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A PROBABLE PARTIAL STRUCTURE OF CIGUATOXIN ISOLATED FROM THE MORAY EEL *Gymnothorax javanicus*

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Summary: A probable partial structure of ciguatoxin obtained from the moray eel *Gymnothorax javanicus* was deduced to be **1** on the basis of MS and NMR spectra. The proposed structure involves 22 carbons, accounting for 37% of the total carbons of the toxin.

Ciguatera is a food poisoning caused by a variety of fishes dwelling in coral reefs and poses serious problems to public health and fisheries in tropic or subtropic regions. Although the first description of the poisoning may date back to the 17th century, the toxic principle, ciguatoxin (CTX), was isolated only in 1980 when Scheuer's group reported its toxicity and some chemical properties, including its polyether feature.1) Despite extensive spectroscopic measurements done by this group,1,2) the structure still remains unknown. Chemical studies have been handicapped by the extreme difficulty in collecting toxic fishes as well as by the complexity of the structure. Previously, we reported isolation of CTX from the moray eel *Gymnothorax javanicus* collected in French Polynesia and presented the probable molecular formula.4) In this paper we wish to report a probable partial structure of CTX.

CTX purified previously4) was used in this study. The toxin (0.35 mg) was obtained as colorless amorphous; LD_{99} 0.40±0.08 μg/kg (mice, ip)4); no UV maximum above 210 nm; IR (film) 3400, 1111, 1042 cm.$^{-1}$ ^1H NMR spectra measured in CD_3OD and in pyridine-d_5 were identical with those reported by Scheuer's group.1,2) High resolution FAB-MS has suggested its composition $C_{60}H_{86}O_{19}$, and the absence of a nitrogen atom in the molecule can be inferred.4)

A partial structure **1** shown in Fig. 1 was established mainly on the basis of ^1H-^1H two dimensional NMR data. The linear skeletons of C1-C10 and C14-C22 correspond to those reported by Tachibana,1) but differ from his assignment in geometries of double bonds and in substituents on C5, C6 and C14. The ^1H-^1H COSY spectra were measured with a 400MHz (JOEL GSX-400) spectrometer in four different solvents: CD_3CN-D_2O 2:1, CD_3OD, pyridine-d_5 and dimethylsulfoxide-d_6, and with a 600 MHz (Brucker AM-600) machine in CD_3CN-D_2O 2:1 (Fig. 2). Proton connectivities from C1 to C22 were grossly indicated by cross peaks on ^1H-^1H COSY as shown in Fig.2. Further confirmation of the connectivities between 20/21or22, 19/18, or 21/22 was done by proton

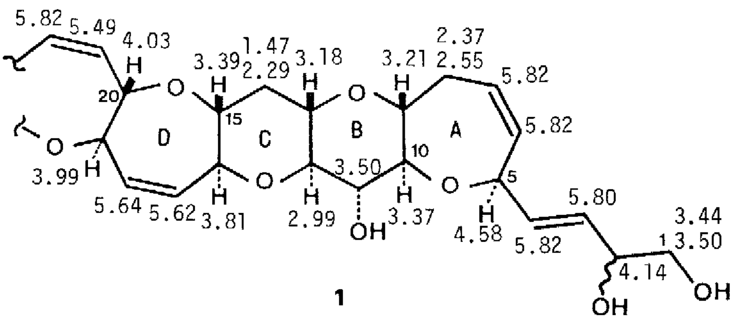

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Fig. 1. Probable Partial Structure of Ciguatoxin from Gymnothorax javanicus The figures are ^{1}H NMR chemical shifts measured in CD_3CN-D_2O (2:1); CD_2HCN as δ 1.99.

decoupling experiments. The cross peak due to H-6/H-7 coupling, overlapping on the diagonal peaks in a $^1H-^1H$ COSY spectrum (Fig.2), was clearly observed at δ 5.90 (H-6) vs. 5.76 (H-7) upon measurement in a pyridine-d_5 solution. The couplings between H-4/H-3 and H-18/H-17 were confirmed by their typical signal shapes for an ABXY spin system (Fig.3). The geometry of the double bonds was determined on the basis of vicinal coupling constants; H-3/H-4, 16.0 Hz; H-6/H-7, ca.12 Hz; H-17/H-18, 12.8 Hz; H-21/H-22,13.3 Hz.

^{1}H NMR spectra in dimethylsulfoxide-d_6 suggested the presence of six hydroxyl groups in the molecule; signals at δ 5.02, 4.84, 4.75, 4.73, 4.50 and 4.06 decreased their intensity upon irradiation at a signal of H_2O (δ 3.27). Their positions were determined by cross peaks on $^1H-^1H$ COSY due to vicinal couplings between a hydroxyl proton and its adjacent methine or methylene.[5)] Consequently, C1, C2 and C11 were assigned to hydroxyl bearing carbons, while the other oxymethines in the partial structure were likely to form ether rings.

The transfusing manner of the ether rings was suggested by the coupling constants of angular protons; H-10 (t 9.0 Hz), H-12 (t 9.2), H-16 (dd 8.9, 2.6) and H-19/H-20 (br.d 9.2). These protons revealed the couplings of ca. 9 Hz, that corresponds to 2,3-diaxial ^3J in a tetrahydropyran. The ether linkages of the rings A, C and D were further confirmed by NOE's on a phase-sensitive NOESY [6)] spectrum. As to the ring B, detection of NOE between H-9 and H-13 was unsuccessful because of their close chemical shifts. The coupling constants of H-10, 11, and 12, however, agreed well with those of H-3, 4 and 5 of glucose,[7)] suggesting the ether linkage between C9/C13 and an equatorial substitution of OH-11, as well as transfusing of the ring B with the ring A.

All these data allow us to deduce the partial structure 1, which contains 22 carbons, 28 hydrogens, and 8 oxygens, accounting for 38% of the molecular

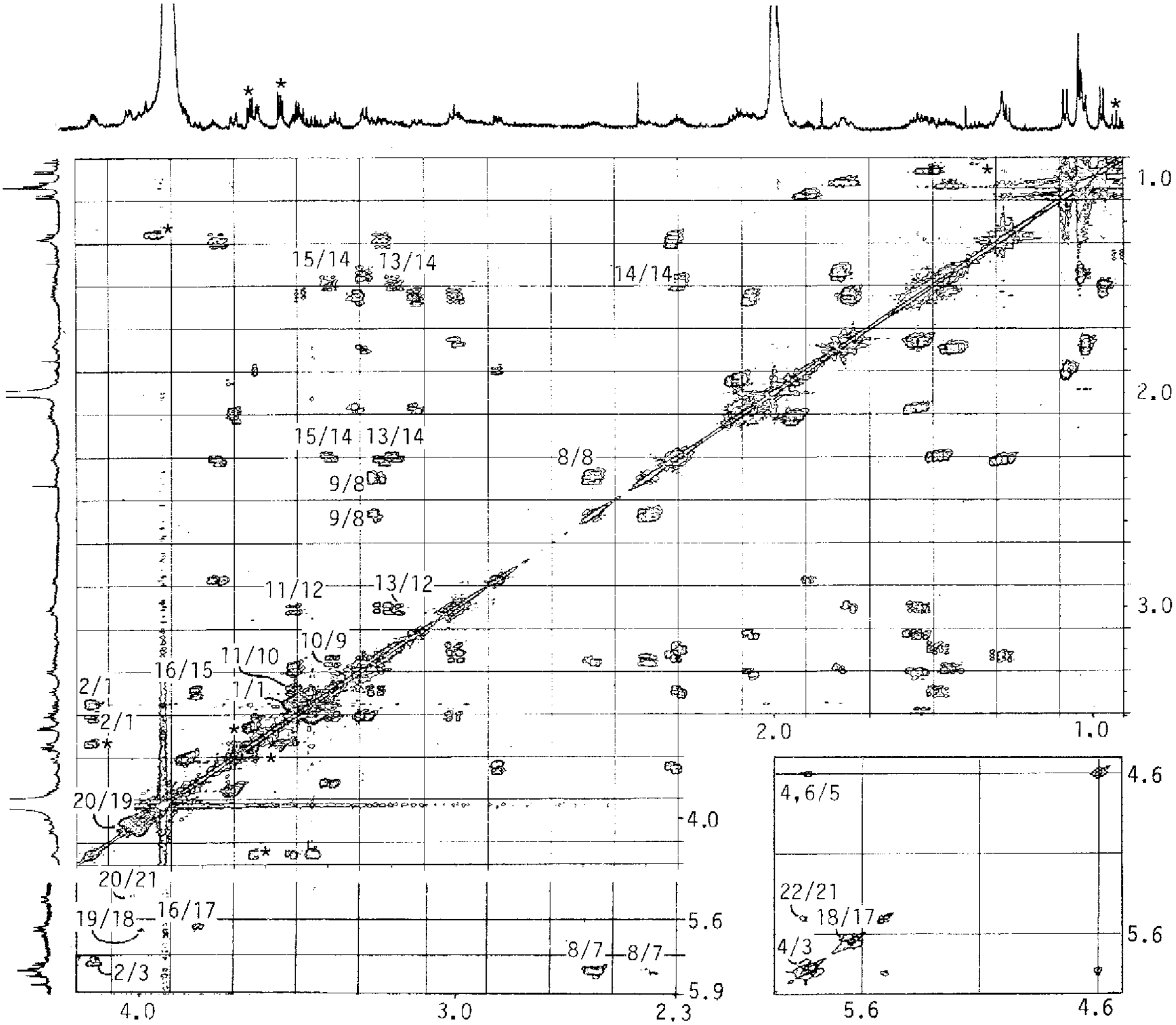

Fig. 2. ^1H-^1H COSY Spectra of Ciguatoxin in CD_3CN-D_2O (2:1). The spectra were meaured on a Bruker AM-600 (600 MHz). Asterisks denote signals or cross peaks due to impurities.

weight. As for the structure after C22, we deduce a similar structure comprising fused ether rings, though their sizes and substituents are not known. The main obstacles to elucidating the whole structure by NMR methods were the interruptions of proton spin systems by quaternary carbons and disappearance or broadening of some signals due to conformational changes of probably eight or nine membered rings, as observed with brevetoxin-A. These problems were compounded by the extremely small quantity of the sample.

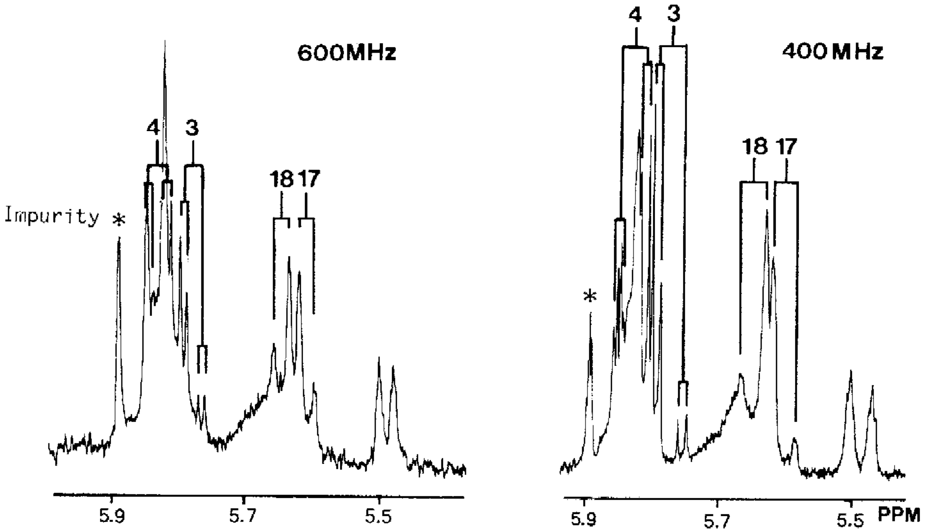

Fig. 3. Partial ^{1}H NMR Spectra of Ciguatoxin Measured at 600 MHz and at 400 MHz in CD_3CN-D_2O (2:1).

References and Notes

1) K. Tachibana "Structural Studies on Marine Toxins", Ph.D. Thesis, University of Hawaii, 1980.

2) M. Nukina, L.M. Koyanagi and P.J. Scheuer, Toxicon, 22, 169 (1984).

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5) In ^{1}H-^{1}H COSY measured in dimethylsulfoxide-d_6, five hydroxyl signals at δ 5.02, 4.73, 4.50, 4.83 and 4.75 gave cross peaks against 11-H (δ 3.30), 2-H (3.95), 1-H_2 (3.27) and two methines (4.89 and 4.22) in the unidentified part of the molecule, respectively.

6) Phase-sensitive NOESY was measured in dimethylsulfoxide-d_6 on a JEOL GSX-400 spectrometer (400 MHz) with a mixing time of 0.3 sec at 21°C. Negative NOE's were detected as clear cross peaks due to H-5/H-10, H-12/H-16 and H-15/H-20 on the spectrum.

7) Glucose, H-3/H-4 10 Hz; H-4/H-5 8.8 Hz; H-5/H-6 8.9 Hz [A. DeBruyn, M. Anteunis. Bull. Soc. Chim. Belg., **84**, 1201 (1975)].

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