A Chemoenzymatic Route to (-)-Pyrenophorin

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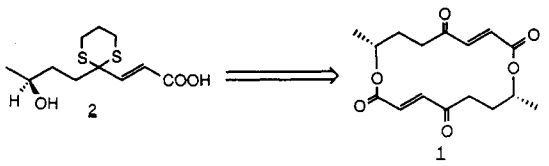
The key penultimate intermediate, (+)-7(*S*)-hydroxy-4,4-(ethylenedioxy)oct-2-enoic acid (**6a**), for the synthesis of (-)-pyrenophorin has been prepared by using a chemoenzymatic approach.

Introduction

(-)-Pyrenophorin (**1**)[1] is a naturally occurring antifungal macrodiolide that possesses a 16-membered macrocyclic ring with C_2 symmetry. Although a number of total syntheses[2] of (±)-pyrenophorin have been reported, to our knowledge only two syntheses[3] of (-)-pyrenophorin (**1**) have been achieved to date. In one of the syntheses, the macrocyclic ring was constructed by the dimerization of the penultimate chiral intermediate, 7(*S*)-hydroxy-4,4-(propylenedithio)oct-2-enoic acid (**2**), by using the Mitsunobu reaction.[4]



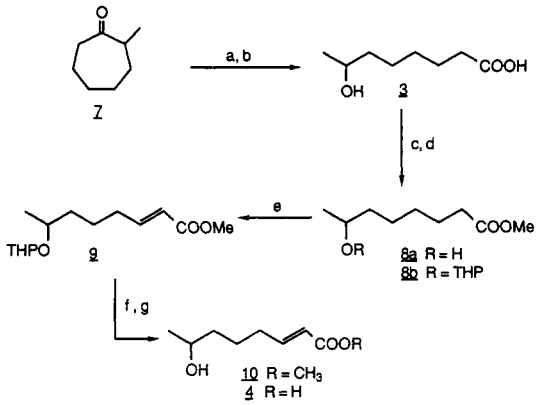
Our interest in (-)-pyrenophorin (**1**) as a synthetic target molecule stemmed from the observation that several lipases are capable of catalyzing the dimerization of (ω - 1)-hydroxy acids to form macrodiolides in anhydrous organic solvents.[5] We envisaged that this biocatalytic method could be an attractive method for the synthesis of diolides with C_2 symmetry if the enzyme possesses the desired stereochemical preference and high degree of enantioselectivity and gives high chemical yield of the product.

Chemical Preparation of Substrates for Enzymatic Studies

To examine the feasibility of this biocatalytic procedure, we synthesized compounds (**3**, **4**, **5**, and **6a**) with varying degrees of functionality as potential substrates for enzymatic macrolactonization (Schemes I and II). As shown in Scheme I, (±)-7-hydroxyoctanoic acid (**3**) was prepared from (±)-2-methylcycloheptanone (**7**) via Baeyer-Villiger oxidation. The resulting lactone was hydrolyzed with aqueous 2 N NaOH in methanol. After column chromatography, (±)-**3** was isolated in 47% overall yield. Further transformation of (±)-**3** to (±)-7-hydroxyoct-2-enoic acid (**4**) was accomplished by using a five-step reaction sequence. Treatment of (±)-**3** with ethereal diazomethane afforded the methyl ester, (±)-**8a** (92%), which in turn was reacted with dihydropyran in the presence of a catalytic amount of *p*-toluenesolfonic acid to yield the THP derivative, (±)-**8b** (78%). Introduction of the Δ^2 double bond was achieved by first reacting (±)-**8b** with lithium diisopropylamide in tetrahydrofuran at -78 °C, and then quenching of the resulting enolate with a solution of phenylselenenyl bromide at -78 °C gave a mixture of diastereomeric selenides, which was immediately oxidized with aqueous 30% H_2O_2 to yield (±)-**9** in 62% yield. The THP-protecting group was cleaved by using acetic acid in aqueous tetrahydrofuran, furnishing (±)-**10** (97%), which upon alkaline hydrolysis yielded (±)-**4** (95%).

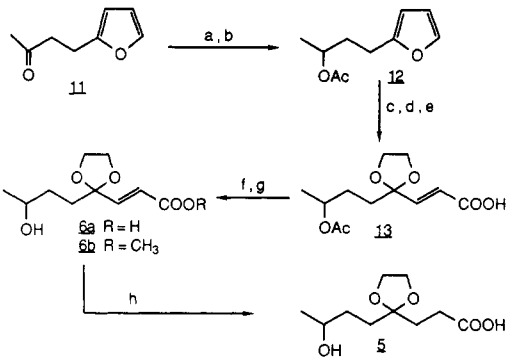
Furfuralacetone (**11**)[6] was used as the starting material for the synthesis of (±)-**5** and (±)-**6a** (Scheme II). The

Scheme I[a]



[a] (a) CH_3CO_3H, $CHCl_3$, 50 °C; (b) NaOH, MeOH, H_2O; (c) CH_2N_2, mEOH; (d) DHP, *p*-TsOH, CH_2Cl_2; (e) i, LDA, PhSeBr, -78 °C; ii, H_2O_2; (f) AcOH, THF, H_2O; (g) LiOH, THF, H_2O.

Scheme II[a]



[a] (a) $NaBH_4$, MeOH; (b) Ac_2O, Py.; (c) Jones reagent; (d) 2-mercaptobenziimidazole; (e) $HOCH_2CH_2OH$, *p*-TsOH; (f) LiOH, THF, H_2O; (g) CH_2N_2; (h) H_2, Pd-C.

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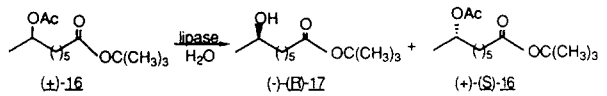
acetate, (±)-**12**, was obtained from **11** in 80% yield by reduction followed by acetylation. The 1,4-dicarbonyl functionality was generated by Jones oxidation of the furan ring of (±)-**12**. The resulting keto acids were found to be unstable and column chromatographic separation of the crude products afforded rather poor yields of a mixture of cis/trans products. However, the crude mixture could be transformed to the ketal acid, (±)-**13**, by first treating the mixture with 2-mercaptobenzimidazole to catalyze the isomerization of the cis to the trans isomer, followed by ketalization. Hydrolysis of (±)-**13** gave the required hydroxy acid, (±)-**6a** (36% overall from (±)-**12**). Conversion of (±)-**6a** to (±)-**5** was achieved by hydrogenation (Pd/C).

Enzymatic Macrolactonization Studies

Having completed the chemical preparation of the requisite substrates, we then turned our attention to enzymatic lactonization. For these studies, we selected the lipase of *Pseudomonas* sp. (AK, Amano) as the source of the biocatalyst, for this enzyme afforded the best yields of macrocyclic lactones among all the commercial lipases examined. While no lactonic products were detected after exposure of (±)-**4**, (±)-**5** and (±)-**6a** to this lipase in anhydrous isooctane even at 65 °C, a mixture of two dilactones was formed from substrate (±)-**3**. These dilactones were separated by column chromatography to yield 5% of the *R,S* dilactone **14** (R_f = 0.53, hexane/ethyl acetate = 5:1) and 12% of *R,R/S,S* dilactone **15** (R_f = 0.50, hexane/ethyl acetate = 5:1). Both dilactones exhibited

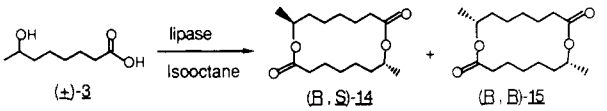


(±)-16 (-)-(R)-17 (+)-(S)-16

(M + 1) peaks of 285 in the mass spectrum and a peak at δ 4.90 (–COOC*H*–) in the ^{1}H NMR spectrum. The stereochemical assignment of the diolide was made by hydrolysis with aqueous 2 N KOH/MeOH (1:1) and conversion into methyl 7-hydroxyoctanoate (**8a**) (CH_2N_2/ether) whose enantiomeric excess (ee) was determined by ^{1}H NMR spectroscopy in the presence of Eu(hfc)$_3$. This analysis[7] revealed that the resulting 7–hydroxyoctanoate (**8a**) derived from the *R,S* dilactone **14** gave a 1:1 mixture of *R* and *S* enantiomers whereas the *R,R/S,S* lactone **15** afforded (–)-methyl 7-hydroxyoctanoate (**8a**) with an *R* to *S* ratio of 96:4. These results demonstrate that the enzymatic macrolactonization reaction proceeded with a high degree of enantioselectivity and possessed the desired stereochemical preference. Unfortunately, the chemical yield of the desired *R,R* diolide was disappointingly low for use as a viable intermediate for transformation into (–)-pyrenophorin.

Biocatalytic Kinetic Resolution of Functionalized Hydroxyalkanoates

Recently, we disclosed a biocatalytic resolution procedure[8] for the preparation of a variety of *R* and *S* hydroxyalkanoic esters of high optical purity. The salient feature of this methodology resides in the introduction of a nonhydrolyzable carboxylic ester such as the *tert*-butyl ester group to enhance the enantioselectivity of the enzymatic hydrolysis of the acyloxy ester. For example, the *Pseudomonas* sp. (K-10) lipase catalyzed the cleavage of the *R* acetoxy ester of (±)-*tert*-butyl 7-acetoxyoctanoate (**16**) with a high degree of enantioselectivity (*E* = >100).[9]



(±)-3 (R,S)-14 (R,R)-15

Thus, it was of interest to further investigate the enantioselectivity of this enzyme on the more advanced intermediates for the synthesis of (–)-**1**. Since the penultimate step in the total synthesis[2] of this diolide entails the chemical dimerization of (+)-7(*S*)-hydroxy-4,4-(ethylenedioxy)oct-2-enoic acid (**6a**), we prepared (±)-**18** from (±)-**6b** as the substrate for our biocatalytic resolution studies. Careful consideration was given to the design of the highly functionalized substrate, (±)-**18**. The introduction of a *tert*-butyl ester grouping at the carboxyl terminus allows the K-10 lipase to achieve highly enantioselective hydrolysis of the acyloxy ester, which suggests that this enzyme possesses a nonpolar active-site, which is inducive to stabilizing a neutral transition state but destabilizing toward a charged carboxylic acid substrate. Because the chemoselective formation of the *tert*-butyl ester in the presence of the ethylenedioxy group would be difficult to achieve, we surmised that the *tert*-butyl ester could perhaps be replaced by a suitable nonhydrolyzable ester at the carboxyl end. Based on the generalized notion that α,β-unsaturated esters are cleaved more slowly by microbial lipases, we selected the simple methyl ester to mask the charged carboxylic function. Finally, the introduction of the activated chloroacetyl ester was to facilitate its enzymatic cleavage, so that the resolution may be completed before competitive cleavage of the methyl ester occurs. It is gratifying to note that the *Pseudomonas* sp. (K-10) lipase was highly enantioselective for the *R* enantiomer of **18** (*E* = >100) and good yield of product was obtained (Scheme III). By terminating the conversion at 42%, methyl (–)-(*R*)-7-hydroxy-4,4-(ethylenedioxy)oct-2-enoate (**19**) was obtained with an enantiomeric purity of >99% ee.[9] By extension of the conversion to 51%, the residual substrate, (–)-(*S*)-**18**, methyl (–)-(*S*)-7-(chloroacetoxy)-4,4-(ethylenedioxy)oct-2-enoate was also obtained with an ee of >99%. Completion of a formal synthesis of (–)-**1** was accomplished by cleavage of the esters in (–)-(*S*)-**18** (2 M LiOH/THF; 25 °C) to afford (+)-(*S*)-**6a** (95% yield), which has been chemically transformed into (–)-**1**.[2]

Experimental Section

^{1}H NMR spectra were recorded at 90 MHz in $CDCl_3$. All compounds that were submitted to mass spectrometric molecular weight determination were of high purity as determined by NMR analysis and TLC. IR spectra were taken in CCl_4.

All solvents were redistilled before use. Thin-layer chromatography (TLC) was performed on plates coated with 0.25-mm thickness on silica gel 60F-254 (E. Merck). Flash chromatography was performed by using Baker silica gel (40 μm). Solvent extracts of aqueous solution were dried over anhydrous $MgSO_4$. Solutions were concentrated under reduced pressure on a rotary evaporator.

(±)-7-Hydroxyoctanoic Acid (3). Peracetic acid (3.9 g of a 35% solution, 17.8 mmol) and boron trifluoride etherate (1.9 g,

(7) From the hydrolysis of *R,S*, $[\alpha]^{22}_D = 0°$ was observed, as expected. In the case of hydrolysis of *R,R/S,S* dilactones, the $[\alpha]^{22}_D = -8.3°$ (*c* 1.8, $CHCl_3$) and ee = 0.92. For absolute configuration assignment, see: Kinoshita, M.; Ishii, K.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3395.

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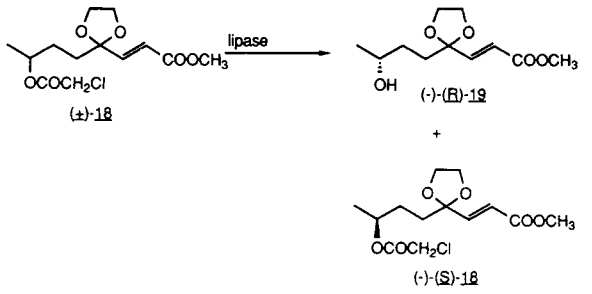
(9) Enantiomeric excess (ee) was determined by ^{1}H NMR experiments in the presence of Eu(hfc)$_3$. Addition of the shift reagent resulted in the resolution and shifting of the methyl doublet at δ 1.10 to δ 5.90 and 6.10 (doublet) for racemic **18**. The enantiomeric ratio (*E* value) is calculated from:

$$E = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]}$$

where $c = ee_s/(ee_s + ee_p)$. See: Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

Scheme III. Lipase-Catalyzed Enantioselective Hydrolysis of (±)-18



13.0 mmol) were sequentially added to a solution of (±)-2-methylcycloheptanone (**7**) (1.5 g, 11.9 mmol) in chloroform (20 mL).

The solution was heated in an oil bath maintained at 50 °C. After 6 h, the reaction mixture was cooled to room temperature and diluted with chloroform. The resulting solution was washed with aqueous ferric sulfate. The organic layer was dried, filtered, and concentrated to give a yellow oil.

The crude oil was dissolved in methanol (20 mL) and aqueous 2 M sodium hydroxide (10 mL) was added. The resulting mixture was stirred at room temperature for 16 h and diluted with water. After being washed with ether, the aqueous layer was acidified with 10% aqueous hydrochloric acid and extracted with ethyl acetate. Drying over $MgSO_4$ and concentration of the organic extracts gave a light yellow oil. Purification by flash chromatography, eluting the column with 70% ethyl acetate in hexane, gave 900 mg (47%) of pure (±)-**3** as a colorless oil: IR 3580, 3450–3300, 3000, 2920, 1760, 1370, 1200, 1105, 1090, 1075, 730 cm^{-1}; ^1H NMR δ 1.17 (d, J = 6.0 Hz, 3 H), 1.50 (m, 8 H), 2.35 (t, J = 7.0 Hz, 2 H), 3.70 (m, 1 H), 6.55 (br s, 2 H, exchangeable with D_2O); MS m/e 161 ($M^{+\cdot}$ + 1), 143, 127, 125, 116, 101, 87, 85, 81, 73, 60, 55, 45 (base); MS $M^{+\cdot}$ − 1 159.1016 (calcd for $C_8H_{15}O_3$ 159.1021).

(±)-Methyl 7-Hydroxyoctanoate (8a). Ethereal diazomethane was added dropwise to a solution of (±)-7-hydroxyoctanoic acid (**3**) (3.4 g, 21 mmol) in methanol (20 mL) until the solution turned slightly yellow. Concentration in vacuo gave an oil, which was purified by flash chromatography. Elution of the column with 30% ethyl acetate in hexane gave 3.4 g (92%) of pure methyl ester (±)-**8a** as a colorless oil: IR 3580, 2995, 2910, 2850, 1720, 1450, 1430, 1370, 1215, 1200, 1165, 1085, 1005, 935 cm^{-1}; ^1H NMR δ 1.13 (d, J = 6.0 Hz, 3 H), 1.37 (m, 8 H), 2.29 (t, J = 7.0 Hz, 2 H), 3.65 (s, 3 H), 3.72 (m, 1 H); MS $M^{+\cdot}$ + 1 175.1338 (calcd for $C_9H_{19}O_3$ 175.1334).

(±)-Methyl 7-(Tetrahydropyranyloxy)octanoate (8b). To a solution of alcohol (±)-**8a** (2.6 g, 14.9 mmol) and p-toluenesulfonic acid (30 mg, 0.16 mmol) in dichloromethane (25 mL) at 0 °C and under an atmosphere of argon was added dihydropyran (1.3 g, 14.9 mmol) via a syringe. After stirring for 1 h saturated aqueous sodium bicarbonate was added. Extraction with dichloromethane, followed by drying over $MgSO_4$ and concentration in vacuo gave an oil. Flash chromatography of the oil and elution of the column with 10% ethyl acetate in hexane gave 3.0 g (78%) of pure THP derivative **8b** as a colorless oil. The diastereomeric mixture of products has the following spectral data: IR 2930, 2860, 1730, 1460, 1450, 1435, 1350, 1190, 1120, 1070, 1030, 1015, 1010 cm^{-1}; ^1H NMR δ 1.09, 1.20 (both d, J = 6.0 Hz each, total 3 H), 1.40–2.00 (m, 14 H), 2.30 (t, J = 7.0 Hz, 2 H), 3.65 (s, 3 H), 3.30–4.20 (m, 3 H), 4.67, 4.96 (both m, total 1 H); MS m/e 259 ($M^{+\cdot}$ + 1), 243, 213, 187, 159, 99, 87 (base), 59, 43; MS $M^{+\cdot}$ 258.1827 (calcd for $C_{14}H_{26}O_4$ 258.1831).

(±)-Methyl 7-(Tetrahydropyranyloxy)oct-2-enoate (9). n-Butyllithium (2.3 mL of 2.5 M, 5.7 mmol) was added via a syringe to a solution of diisopropylamine (581 mg, 5.7 mmol) in tetrahydrofuran (20 mL) at −78 °C and under an atmosphere of argon. After being stirred for 10 min, a solution of (±)-**8** (1.14 g, 4.4 mmol) in tetrahydrofuran (10 mL) was added. After another 20 min, phenylselenenyl bromide (1.4 g, 5.7 mmol) in tetrahydrofuran (10 mL) was added. Stirring was continued for another 1 h and saturated aqueous ammonium chloride solution was added. The resulting mixture was extracted with ether and the extracts were dried, filtered, and concentrated to dryness.

The crude oil (2.1 g) was dissolved in dichloromethane (40 mL) and cooled to 0 °C. A solution of 30% aqueous hydrogen peroxide (20 mL) was added slowly over 40 min. After being stirred for another 1 h at room temperature, saturated aqueous sodium bicarbonate was added. The mixture was extracted with dichloromethane and the organic extracts were washed with brine solution, dried, filtered, and concentrated to give a yellow oil. Flash chromatography of the crude oil and elution of the column with 10% ethyl acetate in hexane gave 701 mg (62%) of pure (±)-**9** as an oil: IR 2930, 2860, 1718, 1650, 1450, 1435, 1260, 1195, 1120, 1110, 1030, 1020, 960 cm^{-1}; ^1H NMR δ 1.10, 1.22 (both d, J = 6.0 Hz each, total 3 H), 1.40–2.00 (m, 10 H), 2.22 (m, 2 H), 3.74 (s, 3 H), 3.40–4.10 (m, 3 H), 4.70 (m, 1 H), 5.87 (d, J = 15 Hz, 1 H), 7.07 (dt, J = 15 Hz, J' = 8.0 Hz, 1 H); MS m/e 256, 225, 199, 171, 143, 98, 87, 74, 69, 55 (base); MS $M^{+\cdot}$ − 1 255.1594 (calcd for $C_{14}H_{23}O_4$ 255.1596).

(±)-Methyl 7-Hydroxyoct-2-enoate (10). A solution of **9** (559 mg, 2.2 mmol) in a solution of acetic acid/tetrahydrofuran/water (4:2:1, 5 mL) was heated in an oil bath maintained at 45 °C. After 4 h, reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by flash chromatography. Elution of the column with 30% ethyl acetate in hexane gave 365 mg (97%) of pure (±)-**10** as an oil: IR 3400, 2930, 1720, 1650, 1430, 1260, 1190, 1030, 1015 cm^{-1}; ^1H NMR δ 1.20 (d, J = 6.0 Hz, 3 H), 1.55 (m, 4 H), 2.28 (m, 2 H), 3.75 (s, 3 H), 4.85 (m, 1 H), 5.87 (d, J = 17 Hz, 1 H), 7.01 (dt, J = 17 Hz, J' = 7.0 Hz, 1 H); MS m/e 172, 159, 131, 122, 107, 101, 87, 81, 50; MS $M^{+\cdot}$ 172.1097 (calcd for $C_9H_{16}O_3$ 172.1099).

(±)-7-Hydroxyoct-2-enoic Acid (4). To a solution of (±)-**10** (410 mg, 2.4 mmol) in 50% aqueous tetrahydrofuran (5 mL) was added anhydrous lithium hydroxide (186 mg, 7.8 mmol). After being stirred for 2 h, the reaction mixture was diluted with water and washed with ether. The aqueous layer was acidified with aqueous 10% hydrochloric acid and extracted with ethyl acetate. The extracts were combined, dried, filtered, and concentrated to give an oil. Flash chromatography of the crude oil and elution of the column with a mixture of ethyl acetate/hexane/acetic acid (50:50:1) gave 359 mg (95%) of pure hydroxy acid (±)-**4**: IR 3450, 3300–3000 (br), 2960, 1700, 1660, 1410, 1375, 1300, 1280, 1200, 1060, 1030, 980, 940 cm^{-1}; ^1H NMR δ 1.17 (d, J = 6.0 Hz, 3 H), 1.50 (m, 4 H), 2.29 (m, 2 H), 3.80 (m, 1 H), 5.82 (d, J = 16 Hz, 1 H), 6.60 (br s, 2 H, exchangeable with D_2O), 7.08 (dt, J = 16 Hz, J' = 6.0 Hz, 1 H); MS m/e 140 ($M^{+\cdot}$ − 18), 123, 95, 81 (base), 55, 53, 44; MS $M^{+\cdot}$ − 18 140.0833 (calcd for $C_8H_{12}O_2$ 140.0837).

(±)-1-(2′-Furyl)-3-acetoxybutane (12). To a solution of furfuralacetone **11** (2.17 g, 15.7 mmol) in methanol (20 mL) at 0 °C and under an atmosphere of argon was added sodium borohydride (600 mg, 15.7 mmol). After being stirred for 30 min, some ice water was added, followed by saturated aqueous ammonium chloride solution. The resulting mixture was extracted with ethyl acetate. Drying over anhydrous $MgSO_4$ and concentration in vacuo gave an oil (2.1 g). The crude oil was placed in pyridine (5 mL) and acetic anhydride (3 g, 29 mmol) was added. The resulting solution was stirred at room temperature for 17 h and concentrated under vacuo. The crude oil was then purified by flash chromatography. Elution of the column with 15% ethyl acetate in hexane gave 2.3 g (80%) of pure acetate (±)-**12** as a colorless oil: IR 3020, 2970, 1730, 1365, 1260, 1235, 1040, 890 cm^{-1}; ^1H NMR δ 1.20 (d, J = 6.0 Hz, 3 H), 1.88 (m, 2 H), 1.98 (s, 3 H), 2.63 (t, J = 8.0 Hz, 2 H), 4.96 (m, 1 H), 6.01 (d, J = 2.0 Hz, 1 H), 6.29 (dd, J = J' = 2 Hz, 1 H), 7.31 (d, J = 2 Hz, 1 H); MS m/e 182, 165, 161, 149, 139, 123, 108, 81 (base), 68, 53; MS $M^{+\cdot}$ 182.0945 (calcd for $C_{10}H_{14}O_3$ 182.0943).

(±)-7-Hydroxy-4,4-(ethylenedioxy)oct-2-enoic Acid (6a). To a solution of acetate (±)-**12** (8.1 g, 44.5 mmol) in acetone (160 mL) at 0 °C was added Jones reagent (40 mL of 1.6 M, 64 mmol) portionwise over a period of 1 h. After being stirred for another 1.5 h, 2-propanol was added to destroy excess oxidant. The resulting greenish solution was diluted with ethyl acetate and decanted. The greenish residue was thoroughly washed with ethyl acetate. The combined organic solution was then successively washed with aqueous 10% hydrochloric acid and water until the organic layer was slightly yellow. Drying and concentration in vacuo gave an oil (8.5 g). The crude oil, which was a mixture of cis/trans isomers, was placed in benzene (85 mL) and 2-

mercaptobenziimidazole (20 mg) was added. The resulting solution was stirred at room temperature for 6 h. TLC indicated that isomerization to the trans isomer was complete.

Ethylene glycol (3.6 g, 58 mmol) and *p*-toluenesulfonic acid (95 mg, 0.5 mmol) were added. The resulting solution was refluxed with an overhead Dean–Stark condensor for 62 h and cooled to room temperature. Water was added and the resulting mixture extracted with ethyl acetate. Drying and concentration gave a yellow oil (±)-**13** (7.6 g).

The crude (±)-**13** was placed in 50% aqueous tetrahydrofuran (70 mL). Lithium hydroxide (2.6 g, 0.1 mol) was added. After being stirred for 19 h, reaction mixture was acidified and extracted with ethyl acetate. Drying and concentration gave a yellow oil, which was purified by flash chromatography. Elution of the column with 70% ethyl acetate in hexane gave 3.5 g (36%, based on acetate **12**) of pure hydroxy acid (±)-**6a** as a light yellow oil: IR 3220, 3000 (br), 2960, 2880, 1690, 1650, 1410, 1320, 1295, 1260, 1050, 1030, 980, 940 cm^{-1}; ^1H NMR δ 1.20 (d, J = 6.0 Hz, 3 H), 1.50 to 2.00 (m, 4 H), 3.90 (m, 4 H), 4.15 (m, 1 H), 6.02 (d, J = 16 Hz, 1 H), 6.88 (d, J = 16 Hz, 1 H), 7.77 (br s, 2 H, exchangeable with D_2O); MS m/e 217, 199, 171, 143 (base), 127, 99, 83, 81, 45; MS $M^{+\cdot}$ 216.0998 (calcd for $C_{10}H_{16}O_5$ 216.0998).

(±)-7-Hydroxy-4,4-(ethylenedioxy)octanoic Acid (5). A solution of unsaturated acid (±)-**6a** (349 mg, 1.6 mmol) in ethyl acetate (5 mL) was hydrogenated over 10% Pd–C (40 mg) at 1 atm of hydrogen pressure. After 2 h, reaction mixture was filtered and concentrated under vacuo. The crude oil was purified by flash chromatography. Elution with 70% ethyl acetate in hexane gave 289 mg (82%) of pure acid (±)-**5** as a colorless oil: IR 3540–3100, 2960, 2910, 1770, 1450, 1418, 1375, 1335, 1115, 1080, 1000, 905, 895 cm^{-1}; ^1H NMR δ 1.18 (d, J = 6.0 Hz, 3 H), 1.50 to 2.60 (several m, 8 H), 3.90 (m, 1 H), 3.99 (s, 4 H), 7.40 (br s, 2 H, exchangeable with D_2O); MS $M^{+\cdot}$ −18 200.1051 (calcd for $C_{10}H_{16}O_4$ 200.1049).

Enzymatic Conversion of (±)-3 into Diolides 14 and 15. To a suspension of 1 g of (±)-**3** in 400 mL of anhydrous isooctane was added 4 g of crude powder (*Pseudomonas* sp. AK lipase). The reaction mixture was incubated on an incubator rotary shaker (200 rpm, 2 in. stroke) at 25 °C for 6 days. After filtration, the residual powder was washed with ethyl acetate, and the combined organic phase was dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude residue was chromatographed over a silica gel (40 μm, J. T. Baker) column (2 × 40 cm). The column was eluted with a solvent mixture consisting of hexane–ethyl acetate (40:1 to 10:1) and 20-mL fractions were collected. Fractions 78–106 were pooled and evaporated to dryness to give 48 mg (5%) of **14**; fractions 114–156 contained 106 mg (12%) of **15**. Both diolides **14** and **15** exhibited identical IR, ^1H NMR, and MS spectra: IR ($CHCl_3$) 2920, 2850, 1710, 1450, 1370, 1200; ^1H NMR δ 1.1 to 1.6 (overlapping d and m, total 22 H), 2.3 (t, J = 7 Hz, 4 H), 4.9 (m, 2 H); MS m/e 285 (M + 1), 267, 143, 125, 55 (base).

Stereochemical Assignment of the Diolides 14 and 15. The diolide **15** (20 mg) was dissolved in 1 mL of a mixture consisting of 0.5 mL of 2 N KOH and 0.5 mL of methanol. The mixture was stirred at 25 °C and the progress of the reaction was followed by TLC analysis (5:1 hexane–ethyl acetate). After 16 h, the reaction mixture was acidified with 1 N HCl and extracted three times with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude residue was chromatographed over a silica gel column (0.8 × 10 cm). Elution of the column with a solvent system consisting of hexane–ethyl acetate (3:1) afforded 18 mg of (−)-7(*R*)-hydroxyoctanoic acid, (−)-**3**, $[\alpha]^{25}_D$ = −8.3° [(*c* 1.8 $CHCl_3$; reported[7] $[\alpha]^{21}_D$ = −7.0° (*c* 2.5, $CHCl_3$)]. Treatment of (−)-**3** with an ethereal solution of diazomethane afforded methyl (−)-7(*R*)-hydroxyoctanoate, (−)-**8a**; its enantiomeric purity was determined by ^1H NMR analysis in the presence of $Eu(hfc)_3$ (0.1 mmol of ester, 0.02 mmol of $Eu(hfc)_3$ in 0.6 mL of CCl_4). The singlet at δ 3.7 (CO_2CH_3) was shifted and split into two peaks at δ 5.0 (*S* enantiomer) and δ 5.1 (*R* enantiomer). The ratio of the two peaks was 4/96 (*S*/*R*), which indicated that **15** was a mixture of *R*,*R* and *S*,*S* with an enantiomeric purity of 0.92 ee (*R*,*R* > *S*,*S*).

Following the same procedure **14** was hydrolyzed and esterified. The resulting methyl 7-hydroxyoctanoate was found to be racemic (1:1 mixture), which indicated that **14** was the *R*,*S* diastereomer.

(±)-Methyl 7-(Chloroacetoxy)-4,4-(ethylenedioxy)oct-2-enoate (18). To a solution of (±)-**6b** (900 mg, 3.90 mmol) in carbon tetrachloride (4 mL) were added pyridine (500 mg) and chloroacetyl chloride (660 mg, 5.60 mmol). The reaction mixture was stirred at 25 °C for 15 min. Excess reagents were evaporated in vacuo. The resulting residue was dissolved in ethyl acetate and washed with aqueous 0.1 N hydrochloric acid and then brine solution. The combined organic portions were dried ($MgSO_4$), filtered, and concentrated. The residue was chromatographed over a silica gel column, which was eluted with 20% ethyl acetate in hexane to give 1.08 g (90%) of pure racemic **18**: IR 2960, 2930, 2880, 1725, 1650, 1430, 1370, 1280, 1115, 1030, 980; ^1H NMR δ 1.18 (d, J = 6 Hz, 3 H), 1.69 (m, 4 H), 3.70 (s, 3 H), 3.88 (m, 4 H), 3.96 (s, 2 H), 4.95 (m, 1 H), 6.30 (d, J = 16 Hz, 1 H), 6.70 (d, J = 16 Hz, 1 H); MS m/e 275 ($M^{+\cdot}$ − OCH_3), 277 ($M^{+\cdot}$ + 2-OCH_3), 263, 210, 157 (base), 137, 123, 122, 121, 103, 101.

(−)-(*S*)-Methyl 7-(Chloroacetoxy)-4,4-(ethylenedioxy)-oct-2-enoate (18). To a suspension of (±)-**18** (96 mg) in phosphate buffer solution (pH = 6.9, 10 mL) was added *Pseudomonas* sp. K-10 (136 mg). After being stirred for 16 h at 34 °C, the reaction mixture was extracted with ethyl acetate. The combined extracts were dried, filtered, and concentrated to give an oil, which was purified by flash chromatography. Elution with 20% ethyl acetate in hexane gave pure (−)-(*S*)-methyl 7-(chloroacetoxy)-4,4-(ethylenedioxy)oct-2-enoate, **18**: $[\alpha]^{22}_D$ = −4.6° (*c* 1.2, $CHCl_3$); ee = 93%.[9] The spectral data and TLC behavior are identical with those of racemic **18**. Further elution gave the hydrolyzed product (−)-(*R*)-**19**: $[\alpha]^{22}_D$ = −7.8° (*c* 1.4, $CHCl_3$); ee = 93%;[9] extent of conversion = 51%; IR 3590, 3010, 2990, 2950, 2920, 2880, 1710, 1655, 1430, 1370, 1300, 1270, 1160, 1130, 1050, 1030, 980, 940; ^1H NMR δ 1.10 (d, J = 6 Hz, 3 H), 1.65 (m, 4 H), 3.65 (s, 3 H), 3.87 (m, 4 H), 6.10 (d, J = 16.5 Hz, 1 H), 6.70 (d, J = 16.5 Hz, 16 H); MS m/e 231 ($M^{+\cdot}$ + 1), 171, 158, 157 (base), 145, 129, 127, 113, 85, 83, 59, 55, 45, 43; MS $M^{+\cdot}$ − 1 229.1060 (calcd for $C_{11}H_{17}O_5$ 229.1076).

(+)-(*S*)-7-Hydroxy-4,4-(ethylenedioxy)oct-2-enoic Acid (2). To a solution of (−)-(*S*)-methyl 7-(chloroacetoxy)-4,4-(ethylenedioxy)oct-2-enoate, (−)-**18** (30 mg, 0.1 mmol), in tetrahydrofuran (3.0 mL) was added aqueous 2 M lithium hydroxide (0.5 mL). The reaction mixture was stirred at 25 °C for 30 min. After being quenched with aqueous 1 N hydrochloric acid, the resulting mixture was extracted with ethyl acetate. The extracts were dried, filtered, and concentrated. The residue was chromatographed over a silica gel column, which was eluted with a solution of hexane/ethyl acetate/acetic acid (8:2:0.1) to give 20 mg (95%) of product, (+)-**6a**: $[\alpha]^{22}_D$ = +9.1° (*c* 1.3, $CHCl_3$); ee > 99%. The spectral data and TLC behavior are identical with those of racemic **6a**.

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