

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231441820>

Structures and configurations of ciguatoxin from the Moray eel, *Gymnothorax-Javanicus* and its likely precursor from the dinoflagellate

ARTICLE in JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · APRIL 2002

Impact Factor: 12.11 · DOI: 10.1021/ja00167a040

CITATIONS

221

READS

4

5 AUTHORS, INCLUDING:



Michio Murata

Osaka University

224 PUBLICATIONS 7,407 CITATIONS

SEE PROFILE

110.9, 96.5, 81.9, 79.5, 72.8, 68.1, 55.3, 49.2, 26.5, 26.3, 24.4, 8.4, 1.58.

(4S,5R,6R,7R)-7-(Hydroxymethyl)spiro[2.4]heptane-4,5,6-triol (5). To a stirred solution of 72 mg (0.27 mmol) of the cyclopropylcarbinol **19** in 2.2 mL of THF at 50 °C under nitrogen was added 0.54 mL of 10% hydrochloric acid solution by pipette. After stirring for 4 h, the green reaction mixture was cooled to room temperature and eluted through an anion exchange column (6 in. × 10 mm column, Dowex AG-1 × 8 resin (hydroxide form, 20–50 mesh), 1 M NH₄OH) with 100 mL of eluant. After lyophilization, the crude product was eluted through a gravity cellulose column (6 in. × 10 mm column, microgranular cellulose, 9% *n*-butanol–water). Fractions of 0.5 mL were collected and each was assayed for product content by TLC employing 4:1 dichloromethane:methanol with KMnO₄ visualization. The product-containing fractions were pooled and concentrated under reduced pressure to yield 20 mg (41%) of pure tetrol **5**: *R_f* 0.31 (SiO₂, 20% MeOH–CH₂Cl₂); [α]_D²⁰ –61.7° (c 1.15 H₂O); ¹H NMR (300 MHz, D₂O) δ 4.03 (dd, 1 H, *J* = 3.7 Hz, *J* = 4.7 Hz), 4.03 (dd, 1 H, *J* = 4.7 Hz, *J* = 9.9 Hz), 3.74 (d, 1 H, *J* = 3.7 Hz), 3.55 (dd, 1 H, *J* = 5.1 Hz, *J* = 11.5 Hz), 3.43 (dd, 1 H, *J* = 4.7 Hz, *J* = 11.6 Hz), 2.13 (dd, 1 H, *J* = 5.1 Hz, *J* = 11.1 Hz), 0.89 (m, 1 H), 0.73 (m, 1 H), 0.34 (m, 2 H); ¹³C NMR (75 MHz, CDCl₃) δ 78.9, 75.9, 75.2, 63.0, 51.7, 26.6, 10.0, 9.5.

(4S,5R,6R,7R)-7-(Hydroxymethyl)spiro[2.4]heptane-4,5,6-triol 7-(Dihydrogen phosphate) (Disodium Salt) (3). To a stirred solution of 20.0 mg (0.097 mmol) of tetrol **5** in 250 μL of triethyl phosphate at 0 °C under nitrogen was added 29.7 mg (0.194 mmol) of phosphorous oxychloride. After stirring for 1.5 h, the solution was treated at 0 °C with 600 μL of 1 M triethylammonium bicarbonate solution (pH 7.5) and

stirred for 5 min. The reaction mixture was then passed through a 6'' × 10 mm column of DEAE sepharose (bicarbonate form) with a gradient of triethylammonium bicarbonate (0 mM to 0.25 M), and the eluant was collected in 0.5 mL fractions. The fractions were analyzed for phosphate by TLC (SiO₂, 50% 1 M NH₄OAc–EtOH) by using an ammonium molybdate/anthranilic acid containing visualization spray. At this stage all carbohydrate-containing fractions were pooled and lyophilized. Proton NMR indicated that the reaction had produced a 3:2:1:1 mixture of 5'-phosphate:3:unknown:unknown. Resubmission of this mixture to a second DEAE sepharose column and chromatography using conditions identical with those used in the first instance provided a pure sample of 5'-phosphate which was passed through a 1'' × 10 mm column of Biorex 70 cation exchange resin (Na⁺ form) and lyophilized to yield 7.1 mg (25%) of pure **3**: [α]_D²⁰ –47.7° (c 0.66 H₂O); ¹H NMR (300 MHz, D₂O) δ 4.13 (m, 2 H), 3.77 (d, 1 H, *J* = 3 Hz), 3.66 (ddd, 1 H, *J* = 5.5 Hz, *J* = 10.4 Hz, *J* = 4.9 Hz), 3.61 (ddd, 1 H, *J* = 5.1 Hz, *J* = 10.3 Hz, *J* = 6.4 Hz), 2.33 (m, 1 H), 0.89 (m, 2 H), 0.48 (m, 1 H), 0.37 (m, 1 H); ¹³C NMR (125 MHz, D₂O) δ 79.3, 75.8, 75.4, 66.1, 50.3, 26.8, 9.4.

Acknowledgment. This work was supported by a grant from the National Institute of Diabetes, Digestive and Kidney Diseases.

Supplementary Material Available: Details of general experimental procedures and actual copies of ¹H and ¹³C nuclear magnetic resonance spectra for each molecule described in the Experimental Section (42 pages). Ordering information is given on any current masthead page.

Structures and Configurations of Ciguatoxin from the Moray Eel *Gymnothorax javanicus* and Its Likely Precursor from the Dinoflagellate *Gambierdiscus toxicus*¹

Michio Murata,^{2a} Anne M. Legrand,^{2b} Yoshihiko Ishibashi,^{2a} Masakazu Fukui,^{2a} and Takeshi Yasumoto^{*2a}

Contribution from the Faculty of Agriculture, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Sendai 981, Japan, and Institut territorial de Mècherches Medicales Louis Malardé, Papeete, Tahiti, French Polynesia. Received November 24, 1989

Abstract: Ciguatoxin (CTX) is the toxic principle of ciguatera, which is responsible for the most widespread food poisoning of nonbacterial origin. The toxin, isolated from the moray eel *Gymnothorax javanicus*, and its congener, from the causative dinoflagellate *Gambierdiscus toxicus*, were used for this study. The structure elucidation was carried out by combined use of ¹H NMR 2D correlation and NOE experiments done with no more than 0.35 mg of CTX and 0.74 mg of the congener. Broadening of ¹H NMR signals due to a slow conformational change around a nine-membered ring was sharpened by measurements at –20 °C, in which all ³J proton connectivities and NOE's around angular protons were clearly indicated. The structure of CTX, which had a molecular formula of C₆₀H₈₆O₁₉, was disclosed to be a brevetoxin-type polyether comprising 13 continuous ether rings (7/6/6/7/7/9/7/6/8/6/7/6/6-spiro-5). The congener was shown to be a less oxygenated analogue of CTX. Their relative stereochemistries, except for C2 of CTX, were clarified by detailed analyses of ¹H NMR NOE experiments, MM2 energy calculations, and spectral simulations.

Ciguatera is a term applied to food poisoning caused by ingestion of coral reef fish. The worldwide occurrence of ciguatera not only endangers public health but also hampers local fisheries in subtropical and tropical regions. It is estimated that roughly 20 000 people suffer annually from the poisoning, making it one of the largest-scale food poisonings of non-bacterial origins.

The toxification mechanism of fish had not been known until one of the authors (T.Y.) identified an epiphytic dinoflagellate, *Gambierdiscus toxicus*, as a causative organism in 1977.³ The

dinoflagellate toxins are transferred through the food chain among coral biota and accumulated most in carnivorous fish.

Ciguatoxin (CTX), the principal toxin causing ciguatera, is extremely potent; its lethal potency against mice is ca. 100 times greater than that of tetrodotoxin on a molar basis. Although many investigators have been attracted by the unique nature of the toxin, lack of knowledge about its structure hampered biological and biochemical research on ciguatera. After a Hawaiian group reported the isolation and partial chemical characterization of the toxin, including its polyether nature,⁴ additional progress was blocked due chiefly to extreme difficulties in obtaining enough

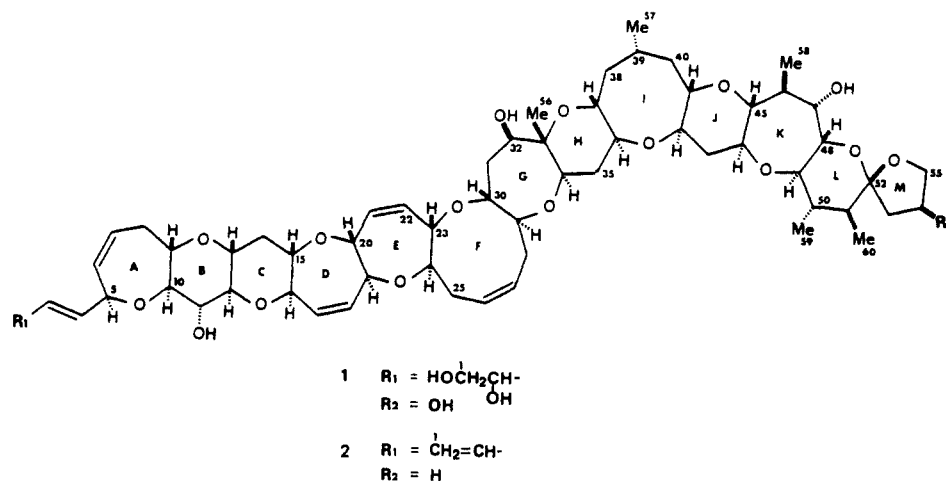
(1) Preliminary announcements of parts of this study have been published: *Tetrahedron Lett.* **1989**, 30, 3793; *J. Am. Chem. Soc.* **1989**, 111, 8929.

(2) (a) Tohoku University. (b) Institut de Louis Malardé.

(3) Yasumoto, T.; Nakajima, I.; Bagnis, R.; Adachi, R. *Bull. Jpn. Soc. Sci. Fish.* **1977**, 43, 1021.

(4) (a) Scheuer, P. J.; Takahashi, W.; Tsutsumi, J.; Yoshida, T. *Science* **1967**, 155, 1267. (b) Tachibana, K. Ph.D. Thesis, University of Hawaii, 1980. (c) Nukina, M.; Koyanagi, L. M.; Scheuer, P. J. *Toxicon* **1984**, 22, 169.

Chart I



samples for structural elucidation. Although *G. toxicus* does produce maitotoxin,⁵ a second important toxin in ciguatera, no CTX is yielded when it is cultured. We were, therefore, forced to extract the toxin from coral fish. Even in moray eels, which are known as the most toxic species, CTX content is extremely low, usually only several ppb in whole bodies.

Within the last decade, NMR spectrometry has advanced with rapid strides in greatly reducing the sample size necessary for structure elucidation. After our isolation work, only 0.35 mg of CTX⁶ and 0.74 mg of the less polar congener, tentatively named gambiertoxin-4b (GT4b), were obtained. We, nevertheless, attempted to solve their structures using NMR methods. In the present paper we wish to report the structures, including the stereochemistries of CTX (1) and GT4b (2).

Results and Discussion

Toxicity and Physicochemical Properties. CTX was obtained as a white solid from 125 kg of moray eel viscera.⁶ The lethal potency against mice (i.p.) was 0.35 $\mu\text{g}/\text{kg}$. From *G. toxicus*, GT4b was isolated as a white, amorphous solid with a potency of ca. 4 $\mu\text{g}/\text{kg}$. Physicochemical properties are as follows. CTX: no UV maximum above 210 nm; IR (film) 3400, 1111, and 1042 cm^{-1} (no bands between 1600 and 1800 cm^{-1}). GT4b: UV_{max} (CH₃CN) 223 nm (ϵ 22000); IR (film) 3400, 1620, and 1040 cm^{-1} ; high-resolution FABMS, m/z 1061.587 (MH)⁺ (calcd for C₆₀H₈₅O₁₆, 1061.584).

Molecular Formula. The molecular weight of CTX was determined by high-resolution FAB mass spectrometry (HR-FABMS). A total of 30 spectra were recorded from approximately four 1- μg samples. The average value for (MH)⁺ with a 95% confidence interval was shown to be 1111.5843 \pm 0.0053.

Previously, the presence of nitrogen(s) in CTX was inferred by the Hawaiian group.⁷ We, however, could find no sign of nitrogen functionality in CTX; amino groups were probably absent since CTX is negative with ninhydrin or Dragendorff's test, and the absence of amides or other nitrogenous carbonyl groups could be inferred by no corresponding absorptions in the IR. The mass number also suggested that CTX bore either zero or an even number of nitrogens. Since the presence of more than two nitrogens seemed to be implausible, we deduced that CTX was comprised of only carbon, hydrogen, and oxygen. On this assumption, only the formula C₆₀H₈₆O₁₉ satisfied the requirement of HR-FABMS. The correctness of this formula was confirmed through the process of structure elucidation; partial structures, deduced from NMR data (see the next section), revealed that CTX had at least 59 carbons, 86 protons, and 18 oxygens. They

accounted for 1082 daltons out of the molecular weight 1110, leaving 28 daltons for the unknown part. It excluded the possible presence of atoms other than carbon, hydrogen or oxygen.

As our next step, we tried to deduce species and numbers of functional groups. As four pairs of sp² protons coupled to each other were observed in the ¹H NMR spectrum of CTX, we initially deduced that CTX had four double bonds. The hydrogenated products of CTX,⁸ however, gave rise to a molecular ion, (M + Na)⁺ at m/z 1143 upon FABMS, suggesting the presence of five double bond equivalents in CTX. As there were no signs of carbonyl, other functional groups bearing unsaturation, or alicyclic rings, we inferred that the unsaturated functional groups of CTX mostly consisted of C=C bonds and ether rings with the number of ether rings being around 13. An average number of carbons necessary for one ether ring in CTX was calculated to be 4.6, comparable to that of brevetoxin B, 4.5.^{9a} Thus, it seemed that CTX might basically resemble the brevetoxins in its ladder-shaped structure.

Proton Connectivity. The ¹H NMR spectra measured in pyridine-*d*₅ and CD₃OD were identical with those reported by the Hawaiian group.^{4a,b} ¹H NMR chemical shifts and coupling constants of CTX and GT4b clearly showed that GT4b was identical with CTX except for the two terminal parts. Thus, the structure elucidation of the common part will be discussed according to the data obtained with GT4b or otherwise noted. The same set of ¹H NMR data was recorded with CTX in the same way.

The skeletal structure was established mainly on the basis of ¹H-¹H 2D NMR data obtained from COSY, relayed COSY (relayed coherence transfer), and 2D-HOHAHA experiments. Interpretations of ¹H-¹H COSY measured in pyridine-*d*₅ at 25 °C are shown in Figure 1. Although the larger part of the whole structure was clarified by these 2D methods, connectivities of some parts were unassignable due to extreme broadening or even disappearance of signals. Regarding the double bond giving no ¹H NMR signals, the same phenomena was reported by Shimizu et al. in brevetoxin A,^{9b} in which a slow conformational change of a nine-membered ring caused disappearance of the signals of the olefinic protons and their neighbors. Therefore, we attempted ¹H NMR measurement at low temperature so as to make the perturbation of the ring slow enough to detect these resonances. As shown in Figure 2, olefinic protons and their neighboring methylenes gave rise to sharp signals when measured at -25 °C in CD₃CN. Since each sharpened peak could be matched with

(5) Yokoyama, A.; Murata, M.; Oshima, Y.; Iwashita, T.; Yasumoto, T. *J. Biochem.* **1988**, *104*, 184.

(6) Legrand, A. M.; Litaudon, M.; Genthon, J. N.; Bagnis, R.; Yasumoto, T. *J. Appl. Phycol.* **1989**, *1*, 183.

(7) Nukina, M.; Tachibana, K.; Scheuer, P. J. *Abstr. Annu. Meeting of Agri. Biol. Chem. Soc. Jpn.* **1987**, 512.

(8) 1 (1 μg) was hydrogenated with H₂ (1.5 atm) on 100 μg of Pd/C in 100 μL of MeOH at 0 °C for 20 min. Hydrogenolysis probably occurred to cleave a C5-O bond because use of PtO₂ as a catalyst led to enhancement of an ion peak at m/z 1145 (CTX + 12H + Na)⁺.

(9) (a) Lin, Y.-Y.; Risk, M.; Ray, S. M.; Engon, D. V.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773. (b) Shimizu, Y.; Chou, H.-N.; Bando, H.; Duyne, G. V.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 514.

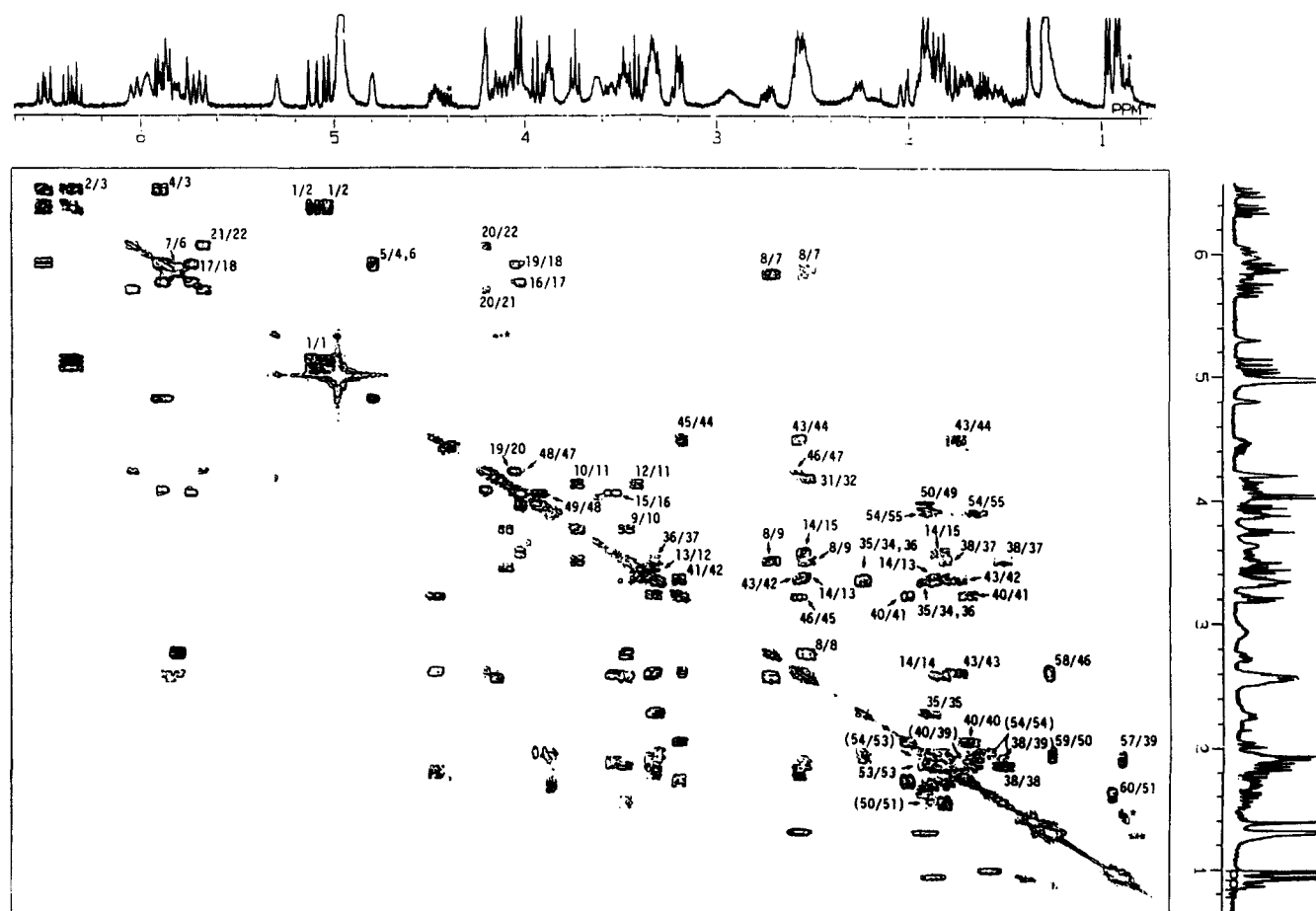


Figure 1. ^1H - ^1H COSY map of GT4b (2) (400 MHz) in pyridine- d_5 at 25 °C and the assignment of cross peaks. Numbers denote the carbon number of coupled protons giving a cross peak. Asterisks express signals and cross peaks due to impurities. Numbers in parentheses were assigned with additional use of 2D-HOHAHA and relayed COSY.

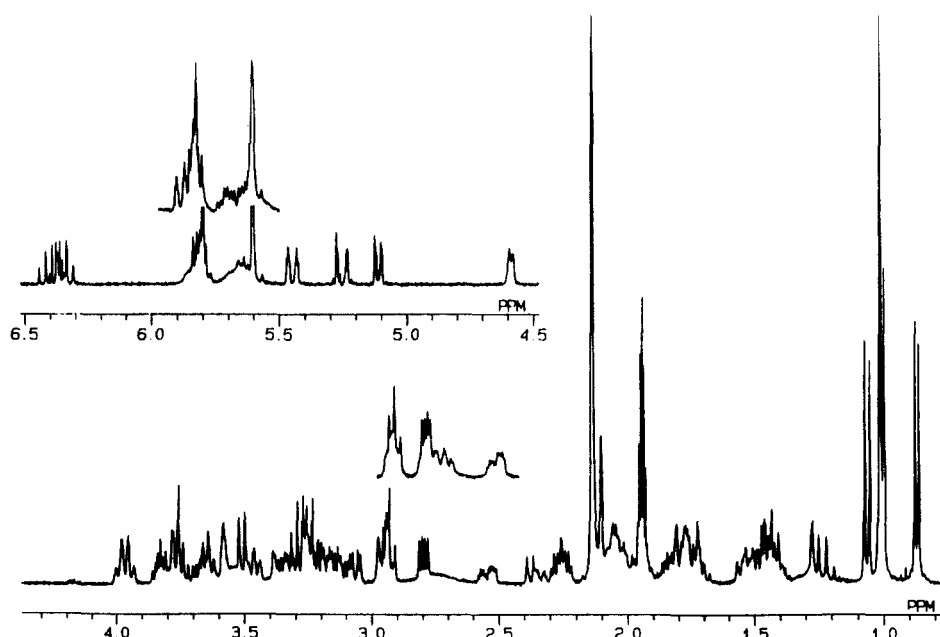


Figure 2. ^1H NMR spectra of GT4b (2) (400 MHz) in CD_3CN at 20 °C. Two inset partial spectra were measured at -25 °C.

a broad signal at room temperature, ring F is assumed to possess mostly a single conformation at low temperatures.

The ^1H - ^1H COSY spectrum measured at -20 °C (CD_3CN and pyridine- d_5) allowed us to assign the structures of four fragments, C1-C32, C34-C38, C40-C51, and C53-C55 (Figure 3A). The positions of hydroxy groups were also clarified by the COSY measured in pyridine- d_5 at 25 or -20 °C. The skeletal chain was, however, interrupted at three points, two quaternary carbons (C33

and C52) and an unassignable methine (C39) bearing a methyl (Me-57).

Close chemical shifts and second-order couplings of H-38/H-39/H-40 prevented us from clarifying their connectivities. In the 2D-HOHAHA experiments (CD_3CN , 20 °C), which could detect the connectivity at six bonds, multiple relayed couplings of H-37/Me-57 and H-41/Me-57 were observed. Moreover, NOE difference spectra revealed an ether linkage between H-36 and

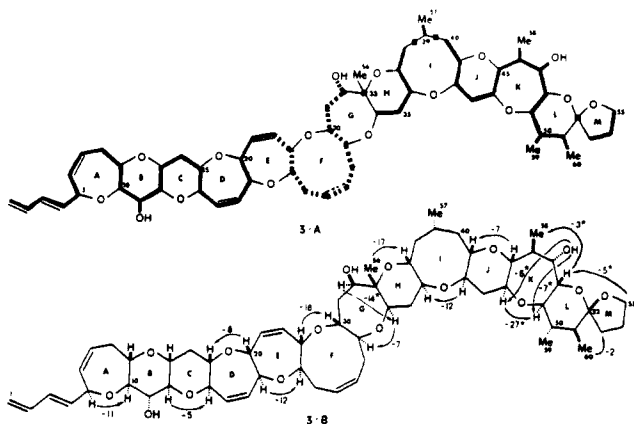


Figure 3. Results of 2D correlation and NOE experiments of GT4b (2). (A) Heavy lines indicate the connectivities assigned on the basis of ^1H - ^1H COSY, relayed COSY, and 2D-HOHAHA at a room temperature, while broken lines denote those in -20°C . 2D maps of relayed COSY and 2D-HOHAHA are given in the supplementary material. (B) Arrows and numbers denote irradiated protons (tail)/observed protons (head) in NOE difference experiments and NOE's in percentage measured in CD_3CN at -25°C . The numbers with an asterisk are those measured in pyridine- d_5 at -25°C . NOE's due to vicinal protons or those bearing no necessary stereochemical information are omitted for clarity. All the NOE difference spectra used in this study are available as supplementary material of this paper and of the previous communication.¹

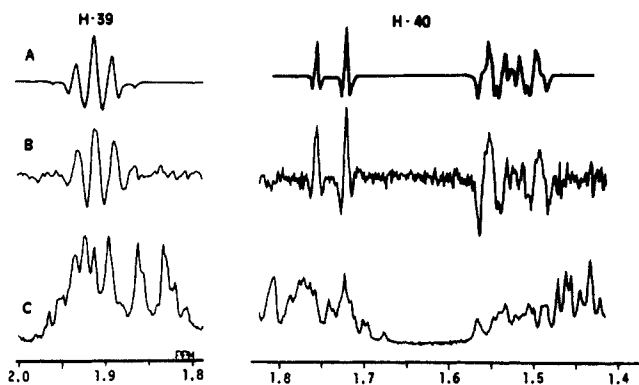


Figure 4. ^1H NMR simulation of decoupling difference spectra for H-39 and H-40 of GT4b (2). Spectra for H-39 were obtained with and without irradiating at Me-57 (δ 0.92) in pyridine- d_5 at 25°C , and those for H-40 were measured with and without decoupling H-41 (δ 2.94) in CD_3CN at 20°C . (A) Simulation spectra; (B) the difference spectra obtained from subtracting a nondecoupling spectrum from a decoupling one; (C) nondecoupling spectra.

H-42 (Figure 3B), suggesting that C39 resided on an ether ring with eight, nine, or ten ring members.

According to the conformational stability of an eight-membered ether ring reported on the brevetoxins,⁹ a crown conformer was likely to be dominant. MM2 calculations done for rings H (chair), I, and J (chair) reached the same conclusion. ^1H NMR spectral simulations were done for H_2 -38/H-39(Me-57)/H-40 and H-39/ H_2 -40/H-41 in a crown conformation with use of the coupling constants obtained from the MM2 calculation and chemical shifts from COSY experiments.¹⁰ As shown in Figure 4, the simulated spectra agreed very well with the observed decoupling difference spectra. It was thus proven that ring I was an eight-membered ether ring bearing a β -methyl at C39.

Connectivity around quaternary carbons is usually assigned with use of NMR sequences designed for detecting $^3J_{\text{C,H}}$ or $^2J_{\text{C,H}}$ (e.g. HMBC, long-range C-H COSY, or COLOC). In this study, the sample sizes were too small to conduct such hetero-2D experi-

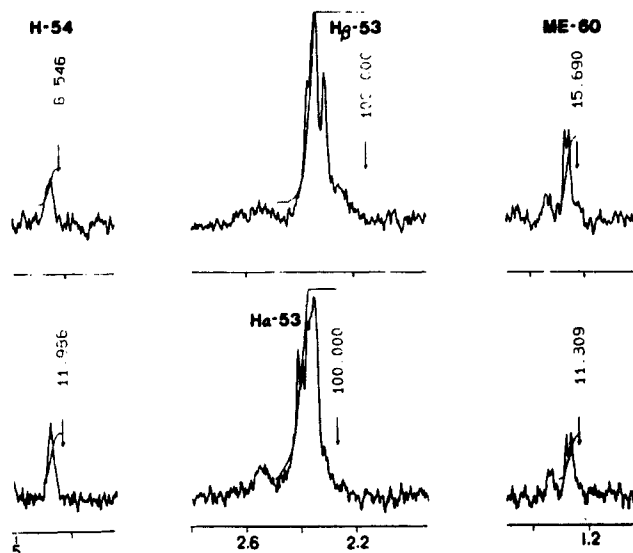


Figure 5. NOE difference spectra of CTX (1) with irradiation at H-53. The spectra were measured in pyridine- d_5 at -25°C . Irradiations were made at δ 2.313 (top) and at 2.440 (bottom).

ments. The structural feature of CTX, however, allowed us to solve the problem by detailed analyses of coupling constants and NOE experiments because relative positions and dihedral angles of protons were relatively fixed in its ladder-shaped structure.

A long-range coupling due to Me-56/H-32 was detected on a DQF (double quantum filter) COSY spectrum (CD_3CN , 23°C). Chemical shifts and coupling constants of H-34, H-35, H-36, and H-37 (Table I) were typical for a 2,2,3,5,6-pentasubstituted tetrahydropyran. Significantly, H-34 was coupled only with H-35, indicating C33 to be a quaternary carbon. A large NOE on Me-56/H-37 (Figure 3B) was due to the axial orientation of Me-56.

A ^{13}C NMR spectrum of GT4b (broad band decoupling), in which some signals were not detectable owing to the extremely small sample size and the conformational mobility of ring F, showed 49 peaks.¹¹ A signal at δ 109.2 was shown to be a quaternary carbon (C52) by the fact that the signal was not observed on a DEPT-45 $^\circ$ experiment. The chemical shift is typical for a ketal carbon of spiro-fused five- and six-membered rings.¹² This ketal carbon plus an oxygen account for the 28-dalton missing part of the molecule. The neighboring carbons of C52 were clarified by the following observations: The decoupling difference spectrum (400 MHz, CD_3CN , 20°C), upon irradiating at Me-60 (δ 0.87), revealed that H-51 was a double quartet (11 and 7 Hz) and it was, therefore, probably in the vicinity of the quaternary carbon. H-53 of CTX showed a three-spin system of an ABX type and was probably adjacent to a quaternary carbon. The orientation of C53 to ring L was further assigned by NOE experiments; prominent NOE's were observed on Me-60/H-53 and on H-48/H-55 (Figures 3B and 5), while no NOE was detectable on H-48/H-53, which would come close to each other if C53 were axially oriented.

These data taken together allowed us to assemble the four fragments into one skeleton but left the positions of the ether linkages and stereochemistry to be assigned.

Ether Linkages and Stereochemistry. Since CTX was thought to have a brevetoxin-like structure consisting of 13 ether rings, we could deduce the positions of ether rings using angular protons on the linear skeleton. The oxymethines other than those bearing hydroxyls should be neighboring to the other oxymethines so as to form a series of ether rings in a ladder-like shape. Thus, we

(10) Coupling constants were obtained from dihedral angles derived from MM2 calculations: $\text{H}_{\text{ax}}\text{-38}/\text{H-39}$, 150.3° ; $\text{H}_{\text{eq}}\text{-38}/\text{H-39}$, 95.2° ; $\text{H-39}/\text{H}_{\text{ax}}\text{-40}$, 153.2° ; $\text{H-39}/\text{H}_{\text{eq}}\text{-40}$, 92.3° . According to the classic Karplus equation ($^3J(\text{Hz}) = 11.8 \cos^2\theta - 0.2 \cos\theta + 0.3$), coupling constants were calculated to be 8.6, -0.2 , 9.1, and -0.3 , respectively.

(11) ^{13}C NMR of 2 at 20°C (100 MHz, CD_3CN): δ 137.5, 136.1, 134.9, 134.2, 132.7, 131.8, 131.4, 128.8, 128.4, 119.3, 109.2, 88.0, 87.4, 84.6, 84.2, 84.0, 83.9, 81.9, 81.4, 80.6, 80.0, 78.7, 78.4, 78.3, 77.6, 76.3, 75.9, 75.0, 74.3, 73.7, 73.7, 72.5, 68.0, 46.7, 46.0, 43.5, 42.7, 41.1, 39.4, 37.4, 36.3, 35.5, 34.6, 28.5, 28.5, 24.9, 20.0, 16.0, 13.6.

(12) Seto, H.; Otake, N. *Heterocycles* **1982**, *17*, 555.

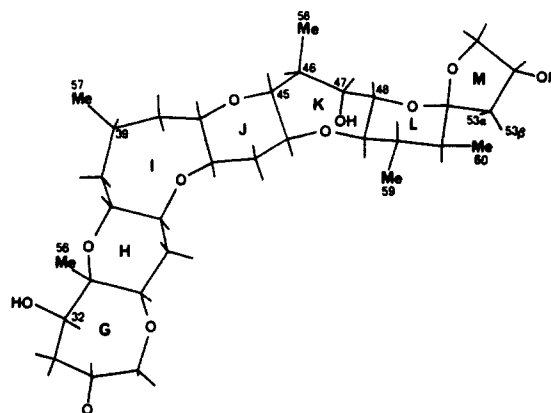
Table I. ^1H NMR Chemical Shifts^a and Coupling Constants^a of Ciguatoxin 1 and GT4b 2

| posn | 2 (pattern) ^b | 1 (pattern) | posn | 2 (pattern) | 1 (pattern) |
|------|--|------------------------------|-------|---------------------------------|------------------|
| 1 | 5.04 (10, 2) | 3.98 (— ^c) | 35 | 1.91 (q, 12) | 1.92 (q, 12) |
| | 5.11 (16, 2) | 3.97 (—) | | 2.26 (12, 4, 4) | 2.25 (12, 4, 4) |
| 2 | 6.35 (16, 10, 10) | 4.69 (m) | 36 | 3.35 (—) | 3.35 (—) |
| 3 | 6.49 (15, 11) | 6.37 (15, 5) | 37 | 3.50 (10, 9, 4) | 3.50 (—) |
| 4 | 5.90 (15, 5) | 6.38 (15, 5) | 38 | 1.54 (13, 11, 8) ^f | 1.53 (—) |
| 5 | 4.80 (m) | 4.86 (m) | | 1.84 (13, 10, 4) ^f | 1.83 (—) |
| 6 | 5.86 (—) | 5.90 (11, 3, 2) | 39 | 1.91 (q, t, 7, 8) ^f | 1.90 (—) |
| 7 | 5.81 (—) | 5.77 (11, 8, 2, 1) | 40 | 1.72 (13, 10, 8) ^f | 1.72 (—) |
| 8 | 2.55 (m) | 2.54 (—) | | 2.03 (13, 10, 3) ^f | 2.03 (—) |
| | 2.73 (15, 7, 4) | 2.72 (15, 7, 4) | 41 | 3.20 (10, 10, 3) | 3.21 (10, 10, 3) |
| 9 | 3.50 (m) | 3.49 (m) | 42 | 3.34 (—) | 3.35 (—) |
| 10 | 3.74 (9, 9) | 3.75 (9, 9) | 43 | 1.77 (q, 12) | 1.77 (q, 12) |
| 11 | 4.11 (9, 9, 2) | 4.10 (—) | | 2.59 (—) | 2.59 (—) |
| 12 | 3.43 (9, 9) | 3.43 (9, 9) | 44 | 4.47 (11, 9, 5) | 4.45 (11, 9, 5) |
| 13 | 3.36 (12, 9, 4) | 3.35 (—) | 45 | 3.19 (9, 5) | 3.21 (9, 5) |
| 14 | 1.85 (q, 12) | 1.85 (q, 12) | 46 | 2.59 (—) | 2.59 (—) |
| | 2.58 (12, 4, 4) | 2.56 (—) | 47 | 4.20 (3, 2) | 4.21 (—) |
| 15 | 3.55 (11, 9, 4) | 3.55 (—) | 48 | 4.03 (9, 1) | 4.06 (10, 1) |
| 16 | 4.03 (br d, 9) | 4.03 (br d, 9) | 49 | 3.94 (10, 10) | 3.96 (10, 10) |
| 17 | 5.74 (13, 2, 2) | 5.73 (br d, 13) | 50 | 1.94 (q, t, 6, 11) ^f | 2.01 (—) |
| 18 | 5.89 (—) | 5.89 (br d, 13) | 51 | 1.60 (q, d, 7, 11) ^f | 1.67 (—) |
| 19 | 4.08 (br d, 9) | 4.07 (br d, 9) | 53 | ~1.85 (—) | 2.35 (13, 5) |
| 20 | 4.22 (br d, 9) | 4.21 (br d, 9) | | ~1.93 (—) | 2.40 (13, 8) |
| 21 | 5.67 (13, 2, 2) | 5.67 (br d, 13) | 54 | ~1.68 (—) | 4.86 (m) |
| 22 | 6.10 ^d (br d, 13) | 6.10 ^d (br d, 13) | | ~1.90 (—) | |
| 23 | 4.03 ^d (br d, ca. 8) ^e | 4.03 ^d (—) | 55 | 3.87 (—) | 4.18 (10, 2) |
| 24 | 3.68 ^d (—) | 3.68 ^d (—) | | 3.88 (—) | 4.19 (10, 5) |
| 25 | 2.20 ^d (—) | 2.20 ^d (—) | 56 | 1.37 (s) | 1.37 (s) |
| | 3.01 ^d (—) | 3.00 ^d (m) | 57 | 0.92 (8) | 0.92 (8) |
| 26 | 6.05 ^d (11, 11, 5) ^h | 6.05 ^d (—) | 58 | 1.29 (8) | 1.30 (8) |
| 27 | 6.07 ^d (11, 11, 5) ^h | 6.07 ^d (—) | 59 | 1.28 (6) | 1.32 (6) |
| 28 | 2.36 ^d (—) | 2.36 ^d (—) | 60 | 0.97 (7) | 1.24 (7) |
| | 2.98 ^d (m) | 2.98 ^d (m) | 1-OH | | 6.40 (6, 4) |
| 29 | 3.86 ^d (br d, 9) ^e | 3.86 ^d (—) | 2-OH | | 6.67 (4) |
| 30 | 3.68 ^d (9, 8, 7) ^e | 3.67 ^d (—) | 11-OH | 7.34 (2) | 7.32 (2) |
| 31 | 2.65 ^d (13, 8, 6) ^f | 2.66 ^d (—) | 32-OH | 5.29 (1) | 5.29 (1) |
| | 2.70 ^d (13, 8, 7) ^f | 2.70 ^d (—) | 47-OH | 6.76 (3) | 6.77 (3) |
| 32 | 4.16 (8, 8, 1) ^f | 4.16 (—) | 54-OH | | 6.53 (4) |
| 34 | 3.34 (12, 4) ^g | 3.33 (—) | | | |

^a Proton NMR spectra were measured with a JEOL GSX 400 (400MHz) spectrometer in pyridine- d_5 at 25 °C except for those with superscripts *d*, *e*, *g*, and *h*. ^b Multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet) and coupling constants in Hz. ^c Couplings were unassignable due to heavy overlappings of signals. ^d Measured in pyridine- d_5 at -20 °C. ^e Obtained from NOE difference spectra in pyridine- d_5 at -20 °C. ^f Obtained from decoupling difference spectra. ^g Coupling constants in CD_3CN at 25 °C. ^h Coupling constants in CD_3CN at -25 °C.

readily construct the probable structure by linking the first ether methine (C5) to the third ether methine (C10) along the linear skeleton and then the second one (C9) to the fifth (C13) and so forth. Confirmation of these ether linkages was done by NOE difference spectra (Figure 3B). In each ring, the angular proton or a methyl (Me-56) gave a prominent NOE when measured in CD_3CN or pyridine- d_5 at -25 °C. All ring fusions were found to be trans, as in the case of the brevetoxins, on the basis of the coupling constants of angular protons, which showed the typical value, ca. 9 Hz, for antiperiplanar substitution on oxycarbons (Table I). Geometry of the double bonds was also assignable by their vicinal coupling constants (Table I).

The stereochemistry of hydroxyls and methyls substituted on the ether rings was clarified by NOE difference experiments and by combined use of MM2 energy calculations and spectral simulations. The substitution pattern in 6-membered rings, B and L, was easily assigned by $^3J_{\text{H,H}}$. The coupling pattern of H-11 (triplet, 9 Hz) indicated the 11-OH to be equatorial, and a coupling constant of H-51 (double quartet, 11, 7 Hz) revealed diequatorial orientations of Me-59 and Me-60. The NOE difference spectra clarified the stereochemistry of 54-OH, which was missing in GT4b. Upon irradiation at H_B-53 of CTX (Figure 5), an observed NOE (-25 °C, pyridine- d_5) was larger on Me-60 than on H-54, and this order in NOE intensities was reversed with irradiation at H_A-54. The coupling pattern of H_B-53 (double doublet, 10, 2 Hz) and H_A-53 (double doublet, 10, 5 Hz) corresponded to trans-vicinal and cis-vicinal couplings, respectively, on tetrahydrofuran. Thus, the hydroxyl group at C-54 was shown to be a β -substituent.

**Figure 6.** Partial stereostructure of CTX (1) deduced from MM2 data.

Because of the ambiguity of the conformations of seven-membered rings, it is difficult to assign the orientation of their substituents. A probable W-type $^4J_{\text{H,H}}$ coupling due to H-32/Me-56, detected on DQF-COSY, suggested that a dihedral angle formed by H-32/C32 and C33/C56 was near 180°. A single conformer of ring G (Figure 6) satisfied such a structural requirement. This is further confirmed by the NOE (-25 °C, pyridine- d_5) observed on H-34 upon irradiation at H-32 (Figure 3B).

The orientation of Me-58 was elucidated to be β in the following manner. A prominent NOE on Me-58 observed upon irradiation at H-48 (Figure 3B) suggested a 1,3-diaxial-like interaction of

the methyl (Figure 6), possibly only for the β -orientation. The α -orientation of OH-47 was inferred by a great deshielding of H-44 and H-49 (Table I) in pyridine- d_5 , compared with those in CD_3CN (H-44, δ 3.67; H-49, δ 3.23); and α OH-47 probably comes so close to H-44 and H-49 that an aromatic solvent affect may act upon them. This was also evidenced by NOE's detected on both H-44/OH-47 and H-49/OH-47 (Figure 3B) and by MM2 energy calculations.¹³

The structural difference between CTX and GT4b was readily elucidated by conventional COSY analyses (25 °C, pyridine- d_5), showing that the latter was a less oxidized entity. GT4b has a *trans*-butadiene moiety at one terminus of the molecule and lacks a hydroxyl group at C54 at the other end.

As we have already assigned the orientation of the methyl (C57) on ring I, and the nature of the spiro-fusion of rings L and M, here we clarified all the relative stereochemistry of GT4b (2) and CTX (1) except for C2 of CTX. The stereochemical assignment of the carbon was unsuccessful because it resided in an acyclic part and was located three-bonds distant from the nearest asymmetric carbon (C5).

The structure of CTX (1) may fall into the same class as brevetoxin or yessotoxin.¹⁵ Moreover, pharmacological studies have disclosed that CTX acts as an agonist against a voltage-sensitive sodium channel¹⁶ as does brevetoxin,¹⁷ and they probably share a common receptor site on the channel.¹⁸ However, the activity of CTX is thought to be some hundred times more potent than brevetoxin. The whole shape and flexibility of the molecule may play an important role in manifesting its extremely potent activity. Studies on structure and activity relationships among this series of compounds would lead to a further understanding of dynamic mechanism in ion channels.

The less oxygenated congener, GT4b, was isolated from the ciguatera causing dinoflagellate, *Gambierdiscus toxicus*. GT4b is, therefore, thought to be a precursor of CTX. We have detected additional minor toxic constituents, probable congeners of CTX from both herbivorous and carnivorous fish. CTX could be one of the metabolites oxidized through detoxification systems in fish. Further studies are necessary to clarify the route and the mechanism of oxidative conversion of CTX congeners through food chains in coral reef biota.

Experimental Section

Chemicals. All solvents and reagents were purchased from Kanto Chemicals Co. in Japan and Merck or Prolab Co. in Tahiti. For extractions and solvent partitions, EP grade solvents were used without further purification. When used for column chromatographies, the solvents were distilled once with use of a glass stiller. Deuterium solvents were purchased from Aldrich Chemical Co. and used without further purification except for CD_3OD , which was obtained from Merck and distilled before use.

Spectral Measurements. UV spectra were run on a Hitachi 124 double beam spectrophotometer. The IR spectrum of 1 was obtained with a Nicolet 7199 FT-IR spectrometer with a film method on a ZnSe window and that of 2 was by a JEOL JIR-3510 FT-IR spectrometer with a KBr pellet. High-resolution mass spectra of 1 were measured by a JEOL JMS HX-110 mass spectrometer in the FAB ionization mode at an accelera-

tion voltage of 10 kV with use of a glycerol matrix. FABMS of hydrogenated products of 1 and those of 2 including high resolution were effected by a JEOL JMS DX-303HF spectrometer with glycerol or 3-nitrobenzyl alcohol as a matrix. For measurements of 2, NaCl was added so as to improve the sensitivity by enhancing the ion peak of (M + Na)⁺.

All 1D and 2D NMR spectra were measured with a JEOL GSX-400 NMR spectrometer (400 MHz) except for several COSY's of 1, which were recorded on a Bruker AM-500 (500 MHz) or a Bruker AM-600 spectrometer (600 MHz). Measurements at low temperatures (mostly -20 or -25 °C) were carried out in the cooling module designed for a GSX-400 instrument (VT system), which cooled the loaded sample with nitrogen gas continuously evaporated by a heater. Conventional ¹H-¹H COSY spectra were recorded by a data matrix of 2K × 512 points at 25 °C and by that of 1K × 256 points at -20 °C. Relayed COSY was obtained at 25 °C with 1K × 256 points. Phase-sensitive NOESY was effected with 1K × 256 points and a mixing time of 150 ms in pyridine- d_5 at -20 °C. 2D-HOHAHA was measured with a 1K × 512 matrix and a mixing time of 63 and 93 ms at 20 °C. DFQ COSY of 2 was taken with a 2K × 512 matrix in CD_3CN at 20 °C. Twofold zero-filling was carried out as to column data of all the 2D NMR spectra. NOE difference spectra were measured at -25 °C in a pyridine- d_5 or in a CD_3CN solution without degassing. ¹³C NMR spectra of 2 were obtained in CD_3CN at 20 °C by a pulse sequence of broad band ¹H decoupling with 2 × 10⁵ scans. The DEPT spectrum of GT4b was effected in CD_3CN at 20 °C by a ¹H-45° pulse sequence with 2 × 10⁵ scans.

Isolation of CTX (1). CTX was extracted from the moray eel *Gymnothorax javanicus* and was purified as previously described.⁶ The moray eels (ca. 4000 kg) were collected from the Tuamotu Archipelago and from the Island of Tahiti in French Polynesia. The viscera (125 kg) were homogenized and extracted with two volumes of acetone twice. After filtration, the extract was left at -20 °C for 1 day to precipitate oily residue. The supernatant was evaporated to dryness and partitioned between diethyl ether and water. The ether layer was condensed and suspended in aqueous 80% MeOH, followed by defatting with hexane. The methanolic layer was condensed, dissolved in acetone, and kept at -20 °C.

After filtration at 0 °C and evaporation, the crude toxin was divided into 4-g portions to be chromatographed on a column of silica gel (Merck, 63–200 μ m, 25 × 500 mm) with $CHCl_3$ -MeOH (97:3) and washed with the same solvent mixture. The column was washed with $CHCl_3$ -MeOH (9:1) to elute the toxin. The crude toxin was further chromatographed over a florisil column (Thouzard et Matignon, 70–140 μ m, 10 × 300 mm) successively washed with EtOAc and EtOAc-MeOH (9:1 and 3:1). The toxin obtained in the second eluate was loaded on a column of DEAE-cellulose (Merck, 25 × 100 mm) and eluted stepwise with $CHCl_3$, $CHCl_3$ -MeOH (1:1), and MeOH, resulting in toxicity detected in the second fraction.

Further purification was carried out by using the following columns and solvent: (1) Sephadex LH-20 (Pharmacia) and $CHCl_3$ -MeOH (6:4); (2) C₁₈ cartridge (Sep-pak C₁₈, Waters) and MeOH-H₂O (6:4, 7:3, 8:2, and 9:1, most toxicity eluted in the third fraction); (3) C₁₈ packed column (LiChrosorb RP-18, Merck) and MeOH-H₂O (8:2); (4) LiChrosorb RP-18 and MeCN-H₂O (13:7). Eluates from the column were monitored with use of mouse lethality tests by intraperitoneal injections.

Isolation of GT4b (2). The ciguatera causative dinoflagellates, *Gambierdiscus toxicus*, were collected in the Gambier Islands in French Polynesia. The cells sticking on the calcareous alga, *Jania sp.*, were collected by shaking in a plastic bag. The detached cells were filtered and extracted with acetone.

The extract was loaded on a column of florisil (Thouzard et Matignon, 26 × 300 mm) and eluted with hexane-acetone (4:1) and acetone-MeOH (9:1 and 1:1). The crude toxin obtained in the second fraction was passed through an ODS column (Develosil lop ODS, Nomura chemical) by successively eluting with MeOH-H₂O (7:3 and 9:1) and MeOH. The toxin eluted in the latter half of the second eluate and that eluted in the former half of the last eluate were combined and passed through a column of Toyopearl HW-40 (Tosoh, fine) with MeOH. After a rechromatography over the same column with MeOH-H₂O (17:3), the crude toxin was purified on a reversed phase HPLC column (Develosil ODS-7, Nomura Chemical) with a linear gradient elution starting from MeOH-H₂O (17:3) and ended at 100% MeOH. The final purification was done with a reversed-phase column of polymer base (Asahipak ODP-50) with MeCN-H₂O (17:3). Location of the toxin in eluates from the column was determined by mouse lethality tests.

Acknowledgment. We are grateful to Drs. J. Roux (Director) and R. Bagnis of Luis Malarde Institute for continuous encouragement, to J. Bennett and P. Cruchet for sample collection and

(13) MM2 energy calculation (version '78) was done as to rings J, K, L, and M, showing dihedral angles of H-45/H-46, 127.0°; H-46/H-47, 63.6°; and H-47/H-48, 72.8°. According to the generalized Karplus equation,¹⁴ coupling constants of H-45/H-46, H-46/H-47, and H-47/H-48 were calculated to be 4.0, 3.0, and 1.2 Hz, respectively, which agreed well with the observed values (4.7, 3.5, and 1.1 Hz).

(14) As ³J_{H,H} around oxycarbons are known to deviate from the classic Karplus equation, the calculation of ³J_{H,H} on ring K was done with the modified equation, which involved the term for evaluation of electronegativity of substituents. An equation and a parameter set used in this study were those for tetrasubstituted ethane: Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Atlona, C. *Tetrahedron* 1980, 36, 783.

(15) Yessotoxin is one of the toxins implicated in diarrhetic shellfish poisoning. Murata, M.; Kumagai, M.; Lee, J.-S.; Yasumoto, T. *Tetrahedron Lett.* 1987, 28, 5869.

(16) Bidard, J.-N.; Vijverberg, H. P. M.; Frelin, C.; Chungue, E.; Legrand, A.-M.; Bagnis, R.; Lazdunski, M. *J. Biol. Chem.* 1984, 259, 8353.

(17) Gallagher, J. P.; Shinnick-Gallagher, P. *Br. J. Pharmacol.* 1980, 69, 367.

(18) Lombet, A.; Bidard, J.-N.; Lazdunski, M. *FEBS Lett.* 1987, 219, 355.

the extraction, and to Prof. M. Hiram of Tohoku University for discussion. This work was supported by a grant-in-aid for overseas research from the Ministry of Education, Science and Culture, Japan, by the French Ministry of Research and Superior Education, by the Pasteur Institute of Paris, and by the French Polynesian Government.

Supplementary Material Available: ^1H NMR assignments of **2** at -25°C in CD_3CN and in pyridine- d_5 , NOE difference spectra of **2**, and 2D maps of relayed COSY and 2D-HOHAHA (8 pages). Ordering information is given on any current masthead page. The other 2D and NOE data are also available as supplementary material for the previous communication.¹

Preparation of Eight-Membered Cyclic Ethers by Lewis Acid Promoted Acetal-Alkene Cyclizations

Todd A. Blumenkopf,^{1a} Matthias Bratz,^{1b} Armando Castañeda,^{1c} Gary C. Look, Larry E. Overman,^{*} Domingo Rodriguez,^{1d} and Andrew S. Thompson^{1e}

Contribution from the Department of Chemistry, University of California, Irvine, Irvine, California 92717. Received November 29, 1989

Abstract: The Lewis acid promoted cyclization of 20 5-alkenyl acetals is reported. Oxocenes with Δ^4 unsaturation (3,6,7,8-tetrahydro-2H-oxocins) can be accessed in this convenient fashion with moderate to excellent efficiency and with perfect regiochemical fidelity. The yield of Δ^4 -oxocene increases as the 5-substituent of a 5-hexenyl acetal is varied from H to SiMe_3 to SPh. High yields (up to 80%) are obtained in vinyl sulfide-acetal cyclizations only. Cyclizations of vinylsilane or vinyl sulfide acetals derived from secondary alcohols proceed with excellent ($>25:1$) stereoselectivity to construct, in a single step, *cis*-2,8-disubstituted- Δ^4 -oxocenes. The competing pathway that is most significant in undermining the yield of Δ^4 -oxocene in cyclizations of 5-(trimethylsilyl)-5-hexenyl acetals is not bimolecular oligomerization reactions, but rather cyclization to form 2-oxocanyl cations. The importance of this latter pathway was established by the isolation of oxocanyl acetals (**47**, **48**, and **50**), alkylated oxocanes (**15** and **16**), and 11-oxabicyclo[5.3.1]undecanes (**20** and **54**). These studies establish that carbon-carbon bond-forming cyclizations that form eight-membered-ring ethers can be high yielding even with simple substrates that lack conformational bias.

Although medium rings are not common in materials of natural origin, eight-membered cyclic ethers are important structural features of several marine natural products,² e.g., brevetoxin A,³ laurencin,⁴ and laurenyne.⁵ Significant biological activity of members of this group,²⁻⁴ in addition to the well-known difficulties in forming medium rings, makes the development of new methods for assembling eight-membered-ring ethers a significant objective.

The construction of medium rings remains one of the more formidable problems in organic synthesis, since cyclization reactions are impeded by developing transannular interactions as well as entropic loss and torsional and angle deformations.⁶ Prior to the 1980s few useful procedures existed for preparing eight-membered cyclic ethers.⁷ However, during the past decade a

number of important new methods have been devised. These approaches fall into three strategy groups: C-O bond-forming cyclizations,⁸⁻¹³ C-C bond-forming cyclizations,¹⁴⁻¹⁶ and transformations of eight-membered lactones.^{17,18}

The objective of our efforts in this area was the development of a cyclization reaction that would (a) assemble eight-membered-ring ethers from simple acyclic precursors and (b) directly install in the cyclic ether product the Δ^4 unsaturation that is found in a number of marine natural products (see Figure 1). Our approach was stimulated by much earlier studies^{19,20} of the thermal

(1) (a) NIH NRSA Postdoctoral Fellow (GM 09444), 1984-1986. Present address: Division of Organic Chemistry, Burroughs Wellcome Co., 3030 Cornwallis Rd., Research Triangle Park, NC, 27709. (b) Feodor Lynen Postdoctoral Fellow of the Alexander von Humboldt Foundation, 1989-1990. (c) COSNET-SEP Mexico Graduate Fellow, 1982-1985. Present address: Syntex S. A. de C. V., Paseo de La Reforma 2822, Mex. D.F. 11000, Mexico. (d) Swiss National Science Foundation Fellow, 1986-1987. Present address: Givaudan SA, 1214 Vernier, Geneva Switzerland. (e) NIH NRSA Postdoctoral Fellow (CA 07787), 1985-1987. Present address: Merck & Co., Inc., R-801-206, Rahway, NJ 07065.

(2) For reviews, see: Faulkner, D. J. *Nat. Prod. Rep.* **1987**, *4*, 539; **1986**, *3*, 1; **1984**, *1*, 251, 551. Moore, R. E. *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 1.

(3) Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773.

(4) Irie, T.; Suzuki, M.; Masamune, T. *Tetrahedron* **1968**, *24*, 4193. Irie, T.; Suzuki, M.; Masamune, T. *Tetrahedron Lett.* **1965**, 1091.

(5) Falshaw, C. P.; King, T. J.; Imre, S.; Islimyeli, S.; Thomson, R. H. *Tetrahedron Lett.* **1980**, 4951.

(6) Sicher, J. *Prog. Stereochem.* **1982**, *3*, 202. Illuminati, G.; Mandolini, L. *Acc. Chem. Res.* **1981**, *14*, 95.

(7) For a brief review, see: Allenbach, H.-J. *Nachr. Chem., Tech. Lab.* **1988**, *36*, 179.

(8) (a) Jackson, W. P.; Ley, S. V.; Morton, J. A. *Tetrahedron Lett.* **1981**, *22*, 2601. (b) Corey, E. J.; Shimoji, K. *J. Am. Chem. Soc.* **1983**, *105*, 1662.

(9) Schreiber, S.; Kelley, S. E. *Tetrahedron Lett.* **1984**, *25*, 1757.

(10) Heslin, J. C.; Moody, C. J.; Slawin, A. M. Z.; Williams, D. J. *Tetrahedron Lett.* **1986**, *27*, 1403.

(11) Nicolaou, K. C.; Duggan, M. E.; Hwang, C.-K. *J. Am. Chem. Soc.* **1986**, *108*, 2468. Nicolaou, K. C.; Prasad, C. V. C.; Hwang, C.-K.; Duggan, M. E.; Veale, C. A. *Ibid.* **1989**, *111*, 5321.

(12) (a) Pornet, J.; Damour, D.; Miginiac, L. *Tetrahedron* **1986**, *42*, 2017. (b) Haseltine, J.; Visnick, M.; Smith, A. B., III *J. Org. Chem.* **1988**, *53*, 6160.

(13) After preparation of this manuscript, Miginiac and co-workers reported the preparation of five Δ^4 -oxocenes in good yields by intramolecular Prins cyclization of allylsilanes; see: Guyot, B.; Pornet, J.; Miginiac, L. *J. Organomet. Chem.* **1989**, *373*, 279.

(14) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4743.

(15) (a) Cockerill, G. S.; Kocienski, P.; Treadgold, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2093. (b) Cockerill, G. S.; Kocienski, P.; Treadgold, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2101.

(16) Overman, L. E.; Blumenkopf, T. A.; Castañeda, A.; Thompson, A. S. *J. Am. Chem. Soc.* **1986**, *108*, 3516.

(17) (a) Carling, R. W.; Holmes, A. B. *J. Chem. Soc., Chem. Commun.* **1986**, 325, 565. (b) Carling, R. W.; Holmes, A. B. *Tetrahedron Lett.* **1986**, *27*, 6133.

(18) Nicolaou, K. C.; McGarry, D. G.; Somers, P. K.; Veale, C. A.; Furst, G. T. *J. Am. Chem. Soc.* **1987**, *109*, 2504.

(19) Blomquist, A. T.; Taussig, P. R. *J. Am. Chem. Soc.* **1957**, *79*, 3505. Roth, W. R. *Chimia* **1966**, *20*, 229.

(20) Crandall, J. K.; Watkins, R. J. *J. Org. Chem.* **1971**, *36*, 913. Lambert, J. B.; Fabricius, D. M.; Napoli, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 1793.