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Conformational Analysis of Natural Products Using Long-Range Carbon-Proton Coupling Constants: Three-Dimensional Structure of Okadaic Acid in Solution

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Abstract: Long-range carbon-proton coupling constants ($^{2,3}J_{C,H}$) are known to depend on the dihedral angles as is the well-known case with interproton couplings. The precise measurement of these *J* values were carried out using hetero half-filtered TOCSY and phase-sensitive HMBC on a marine natural product, okadaic acid. From the obtained values together with $^3J_{H,H}$ we disclosed the conformations of okadaic acid in organic and aqueous solutions (CD_3OD-$CDCl_3$ or NaOD/D_2O), which resembled each other and that obtained for its crystalline *o*-bromobenzyl ester. The successful result in the present conformational study, even though as an exmaple, demonstrated this methodology to be powerful in elucidation of conformations and configurations of acyclic or macrocyclic portions in natural products of this size.

INTRODUCTION

Elucidating the three-dimensional structures of bioactive natural products is essential if we are to understand their mode of action at the molecular level. Currently, the conformational analysis of these complicated molecules in solution relies mainly on NOE measurements, as is the case with proteins or peptides. However, for flexible molecules such as acyclic or macrocyclic compounds, it is difficult to determine the dominant conformation from NOE-derived distances in a rapidly fluttering system, since minor conformations can often make disproportionally large contributions to NOE intensities. On the other hand, the interproton or heteronuclear coupling constant ($^3J_{H,H}$ or $^3J_{C,H}$) is obtained as a weighted average over that derived from each conformer. The dihedral angles obtained from these coupling data should therefore provide more reliable information regarding a major conformer for flexible molecules.

Although long-range heteronuclear coupling constants ($^{2,3}J_{C,H}$) are known to depend on the dihedral angle, being analogous to $^3J_{H,H}$, they are seldom used because of the difficulty in detecting the ^{13}C-coupled ^{1}H-resonances. As for $^3J_{C,H}$, a Karplus type equation has been proposed[1] and proven to be useful for conformational analyses of particular molecules such as polysaccharides. The use of $^2J_{C,H}$ in conformational studies has been further limited because of the absence of clear dependence on structural parameters. However, when the α-carbon carries an electronegative substituent (X) such as an oxygen or halogen atom (^{1}H-C-^{13}C-X systems), a clear relation has been reported between the magnitude of $^2J_{C,H}$ and the dihedral angle between the proton and the heteroatom.[2-4] Namely, the $^2J_{C,H}$ value is around -6 Hz when H is gauche to OH in a rigid cyclohexanol system, while being *ca.* 0 Hz for the antiperiplanar orientation.[5] This reliance of $^2J_{C,H}$ on the orientation of an oxygen functionality should be very useful for conformational analysis for highly oxygenated natural products.

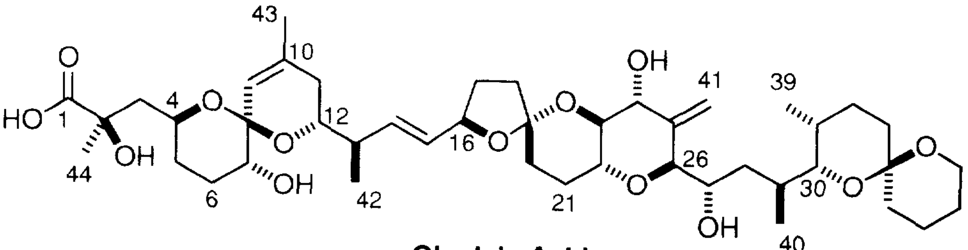
Recently, several NMR experimental techniques designed to measure $^{2,3}J_{C,H}$ have been proposed, thus making it possible to determine these values with much greater accuracy and sensitivity than with conventional methods, such as the C-H long-range J resolved spectrum.[6] Two-dimensional hetero half-filtered TOCSY experiments (HETLOC),[7-9] in which the pulse sequence contains a hetero nuclear half-filter after an excitation pulse, is probably the most powerful method for this purpose. However, this method requires that 1) the carbons in the concerned system must bear protons, and 2) the magnetization of the carbon-bound protons should be relayed to the long-range-coupled protons by TOCSY coherence transfer. Therefore, partial structures containing a quaternary carbon or a weakly coupled pair of protons are not suited for this method. In these cases, an alternative methodology based on a different principle should be used to obtain the values of $^{2,3}J_{C,H}$. As Zhu *et al.* reported, $^{2,3}J_{C,H}$ can be calculated from the relative intensities of a cross-peak in the phase-sensitive HMBC spectrum to that in the reference spectrum.[10] However, with molecules which give overlapping signals in the 1D ^{1}H spectrum, as is often seen with contemporary natural products, this method does not work well due to superposition of signals in the reference spectrum. The use of this method in combination with hetero half-filtered experiments, however, overcomes this difficulty as described below, and thus should be effective for measuring $^{2,3}J_{C,H}$ in systems which involve quaternary carbons or weakly coupling protons.

In the phase-sensitive HMBC spectrum, the coupling patterns of cross-peaks arising from a proton signal are identical, but the intensity (I) ratio of the cross-peaks depends on $^{2,3}J_{C,H}$, as given by the following equation:

$$I_{Ca,H} / I_{Cb,H} = \sin^2(\pi J_{Ca,H} \cdot \Delta) / \sin^2(\pi J_{Cb,H} \cdot \Delta) \qquad \text{Eq.1}$$

where Ca and Cb are the carbons coupling with the proton, and Δ is an interval in the phase-sensitive HMBC pulse sequence (see Experimental).[10a] Provided that the $J_{Cb,H}$ is known from another method, such as a HETLOC spectrum, the intensity ratio of the cross-peaks in the HMBC spectrum gives the $J_{Ca,H}$ from Eq.1. The relative intensities corresponding to the left side of Eq.1 can be obtained by simply comparing the heights of the cross-peaks without consulting with the congested reference spectrum.

In this paper, we demonstrate that the combined use of $^{2,3}J_{C,H}$ and $^3J_{H,H}$ efficiently provides detailed information on the structure of the acyclic portions of natural products. Okadaic acid,[11] which is a well known tumor promoter[12] and a potent inhibitor of protein phosphatase 2A,[13] was chosen as a target, since its structure in solution, especially of its acyclic portions, has not yet been fully established despite extensive NOE experiments coupled with distance geometrical calculations disclosing its conformation partly.[14]



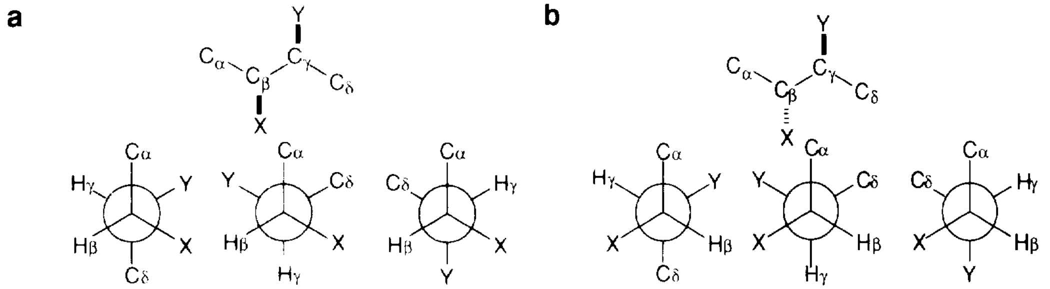
Okadaic Acid

RESULTS AND DISCUSSION

Determination of local conformations from coupling constants. Three rotamers staggered around a C-C bond between methine/methine or methine/methylene are shown in Figs. 1 and 2, respectively, together with the expected magnitudes of the J values. A Karplus-type equation can be applied also to ${}^3J_{C,H}$.[3] In systems with no electronegative substituents, the values of ${}^3J_{C,H}$ are *ca.* 8 Hz for the antiperiplanar orientation and *ca.* 2 Hz for the gauche orientation. For methine-methine systems (Fig. 1), the type I-a and II-a rotamers can not be distinguished using only ${}^3J_{H,H}$, since the arrangements between Hβ and Hγ are both gauche. However, if either ${}^3J$(Cα, Hγ) or ${}^3J$(Hβ, Cδ) is measurable, the two rotamers can be differentiated.

With regard to the methine-methylene systems (Fig. 2), rotamers 1 and 2 can be differentiated by ${}^3J_{H,H}$ only when the prochiral methylene protons have been assigned. ${}^3J$(Cα, Hγ1) and/or ${}^3J$(Cα, Hγ2) determine the stereospecific assignments here. When substituent X is an oxygen functionality such as a hydroxyl or alkoxyl group, ${}^2J_{C,H}$ can be also used for this purpose in addition to ${}^3J_{C,H}$ (Fig. 2d).

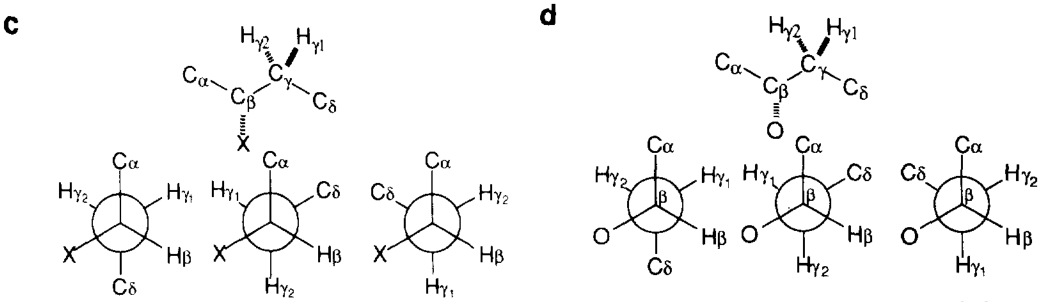
Possible co-existence of rotamers must also be taken into account. When one of the three rotamers is predominant in an equilibrium, in which typical J values for the gauche or anti orientation should be observed, we can safely rule out the contribution from the other rotamers and determine the major one by the strategy shown in Figs. 1 and 2. If there are two or more dominant rotamers, at least one of these coupling constants must be an intermediate value. In this case, some simulation studies will be required to determine those multiple rotamers. In addition, when the rotational conformer is deviated from the staggered one over 15°, which is relatively unusal in acyclic systems, this method cannot be directly applied.



	I-a	II-a	III-a
${}^3J$(Hβ, Hγ)[a]	Small	Small	Large
${}^3J$(Cα, Hγ)	Small	Large	Small
${}^3J$(Cδ, Hβ)	Small	Large	Small

	I-b	II-b	III-b
${}^3J$(Hβ, Hγ)	Large	Small	Small
${}^3J$(Cα, Hγ)	Small	Large	Small
${}^3J$(Cδ, Hβ)	Small	Small	Large

Fig. 1. Three rotamers staggered with respect to a methine-methine bond, together with the expected magnitudes of the coupling constants. [a] The typical 'Large' values for ${}^3J_{H,H}$ and ${}^3J_{C,H}$ are around 10 Hz and 8 Hz, respectively. The typical 'Small' values for ${}^3J_{H,H}$ and ${}^3J_{C,H}$ are both 2–3 Hz.



	1–c	2–c	3–c
^3J(Hβ, Hγ1)[a]	Small	Large	Small
^3J(Hβ, Hγ2)	Large	Small	Small
^3J(Cα, Hγ1)	Small	Small	Large
^3J(Cα, Hγ2)	Small	Large	Small

	1–d	2–d	3–d
^3J(Hβ, Hγ1)[a]	Small	Large	Small
^3J(Hβ, Hγ2)	Large	Small	Small
^2J(Cβ, Hγ1)[b]	Small	Large	Large
^2J(Cβ, Hγ2)	Large	Large	Small

Fig. 2. Three rotamers staggered with respect to a methine-methylene bond, together with the expected magnitudes of the coupling constants (**c**). When X is an oxygen functionality such as a hydroxyl group, $^2J_{C,H}$ can be used in addition to $^3J_{C,H}$ (**d**). [a] The typical 'Large' values for $^3J_{H,H}$ and $^3J_{C,H}$ are around 10 Hz and 8 Hz, respectively, and the typical 'Small' values for $^3J_{H,H}$ and $^3J_{C,H}$ are both 2–3 Hz. [b] The values of $^2J_{C,H}$ for the gauche and trans arrangements between Hγ and an oxgen atom are *ca.* -6 (Large) and 0 to -1 (Small) Hz, respectively.

Determination of Relevant Coupling Constants. $^3J_{H,H}$ were extracted from the E.COSY[15] spectrum or 1D selective ^{1}H-decoupling experiments. All of the relevant $^3J_{H,H}$ in the chain portions of okadaic acid were obtained (Table 1).

$^{2,3}J_{C,H}$ values were measured mainly by HETLOC experiments, which were carried out with 25 mg of a sample dissolved in 0.5 mL of CD_3OD-$CDCl_3$ (1:2). The HETLOC spectrum exhibits an E.COSY-like cross-peak structure, which is split by large $^1J_{C,H}$ in the *F*1 dimension and displaced by the small $^{2,3}J_{C,H}$ in the *F*2 (Fig. 3). The direction of the offset along *F*2 represents the relative sign of the coupling constant. In this study, mixing times of 30 ms and 60 ms were used to determine $^2J_{C,H}$ and $^3J_{C,H}$, respectively.

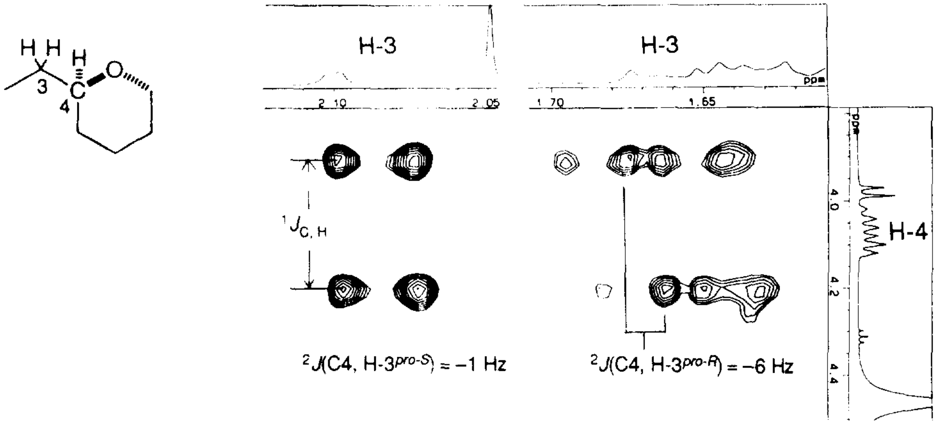


Fig. 3. The expanded HETLOC spectrum of okadaic acid, showing the cross-peaks between H_2-3 and H-4.

Since HETLOC experiments cannot be applied to systems containing quaternary carbons, the phase-sensitive HMBC experiments was carried out for the C1-C3 portion of okadaic acid, which involved a carboxylic carbon (C1) and a quaternary sp^3 carbon (C2). The values of ^3J(C1, H-$3^{pro\text{-}R}$) and ^3J(C44, H-$3^{pro\text{-}R}$) for okadaic acid were determined using Eq. 1 with the intensity of the cross-peaks in the phase-sensitive HMBC spectrum and ^2J(C4, H-$3^{pro\text{-}R}$) as the reference value, which was obtained from the HETLOC spectrum (Fig. 4). Table 2 shows $^{2,3}J_{C,H}$ for the acyclic portions of okadaic acid.

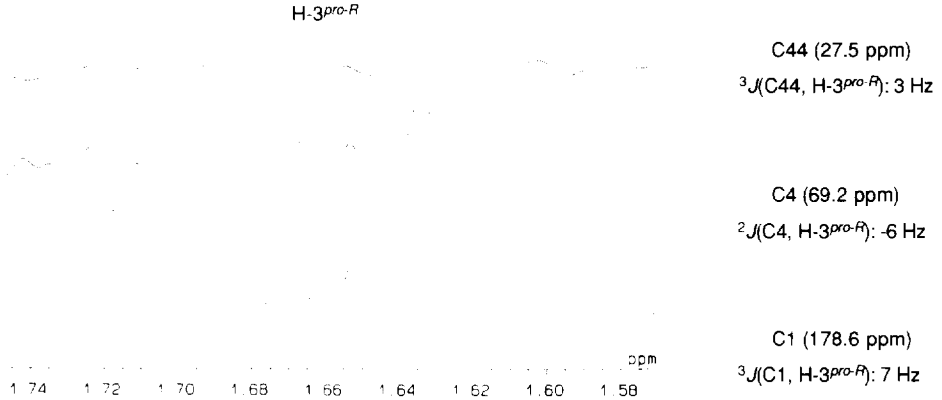


Fig.4. *F*2 cross-sections of the phase-sensitive HMBC spectrum. Cross-peaks of H-3 (*pro-R*) with C44, C4 and C1 are shown.

Table 1. $^3J_{H,H}$ (Hz) of the acyclic portions of okadaic acid in CD_3OD-$CDCl_3$ and D_2O-NaOD[a]

$^3J_{H,H}$	CD_3OD-$CDCl_3$	D_2O-NaOD
H-$3^{pro\text{-}R}$, H-4	11	8
H-$3^{pro\text{-}S}$, H-4	2	3
H-12, H-13	9	9
H-13, H-14	9	9
H-15, H-16	8	9
H-26, H-27	8	9
H-27, H-$28^{pro\text{-}R}$	11	11
H-27, H-$28^{pro\text{-}S}$	3	3
H-$28^{pro\text{-}R}$, H-29	3	3
H-$28^{pro\text{-}S}$, H-29	11	12
H-29, H-30	10	11

[a] The digital resolution of E.COSY spectra is 0.7 Hz.

Table 2. $^{2,3}J_{C,H}$ of the acyclic portions of okadaic acid in CD_3OD-$CDCl_3$[a]

$^2J_{C,H}$	value (Hz)	$^3J_{C,H}$	value (Hz)
C4, H-$3^{pro\text{-}R}$	-6	C1, H-$3^{pro\text{-}R}$	7[b]
C4, H-$3^{pro\text{-}S}$	-1	C44, H-$3^{pro\text{-}R}$	3[b]
C12, H-13	-6	C11, H-13	3
C26, H-27	-3	C14, H-12	2
C27, H-26	-6	C42, H-12	2
C27, H-$28^{pro\text{-}R}$	-7	C26, H-$28^{pro\text{-}R}$	3
C27, H-$28^{pro\text{-}S}$	-1	C26, H-$28^{pro\text{-}S}$	1
		C27, H-29	1
		C29, H27	1
		C30, H-$28^{pro\text{-}S}$	2
		C40, H-$28^{pro\text{-}R}$	8
		C40, H-30	2

[a] The digital resolution of HETLOC spectra is 0.7 Hz. [b] Determined by the combination of phase-sensitive HMBC and HETLOC experiments (see text). The other values were obtained from the HETLOC spectra.

Conformation of okadaic acid in CD_3OD-$CDCl_3$. We first investigated the conformation of the C1-C4 portion of okadaic acid in conjunction with the stereospecific assignment of the H_2-3 methylenes. For the C3-C4 bond, one large and one small $^3J_{H,H}$ indicate that the rotamer is either type 1-d or 2-d (see Fig. 2). Unfortunately, relevant $^3J_{C,H}$ for discriminating these rotamers were not obtained due to insufficient TOCSY transfer from H-3 to H-5. Thus, we resorted to $^2J_{C,H}$, which represented the orientation between the H_2-3 protons and the ether oxygen. The ^2J(C4, H_2-3) are -6 Hz for one of the H_2-3 at δ 1.66 and -1 Hz for the other at δ 2.09, indicating the predominance of the 1-d rotamer. Accordingly, the diastereotopic H_2-3 protons are assigned as follows; the high-field resonanced proton is *pro-R*, and the low-field is *pro-S*. With respect to the C2-C3 bond, the large coupling between C1 and H-$3^{pro\text{-}R}$ implies their anti arrangement. The resultant conformation of the C1-C4 portion is shown in Fig. 5a.

In the C12-C16 portion of okadaic acid, fairly large $^3J_{H,H}$ values (8-9 Hz) are observed for all of the vicinal pairs of protons. This implies that the relations between the hydrogens on the C12-C16 moiety are all anti (Fig. 5b).

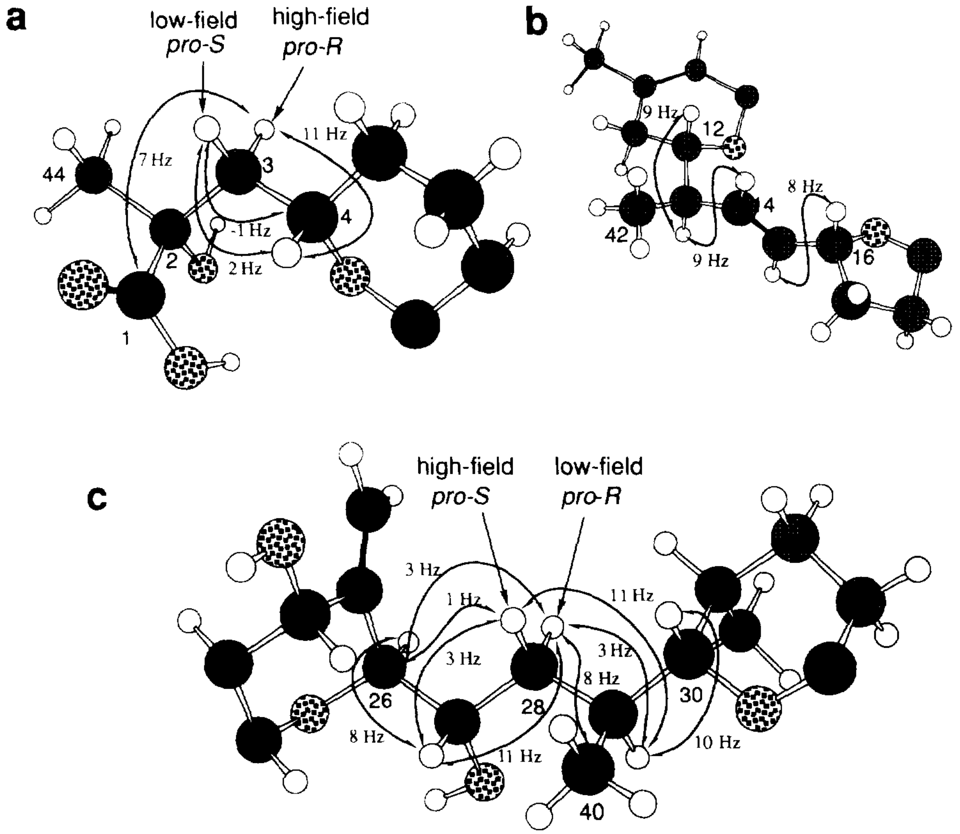


Fig.5. Stereostructures of three acyclic regions of okadaic acid constructed by $^3J_{H,H}$ and $^{2,3}J_{C,H}$. a) The C1-C4 region with the stereospecific assignment of H_2-3 protons. b) The C12-C16 region. c) The C26-C30 region with the stereospecific assignment of H_2-28 protons.

The conformation of the C26-C30 region of okadaic acid is also determined similarly. For the C26-C27 and C29-C30 bonds, fairly large $^{3}J_{H,H}$ (8 and 10 Hz, respectively) were observed, which indicates that type III-a rotamers prevail in both cases (Fig.1a). For the C27-C28 bond, one small and one large values were obtained for ^{3}J(H-27, H_2-28), and two small values were obtained for ^{3}J(C26, H_2-28). These data suggest that the rotamer around the C27-C28 bond is type 1-c (Fig. 2c), and the high-field H_2-28 proton is assigned to *pro-S*, which leads to the stereospecific assignment of the H_2-28 protons . The rotamer around the C28-C29 bond can be determined on the basis of the $^{3}J_{H,H}$ using thus stereospecifically assigned H_2-28; a large coupling between H-$28^{pro\text{-}S}$ and H-29 is indicative of rotamer 1-c. Together, these rotamers give the conformation shown in Fig. 5c for the C26-C30 portion.

Based on the conformations of the acyclic portions determined as above, and those of polycyclic ethers, which were easily derived from $^{3}J_{H,H}$ and NOE data by conventional methods, the entire 3-dimensional structure of okadaic acid in CD_3OD-$CDCl_3$ was generated. To adjust minute rotational angles, energy minimization calculations were performed using the CFF91 force field in DISCOVER/INSIGHT II (Biosym) software. During minimization, no drastic conformational changes were observed and the maximum deviations from the initial staggered rotamers constructed by $^{3}J_{H,H}/^{2,3}J_{C,H}$ was seen at the C26-C27 bond by less than 10°. It should be noted that the resulting structure in Fig. 6 closely resembles to the crystal structure obtained for its *o*-bromobenzyl ester.[11]

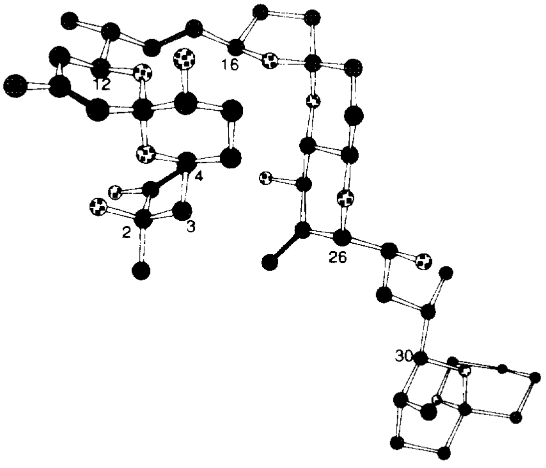


Fig.6. Stereostructure of okadaic acid in organic solution, obtained after the minute adjustment (using the CFF91 force field) of the conformation derived from coupling constants. Hydrogen atoms are omitted for clarity.

Conformation of okadaic acid in aqueous solution. We also investigated the conformation of okadaic acid in an aqueous solution to deduce the stereostructure under physiological conditions. Okadaic acid was dissolved in 0.1M NaOD-D_2O, and $^{3}J_{H,H}$ were measured by E.COSY or selective proton decoupling spectra. The *J* values observed in the aqueous solution (Table 1) are consistent with those in the organic solvent (Table 1) except for ^{3}J(H_2-3, H-4), which indicates a small clockwise rotation around the C3-C4 bond. The rest of the molecule turned out to take the nearly identical conformation in the two solvents. One possible explanation for this small change in the carboxyl terminus is that the solvent change and/or the ionization of the carboxylic acid affect a hydrogen bond between 2-OH and the ether oxygen on C4/C8.

In this study, $^{2,3}J_{C,H}$ has been demonstrated to be a useful parameter for determining the conformation of organic compounds possessing acyclic moieties, using okadaic acid as an example. We are currently attempting to use this method to determine the relative configurations for acyclic moieties. The diastereomeric relationships among adjacent asymmetric centers or asymmetric centers separated by methylene can be umambiguously established on the basis of the dihedral angles of H/H, O/H and C/H derived from $^3J_{H,H}$ and $^{2,3}J_{C,H}$. To see how this method works, we pretend not to know the diastereomeric relation between C27 and C29 of okadaic acid. As shown in Fig. 2c, there are three possible rotamers with respect to the C27-C28 bond for the (S*)-C27 configuration. The two H_2-28 protons at δ 0.99 and δ 1.38 can be unambiguously assigned as *pro-S** and *pro-R**, respectively, based on the vicinal H-H and C-H coupling constants (large/small $^3J_{H,H}$ and small/small $^3J_{C,H}$) which can only be explained by a 1-c type rotamer around the C27-C28 bond (Fig. 2c) with the *pro-R** proton (Hγ2) coupling by 11 Hz with H-27(Hβ) and the *pro-S** proton (Hγ1) coupling by 3 Hz (Fig. 5c). Regarding the C28-C29 bond, three rotamers may also be possible for each C29 configuration. Using the three coupling constants: $^3J(\text{H-28}^{pro\text{-}R*}, \text{H-29}) = 3$ Hz, $^3J(\text{H-28}^{pro\text{-}S*}, \text{H-29}) = 11$ Hz and $^3J(\text{C40}, \text{H-28}^{pro\text{-}R*}) = 8$ Hz, the dominant rotamer can be determined as type 1-c (in this case, C40 is a substituent depicted as 'X' and oriented *anti* to Hγ1, and the H_2-28 protons carry the inverted prochiral relations: Hγ1 is *pro-R** and Hγ2 is *pro-S**. This assignment for H_2-28 coincides with that from the C27-C28 bond only when C29 carries the S* configuration). The diastereomeric relation between C27 and C29 is thus clearly established using the dual stereospecific assignments between H_2-28/C27 and H_2-28/C29. This successful attempt suggests that unknown stereogenic centers in natural products can be assigned by the use of coupling constants, $^3J_{H,H}$ and $^{2,3}J_{C,H}$. Further attempts to determine relative configurations of acyclic portions of larger natural products such as maitotoxin[16] and amphidinols[17] are now in progress.

EXPERIMENTAL

Isolation of okadaic acid: Okadaic acid was isolated from the black sponge *Halichondria okadai* collected on the seashore in Tokushima Prefecture, Japan. A slight modification of the procedure used to isolate diarrhetic shellfish toxins was used in the present isolation.[18] An acetone extract of the sponge (8.0 kg, wet) was condensed to an aqueous suspension and extracted with EtOAc. The concentrated organic layer was diluted with aqueous 90% MeOH, and extracted with hexane and then with CH_2Cl_2. The CH_2Cl_2 extract was loaded on a column of deactivated alumina gel (Merck 90) with CH_2Cl_2-MeOH (2:1), MeOH, and then aqueous 1% NH_4OH-MeOH (1:1). The residue obtained in the last eluate was further purified by successive column chromatography on Sephadex® LH-20 (Pharmacia) with MeOH, ODS (YMC, ODS-AM) with MeCN-water-AcOH (50:50:1 and then 60:40:1), and silica gel (Merck 60) with CH_2Cl_2-MeOH (19:1). The eluate from each column chromatography was monitored by antifungal assays against *Aspergillus niger* or silica-gel TLC. The final purification was achieved by recrystallization from 50 % aqueous MeCN to give 29.4 mg of okadaic acid.

NMR experiments: 2D-NMR measurements were performed on a JEOL α500 spectrometer (500 MHz) at 300 K and recorded in the phase-sensitive mode using the method by States *et al.*[19] Okadaic acid samples were prepared by dissolving 25 mg in 0.5 mL of CD_3OD-$CDCl_3$ (1:2) or 1.5 mg in 0.5 mL of 0.1 M NaOD-D_2O, and the ^1H and ^{13}C signals were assigned in both solvent systems.[20]

E.COSY experiments were carried out in both solvents with a data size of $4K(t_2) \times 512(t_1)$ points for a spectral width of 3000 Hz (6 ppm, 24 scans per t_1 increment), and two-fold zero-filling was carried out in $F1$ to afford data sets of $4K(t_2) \times 1K(t_1)$ points. A squared sine-bell window function shifted by $-2\pi/7$ was applied in both dimensions prior to Fourier transformation. The resultant digital resolution in $F2$ was 0.74 Hz/point.

The hetero half-filtered TOCSY (HETLOC) spectra were obtained with the following pulse sequence proposed by Wollborn and Leibfritz;[9] $\mathrm{BIRD}[90°(^1\mathrm{H})\text{–}\Delta\text{–}180°(^1\mathrm{H},^{13}\mathrm{C})\text{–}\Delta\text{–}90°(^1\mathrm{H})\text{–}\tau]\text{–}90°(^1\mathrm{H})\text{–}\Delta\text{–}90°(^{13}\mathrm{C})\text{–}180°(^1\mathrm{H})\text{–}90°(^{13}\mathrm{C})\text{–}\Delta\text{–}t_1$–trim pulse–MLEV17–AQ(^{1}H). The BIRD pulse[21,22] before the half-filter sequence serves to suppress the signals due to protons on ^{12}C. For quadrature detection in the $F1$ dimension, States-type phase alteration was applied to the 90°(^{1}H) pulse after the BIRD pulse. The duration of the trim pulse was 2.5 ms; the MLEV17 spin-lock period was set to 30 ms for $^2J_{\mathrm{C,H}}$ and to 60 ms for $^3J_{\mathrm{C,H}}$; the delay in the BIRD pulse (τ) was 330 ms; and the delay time Δ was 3.45 ms. Two hundred fifty-six t_1 increments, each with 64 repetitions, were carried out with $2K$ points in $F2$. The sweep width in both dimensions was 3000 Hz. Data were zero-filled to yield $4K(t_2) \times 512(t_1)$ matrices, and a squared sine-bell window function shifted by $-2\pi/7$ was applied in both dimensions.

The pulse sequence of phase-sensitive HMBC[23] is $90°_x(^1\mathrm{H})\text{–}\Delta\text{–}90°_{\phi1}(^{13}\mathrm{C})\text{–}t_1/2\text{–}180°_y(^1\mathrm{H})\text{–}t_1/2\text{–}90°_{\phi2}(^{13}\mathrm{C})\text{–}\Delta\text{–}\mathrm{AQ}_{\phi3}(^1\mathrm{H},\ ^{13}\mathrm{C}$-decoupling); $\phi1 = x, -x$; $\phi2 = 2(x), 2(-x)$; $\phi3 = x, 2(-x), x$. A States-type phase alteration was applied to the first 90° (^{13}C) pulse. Δ was set to 40 ms to optimize 12.5 Hz of $^{2,3}J_{\mathrm{C,H}}$. Five hundred twelve experiments, each with 64 scans, were performed with $2K$ data points in $F2$. The spectral width in $F1$ and $F2$ was 23750 and 3000 Hz (190 and 6 ppm), respectively. Two-fold zero-filling was carried out in $F1$ and $F2$ to give data sets of $4K\ (t_2) \times 1K\ (t_1)$ points. The data were processed using a squared sine-bell window function in both dimensions before transformation.

Molecular Mechanics Calculation: The molecular mechanics calculation was carried out on a Silicon Graphics Indigo 2® computer using the CFF91 force field in the DISCOVER® software package (Ver. 2.9.0; Biosym) together with INSIGHT II® (Ver. 2.3; Biosym) as a graphic interface. The conformation of okadaic acid constructed by the coupling data was minimized by the steepest descents algorithm (500 steps), the conjugate gradients algorithm (500 steps), and the Newton-Raphson algorithm (500 steps), which led to the conformation shown in Fig. 6.

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20. Chemical shifts (ppm) for the acyclic portions of okadaic acid are shown below.

Position	δ_C (CD_3OD-$CDCl_3$)	δ_H (CD_3OD-$CDCl_3$)	δ_H (NaOD-D_2O)
1	178.6	-	-
2	75.8	-	-
3	44.2	1.66 (*pro-R*), 2.09 (*pro-S*)	1.58, 2.10
4	69.2	4.04	3.93
44	27.5	1.38	1.19
12	71.2	3.62	3.61
13	42.4	2.30	2.25
14	137.1	5.70	5.85
15	131.1	5.50	5.43
16	79.5	4.54	4.50
42	16.1	1.05	0.92
26	85.2	3.98	3.97
27	65.4	4.08	4.08
28	35.7	0.99 (*pro-S*), 1.38 (*pro-R*)	0.88, 1.28
29	31.3	1.91	1.69
30	75.8	3.29	3.27
40	16.3	1.06	0.93

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