Chiral Anisotropic Reagents for Determining the Absolute Configuration of Secondary Alcohols and Carboxylic Acids

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Abstract: Based on the modified Mosher's method, we have developed several chiral anisotropic reagents. Together with a unique application of the modified Mosher's method to an enantiomeric mixture, utility of methoxy-(1- and 2-naphthyl)acetic acids (1NMA, 2NMA and other analogues) and phenylglycine methyl ester (PGME) in elucidation of the absolute configuration of secondary alcohols, amines, and carboxylic is summarized.

1. Introduction

Most natural products have stereogenic centers and they exist as enantiomers. Since the building blocks of organisms such as amino acids and sugars are chiral, only one enantiomer has biological activity and, in many cases, another enantiomer may be seriously hazardous. Therefore, information on the absolute configuration of organic compounds is important especially in pharmaceutical drug development and research on the biological significance of natural products.

The modified Mosher's method using 1-methoxy-1-phenyl-1-trifluoromethylacetic acid (MTPA: Mosher's reagent) has been developed as a simple and reliable method for determining the absolute configuration of secondary alcohols.¹ This method is based on Mosher's ingenious finding that an MTPA ester has a certain preferable conformation (see Section 2-1) and the phenyl ring of the MTPA moiety may cause characteristic anisotropic effects in the ¹H NMR spectrum of the MTPA ester.^{2a} Mosher also developed a unique method utilizing ¹⁹F NMR chemical shifts of the MTPA diastereomers.^{2b} Because this method is simple [comparing the CF₃ chemical shifts between (*R*)- and (*S*)-MTPA diastereomers], it was long used as a 'Mosher's method' for absolute configuration determination until we indicated that the ¹⁹F method might result in wrong configurations.¹

The modified Mosher's method, a new version of Mosher's method (¹H NMR), depends on the remarkable development of FT-NMR techniques, and, because the FT-NMR instruments equipped with superconductive magnets are the most popular in the laboratories of many scientific research institutes, universities, and companies, the method has become a textbook methodology for the stereochemical study of natural and synthetic compounds.

The modified Mosher's method seems to have triggered a growing number of new NMR reagents and techniques that can predict the absolute configuration of various types of compounds.³ We have developed 1- and 2NMA [1-methoxy-1-(1- and 2-naphthyl)acetic acids]⁴ that are effectively appli-

cable to long-chain secondary alcohols, PGME (phenyl glycine methyl ester) for chiral carboxylic acids,⁵ and a sulfoximine method that can elucidate the absolute configuration of optically active sulfoxides,⁶ and MPPO (5-methyl-5-phenylpyrroline *N*-oxide) for chiral 1,3-disubstituted allenes.⁷

This paper summarizes our work focusing on 1- and 2NMA as well as PGME that enables clarification of the absolute configuration of secondary alcohols and carboxylic acids, respectively.

2. The Modified Mosher's Method

2.1 Principle of the Modified Mosher's Method

Figure 1 illustrates the conformation of an MTPA ester of a secondary ester, which was proposed by Mosher as the most preferable one.^{2a} In this conformation [A], the carbinyl proton (α -H), carbonyl oxygen and trifluoromethyl group are located on the same plane (MTPA plane). The protons oriented on the same side of the phenyl group [*H*_X, *H*_Y, *H*_Z of (*S*)-MTPA ester] are shielded and give the signals at more upfield than those of another diastereomer [*H*_X, *H*_Y, *H*_Z of (*R*)-MTPA ester]. The signals of as many protons as possible are assigned, $\Delta\delta = \delta_S - \delta_R$ are calculated for the respective protons, and the protons with positive and negative $\Delta\delta$ values are placed in Model A, which gives the absolute configuration of the secondary alcohol in question.

The upfield shift of the chemical shifts of the protons situated on the same plane with the phenyl group is rationalized by a particular orientation of the phenyl group: Several X-ray crystal data of MTPA esters reveal that the phenyl group takes a conformation, in which the phenyl plane

Figure 1. [A] Preferable conformation of an MTPA ester of a secondary alcohol.
[B] Model A to determine the absolute configuration of secondary alcohols.

directs perpendicularly toward the carbonyl moieties.⁸

2.2 Application to Natural Products

The typical results obtained for natural products to which the modified Mosher's method has been applied are shown in Figure 2.¹ In all of the compounds, the protons having positive and negative $\Delta\delta$ values are found on the right and left side of the MTPA plane, respectively.

Compounds 2–3 are diterpenes isolated from the *Dictyota* brown alga, and their absolute configurations are established by this method for the first time. Later, the absolute configuration of 4 was confirmed by total synthesis.⁹

The absolute configurations of an enormous numbers of organic compounds have been determined by the modified Mosher's method, and the number is still increasing. A lengthy summary of the published results is skipped in this article, although it should be noted that the method is applicable even to compounds without a secondary hydroxy group: a hydroxy group can be produced by *e.g.* reduction of a carbonyl group,¹⁰ hydroboration of an olefin, a Baeyer-Villiger reaction of a ketone followed by hydrolysis.¹¹

2.3 Application to Enantioselective Synthesis

Enantioselective synthesis has been a major target of organic chemists, and there have been remarkable advances in

this particular area. When a chemist obtains a single enantiomer by enantioselective synthesis, the absolute configuration can be determined by the modified Mosher's method. If the reaction is not 'enantioselective' enough, it may lead to a mixture of enantiomers. In such cases, the absolute configuration of the major and minor components can be determined by preparing only (*S*)-MTPA esters of both enantiomers (Figure 3).¹²

Before interpreting the process, the reader should bear in mind that the ¹H NMR spectrum of (*S*)-MTPA ester of one product is identical with that of the (*R*)-ester of its enantiomer. Esterification of the hypothetical product mixture consisting of the major enantiomer (*R'*) and its minor antipode (*S'*) with (*S*)-MTPA acid will give the *S*:*R'* (major diastereomer) and *S*:*S'* (minor diastereomer) esters, which are separated by chromatography. Because the ¹H NMR spectrum of *S*:*S'* is identical with that of its enantiomer *R*:*R'*, subtraction of the chemical shifts of *S*:*R'* from those of *S*:*S'* (= *R*:*R'*) will give $\Delta\delta$ values for assigning the absolute configuration (*R'*) of the major product: [$\Delta\delta = \delta_{(S)\text{-MTPA ester}} - \delta_{(R)\text{-MTPA ester}} = \delta_{S:R'} - \delta_{R:R'} (= \delta_{S:S'})$]. This idea has been actually adopted in the total syntheses of natural products.¹³

3. 1NMA, 2NMA and Their Analogues

3.1 1NMA and 2NMA, Chiral Anisotropic Reagents

The modified Mosher's method is based on the anisotropic effect by the phenyl group of the MTPA. When the phenyl group is replaced with a certain aromatic moiety that shows an anisotropic effect stronger than that of the benzene ring, the resulting reagent would be more effective than MTPA. This consideration has led to development of methoxy-(1-naphthyl)acetic acid (1NMA: 5) and methoxy-(2-naphthyl)acetic acid (2NMA: 6) (Figure 4).^{4a,b} These reagents are the analogues of 'Trost's reagent' [methoxy(phenyl)acetic acid or *O*-methylmandelic acid (MPA) (7)],¹⁴ which has been used as a chiral reagent for determining the absolute configuration of secondary alcohol, although the former turn out to give stronger anisotropic effects than 7. Reagents 5 and 6 were also synthesized by Riguera's group.¹⁵

Figure 4[A] illustrates a preferable conformation of a 2NMA (and 1NMA) ester of a secondary alcohol, in which the carbonyl proton, carbonyl oxygen, and methoxy group are on the same plane (1NMA/2NMA plane), as has been originally proposed by Trost for *O*-methylmandelate.¹⁴ This conformation was verified by X-ray crystallography and computer calculation. Model B in Figure 4[B] can be used for absolute configuration of secondary alcohols in the same way as in Figure 1[B], although it should be noted the definition of $\Delta\delta (= \delta_R - \delta_S)$ is different from that in the modified Mosher's method ($\Delta\delta = \delta_S - \delta_R$).

Figure 5 demonstrates comparison of the $\Delta\delta$ values among the MTPA (8), 1NMA (9), and 2NMA (10) esters of (–)-menthol. It is noteworthy that the values observed for the protons of 9 and 10 are four to five times larger than those of 8.

This finding may lead to the conclusion that 1NMA and 2NMA are more versatile reagents than MTPA. The situation, however, is not that

Figure 2. Examples to which the modified Mosher's method was applied; 1: (–)-menthol, marine terpenoids 2–4.

Figure 3. The modified Mosher's method applied to an enantiomeric mixture obtained by an enantioselective reaction.

Figure 4. Structure of 1NMA (5), 2NMA (6), and *O*-methyl-mandelic acid (7). [A] A preferable conformation of a 2NMA ester of a secondary alcohol. [B] Model B to determine the absolute configuration of secondary alcohols.

Figure 5. The $\Delta\delta$ values obtained for MTPA (8), 1NMA (9), and 2NMA (10) esters of (-)-menthol.

simple. Certain proton signals, which are well separated in the spectrum of an MTPA ester, may become perversely overlapped due to the large anisotropic effect of the naphthyl ring. Therefore, we prefer MTPA to 1NMA and 2NMA when we deal with cyclic compounds of relatively small sizes.

We have proposed the NMR reagents such as MTPA, 1NMA, and 2NMA to be classified as *chiral anisotropic reagents*, since they are chiral and in common have an aromatic ring with strong anisotropic effect.^{4a}

3.2 Chiral Anisotropic Reagents for Long-chain Compounds

When a compound with a long chain has a hydroxy group in the middle of the chain, the modified Mosher's method is of little use since the anisotropic effect of the phenyl group of MTPA is so short-ranged that it is difficult

to differentiate even the protons β to the oxy group (7- and 11-H₂ of **11** in Figure 6). For such type of acyclic alcohol, 1- and 2NMA, especially the latter, turned out to be quite useful for determining the absolute configuration.^{4a} Figure 7[A] shows the ¹H NMR spectrum (1.62–0.80 ppm) of 9(*S*)-hydroxyoctadecanoic acid (**12**). As typical for hydroxy fatty acid, a quintet due to 3-CH₂ appears at 1.61 ppm, 8- and 10-CH₂ shows a hamper at around 1.42 ppm, and other signals except for the methyl signal appear as seriously overlapped envelopes centered at 1.3 ppm.

Figure 7[B] (above) demonstrates the spectrum of the (*R*)-2NMA ester (**13**). Because the 2-naphthyl group is directed toward the methyl terminal (refer Figure 4[A]), the CH₂ protons on that side of the chain show remarkable upfield shifts. Most significant are the chemical shifts of 11-H₂ (δ 0.84) and 13-H₂ (δ 0.86), appearing at higher field than 18-methyl. On the contrary, the CH₂ protons on the methoxycarbonyl chain of the (*S*)-2NMA ester (**14**) show distinct upfield shifts as shown in Figure 7[B] (below). It should be noted that, in both spectrum, the signals that move upfield are all well separated and their quintet patterns, but for 17-H₂ in the (*R*)-2NMA derivative, can be clearly seen. Assignment of the upfield-shifted signals is quite easy by analyzing their ¹H-¹H COSY spectra. Especially important is the fact that the absolute configuration at C-9 of **12** can be deduced by either the (*R*)- or (*S*)-2NMA derivative, because the connectivity 17-H₂/18-Me and 3-H₂/2-H₂ is observable in the COSY spectra of **13** and **14**, respectively. In other words, preparation of only one diastereomer can lead to the absolute configuration of the long-chain secondary alcohol, when the number of CH₂ from the hydroxylated carbon is less than eight.

Figure 8 shows the long-chain secondary alcohols **15–18**, the absolute configurations of which have been elucidated by the $\Delta\delta$ values obtained by their (*R*)- and (*S*)-2NMA diastereomers.^{4a} Even the configuration of 10-hydroxyeicosanone (**18**), ginnol isolated from the leaves of the ginkgo tree, was determinable by the 2NMA method.^{4b}

Figure 9 is to show the effectiveness of 2NMA as a chiral anisotropic reagent. In the spectrum (above) of the (*R*)-diastereomer (**18**), two methyl signals appear separately while the methyl signals are overlapped in the spectrum of

Figure 6. The ¹H NMR spectrum (400 MHz, CDCl₃) of the (*R*)-MTPA ester of methyl *rac*-9-hydroxyoctadecanoate (**11**).

Figure 7. [A] The ^1H NMR spectrum of 9(*S*)-hydroxyoctadecanoic acid (**12**). [B] The ^1H -NMR spectra of (*R*)- (**13**) and (*S*)-2NMA (**14**) esters of **12**. The spectra were recorded at 400 MHz in CDCl_3 .

Figure 8. Examples of the long-chain alcohols whose absolute configurations were determined by applying 2NMA method. $\Delta\delta$ values are denoted in each structure. The size of the circles roughly corresponds to the magnitude of the $\Delta\delta$ values.

the (*S*)-isomer (below). These suggest that the anisotropic effect of the 2-naphthyl group reaches as far as the 1-Me group (ten bonds apart from 10-C) in the former, in which the naphthyl group is oriented on the same side of 1-Me. In the latter, 29-Me is too far from 10-C, and, thus, the chemical shift of 29-Me is coincident with that of 1-Me.^{4b}

Interesting is the fact that the $\Delta\delta$ values obtained for the long-chain compounds **15**–**18** in Figure 8 change small and large alternatively: the values of β -protons are smaller than those of γ -protons, which show larger values than δ -protons and so on. These characteristics may reflect the zigzag conformation of the aliphatic chains in the solutions.

The absolute configuration of yessotoxin, a marine toxin with polycyclic ether, was also determined by the 2NMA method (Figure 10).¹⁶

3.3 Preparation and Resolution of 1NMA and 2NMA

Racemic 2NMA is easily prepared by one-step synthesis: treatment of 2-naphthaldehyde with a mixture of potassium hydroxide and tribromomethane in methanol¹⁷ affords 2NMA (**6**) as racemate in high yield. Use of dry methanol remarkably decreases the yield. A plausible reaction mechanism is shown in Figure 11. Racemic 1NMA (**5**) was also prepared by the same reaction.

Resolution of *rac*-2NMA (**6**) was successfully achieved by converting it to a diastereomeric mixture of *L*-phenylalaninol amides (**19**), which was easily separated by flash column chromatography (FCC). Pure enantiomers are obtained by

Figure 10. Yessotoxin and the $\Delta\delta$ values obtained from its 2NMA esters, which solved the absolute configuration.

Figure 9. The ^1H NMR spectra of the (*R*)- (above) and (*S*)-2NMA esters of ginnol (**18**) (400 MHz, CDCl_3).

hydrolysis under relatively mild condition (heat in 3 M hydrochloric acid for 1 h). Enantiomeric excess of both products exceeds 99 %. L-Phenylalaninol can be recovered in 50–75 % yield. The facile hydrolysis of the amide (**19**) may be owing to the participation of the neighboring hydroxy group (Figure 12).¹⁸

As has been described above, all the processes to obtain (*R*)- and (*S*)-2NMA from 2-naphthaldehyde are simple and can be carried out in large scale within a week.

3.4 2ATMA and 9ATMA Possessing an Anthracene Ring

Ring

As discussed above, 1NMA and 2NMA having a naphthalene ring exhibit anisotropic effects stronger than MTPA with a phenyl group. It would be natural to expect a compound possessing an anthryl group as a more powerful chiral anisotropic reagent. Because 9-anthraldehyde is commercially available, we at first attempted to prepare 9-anthryl (methoxy)acetic acid (9ATMA; Figure 13). Treatment of 9-anthraldehyde with $\text{CHBr}_3/\text{KOH}/\text{MeOH}$ as in Figure 11 gave no trace of 9ATMA. Therefore, the stepwise synthesis summarized in Figure 13[A] was performed giving racemic

9ATMA. The total yield was poor (10 % at best) mainly because the last oxidation gave 9ATMA together with anthraquinone and its degradation products, which obstructed purification of the desired product. Oxidation of the alcohol with metal reagents gave only anthraquinone.

Resolution of 9ATMA proceeded smoothly. 9ATMA was esterified with (+)-*sec*-phenethyl alcohol (expensive!) and the diastereomers were separated by chromatography. Hydrolysis of the separated diastereomer (3 M HCl in 1,4-dioxane, reflux, 3 h) afforded (*R*)- and (*S*)-9ATMA (e.e. 85–90 %).⁴

Figure 13[B] shows a comparison of the $\Delta\delta$ values observed for the 2NMA (bold) and 9ATMA (bold and italics) esters of (–)-menthol (**20**) and the MTPA (plain), 1NMA (plain and italics), 2NMA, and 9ATMA esters of a seaweed metabolite (**21**).^{4,19} The large anisotropy of 9ATMA is clearly recognized in **20**, where the $\Delta\delta$ values of the 9ATMA ester are two or three times larger than those of 2NMA (that is, almost ten times larger than those of MTPA; see Figure 5), particularly for the protons close to the moiety. The $\Delta\delta$ values, however, are not significantly larger for the protons

located far from the 9ATMA moiety, and they fell to the same order with those of 2NMA. The same tendency is observed in the case of **21**. The values of 9ATMA are remarkably large for the protons close to this moiety. But the effect quickly fades as the distance from the moiety increases. For a long-chain compound, therefore, 2NMA seems to be preferable to 9ATMA, considering the easier preparation of the former.

The long-range anisotropic effect of 2NMA in a long-chain compound may be interpreted by the scheme illustrated in Figure 14. Since the Trost's type esters of secondary alcohols exist in the conformation [A], the perspective views of MPA, 1NMA, 9ATMA, and 2NMA esters can be figured as in Figure 14. When the width of the benzene ring is defined as 'a', the widths of the respective aromatic rings are 1.5a, 1.5a, and 2a. It may be clear, for determination of the long-chain alcohols, that 2NMA would be the best since the width, in other words, the distance to which the anisotropic effect of the aromatic ring reaches, is the largest.

Then, what will be the results if the secondary alcohol is esterified with 2ATMA (2-anthrylmethoxyacetic acid)? The 2ATMA has the width of 3a (Figure 14), and, therefore, it may be the most powerful chiral anisotropic reagent (for a long-chain compound) that ever exists.

Racemic 2ATMA (**22**) was prepared from 2-methylantraquinone, which had been the only 2-substituted anthracene compound purchasable, as shown in Figure 15. Yield of the last step was <30 % due to the poor solubility of 2-anthrylaldehyde in any organic solvent. The racemate was resolved by use of (+)-*sec*-phenethyl alcohol and hydrolysis as in

Figure 11. Preparation of racemic 2NMA (**6**) from 2-naphthaldehyde.

Figure 12. Resolution of racemic 2NMA (**6**) by separation of diastereomeric derivatives with L-phenylalaninol (**19**). Facile hydrolysis of **19** by neighboring group participation of the hydroxy group is interpreted at the bottom.

Figure 13. [A] Synthetic scheme of 9ATMA. [B] Comparison of $\Delta\delta$ values of MTPA (plain), 1NMA (plain italics), 2NMA (bold), and 9ATMA (bold italics) esters obtained for (-)-menthol and a derivative of an algal product (**21**). The values smaller than 0.003 ppm are abbreviated. For (-)-menthol, only the values of 2NMA and 9ATMA esters are described (**20**). The $\Delta\delta$ values marked by asterisk are absolute ones.

Figure 14. Comparison of the 'width' of the aromatic ring among MPA, 1NMA, 2NMA, 9ATMA, and 2ATMA. Perspective views from the direction illustrated in [A] are drawn. "a" in the drawings corresponds to the width of a benzene ring.

the case of 9ATMA. The absolute configuration of the enantiomers was determined by comparing their CD spectra with (*R*)- and (*S*)-2NMA.²⁰

As anticipated by the scheme in Figure 14, 2ATMA (**22**) proved to be a potent reagent for determination of the absolute configuration of long-chain secondary alcohols (Figure 16). It was also confirmed that, thanks to the enormous upfield shifts, esterification of a linear secondary alcohol with only one enantiomer is enough to elucidate the absolute configuration (Section 3-5).

Figure 15. Preparation of rac-2ATMA (**22**) from 2-methylantraquinone.

3.5 Absolute Configuration of Taurolipid B Determined with 2ATMA

Taurolipid B (**25**) is a unique lipid produced by a fresh-water protozoan *Tetrahymena thermophila*.²¹ It exhibits growth-inhibitory activity against HL-60. In order to determine the absolute configuration of four asymmetric carbons, it was converted to methyl tetrahydroxyoctadecanoate (**26**).

The strategy that the professor, the first author of this article, gave a graduate student to determine all the four stereogenic centers of **26** was quite primitive:

"Why bother manipulating the molecule? Just add one equivalent of (*R*)-2ATMA, which will give four monoesters. Then separate them by HPLC and measure their ¹H NMR spectra."

As a general rule, a student is smarter and more practical than the boss, because he/she has to do the job by him(her)self.

"I'm afraid that you are far too optimistic. The four OH groups are oriented separately, and, even if one equivalent of the (*R*)-acid were added, there would be a good chance to obtain di- and tri-esters besides monoesters, which would make separation of the product impossible."

"Well.....?"

As a compromise, the vicinal diol of the tetraol (**26**) was protected as an acetonide (acetone, CuSO₄, H⁺) to give **27**, and one equivalent of (*R*)-2ATMA was added with a condensing reagent (EDC). By HPLC separation, the monoesters [**28(R)** and **29(R)**] were obtained together with a small amount of diester. The positions of the 2ATMA in **28(R)** and **29(R)** were easily determined by analysis of the ¹H-¹H COSY spectra. (*S*)-2ATMA was condensed in the same manner to give **28(S)** and **29(S)**.

The 7(*R*)- and 13(*R*)-configurations were almost instantly deducible by inspecting the 1D ¹H NMR spectra of **28(R)** and **29(R)** considering the ideal conformation of the mande-

Figure 16. Comparison of the $\Delta\delta$ values among 2ATMA (**23**, **24**) and 2NMA (**17**, **18**) esters.

Figure 17. Taurolipid B (**25**) and its derivatives (**26–29**) that were prepared to determine the stereochemistry of **25**.

Figure 18. $\Delta\delta'$ values obtained for 7-[(*R*)-2ATMA] [**28(R)**], 13-[(*R*)-2ATMA] [**29(R)**], 7-[(*S*)-2ATMA] [**28(S)**], and 13-[(*S*)-2ATMA] [**29(S)**] esters. $\Delta\delta'$ is defined as $\Delta\delta' = \delta_{\text{alcohol}} - \delta_{2\text{ATMA}}$, and the protons whose values exceed 0.2 ppm are circled. The structure **30** shows the absolute configuration of the tetraol (**26**).

late-type ester (Figure 4[A]). In **28(R)**, the protons whose $\Delta\delta'$ values ($\Delta\delta' = \delta_{\text{alcohol}} - \delta_{2\text{ATMA}}$) exceed 0.2 ppm (circled) are at C-2, 3, 4, 5 and the acetonide methyl, and, therefore, the 2-anthryl group is directed toward the CO_2Me terminus, which necessitates the (*R*)-configuration at C-7. The same discussion stands for **29(R)**, establishing (*S*)-configuration at C-13. The same conclusion was obtained from the (*S*)-2ATMA pairs **28(S)** and **29(S)**.

Finally, the stereochemistry of the remaining 2- and 3-OH was determined by i) acetylation of **27** to give a diacetate, ii) deprotection of acetonide, iii) applying the monoesterification with (*R*)-2ATMA. The absolute configuration of

the tetraol thus determined is shown in **30**.²² The stereochemistry of 2- and 3-position was further confirmed by the dibenzoate CD experiment.

4. PGME Method for Chiral Carboxylic Acid

4.1 Principle of the PGME Method

Application of the chiral anisotropic reagents hitherto mentioned is restricted in their usage to secondary alcohols and primary amines. In principle, the concept of chiral anisotropic reagents, composed of an aromatic ring and other substituents that serve to constrain the conformation of the chiral moiety, can be extended to stereochemical study of a variety of compounds possessing a functional group other than a secondary hydroxy group. In this context, we developed a new chiral anisotropic reagent that can be applied to the absolute configuration study of chiral carboxylic acids.

The molecular design to create the new reagents is depicted in Figure 19: A carboxylic acid of type **I** is converted to an ester ($\text{X}=\text{O}$) or an amide ($\text{X}=\text{NH}$) (**II**). The latter seems preferable since the conformation of an amide is fixed so that C_1 and C_4 exist in the $s(\text{C}_2-\text{X}_3)$ -*trans* orientation, which is general in peptide chemistry. The carbon at the 4-position must bear a phenyl group that is essential for a chiral anisotropic reagent. Group Y in **II** can be either alkoxy-carbonyl (ester) or an alkylaminocarbonyl (amide) group ($\text{Z} = \text{OR}$ or NRR' in **III**). Its carbonyl oxygen may fix the conformation of the chiral moiety by i) forming a hydrogen bond with NH, or ii) forcing the $s(\text{X}_3-\text{C}_4)$ -*trans* orientation of C_2

Figure 19. Molecular design of the chiral anisotropic reagents applicable to chiral carboxylic acids of type **I**. Model C is for the absolute configuration of a PGME (or PGDA) amide.

Figure 20. An X-ray structure of the (*R*)-PGME amide of 2(*R*)-methyl-4-phenylbutanoic acid.

(δ_+) and Y_5 (C=O) (δ_+) by electrostatic repulsion.

What came from this modeling were phenylglycine methyl ester (PGME) and phenylglycine dimethylamide (PGDA) (IV). The former is purchasable while the latter must be synthesized. Although PGDA turned out to work excellently as expected, partial epimerization was observed during preparation. Therefore, we selected PGME as a candidate for the chiral anisotropic reagent. Validity of the supposed stable conformation **III** in Figure 19 was confirmed by an X-ray analysis performed on the (*R*)-PGME amide of 2(*R*)-methyl-4-phenylbutanoic acid (Figure 20).²³

4.2 Application to Secondary Carboxylic Acids

Figure 21 shows the results of the PGME method⁵ applied to the compounds (**31–34**), the absolute configurations of which are known. Positive and negative $\Delta\delta$ ($= \delta_S - \delta_R$) values are systematically arranged on the right and left side of Model C (Figure 19), respectively.

A carboxyl group can be created from various types of functional groups. Therefore, the PGME method can be extended to a compound that originally has no carboxyl group. As an example, an experiment to confirm the configuration of the side-chain methyl group of ergosterol (**35**) is shown in Figure 22.²⁴ Ozonolysis of **35** followed by oxidation with hydrogen peroxide afforded a complex mixture, to which (*R*)-PGME was added with a condensation reagent (PyBOP). By preparative TLC and HPLC, the (*R*)-PGME amide of 2,3-dimethylbutanoic acid was isolated. Similarly, the (*S*)-PGME amide was prepared, and the $\Delta\delta$ values were calculated (**36**), which supported the (*R*)-configuration of the methyl group. We have isolated various types of steroids that have a 5,6-dimethyl (or 5-ethyl-6-methyl)-3-hepten-2-yl side chain from marine sponges. The above experimental result may be generally used to assign the stereochemistry of such steroid side-chains.

Another example to show the utility of the PGME method is outlined in Figure 23. Compound **37** is a sesquiterpene isolated from a fungus.²⁵ The tertiary carboxyl group was first methylated, and the alcohol was oxidized, affording a secondary carboxylic acid (**38**), to which the

PGME method was applied. Structure **39** exhibits the systematically arranged positive and negative $\Delta\delta$ values leading to the absolute configuration of **37**.²⁶

4.3 Extension of the PGME Method to α -Oxy Carboxylic Acids

The idea to extend the application of the PGME method to α -oxy carboxylic acids came from an experiment carried out on yohimbic acid (**40**) (PGME = OH) (Figure 24). The $\Delta\delta$ values are distributed regularly, and the absolute configuration deduced from the $\Delta\delta$ values is compatible with the known one.

In the $\Delta\delta$ values obtained for the PGME amide (**40**), we noticed an abnormally large $\Delta\delta$ value (+0.916) of the *pro*-(*S*) proton at the γ -position. This value is 5 times larger than that of *pro*-(*R*) proton (+0.176). Moreover, a very small (+0.001) $\Delta\delta$ value was observed for the proton at the β position. These findings can be rationalized by assuming an intramolecular hydrogen bond between the (*R*)-PGME amide carbonyl oxygen and β -hydroxy groups as shown in **41**, which restricts the rotation around the C_α -C($=O$) bond to force the phenyl group to approach the *pro*-(*S*) proton of the γ -methylene group. The larger $\Delta\delta$ values found for **40** than those of other PGME amides are also supportive of the fixed conformation of the PGME moiety.²⁶

Figure 21. The $\Delta\delta$ values obtained for compounds **31–34**, the absolute configurations of which are known.

Figure 22. An experiment to confirm the (*R*)-configuration of the side-chain of ergosterol (**35**) by the PGME method. The $\Delta\delta$ values of the reaction product (**36**) are shown.

Figure 23. Experiments to determine the absolute configuration of **37**, a sesquiterpene produced by a fungus, by means of the PGME method. Structure **39** shows the $\Delta\delta$ values.

Suppose that a certain functional group is introduced to a secondary carboxyl group and the new group plays a role to fix the conformation of the PGME, the application of the present method can be extended to other types of carboxylic acids. This assumption led to the idea that the PGME method would be applicable to α -oxy carboxylic acids, since the polar group at the α -position would fix the ideal conformation of the PGME group by dipolar interaction as interpreted in Figure 25.²⁷

When conformations A and B of the PGME amide of a secondary carboxylic acid are compared, A is clearly more stable because B suffers steric interaction (denoted by *) between N-H and two substituents (R_1 and R_2). In the case of the PGME amide of α -hydroxycarboxylic acid, conformation D is far more unstable than C owing to the presence of a repulsive interaction between the polar hydroxy and carbonyl oxygens (**) in addition to the steric interaction (*). In the same context with the secondary carboxylic acids, the absolute configuration of an α -oxy carboxylic acid should be determined by Model D ($\Delta\delta = \delta_S - \delta_R$),²⁷ and the $\Delta\delta$ values are expected to be larger than those obtained for the secondary carboxylic acids described in Section 4-2 because of the predominant conformation C.

Figure 26 summarizes the results of the PGME method applied to (A) α -hydroxycarboxylic acids, (B) α -acyloxycarboxylic acids, and (C) α -alkoxycarboxylic acids.²⁷ It should be noted that the PGME method is generally applicable to these types of α -oxycarboxylic acids, even though the oxycarboxylic group is involved in rather complex molecules

such as **47**, **50**, **54**, and **60**. Comparing the results shown in Figure 26 with those appearing in Figure 21, we can notice that (i) the $\Delta\delta$ values of α -hydroxy- (A) and α -acyloxycarboxylic acids (B) are in average larger than those observed for **31-34** and (ii) the values of α -alkoxycarboxylic acids are the largest of the carboxylic acids hitherto argued. The reason for (ii) is not clear, although we presume that the alkoxy oxygen is more electronegative than the hydroxyl and acyloxyl oxygens so that the electrostatic repulsion represented in conformation D of Figure 25 is larger than the others.

We next studied if the PGME method might be applied to an α -oxy primary carboxylic acid by utilizing Model D, in which one of the α -substituents is a hydrogen (Figure 27). Compounds **61-64**, whose absolute configurations are known, were subjected to the PGME method. The $\Delta\delta$ values ($= \delta_S - \delta_R$) denoted in Figure 27 are in accordance with those expected from their configurations based on Model D. It should be noted that if Model C is applied to α -oxy primary carboxylic acids, the predicted absolute configurations are antipodal to the correct ones.

The methyl vinyl carbinol (3-hydroxy-3-methylpent-4-en-1-yl) moiety, included in compounds such as sclareol (**65**), is frequently encountered particularly in terpenoids (Figure 28). Because it usually exists as a side chain, even its stereochemistry relative to the cyclic residue is extremely difficult to be solved. In many terpenoids, the stereochemistry of the methyl vinyl carbinol moiety has remained unsolved. An oxidative cleavage of the vinyl group, however, will give an α -hydroxy carboxylic acid, the absolute configuration of which will be conveniently determined by the present method.

Sclareol (**65**) was ozonized followed by treatment with hydrogen peroxide in methanol/acetic acid, producing **66** possessing an α -hydroxycarboxylic acid. The PGME method was applied to this acid and the $\Delta\delta$ values were assigned (**67**). The absolute configuration determined by Model D is accordant with the known one.

This finding is important in that the thus-far-undeterminable configuration at the methyl vinyl carbinol carbon of many terpenes will be clarified by applying the PGME method.

4.4 Conformation of the PGME Amide of *N*-Acyl Amino Acids

As discussed in Section 4-3, the PGME amide of an α -hydroxy primary carboxylic acid such as **61-64** possesses the conformation C exhibited in Figure 25, and thus Model D instead of Model C must be used for determination of the absolute configuration. It should be interesting to study which of Model C-type or Model D-type conformation an *N*-substituted α -amino primary carboxylic acid, that is, an *N*-substituted α -amino acid will favor. Our anticipation was that the amino acid derivative would prefer the Model D-type conformation because the electrostatic repulsion between nitrogen and carbonyl oxygen would be less in the conformation as discussed in Figure 25.

Figure 24. The PGME amide of yohimbic acid (**40**) and a molecular model of the (*R*)-PGME amide (**41**) constructed based on the $\Delta\delta$ values depicted in **40**.

Figure 25. Conformations A and B of the PGME amide of a secondary carboxylic acid. C and D are those of an α -oxycarboxylic acid. A and C are supposed to be more stable than B and D, respectively, owing to destabilization of the latter ones by steric (*) and dipole-dipole (**) interactions. Model D is for absolute configuration of α -oxy carboxylic acids ($\Delta\delta = \delta_S - \delta_R$).

Figure 26. $\Delta\delta$ values obtained for (A) α -hydroxy-, (B) α -acyloxy-, and (C) α -alkoxycarboxylic acids.

Figure 27. Application of the PGME method to L-leucic acid (61), (S)-(+)-5-oxo-2-tetrahydrofurancarboxylic acid (62), L-leucic acid acetate (63), and L-glycric acid diacetate (64). The $\Delta\delta$ value are based on Model D ($\Delta\delta = \delta_S - \delta_R$).

Figure 28. Sclareol (65) and its oxidation products (66). $\Delta\delta$ values are assigned to structure 67.

Figure 29. (above) Plausible conformations of the PGME amide of an *N*-acyl (R' = acyl) amino acid, Model D-type and Model C-type. (below) $\Delta\delta$ values ($= \delta_S - \delta_R$) observed for the PGME amides of *N*-acyl amino acids 68–70.

Figure 30. Ozonolysis of cyclohexene [A] gives β,β -disubstituted propionic acid [B]. Four terpenoids composed of fragment [A] are shown.

This, however, turned out to be wrong (Figure 29).²⁷

The PGME amides of compounds 68–70, *N*-acyl amino acids derivatives, all showed the $\Delta\delta$ values that can be verified by Model C-type conformation. This rather puzzling result could be interpreted by repellent interaction between the pos-

itively charged acyl nitrogen and the amide nitrogen which is greater in Model D-type conformation.

4.5 Application to β,β -Disubstituted Propionic Acids

A carboxyl group can be produced by various types of reactions, and, in principle, the PGME method can be applied to a wide range of compounds that have a functional group convertible to a carboxyl group.

A cyclohexene of type [A] is a fragment involved in the structure of some terpenes as exemplified by limonene, carene, α -patchoulene, and copaene (Figure 30). Oxidative cleavage of the double bond of [A] will give rise to a carboxylic acid, β,β -disubstituted propionic acid [B]. We examined if the PGME method could be used for determination of the absolute configuration of [B].

In Figure 31, three staggered conformations [I], [II], and [III] of the (*R*)-PGME amide of β,β -disubstituted propionic acid resulting from rotation around C_1 – C_2 and viewed from the arrow in [C], are illustrated. Of the three rotamers, [III] must be the least stable because the bulky PGME group is placed between R_1 and R_2 . Rotamers [I] and [II] would be in almost the same energy level. Therefore, the PGME amide

Figure 31. The possible conformations of the (*R*)-PGME amide of β,β -disubstituted propionic acid [B]. Newman projection models [I], [II], and [III], resulting from rotation around C_1 – C_2 , are shown. The gray circle represents the PGME moiety. The conformation [D] is the averaged conformation of two stable conformers [I] and [II], and is expressed as [C]. Model E, in which $\Delta\delta = \delta_R - \delta_S$, shows the absolute configuration of the β,β -disubstituted propionic acid [B].

of β,β -disubstituted propionic acid [B] can be considered to exist in conformer [D], which is an averaged conformation of [I] and [II]. This conformation is expressed as [C]. In other words, when the PGME amide of chiral β,β -disubstituted propionic acid takes the conformation [C], the absolute configuration at the β -position of the propionic acid will be determined in an analogous way to the modified Mosher's method using Model E [Note: $\Delta\delta = \delta_R - \delta_S$].

Four terpenoids were converted into β,β -disubstituted propionic acids; (i) (1*R*)-(+)-pinene to **71** by ozonization/ H_2O_2 oxidation, (ii) D- Δ^3 -carene to **72** in the same way as pinene, (iii) (3*aR*)-(+)-sclareolide to **73** by alkaline hydrolysis, and (iv) (*R*)-(+)-citronellol to **74** by Jones oxidation. These carboxylic acids were subjected to the PGME method, the results of which are shown in structures **75–78** of Figure 32. Positive and negative $\Delta\delta$ values ($\delta_R - \delta_S$) are distributed on the right and left side of the PGME plane (Figure 31[C]), respectively, and the absolute configurations predicted by Model E were all in accordance with the known ones. It should be noted that these terpenes originally do not have a carboxyl group.

From a scale insect *Ceroplastes pseudoceriferus* Green, we

have isolated a diterpenoid **79** (Figure 33). Although its relative stereochemistry was easily deduced by the NMR analyses, the absolute configuration was difficult to be solved. Finally, we succeeded in overcoming the problem by converting **79** into **82** possessing the β,β -disubstituted propionyl moiety.²⁸

The ω -hydroxy fatty acid group of **79** was removed by hydrolysis, and the resulting diol was acetylated affording diacetate **80**. Ozonolysis of the product gave an intractable mixture, and, therefore, the oxygen functions were eliminated by Birch reduction to give **81**. Cleavage of the double bonds by ozonolysis gave the desired carboxylic acid **82**, to which the PGME method was applied. The result is shown in **83**. On the basis of the $\Delta\delta$ ($\delta_R - \delta_S$) values and Model E, the absolute configuration was determined as shown in **79**. This compound proved to be an *ent*-diterpene that was unusual in nature. Compound **84**, obtained from the same insect, was also an *ent*-diterpene, which was confirmed by the modified Mosher's method using 3-OH as a handhold.

5. Conclusion

Starting from the modified Mosher's method, we have

Figure 32. (above) Conversion of (1*R*)-(+)- α -pinene, D- Δ^3 -carene, (3*aR*)-(+)-sclareolide, and (*R*)-(+)-citronellol to the corresponding β,β -disubstituted propionic acids, **71–74**. (below) The $\Delta\delta$ ($= \delta_R - \delta_S$) values obtained for the PGME amides **75–78**.

Figure 33. Diterpenoids **79** and **84** obtained from a scale insect *Ceroplastes pseudoceriferus* Green. The procedures leading to the absolute configuration of **79** via **80–82** are shown. $\Delta\delta$ values ($\delta_R - \delta_S$) are assigned in **83**.

developed several new reagents that can clarify the absolute configurations of various types of compounds. There are, however, so many kinds of organic compounds whose absolute configurations cannot be solved by the existing analytical means and reagents. In this respect, new methods must be further developed by organic chemists to meet the industrial and academic requirement that information about chirality of as many substances such as drugs, biochemical reagents, and organic metabolites should be given.

This article has indicated that combination of chemistry and instrumental analysis will enable us to elucidate the absolute configurations of a variety of chemical substances even now when the number of useful methodologies is limited.

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PROFILE

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