Pergamon

Tetrahedron Letters 39 (1998) 1197–1198

TETRAHEDRON LETTERS

Isolation and Structures of New Ciguatoxin Analogs, 2,3-DihydroxyCTX3C and 51-HydroxyCTX3C, Accumulated in Tropical Reef Fish

Masayuki Satake,[a] Masakazu Fukui,[a] Anne-Marie Legrand,[b] Philippe Cruchet,[b] Takeshi Yasumoto[a]*

[a]Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiya, Aoba-ku, Sendai 981, Japan.

[b]Institut Territorial de Recherches Medicales Louis Malarde, BP 30, Papeete, Tahiti, French Polynesia.

Received 18 November 1997; revised 1 December 1997; accepted 6 December 1997

Abstract: Two new ciguatoxin analogs, 2,3-dihydroxyCTX3C and 51-hydroxyCTX3C, were isolated from the moray eel *Gymnothorax javanicus*. Their structures including relative configurations were elucidated on the basis of ^{1}H-NMR data.

Ciguatera is the name of seafood poisoning prevalent in tropical areas. Previously, we determined the structure of four causative toxins, ciguatoxin (CTX),[1] CTX3C,[2] CTX4B[1] and CTX4A[3] isolated from either toxic fish or the epiphytic dinoflagellate *Gambierdiscus toxicus*. In this study we report the isolation and structural determination of two new analogs of CTX3C, 2,3-dihydroxyCTX3C (**1**) and 51-hydroxyCTX3C (**2**).

HPLC purification of methanolic extracts of the viscera of the moray eel *Gymnothorax javanicus* was carried out by slightly modifying the conditions applied to other analogs.[1, 2] The retention times of **1** (9.5-11.7 min) and **2** (15.0-18.2 min) on an Asahipak ODP-50 column (4.6 x 150 mm) with MeCN/H_2O (6:4) indicated that the two analogs were more polar than CTX3C (44.8-48.1 min). Separation of toxins was monitored with a UV monitor at 210 nm and by mouse bioassays.

The yield of **1** was 0.19 mg: HR-FABMS, $(M+Na)^+$ 1079.5510 (calcd. for $C_{57}H_{84}O_{18}Na$ is 1079.5560), mouse lethality: ca. 1.8 μg/kg. The molecular formula deduced from the HR-FABMS data indicated that **1** was larger than CTX3C ($C_{57}H_{82}O_{16}$) by two hydroxyl groups. ^{1}H-NMR signals of **1** closely resembled those of CTX3C in C_5D_5N, but two olefinic proton signals due to H-2 and H-3 in CTX3C were replaced by new oximethylene and oximethine signals at around 4.3 ppm in **1**. These signals were assigned to H_2-1, H-2 and H-3. Connectivities from H_2-1 to H-29, H-31 to H-48, and H_2-50 to H $_2$-52 in **1** were easily assigned by ^{1}H-^{1}H COSY and TOCSY measured at 20 °C and -10 °C. Cross peaks from H-2 to C2-O*H* (6.82 ppm) and from H-3 to C3-O*H* (6.49 ppm) were also observed. Chemical shifts, coupling constants and NOE correlations of protons from H-6 to H-29, H-31 to H-48 and H_2-50 to H_2-52 and chemical shifts of five methyls were virtually unchanged between **1** and CTX3C. These results indicated that the partial structure from ring B to ring M of **1** was identical with that of CTX3C including the relative stereostructure. NOE correlations from H-2 to both

0040-4039/98/$19.00
PII: S0040-4039(97)10808-5

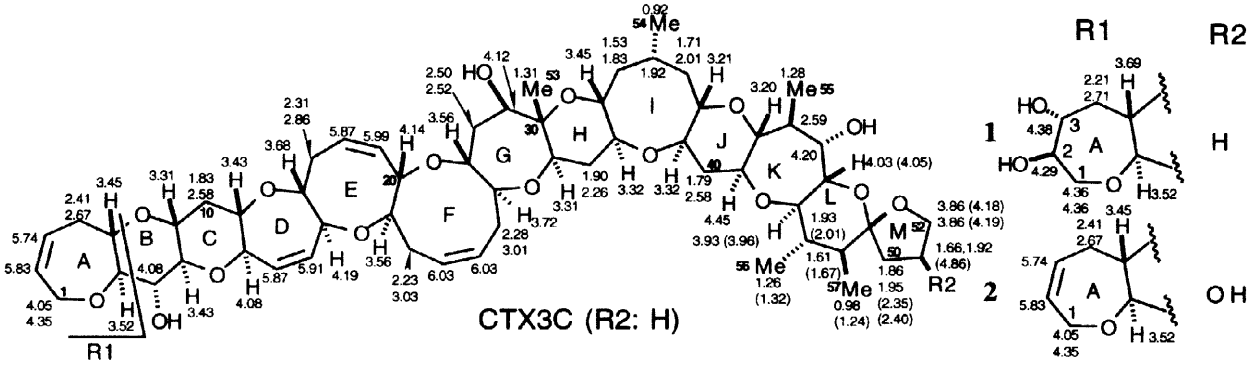

Fig. Structures and ^1H chemical shifts (δ) of CTX3C, 2,3-dihydroxyCTX3C (**1**) and 51-hydroxyCTX3C (**2**). NMR spectra were measured in C_5D_5N at 20 °C and -10 °C. Numbers in parenthesis on rings L and M indicate ^1H chemical shifts of **2**.

H-4α and H-6, and from H-3 to H-5 inferred that the configurations of C2-OH and C3-OH were β and α, respectively. Thus, **1** was concluded to be 2, 3-dihydroxyCTX3C.

The analog **2** (0.08 mg) was isolated as a colorless amorphous solid; HR-FABMS, $(M+Na)^+$ 1061.5460 (calcd. for $C_{57}H_{82}O_{17}Na$ is 1061.5450), mouse lethality: 0.27 μg/kg. Obviously, **2** was larger than CTX3C by one oxygen atom. The structure of **2** was determined by comparing the ^1H-NMR data with those of CTX and CTX3C. Proton connectivities of **2** agreed well with those of CTX3C except for that of a new signal assignable to an oximethine proton at H-51 (4.86 ppm). The chemical shifts and the coupling patterns of protons on rings L and M were identical with those of CTX, implying that the stereostructure of rings L and M in **2** was identical with that of CTX. Therefore, the analog **2** was deduced to be 51-hydroxyCTX3C.

This study is the first to report the analogs of CTX3C. Their occurrence in fish but not in *G. toxicus* implies that CTX3C produced by the dinoflagellate was oxidized in fish to **1** and **2**, and thus supports our previous theory for oxidative modification of ciguatera toxins during the food chain transmission. Knowledge of the structural diversity of ciguatera toxins[4] is important not only in developing an immunoassay method but also in elucidating the action mechanism of these intriguing molecules on the target protein.

ACKNOWLEDGEMENT. This work was supported by grants from the Ministry of Education, Science, Sports and Culture, Japan (No. 07102002) and the Ministry of Health and Research of French Polynesia.

REFERENCES AND NOTES

1) M. Murata, A.-M. Legrand, Y. Ishibashi, M. Fukui, and T. Yasumoto, *J. Am. Chem. Soc.*, **1990**, *116*, 4380-4386.

2) M. Satake, M. Murata, and T. Yasumoto, *Tetrahedron Lett.*, **1993**, *34*, 1975-1978.

3) M. Satake, Y. Ishibashi, A.-M. Legrand, T. Yasumoto, *Biosci. Biochem. Biotech.*, **1997**, *60*, 2103-2105.

4) A.-M. Legrand, M. Fukui, P. Cruchet, and T. Yasumoto, *Bull. Soc. Path. Ex.*, **1992**, *85*, 467-469.