Chromatographic Separation Based on Isotopic Chirality

Kazuhiro Kimata,[†,‡] Mariko Kobayashi,[†] Ken Hosoya,[‡] Takeo Araki,[‡] and Nobuo Tanaka*[,‡]

Contribution from the Nacalai Tesque, 17 Ishibashi, Kaide-cho, Muko-shi, Kyoto 617, Japan, and Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan

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Abstract: The first example of chromatographic separation based on isotopic chirality is shown. Diastereomers of methyl 3-phenyl-3-phenyl-d_5-glycidate were separated by reversed-phase high-performance liquid chromatography based on the isotopic chirality provided by the presence of phenyl and phenyl-d_5 groups at one of the two chiral centers. Each of the two separated peaks was fractionated and subsequently separated into two peaks representing individual enantiomers having isotopic chirality by using a chiral stationary phase.

Chiral compounds and the recognition of enantiomers are ubiquitous in nature and essential for living species. Various chromatographic methods are available for the separation of enantiomers. Differentiation of isomeric compounds based on isotopic chirality, however, presents one of the most difficult problems even for spectroscopic detection. It has been believed that enantiomers or diastereomers owing their chirality to isotopic substitution are not amenable to separation by known methods[1−3] including chromatography or fractional recrystallization. Old reports[4] describing the separation of diastereomers based on isotopic chirality have been disputed by later studies.[3,5] We report here a successful separation based on isotopic chirality achieved by reversed-phase liquid chromatography (RPLC) with diastereomers of methyl 3-phenyl-3-phenyl-d_5-glycidate (**1**−**4**) having phenyl and phenyl-d_5 groups at one of the two chiral centers.

Experimental Section

Benzophenone-d_5 and benzophenone-d_{10} were prepared from benzene-d_6 by reacting with benzoyl chloride and benzoyl chloride-d_5, respectively. Methyl 3-phenyl-3-phenyl-d_5-glycidate (**1**−**4**), methyl 3,3-di(phenyl-d_5)glycidate (**5**; a racemic mixture of 2*R* and 2*S*), and methyl 3,3-diphenylglycidate (**6**; a racemic mixture of 2*R* and 2*S*) were prepared by Darzen reaction with methyl chloroacetate from benzophenone-*2,3,4,5,6*-d_5, benzophenone-d_{10}, and benzophenone without deuterium, respectively, in the presence of potassium *tert*-butoxide.[6] Alkyl 3-alkyl-3-phenylglycidates were prepared by a similar method. The products were purified by column chromatography on silica gel using a hexane−ethyl acetate (9/1) mixture as solvent.

The structures of the products were confirmed by NMR (200 MHz, $CDCl_3$) and MS. **1**−**4**: ^{1}H NMR δ 3.52 (s, 3H), 3.99 (s, 1H), 7.30−7.44 (m, 5H); MS *m/e* 77 (16), 82 (17), 105 (17), 110 (19), 169 (76), 170 (100), 198 (63), 199 (99); high-resolution MS calcd for $C_{16}H_9D_5O_3$ 259.1254, found 259.1252. **5**: ^{1}H NMR δ 3.52 (s, 3H), 4.00 (s, 1H); MS *m/e* 82 (16), 110 (24), 174 (100), 203 (84); high-resolution MS calcd for $C_{16}H_4D_{10}O_3$ 264.1569, found 264.1574. **6**: ^{1}H NMR δ 3.51 (s, 3H), 4.00 (s, 1H), 7.29−7.50 (m, 10H); MS *m/e* 77 (29), 105 (27), 165 (100), 194 (96); high-resolution MS calcd for $C_{16}H_{14}O_3$ 254.0939, found 254.0950.

The structures of alkyl 3-alkyl-3-phenylglycidates were confirmed by ^{1}H-NMR (200 MHz, $CDCl_3$).[6,7] Methyl (*E*)-3-methyl-3-phenylglycidate: δ 1.77 (s, 3H, CH_3), 3.47 (s, 1H, CHCOO), 3.84 (s, 3H, CH_3O), 7.27−7.41 (m, 5H, C_6H_5). Methyl (*Z*)-3-methyl-3-phenylglycidate: δ 1.75 (s, 3H, CH_3), 3.44 (s, 3H, CH_3O), 3.70 (s, 1H, CHCOO), 7.26−7.41 (m, 5H, C_6H_5). Ethyl (*E*)-3-phenylglycidate: δ 1.33 (t, 3H, CH_3CH$_2$O), 3.51 (d, 1H, CHCOO), 4.09 (d, 1H, CHC$_6$H$_5$), 4.15−4.43 (m, 2H, CH$_3$CH_2O), 7.16−7.54 (m, 5H, C_6H_5). Ethyl (*Z*)-3-phenylglycidate: δ 1.01 (t, 3H, CH_3CH$_2$O), 3.82 (d, 1H, CHCOO), 3.88−4.12 (m, 2H, CH$_3$CH_2O), 4.27 (d, 1H, CHC$_6$H$_5$), 7.25−7.45 (m, 5H, C_6H_5). Ethyl (*E*)-3-methyl-3-phenylglycidate: δ 1.33 (t, 3H, CH_3CH$_2$O), 1.77 (s, 3H, CH_3), 3.46 (s, 1H, CHCOO), 4.24−4.36 (m, 2H, CH$_3$CH_2O), 7.31−7.41 (m, 5H, C_6H_5). Ethyl (*Z*)-3-methyl-3-phenylglycidate: δ 0.89 (t, 3H, CH_3CH$_2$O), 1.74 (s, 3H, CH_3), 3.67 (s, 1H, CHCOO), 3.67−3.97 (m, 2H, CH$_3$CH_2O), 7.25−7.35 (m, 5H, C_6H_5). Ethyl (*E*)-3-ethyl-3-phenylglycidate: δ 0.95 (t, 3H, CH_3CH$_2$), 1.34 (t, 3H, CH_3CH$_2$O), 1.81−1.99 (m, 1H, CH$_3$CH_2), 2.08−2.27 (m, 1H, CH$_3$CH_2), 3.47 (s, 1H, CHCOO), 4.25−4.36 (m, 2H, CH$_3$CH_2O, 7.30−7.45 (m, 5H, C_6H_5). Ethyl (*Z*)-3-ethyl-3-phenylglycidate: δ 0.87 (t, 3H, CH_3CH$_2$O), 0.93 (t, 3H, CH_3CH$_2$), 1.72−1.90 (m, 1H, CH$_3$CH_2), 2.10−2.28 (m, 1H, CH$_3$CH_2), 3.69 (s, 1H, CHCOO), 3.78−3.98 (m, 2H, CH$_3$CH_2O), 7.25−7.42 (m, 5H, C_6H_5). Ethyl (*E*)-3-phenyl-3-propylglycidate: δ 0.89 (t, 3H, CH_3CH$_2$CH$_2$), 1.34 (t, 3H, CH_3CH$_2$O), 1.20−1.55 (m, 2H, CH$_3$CH_2CH$_2$), 1.75−1.90 (m, 1H, CH$_3$CH$_2$CH_2), 2.06−2.21 (m, 1H, CH$_3$CH$_2$CH_2), 3.44 (s, 1H, CHCOO), 4.25−4.37 (m, 2H, CH$_3$CH_2O), 7.27−7.43 (m, 5H, C_6H_5). Ethyl (*Z*)-3-phenyl-3-propylglycidate: δ 0.87 (t, 3H, CH_3CH$_2$O), 0.90 (t, 3H, CH_3CH$_2$CH$_2$), 1.20−1.53 (m, 2H, CH$_3$CH_2CH$_2$), 1.62−1.78 (m, 1H, CH$_3$CH$_2$CH_2), 2.09−2.24 (m, 1H, CH$_3$CH$_2$CH_2), 3.66 (s, 1H, CHCOO), 3.83−3.95 (m, 2H, CH$_3$CH_2O), 7.24−7.42 (m, 5H, C_6H_5).

RPLC separation was carried out by using a recycle column system consisting of four columns of 6 mm i.d., 15 cm in length packed with 5 μm octadecylsilylated (C_{18}) silica (Cosmosil C_{18} MS), in 65% methanol at 30 °C. A pump with two 10 μL displacement heads (Shimadzu LC-9A) was used to minimize band broadening, allowing the recycle operation through the pump. Sample bands passed through the cell (8 μL) of a variable-wavelength UV detector (Shimadzu SPD-6A) in each cycle. Solutes were detected at 254 nm. Consistent peak areas were observed for the solutes **1**−**4**, **5**, and **6** during recycle

* Author to whom correspondence should be addressed.
† Nacalai Tesque.
‡ Kyoto Institute of Technology.
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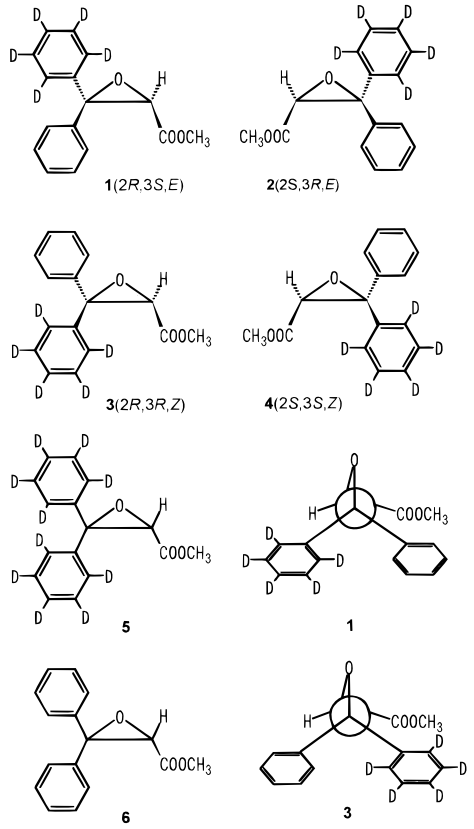

Figure 1. Structures of methyl 3-phenyl-3-phenyl-d_5-glycidate (**1**−**4**), methyl 3,3-di(phenyl-d_5)glycidate (**5**), and methyl 3,3-diphenylglycidate (**6**).

separation, indicating the stability of these compounds under the separation conditions. A polarimetric detector (Shodex OR-1) was also used for chiral separation.

Another recycle system consisting of four columns of 10 mm i.d., 15 cm in length packed with the C_{18} silica, was used for the fractionation of the diastereomers in **1**−**4**. The collected fractions each containing a pair of enantiomers were subjected to chiral separation. Cellulose *p*-methylbenzoate-coated silica was prepared from 5 μm silica particles with 100 nm pore size (Nucleosil)[8] and used for chiral separation.

Results and Discussion

The structures of the four stereoisomers (**1**−**4**), having isotopic chirality, are shown in Figure 1. A chiral center at C_2 and deuterium substitution on one of the two phenyl groups on C_3 create diastereomers, (*E*)-isomers **1** (2*R*,3*S*) and **2** (2*S*,3*R*), possessing the deuterated phenyl group close to α-hydrogen, and (*Z*)-isomers **3** (2*R*,3*R*) and **4** (2*S*,3*S*), with the opposite configuration. The 500 MHz ^{1}H NMR spectrum of a mixture of **1**−**4**, however, only showed singlets for the α-hydrogen and the methyl group in $CDCl_3$, indicating a very similar electronic environment for these groups in spite of the deuterium substitution. We attempted the total separation of **1**−**4**, first into two pairs of enantiomers, **1** and **2**, and **3** and **4**, by RPLC using C_{18} stationary phase that is achiral, then into individual enantiomers by the discrimination of chirality at C_2 using a chiral stationary phase.

Recycle chromatography using the 60 cm column system packed with C_{18} silica resulted in a separation of a mixture of **1**−**4** into two peaks with ca. 600 000 theoretical plates at 18 cycles, as shown in Figure 2.[9] The two peaks are presumably provided by the diastereomer separation between (*E*)-isomers

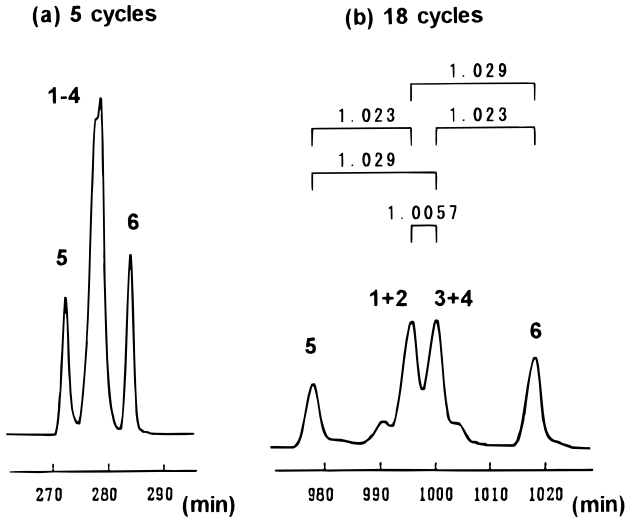

Figure 2. Separation of diastereomers of methyl 3-phenyl-3-phenyl-d_5-glycidate (**1**−**4**) based on isotopic chirality. Compounds **5** and **6** were included in the sample mixture. Stationary phase: C_{18} silica, four columns of 6 mm i.d., 15 cm in length. Mobile phase: methanol−water = 65/35. Detection: UV 254 nm. Solutes were recycled through the column system, the detector, and the pump.

(**1** and **2**) and (*Z*)-isomers (**3** and **4**), because the achiral C_{18} stationary phase cannot differentiate the enantiomers (**1** and **2**, or **3** and **4**). This is the first example of chromatographic separation based on isotopic chirality, and was achieved by the differentiation between the phenyl and phenyl-d_5 groups by the neighboring chiral group that produced differences in chromatographic behavior between the diastereomers. A mechanism of differentiation of the isotopic chirality and structural assignment of the two peaks are provided as follows.

The mixture of **1**−**4** was easily separated from **5** and **6** on the basis of the difference in the number of deuterium atoms present on the phenyl groups. Solute retention in RPLC is dominated by hydrophobic effects[10−13] which include unfavorable interactions between hydrophobic species and aqueous solvents. Since an aqueous phase favors CD groups over CH groups, deuterated species elute earlier than protiated species in RPLC.[13−15] Greater deuterium isotope effects were observed for compounds exposing the larger molecular surface area of deuterated moieties to aqueous solvents.[13] The early-eluting compounds in a mixture of **1**−**4** are supposed to have the deuterated phenyl group close to α-hydrogen to produce the greater isotope effect due to the less steric hindrance of hydrophobic solvation. The assignment that the first peak is a racemic mixture of **1** (2*R*,3*S*) and **2** (2*S*,3*R*), and the second peak that of **3** (2*R*,3*R*) and **4** (2*S*,3*S*), agreed with the results obtained with several alkyl 3-alkyl-3-phenylglycidates. As

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(9) The minor peaks seen in the leading edge of the first peak (**1** + **2**) and the trailing edge of the second peak (**3** + **4**) for the mixture of **1**−**4** were presumably caused by methyl 3,3-diphenylglycidate with deuterium substitution greater and fewer than phenyl-d_5 by one deuterium atom, respectively, coproduced during the preparation of **1**−**4**, as substantiated by a small extent of deuterium scrambling on benzophenone-*2,3,4,5,6*-d_5 in the presence of potassium *tert*-butoxide.

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Table 1. Separation Factors between Diastereomers of Alkyl 3-Alkyl-3-phenylglycidate[a]

R_1[b]	R_2[c]	$k'(E)$[d]	$k'(Z)$[e]	α[f]
CH_3	CH_3	1.12	0.72	1.55
H	C_2H_5	1.44	0.96	1.51
CH_3	C_2H_5	1.90	1.23	1.54
C_2H_5	C_2H_5	2.65	1.93	1.37
n-C_3H_7	C_2H_5	4.12	3.20	1.29

[a] Column: Cosmosil C_{18}-MS, 4.6 mm i.d., 15 cm long. Mobile phase: 70% methanol. 30 °C. [b] Alkyl group on C_3. [c] Alkyl group on ester oxygen. [d,e] k' values of (*E*)- and (*Z*)-alkyl 3-alkyl-3-phenylglycidate, respectively. [f] Separation factor between (*E*)- and (*Z*)-alkyl 3-alkyl-3-phenylglycidate, $k'(E)/k'(Z)$.

shown in Table 1, an isomer having the smaller alkyl group close to the α-hydrogen was eluted earlier. Incidentally, the size of a phenyl-d_5 group should be slightly smaller than that of a phenyl group because of the shorter C−D bond than C−H.

The elution of **1**−**4** with **5** and **6** permits the estimation of the isotope effect caused by the presence of a deuterated phenyl group. The deuterium isotope effects on retention (k'_H/k'_D) observed between (**1** + **2**) and **6**, and between (**3** + **4**) and **5** are 1.029 per phenyl-d_5 group that is more exposed to the aqueous phase. This is slightly greater than the isotope effects of 1.023 seen between (**1** + **2**) and **5**, and between (**3** + **4**) and **6** per phenyl-d_5 group that is eclipsed with the methoxycarbonyl group. These isotope effects are comparable with the isotope effects per phenyl-d_5 group on the retention of benzophenone (1.026) or benzhydrol (1.024) found in the same mobile phase. The separation factor between the diastereomers (**1** + **2**) and (**3** + **4**) provided by the isotopic chirality based on the positioning of a phenyl and a phenyl-d_5 group is much smaller, ca. 1.0057. This corresponds to the difference in a free energy change of ca. 3.4 cal/mol associated with the transfer of these molecules from the methanol−water (65/35) mixture to the C_{18} stationary phase.

Each of the two peaks corresponding to (**1** + **2**) and (**3** + **4**) was fractionated by recycle chromatography on 10 mm i.d. semipreparative columns under similar conditions as in Figure 2. The recovered materials showed consistent chromatographic properties in RPLC as well as in chiral separations. No peak splitting was observed with **5** or **6** during the recycle chromatography. These facts indicate the stability of the epoxy ester during the recycle separation. The two fractionated enantiomer pairs were subjected to chiral separation. Figure 3 shows the separation on a chiral stationary phase between **1** and **2**, and between **3** and **4** contained in separated peaks in Figure 2. The chiral stationary phase provided an easy separation based on the chirality at the α-carbon between 2*R* and 2*S* forms, resulting in the total separation of **1**−**4**. The chiral stationary phase, however, could not separate the diastereomers based on the isotopic chirality at C_3. The polarimetric detection indicates the same elution orders between the enantiomers for (*E*)- and (*Z*)-isomer mixtures as seen with the direct separation of **1**−**4**, (−)-form followed by the (+)-form at the α-carbon, although the absolute configuration has not been assigned for each peak. The total separation of **1**−**4** was cross-checked by first separating the (−)-form and (+)-form on the chiral stationary phase, followed by the separation into the four individual stereoisomers by RPLC. It will be possible to obtain an enantiomer of β-hydroxy carboxylic acid[16] having chirality based only on isotopic substitution from each of the separated stereoisomers (**1**−**4**).

The minute but clear separation based on the isotopic chirality was enabled by careful selection of the conditions. First of all,

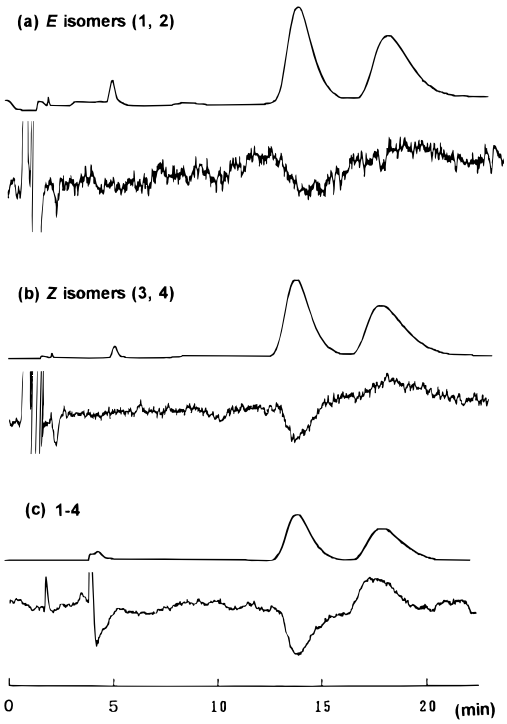

Figure 3. Separation of enantiomers in each of the two peaks in Figure 2 [(a) **1** and **2**, (b) **3** and **4**] and of **1**−**4** on a chiral stationary phase (c). Upper trace: UV detection at 254 nm. Lower trace: polarimetric detection. Column: cellulose *p*-methylbenzoate-coated silica gel[8] (5 μm particles, 100 nm pore size), 4.6 mm i.d., 15 cm long. Mobile phase: 2-propanol−hexane = 20/80. The samples for (a) and (b) were obtained by RPLC separation of **1**−**4** by using semipreparative C_{18} columns (10 mm i.d., 15 cm × 4) operated as in Figure 2, prior to this separation of enantiomers.

RPLC was employed to provide high efficiency that had enabled isotope separations[13−15,17] for H/D, $^{14}N/^{15}N$, and $^{16}O/^{17}O/^{18}O$. Chiral stationary phases to be used for the separation of enantiomers are not as efficient as those for RPLC. Secondly, the substrate was prepared to have aromatic CH(CD) groups rather than aliphatic CH(CD) to provide maximum isotope effects in RPLC, and to have a chiral group directly bonded to the isotopic chiral center to form diastereomers. Finally, chromatographic separation was carried out in a mobile phase with a water content as high as possible to maximize the isotope effect[13,14] while keeping the k' range at around 3 for separation efficiency. The separation factor between (**1** + **2**) and (**3** + **4**) was 1.0055 in 70% methanol, and smaller at higher methanol contents. Increase in the size of a substituent on C_2 adversely affected the differentiation of the isotopic chirality. The separation factor of 1.0048 was obtained for diastereomers of ethyl (*E*)- and (*Z*)-3-phenyl-3-phenyl-d_5-glycidate.

Chromatographic separation of chiral compounds owing their chirality to isotopic substitution has been believed to be impossible, and such individual isomers have been prepared with considerable difficulty. The present results have proved the possibility of differentiation of isotopic chirality, and suggest further study aiming at a direct separation of enantiomers owing their chirality to isotopic substitution. We can anticipate some difficulties for such true recognition of isotopic chirality that will be one of the extremes of molecular recognition. First of all, it will require a chiral stationary phase with very high efficiency. The efficiencies of chiral stationary phases currently

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available are much lower than those for RPLC. Secondly, direct recognition of isotopic chirality will be accomplished on the basis of intermolecular interactions between the substituents on chiral centers at a greater distance than the present case, which would be less effective for discrimination of isotopic groups. In the present case, a separation factor of 1.0057 was obtained with the phenyl and phenyl-d_5 groups on one chiral center with the neighboring chiral center about 1.5 Å away having a hydrogen and a methoxycarbonyl group at the eclipsed positions that would be a favorable arrangement for discrimination. Because intermolecular interactions will rarely involve eclipsed arrangements between the interacting groups such as phenyl, the separation of noncyclic diastereomers with isotopic chirality will allow further estimation for the possibility of the direct separation based on isotopic chirality. It will be of much interest to examine the recognition of isotopic chirality with various host molecules as well as naturally occurring chiral selectors such as proteins in addition to conventional chromatographic stationary phases.

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