4) but with the concomitant protonation of a third group absorbing around 1710 cm^{-1}, keeping the total number of charges constant.[17] It is not clear which mechanism shields the additional charge in 5,6-diH-META-I.

The data presented in this work provide evidence that saturation of the 5,6-double bond of the retinal chromophore leads to the formation of a new intermediate (BSI) at 80 K, in which the retinal chromophore adopts a relaxed conformation, although the protein can be assumed to be rather rigid at this temperature. This phenomenon is indicative of a tightly packed protein geometry in the vicinity of the ionylidene ring which hinders the relaxation of the twists caused by the cis-trans isomerization at the BATHO stage of the unmodified pigment. Due to the greater flexibility of the chromophore in 5,6-diH-ISORHO, the relaxation process can occur already at lower temperatures. In the native pigment, since the relaxation process leading to the BSI intermediate is slower, it can be resolved at room temperature with use of nanosecond spectroscopy.[12] These results show that the steric interaction of the chromophore with the protein is important in regulating the relaxation process during the reaction cascade. This agrees well with the previous results on the photoreactions of 9-demethylrhodopsin[17] and of 13-demethylrhodopsin,[18,19] which also possess a blue-shifted intermediate either at the BATHO state (9-demethylrhodopsin) or between BATHO and LUMI (13-demethylrhodopsin). The similarity between the BSI intermediate and the additional intermediate of 13-demethylrhodopsin has been emphasized recently.[20]

In addition, in the LUMI and META-I intermediates of the artificial pigment, *cis*-5,6-diH-ISORHO, molecular changes of the protein occur which differ from those observed for the corresponding unmodified intermediates. Some of these molecular changes take place already at lower temperature. Again, this can be explained by the greater flexibility of the retinal chain. The possibility that these alterations affect the G-protein activation is under investigation in our laboratory.

Acknowledgment. We acknowledge support from the Deutsche Forschungsgemeinschaft, SFB 60 G-9 and A-4, from the Fund for Basic Research administered by the Israel National Academy of Science and Humanities, and from the U.S.-Israel Binational Science Foundation.

High-Field FT NMR Application of Mosher's Method. The Absolute Configurations of Marine Terpenoids

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Abstract: Mosher's (^1H) method to elucidate the absolute configuration of secondary alcohols was reexamined by use of high-field FT NMR spectroscopy, which enables assignment of most of the protons of complex molecules. There is a systematic arrangement of $\Delta\delta$ ($\delta_S - \delta_R$) values obtained for the (*R*)- and (*S*)-MTPA esters of (−)-menthol, (−)-borneol, cholesterol, and ergosterol, the absolute configurations of which are known. Analysis of the $\Delta\delta$ values of these compounds led to a rule that could predict the absolute configurations of natural products. When this rule was applied to some marine terpenoids including cembranolides and xenicanes, their absolute configurations were assigned and a part of the results were confirmed by X-ray structural analyses. In the case of sipholenol A, which has a sterically hindered OH group, this rule is inapplicable. But the problem is overcome by inverting the OH group to a less sterically hindered position; the resulting epimer gives systematically arranged $\Delta\delta$ values, which enabled the elucidation of the absolute configuration. Comparison of the present method with Mosher's ^{19}F method indicates that the latter one using ^{19}F NMR lacks in reliability.

Introduction

Determination of the absolute configurations of organic compounds has become an important task of the natural products chemist as well as the synthetic chemist. There are a few physical methods, e.g., exciton chirality method[1] and X-ray crystallography, that fill this need, but they have some limitations. There are also several chemical methods used to predict the absolute configurations of organic substances.[2] Among them, Mosher's method[3] using 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters has been most frequently used.

Mosher proposed[3] that, in solution, the carbinyl proton and ester carbonyl and trifluoromethyl groups of the MTPA moiety lie in the same plane (Figure 1A).[4] The PCILO calculation[5] on this MTPA derivative demonstrates that the proposed conformation is one of the two stable conformations. Another stable conformation is the one in which the methoxy group comes close to the ester carbonyl. On the other hand, the X-ray studies on the (*R*)-MTPA esters of 4-*trans*-*tert*-butylcyclohexanol[6a] and 1-(*R*)-hydroxy-2(*R*)-bromo-1,2,3,4-tetrahydronaphthalene[6b] reveal that the MTPA moiety is in the conformation that is almost identical with the one proposed by Mosher. Furthermore, the band profile analysis[5] of the IR adsorptions (in CCl_4) on the (*R*)-MTPA esters of several cyclohexanols exhibits that the Mosher's conformation of the MTPA moiety is much more preferable (7:3) than the one with CF_3 group anti ($\theta = 180°$) to the ester carbonyl.

When the MTPA group is in the hypothesized conformation, Mosher pointed out that the ^1H NMR signal of L^2 of the (*R*)-MTPA ester will appear upfield relative to that of the (*S*)-MTPA ester due to the diamagnetic effect of the benzene ring. When Mosher first put forward this analysis, the NMR instruments most commonly available were 60–100-MHz instruments and the complete assignment of protons of complex organic molecules was practically impossible. This is mainly why the modifications using ^{19}F NMR[3b] or lanthanide-shift reagents[7] have been used instead of the original method.

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[‡] Tel-Aviv University.

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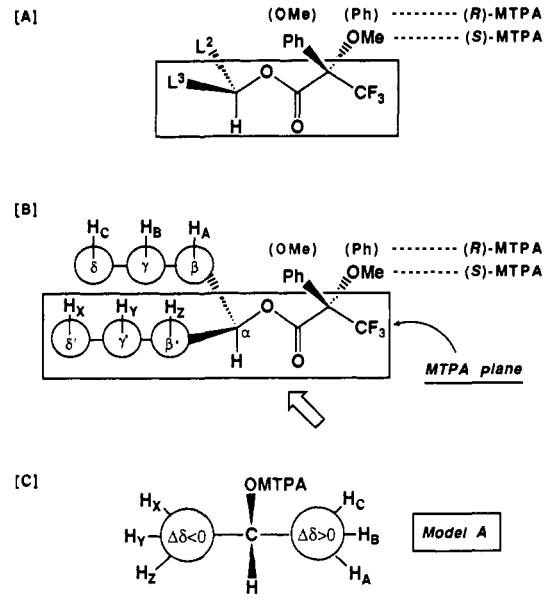

Figure 1. (A) Configurational correlation model for the (*R*)-MTPA derivatives and the (*S*)-MTPA derivatives proposed by Mosher. (B) MTPA plane of an MTPA ester is shown. $H_{A,B,C}$ and $H_{X,Y,Z}$ are on the right and left sides of the plane, respectively. (C) Model A to determine the absolute configurations of secondary alcohols is illustrated. Model A is a view of the MTPA ester drawn in (B) from the direction indicated by the outlined arrow.

The most important factor in use of these modified methods is the difference in steric bulkiness of the substituents on the β- and β'-carbons (Figure 1B); the steric repulsion between the phenyl group of the MTPA moiety and the β-substituents is essential to bring about the chemical shift difference of the CF_3 (^{19}F) or OMe (^1H) group. However, as we pointed out previously,[8] certain compounds (e.g., **5** and **6**) can adopt a conformation in which β-substituents are placed far from the MTPA moiety and other substituents (on the γ-, δ-, or ϵ-position) may have greater steric interaction with the MTPA group. In such cases, a simple comparison of the bulkiness between the β-substituents is meaningless. The intrinsic drawback of these methods, however, is that they depend on only *two data points*; that is, they are concerned with the chemical shift difference between two CF_3's (^{19}F) (or two OMe's (^1H)) of the (*R*)- and (*S*)-MTPA esters.

Recently, we have reported on the high-field FT NMR application of Mosher's method[8] to elucidate the absolute configurations of secondary alcohols by using high-field ^1H NMR spectroscopy. This method enables one to examine the chemical shift differences of as many protons as can be assigned by means of up to date NMR techniques including 2D spectra such as H,H COSY and HOHAHA spectra. We feel that, as this method relies on *a larger number of data points* (i.e., all of the protons assignable by 2D NMR techniques), it is more reliable than the above-mentioned variations of Mosher's method. Two analogous methods, one using *O*-methylmandelates[9] and the other using MTPA esters,[10] have been reported.

The basic concept of the present method is essentially the same as Mosher proposed: The idealized conformation is depicted in Figure 1B. (For convenience, the plane and the conformation of the MTPA group will be called the *MTPA plane* and the *ideal conformation*, respectively.) Due to the diamagnetic effect of the benzene ring, the $H_{A,B,C,...}$ NMR signals of the (*R*)-MTPA ester should appear upfield relative to those of the (*S*)-MTPA ester. The reverse should hold true for $H_{X,Y,Z,...}$. Therefore, when $\Delta\delta = \delta_S - \delta_R$, protons on the right side of the MTPA plane (Figure 1B) must have positive values ($\Delta\delta > 0$) and protons on the left side of the plane must have negative values ($\Delta\delta < 0$). This is illustrated in Figure 1C. Now, Mosher's method can be extended as follows: (1) Assign as many proton signals as possible with respect to each of the (*R*)- and (*S*)-MTPA esters. (2) Obtain $\Delta\delta$ values for the protons. (3) Put the protons with positive $\Delta\delta$ on the right side and those with negative $\Delta\delta$ on the left side of model A (Figure 1C). (4) Construct a molecular model of the compound in question and confirm that all the assigned protons with positive and negative $\Delta\delta$ values are actually found on the right and left sides of the MTPA plane, respectively. The absolute values of $\Delta\delta$ must be proportional to the distance from the MTPA moiety. When these conditions are *all* satisfied, model A will indicate the correct absolute configuration of the compound.[11]

This paper describes the scope of the high-field FT NMR application of Mosher's method and focuses on the elucidation of the absolute configurations of some marine terpenoids.

Experimental Section

Materials. Commercially available (+)- and (−)-MTPA acids (Aldrich, No. 15,526-8, $[\alpha]_D$ 72°; No. 31,803-5, $[\alpha]_D$ −72°, respectively), (−)-menthol, (−)-borneol, cholesterol, and ergosterol (Tokyo Kasei) were used without purification. (+)- and (−)-MTPA chlorides were prepared according to literature.[12] Other natural products are those obtained in our laboratory. 3α-Cholesterol (**15a**) was prepared according to ref 31. Friedelan-3β-ol (**14a**) and friedelan-3α-ol (**16a**) were prepared by reduction of friedelin[13] with $NaBH_4$[14] and lithium in liquid ammonia,[15] respectively. Usually 1–10 mg of secondary alcohols was used in the MTPA ester preparation.

Preparation of (*R*)- and (*S*)-MTPA Esters. The followings are the typical procedures.

Method A. (*R*)-MTPA Ester of Sanadaol (9a). To a solution of sanadaol (1.9 mg, 6 μmol) in pyridine (20 μL) was added (+)-MTPA chloride (2.3 μL, 12 μmol), and the solution was allowed to stand at room temperature for 13 h. 3-[(Dimethylamino)propyl]amine (1.4 μL, 12 μmol) was added, and after 10 min of standing, the solvent was evaporated. The residue was subjected to preparative TLC (Merck, Kieselgel 60, F_{254}, CH_2Cl_2), affording the pure (^1H NMR) (*R*)-MTPA ester **9b** (1.4 mg, 44%).

Method B. (*R*)-MTPA Ester of Friedelan-3β-ol (14a). A solution of friedelan-3β-ol (6.6 mg, 15 μmol), (dimethylamino)pyridine (7.5 mg, 61 μmol), and triethylamine (3.1 μL, 22 μmol) in 0.5 mL of dichloromethane (distilled from P_2O_5) was treated with (+)-MTPA chloride (5.6 μL, 30 μmol), and the mixture was allowed to stand at room temperature for 15 h. 3-[(Dimethylamino)propyl]amine (3.7 μL, 29 μmol) was added, and the residue obtained after evaporation of the solvent was applied to preparative TLC (hexane–CH_2Cl_2 (2:3)) to give pure (^1H NMR) (*R*)-MTPA ester **14b** (8.7 mg, 77%).

The MTPA esters of **1a**, **2a**, **3a**, **4a**, and **9a** were prepared by method A, and those of **5a**, **6a**, **7a**, **8a**, **12a**, **13a**, **14a**, **15a**, and **16a** were obtained by method B. The NMR properties of the products are listed in Table I (see supplementary material).

NMR Measurement. A Bruker AM-500 spectrometer was used. All 1D ^1H NMR spectra were recorded in $CDCl_3$. The data size was 32K, and the spectral width was 15 ppm (digital resolution per point 0.46 Hz). For determination of chemical shifts, a Gaussian window function (LB = −2, GB = 0.2) was used to transform the FIDs. 2D H,H COSY spectra were recorded with an F2 size of 2K and an F1 size of 512W when FIDs were collected, and only the F1 direction was zerofilled to 1K when they were transformed. Spectral widths were ambient but were narrowed to 1.5 ppm when the spectra were measured for **14** and **16** to enhance the digital resolutions. Phase-sensitive NOESY spectra were taken with use of a microprogram (NOESYPH) with pulse delay of 2–3 s and mixing time of 1–3 s. The data size was usually 4K (F2) × 256W

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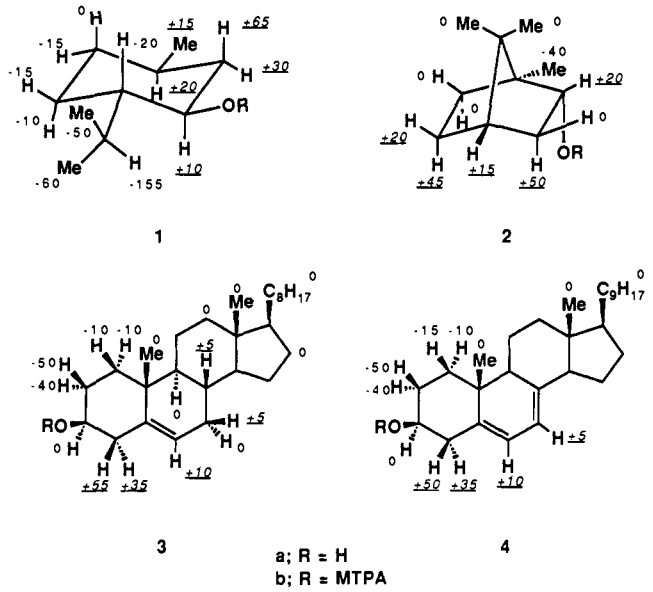


Figure 2. $\Delta\delta$ values obtained for the MTPA esters of (−)-menthol (**1a**), (−)-borneol (**2a**), cholesterol (**3a**), and ergosterol (**4a**). $\Delta\delta$ values are expressed in hertz (500 MHz).

(F1) when FIDs were collected, and the F1 direction was zerofilled to 1K when the FIDs were transformed. NOE difference spectra were recorded with use of a microprogram (NOEDIFF) with a pulse delay of 3 s and an irradiation period of 1–2 s. Usually 3–30 mM solutions were used for the ^{1}H NMR spectra.

Results and Discussions

Validity of the Method (Use of Known Compounds). For the purpose of confirming if the chemical shift behaviors of the protons actually accord with the rule, the following compounds **1a–4a**, the absolute configurations of which are known, were examined (Figure 2). In all cases, assignments of the protons were established by H,H COSY, phase-sensitive NOESY, and decoupling and NOE difference spectra at 500 MHz ($CDCl_3$). The concentrations of (*R*) and (*S*)-MTPA esters of a compound were adjusted to be the same, and the measurement of the 1D spectra was performed on the same day to minimize chemical shift change. Due to the extraordinary stability of the superconducting magnet, the chemical shift of the internal standard $CHCl_3$ did not vary (<1 Hz; <0.002 ppm) irrespective of sample solutions.

As an example, (−)-menthol derivative **1b** can be used to demonstrate the validity of the present methodolgy: It is evident that protons with $\Delta\delta > 0$ are located on the right side of the MTPA plane and the ones with $\Delta\delta < 0$ on the left side. Also, $\Delta\delta$ values are proportional to the distance between the protons and the MTPA moiety. The results obtained for compounds **2b**, **3b**, and **4b** again show the validity of the rule. In the steroid derivatives **3b** and **4b**, and $\Delta\delta$ values for methyls on C-10 and -13 are zero, possibly because they lie on the MTPA plane. The methyls on the side chains of **3b** and **4b** are too far from the MTPA group and so $\Delta\delta$ is zero. It should be noted that the β-carbons of these compounds are both secondary. In such a case, the conventional methods using ^{19}F NMR or lanthanide-shift reagents are not applicable. It is interesting to compare the values observed for the methine protons (α) on the carbons bearing MTPA moieties of **1b-4b**; $\Delta\delta$'s of **1b** and **2b** have positive values, whereas those of **3b** and **4b** are zero. If the (*R*)- and (*S*)-MTPA groups exist in the *ideal* conformation (i.e., the methine proton is just in the MTPA plane), the $\Delta\delta$ values should be zero. We think that the methine proton should be anisotropically affected more by the carbonyl group than by the phenyl group; the distance between the methine proton and the carbonyl oxygen is 0.18 nm, and that between the proton and the center of the benzene ring is 0.55 nm. Conformational deviation of the MPTA moiety from the *ideal* position would be less in **3b** and **4b**, in which both of the β-carbons are secondary and the steric compression from them to (*R*)- and (*S*)-MTPA is nearly equal ($\Delta\delta \approx 0$), than in **1b** and **2b**, in which the nonequivalence of the substitution grade of the β-carbons (one is secondary and the other is tertiary or quaternary) would result in distortion of the conformation(s) of either of the (*R*)- and (*S*)-MTPA groups ($|\Delta\delta| \gg 0$).

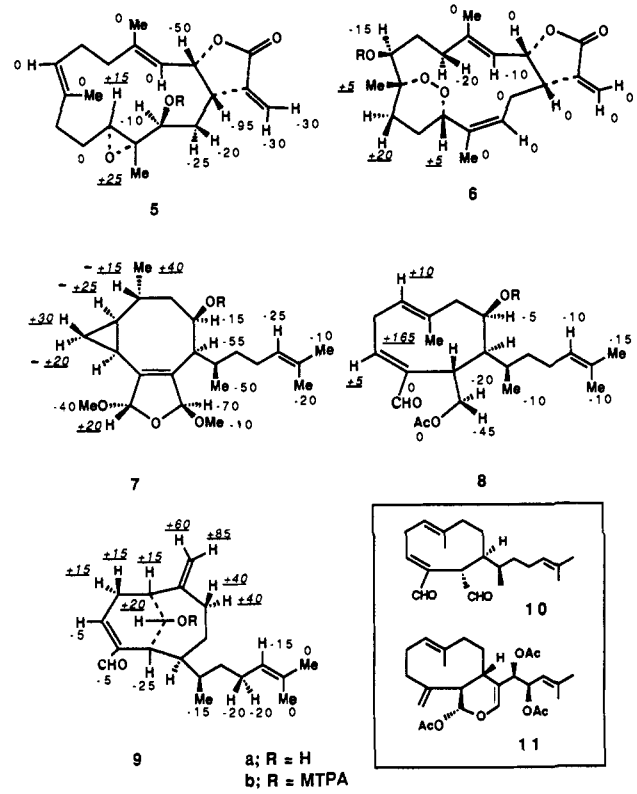
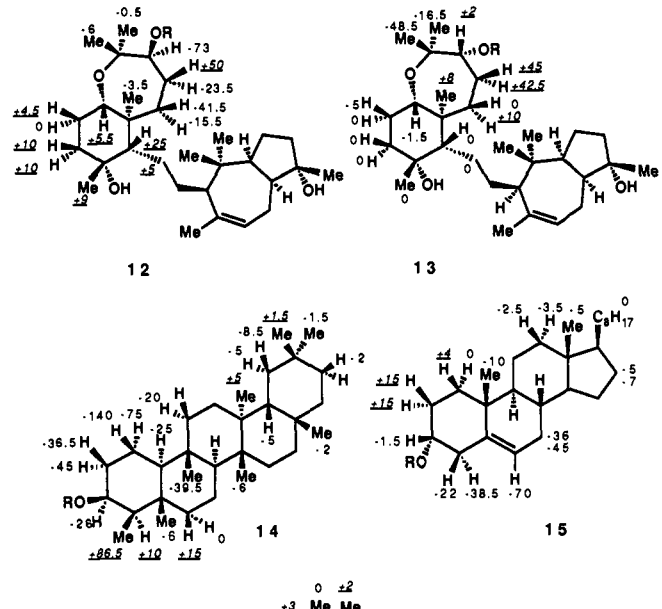


Figure 3. $\Delta\delta$ values obtained for the MTPA esters of marine terpenoids **5a–9a**.



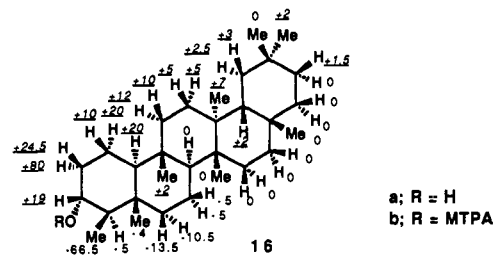


Figure 4. $\Delta\delta$ values obtained for the MTPA esters of sipholenol A (**12a**), episipholenol A (**13a**), friedelan-3β-ol (**14a**), 3α-cholesterol (**15a**), and friedelan-3α-ol (**16a**).

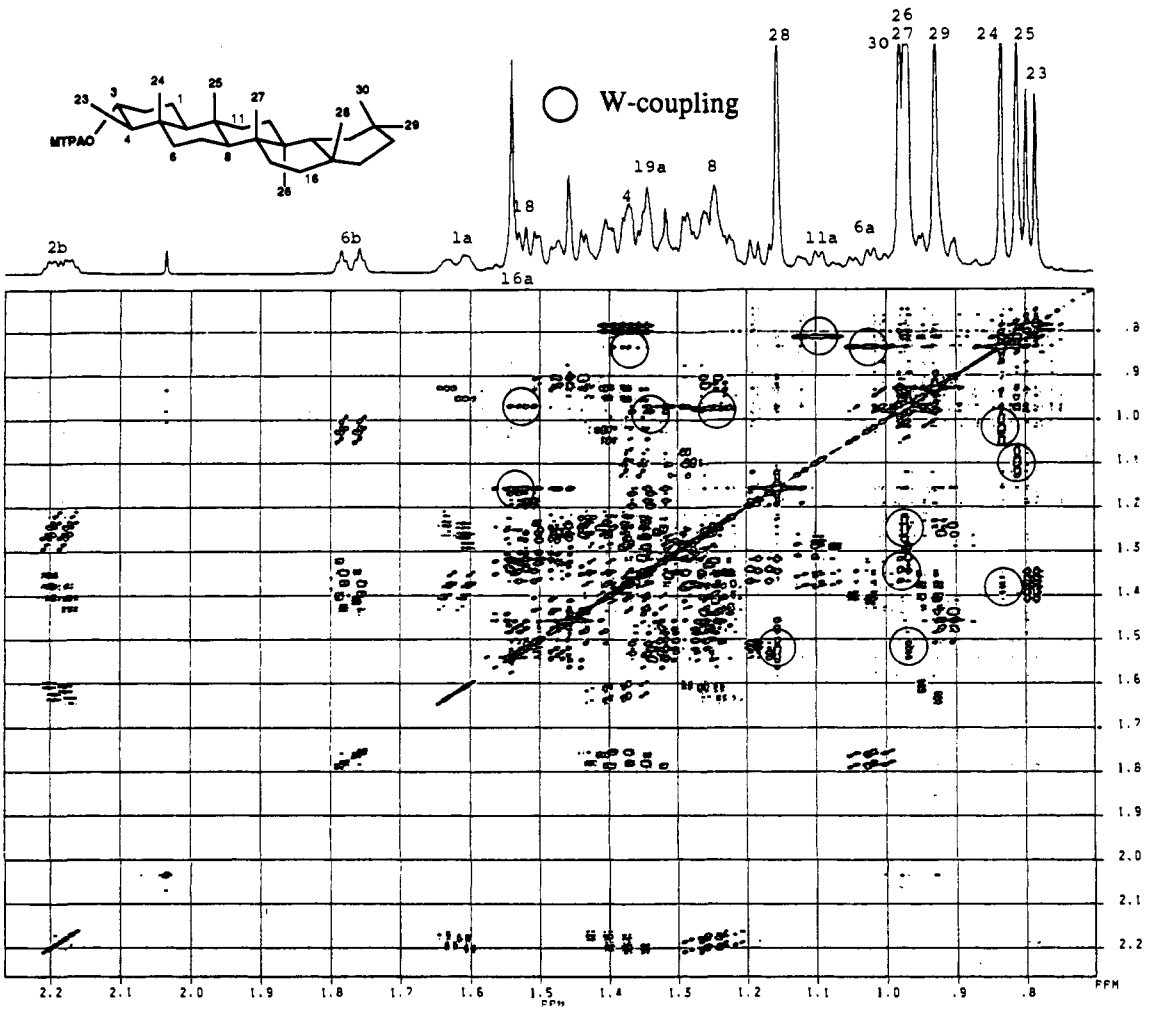
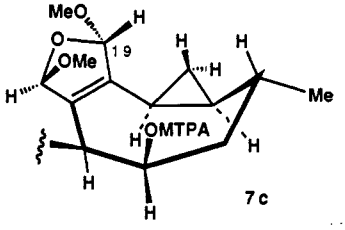


Figure 5. COSY 45 spectrum (500 MHz, $CDCl_3$) of the (*R*)-MTPA ester **16b**.

Application to Marine Terpenoids. During this decade, the secondary metabolites of marine organisms have been studied because many of them exhibit important biological activities and their chemical structures are often unique.[16] It occasionally happened that marine organisms, e.g., sponges, soft corals, and seaweeds, produce organic substances that are enanthiomeric to the ones found in terrestrial creatures.[17] Thus, determination of the absolute configurations of marine natural products are extremely important to deduce, for instance, the difference of biosynthetic pathways between the marine and terrestrial organisms.

In Figure 3 are summarized the results of the present method, which was successfully applied to marine diterpenes **5a**,[8,18] **6a**,[8,19] **7a**,[20] **8a**,[21] and **9a**,[22] the absolute configurations of which had been unknown. Without exception, protons with positive and negative $\Delta\delta$ values are found on the right and left sides of the MTPA planes, respectively, when their absolute structures are drawn as in Figure 3. (When viewing the structure, one should remember to view it from the direction so that the structure may seem as illustrated in model A (Figure 1C).) The absolute configuration of the cembranoid **6a** was also confirmed by X-ray analysis on its *p*-bromobenzoate.[8b] In the MTPA derivative of crenulacetal B (**7a**), $\Delta\delta$ of the methine proton on C-19 is +20. The proton may seem to be located on the left side of the MTPA plane in structure **7b**, but actually it exists on the right side of the plane (see the stereochemical structure **7c**).



7c

Among the compounds, sanadaol (β-crenulal[23]) (**9a**) may be worth mentioning: Sanadaol has been chemically correlated[23,24] with dictyodial (**10**),[25] a major component of some brown algae of the Dictyota family. The absolute configuration of the carbon framework of **9a**, determined by the present method, turned out to be enantiomeric to the one of xenicine (**11**), which was isolated from a marine soft coral.[26] The absolute configuration of **8a**, which coexists with dictyodial in *Pachydictyon coriaceum* and has the same xenicane skeleton, reinforces the validity of the

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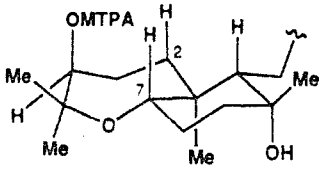
conclusion. The same absolute configuration of **9a** has been established by the total synthesis.[27]

Exceptions to the Rule and a Countermeasure. In our attempt to elucidate the absolute configuration of the marine triterpene sipholenol A (**12a**),[28] we noticed that $\Delta\delta$ values of the protons on the A and B rings of the MTPA derivatives are irregularly arranged as seen in structure **12b**. The data, of course, could not be used to determine the absolute configuration. However, this case shows another advantage of the present method. It has *a self-examination mechanism to know if the observed data can be used or if they should be abandoned.*

Examination of the molecular models of **12b** indicates that the OMTPA group takes an axiallike orientation and is sterically compressed by two axial protons on C-2 and -7 (**12c**), which may compel the MTPA group to take the conformation greatly different from the *ideal* one. Therefore, **12a** was converted into **13a**,[28b] in which the OH group exists in a sterically freer equatorial position, by oxidation of **12a** (PDC in dichloromethane[29]) followed



12c

by reduction with sodium borohydride. Delightfully, the positive and negative $\Delta\delta$ values of **13b** were found to be systematically arranged, which enabled us to assign the absolute configurations of **13a** and **12a** as shown in the respective structures.[30] The absolute configuration was verified by X-ray crystallography work on the (*S*)-MTPA ester of sipholenol A (**12a**).[31]

In order to confirm that steric compression to the MTPA group may cause an irregular arrangement of $\Delta\delta$ values, friedelan-3β-ol[14] (**14a**) and 3α-cholesterol[32] (**15a**) were examined. Owing to the rigidity of the rings, the hydroxy groups are oriented in axial directions in these compounds. The $\Delta\delta$ values of the MTPA derivatives **14b** and **15b** are, as anticipated, irregularly distributed (Figure 4) and are in good contrast to the results obtained for their epimers **3b** (Figure 2) and **16b** (Figure 4), $\Delta\delta$ value arrangements of which completely accord with the rule. It should be emphasized here that recent NMR techniques made it possible to assign all the protons of **16b**, a highly saturated triterpene. The H,H COSY spectrum of the (*R*)-MTPA ester of **16a** is shown in Figure 5. The long-range (^4J) couplings (W-type[33]) from a methyl to the vicinal axial protons (e.g., from H_3-24 to H-4 and H-6; the cross peaks due to the long-range coupling are encircled) were quite helpful for the assignments.

Table II. Absolute Configurations Predicted by the Mosher's Method Using ^{19}F NMR and the Present Method Using ^{1}H NMR Spectroscopy

compound	^{19}F chemical shift (δ)[a] (*S*)-MTPA	(*R*)-MTPA	$\delta_S - \delta_R$	configuration ^{19}F	^{1}H	agreement
1b[b]	4.34	4.46	−0.12	*R*	*R*	O
2b[b]	4.25	4.15	0.10	*S*	*R*	X
5b[c]	7.27	7.51	−0.24	*R*	*S*	X
6b[d]	7.08	7.27	−0.19	*R*	*S*	X
7b[c]	5.21	5.44	−0.23	*R*	*R*	O
8b[c]	7.53	7.43	0.10	*S*	*R*	X
12b[d]	6.03	5.25	0.78	*S*	*S*[e]	O
13b[c]	4.53	4.59	−0.06	*R*	*R*	O
14b[b]	4.88	4.97	−0.09	*R*	*S*[e]	X
16b[b]	4.53	4.41	0.12	*S*	*R*	X

[a] The ^{19}F NMR spectra were recorded on a Bruker AM-270 (**5b** and **6b**) and a JEOL FX-90Q spectrometer in $CDCl_3$. Chemical shifts are from that of TFA, a solution of which was measured separately. [b] Absolute configuration is already known. [c] Absolute configuration had been unknown. [d] Absolute configuration was confirmed by X-ray analysis by us. [e] The present Mosher's method is inapplicable to this compound.

The above results may suggest a general device to overcome the problem of the present method: If the $\Delta\delta$ values of the MTPA derivatives of a secondary alcohol are irregularly arranged and cannot be used, conversion of the hydroxy group (by use of, e.g., the Mitsunobu method,[32] hydrolysis of the tosylate,[34] oxidation to the ketone followed by reduction[35]) and application of the MTPA method to the epimeric alcohol may work.

Comparison with the Conventional Method Using ^{19}F NMR. Of the 14 compounds prepared for the present study, 10 are suitable for the conventional method using ^{19}F NMR spectroscopy,[3b] because the substitution grade of the 2 β-carbons is different in the respective compounds (**3b**, **4b**, **9b**, and **15b** are expections). It is evident from Table II that the predicted absolute configurations by the ^{19}F method are correct for compounds **1b**, **7b**, **12b**, and **13b** but wrong for compounds **2b**, **5b**, **6b**, **8b**, **14b**, and **16b**. Thus, probability of the correct prediction made by the ^{19}F method is less than 50%! This finding indicates that *the absolute configurations of the natural products in the literature determined by ^{19}F NMR spectra of MTPA esters ought to be all reexamined.*

Supplementary Material Available: Table I, listing ^{1}H NMR spectral data of the MPTA esters, and Figure 6, showing the numberings of the compounds used in this experiment (4 pages). Ordering information is given on any current masthead page.

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