Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb20

Isolation and Structural Assignment of 11-Nortetrodotoxin-6(*S*)-ol from the Puffer *Arothron nigropunctatus*

Mari Yotsu, Yoshiko Hayashi, Samanta S. Khora, Shin'ichi Sato & Takeshi Yasumoto

To cite this article: Mari Yotsu, Yoshiko Hayashi, Samanta S. Khora, Shin'ichi Sato & Takeshi Yasumoto (1992) Isolation and Structural Assignment of 11-Nortetrodotoxin-6(*S*)-ol from the Puffer *Arothron nigropunctatus*, Bioscience, Biotechnology, and Biochemistry, 56:2, 370-371, DOI: 10.1271/bbb.56.370

To link to this article: https://doi.org/10.1271/bbb.56.370

Biosci. Biotech. Biochem., **56** (2), 370—371, 1992

Short Communication

Isolation and Structural Assignment of 11-Nortetrodotoxin-6(*S*)-ol from the Puffer *Arothron nigropunctatus*

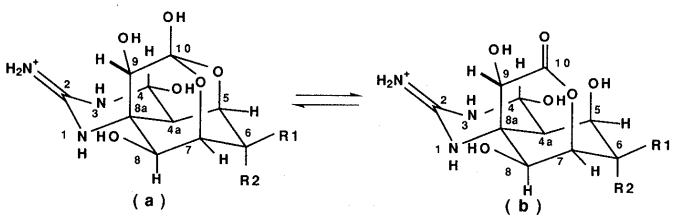
Mari YOTSU, Yoshiko HAYASHI, Samanta S. KHORA, Shin'ichi SATO, and Takeshi YASUMOTO*

Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiya, Aoba-ku, Sendai 981, Japan

Received September 25, 1991

Naturally occurring analogs of tetrodotoxin (TTX, **1**), a well known neurotoxin, provide valuable information on its biosynthetic and metabolic pathways.[1,2] Previously, we have isolated 6-*epi* TTX (**2**) and 11-deoxyTTX (**3**) from newts[1] and puffers,[2] 11-norTTX-6(*R*)-ol (**4**)[2] and 11-oxoTTX (**5**)[3] from puffers, and chiriquitoxin from frogs.[4] These analogs modified at C-6 or C-11 still retained significant potency to block sodium channels, and thus were useful to probe the sodium channel molecule.[5-7] We now report the isolation and structural assignment of 11-norTTX-6(*S*)-ol (**6**) from the southern puffer, and the preparation of **6** from **1**.

The puffer specimen, *Arothron nigropunctatus* (Yogorefugu in Japanese, 2.8 kg, whole body), collected in Okinawa was extracted with 0.1% HOAc. An analysis of analogs in the extract was conducted by fluorometric HPLC analysis[8,9] under the following condition: column, Develosil ODS-5 (4.6 × 250 mm); mobile phase, 0.05 M HOAc/NH_4OH buffer containing 3% MeCN and 0.06 M heptafluorobutyric acid (pH 5.0); flow rate, 0.5 ml/min. The presence of **6**, in addition to **1** and **5**, was revealed. The extract was successively treated on charcoal, Bio-Gel P-2, Bio-Rex 70 and Hitachi Gel 3011C, as previously described for **1** and other analogs.[1] Elution of **6** was monitored by fluorometric HPLC analysis. The final purification of **6** was accomplished under the same chromatographic conditions as those for analysis, and the eluates were desalted in a Hitachi Gel 3011C column.



(a) (b)

		R1	R2
1	TTX	CH_2OH	OH
2	6-*epi*TTX	OH	CH_2OH
3	11-deoxyTTX	CH_3	OH
4	11-norTTX-6(*R*)-ol	OH	H
5	11-oxoTTX	CHO	OH
6	11-norTTX-6(*S*)-ol	H	OH
7	11-norTTX-6,6-diol	OH	OH

The Carbon in the **R1** or **R2** Unit is Numbered to 11.

The yield of **6** was 0.1 mg. **6** was found to have the same molecular weight as that of **4** by FABMS [*m/z* 290 $(M+H)^+$], but was distinguishable from **4** by TLC (R_f 0.73 for **6** and 0.57 for **4**; silica gel 60, pyridine/EtOAc/HOAc/H_2O = 15 : 7 : 3 : 6) and by HPLC analysis (t_R 12′7″ for **6** and 14′2″ for **4**). Analogous with **4**,[2] **6** showed tautomerism between the hemilactal and lactone forms (ratio 3 : 2), as indicated by two sets of signals in the ^{1}H-NMR spectra. Each set had seven oxymethine signals and lacked signals due to 11-CH_2OH of **1**, analogous with **4**. Coupled signals corresponding to the characteristic signals of 1,2-diaxial H-4a and H-4 of the tetrodotoxin skeleton were observed at δ 5.50 (d 9.4 Hz) and δ 2.18 (d 9.4 Hz). These signals, the tautomerism and FABMS data strongly suggested that **6** was an isomer of **4**. Of the two sets of proton signals, the one with low intensity was assigned to the lactone form by analogy with **4**. The same spin systems, H-4/H-4a, H-4a/H-5, H-5/H-6, H-6/H-7, and H-7/H-8, were shown by the ^{1}H–^{1}H COSY spectrum of the lactone form (**6b**). Downfield shifts of H-5 (0.13 ppm) and H-7 (0.09 ppm), and upfield shifts of H-4a (0.11 ppm) and H-8 (0.12 ppm) compared with those of **1b**, being analogous with those of **4b**, are explainable by the loss of the CH_2OH unit from **1b**. Assignment of the proton signals corresponding to H-5, H-6, H-7, and H-8 of the hemilactal form (**6a**) was difficult, because no coupling between H-4a and H-5 was observed. However, the W-type coupling between H-9 and H-4a observed in the ^{1}H–^{1}H COSY spectrum of **6** indicated the stereochemistry at C-9 to be the same as that of **4**. The stereochemistry at C-6 and C-8 of **6b** was determined by measuring NOE difference spectra (4% CD_3COOD/D_2O, 400 MHz, 23°C).[1,2] Irradiation of a signal at δ 2.24 (H-4a) enhanced the signal intensity of H-8 (15.7%), but not that of H-6, whereas irradiation of the signal at δ 2.00 (H-4a) of **4b** enhanced the signal intensities of H-6 (14.0%), H-8 (16.7%), and H-5 (12.6%). These data suggest that **6b** was an epimer of **4b** at C-6. The equatorial configuration of H-6 in **6b** was further supported by its downfield shift (0.33 ppm) compared with the corresponding signal in **4b**. The anisotropic effect of the axial OH at C-6 in **6b** also explains the downfield shifts of H-4a (0.24 ppm) and H-8 (0.17 ppm). All these data unanimously support structure **6** for the new analog. The proton signals of **6b** were assigned as shown in Table I.

To confirm structure **6** and assign the proton signals of **6a**, **6** was derived from **1**. **1** (3.21 mg, 1.0 μmol) was oxidized with 1.1 μmol of H_5IO_6 in 4% CD_3COOD/D_2O,[10-12] the reaction being run in an NMR tube and monitored by ^{1}H-NMR. Assignment of the proton signals of the resulting 11-norTTX-6,6-diol (**7**) by ^{1}H–^{1}H COSY are shown in Table I (**7a** : **7b** = 1 : 1). Reduction of **7** with 5 μmol of $NaBH_3CN$[12] in 0.2 N HOAc at 37°C for 5 hr yielded two products in the ratio of 1 : 6. Each product was isolated by the same method as that described for the final purification

* To whom correspondence should be addressed.

Table I. ^{1}H-NMR Spectral Data for **6**, **1**, **4**, and **7**

	6	**1**[1]	**4**[2]	**7**
Hemilactal (**a**)				
4	5.50 (d 9.4)	5.50 (d 9.4)	5.53 (d 9.1)	5.50 (d 9.6)
4a	2.18 (d 9.4)	2.35 (d 9.4)	1.84 (br. d 9.2)	2.23 (d 9.6)
5	4.36 (br. s)	4.25 (br. s)	4.42 (ddd, 1.0, 1.5, 1.8)	4.17 (t 1.2)
6	4.37 (br. s)	—	3.73 (t 1.7)	—
7	4.20 (br. s)	4.08 (t 1.8)	4.24 (dt 1.2, 1.8)	4.00 (t 2.0)
8	4.17 (br. s)	4.30 (d 1.5)	4.05 (d 1.2)	4.21 (d 2.0)
9	3.98 (s)	3.96 (s)	3.98 (s)	3.97 (s)
11	—	4.02 (d 12.6) 4.04 (d 12.6)	—	—
Lactone (**b**)				
4	5.52 (d 9.4)	5.50 (d 9.4)	5.55 (d 9.3)	5.52 (d 9.6)
4a	2.24 (dd 2.2, 9.4)	2.35 (d 9.5)	2.00 (d 9.8)	2.25 (dd 9.6, 3.0)
5	4.16 (br. s)	4.03	4.32 (br. s)	3.98 (t 3.0)
6	4.27 (t 3.2)	—	3.94 (br. s)	—
7	4.64 (br. t)	4.55 (br. s)	4.72 (br. s)	4.43 (t 2.0)
8	4.32 (br. d 2.0)	4.44 (br. s)	4.15 (d 1.4)	4.38 (d 2.0)
9	4.58 (s)	4.57 (s)	4.56 (s)	4.57 (s)
11	—	3.77 (d 12.6)	—	—
11	—	4.04 (d 12.6)	—	—

6 and **7**, 400 MHz (GSX-400); **1** and **4**, 360 MHz (NT360). Solvent: **6**, **4**, and **7**, 4% CD_3COOD/D_2O; **1**, 1% CF_3COOD, 4% CD_3COOD/D_2O. CHD_2COOD as 2.06 ppm.

of **6** from puffers. The minor product (60 μg) was assigned as **4** on the basis of ^{1}H-NMR and HPLC data.[2] The major product [420 μg; FABMS m/z 290 $(M+H)^+$] was thus concluded to be its epimer at C-6. The synthetic product was indistinguishable from the analog isolated from the puffers by ^{1}H-NMR, HPLC and TLC data. The new analog was thus confirmed to have structure **6**. By analogy with **1**,[1] the NOESY spectrum of **6** thus prepared allowed us to assign the proton signals of **6a** by the crosspeaks due to saturation transfer between corresponding protons of the tautomers (**6a** and **6b**; Table I). LD_{50} of **6** to mice was 54 μg/kg (i.p., acetate form).

The occurrence of **4** in *Fugu niphobles*[2] and **6** in *A. nigropunctatus* suggests that the metabolic pathway for **1** is species specific. The predominance of **6** over **4** in the reduction products of **7** is contradictory to the result in the literature.[12] However, the previous assignment of the reaction product as **4**, made on the basis of unobservable coupling between H-5/H-6/H-7, is questionable because of the difficulty in distinguishing between $J_{a,a}$ and $J_{a,e}$ in a substituted cyclohexane. The prevailing equatorial attack of the reductant on the carbonyl group may be explained by the affinity of boron to the two ether oxygens of **7**. The high yield (55%) of **4** and **6** from **1**, their significant potency, and the ease of radiolabeling with NaB^3HCN suggest the usefulness of **4** and **6** for sodium channel studies.

Acknowledgments. We are grateful to Mr. J. Isa (Okinawa Prefectural Institute of Fisheries) for collecting the samples. The present work was supported by the Ministry of Agriculture, Forestry, and Fishery, and by the Inamori Foundation.

References

1) T. Yasumoto, M. Yotsu, M. Murata, and H. Naoki, *J. Am. Chem. Soc.*, **110**, 2344—2345 (1988).
2) A. Endo, S. S. Khora, M. Murata, H. Naoki, and T. Yasumoto, *Tetrahedron Lett.*, **29**, 4127—2128 (1988).
3) S. S. Khora and T. Yasumoto, *Tetrahedron Lett.*, **30**, 4393—4394 (1988).
4) M. Yotsu, T. Yasumoto, Y. H. Kim, H. Naoki, and C. Y. Kao, *Tetrahedron Lett.*, **31**, 3187—3190 (1990).
5) H. S. Mosher, *Ann. N.Y. Acad. Sci.*, **479**, 32—43 (1986).
6) L. Yang, S. Hu, C. Y. Kao, and T. Yasumoto, *J. Gen. Physiol.*, **49**, 38a (1989).
7) E. Yashida, H. Nakayama, Y. Hatanaka, and Y. Kanaoka, *Chem. Pharm. Bull.*, **38**, 982—987 (1990).
8) T. Yasumoto and T. Michishita, *Agric. Biol. Chem.*, **49**, 3077—3080 (1985).
9) M. Yotsu, A. Endo, and T. Yasumoto, *Agric. Biol. Chem*, **53**, 893—895 (1989).
10) K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, K. Sakai, C. Tamura, and O. Amakatsu, *Chem. Pharm. Bull. Jpn.*, **12**, 1357—1374 (1964).
11) T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron*, **21**, 2059—2088 (1965).
12) L. A. Pavelka, F. A. Fuhrman, and H. S. Mosher, *Heterocycles*, **17**, 225—230 (1982).