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THE STRUCTURE OF CHIRIQUITOXIN FROM THE COSTA RICAN FROG ATELOPUS CHIRIQUIENSIS

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Summary: The structure of chiriquitoxin, a tetrodotoxin analog isolated from the Costa Rican frog Atelopus chiriquiensis, was elucidated on the basis of NMR data. In the structure 11-CH_2OH of tetrodotoxin was replaced by a $CH(OH)CH(NH_2)COOH$ group.

Chiriquitoxin (CHTX, **1**) was first isolated in 1975 from the Costa Rican frog Atelopus chiriquiensis.[1] On the basis of ^1H NMR[2] and mass spectra[3], its structure was postulated to differ from that of tetrodotoxin (TTX, **2**) only with respect to the substituent at C-6. **2** is a potent neurotoxin of puffers[4] and newts,[5] and is an important neurobiological tool.[6] Among derivatives and natural analogs of **2**[7], **1** is unique in being as potent as **2** in lethality to mice[1] and in blocking the voltage-gated sodium channel,[8] whereas all others have markedly reduced biological activities.[9] Earlier work with **1** was hampered by a scarcity of material and by difficulties in separating it from co-existing **2**. In late June 1988, renewed collection of the frogs was successful. Using the paradigm which led to structural determination of natural analogs of **2** in newts[7a] and in puffers, [7b,c,d] we report here the structure of **1**.

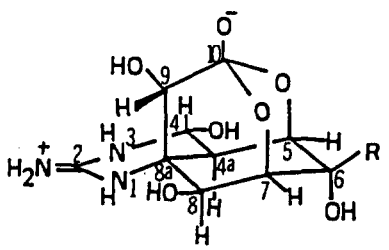
The skin (100 g) of A. chiriquiensis collected in Costa Rica was extracted with 3% HOAc. The extracts were chromatographed successively on columns of Bio-Gel P-2, Hitachi cation exchange gels 3011C and 3013C with 0.1 % HOAc-pyridine buffer (pH 6.5), and finally on a TSK Gel G1000 PW column with 0.05N HOAc. Separation of **1** from **2** was monitored by a TTX analyzer,[10] by mouse lethality bioassays, and by TLC (silica gel 60 with pyridine-EtOAc-HOAc-H_2O, 15:7:3:6).

1 (6 mg) was eluted before **2** (2 mg)[11] on Hitachi gels 3011C and 3013C, and isolated as a colorless amorphous solid: $[\alpha]_D^{22}$ -17.3, (c 0.075, 0.05N HOAc). HR-FABMS (JEOL JMS DX-303HF) pointed to a probable molecular formula, $C_{13}H_{20}N_4O_{10}$ (MH^+, m/z 393.1258, found 393.1310). A positive ninhydrin reaction suggested the presence of NH_2 in the molecule. The band at 1667 cm^{-1} in FT-IR spectra (Nicolet 7199) indicated the presence of a

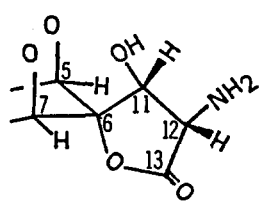
Table 1 NMR spectral data of CHTX and CHTX-13,6-lactone

	CHTX (1)*		CHTX-13,6-lactone (4)**	
	C	H	C	H
2	156.6		156.7	
4	75.2	5.51 (d 9.4)	75.3	5.53 (d 8.5)
4a	40.5	2.31 (d 9.4)	41.7	2.16 (d 8.5)
5	73.5	4.40 (br s)	70.6	4.71 (br s)
6	72.1		84.3	
7	81.1	4.39 (br t)	77.7	4.30 (br t)
8	72.7	4.40 (br s)	72.8	4.19 (d 1.5)
8a	59.3		59.5	
9	70.9	4.00 (s)	70.7	4.03 (s)
10	111.0		111.1	
11	70.3	4.90 (d 1.8)	69.7	5.20 (d 5.5)
12	58.1	4.27 (d 1.8)	-	4.85 (d 5.5)
13	174.1		173.0	

^{13}C NMR *75.5 MHz (GN-300), **100 MHz (JEOL GSX-400)
$^{13}CD_3COOD$ as 22.4 ppm. ^{1}H NMR *300 MHz (GN-300),
**400 MHz (JEOL GSX-400), CHD_2COOD as 2.06ppm.
Solvent: * 4%CD_3COOD/D_2O, ** 1%TFA, 4%CD_3COOD/D_2O (45°C)
- Unassignable carbon due to an exchange of H-12 with D.
(^{13}C NMR spectrum of 4 was measured after keeping 1 in 1%TFA, 4%CD_3COOD/D_2O for one month at 5°C.)



R



4 CHTX-13,6-lactone

	R
	11 12 13
1. CHTX	$CH(OH)CH(NH_2)COOH$
	R *S*
	11
2 TTX	CH_2OH
3 11-norTTX-6,6-diol	OH

guanidinium group, and two C=O bands appeared at 1741 and 1800 cm^{-1} in the spectrum measured with HCl. Comparison of COSY, HETCOR and DEPT spectra (GN-300) of **1** with those of **2** allowed assignment of all ^{1}H and ^{13}C signals of **1** (Table 1). Unlike **2**, which exists in solution as hemilactal and 10,7-lactone tautomers,[4] **1** existed mainly in the hemilactal form. The COSY spectrum of **1** displayed couplings between H-4/H-4a, H-5/H-7(W-type), H-7/H-8, and H-4a/H-9 (W-type). This supports the previous suggestion[1,2] that **1** has the same skeleton as **2**. In the ^{1}H NMR spectrum of **1**, no methylene signal due to CH_2-11 of **2** was present, but two new methine signals coupled to each other (δ4.27, J=1.8Hz, d and δ4.90, J=1.8Hz, d) were present. The signal at 174.1 ppm on ^{13}C NMR spectrum of **1** and the band at 1741cm^{-1} in FT-IR spectrum (+HCl) suggested the presence of COOH. Oxidation (H_5IO_6) of **1** yielded 11-norTTX-6,6-diol (**3**), which was identified by HR-FABMS($C_{10}H_{15}N_3O_8$, MH^+, m/z 306.0937, found 306.0861), as does **2**.[12] Carbon spectra of **1** obtained with long-range selective proton decoupling of the signal δ4.90 (an oxymethine proton) demonstrated that a three-bond coupling with C-7 was present. These data are in accord with the conclusion that **1** is an analog of **2** in which the 11-CH_2OH is replaced by CH(OH)CH(NH_2)COOH. In a D_2O solution containing 1% CF_3COOD and 4% CD_3COOD, the ^{1}H signals assigned to H-12 and H-11 were shifted downfield by 0.11 and 0.07 ppm, respectively, from those in D_2O. The shift-dependencies on the COOH dissociation also supported the α-amino acid structure of **1**. The pKas of COOH and NH_2 were estimated to be 2.0 and 9.3, respectively, based on the signal displacements of H-12 depending on pH.[13]

Keeping **1** in a D_2O solution containing 1% CF_3COOD and 4% CD_3COOD led to the formation of a 13,6-lactone (**4**) as evidenced by FABMS measurement (MH^+, m/z, 375). Assignments of ^{1}H and ^{13}C signals of **4** were achieved by COSY and HETCOR (Table 1). The downfield shifts of C-6 (12.2 ppm), H-11 (0.30 ppm) and H-12 (0.58 ppm) of **4** and identical chemical shift of C-10 in **1** and **4** supported the conclusion that the lactone band at 1800 cm^{-1} in the FT-IR spectrum (+HCl) of **1** was derived from a 13,6-lactone form and not from a 10,7-lactone form. The relative configuration of **4** at C-6, C-11 and C-12 was suggested by NOE measurements and by difference spectra to be (S), (R), and (S), respectively. The absence of NOEs between H-11/H-4a or H-11/H-8 suggested the stereochemistry of C-6 to be (S), analogous with that in **2**. Irradiation of H-7 (δ4.30) of **4** enhanced signal intensities of H-11 (5.0 %) and H-12 (7.9 %), while irradiation of H-5 (δ4.71) gave no NOE enhancement. These observed NOE enhancements suggested an erythro configuration at C-11 and C-12, which was also supported by the 5.5 Hz coupling of the signals from H-11 and H-12.[14] If a threo configuration was present, a larger coupling constant would be expected.[14] All these data support the structural assignment of **1**.

Biosynthesis of **1** may involve **2** or its analog oxidized at C-11[7d] and glycine. The high potency of **1** suggests that the sodium channel protein has specific binding sites for the C-12 NH_2 and/or the C-13 COOH. In addition, functionalities within the channel which

interact with the guanidinium and the C-9 and C-10 hydroxyls of **2** may be operative.[15] ^{1}H and ^{13}C NMR data indicate that, under acidic conditions, H-12 of **1** and **4** slowly exchanges within one month with deuterium of solvents. However, the configuration at C-12 was not changed. This observation may lead to developing a specifically ^{3}H-labeled **1** for biochemical studies of the sodium channel.

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