the presence of Eu(tfc)$_3$ (1.0 eq).

(−)-**3**: (46.5 mg, 54.0%), $[\alpha]_D^{24}$ −33° (c=2.3, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with authentic spectra. Its enantiomeric excess was determined to be 47% from its ^{1}H NMR spectrum in the presence of Eu(tfc)$_3$ (1.0 eq).

(+)-**4**: (33.4 mg, 32.0%), $[\alpha]_D^{24}$ +50° (c=1.7, $CHCl_3$); Its IR and ^{1}H NMR spectra were identical with authentic spectra. Its enantiomeric excess was determined to be over 99% from its ^{1}H NMR spectrum in the presence of Eu(hfc)$_3$ (1.0 eq).

Results obtained under other conditions were listed in Table 2.

Preparative scale hydrolysis of (±)-**4** with lipase OF. A mixture of (±)-**4** (2.2 g, 9.2 mmol) and lipase OF (2.2 g) in 0.1M phosphate buffer (pH 7.0, 1100 ml) was stirred on gyrorotary shaker (150 rpm) at 30°C for 90 h. After the addition of EtOAc (1000 ml) and NaCl (300 g), the mixture was stirred and filtered through a pad of Radiolite (see above). The filtrate was extracted with EtOAc (800 ml x 3). The extract was dried with Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography.

(+)-**2**: (133 mg, 7.4%), $[\alpha]_D^{26}$ +84.7° (c=1.2, $CHCl_3$); 63% e.e. [NMR, Eu(tfc)$_3$ (1.0 eq)].

(−)-**3**: (906 mg, 49.9%), $[\alpha]_D^{27}$ −36.7° (c=1.5, $CHCl_3$); 52% e.e. [NMR, Eu(tfc)$_3$ (1.0 eq)].

(+)-**4**: (720 mg, 32.7%), b.p. 110~113°C/3.0 Torr (bulb-to-bulb); $[\alpha]_D^{27}$ +54.2° (c=3.38, $CHCl_3$); >99% e.e. [NMR, Eu(hfc)$_3$ (1.0 eq)]; MS: m/z 240 (M^+, 40%).

Hydrolysis of (±)-**4** with lipase OF at −3°C. A mixture of (+)-**4** (104.4 mg, 0.434 mmol) and lipase OF (200 mg) in 0.1M phosphate buffer (pH 7.0, 50 ml) was stirred at −3°C (bath temp) for 100 h. After 36% conversion, the reaction was worked-up in the same manner as before, and the crude extract was purified by silica gel preparative TLC (hexane-EtOAc, 30:1)

(−)-**3**: (30.1 mg, 35.0%), $[\alpha]_D^{25}$ −53.6° (c=1.2, $CHCl_3$); 77% e.e. [NMR, Eu(tfc)$_3$ (1.0 eq)].

(+)-**4**: (55.2 mg, 52.9%), $[\alpha]_D^{27}$ +36.4° (c=1.2, $CHCl_3$). Comparing its optical rotation with an authentic one, its e.e. was determined to be 67%.

(2R,6R)-2-Ethoxycarbonyl-2,6-dimethylcyclohexanone (−)-**2** and (2R,6S)-2-ethoxycarbonyl-2,6-dimethylcyclohexanone (+)-**3**. To a soln of (+)-**4** (688 mg, 2.86 mmol) in dry EtOH (7 ml) was added a catalytic amount of $HClO_4$ (70% aq soln), and the mixture was stirred and heated under reflux for 13 h. After cooling to 0°C, the mixture was neutralized by the addition of powdered $NaHCO_3$ and filtered. The filtrate was concentrated in vacuo and extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography.

(−)-**2**: (381.5 mg, 67.3%), $[\alpha]_D^{24}$ −135.2° (c=0.91, $CHCl_3$); >99% e.e. [NMR, Eu(tfc)$_3$ (1.0 eq)]. Its IR and ^{1}H NMR spectra were identical with authentic spectra.

(+)-**3**: (65.0 mg, 11.5%), $[\alpha]_D^{24}$ +54.0° (c=1.2, $CHCl_3$); 77% e.e. [NMR, Eu(tfc)$_3$ (1.0 eq)]. Its IR and ^{1}H NMR spectra were identical with authentic spectra.

(2R,6R)-2-Ethoxycarbonyl-2,6-dimethyl-1-phenylethynylcyclohexan-1-ol (−)-**6**. To a soln of phenylacetylene (266 mg, 2.61 mmol) in dry THF (1 ml) was added n-BuLi (1.57 M soln in hexane, 1.54 ml, 2.42 mmol) at −20°C under Ar. After stirring for 2 h, the mixture was cooled to −78°C and then a soln of (−)-**2** (369 mg, 1.86 mmol) in dry THF (2 ml) was slowly added. The reaction temp was gradually raised to room temp and the mixture was stirred overnight

at room temp. Sat NH_4Cl soln was added at 0°C and the mixture was extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel preparative TLC (hexane-EtOAc, 30:1) to give **5** (462 mg, 82.8%) as an oil, $[\alpha]_D^{24}$ -29.8° (c=1.9, $CHCl_3$); νmax 3470, 2240, 1695, 1600, 1240, 1180, 1150, 1070, 755, 690 cm^{-1}; δ (^1H, 400 MHz, $CDCl_3$) 1.18 (3H, d, J=6.2 Hz), 1.30 (3H, t, J=7.0 Hz), 1.53 (3H, s), 1.44~2.22 (7H, m), 4.14 (2H, q, J=7.0 Hz), 4.60 (1H, s), 7.26~7.60 (5H, m); MS: m/z 300 (M^+, 43%).

(2R,6R)-2-Ethoxycarbonyl-2,6-dimethyl-1-(2-phenyl)ethylcyclohexan-1-ol (-)-**7**. A mixture of **6** (459 mg, 1.53 mmol), and Pd-C (10%, 135 mg) in EtOH (25 ml) was vigorously stirred overnight at room temp under H_2. The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified by silica gel preparative TLC (hexane-EtOAc, 20:1) to give **7** (416 mg, 89.4%) as an oil, $[\alpha]_D^{24}$ -32.5° (c=1.1, $CHCl_3$); νmax 3500, 1695, 1600, 1240, 1170, 1150, 1080, 1020, 755, 695 cm^{-1}; δ (^1H, 400 MHz, $CDCl_3$) 1.05 (3H, d, J=6.2 Hz), 1.28 (3H, t, J=7.0 Hz), 1.35 (3H, s), 1.42~2.25 (8H, m), 2.78 (2H, t, J=8.2 Hz), 4.18 (2H, q, J=7.0 Hz), 4.66 (1H, s), 7.15~7.40 (5H, m); MS: m/z 304 (M^+, 77%).

(1R,4aR,10aS)-(-)-1-Methoxycarbonyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene [(-)-deoxypodocarpic acid methyl ester] **1**. According to a reported procedure, a mixture of **7** (100 mg, 0.33 mmol) and conc H_2SO_4 (0.2 ml) in AcOH (2 ml) was stirred and heated under reflux for 2 h. Then the mixture was poured into ice-water and extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was diluted with Et_2O (2 ml) and to it was added a ethereal soln of CH_2N_2. The mixture was concentrated in vacuo and the residue was purified by silica gel preparative TLC (hexane-EtOAc, 9:1) followed by recrystallization from hexane to give **1** (58.6 mg, 64.6%) as prisms, m.p. 141~142°C (lit.[1] m.p. 141~142°C); $[\alpha]_D^{26}$ -149° (c=0.20, EtOH); [lit.[1] $[\alpha]_D$ -149° (EtOH)]; νmax 3450, 3075, 2960, 3860, 1725, 1460, 1445, 1435, 1380, 1330, 1240, 1230, 1190, 1145, 1030, 760, 730 cm^{-1}; δ (^1H, 400 MHz, $CDCl_3$) 1.04 (3H, s), 1.09 (1H, dt, J=3.9, 13.7 Hz), 1.28 (3H, s), 1.39 (1H, dt, J=3.9, 13.7 Hz), 1.54 (1H, br.s), 1.63 (1H, d, J=14.2 Hz), 1.94~2.03 (2H, m), 2.19 (1H, dd, J=5.9, 13.9 Hz), 2.28 (2H, d, J=13.2 Hz), 2.80 (1H, ddd, J=5.9, 12.7, 16.8 Hz), 2.92 (1H, dd, J=5.9, 16.8 Hz), 7.04 (1H, d, J=7.3 Hz), 7.08 (1H, t, J=7.3 Hz), 7.13 (1H, t, J=7.3 Hz), 7.27 (1H, d, J=7.3 Hz). (Found: C, 79.54; H, 8.92. Calc for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88%).

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