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Long-range Carbon-Proton Coupling Constants for Stereochemical Assignment of Acyclic Structures in Natural Products: Configuration of the C5-C9 Portion of Maitotoxin

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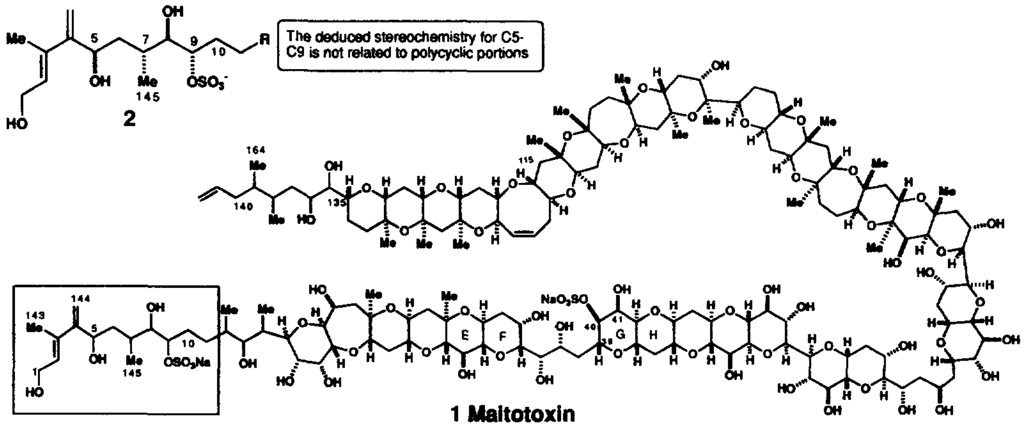
Abstract: Long-range carbon-proton coupling constants ($^{2,3}J_{C,H}$) were measured for maitotoxin (MTX), one of the largest natural non-biopolymers, by hetero-half filter experiments and phase-sensitive HMBC with use of 9 mg of a 4% ^{13}C-enriched sample. The necessary coupling constants within the terminal acyclic portions of MTX, where NOE analysis was not successful owing to the presumed coexistence of multiple conformers, were thus obtained for the resultant elucidation of relative configurations for the acyclic stereogenic centers to be 5*R**, 7*R**, 8*R**, and 9*S**.

Long-range carbon-proton coupling constants ($^{2,3}J_{C,H}$) have been seldom utilized for structural analysis of natural products, chiefly owing to their poor detectability. Recently, a series of inverse-detected methods to measure coupling constants between a proton and other nuclei were proposed mainly for the conformational analysis of biopolymers without ^{13}C- or ^{15}N-enrichment,[1] somewhat facilitating measurements of $^{2,3}J_{C,H}$ for natural products. We have been attempting to utilize $^{2,3}J_{C,H}$ for the configurational assignment of acyclic asymmetric carbons in natural products,[2] where unlike cyclic systems NOEs often lead to an incorrect result.

We have recently succeeded in elucidation of the entire planar structure of maitotoxin (MTX, **1**), the largest natural non-biopolymer.[3] The stereochemistry for acyclic parts was further determined by spectroscopic comparison between MTX and synthetic fragments.[4,5] In the course of this structural study,



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The deduced stereochemistry for C5-C9 is not related to polycyclic portions

1 Maitotoxin

^{13}C-^{1}H coupling constants turned out to provide invaluable information for the stereochemistry of acyclic portions, where NOEs sometimes caused confusing results owing to the coexistence of multiple conformers. In particular, both terminal chains of MTX, where all the configurations still remain unknown, probably undergo conformational alteration and prevented us from NOE analysis. In this communication we wish to report measurements and interpretations of long-range ^{13}C-^{1}H coupling constants ($^{2,3}J_{C,H}$), which lead to the stereochemical assignment of an acyclic portion in MTX.

MTX (9 mg, enriched with ^{13}C isotope at 4%)[6] isolated from the dinoflagellate *Gambierdiscus toxicus* cultured with $Na_2{}^{13}CO_3$ was used for the NMR measurements. $^{2,3}J_{C,H}$ values were determined by hetero half-filtered TOCSY (HETLOC),[7] where $^{2,3}J_{C,H}$ appeared as dislocation of a ^{1}H-^{1}H cross peak, and by phase-sensitive HMBC spectra,[8] where the relative values of $^{2,3}J_{C,H}$ could be calculated from the intensity of cross peaks. Acquisition data (2k x 256) of the both spectra were converted to 2D charts after 2-fold zerofilling for f1 and f2 dimensions. $^{3}J_{H,H}$ values were determined by E. COSY.[9] All the spectra were measured in C_5D_5N-CD_3OD (1:1 v/v) at 500 MHz (JEOL, A 500).

Some $^{2,3}J_{C,H}$ values for MTX could be determined by the HETLOC as shown in Table I, although heavy overlap and broadening of cross peaks largely hampered the $^{2,3}J_{C,H}$ measurements, particularly for the polyether parts. Informative $^{2,3}J_{C,H}$ data could be obtained for the both terminal chains because their

Table I. $^{3}J_{H,H}$ **and** $^{2}J_{C,H}$ **for C5-C10 of Maitotoxin**

position	$^{3}J_{H,H}$	position	$^{2}J_{C,H}$	position	$^{3}J_{C,H}$
H-5/H-6a	3.2	C5/H-6a	-1.5	C5/H-7	2.3
H-5/H-6b	10.3	C5/H-6b	-6.0	C7/H-5	1.5
H-6a/H-7	10.3	C8/H-7	-3.0	C145/H-6b	6.8
H-6b/H-7	3.2	C8/H-9	-1.5	C145/H-8	3.0
H-7/H-8	6.3	C9/H-8	-4.5		
H-8/H-9	4.8				
H-9/H-10a	7.9				
H-9/H-10b	3.2				

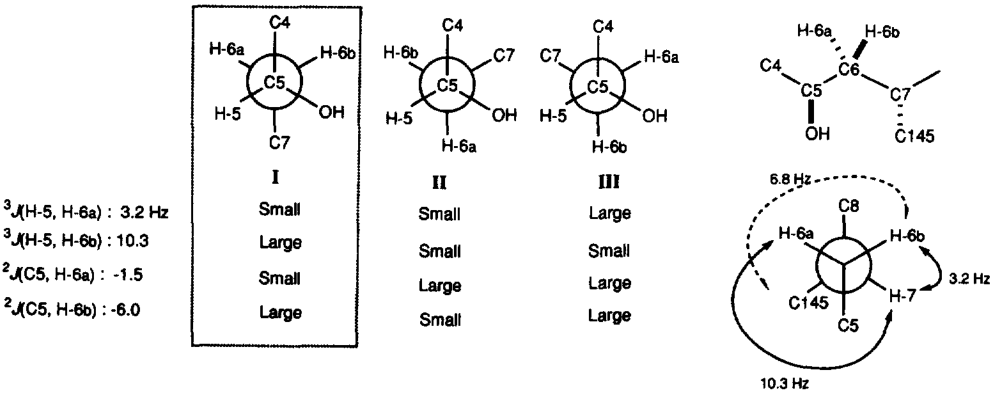
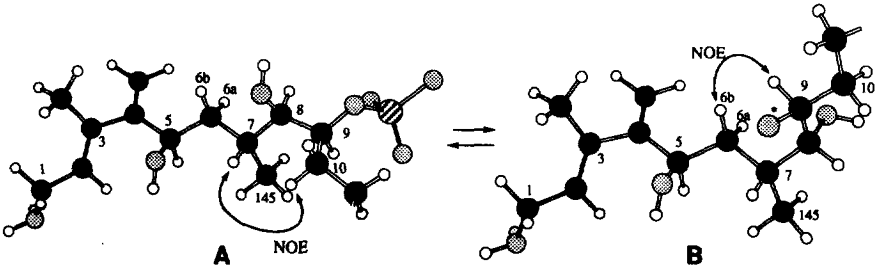


Figure 1. Three possible rotamers around C5-C6 and relative stereochemistry between C5 and C7

relatively long T_2-relaxation time resulted in enhancement of signal intensity. The stereochemical relation between C5 and C7 could be determined in the following manner via the prochiral assignment for methylene protons at C6. Since ^{13}C-^1H geminal coupling constants ($^2J_{C,H}$) were known to be dependent on the dihedral angle between a proton and an oxygen atom at vicinal position[10] (Fig. 1), dihedral analysis around an oxygen-bearing carbon such as C5, C8 and C9 was carried out mainly using $^2J_{C,H}$ values, which could be often measured with greater accuracy than $^3J_{C,H}$ in the HETLOC spectrum. As depicted in Fig. 1, there are three possible rotamers with respect to a C5-C6 bond. While with $^3J_{H,H}$ values alone one cannot specify a rotamer (both I and III in Fig. 1 give a pair of the large and small coupling constants), additional $^2J_{C,H}$ values enable one to identify the rotamer, which also allows the relative prochiral assignment for H_2-6. The diastereomeric relation between C5-C7 could be established by the similar rotation analysis around the C6-C7 bond; H-7 could be placed at the *anti* position to H-6a on the basis of a large $^3J_{(H\text{-}6a,\ H\text{-}7)}$; and C145 is *anti* to H-6b based on a large $^3J_{(C145,\ H\text{-}6b)}$, thereby resulting in the configurational and conformational assignment between C5 and C7 (Fig. 1 and Fig. 2).

Regarding the C7-C8 and C8-C9 diastereomeric relations, two dominant conformations were suggested to be present by NOESY,[11] since some NOEs such as those due to H-7/H-10 and H-6b/H-9 (Fig. 2) could not be accounted for by a single conformer. This conformational alteration was also inferred by coupling constants with intermediate values between *gauche* and *anti* orientations such as H-7/H-8 (6.3 Hz) and H-8/H-9 (4.8 Hz). Even for such a complicated system, $^{2,3}J_{C,H}$ gave invaluable information as to the stereochemistry. Newman projections for C7-C8 and C8-C9 bonds are shown in Fig. 3. Since $^3J_{H\text{-}7,H\text{-}8}$ of 6.3 Hz and $^2J_{H\text{-}7,C8}$ of -3.0 Hz were intermediate values and thought to be derived from two conformations as a weighted average, H-7 and H-8 should be *anti* (thus H-7/8-OH being *gauche*) in one conformer, while in the other H-7 and H-8 should be *gauche* (thus H-7/8-OH being *anti*). Furthermore, $^3J_{C145,H\text{-}8}$ of 3 Hz indicated that in the both conformers C145 is *gauche* to H-8. Only a pair of the rotamers in Fig. 3 out of six possible ones arising from both diastereomers for C7/C8 satisfied all these requirements. By the same logic, the diastereomeric relation of C8/C9 could be assigned on the basis of $^3J_{H\text{-}8,H\text{-}9}$, $^2J_{H\text{-}8,C9}$, and $^2J_{C8,H\text{-}9}$ as shown in Fig. 3. Based on these data we successfully deduced the stereochemistry of asymmetric carbons in the side chain of MTX to be 5*R**, 7*R**, 8*R** and 9*S** (**2**).

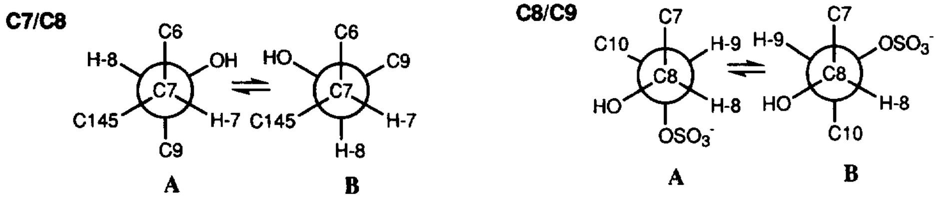
Synthetic and spectroscopic studies are now in progress for the stereochemical assignment of the remaining extension which connects to the cyclic portion. The present study has revealed that long-range ^{13}C-^1H coupling constants potentially serve as a powerful tool for elucidating the stereochemistry of acyclic structures[2] in complicated natural products. The amount necessary for the HETLOC and phase-sensitive



*Sulfate is omitted for clarity

Figure 2. Two alternating conformers for the C7-C9 portion of MTX

HMBC is usually about 5 μmol and thus we believe that these methods will possibly become a standard method for stereochemical analysis of natural products.



^3J(H-7, H-8) : 6.3 Hz	Large	Small	^3J(H-8, H-9) : 4.8 Hz	Small	Large
^2J(C8, H-7) : -3.0 Hz	Large	Small	^2J(C8, H-9) : -1.5 Hz[b]	Small	Large
^3J(C145, H-8) : 3.0 Hz[a]	Small	Small	^2J(C9, H-8) : -4.5 Hz	Large	Large

Figure 3. Two pairs of rotamers for C7/C8 and C8/C9.

[a] $^3J_{C,H}$ values were determined by phase-sensitive HMBC; [b] In a vicinal dioxygenated system such as C8/C9, $^2J_{C,H}$ value for *gauche* is smaller than that for non- or mono-oxygenated systems, and $^2J_{C,H}$ for *anti* often take a positive value (a typical value for *gauche* is -4 to -5 Hz, and that for *anti* is +1 to +2 Hz).[12] Thus the $^2J_{C,H}$ for C8/H9 can be ragarded as the intermediate value between *gauche* and *anti* conformers (the sulfate substitution appeared to give no significant effect to $^2J_{C,H}$).[13]

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REFERENCE AND NOTES

1. For a review, Eberstandt, M.; Gemmechker, G.; Mierke, D.G.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **34**, 1671-1695 (1995).
2. Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron.* **51**, 12229-12238 (1995).
3. (a) Yokoyama, A.; Murata, M; Ohshima, Y.; Iwashita, T.; Yasumoto, T. *J. Biochem.* **104**, 184-187 (1988); (b) Murata, M.; Naoki, H.; Matsunaga, S.; Satake, M.; Yasumoto, T. *J. Am. Chem.. Soc.* **116**, 7098-7107 (1994).
4. Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **35**, 5023-5026 (1994).
5. (a) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* in press; (b) Sasaki, M.; Matsumori, N.; Murata, M.; Tachibana, K.; Yasumoto, T. *Tetrahedron Lett.* in press.
6. Satake, M.; Ishida, S.; Yasumoto, T.; Murata, M.; Utsumi, H.; Hinomoto, T. *J. Am. Chem. Soc.* **117**, 7019- 7020 (1995).
7. (a) Otting, G.; Wüthrich, K. *Quart. Rev. Biophys.* **23**, 39-96 (1990); (b) Kurz, M.; Schmieder, P.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **30**, 1329-1331 (1991); (c) Wollborn, U.; Leibfritz, D. *J. Magn. Reson.* **98**, 142-146 (1992).
8. Zhu, G.; Bax, A. *J. Magn. Reson.* **104A**, 353-357 (1993).
9. Griesinger, C.; Sørensen, O.W.; Ernst, R. R. *J. Am. Chem. Soc.* **107**, 6394-6396 (1985).
10. (a) Schwarcz, J.A.; Cyr, N.; Perlin, A.S. *Can. J. Chem.* **53**, 1872-1875 (1975); (b) Hines, J.V.; Varani, G.; Landry, S.M.; Tinoco, I. Jr. *J. Am. Chem. Soc.* **115**, 11002-11003 (1993); (c) Hines, J.V.; Landry, S.M.; Varani, G.; Tinoco, I. Jr. *J. Am. Chem. Soc.* **116**, 5823-5831 (1994).
11. NOESY's were measured at 25 °C (600 MHz instrument) and -20 °C (500 MHz) with the same sample in C_5D_5N-CD_3OD (1:1).
12. Podlasek, C. A.; Wu, J. Stripe, W. A.; Bondo, P. B.; Serianni, A.S. *J. Am. Chem. Soc.* **117**, 8635-8644 (1995).
13. The effect of a sulfate group to $^2J_{C,H}$ was evaluated with use of a synthetic fragment of rings EF/GH of maitotoxin, in which a sulfate was substituted at C40.[5b] $^2J_{C,H}$ values for a vicinal dioxygenated system similar to C8/C9 in Fig. 3 could be determined; the $^2J_{C,H}$ values for the *gauche* H/O orientation between C41(OH)-**H** and C40-**OSO$_3^-$** was -3.7 Hz, and those for *gauche* H/O (C40(OSO_3^-)-**H** and C39-**OH**) and for *anti* H/O (C40(OSO_3^-)-**H** and C41-**OH**) were -5.1 Hz and +2.2 Hz, respectively, which revealed that the effect of a sulfate substitution to $^2J_{C,H}$ was insignificant in a system like C8/C9.

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