Separation of Stereoisomers of Some Terpene Derivatives by Capillary Gas Chromatography-Mass Spectrometry and High-Performance Liquid Chromatography Using β-Cyclodextrin Derivative Columns

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Gas chromatographic separations of the stereoisomers of menthol derivatives, important intermediates in the synthesis of physiologically active natural products, were carried out on several substituted β-cyclodextrin (CD) columns, including *per-O*-methyl-β-cyclodextrin (PME-β-CD), heptakis(2,3-di-*O*-acetyl-6-*tert*-butyldimethylsilyl)-β-CD (DIAC-6-TBDS-β-CD), and heptakis(2,3-di-*O*-methyl-6-*tert*-butyldimethylsilyl)-β-CD (DIME-6-TBDS-β-CD) as chiral stationary phases (CSPs). With the DIME-6-TBDS-β-CD column, a separation of the *Z*- and *E*-isomers of methylidenementhol was accomplished; no separation was achieved with the other columns. The stereoisomers of methylidenementhol and the corresponding *tert*-butyldimethylsilyl (TBS) ether were separated on both the β-CD and the heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TME-β-CD) columns by high-performance liquid chromatography (HPLC) with a mobile phase involving acetonitrile and H_2O. For the separation of the *Z*- and *E*-isomers of methylidenementhol, the TME-β-CD column was superior. In contrast, the β-CD column was preferable in the case of the corresponding TBS ether.

Key words stereoisomers separation; β-cyclodextrin derivative; menthol; terpene; GC-MS; HPLC

Modified cyclodextrins (CDs) are well established as reagents for the chiral discrimination of enantiomers in the form of chiral stationary phases.[1—3] These oligosaccharides effect the separation of stereoisomers by forming diastereomeric inclusion complexes with them. The derivatization of the hydroxyl groups, primarily at the 6-position and secondarily at the 2- and 3-positions on the CD ring, appears to determine the chiral recognition. Branch *et al.*[4] proposed that the chiral discrimination is brought about by differences in the interactions of each of the enantiomers with the secondary hydroxyl groups (at the 2- and 3-positions of the glucose monomers), which line the outer rim of the CD cavity. The inclusion complexation by CD is of particular interest, since it occurs mostly through weak interactions, *i.e.*, van der Waals, hydrogen bonding, and/or dipole–dipole interactions.

Optically active compounds have recently aroused wide interest in many fields, for example, natural products, agrochemicals, and pharmaceuticals,[5] therefore, their preparation and analysis are of increasing importance. Terpenes are important not only because of their widespread occurrence in natural products but also because of their role as versatile intermediates in synthetic processes.[6—11] In particular, monoterpenes, because of their availability, low-price, and unique composition, consisting of C10 units, are commonly used for syntheses of natural products as chiral building blocks.[12] Chemically synthesized compounds are often not stereochemically 100% pure, hence, the unambiguous determination of stereoisomeric composition is an important analytical task. Chromatographic enantioseparations have considerably advanced the technology for determining their optical purity.

Modified CDs are widely used as chiral stationary phases (CSPs) in GC. Dietrich *et al.* reported that the selectivity of DIME-6-TBDS-β-CD was superior to that of PME-β-CD for separations of various furanones,[13] and pointed out the usefulness of DIAC-6-TBDS-β-CD for the separation of flavor compounds[14] with GC.

With HPLC, CDs are also known to be useful for the separation of stereoisomers. Ryu *et al.*[15] examined β-CD, heptakis(3-*O*-methyl)-β-CD, and heptakis(2,3-di-*O*-methyl)-β-CD for separations of several racemic compounds.

In our previous work,[16] we performed separations of the stereoisomers (enantiomers and geometric isomers) of several furan derivatives, classified as terpenes, by capillary GC-MS, supercritical fluid chromatography, and HPLC using CSPs, including modified CDs, and also determined the ratios of the isomers. Based on those results, the conditions required for the separation of the enantiomers of terpene compounds tended to correspond well to those required for separation of the geometric isomers. Thus, it is worth examining several kinds of CSPs for separations of geometric isomers, because they should also be useful in the chiral recognition of related terpene compounds.

Here, we describe the direct separation of the *Z*- and *E*-isomers of a menthol derivative (**1**) and that of the corresponding TBS ether (**2**), prepared from optically active (−)-menthone, which is a monoterpene, as a starting material, by use of HPLC with β-CD and TME-β-CD columns and also by use of capillary GC-MS with PME-β-CD, DIAC-6-TBDS-β-CD, and DIME-6-TBDS-β-CD columns. By calculating the area of each peak appearing on the chromatogram, the ratio of isomers in the sample can be determined.

Results and Discussion

It is well known that chiral selectivity is influenced by the nature of the substitution pattern at the hydroxyl rim of the CD cavity. In the present work, several kinds of modified CDs were examined, and their separations were compared.

Separation of *Z*- and *E*-Isomers of Compound (1) by Capillary GC-MS Gas chromatographic separations of

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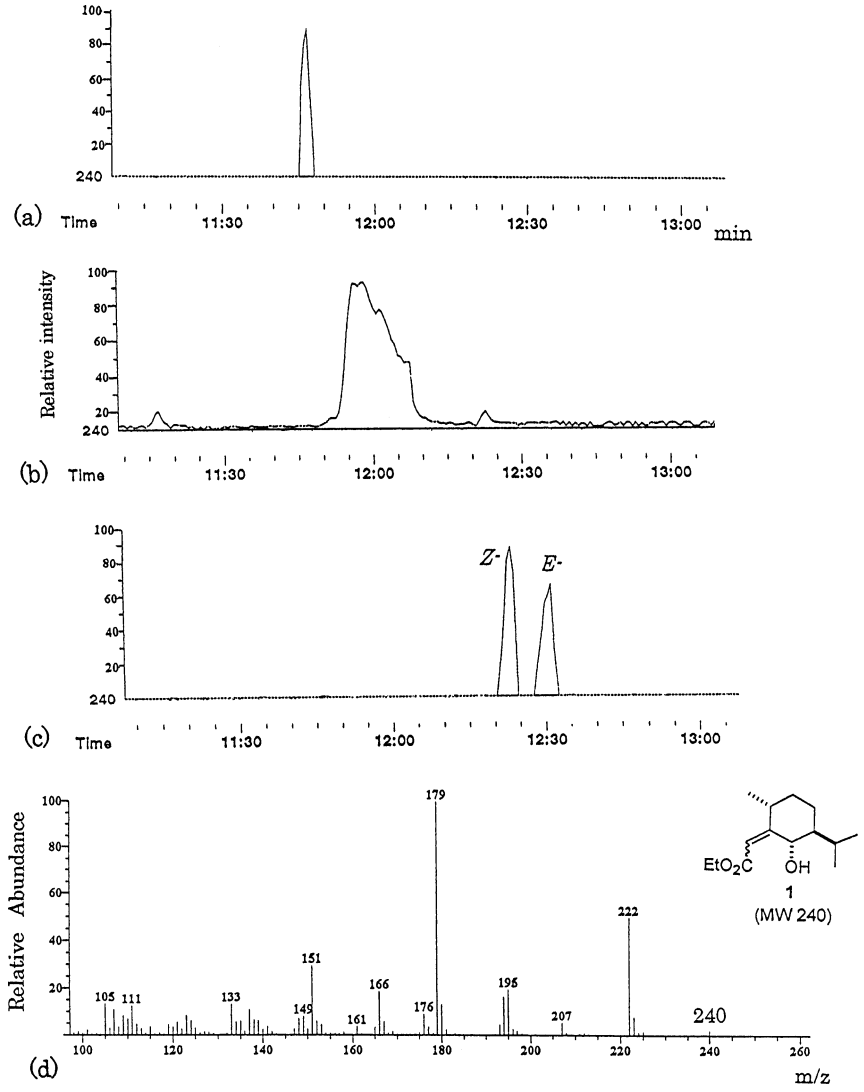

Fig. 1. Separation of *Z*- and *E*-Isomers of Compound (**1**) by GC-MS at 20 °C/min Heating Rate from 60 to 200 °C and Subsequent 10 °C/min from 200 to 220 °C with a 30 m×0.25 mm i.d. Capillary, 0.25-μm Film Thickness, (a) PME-β-CD, (b) DIAC-6-TBDS-β-CD, (c) DIME-6-TBDS-β-CD Columns and (d) Mass Spectrum of **1** with Electron Ionization

compound (**1**) were examined on several modified β-CD columns, including PME-β-CD, DIAC-6-TBDS-β-CD, and DIME-6-TBDS-β-CD. A sample (1 μl) was injected, and the column temperature was programmed to increase by 20 °C/min from 60 to 200 °C and subsequently at 10 °C/min from 200 to 220 °C. Furthermore, a slower approach to the final column temperature, at 2 °C/min from 200 to 220 °C, was also tried. Adequate resolution was not achieved on the PME-β-CD and DIAC-6-TBDS-β-CD columns. However, the DIME-6-TBDS-β-CD column was found to be suitable for the separation of **1**. A complete base-line separation of the isomers was achieved, with the elution order such that the *Z*-isomer eluted first, followed by the *E*-isomer. Their elution order may be affected by a difference in their geometric characteristics of this type.

Chromatographic separation of the *Z*- and *E*-isomers of compound (**1**) was accomplished with DIME-6-TBDS-β-CD as a stationary phase, even though a separation was not achieved with DIAC-6-TBDS-β-CD, which differs only in the 2- and 3-substituted groups. DIME-6-TBDS-β-CD exhibited superior recognition ability for stereoisomers of **1**. These results can be explained by a difference in the interactions between **1** and the methoxy or acetoxy groups at 2- and 3-positions of the modified β-CDs. Both the methoxy and acetoxy groups on the CD rim could act as hydrogen bond acceptors for the hydroxyl group of **1**. Differences in the hydrogen bonding patterns supported by the fact that Lewis basicity of the methoxy group is generally stronger than that of the acetoxy group may be one possible explanation for why DIME-6-TBDS-β-CD is superior to DIAC-6-TBDS-β-CD in discriminating between the isomers of **1**.

Separation of *Z*- and *E*-Isomers of Compound (1) by HPLC Two kinds of stationary phases were examined, β-CD and TME-β-CD, using mobile phases consisting of acetonitrile and H_2O. Base-line separations were achieved on both of these columns, with an elution order such that the *E*-isomer eluted first, followed by the *Z*-isomer.

Within the range of acetonitrile/water volume ratios from 40/60 to 90/10 in the mobile phase, the peak resolution (*Rs*) values obtained with TME-β-CD were commonly higher than those obtained with β-CD. Compound (**1**) contains a hydroxyl group that can form an inclusion complex and a hy-

drogen bonding interaction with the methoxy group of TME-β-CD or the hydroxyl group of β-CD. We propose that the ability of TME-β-CD to recognize differences in the interactions with each isomer of **1** should be superior to that for β-CD.

As a mobile phase, methanol–H_2O was also examined with the TME-β-CD column. With increasing methanol volume ratio in the mobile phase, the *Rs* values decreased. One explanation for this finding is that the hydroxyl group in methanol should reduce the ability of **1** to form hydrogen bonding with β-CD, which is supposed to be one of the important interactions. As a result, the degradation of the interaction in the β-CD cavity leads to a decrease in the *Rs* value. In contrast, within the range of acetonitrile/water volume ratios from 50/50 to 100/0 in the mobile phase, the *Rs* values were constantly greater than 1.8. Relating to the effect of the acetonitrile volume ratio in the mobile phase on the retention factor (k) with TME-β-CD column, within the range of acetonitrile/water volume ratios from 50/50 to 100/0, the k values decreased as acetonitrile volume ratio increased.

Separation of *Z*- and *E*-Isomers of Compound (2) by Capillary GC-MS For the separation of the *Z*- and *E*-isomers of compound (**2**) by capillary GC-MS, reasonable separations were obtained with each of the columns: PME-β-CD,

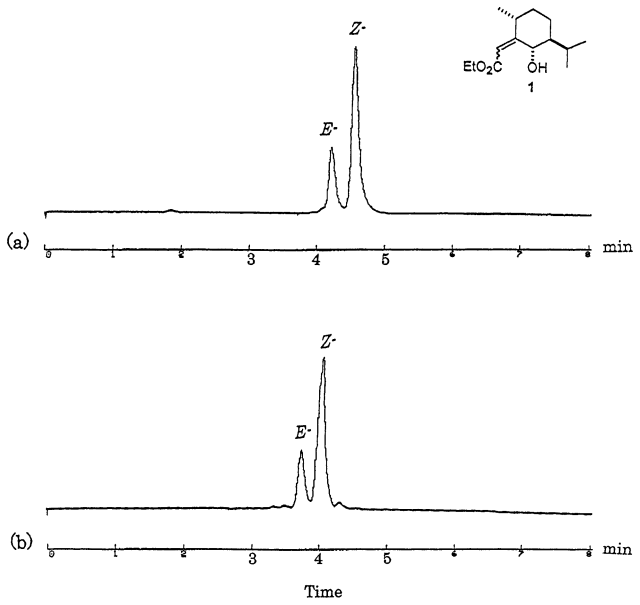

Fig. 2. Separation of *Z*- and *E*-Isomers of Compound (**1**) by HPLC with Acetonitrile–H_2O 80 : 20 v/v Mobile Phase at a Flow-Rate of 0.8 ml/min

Column: 250×4.6 mm i.d., packed with (a) TME-β-CD, (b) β-CD, bound on 5-μm silica-gel support.

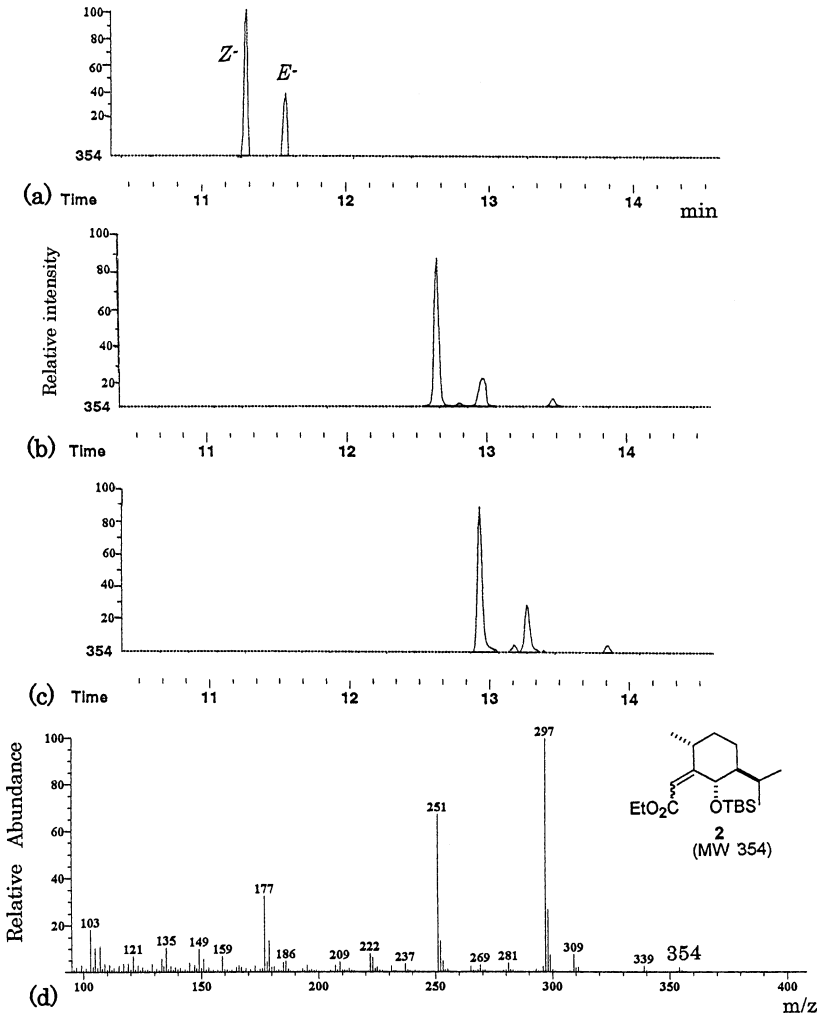

Fig. 3. Separation of *Z*- and *E*-Isomers of Compound (**2**) by GC-MS at 20 °C/min Heating Rate from 60 to 200 °C and Subsequent 10 °C/min from 200 to 220 °C with a 30 m×0.25 mm i.d. Capillary, 0.25-μm Film Thickness, (a) PME-β-CD, (b) DIAC-6-TBDS-β-CD, (c) DIME-6-TBDS-β-CD Columns and (d) Mass Spectrum of **2** with Electron Ionization

DIAC-6-TBDS-β-CD, and DIME-6-TBDS-β-CD. Complete base-line separations of each isomer were achieved, with the *Z*-isomer eluted first, followed by the *E*-isomer. The retention times were shortest with the PME-β-CD column, followed by the DIAC-6-TBDS-β-CD column, and finally the DIME-6-TBDS-β-CD column.

Separation of *Z*- and *E*-Isomers of Compound (2) by HPLC β-CD and TME-β-CD columns were examined as stationary phases with mobile phases consisting of acetonitrile and H_2O. The separations performed on β-CD resulted in an elution order in which the *Z*-isomer eluted first, followed by the *E*-isomer. However, sufficient separation was not achieved with TME-β-CD.

It is reasonable to suppose that compound (**2**), which is the TBS ether, could not form either an inclusion complex or a hydrogen bonding interaction with the methoxy group of TME-β-CD, leading to the result of incomplete separation.

Although methanol–H_2O, as a mobile phase, was also examined with the β-CD column, acetonitrile–H_2O was superior to the other from the viewpoint of both *Rs* and *k* values. Within the range of acetonitrile/water volume ratios from 50/50 to 80/20, the *k* values with β-CD column decreased with increasing of acetonitrile volume ratio in the mobile phase.

Conclusions

In the gas chromatographic separation of stereoisomers of menthol derivatives, the selectivity of the DIME-6-TBDS-β-CD column was superior to that of either DIAC-6-TBDS-β-CD or PME-β-CD. In contrast, for the corresponding TBS ether, the PME-β-CD column was superior to the others from the viewpoint of both retention factor and peak resolution values.

In high-performance liquid chromatographic separations, the TME-β-CD and native β-CD columns were suitable for the menthol derivative and the corresponding TBS ether, respectively, utilizing a mobile phase consisting of acetonitrile and H_2O.

The ratios of each peak area in the ion chromatogram or the absorption values that were obtained using GC-MS or HPLC were identical to the ratios of each isomer that were determined by ^{1}H-NMR spectroscopy.

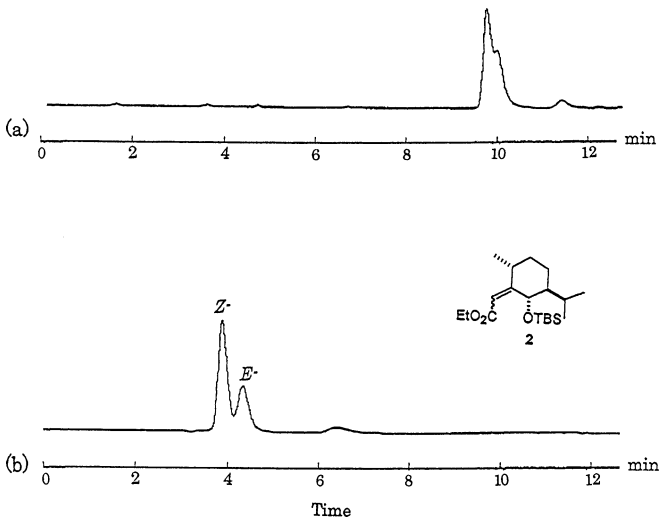

Fig. 4. Separation of *Z*- and *E*-Isomers of Compound (**2**) by HPLC with Acetonitrile–H_2O 65 : 35 v/v Mobile Phase at a Flow-Rate of 0.8 ml/min

Column: 250×4.6 mm i.d., packed with (a) TME-β-CD, (b) β-CD, bound on 5-μm silica-gel support.

Experimental

Reagents (−)-Menthone was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, U.S.A.). Acetic anhydride (Ac_2O), pyridine, *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), cerium(III) chloride heptahydrate, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), diisopropylamine, 4-(dimethylamino)pyridine (DMAP), 2,6-lutidine, and sodium borohydride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and *n*-BuLi (1.57 M hexane solution) from Kanto Chemical Co., Inc. (Tokyo, Japan). Ethyl glyoxylate was prepared by ozonolysis of diethyl maleate. Dehydrated THF and CH_2Cl_2, purchased from Wako Pure Chemical Industries, Ltd., were used for the reaction.

Synthesis IR spectra were obtained using a JASCO FT/IR-200 spectrometer (Tokyo, Japan). ^{1}H- and ^{13}C-NMR spectra were obtained on JEOL LAMBDA-270 and 500 instruments (Tokyo, Japan), and chemical shifts are reported on the δ scale from internal TMS. Mass spectra were measured with a JEOL JMS-600W spectrometer. Elemental analyses were performed on a YANACO-MT5 instrument (Kyoto, Japan).

Menthol derivatives (**1**) and (**2**) were synthesized as shown in Chart 1.

(1*R*,4*S*)-2-(Ethoxycarbonylhydroxymethyl)-*p*-menthan-3-one To a stirred solution of lithium diisopropylamide [prepared from 14.5 ml (104 mmol) of diisopropylamine and 62 ml (97.4 mmol) of *n*-BuLi (1.57 M hexane solution) in 150 ml of THF] was added a solution of 10.0 g (64.9 mmol) of (−)-menthone in 10 ml of THF at −78 °C under argon, and stirring was continued at the same temperature for 0.5 h. To the reaction mixture was added a solution of 15.8 g (156 mmol) of ethyl glyoxylate in 20 ml of THF, and stirring was further continued at the same temperature for 0.5 h. The mixture was quenched with brine and extracted three times with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (hexane–AcOEt, v/v=4 : 1) to give (1*R*,4*S*)-2-(ethoxycarbonylhydroxymethyl)-*p*-menthan-3-one (12.1 g, 73%) as a colorless oil. IR ν_{max} (1710, 1740, 3530 cm^{-1}); ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.83 and 0.88 (each 3H, each d, *J*=6.4 Hz, ($C\underline{H}_3$)$_2$CH), 1.15 (3H, d, *J*=6.3 Hz, 7-CH_3), 1.26 (3H, t, *J*=7.2 Hz, $CH_2C\underline{H}_3$), 1.37—1.62 (2H, m, 5-CH_2), 1.95—2.09 (5H, m, 6-CH_2, 8-CH, 1′-CH, OH), 2.63 (1H, d, *J*=0.7 Hz, 4-CH), 3.13 (1H, d, *J*=8.4 Hz, 2-CH), 4.16 (1H, d, *J*=7.9 Hz, C$\underline{H}$OH), 4.22 (2H, t, *J*=7.2 Hz, $C\underline{H}_2CH_3$); ^{13}C-NMR ($CDCl_3$; 67.8 MHz) δ 14.0, 18.5, 20.2, 21.0, 25.7, 27.9, 33.9, 36.1, 56.3, 60.5, 61.3, 67.9, 174.3, 212.4; MS (EI): 256 (M^+); HR-MS (EI) Calcd for $C_{14}H_{24}O_4$: 256.1674.

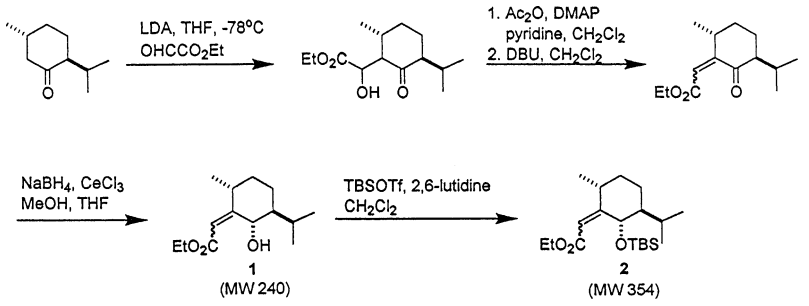

Chart 1. Preparation of Ethoxycarbonylmethylidenementhol Derivatives

Found: 256.1698. *Anal.* Calcd for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44. Found: C, 65.20; H, 9.44.

(1*R*,4*S*)-2-(Ethoxycarbonylmethylidene)-*p*-menthan-3-one A solution of 11.1 g (43.8 mmol) of the β-hydroxy ketone, 40 ml (424 mmol) of pyridine, 60 ml (742 mmol) of acetic anhydride, and 2.7 g (21.9 mmol) of 4-(dimethylamino)pyridine in 165 ml of CH_2Cl_2 was stirred at room temperature for 0.5 h. The mixture was diluted with water and extracted three times with Et_2O. The combined organic layer was washed with saturated aqueous potassium hydrogen sulfate solution and then with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated to give the corresponding acetate, which was further used for the next reaction without purification due to its instability.

A solution of the above acetate and 12.9 g (84.7 mmol) of DBU in 120 ml of CH_2Cl_2 was stirred at room temperature for 5 h. The mixture was diluted with water and extracted three times with Et_2O. The combined organic layer was washed with saturated aqueous potassium hydrogen sulfate solution and then with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (hexane–Et_2O, v/v=93 : 7) to give a geometric mixture of (1*R*,4*S*)-2-(ethoxycarbonylmethylidene)-*p*-menthan-3-one (6.7 g, 64%) as a colorless oil. The ^{1}H-NMR spectra of the enone showed the *Z*/*E* ratio to be 67 : 33.

Major (*Z*)-Enone: IR ν_{max} (1695, 1720 cm^{-1}); ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.88 and 0.95 (each 3H, each d, J=7.0 Hz, $(C\underline{H}_3)_2CH$), 1.09 (3H, d, J=7.3 Hz, 7-CH_3), 1.29 (3H, t, J=7.1 Hz, $CH_2C\underline{H}_3$), 1.77—2.00 (4H, m, 5-CH_2, 6-CH_2), 2.20 (1H, ddd, J=3.6, 6.1, 12.2 Hz, 4-CH), 2.42 (1H, d septet, J=3.6, 7.0 Hz, 8-CH), 4.07—4.16 (1H, m, 1-CH), 4.20 (2H, q, J=7.1 Hz, $C\underline{H}_2CH_3$), 6.18 (1H, d, J=0.8Hz, CH=C); ^{13}C-NMR ($CDCl_3$; 67.8 MHz) δ 14.1, 17.9, 19.1, 19.2, 20.4, 26.3, 30.1, 32.3, 56.9, 60.4, 119.7, 158.8, 166.0, 204.1; MS (EI): 238 (M^+); HR-MS (EI) Calcd for $C_{14}H_{22}O_3$: 238.1569. Found: 238.1579. *Anal.* Calcd for $C_{14}H_{22}O_3$: C, 70.55; H, 9.31. Found: C, 70.35; H, 9.31.

Because the (*E*)-enone could not be purified as a sole product, only the ^{1}H- and ^{13}C-NMR spectral data of the minor (*E*)-enone are shown.

Minor (*E*)-Enone: ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.85 and 0.91 (each 3H, each d, J=6.6 Hz, $(C\underline{H}_3)_2CH$), 1.13 (3H, d, J=7.1 Hz, 7-CH_3), 1.30 (3H, t, J=7.1 Hz, $CH_2C\underline{H}_3$), 1.35—1.45 (1H, m), 1.70—1.85 (1H, m), 1.90—2.20 (4H, m), 3.98—4.05 (1H, m, 1-CH), 4.19 (2H, q, J=7.1 Hz, $C\underline{H}_2CH_3$), 6.10 (1H, d, J=0.9Hz, CH=C); ^{13}C-NMR ($CDCl_3$; 67.8 MHz) δ 14.0, 19.1, 20.3, 20.4, 21.6, 27.2, 27.3, 32.5, 56.4, 60.3, 120.1, 159.9, 165.5, 206.9.

(1*R*,3*S*,4*S*)-2-(Ethoxycarbonylmethylidene)-*p*-menthan-3-ol (1) To a stirred solution of 2.53 g (10.6 mmol) of the enone and 26.5 ml (10.6 mmol) of 0.4 M $CeCl_3$ methanol solution in 60 ml of THF was added 0.403 g (10.6 mmol) of sodium borohydride at −78 °C, and stirring was continued at the same temperature for 1.5 h. The mixture was quenched with brine and extracted three times with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (hexane–Et_2O, v/v=85 : 15) to give a geometric mixture of (1*R*,3*S*,4*S*)-2-(ethoxycarbonylmethylidene)-*p*-menthan-3-ol (2.54 g, 100%) as a colorless oil. The ^{1}H-NMR spectrum of the allylic alcohol showed the *Z*/*E* ratio to be 67 : 33.

Major (*Z*)-Alcohol: IR ν_{max} (1715, 3450 cm^{-1}); ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.90 and 0.95 (each 3H, each d, J=7.0 Hz, $(C\underline{H}_3)_2CH$), 1.12 (3H, d, J=7.3 Hz, 7-CH_3), 1.25—1.34 (1H, m, 5-CHa), 1.28 (3H, t, J=7.1 Hz, $CH_2C\underline{H}_3$), 1.42 (1H, d, J=6.4 Hz, OH), 1.48—1.72 (4H, m, 6-CH_2, 4-CH, 5-CHb), 2.20 (1H, d septet, J=2.8, 7.0 Hz, 8-CH), 4.16 (2H, q, J=7.1 Hz, $C\underline{H}_2CH_3$), 4.17—4.24 (2H, m, 1-CH, 3-CH), 6.02 (1H, d, J=2.0 Hz, CH=C); ^{13}C-NMR ($CDCl_3$; 67.8 MHz) δ 14.3, 15.7, 18.2, 18.7, 23.0, 26.8, 31.0, 31.9, 59.6, 70.5, 73.8, 100.5, 109.9, 112.5; MS (EI): 240 (M^+); HR-MS (EI) Calcd for $C_{14}H_{24}O_3$: 240.1725. Found: 240.1716. *Anal.* Calcd for $C_{14}H_{24}O_3$: C, 69.96; H,10.07. Found: C, 69.85; H, 9.95.

Since the minor (*E*)-alcohol could not be purified at all, only the characteristic ^{1}H-NMR spectral data are shown.

Minor (*E*)-Alcohol: ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.87 and 1.02 (each 3H, each d, J=6.3 Hz, $(C\underline{H}_3)_2CH$), 1.17 (3H, d, J=7.3 Hz, 7-CH_3), 1.29 (3H, t, J=7.1 Hz, $CH_2C\underline{H}_3$), 5.94 (1H, br s, CH=C).

(1*R*,3*S*,4*S*)-2-(Ethoxycarbonylmethylidene)-3-(*tert*-butyldimethylsilyloxy)-*p*-menthane (2) A solution of 1.3 g (5.3 mmol) of the allylic alcohol, 2.3 g (21.4 mmol) of 2,6-lutidine, and 2.8 g (10.7 mmol) of *tert*-butyldimethylsilyl trifluoromethanesulfonate in 80 ml of CH_2Cl_2 was stirred at room temperature for 4 h. The mixture was diluted with water and extracted three times with Et_2O. The combined organic layer was washed with saturated aqueous potassium hydrogen sulfate solution and then with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (hexane–Et_2O, v/v=95 : 5) to give a geometric mixture of (1*R*,3*S*,4*S*)-2-(ethoxycarbonylmethylidene)-3-(*tert*-butyldimethylsilyloxy)-*p*-menthane (1.9 g, 99%) as a colorless oil. The ^{1}H-NMR spectrum of the silyl ether showed the *Z*/*E* ratio to be 67 : 33.

Major (*Z*)-Silyl Ether: IR ν_{max} (1715 cm^{-1}); ^{1}H-NMR ($CDCl_3$; 270 MHz) δ 0.00 and 0.05 (each 3H, each s, $Si(CH_3)_2$), 0.79 and 0.88 (each 3H, each d, J=7.0 Hz, $(C\underline{H}_3)_2CH$), 0.95 (9H, s, $(CH_3)_3C$), 1.10 (3H, d, J=7.1 Hz, 7-CH_3), 1.25 (3H, t, J=7.2 Hz, $CH_2C\underline{H}_3$), 1.29—1.59 (5H, m, 4-CH, 5-CH_2, 6-CH_2), 2.20 (1H, d septet, J=2.0, 7.0 Hz, 8-CH), 4.08—4.20 (4H, m, 1-CH, 3-CH, $C\underline{H}_2CH_3$), 5.96 (1H, d, J=1.7 Hz, CH=C); ^{13}C-NMR ($CDCl_3$; 67.8 MHz) δ −4.75, −4.21, 14.3, 15.9, 18.0, 18.2, 18.3, 21.5, 25.6, 26.1, 31.4, 32.5, 53.7, 59.4, 71.0, 110.6, 167.3, 168.8; MS (EI): 354 (M^+); HR-MS (EI) Calcd for $C_{20}H_{38}O_3Si$: 354.2590. Found: 354.2589. *Anal.* Calcd for $C_{20}H_{38}O_3Si$: C, 67.74; H, 10.80. Found: C, 67.70; H, 10.68.

Since the minor (*E*)-silyl ether could not be purified at all, only the characteristic ^{1}H-NMR spectral data are shown.

Minor (*E*)-Silyl Ether: ^{1}H-NMR ($CDCl_3$; 270 MHz) δ −0.01 and 0.02 (each 3H, each s, $Si(CH_3)_2$), 0.80 and 0.97 (each 3H, each d, J=6.7 Hz, $(C\underline{H}_3)_2CH$), 0.90 (9H, s, $(CH_3)_3C$), 1.14 (3H, d, J=7.3 Hz, 7-CH_3), 1.28 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 5.93 (1H, d, J=1.5 Hz, CH=C).

GC-MS Analysis A HP-6890 GC system from Agilent (Palo Alto, CA, U.S.A.) equipped with a JEOL JMS-BU20 mass spectrometer was used. A methanol solution of **1** or **2** was injected in the splitless mode. The injection port was set at 220 °C. Helium was used as the carrier gas in the constant flow mode. The mass spectrometer was operated at a filament current of 300 μA, an accelerating voltage of 2.5 kV, an electron energy of 70 V, an ion chamber temperature of 200 °C, and a resolution of 500.

The capillary columns used were β-DEX120, β-DEX225, and β-DEX325 (30-m×0.25-mm internal diameter), coated with 0.25-μm films of PME-β-CD, DIAC-6-TBDS-β-CD, and DIME-6-TBDS-β-CD, purchased from Supelco (Bellefonte, PA, U.S.A.).

HPLC Analysis A Shimadzu HPLC system (Kyoto, Japan) consisting of an LC-9A pump, a Model SPD-6AV UV-VIS detector operating at 230 nm, and a C-R4A Chromatopac data system was utilized. The mobile phase, consisting of water and methanol or acetonitrile, was pumped into the column at a flow-rate of 0.8 ml/min. A 2-μl aliquot of a methanol or acetonitrile solution of **1** or **2** was injected.

The columns used were OA7100 and OA7500 (250×4.6 mm i.d.), packed with β-CD and TME-β-CD, respectively, bound on 5-μm silica-gel support. Both of these columns were purchased from Sumika Chemical Analysis Service (Osaka, Japan).

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