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Preparation of the enantiomers of hydroxy-C18 fatty acids and their anti-rice blast fungus activities

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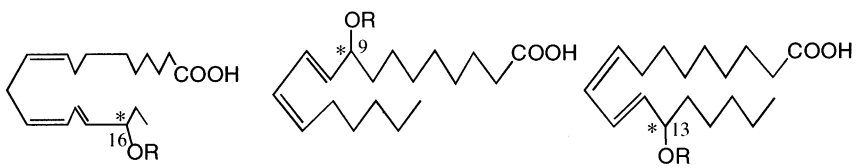
Abstract—In order to examine the correlation between the anti-rice blast fungus activity and the chirality of allylic alcohols **1**–**3**, which were characterized from the infected rice plants as an enantiomeric mixture with <10% e.e., a procedure for the chemical preparation of both enantiomers was explored, starting from the original fatty acids **4** and **5**. The anti-rice blast fungus activities of both enantiomers of the allylic alcohols **1**–**3** and epoxy α-linolenic acids **12** and **13** demonstrate no noticeable correlation between the activity and chirality.

1. Introduction

Our previous research concerning anti-rice blast fungus materials from rice plants has led to the isolation of various types of oxygenated unsaturated fatty acids[1] playing an important role in the defense of the rice plants against rice blast fungus.[2] Among the fatty acids isolated as defense substances are the allylic alcohols as exemplified by 16-hydroxy-α-linolenic acid **1** and 9- and 13-hydroxylinoleic acids **2** and **3**, respectively, which are accumulated as defense substances in the infected rice plants belonging to the susceptible varieties.

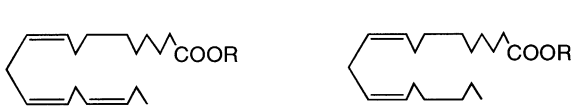
Our continuing studies[3] have further demonstrated that the activity of lipoxygenase (LOX) increases markedly in rice plants in response to infection with the pathogens. The endogeneous α-linolenic and linoleic acids **4** and **5** (R = H) are oxidized to the corresponding hydroperoxy fatty acids **6**–**8** by the in vitro oxidation with crude LOX solution obtained from the infected rice plants. The $NaBH_4$ reduction of the in vitro oxidation products provides the allylic alcohols **1**–**3**, all of the stereogenic carbons being *S*-configured and possessing over 99% e.e.[4] Although the bioactive allylic alcohols isolated from the infected rice plants may be



1 R = H
6 R = OH

2 R = H
7 R = OH

3 R = H
8 R = OH



4 **5**

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derived from the corresponding hydroperoxides produced by the in vivo oxidation with the increased LOX, the characterized alcohols are found to be largely racemic, the e.e. being <10% with the same *S*-configuration.[1,5]

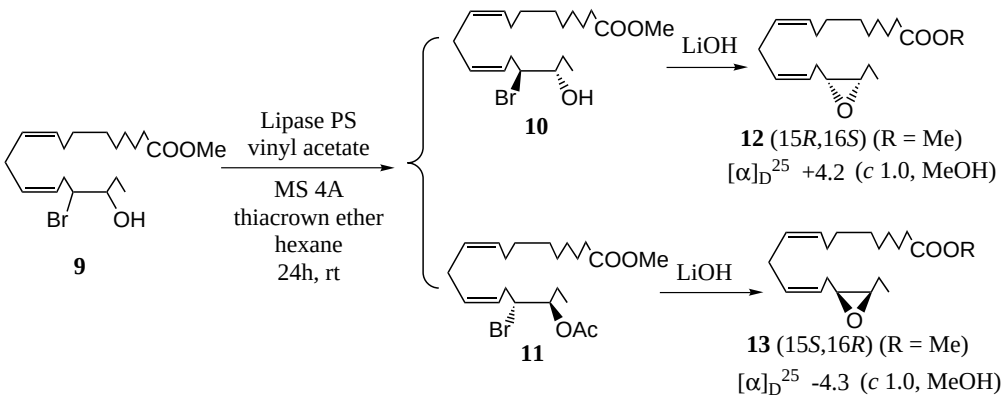
It is of particular interest to examine the anti-rice blast fungus activity of the enantiomerically pure allylic alcohols. Herein, we report the chemical preparation and comparison of the biological activities of both enantiomers of the allylic alcohols **1**–**3**, starting from the original fatty acids **4** and **5**.

2. Results and discussion

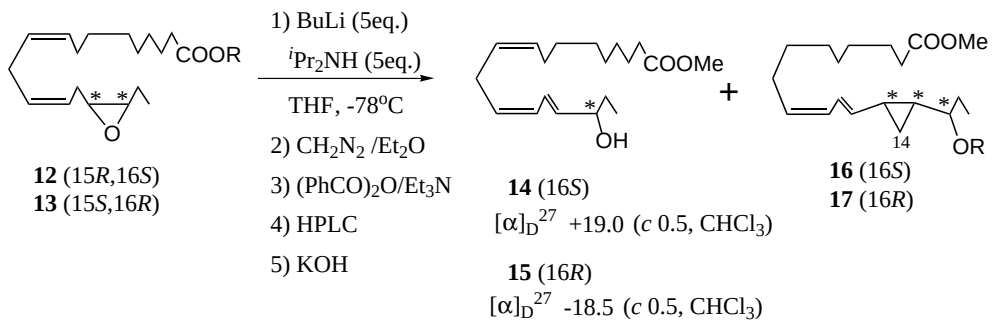
2.1. Preparation of both enantiomers of the allylic alcohol 1

We have already established conditions for the regioselective oxidation of the terminal double bond of ω-3 polyunsaturated fatty acids with NBS in aqueous DME solution.[6] The application of the method to methyl α-linolenate **4** (R = Me) furnishes the corresponding DL-bromohydrin **9**. The efficient resolution of **9** was performed by treatment with vinyl acetate and lipase PS in the presence of thiacrown ether,[7] providing the resolved compounds **10** and **11** with over 97% e.e. (Scheme 1). Both enantiomers of epoxy α-linolenic acids **12** and **13** (R = H) are easily prepared from the resolved compounds by treatment with aqueous LiOH.[8] Each enantiomer (R = H) was treated with LDA in THF at −78°C, followed by esterification with CH_2N_2, to give a mixture of two types of alcohols in 72% yield, one being the expected allylic alcohols **14** and **15** and the other, cyclopropane derivatives **16** and **17** (R = H), respectively (Scheme 2).

Completing the epoxide-ring opening with LDA at low temperature was essential since the reaction led to complete decomposition when carried out at 0°C. The mixture of hydroxy esters **14** and **16** (R = H) from **12**, and **15** and **17** (R = H) from **13** showed a single peak in the HPLC analysis. We managed to obtain each compound by separating with HPLC after the free hydroxyl group was esterified to the corresponding benzoate with benzoic anhydride. The conjugated (*Z*,*E*)-diene moieties of the alcohols **14** and **15**, and **16** and **17**, in particular those of **16** and **17** are fairly unstable and apt to isomerize to the stable (*E*,*E*)-geometry. The conjugated diene moiety possessing (*Z*,*E*)-geometry in each pair was confirmed by comparison of the NMR spectra with those of the related fatty acids.[4] The cyclopropane ring appended by the conjugated diene moiety and α-hydroxypropyl group was estimated from the detailed H–H COSY NMR spectra, in which the methylene protons at the 14-position and two methine protons at the 13- and 15-positions appeared at 0.80, 1.60 and 1.18 ppm, respectively. The stereochemistry of the resulting cyclopropane ring of the benzoates **16** and **17** (R = Bz) was estimated as (13*R*,15*R*,16*S*) for **16** and (13*S*,15*S*,16*R*) for **17** on the basis of the formation of the cyclopropane ring by S_N2-type ring-opening of the starting epoxides **12** and **13**.



Scheme 1.



Scheme 2.

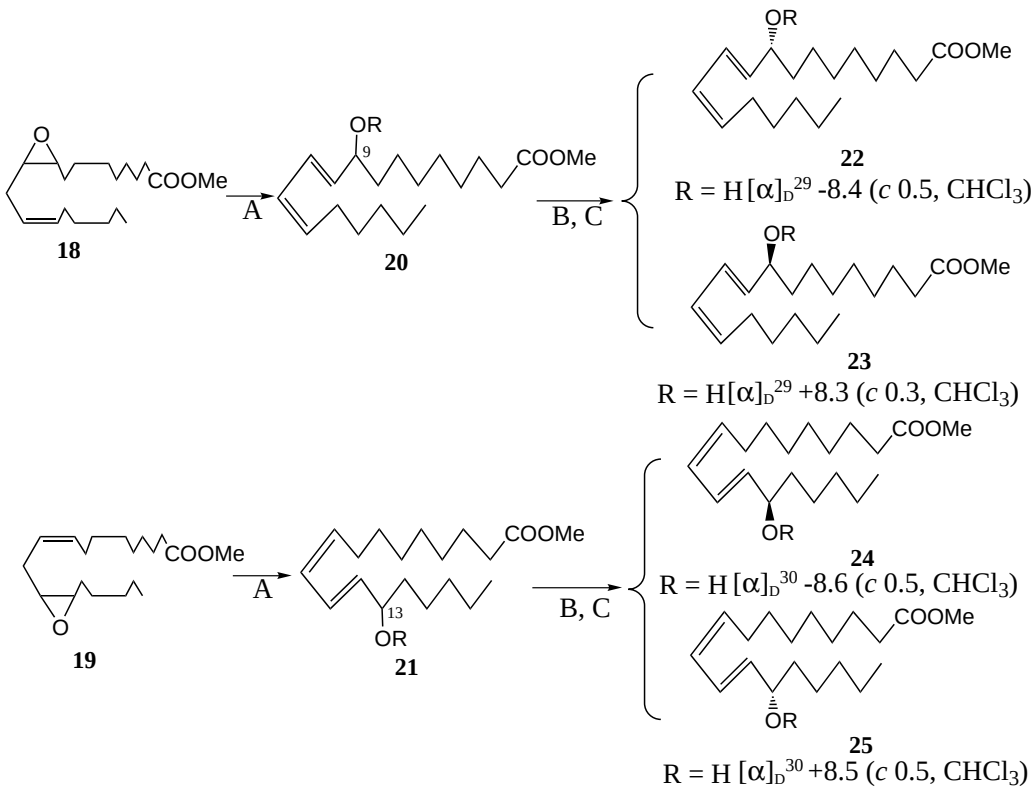
2.2. Preparation of both enantiomers of the allylic lcohols 2 and 3

For the preparation of both enantiomers of 9-hydroxy- and 13-hydroxylinoleic acids **2** and **3**, the easily available methyl DL-(*Z*)-9,10-epoxy- and 12,13-epoxy linoleic acids **18** and **19** were chosen as the starting materials, which were prepared from methyl linoleate by epoxidation with *m*-chloroperbenzoic acid and purification by flash column chromatography[1,9] (Scheme 3). Each epoxide was subjected to epoxide ring-opening with LDA, affording the (*Z*,*E*)-diene alcohols **20** and **21** (R = H) in good yields. On treatment of 9-hydroxy-diene alcohol **20** with lipase PS and vinyl acetate in the presence of thiacrown ether in hexane solution, partial resolution took place to give the resolved acetate **22** (R = Ac) and alcohol **23** (R = H) with ca. 60% e.e. When the resolution experiment was applied to the 13-hydroxy isomer **21** under the same conditions, less efficient resolution was observed, giving compounds **24** (R = Ac) and **25** (R = H) with ca. 15% e.e. The partially resolved hydroxy compounds **22** and **23**, and **24** and **25** were converted to the corresponding benzoates and the absolute configuration of each pair was estimated from the optical rotations of the resultant benzoates. As reported previously, the *S* configuration of the benzoates **23** and **25** (R = Bz) possessing positive optical rotations was determined by CD spectra, both showing a positive Cotton effect.[4] Changing the solvent from hexane to $^{i}Pr_2O$, changing the lipase from lipase PS to the lipases-AK or -CRL, or kinetic resolution mediated by Ru(II) and the lipase-based catalysts discovered recently by Kim and Park[10] gave no improvement in the resolution. The deacetylation–resolution of DL-acetyl compounds **20** and **21** (R = Ac) also gave unsatisfactory results with the lipases mentioned above. Fortunately, it was found that the benzoates **20** and **21** (R = Bz) could be resolved by the use of a chiral HPLC (chiral OD column) eluting with hexane:^{i}PrOH, 500:1. Each benzoate derived from the partially resolved compounds was submitted to semi-preparative chiral HPLC to afford the enantiomerically pure benzoates, which were then hydrolyzed to the free hydroxy compounds **22**–**25** (R = H).

2.3. Anti-rice blast fungus activities of both enantiomers

The anti-rice blast fungus activity of both enantiomers of three kinds of allylic alcohols was examined using spores of the same isolate [Kyu86-238 (race 003) or Kyu89-22-246] for the individual series by the established procedure.[11] The ED_{50} values in ppm of the inhibition of germination of the spores are summarized in Table 1, in which those of epoxides, one of the anti-fungus natural products in rice leaves, were added for comparison.

The results in Table 1 indicate that no noticeable differences in activity were detected between the enantiomers. The ED_{50} values are almost identical with those of each substance possessing <10% e.e. isolated from rice plant. This phenomenon is quite different from that encountered in nature as typified by the

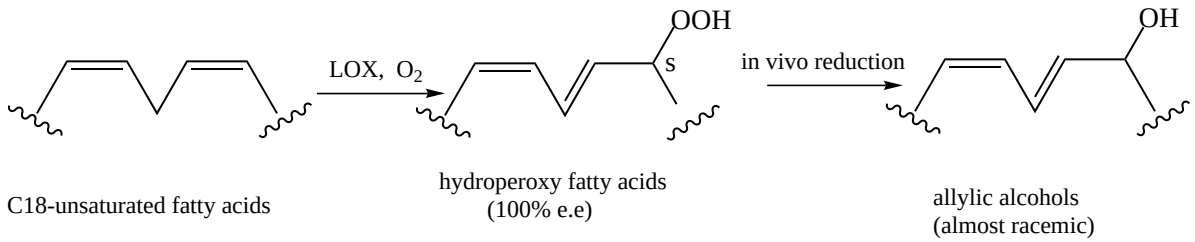


Scheme 3. (A) (1) 0.5N LiOH/H_2O–dioxane, (2) *n*-BuLi (5 equiv.) and $^{i}Pr_2NH$ (5 equiv.), THF, (3) CH_2N_2, (ca. 80% overall yields); (B) lipase PS, AcOCH=CH_2; (C) chiral column separation of benzoates of **22**–**25**.

Table 1. Anti-rice blast fungus activities of enantiomers of fatty acids[a]

Compounds	Anti-rice blast fungus activity ED_{50} (ppm)	Compounds	Anti-rice blast fungus activity ED_{50} (ppm)
(15*R*,16*S*)-Epoxy α-linolenic acid **12** (R = H)	37	(9*R*)-Hydroxy linoleic acid **2** (9*R*)	50
(15*S*,16*R*)-Epoxy α-linolenic acid **13** (R = H)	23	(9*S*)-Hydroxy linoleic acid **2** (9*S*)	75
(16*S*)-Hydroxy α-linolenic acid **1** (16*S*)	40	(13*R*)-Hydroxy linoleic acid **3** (13*R*)	30
(16*R*)-Hydroxy α-linolenic acid **1** (16*R*)	35	(13*S*)-Hydroxy linoleic acid **3** (13*S*)	25

[a] Spores of the same isolate of *Pyricularia oryzae* Cavara were used in the activity experiment of both enantiomers.



Scheme 4.

insect world, in which the biological activity is observed in only one enantiomer, the antipode being inactive.

Our previous study demonstrates clearly that the enantiomerically pure hydroperoxy fatty acids are produced by lipoxygenase (LOX), the activity of which increased after infection with the pathogens[3] (Scheme 4). Elucidation of the reaction mechanism of asymmetry destruction of the hydroperoxy fatty acids in the in vivo reduction is a fascinating subject for future studies.

3. Experimental

3.1. General

Unless otherwise noted, ^1H and ^{13}C NMR spectra were recorded on solution in $CDCl_3$ with $SiMe_4$ as an internal standard by JEOL spectrometers with the indicated MHz. Chemical shifts are reported in δ_H and δ_C, and J values in hertz. The following multiplicities were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on a Hitachi 270-30 spectrophotometer. The mass spectra were measured with a Hitachi M-80B spectrometer including EIMS (electron ionization, 70 eV) and HRMS mass spectrometries. Optical rotations were measured on a JASCO DIP-370 polarimeter with a path length of 1 dm. Concentrations are given in g/100 mL. Column chromatographic purification was carried out using Kiesel gel 60, Art 7734 (70–230 mesh). HPLC was performed with Waters Associates equipment and Guard-Pak Cartridge Prep Novapak HR (Waters Associates) and μ-porasil columns, respectively. Thin-layer chromatography was carried out on aluminium sheets coated with $60F_{254}$ silica. Plates were developed using a spray of 0.5% anisaldehyde in 2 M sulfuric acid. The starting methyl esters of α-linolenic and linoleic acids were purified using silica gel impregnated with 5% $AgNO_3$ column chromatography eluted with mixed solvents of hexane and AcOEt by changing the ratio from 60:1 to 30:1 and finally to 10:1. Solvents and commercially available reagents were dried and purified if necessary before use according to standard procedures. All the derivatives described in this paper were pale yellow oily substances and the purities were confirmed by ^{13}C NMR spectra. The usual work-up involved dilution of the reaction mixture with water, extraction with ether and evaporation after washing the organic extracts with water and brine, followed by drying over Na_2SO_4. The experiments concerning resolution of the indicated compounds were carried out more than three times, and the average values of optical rotations are shown, the deviation of optical rotation being ±0.2 in each experiment.

3.2. (16*S*)- and (16*R*)-Hydroxy-α-linolenic acids 1 (16*S* and 16*R*)

Under an argon atmosphere, a solution of (15*S*,16*R*)-epoxy-α-linolenic acid **13** (R = H, 29 mg, 0.1 mmol) in THF (1.62 mL) was added over 5 min at −78°C to a solution of LDA, [freshly prepared by treatment of a solution of diisopropylamine (0.11 mL, 0.6 mmol) in THF (0.8 mL) with *n*-BuLi in hexane (6.43 mL, 0.5 mmol) at −78°C]. The mixture was stirred for 90 min at −78°C. After addition of aq. NH_4Cl solution to make the pH 6–7, the mixture was worked up as usual. Excess CH_2N_2 in ether was added to the resulting residue. After evaporation of volatile materials, the residue was passed through a SiO_2 (4 g) column eluting with hexane:AcOEt 20:1 and then subjected to prepara-

tive HPLC (μ-porasil; hexane:EtOH 300:1; flow 3.0 mL/min) to obtain a yellowish oily product (21 mg) of allylic alcohol **15** and cyclopropane alcohol **17** (R = H) as an inseparable mixture by HPLC under several conditions.

A solution of Et_3N (27 mL, 0.2 mmol) in CH_2Cl_2 (1 mL) was added to the mixture of **15** and **17** (21 mg) and then benzoic anhydride (44 mg, 0.2 mmol) was added. After stirring for 25 h at rt under an argon atmosphere, aq. NH_4Cl solution was dropped to make the pH 6–7, and then the mixture was treated as usual. The resulting residue was first passed through a SiO_2 (4 g) column eluted with hexane:AcOEt 40:1 and then subjected to a preparative HPLC (μ-porasil; hexane:AcOEt 40:1, flow 3.0 mL/min) to isolate the yellowish oily methyl ester of the benzoates of **15** (16 mg, 56%) and **17** (R = Bz, 10 mg, 37%), respectively.

Similarly, (15*R*,16*S*)-epoxy-α-linolenic acid **12** (R = H, 29 mg, 0.1 mmol) provided a mixture of allylic alcohol **14** and cyclopropane alcohol **16** (R = H) (15 mg) as a single peak in HPLC analysis. The benzoylation of the mixture (20 mg) under the same conditions followed by HPLC separation afforded the corresponding methyl ester benzoates of **14** (18 mg, 89%) and **16** (2 mg, 10%) as yellow oil, respectively. Benzoates of **14** and **15**: δ_H (270 MHz, $CDCl_3$) 0.98 (3H, t, J = 7.4 Hz), 1.29 (8H, brs), 1.61 (2H, m), 1.80 (2H, m), 2.02 (2H, m), 2.30 (2H, t, J = 7.4 Hz), 2.92 (2H, t, J = 6.4 Hz), 3.66 (3H, s), 5.37 (4H, m), 5.73 (1H, dd, J = 7.3, 15.2 Hz), 5.98 (1H, t, J = 11.1 Hz), 6.64 (1H, dd, J = 11.1, 15.0 Hz), 7.52 (3H, m) and 8.08 (2H, m). δ_C (68 MHz, $CDCl_3$) 174.3 (s), 165.9 (s), 132.8 (d), 131.5 (d), 131.1 (d), 130.8 (d), 130.7 (s), 129.6 (d)×2, 128.3 (d)×2, 127.8 (d), 127.5 (d), 127.0 (d), 76.4 (d), 51.4 (q), 34.1 (t), 29.45 (t), 29.1 (t), 29.1 (t), 29.0 (t), 27.7 (t), 27.2 (t), 26.1 (t), 24.9 (t), and 9.6 (q). HRMS of benzoates of **14** and **15** calcd for $C_{26}H_{36}O_4$: 412.2614. Observed: 412.2607 for **14** and 412.2611 for **15**. $[\alpha]_D^{27}$ +96.5 (*c* 1.0, $CHCl_3$) for benzoate of **14** and −90.6 (*c* 1.0, $CHCl_3$) for that of **15**. Benzoates of **16** and **17** (R = Bz) (270 MHz, $CDCl_3$) δ_H 0.80 (1H, dt, J = 8.5, 5.4, 1H, dt, J = 8.8, 5.1, C-14), 1.00 (3H, t, J = 7.3, C-18), 1.18 (1H, m, C-15), 1.23 (8H, brs, C-4,5,6,7), 1.60 (1H, m, C-13), 1.61 (2H, m, C-3), 1.80 (2H, m, C-17), 2.12 (2H, m, C-8), 2.29 (2H, t, J = 7.6, C-2), 3.66 (3H, s, OMe), 4.64 (1H, dt, J = 6.3, 8.1, C-16), 5.20 (2H, m, C-9,12), 5.87 (1H, dd, J = 10.1, 11.0, C-10), 6.32 (1H, dd, J = 11.1, 14.9, C-11), 7.45 (3H, m, Ph), and 8.06 (2H, m, Ph). δ_C (100 MHz, $CDCl_3$) 174.4 (s), 166.3 (s), 136.1 (d), 133.7 (d), 132.8 (d), 130.7 (s), 130.2 (d), 129.9 (d), 129.6 (d), 128.5 (d), 128.2 (d), 124.1 (d), 78.7 (d), 51.5 (q), 34.1 (t), 29.6 (t), 29.1 (t), 29.0 (t)×2, 27.7 (t), 27.6 (t), 24.9 (d), 24.8 (t), 20.0 (d), 12.2 (t), and 9.8 (q). HRMS of benzoates of **16** and **17** calcd for $C_{26}H_{36}O_4$: 412.2614. Observed: 412.2616 for **16** and 412.2616 for **17**. $[\alpha]_D$ of benzoates of **16** and **17**, not measured.

A mixture of the methyl ester of the (16*S*)-benzoate of **14** (18 mg, 0.04 mmol) and 0.5N KOH in dioxane/H_2O (1:1, 0.8 mL) was stirred at rt for 30 min under an argon atmosphere, the mixture was diluted with water and 0.5N aq. oxalic acid solution was added to make the pH 3–4. After usual work-up, excess CH_2N_2 in ether was added and volatile materials were removed to obtain a residue. The residue was passed through a SiO_2 column eluting with hexane:AcOEt 20:1 and then subjected to preparative HPLC (μ-porasil; hexane:EtOH 300:1; flow 3.0 mL/min) to obtain a yellowish oily allylic alcohol **14** (10 mg, 81%). Similarly, the (16*R*)-benzoate of **15** (15 mg) was treated to obtain (16*R*)-allylic alcohol **15** (9 mg, 73%). Allylic alcohols **14** and **15** δ_H (300 MHz, $CDCl_3$) 0.94 (3H, t, J = 7.4 Hz), 1.31 (8H, brs), 1.62 (4H, brs), 2.04 (2H, m), 2.30 (2H, t, J = 7.5 Hz), 2.93 (2H, t, J = 7.0 Hz), 3.67 (3H, s), 4.09 (1H, m), 5.39 (3H, m), 5.67 (1H, dd, J = 6.8, 15.1 Hz), 5.98 (1H, t, J = 10.8 Hz), 6.52 (1H, dd, J = 11.0, 15.1 Hz). δ_C (76 MHz, $CDCl_3$) 174.4 (s), 136.0 (d), 130.7 (d), 130.6 (d), 127.8 (d), 127.1 (d), 125.6 (d), 74.1 (d), 51.5 (q), 34.1 (t), 30.2 (t), 29.5 (t), 29.1 (t), 29.1 (t), 29.0 (t), 27.2 (t), 27.1 (t), 24.9 (t), and 9.7 (q). HRMS of **14** and **15** calcd for $C_{19}H_{32}O_3$: 308.2351. Observed: 308.2342 for **14** and 308.2360 for **15**. $[\alpha]_D^{27}$ +19.0 (*c* 0.5, $CHCl_3$) for **14** and −18.5 (*c* 0.5, $CHCl_3$) for **15**.

3.3. DL-9- and 13-Hydroxylinoleic methyl esters

A solution of methyl 9-epoxylinoleic ester **18** (180 mg, 0.57 mmol) in 0.5N LiOH/dioxane H_2O (1:1, 11.4 mL) was stirred at rt for 24 h, dioxane was removed under reduced pressure at 40°C and the residue was diluted with water and then 0.5N aq. oxalic solution was added to make the pH 3∼4. The usual work-up afforded the crude epoxy acid. A solution of the crude 9-epoxylinoleic acid **18** (H instead of Me) in THF (1.62 mL) was added at −78°C under an argon atmosphere to an LDA solution, freshly prepared by treatment of a solution of diisopropylamine (0.66 mL, 5.1 mmol) in THF (3 mL) with *n*-BuLi in hexane (2.45 mL, 4.01 mmol) at 0°C for 5 min, and the mixture was stirred for 1 h at 0°C. After addition of aq. NH_4Cl solution to make the pH 6∼7, the mixture was worked up as usual. An excess CH_2N_2 in ether was added to the resulting residue. After evaporation of volatile materials, the residue was passed through a SiO_2 (18 g) column eluted with hexane:AcOEt 20:1 and then subjected to a preparative HPLC (μ-porasil; hexane:EtOH 300:1; flow 3.0 mL/min) to afford allylic alcohol **20** (R = H, 140 mg, 78%) as a yellowish oil.

By similar treatment, 12-epoxylinoleic methyl ester **19** (134 mg, 0.45 mmol) provided the allylic alcohol **21** (R = H, 106 mg, 76%). Compound **20** (R = H) δ_H (270 MHz, $CDCl_3$) 0.89 (3H, t, J = 6.5 Hz), 1.31 (14H, brs), 1.61 (4H, m), 2.16 (2H, m), 2.30 (2H, t, J = 7.4 Hz), 3.66 (3H, s), 4.13 (1H, dt, J = 6.1, 6.4 Hz), 5.44 (1H, m), 5.67 (1H, dd, J = 6.8, 15.1 Hz), 5.97 (1H, t, J = 11.1 Hz), and 6.48 (1H, dd, J = 10.9, 15.1 Hz). δ_C (68 MHz, $CDCl_3$) 174.3 (s), 135.7 (d), 133.0 (d), 127.6 (d), 125.8 (d), 72.8 (d), 51.4 (q), 37.3 (t), 34.0 (t), 31.4 (t), 29.3 (t)×2, 29.1 (t), 29.0 (t), 27.7 (t), 25.3 (t), 24.9 (t), 22.5 (t), and 14.0 (q). Compound **21** (R = H) δ_H (500 MHz, $CDCl_3$) 0.89 (3H, t, J = 6.6 Hz), 1.31 (12H, brs), 1.39 (2H, m), 1.59 (4H, m), 2.17 (2H, m), 2.30 (2H, t, J = 7.4 Hz), 3.66 (3H, s), 4.14 (1H, dt, J = 6.6, 6.4 Hz), 5.44

(1H, m), 5.66 (1H, dd, $J=7.0$, 15.3 Hz), 5.97 (1H, t, $J=11.0$ Hz), and 6.48 (1H, dd, $J=11.0$, 15.3 Hz). δ_C (126 MHz, $CDCl_3$) 174.3 (s), 136.0 (d), 132.8 (d), 127.9 (d), 125.7 (d), 72.9 (d), 51.5 (q), 37.3 (t), 34.1 (t), 31.8 (t), 29.5 (t), 29.1 (t)×2, 29.0 (t), 27.7 (t), 25.1 (t), 24.9 (t), 22.6 (t), and 14.1 (q). HRMS calcd for $C_{19}H_{34}O_3$: 310.2508. Observed: 310.2501 for **20** and 310.2516 for **21**.

3.4. Lipase PS treatments of 9-hydroxy- and 13-hydroxylinoleic methyl esters 20 and 21 (R=H)

A mixture of 9-hydroxylinoleic methyl ester **20** (R=H, 80 mg, 0.26 mmol), lipase PS (80 mg), 4 Å molecular sieves (ca. 50 mg), 1,4,8,11-tetrathiacyclotetradecane (ca. 5 mg) and vinyl acetate (0.3 mL) in hexane (3 mL) was stirred at rt for 3 days under an argon atmosphere, the mixture was passed through a pad of SiO_2 and the SiO_2 was washed repeatedly with ether. The volatile materials were removed and the residue was passed through a SiO_2 (8 g) column eluted with hexane:AcOEt 40:1 to obtain the acetate **22** (R=Ac) and resolved alcohol **23** (R=H). The acetate **22** was purified with HPLC (μ-porasil, hexane:AcOEt 40:1, flow=3.0 mL/min) to obtain (9*R*)-acetate **22** (R=Ac, 39 mg, 43%); the resolved alcohol was subjected to HPLC (μ-porasil, hexane:EtOH 300:1, flow=3.0 mL/min) to get 9*S*-alcohol **23** (R=H, 40 mg, 49%), respectively.

Similarly, DL-alcohol **21** (R=H, 90 mg, 0.29 mmol) furnished the (13*R*)-acetate **24** (R=Ac, 41 mg, 40%) and 13*S*-alcohol **25** (R=H, 44 mg, 49%) after purification with HPLC under the same conditions. 9*R*-Acetate **22** (R=Ac) δ_H (270 MHz, $CDCl_3$) 0.89 (3H, t, $J=6.6$ Hz), 1.29 (14H, brs), 1.60 (4H, m), 2.05 (3H, s), 2.15 (2H, m), 2.30 (2H, t, $J=7.4$ Hz), 3.67 (3H, s), 5.26 (1H, dd, $J=6.6$, 13.7 Hz), 5.50 (2H, m), 5.94 (1H, t, $J=11.1$ Hz), and 6.49 (1H, dt, $J=11.1$, 15.1 Hz). δ_C (126 MHz, $CDCl_3$) 174.3 (s), 170.4 (s), 133.7 (d), 131.0 (d), 128.0 (d), 127.6 (d), 74.8 (d), 51.4 (q), 34.5 (t), 34.1 (t), 31.6 (t), 29.5 (t), 29.1 (t), 29.1 (t), 29.0 (t), 27.8 (t), 24.9 (t), 24.8 (t), 22.5 (t), 21.4 (q), and 14.0 (q). HRMS calcd for $C_{21}H_{36}O_4$: 352.2614. Observed: 352.2616 $[\alpha]_D^{29}$ +9.46 (*c* 1.0, MeOH). 9*S*-Alcohol **23** δ_H (270 MHz, $CDCl_3$) and δ_C (68 MHz, $CDCl_3$) were identical with those of **20**. HRMS calcd for $C_{19}H_{34}O_3$: 310.2508. Observed: 310.2500. $[\alpha]_D^{27}$ +5.22 (*c* 1.0, MeOH). 13*R*-Acetate **24** (R=Ac) δ_H (270 MHz, $CDCl_3$) 0.88 (3H, t, $J=6.5$ Hz), 1.31 (14H, brs), 1.60 (4H, m), 2.1 (3H, s), 2.1 (2H, m), 2.3 (2H, t, $J=7.3$ Hz), 3.67 (3H, s), 5.28 (1H, dd, $J=6.8$, 13.8 Hz), 5.48 (2H, m), 5.94 (1H, t, $J=11.1$ Hz), and 6.50 (1H, dd, $J=11.1$, 15.1 Hz). δ_C (126 MHz, $CDCl_3$) 174.1 (s), 170. (s), 133.7 (d), 131.0 (d), 128.0 (d), 127.5 (d), 74.8 (d), 51.4 (q), 34.5 (t), 34.1 (t), 31.5 (t), 29.5 (t), 29.1 (t)×2, 29.0 (t), 27.7 (t), 24.9 (t), 24.8 (t), 22.5 (t), 21.4 (q), and 14.0 (q). HRMS calcd for $C_{21}H_{36}O_4$: 352.2614. Observed: 352.2609. $[\alpha]_D^{28}$ +3.1 (*c* 1.0, MeOH). 13*S*-Alcohol **25** δ_H (500 MHz, $CDCl_3$) and δ_C (126 MHz, $CDCl_3$) were identical with those of **21**. HRMS calcd for $C_{19}H_{34}O_3$: 310.2508. Observed: 310.2513. $[\alpha]_D^{28}$ +1.3 (*c* 1.0, MeOH).

3.5. Benzoylation of methyl hydroxylinoleates 20 and 21 (R=H)

A mixture of DL-9-hydroxy linoleic acid methyl ester **20** (R=H, 20 mg, 0.06 mmol), benzoic anhydride (41 mg, 0.18 mmol), DMAP (ca. 5 mg) and Et_3N (25 mL, 0.18 mmol) in CH_2Cl_2 (1 mL) was stirred at rt for 30 h under an argon atmosphere, aq. NH_4Cl solution was added to make the pH 6–7. After usual work-up, the residue was first passed through a SiO_2 (3 g) column eluting with hexane:AcOEt 40:1 and then purified by HPLC (μ-porasil, hexane:AcOEt 40:1, flow=3.0 mL/min) to obtain the benzoate **20** (R=COC_6H_5, 22 mg, 90%). The DL or partly resolved benzoate was separated by preparative chiral column (Daisel CHIRAL OD; hexane:^{i}PrOH 500:1). Similarly, DL-13-alcohol **21** (R=H, 20 mg, 0.06 mmol) was converted to the corresponding benzoate **21** (R=COC_6H_5, 22 mg, 90%) and then the pure (13*R*)- and (13*S*)-benzoates were obtained by CHIRAL OD column separation under the same conditions. (9*R*)- and (9*S*)-Benzoates **22** and **23** (R=COC_6H_5) δ_H (270 MHz, $CDCl_3$) 0.87 (3H, t, $J=6.6$ Hz), 1.30 (16H, brs), 1.63 (4H, m), 2.15 (2H, m), 2.29 (2H, t, $J=7.3$ Hz), 3.66 (3H, s), 5.53 (2H, m), 5.69 (1H, dd, $J=7.3$, 14.9 Hz), 5.96 (1H, t, $J=10.9$ Hz), 6.60 (1H, dd, $J=11.2$, 15.1 Hz), 7.44 (2H, m), 7.55 (1H, m), and 8.05 (2H, m). δ_C (76 MHz, $CDCl_3$) 174.3 (s), 165.9 (s), 134.0 (d), 132.8 (d), 130.8 (d), 130.7 (s), 129.5 (d)×2, 128.3 (d)×2, 128.1 (d), 127.4 (d), 75.3 (d), 51.4 (q), 34.7 (t), 34.0 (t), 31.4 (t), 29.2 (t)×2, 29.1 (t), 29.0 (t), 27.7 (t), 25.1 (t), 24.9 (t), 22.5 (t), and 14.0 (q). HRMS calcd for $C_{26}H_{38}O_4$: 414.2770. Observed: 414.2771 for **22** and 414.2765 for **23** (each R=COC_6H_5). $[\alpha]_D^{29}$ −77.6 (*c* 0.7, $CHCl_3$) for **22** and $[\alpha]_D^{29}$ +72.5 (*c* 0.5, $CHCl_3$) for **23** (each R=COC_6H_5). 13*R*- and 13*S*-Benzoates **24** and **25** (R=COC_6H_5) δ_H (500 MHz, $CDCl_3$) 0.88 (3H, t, $J=6.7$ Hz), 1.29 (12H, brs), 1.60 (2H, m), 1.75 (2H, m), 2.14 (2H, m), 2.30 (2H, t, $J=7.4$ Hz), 3.66 (3H, s), 5.49 (1H, dt, $J=10.4$, 7.8 Hz), 5.55 (1H, dt, $J=7.0$, 6.8 Hz), 5.68 (1H, dd, $J=7.4$, 15.0 Hz), 5.96 (1H, t, $J=11.1$ Hz), 6.59 (1H, dd, $J=11.3$, 15.3 Hz), 7.44 (2H, t, $J=8.0$ Hz), 7.55 (1H, t, $J=7.3$ Hz), and 8.06 (2H, m). δ_C (68 MHz, $CDCl_3$) 174.3 (s), 165.9 (s), 133.8 (d), 132.8 (d), 131.0 (d), 130.8 (s), 129.6 (d)×2, 128.3 (d)×2, 128.1 (d), 127.6 (d), 75.4 (d), 51.4 (q), 34.7 (t), 34.1 (t), 31.6 (t), 29.5 (t), 29.1 (t), 29.0 (t)×2, 27.8 (t), 24.9 (t), 24.9 (t), 22.5 (t), and 14.0 (q). HRMS calcd for $C_{26}H_{38}O_4$: 414.2770. Observed: 414.2767 for **24** and 414.2775 for **25** (each R=COC_6H_5). $[\alpha]_D^{30}$ −74.4 (*c* 1.0, $CHCl_3$) for **24** and +78.3 (*c* 1.0, $CHCl_3$) for **25** (each R=COC_6H_5).

3.6. (9*R*)- and (9*S*)- and (13*R*)- and (13*S*)-Hydroxylinoleic methyl esters 22–25

A mixture of (9*R*)-benzoate **22** (R=COC_6H_5, 21 mg, 0.05 mmol) and 0.5N LiOH/H_2O–dioxane (1:1, 1.0 mL) was stirred at rt for 30 h, the mixture was diluted with water and then evaporated to half of its original volume to remove dioxane. 0.5N aq. oxalic acid solution was added to make the pH 3∼4 and the mixture was extracted with ether. Excess CH_2N_2 in ether was added to the ether solution and the volatile materials were removed. The residue was passed through a SiO_2 (2 g)

column eluted with hexane:AcOEt 20:1 and then submitted to HPLC purification (μ-porasil, hexane:EtOH 300:1, flow = 3.0 mL/min) to obtain the methyl ester of (9*R*)-hydroxylinoleic acid **22** (R = H, 14 mg, 80%). Similarly, the (9*S*)-benzoate **23** ($R = COC_6H_5$, 17 mg, 0.04 mmol) was treated with aq. $LiOH/H_2O$–dioxane to give (9*S*)-alcohol **23** (R = H, 11 mg, 85%) after purification via HPLC. (9*R*)- and (9*S*)-Alcohols **22** and **23** (each R = H) δ_H (270 MHz, $CDCl_3$) and δ_C (68 MHz, $CDCl_3$) were identical with those of **20** (R = H). HRMS calcd for $C_{19}H_{34}O_3$: 310.2508. Observed: 310.2517 for **22** and 310.2501 for **23** (R = H). $[\alpha]_D^{29}$ −8.4 (*c* 0.5, $CHCl_3$) for **22** and +8.3 (*c* 0.3, $CHCl_3$) for **23** (each R = H). Similarly, 13*R*- and 13*S*-alcohols were obtained from the corresponding benzoates. 13*R*- and 13*S*-Alcohols **24** and **25** (R = H) δ_H (300 MHz, $CDCl_3$) and δ_C (126 MHz, $CDCl_3$) were identical with those of **21** (R = H). HRMS calcd for $C_{19}H_{34}O_3$: 310.2508. Observed: 310.2509 for **24** and 310.2513 for **25**. $[\alpha]_D^{30}$ −8.6 (*c* 0.5, $CHCl_3$) for **24** and +8.5 (*c* 0.5, $CHCl_3$) for **25** (R = H).

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References

1. Kato, T.; Yamaguchi, Y.; Namai, T.; Hirukawa, T. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 283–287.
2. Namai, T.; Kato, T.; Yamaguchi, Y.; Hirukawa, T. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 611–613.
3. Namai, T.; Kato, T.; Yamaguchi, Y.; Togashi, J. *Ann. Phytopath. Soc. Jpn.* **1990**, *56*, 26–32.
4. Kato, T.; Watanabe, T.; Hirukawa, T.; Tomita, N.; Namai, T. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1663–1666.
5. (a) Kato, T.; Yamaguchi, Y.; Hirano, T.; Yokoyama, T.; Uyehara, T.; Namai, T.; Yamanaka, S.; Harada, N. *Chem. Lett.* **1984**, 409–413; (b) See also Ref. 1.
6. Kato, T.; Hirukawa, T.; Namiki, T. *Tetrahedron Lett.* **1992**, *33*, 1475–1478.
7. Kato, T.; Ishimatsu, T.; Aikawa, A.; Taniguchi, K.; Kurahashi, T.; Nakai, T. *Tetrahedron: Asymmetry* **2000**, *11*, 851–860.
8. Kato, T.; Nakai, T.; Ishimatsu, T.; Hoshikawa, M.; Namai, T. *Heterocycles* **2000**, *52*, 1353–1358.
9. Kato, T.; Hirukawa, T.; Yano, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 834–842.
10. Lee, D.; Huh, E. A.; Kim, M.-J.; Jung, H. M.; Koh, J. H.; Park, J. *Org. Lett.* **2000**, *2*, 2377.
11. Namai, T.; Kato, T.; Yamaguchi, Y.; Togashi, J. *Ann. Phytopath. Soc. Jpn.* **1991**, *57*, 339–344.