Absolute Configuration of Amphidinol 3, the First Complete Structure Determination from Amphidinol Homologues: Application of a New Configuration Analysis Based on Carbon−Hydrogen Spin-Coupling Constants

Michio Murata,* Shigeru Matsuoka, Nobuaki Matsumori, Gopal K. Paul, and Kazuo Tachibana

Department of Chemistry, School of Science
The University of Tokyo, Hongo, Bunkyo-ku
Tokyo 113-0033, Japan

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Dinoflagellates, a type of primitive unicellular algae, are a rich source of structurally and biologically intriguing natural products; e.g., okadaic acid, brevetoxins, ciguatoxins, and maitotoxin. Among these polyether-cyclic compounds, amphidinols are unique dinoflagellate metabolites since they are primarily made up of linear polyhydroxy structures. The first member of this group was isolated from the dinoflagellate *Amphidinium klebsii* as a potent antifungal substance by Yasumoto's group.[1a] A series of homologues[1b,c] has since been found in the same genus, and Kobayashi's group reported closely related compounds, luteophanols.[2] These long-chain polyhydroxy compounds may be one of the most challenging targets for stereostructural elucidation since chiral centers are scattered over a flexible acyclic structure. Thus, little is known of the nature of the stereogenic centers in amphidinols.

We recently developed *J-based configuration analysis*,[3] which has been proven to be a powerful tool for the stereochemical determination of acyclic structures.[3b] In this method, 1,2-diastereomeric relationships between chiral centers are determined by choosing a correct staggered rotamer among six possibilities arising from *erythro* and *threo* configurations using spin-coupling constants ($^3J_{H,H}$ and $^{2,3}J_{C,H}$, e.g., see Figure 1c for $^2J_{C,H}$).[3a] In this paper, we report the complete configuration of amphidinol 3 (**1**), a representative homologue of the amphidinol family, mainly using this *J*-based method.

From cellular extracts of *A. klebsii* obtained from 440 L of culture, 12 mg of **1** was isolated together with other amphidinol homologues.[1b,c] To facilitate measurements of $^{2,3}J_{C,H}$, we prepared a ^{13}C-enriched sample of **1** (25% ^{13}C, 8 mg) by making another culture (200 L) in the presence of 12 mM $NaH^{13}CO_3$.

The stereochemical assignment of **1** was accomplished as follows; (a) the *J*-based method[3] was used for acyclic parts with 1,2- and 1,3-chiral centers, C20−C27, C32−C34, C38−C39, C44−C45, and C50−C51; (b) NOE analysis combined with *J* analysis was used for two ether cycles and their linkage C39−C44; (c) the modified Mosher method[4] and chromatographic/NMR comparison were used for degradation products to determine the absolute stereochemistry at C2, C6, C10, C14, C23, and C39.

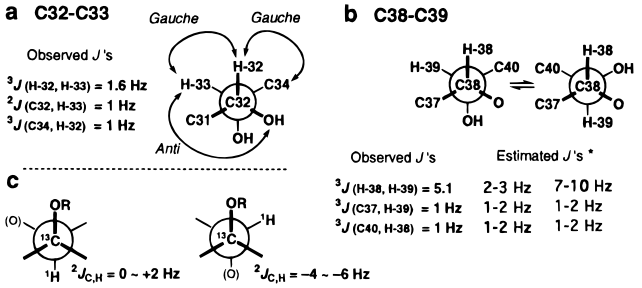
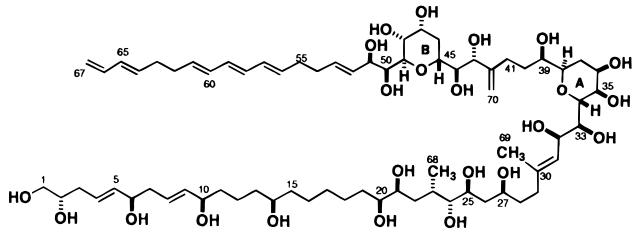


Figure 1. Rotamers and coupling constants for C32−C33 (a) and C38−C39 (b). **J* values for each rotamer estimated from stereochemically known compounds.[3a] (c) $^2J_{C,H}$ values depend on the dihedral angle between an oxygen atom on a relevant carbon and a proton on a neighboring carbon; in 1,2-dioxygenated systems such as C32−C33 (a), the anti O/H orientation gives $^2J_{C,H}$ of 0 to +2 Hz while the gauche orientation gives $^2J_{C,H}$ of −4 to −6 Hz.[8]

$^3J_{H,H}$ and $^{2,3}J_{C,H}$ values of intact **1** were measured by E.COSY[5]



1 Amphidinol 3

and hetero half-filtered TOCSY (HETLOC),[6] respectively; phase-sensitive HMBC[7] was also used for parts where the small magnetization transfer by TOCSY hampered the accurate measurement of $^{2,3}J_{C,H}$ by HETLOC. C32−C33 and C38−C39 can be used as examples to see how the *J*-based analysis works in configuration assignments. As shown in Figure 1a, ^3J(H-32, H-33) revealed a value that is typical of gauche interaction for a 1,2-diol system.[3a] The values for ^2J(C32, H-33) and ^3J(C34, H-32) indicate that H-33 is anti to C32-OH[8] and H-32 is gauche to C34, respectively. These interactions unambiguously establish the *threo* configuration for C32−C33, as depicted in Figure 1a. For C38−C39, ^3J(H-38, H-39), which is intermediate between anti and gauche, suggests that this bond undergoes a conformational change. The *J*-based analysis can even be applied to such a flexible system. The two small values for ^3J(C37, H-39) and ^3J(C40, H-38) indicate gauche C37/H-39 and gauche C40/H-38 interactions in both conformers (Figure 1b). Of the six possible pairs of alternating rotamers arising from *erythro* and *threo* configurations, only one pair in Figure 1b satisfies all of these requirements. The relative configurations of the consecutive stereogenic center in C20−C27 can be determined using this method, as shown in Figure 2. The diastereomeric relationships of C44−C45 and C50−C51 were assigned in the same manner on the basis of $^3J_{H,H}$ and $^{2,3}J_{C,H}$. The configurations of rings A/B and their linkage (C39−C44) were elucidated using NOEs[9] in combination with $^3J_{H,H}$ and $^{2,3}J_{C,H}$ data (see Supporting Information).

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(8) Dependence of $^2J_{C,H}$ on the dihedral orientation of oxygen functions has been investigated (e.g., Schwarcz, J. A.; Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1872−1875).

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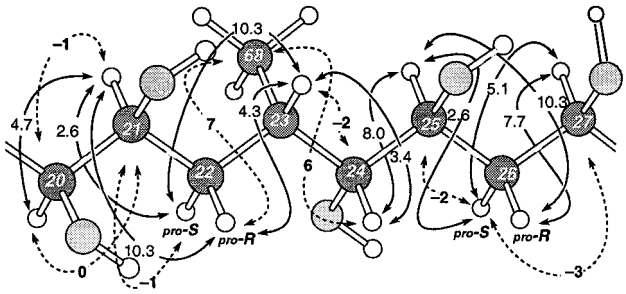
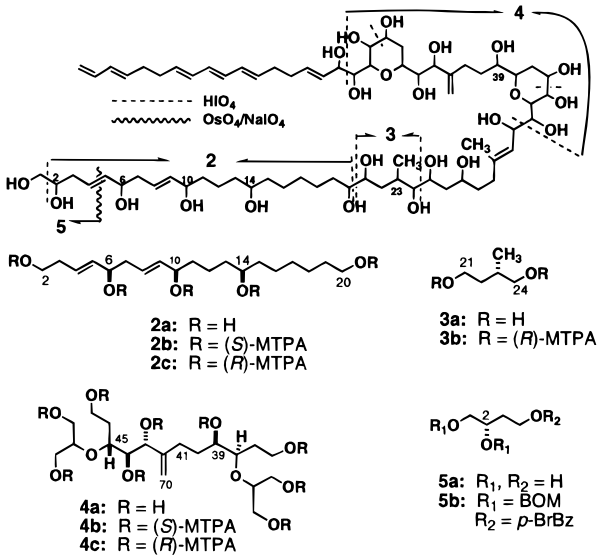


Figure 2. Configuration and conformation for C20−C27 of **1** established on the basis of $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$. Broken lines with arrows and numbers indicate $^{2,3}J_{C,H}$ in Hz, and plain lines denote $^{3}J_{H,H}$ in Hz. For pairs of vicinal carbons, except C24−C25,[9] two anti orientations of H/H, C/H, and/or O/H can be assigned on the basis of $^{2,3}J_{C,H}/^{3}J_{H,H}$, which results in elucidation of the 1,2-diastereomeric relationship (for methine−methylene pairs such as C21−C22, the stereospecific assignment of methylene protons can also be attained).[3a]

These NMR-based analyses using intact **1** have revealed the relative configurations of C20−C27 and C32−C51. Next, their absolute configurations and those at C2, C6, C10, and C14 were investigated using degradation products; treatment of **1** with HIO_4/$NaBH_4$, followed by esterification with (*R*)- and (*S*)-MTPA (2-methoxy-2-trifluoromethyl-2-phenylacetic acid) and separation by HPLC, furnished MTPA esters of fragments corresponding to C2−C20 (**2b**,**c**), C21−C24 (**3b**) and C33−C50 (**4b**,**c**).[10] The



absolute stereochemistries of C6, C10, C14,[11] and C39[12] were elucidated by the modified Mosher method using **2b**/**2c** and **4b**/**4c**.[4] The configuration of **3a** was determined to be 23*S* by comparison of the NMR data of the bis-(*R*)-MTPA esters **3b** with (*S*)- and (*R*)-MTPA esters of authentic (*R*)-methyl-1,4-butanediol.[13] The configuration of C2 was determined using the C1−C4 fragment obtained from the *O*-benzyloxy-methyl derivative of **1** by treatment with $OsO_4/NaIO_4$.[14] The resulting 1,2-dibenzyloxylmethoxy-butyl *p*-bromobenzoate (**5b**) was chromatographed on a chiral resolution column[15] and determined to be an (*S*)-enantiomer. Considering all of these partial configurations led to the complete structure of amphidinol 3 with the absolute stereochemistry of 2*S*, 6*R*, 10*R*, 14*R*, 20*S*, 21*S*, 23*S*, 24*R*, 25*S*, 27*S*, 32*R*, 33*S*, 34*R*, 35*R*, 36*R*, 38*R*, 39*R*, 43*R*, 44*R*, 45*R*, 47*R*, 48*R*, 49*R*, 50*S*, and 51*R*.

Despite recent progress in the NMR-based configuration analysis of natural products, chemical degradation in combination with partial synthesis is still considered the sole reliable method for determining the stereochemistry in acyclic structures, which often requires a fairly large amount of sample, as in the pioneering studies of mycoticins by Schreiber's group,[16] nystatin by Beau's group,[17] and roflamycoin by Rychnovsky's group.[18] In this study, we were able to perform a complete structural determination using only 3 mg of the sample for all of the chemical degradations and 8 mg of the ^{13}C-enriched sample for NMR measurements. This considerable reduction in sample size is mainly attributable to the *J*-based configuration analysis, since 12 of 17 chiral centers in the acyclic parts of **1** were established by this method. Therefore, $^{2,3}J_{C,H}$ should be reevaluated as a useful parameter for configuration assignments of acyclic stereogenic centers in natural products.

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Supporting Information Available: Selected spectra used for configuration assignments of **1** and their interpretations; HETLOC and HMBC for measurement of $^{2,3}J_{C,H}$, NOESY and ROESY (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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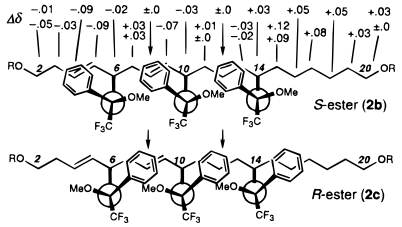
(9) Among 14 vicinal methine−methine interactions in the acyclic parts of **1**, there are four H/H-anti orientations, which were indicated by ^{3}J (H-24, H-25) = 8.0 Hz, ^{3}J(H-33, H-34) = 9.5 Hz, ^{3}J(H-43, H-44) = 9.0 Hz, and ^{3}J(H-49, H-50) = 10.0 Hz. For configuration assignments of these parts, additional NOE information is necessary.[3a] Diastereomeric relations between C24 and C25, C33 and C34, C43 and C44, and C49 and C50 were established by key NOEs between H-23 and C25−OH, H-32 and H-38, C43−OH and H-45, and H-45 and H-51, respectively. See Supporting Information for details.

(10) MTPA esters **2b** (690 μg), **2c** (520 μg), **3b** (30 μg), **4b** (400 μg), and **4c** (150 μg) were obtained from 3.0 mg of **1**. See Supporting Information.

(11) As shown below, the stereochemistry at C6 and C14 can be determined to be 6*R* and 14*R* based on the distribution of $\Delta\delta$ (**2b** − **2c**), in which positive values are observed in C15−C20, while negative ones are seen in C2−C5. The configuration of C10 could also be assigned since $\Delta\delta$ of C8 and C12 are zero. This can only be accounted for by the *R* configuration at C10, since the shielding effect at C8 (or C12) from a phenyl group of (*S*)-MTPA at C10 (or C14) should be equal to that from an (*R*)-MTPA at C6 (or C10).



(12) The $\Delta\delta$ values of (**4b** − **4c**) were as follows: $CDCl_3$, 500 MHz, H_2-37, −0.12/−0.06; H-38, −0.57; H-39, −0.14; H-40, +0.37; H_2-41, +0.30/+0.37; H_2-70, +0.33/+0.42. These data show that C39 has an *R* configuration, since the clear separation of negative and positive values with respect to C39 shows that the $\Delta\delta$ values were predominately affected by the MTPA group on C39 rather than by other MTPA groups on relatively remote sites.

(13) (*R*)-Methyl-1,4-butanediol was prepared from dimethyl-(*R*)-methylsuccinate by $LiAlH_4$ reduction, followed by esterification with (*R*)- and (*S*)-MTPA. ^{1}H NMR of **3b** was identical to that of the (*S*)-ester of the authentic diol; **3b** and (*S*)-ester ($CDCl_3$, 500 MHz) δ 3.98, 3.92, 3.82, 3.73, 1.53, 1.38, 1.05, 0.52. (*R*)-ester δ 3.97, 3.93, 3.83, 3.70, 1.52, 1.38, 1.05, 0.52.

(14) A procedure that was previously reported for ciguatoxin was followed: Satake, M.; Morohashi, A.; Oguri, H.; Oishi, T.; Hirama, H.; Harada, N.; Yasumoto, T. *J. Am. Chem. Soc.* **1997**, *119*, 11325−11326.

(15) Chiral resolution was performed on CHIRALPAK AD (ϕ 4.6 × 250 mm, DAICEL) eluted with hexane-1-propanol (25: 1). Authentic (*S*)-ester and the derivative from **1** (**5b**) were eluted at 16 min 50 sec and 16 min 59 sec, respectively, while (*R*)-ester appeared at 19 min 13 sec.

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