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Diastereomer Method for Determining % ee by ^{1}H NMR and/or MS Spectrometry with Complete Removal of the Kinetic Resolution Effect

Hiromi Taji,[†] Masataka Watanabe,[†] Nobuyuki Harada,*[,†] Hideo Naoki,[‡] and Yoshihisa Ueda[§]

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba, Sendai 980-8577, Japan, Suntory Institute for Bioorganic Research, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503, Japan, and JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan

n-harada@tagen.tohoku.ac.jp

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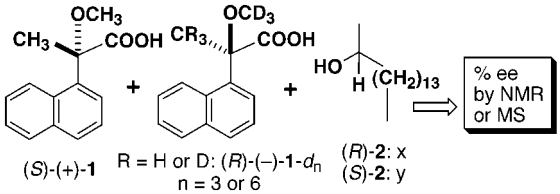
ABSTRACT



Using chiral auxiliaries, 2-methoxy-2-(1-naphthyl)propionic acid (MαNP acid) (*S*)-(+)-1 and its deuterium-labeled enantiomer (*R*)-(−)-1-d_n (n = 3 or 6), we have developed a new diastereomer method for determining enantiomeric excess (% ee) of chiral alcohols by ^{1}H NMR and/or MS spectrometry, where the kinetic resolution effect is completely excluded. The data of % ee determined by this method agree well with those calculated by weight, the average error being ca. ±1.08% ee.

Recently we have reported that 2-methoxy-2-(1-naphthyl)-propionic acid (MαNP acid **1**, Scheme 1) is very powerful for determining the absolute configurations of chiral alcohols by the ^{1}H NMR anisotropy method.[1−3] This acid **1** is superior to conventional chiral acids, e.g., Mosher's α-methoxy-α-trifluoromethylphenylacetic acid (MTPA acid)[4] and Trost's α-methoxyphenylacetic acid (MPA acid),[5] because of its stronger anisotropy effect. Unlike methoxy(1- and 2-naphthyl)acetic acids (1-NMA and 2-NMA),[6,7] the MαNP acid **1** has the advantage of not racemizing, and therefore enantiopure MαNP acid **1** is easily accessible. In addition, the acid **1** has a great power to enantioresolve racemic alcohols, especially acyclic aliphatic alcohols, by HPLC of MαNP esters.[3] For example, racemic 2-hexadecanol (±)-**2** was esterified with acid (*S*)-(+)-**1**, yielding a diastereomeric mixtures of esters, which was easily separated by HPLC on silica gel (hexane/EtOAc 20:1): separation factor $\alpha = 1.93$; $Rs = 3.68$. These characteristics of acid **1** enabled us to develop a new diastereomer method for determining enantiomeric excess (% ee) of chiral alcohols by ^{1}H NMR and/

[†] Tohoku University.
[‡] Suntory Institute.
[§] JEOL Ltd.

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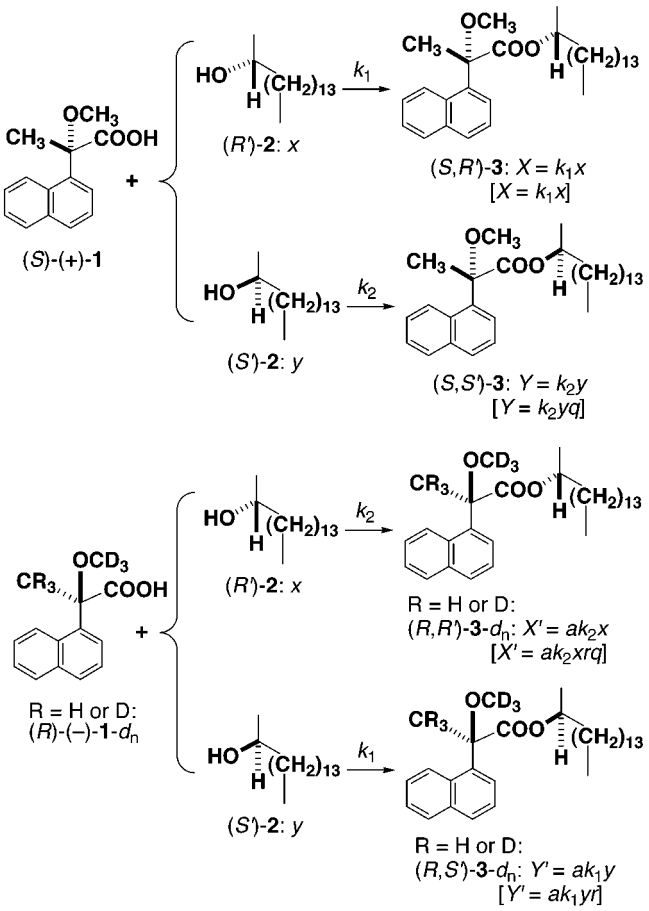
Scheme 1[a]



[a] $n = 3$ or 6. Parameters k_1 and k_2 are proportional coefficients including kinetic resolution factors. Parameter a is coefficient reflecting the abundance of labeled (*R*)-(−)-**1**-d_n and isotope effects. The equations in brackets are for MS spectrometry, where q and r are the parameters of ionization efficiency for diastereomer and deuterium labeled isomer, respectively.

or MS spectrometry. We report here the principle and practice of this method.

There are many methods for determining the enantiomeric excess (% ee) of chiral compounds: (1) chiroptical method to compare $[\alpha]_D$ or CD intensity; (2) HPLC or GC with chiral stationary phase,[8] or electrophoresis with chiral supporting electrolyte; (3) ^{1}H NMR spectroscopy with chiral organometallic shift reagents;[9] (4) HPLC or ^{1}H NMR of diastereomers prepared with chiral derivatizing agents;[10] (5) MS spectrometry with chiral derivatizing agents or chiral host compounds;[11−14] and (6) kinetic methods.[15] These methods have both advantages and disadvantages. In some cases, the calibration curves have to be made using the standard samples of known enantiopurity. In other cases, peak broadening disturbs exact determination of peak intensity. For the diastereomer methods using chiral derivatizing agents, the most essential problem is how to evaluate the effect of kinetic resolution. If the reaction with derivatizing agent proceeds in 100% yield, the term of kinetic resolution is excluded. However, the reaction of 100% yield is not practical. On the other hand, the method based on the kinetic resolution effect contains approximations and needs calibration curves. Therefore the diastereomer methods for determining % ee have been always troubled by the kinetic resolution effect. However, as shown below, we have first succeeded in the development of a diastereomer method for determining % ee by ^{1}H NMR and/or MS spectrometry with complete removal of the kinetic resolution effect.

The principle and procedure of this method are explained using a model compound of 2-hexadecanol **2** as follows. A mixture (ca. 1:1) of MαNP acid (*S*)-(+)-**1** and deuterium labeled (*R*)-(−)-**1**-d_3 was allowed to react with chiral alcohol (**2**, 0−100% ee) to give a diastereomeric mixture of esters (Scheme 1, R = H, $n = 3$). The compositions of (*R*′)-**2** and (*S*′)-**2** are defined as x and y, respectively, and $x + y = 1$. Similarly the compositions of esters formed are defined: ester (*S*,*R*′)-**3**, X; (*S*,*S*′)-**3**, Y; (*R*,*R*′)-**3**-d_3, X'; (*R*,*S*′)-**3**-d_3, Y'. The amounts of esters are formulated as $X = k_1x$ and $Y = k_2y$, where k_1 and k_2 are proportional coefficients including kinetic resolution factor (note that those are not rate constants). Since esters (*S*,*S*′)-**3** and (*R*,*R*′)-**3**-d_3 are enantiomers of each other, the same coefficient k_2 can be used to define the amount of (*R*,*R*′)-**3**-d_3: $X' = ak_2x$, where a is coefficient reflecting the abundance of (*R*)-(−)-**1**-d_3 and isotope effects of ester formation. For the remaining ester (*R*,*S*′)-**3**-d_3, $Y' = ak_1y$. By taking the ratio X/Y', those equations are reformed as shown in eq 1, where k_1, proportional coefficient including kinetic resolution factor, is canceled:

$$X'/Y = (k_1x)/(ak_1y) = (1/a)x/y \quad (1)$$

Similarly k_2 is also canceled:

$$X/Y' = (ak_2x)/(k_2y) = (a)x/y \quad (2)$$

The product of eqs 1 and 2 gives eq 3, where a, coefficient reflecting the abundance of (*R*)-(−)-**1**-d_3 and isotope effects, is also canceled

$$(X/Y')(X'/Y) = [(1/a)x/y][(a)x/y] = (x/y)^2 = [(1st,M)/(1st,M+3)][(2nd,M+3)/(2nd,M)] \quad (3)$$

A mixture of those diastereomers is separable by HPLC on silica gel (22ϕ × 300 mm, hexane/EtOAc 20:1) (Figure

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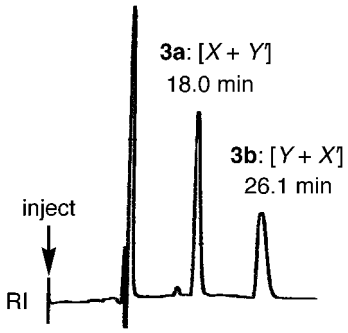

Figure 1. HPLC separation of diastereomeric esters **3a** and **3b**: silica gel glass column (22ϕ × 300 mm); n = 9500−11600; hexane/EtOAc 20:1; α = 1.96, *Rs* = 4.15.

1). The first-eluted fraction contains esters (*S*,*R*′)-**3** and (*R*,*S*′)-**3**-d_3, and the ratio of components X/Y' = (1st,M)/(1st,M+3) can be determined from the ^{1}H NMR intensities of methoxyl and methyl groups of MαNP moiety. Namely, the intensity of the methoxyl group corresponds to X, while that of the methyl group to $X + Y'$. Similar treatment is applicable to the second-eluted fraction containing (*S*,*S*′)-**3** and (*R*,*R*′)-**3**-d_3. The ratio X'/Y = (2nd,M+3)/(2nd,M) is obtainable by ^{1}H NMR. By substituting the observed ratio into eq 3, $(x/y)^2$ is calculated, and since $x + y = 1$, the enantiomeric excess (% ee) of alcohol **2** is obtained.

Deuterium labeled MαNP acid (*R*)-(−)-**1**-d_3 was prepared by methylation of methyl 2-hydroxy-2-(1-naphthyl)-propionate with CD_3I (D content >99.5 atom %) followed by enantioresolution with (−)-menthol. A mixture of (*S*)-(+)-**1** and (*R*)-(−)-**1**-d_3 (ratio, 1:0.987, total 8.1 mg, 0.0349 mmol) was allowed to react with the test sample of chiral 2-hexadecanol (**2**, 9.237 mg, 1.09 × 0.0349 mmol, 60.9% ee calculated by weight) yielding a diastereomeric mixture of esters, which was separated by HPLC on silica gel (hexane/EtOAc 20:1) (Figure 1). From the ^{1}H NMR of the first-eluted fraction, the compositions of ester (*S*,*R*′)-**3** and (*R*,*S*′)-**3**-d_3 were determined: X = 1.00, Y' = 0.22, X/Y' = 4.54. Similarly from the ^{1}H NMR of the second fraction, the compositions of (*S*,*S*′)-**3** and (*R*,*R*′)-**3**-d_3 were determined: Y = 1.00, X' = 3.60, X'/Y = 3.60. The product $(X/Y')(X'/Y)$ = 16.3636, and so x/y = 4.045. Since $x + y = 1$, x = 0.8017 and y = 0.1982, leading to 60.4% ee, which reasonably agrees with 60.9% ee calculated by weight. This method has been applied to 10 test samples **2** with 0−90% ee. As seen in Figure 2, the % ee values obtained by ^{1}H NMR agree well with those calculated by weight: average error, ±0.4% ee; maximum error, 1.3% ee. Those results clearly indicate that our % ee determination method is successful. When expanding to 5 test samples with 92−100% ee, however, the deviation became larger: average error, ±1.2% ee; maximum error, 6.3% ee. Such larger deviation may be due to the limit of intensity determination for weak peaks in ^{1}H NMR. To overcome those difficulties, we next employed MS spectrometry to determine the composition, because MS is much more sensitive than NMR.

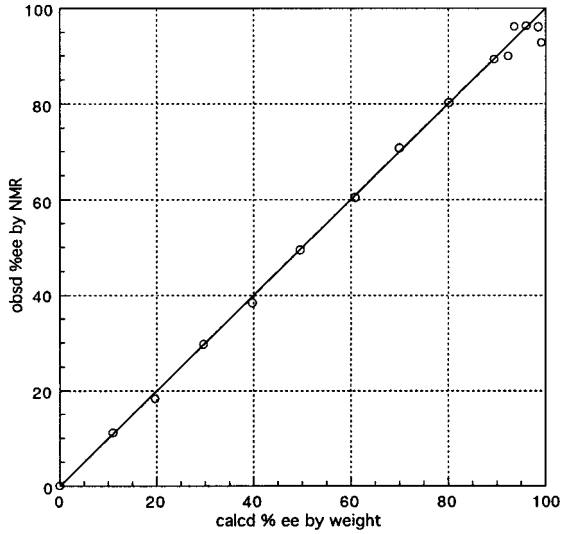

Figure 2. Comparison of % ee values determined by NMR with those calculated by weight: (*R*)-2-hexadecanol **2** > (*S*)-**2**.

In the case of MS spectrometry, the factor of ionization efficiency (f) for each isomer was added as follows: for ester (*S*,*R*′)-**3**, $f = 1$ and so $X = k_1x$; for (*S*,*S*′)-**3**, $f = q$ and $Y = k_2yq$; for (*R*,*S*′)-**3**-d_n (n = 3 or 6), $f = r$ and $Y' = ak_1yr$; for (*R*,*R*′)-**3**-d_n (n = 3 or 6), $f = rq$ and $X' = ak_2xrq$, where q is the relative ionization efficiency due to the diastereomeric structure difference, and r is that due to the deuterium substitution. By taking the ratio X/Y', eq 1 is reformed as

$$X/Y' = (k_1x)/(ak_1yr) = (1/ar)x/y \qquad (1')$$

Similarly eq 2 is reformed as

$$X'/Y = (ak_2xrq)/(k_2yq) = (ar)x/y \qquad (2')$$

Equation 3 becomes

$$(X/Y')(X'/Y) = [(1/ar)x/y][(ar)x/y] = (x/y)^2 = [(1\text{st,M})/(1\text{st,M}+n)][(2\text{nd,M}+n)/(2\text{nd,M})] \qquad (3')$$

The same scheme for determining % ee is thus applicable also to MS spectrometry.

This % ee determination method using MS spectrometry is essentially applicable to trisdeuterated MαNP acid (*R*)-(−)-**1**-d_3. However the difference of three m/z units (M+3 vs M) in MS is not enough to avoid the overlap with isotope peaks of natural abundance. Therefore we have had to make several corrections. After corrections for background, overlap with isotope peaks, deuterium content, and racemate (0% ee), a good agreement was obtained in all regions (0−100% ee): average error, ±0.7% ee; maximum error, 1.9% ee. However, those corrections are inconvenient for practical use. Therefore, we next selected hexadeuterated MαNP acid (*R*)-(−)-**1**-d_6 (Scheme 1, R = D, n = 6).

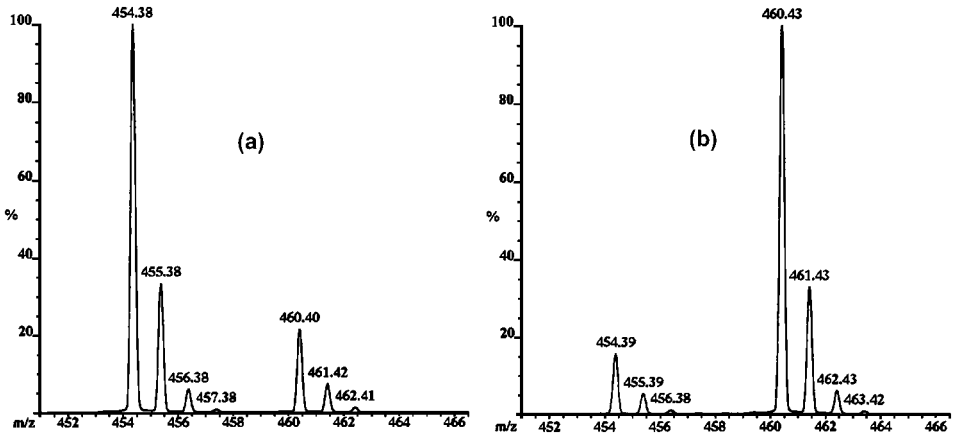

Figure 3. MS spectra of diastereomeric MαNP esters of 2-hexadecanol (69.77% ee); JEOL, JMS GC mate II, EI 40 eV: (a) the first-eluted **3a** containing (*S*,*R*′)-**3** and (*R*,*S*′)-**3**-d_6; (b) the second-eluted **3b** containing (*S*,*S*′)-**3** and (*R*,*R*′)-**3**-d_6.

Chiral acid (*R*)-(−)-**1**-d_6 was prepared by the Grignard reaction of ethyl 2-(1-naphthyl)-2-oxoacetate using CD_3I (D content >99.5 atom %) followed by methylation with CD_3I (D content >99.5 atom %) and enantioresolution with (−)-menthol. On the basis of six *m*/*z* units difference (M+6 vs M), the term of overlap with isotope peaks of natural abundance is completely negligible.

The MS spectrometric method was next checked using a test sample **2** (69.77% ee); a mixture of diastereomeric esters prepared from (*S*)-(+)-**1** and (*R*)-(−)-**1**-d_6 (ratio, 1:0.994) was separated by HPLC on silica gel (hexane/EtOAc 20:1). From the MS of the first-eluted fraction, the compositions of esters (*S*,*R*′)-**3** (M^+ *m*/*z* 454) and (*R*,*S*′)-**3**-d_6 (M^+ *m*/*z* 460) were determined with background correction: $X = 52.284$, $Y' = 11.221$, $X/Y' = 4.6594$ (Figure 3). Similarly from the MS data of the second fraction, the compositions of (*S*,*S*′)-**3** (M^+ *m*/*z* 454) and (*R*,*R*′)-**3**-d_6 (M^+ *m*/*z* 460) were determined: $Y = 8.607$, $X' = 55.278$, $X'/Y = 6.4224$. The product $(X/Y')(X'/Y) = 29.9245$, and so $x/y = 5.4703$. Since $x + y = 1$, $x = 0.845448$ and $y = 0.154552$, leading to 69.09% ee, which reasonably agrees with 69.77% ee calculated by weight. This MS method has been applied to 15 test samples **2** with 0−100% ee. As shown in Figure 4, the % ee values obtained by MS agree well with those calculated by weight: average error, ±1.08% ee; maximum error, 1.79% ee. All data points obtained follow a straight line even in the region of 90−100% ee unlike the case of ^{1}H NMR. The MS method, which is much more sensitive than ^{1}H NMR, is thus applicable to samples with higher % ee. We have now obtained a diastereomer method for determining % ee with complete removal of the kinetic resolution effect. It should be emphasized that in this method, calibrations are unnecessary, and kinetic resolution efficiency is not required. These excellent qualities are purchased at the cost of separating diastereomers by HPLC.

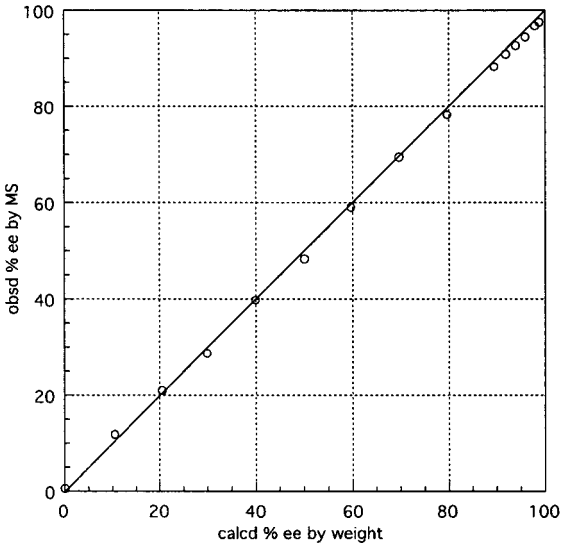

Figure 4. Comparison of % ee values determined by MS with those calculated by weight: (*R*)-2-hexadecanol **2** > (*S*)-**2**.

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Supporting Information Available: Synthetic schemes, preparation procedure of MαNP ester, ^{1}H NMR spectra, and tables of ^{1}H NMR and MS spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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