Structure of Caribbean Ciguatoxin Isolated from *Caranx latus*

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Abstract: Caribbean ciguatoxins (C-CTXs) are responsible for the widespread occurrence of ciguatera in the Caribbean Sea. The structure and configuration of C-CTX-1 (**1**), the major ciguatoxin isolated from the horse-eye jack *(Caranx latus)*, has been determined from DQF-COSY, E-COSY, TOCSY, NOESY, ROESY, ge-HSQC, and HMQC experiments performed at 750 MHz and 500 MHz on a 0.13 μmol sample. C-CTX-1 ([M + H]$^+$ *m*/*z* 1141.6 Da, molecular formula $C_{62}H_{92}O_{19}$) has a ciguatoxin/brevetoxin ladder structure comprising 14 trans-fused, ether-linked rings (7/6/6/7/8/9/7/6/8/6/7/6/7/6) assembled from 6 protonated fragments. The relative stereochemistry and ring configuration of **1** was determined from an analysis of coupling constant and NOE data. Like ciguatoxins in the Pacific Ocean (P-CTX), C-CTX-1 possesses a flexible nine-membered ring which may be a conserved feature among ciguatoxins. However, C-CTX-1 has a longer contiguous carbon backbone (57 vs 55 carbons for P-CTX-1), one extra ring, and a hemiketal in ring N but no spiroketal as found in P-CTX. C-CTX-1 possesses a primary hydroxyl which may allow selective derivatization. A minor analogue, C-CTX-2, was also isolated from fish and assigned the structure 56 epi-C-CTX-1 (**2**), since it slowly rearranged to C-CTX-1 in solution. Given the structural similarities between Caribbean and Pacific ciguatoxins, we propose that C-CTX-1 and C-CTX-2 arise from a Caribbean strain of the benthic dinoflagellate, *Gambierdiscus toxicus*.

Introduction

Ciguatera is an illness that follows the consumption of warm water fish contaminated with sodium channel toxins known as ciguatoxins (CTX).[1] Ciguatera is a major problem in the Pacific and Indian Oceans and the Caribbean Sea, affecting >25 000 persons annually. Structures have been determined for a number of ciguatoxins isolated from Pacific fish.[2] Ciguatoxins are potent sodium channel activator toxins that bind quasi-irreversibly to site 5 on the voltage sensitive sodium channel (VSSC), a site overlapping the brevetoxin binding site.[2b,3] Pacific CTX-1 (P-CTX-1) remains the most potent sodium channel toxin known with a mouse i.p. LD_{50} of 0.25 μg/kg.[2b] All ciguatoxins accumulate through marine food chains, often undergoing oxidative biotransformations to more potent forms in fish.[4] The Pacific ciguatoxins are produced by certain strains of the benthic dinoflagellate *Gambierdiscus toxicus*,[4] while the origins of ciguatoxins contaminating fish in the Indian Ocean and Caribbean Sea have not been determined. Acute ciguatera is treatable with hyperosmotic mannitol infusions which may reduce Schwann cell oedema[5] that has been observed in severe cases of ciguatera.[6] Presently there are no routine methods for detecting ciguateric fish, which accumulate CTX in their flesh and viscera to levels above 0.1 ppb.

Ciguatera in the Caribbean is characterized by a similar range of symptoms as the disease in the Pacific but the frequency of symptoms differs and recent studies suggest that it may be caused by a new class of ciguatoxins.[7] A partial structure of Caribbean ciguatoxin was determined on a 0.1 μmol sample using a Shigemi microprobe and 500-MHz NMR spectroscopy.[7a] Using an NMR approach to the structure elucidation of ciguatoxins pioneered by Yasumoto's group,[2a] we report the structure of C-CTX-1 (**1**) based on proton and carbon assign-

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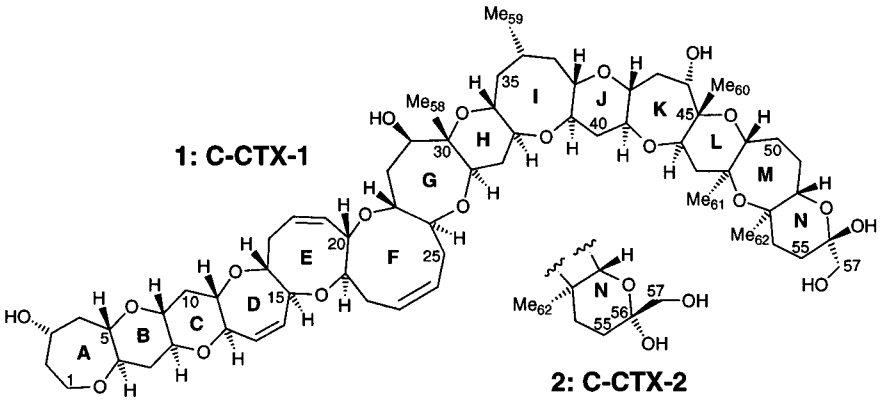
Chart 1



ments, except for five quaternary carbons, determined on a 0.13 μmol sample using 750-MHz NMR. The structure of C-CTX-2 (**2**), a minor ciguatoxin also isolated from fish, is also proposed (Chart 1).

Results

Isolation and Toxicity. C-CTX-1 and -2 were isolated in a ~20:1 ratio as major and minor ciguatoxins from *Caranx latus*.[7b] From 51 kg of tissue from ciguateric *C. latus*, 150 μg of C-CTX-1 was obtained as a white amorphous solid with an i.p. LD_{50} in 20 g mice of 3.6 μg/kg.[7b]

Molecular Formula. The molecular weight of C-CTX-1 determined by ion spray mass spectrometry (ISMS) was $[M + H]^+$ *m*/*z* 1141.6, 30 Da larger than P-CTX-1 ($[M + H]^+$ 1111.6, $C_{60}H_{86}O_{19}$).[2a] The even mass for C-CTX-1 indicated it contained zero or an even number of nitrogens. Similar to other polyether toxins,[8] C-CTX-1 was observed as $[M + NH_4]^+$ and $[M + Na]^+$ ions and lost up to five waters as 18 Da neutral species. Assuming that C-CTX-1 is comprised of only carbon, hydrogen, and oxygen, $C_{62}H_{92}O_{19}$ satisfies the ISMS data. This formula was independently confirmed through the process of structure elucidation. The mass of C-CTX-2 determined by ISMS was also $[M + H]^+$ *m*/*z* 1141.6, indicating that C-CTX-2 may be an epimer of C-CTX-1.[7b]

Purity. C-CTX-1 was estimated to be >90% pure by HPLC[7b] and NMR (see Figure 1). Resonances identified as impurities did not give rise to NOESY or TOCSY cross-peaks to any proton resonances assigned to C-CTX-1, at both 2 °C and 27 °C (see Supporting Information).

Proton Connectivities and Ether Rings. The backbone structure of C-CTX-1 was established from DQF-COSY and TOCSY experiments in pyridine-d_5 at 27 and 2 °C (Figures 1 and 2A). Connectivities C1−C21, C26−C29, C31−C44, C46−C47, C49−C52, and C54−C55 and the location of Me-59 were independently established at 27 and 2 °C (Figure 2A). The resolution obtained using 750-MHz NMR spectroscopy allowed unambiguous assignment of each proton in pyridine-d_5 (see Supporting Information for further details). An additional set of connectivities C21−C26 was established from DQF-COSY and TOCSY spectra in pyridine-d_5 at 2 °C, since these proton resonances were too broad to be observed in the DQF-COSY spectrum at 27 °C. The fragments C22−C25 and C31−C42 were also proposed by Crouch et al.[7a] The location of OH-3 and OH-44 were established from TOCSY and 2 °C DQF-COSY spectra, while the location of OH-29 was established from TOCSY and −25 °C NOESY spectra (Figure 2B,C). Finally, a connectivity between OH-57 (δ 6.88) and H,H-57 (δ 4.01) was determined from a TOCSY spectrum at 2 °C. Each hydroxyl proton was detected as a cross-peak to the H_2O resonance.[2c] Thus C-CTX-1 was composed of six protonated fragments C1−C29, C31−C44, C46−C47, C49−C52, C54−C55, and C57. The full proton assignment of C-CTX-1 (**1**) at 27 °C is given in Table 1. A similar set of assignments was obtained at 2 °C (Supporting Information).

C-CTX-1 in pyridine-d_5 gave relatively strong negative NOEs in NOESY spectra at 750 MHz that simplified structure elucidation. ROESY experiments, performed at two transmitter offset frequencies to identify false NOEs, supported the NOESY assignments. Oxymethine NOEs identified from a 200 ms mixing time NOESY spectrum readily established the ladder-like structure of C-CTX-1, revealing the existence of nine ether-fused rings A−G and J−K (Figure 2B). An NOE between H-33/H-39 could not be detected because these protons had similar chemical shifts (δ 3.35/3.34); however, a prominent NOE between α H-32/α H-40 established the presence of the ether-fused ring I (Figure 2B). NOEs from oxymethines to bridgehead methyls established rings H and L, and an NOE between a pair of bridgehead methyls established ring M (Figure 2B). Thus 13 rings and connections between C29−C31, C44−C46, C47−C49, and C52−C54 were established, four quaternary carbons were identified, and five of the six protonated fragments of C-CTX-1 were assembled into a contiguous 55 carbon backbone (see Supporting Information for further discussion). Insufficient material was available to obtain supporting data on quaternary carbons from HMBC experiments. However, the more sensitive HSQC (27 °C) and HMQC (2 °C) experiments on **1** yielded ^{13}C assignment for 57 carbons that had proton resonances which corresponded to the 5 methyls, 20 methylenes, 26 methines, and 6 vinyl protons identified from the 2D homonuclear spectra (Table 1). This assignment, which includes 1 primary and 3 secondary hydroxyls, 4 quaternary carbons and 13 ether oxygens, gave the formula $C_{61}H_{91}O_{17}$, 45 Da less than the mass determined by ISMS and indicating that the missing piece was CHO_2. The location of this piece was identified from a low temperature 300-ms NOESY spectrum (−25 °C in pyridine-d_5) which revealed NOEs between H-52/OH-56, OH-56/OH-57, and H,H-57/OH-56, in addition to the NOE between H,H-57/α H-55 which was observed at 27, 2, and −25 °C (Figure 2B). The missing piece was thus a hemiketal and ring N was formed through an ether bridge between the oxymethine at C52 and the anomeric carbon C56. Thus the planar structure of C-CTX-1 was established as **1**.

Stereochemistry and Ring Configuration of C-CTX-1. DQF-COSY and E-COSY spectra allowed the measurement of

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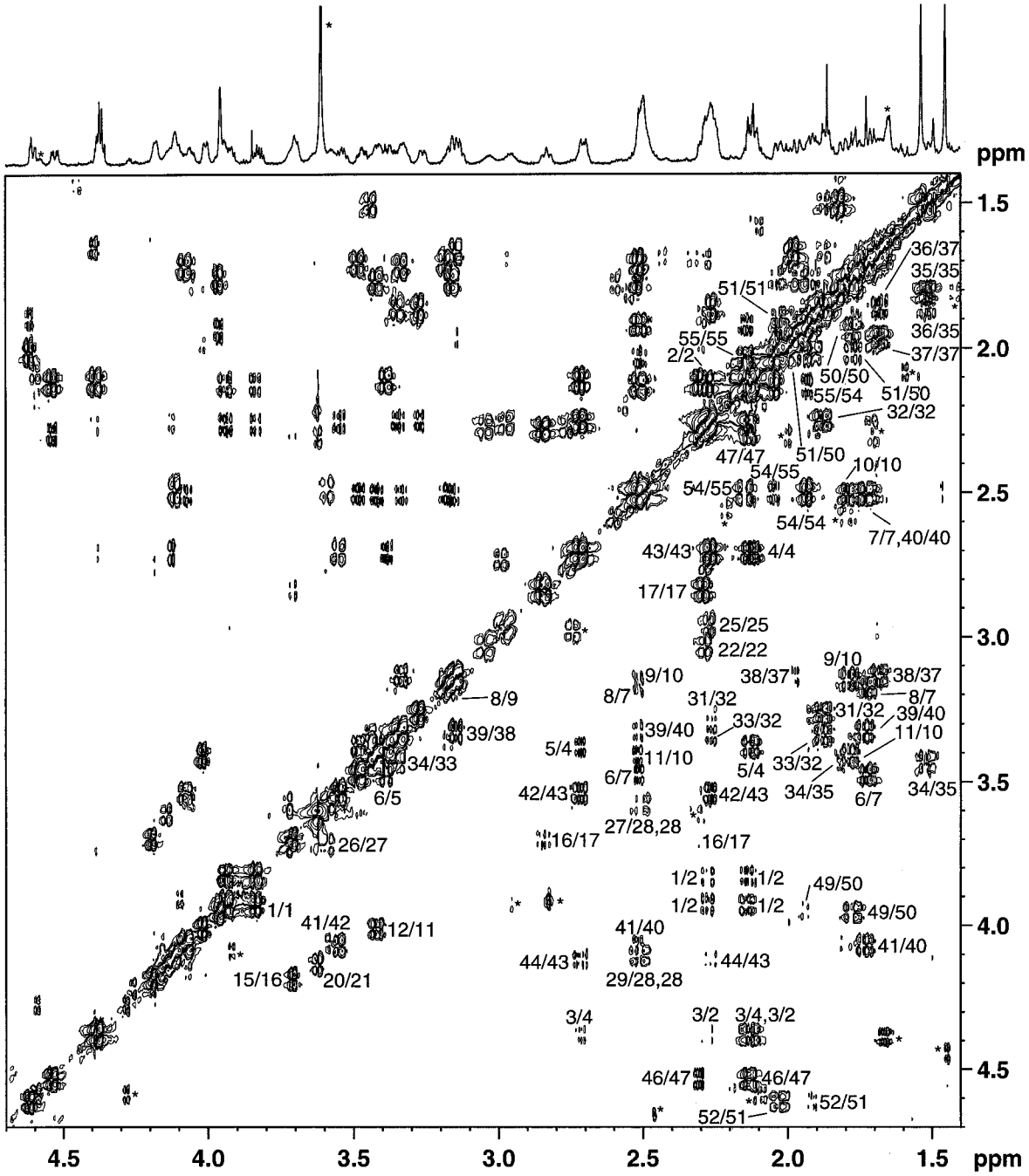

Figure 1. Part of a DQF-COSY spectrum of C-CTX-1 (**1**) obtained at 750 MHz in pyridine-d_5 (27 °C) with the corresponding 1D spectrum. Numbers indicate the carbon number of scalar coupled protons (see Table 1). Asterisks indicate impurities, including a trace of methanol at δ 3.62.

most $^3J_{H,H}$ couplings between protons (Table 1). Couplings of between 9 and 12 Hz, typical for an antiplanar substitution on oxycarbons,[2a] indicated that all rings of C-CTX-1 were trans-fused. The NOE data showed that Me-58, Me-60, Me-61, and Me-62 were also trans-substituted (Figure 2B). As observed for ring I of P-CTX-1,[2a] Me-59 was α to H-36 but had a β orientation to the eight-membered ring I, which adopted a crown conformation in C-CTX-1 (Figures 2B,C and 3). Coupling constant data indicated that the double-bond geometry in rings D and E were cis (Table 1), while NOE data supported a cis geometry for the double bond in ring F (Figure 2C).

The orientation of the hydroxyls of C-CTX-1 was determined from an analysis of coupling constant and NOE data (Table 1, Figure 2C) and molecular modeling (Figure 3). The large scalar coupling between H-3/α H-2 (δ 2.12) and H3/α H-4 (δ 2.11) determined from an E-COSY spectrum, and the NOEs between OH-3/α H-1 (weak) and H-3/H-5 indicated that OH-3 was an α substituent. NOEs between OH-29/Me-58, H-29/H-26, and H-29/H-31 indicated that OH-29 was a β substituent. The NOEs between OH-44/H-46 (weak) and H-44/Me-60 indicated that OH-44 was an α substituent. NOEs also revealed that the anomeric carbon C56 was equatorially substituted, with OH-56 being β in **1** (Figure 2B).

Stability of C-CTX-1 and C-CTX-2. Gradient reverse-phase HPLC/mass spectrometry (HPLC/MS) was used to assess the stability of C-CTX-1 and C-CTX-2 in solution. C-CTX-1 remained unchanged after 6 month's storage at −10 °C in 50% aqueous acetonitrile or after 4 h in 50% acetonitrile/5% trifluoracetic acid (TFA). However, C-CTX-2 after 6 month's storage at −10 °C in 50% aqueous acetonitrile was no longer

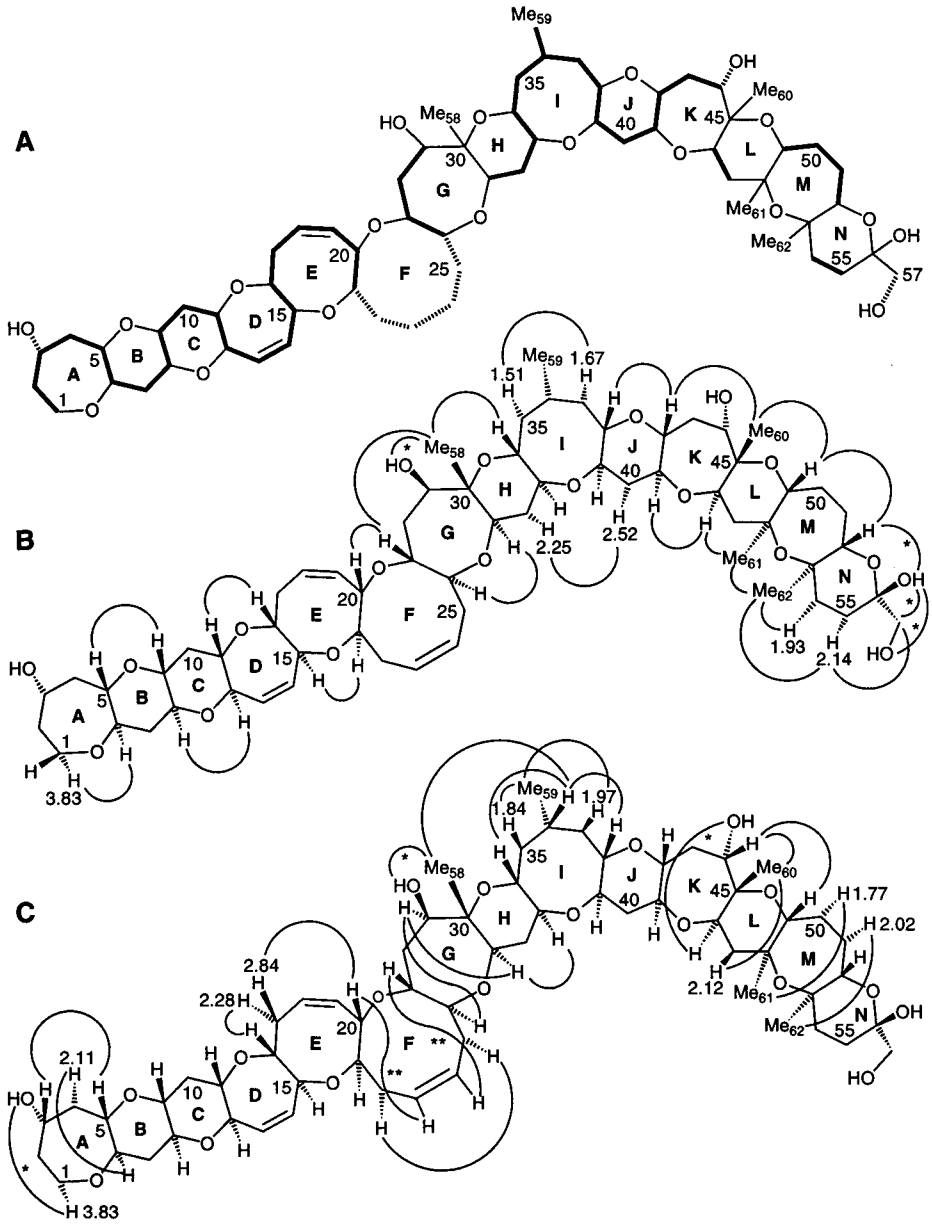

Figure 2. Summary of data derived from DQF-COSY, TOCSY, and NOESY spectra of C-CTX-1 (**1**). (A) Connectivities determined from DQF-COSY spectra at 27 °C (bold lines) and 2 °C (dashed lines). (B) NOE data used to assemble the six protonated fragments and establish the 14 ether-fused rings of **1**. (C) Additional NOE data used to assign the relative stereochemistry and ring configuration of **1**. All NOEs were determined from a 200 ms mixing time NOESY spectrum (750 MHz, 27 °C), except those marked with a single asterisk (300 ms NOESY, 500 MHz, −25 °C) or a double asterisk (400 ms NOESY, 750 MHz, 2 °C). C-CTX-1 (5*S*) was drawn with the same absolute stereochemistry as P-CTX.[11]

detected and a new peak was observed that eluted at a retention time and mass indistinguishable from C-CTX-1.

Discussion

The structure of C-CTX-1 (**1**) isolated from Caribbean fish has been determined from NMR experiments at 750 MHz on a 0.13 μmol sample. C-CTX-1 is an exclusively trans-fused polyether, with a contiguous 57-carbon skeleton, 14 rings, 3 double bonds, 5 methyls, and 5 hydroxyls. The single primary hydroxyl in C-CTX-1 may allow selective addition of cross-linkers or labels. C-CTX-1 is identical to P-CTX-1[2a] at rings C and D and from ring F to ring J and identical to CTX-3C[2d] from ring C to ring J. In support, chemical shifts for protons and carbons were within 0.1 and 1.0 ppm, respectively, from position 10 to 39 of C-CTX-1 and CTX-3C.[2d] Like CTX-3C, C-CTX-1 does not possess the aliphatic side chain seen on ring A of P-CTX-1. Thus C-CTX-1 resembles the middle section of P-CTXs, but has a longer contiguous carbon backbone, one extra ring, and a hemiketal in ring N but no spiroketal as found in P-CTXs (Figure 4). Comparison of our 1D ^{1}H NMR and HSQC spectra of C-CTX-1 in pyridine-d_5 with equivalent spectra reported by Crouch et al.,[7a] indicated that the Caribbean CTX analyzed were similar, if not identical, with differences between the spectra explained by the presence of different impurities in the two samples. HPLC/MS and HPLC/MS/MS analyses have also identified C-CTX-1 from *Seriola dumerili* and a range of other Caribbean fish species,[3b,7c] indicating that C-CTX-1 may be the major ciguatoxin in carnivorous fish from the Caribbean Sea.

The proton connectivities in C-CTX-1were interrupted by five quaternary carbons, whereas P-CTX-1 is interrupted by only two quaternary carbons. This potential obstacle to structure

Table 1. ^{1}H and ^{13}C Chemical Shifts and Active Couplings of C-CTX-1 (**1**)[a]

position	^{1}H ($^3J_{H,H}$)[c]	^{13}C	position	^{1}H ($^3J_{H,H}$)[c]	^{13}C
1	3.83 (5, −)	66.0	33	3.35 (12)	84.0
	3.94 (4, 10)		34	3.44 (12, 5)	73.2
2	2.12 (>10)	39.6	35	1.51 (−)	46.8
	2.27 (−)			1.85 (−)	
3	4.39 (11, 6)	65.7	36	1.88 (−, −)	28.2
4	2.11 (13)	45.0	37	1.67 (11)	46.1
	2.71 (5)			1.97 (5)	
5	3.39 (9)	79.0	38	3.14 (11)	81.4
6	3.48 (12, 5)	77.2	39	3.34 (12, 5)	84.4
7	1.71 (−)	37.7	40	1.72 (11)	37.7
	2.51 (5)			2.52 (5)	
8	3.18 (9)	76.4	41	4.08 (11)	77.6
9	3.16 (12, 5)	76.2	42	3.55 (−, −)	79.6
10	1.79 (11)	37.7	43	2.26 (7)	37.0
	2.52 (5)			2.71 (−)	
11	3.42 (10)	79.1	44	4.12	74.8
12	4.02 (−)	81.0	46	4.54 (12, 5)	73.2
13	5.94 (13)	131[d]	47	2.12	44.0
14	5.82 (7)	131[d]		2.30	
15	4.19 (10)	82.7	49	3.96 (11, 5)	73.8
16	3.71 (−, 6)	85.4	50	1.77 (−, −)	24.6
17	2.28 (8)	32.8		1.95 (−, −)	
	2.84 (9)		51	1.91 (5)	24.2
18	5.84 (12)	126[d]		2.02 (12)	
19	5.98 (7)	138[d]	52	4.62	72.5
20	4.14 (11)	84.0	54	1.93 (−, 6)	37.2
21	3.62 (−, −)	86.4		2.51 (5, −)	
22	2.28[b] (−)	32.2	55	2.08	29.0
	3.02[b] (−)			2.14	
23	6.01[b] (−)	128.9	57	3.96	69.6
24	6.01[b] (−, −)	128.9		3.96	
25	2.27[b] (−)	32.8	58	1.27 (s)	9.8
	2.96[b] (−)		59	0.93 (7)	28.2
26	3.72 (12)	83.8	60	1.24 (s)	14.0
27	3.58 (−, −)	83.5	61	1.55 (s)	21.0
28	2.49 (−)	40.2	62	1.47 (s)	20.4
	2.53 (−)		3-OH	6.31 (−)	
29	4.11	74.9	29-OH	5.12 (−)	
31	3.27 (11, 5)	81.2	44-OH	6.20 (−)	
32	1.86 (−)	36.5	56-OH	7.08 (s)	
	2.25 (5)		57-OH	6.54 (−)	

[a,b] ^{1}H and ^{13}C chemical shifts (ppm) were obtained from spectra of **1** in pyridine-d_5 at 27 °C, with pyridine-d_5 taken as δ 7.21 and δ 123.5, respectively. Spectra were acquired on a DMX750 spectrometer (Bruker). ^{1}H chemical shifts were obtained from a DQF-COSY spectrum, except those identified with superscript *b*, which were measured from a TOCSY spectrum at 27 °C, with connectivities determined from a DQF-COSY spectrum at 2 °C. [c,d] Active couplings (Hz) from DQF-COSY and E-COSY spectra are indicated at the first positioned proton e.g. for the proton at position 9, scalar couplings of 12 and 5 Hz were observed to α H-10 (δ 1.79) and β H-10 (δ 2.52), respectively. Couplings between methylene protons are excluded and dashes indicate those active couplings which were not measureable due to spectral complexity, line broadening, or overlap. The carbon chemical shifts were determined from HSQC and HMQC spectra at 27 °C and 2 °C, respectively, except those identified with the superscript *d* which gave weak signals that were confirmed from the HMQC spectrum shown by Crouch et al.[7a]

elucidation was overcome through the extensive use of NOE data that unambiguously assembled the six protonated fragments of C-CTX-1 into the final structure **1**. C-CTX-1 is the first ciguatoxin identified that possesses a hemiketal moiety. The isolation from fish of C-CTX-2, a minor ciguatoxin which slowly converted to C-CTX-1 in solution, was not unexpected given the propensity of hemiketals to undergo interconversion. From this evidence we propose that C-CTX-2 is 56 epi-C-CTX-1 (**2**). The rearrangement of C-CTX-2 to the lower energy C-CTX-1 is predicted from anomeric and steric effects.[9]

NOE and coupling constant data (Figure 2C, Table 1), together with molecular modeling, were used to establish the configuration of the medium sized rings A, E, F, G, I, K, and M of C-CTX-1 and thus the overall configuration of C-CTX-1 (Figure 3A,B). A comparison of the modeled structures of C-CTX-1 and P-CTX-1 indicated that both toxins are mostly flat and of similar length (32 Å), with rings M and N of C-CTX-1 extended in a bent conformation (Figure 3). Modeling also indicated that OH-44 of **1** adopted an equatorial position, whereas the equivalent hydroxyl in P-CTX-1 (OH-47) is axial (Figure 3).

The similarity in structure between Pacific and Caribbean ciguatoxins indicates that a Caribbean strain of *G. toxicus* is the likely origin of C-CTX-1 and C-CTX-2. The extent to which C-CTX-1 has been modified as it passes through the marine food chain remains to be determined. Pacific and Caribbean CTX appear to act similarly at site 5 on the voltage-sensitive sodium channels.[3,10] Broadened proton resonances on ring F indicated that the middle part of C-CTX-1 changed conformation on a millisecond time scale. A similar explanation has been forwarded to explain the broadened resonances of ring F of P-CTX.[2] The flexible nine-membered ring may be a conserved feature among ciguatoxins, perhaps being important for their high potency. Comparison of the structure of C-CTX-1 with the structures of P-CTXs and brevetoxins may advance our understanding of the structure−activity relationships among polyether sodium channel toxins. Given their similar pharmacology and origin, C-CTX-1 is expected to have the same absolute stereochemistry as P-CTX.[11]

Rapid screening methods are required to minimize the risk of ciguatera in tropical and subtropical waters. Progress is being made toward the development of rapid screens that utilize high-affinity antibodies raised to synthetic fragments of P-CTX-1.[12] Alternative approaches that measure the effect of ciguatoxin binding to sodium channels[2b,3b,10a,b] are also being developed. Given the structural differences between the Pacific and Caribbean ciguatoxins, antibodies recognizing the terminal rings of P-CTX are unlikely to have a high affinity for C-CTX-1. The A/B/C and K/L/M/N rings of C-CTX-1 provide new synthetic targets which might be used in the production of antibodies that recognize Caribbean ciguatoxins.

Materials and Methods

Chemicals. All solvents used were analytical grade or equivalent. Pyridine-d_5 (Cambridge Isotope Laboratories) was used without further purification.

Isolation and Mass Spectrometry (MS). CTX-1 and C-CTX-2 were isolated from 51 kg of liver, viscera, and flesh of the horse-eye jack (*Caranx latus*) collected from St. Barthelemy in the Caribbean Sea. C-CTX-1 and -2 were purified to homogeneity by HPLC as previously described.[7b] Mass spectra were aquired on a PE-Sciex API-III (Toronto, Canada) atmostpheric pressure ionization MS (ISMS). C-CTX-1 and C-CTX-2 were infused with 50% acetonitrile/0.05% TFA

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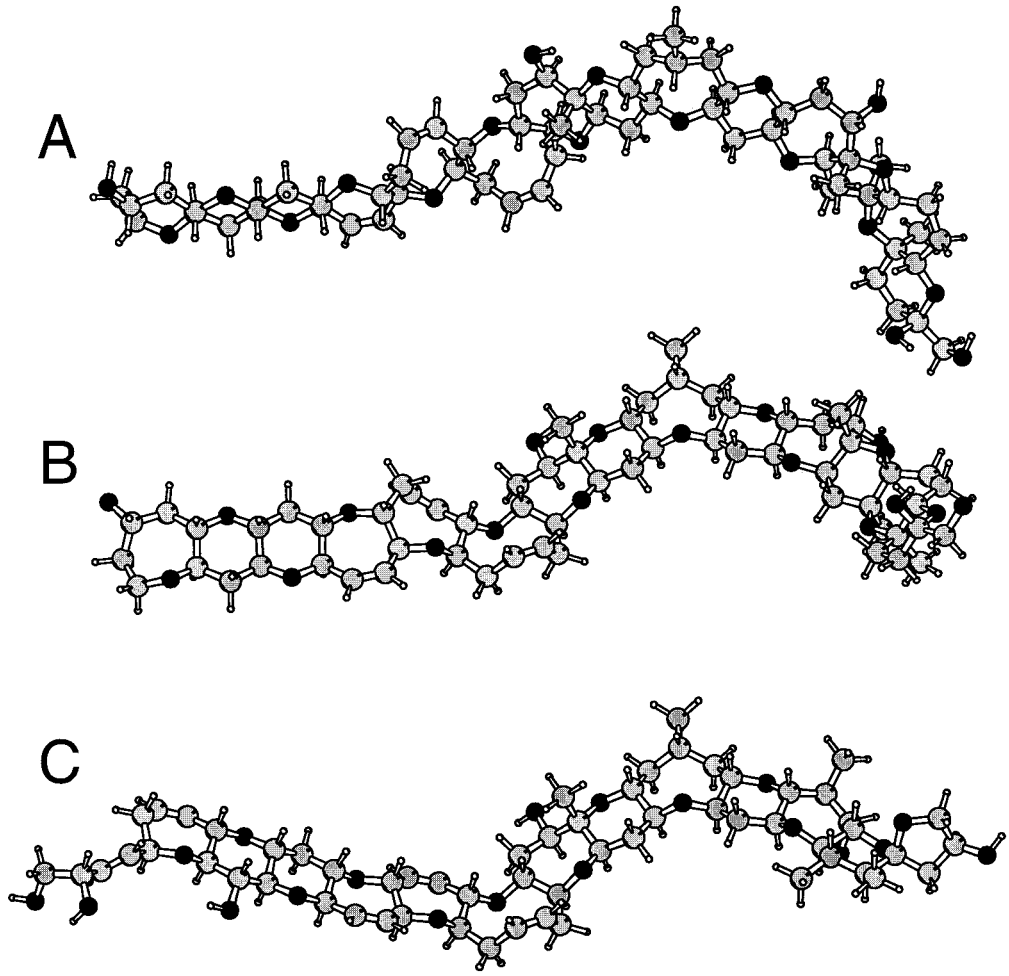

Figure 3. Modeled structures of C-CTX-1 (**1**) and P-CTX-1. (A) The low-energy conformer of C-CTX-1. This conformer satisified the NOE and scalar coupling data for all medium sized rings. (B) The conformer in A rotated through −90° about the *x*-axis. (C) The low-energy conformer of P-CTX-1[20] orientated with rings F−I superimposed on the corresponding rings of C-CTX-1 in B. Structures were minimized in vacuo (dielectric constant = 1) and presented using MolScript (v1.4).[21] Similar results were obtained at a dielectric constant of 13 to simulate pyridine. C-CTX-1 (5*S*) was modeled with the same absolute stereochemistry as P-CTX.[11]

and positive ions detected at orifice potentials of 30−120 V over *m*/*z* 1050−1200 at 0.1 Da steps.

Nuclear Magnetic Resonance (NMR) Spectroscopy. NMR spectra of C-CTX-1 were acquired at 750 MHz on Bruker DMX750 spectrometer (27 and 2 °C) and at 500 MHz on a Bruker DRX500 spectrometer (−25 °C). 1D and 2D NMR spectra of a 0.15 mg sample of C-CTX-1 were acquired in 100 μL of pyridine-d_5 using a micro probe (400−600 t_1 increments, each with 2K complex data points) over a spectral width of 11 ppm in the ^{1}H dimension and 220 ppm in the ^{13}C dimension. DQF-COSY,[13] E-COSY,[14] 55 ms mixing time TOCSY,[15] 200, 300 and 400 ms mixing time NOESY,[16] gradient enhanced ge-HSQC,[17] HMQC,[18] and 200 ms mixing time ROESY[19] data were processed and analyzed using XWINNMR software (Bruker) or 0.1 ppm/cm plots of the spectra. Raw data were zero-filled to produce 4K × 1K complex matrixes, except in the case of DQF-COSY and E-COSY spectra used to measure active couplings and to resolve partially overlapping resonances, where 8K × 0.5K strip transforms were analyzed using the Aurelia processing program (Bruker). Selected DQF-COSY, TOCSY, NOESY, and ge-HSQC spectra are supplied as Supporting Information.

HPLC Mass Spectrometry (HPLC/MS). The stability of C-CTX-1 and C-CTX-2 was monitored by HPLC/MS using a 5 μm Vydac HS201 C-18 column (2.1 × 250 mm) eluted with 50% B in A for 5 min followed by a linear gradient from 50% B to 100% B over a 25 min period: A, 0.05% trifluoracetic acid (TFA); B, 90% acetonitrile/0.05% TFA. Positive ions were detected by turbo-assisted ISMS at an orifice potential of 80 V over *m*/*z* 1050−1200 at 0.1 Da steps. Freshly isolated C-CTX-2 eluted at 12.8 min, compared with 10.2 min for C-CTX-1. HPLC/MS was also used to analyze samples of C-CTX-1 and -2 stored at −10 °C in 50% acetonitrile−H_2O for 6 months or at room temperature in 50% acetonitrile/5% TFA for 4 h.

Molecular Modeling. C-CTX-1 was minimized with the *Discover* program using the CVFF force field (v. 2.9.7, Biosym/MSI) on a Silicon Graphics Indigo workstation. For these calculations we matched the configuration of C-CTX-1 to the 5*R* configuration of P-CTX-1 determined from circular dichroism studies.[11] C-CTX-1 was constructed so that the seven-, eight-, and nine-membered rings adopted a configuration supported by NOE and coupling constant data (Figure 3, Table 1). This conformer was minimized in the gas phase or in simulated pyridine (dielectric constants of 1 and 13, respectively) first

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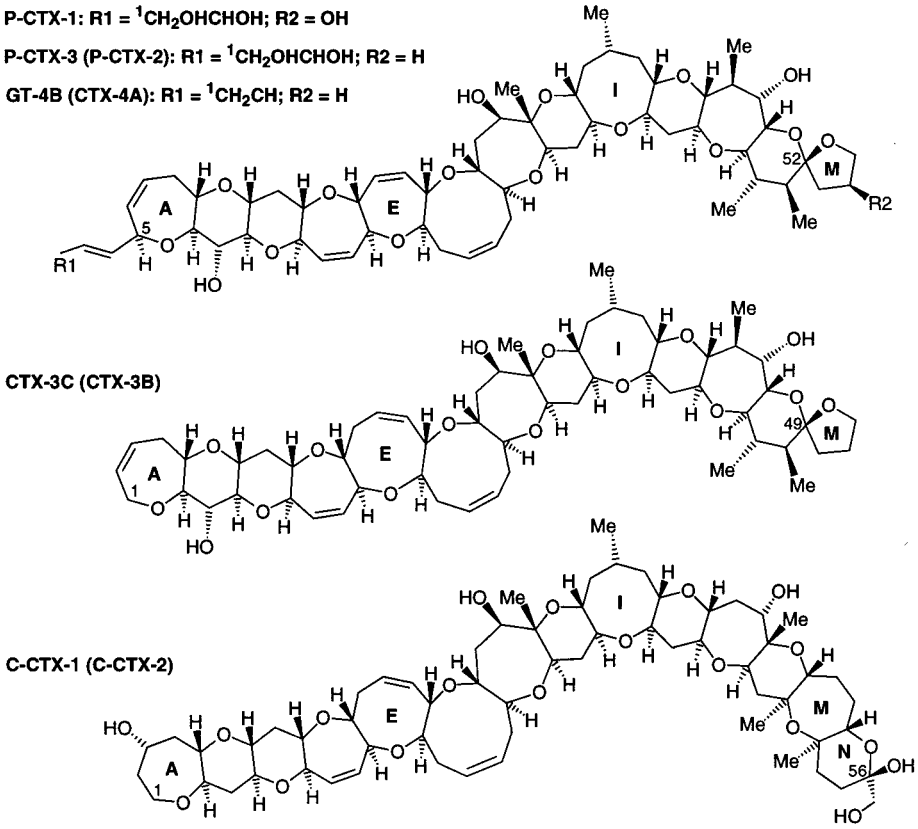

Figure 4. Comparison of Pacific and Caribbean ciguatoxins. Shown are P-CTX-1,[2a] P-CTX-3,[2c] GT-4B,[2a] CTX-3C[2d] from the Pacific, and C-CTX-1. The less energetically favorable epimers P-CTX-2 (52-epi P-CTX-3),[2c] GT-4A (CTX-4A = 52-epi GT-4B),[2e] CTX-3B (49-epi CTX-3C),[2f] and C-CTX-2 (56-epi C-CTX-1) are indicated in parentheses. 2,3-DihydroxyCTX3C and 51-hydroxyCTX3C have recently been isolated from Pacific fish.[2g]

by steepest descent (100 iterations) followed by conjugate gradient algorithms to a maximum derivative of <0.01 kcal/mol-Å. Graphical displays were viewed using *Insight II* molecular modeling system (v. 95.0.3, MSI).

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Supporting Information Available: Spectra of **1** in pyridine-d_5 provided include TOCSY and NOESY at 27 °C and 2 °C, DQF-COSY and HMQC at 2 °C, NOESY at −25 °C, ge-HSQC and 1D ^{1}H NMR at 27 °C, a list of proton chemical shifts at 2 °C, and a discussion of assignments of partially overlapping resonances (18 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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