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# THE STRUCTURE AND STEREOCHEMISTRY OF TETRODOTOXIN

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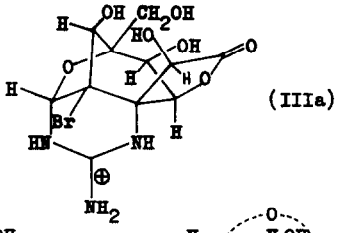
The complete structure and stereochemistry of tetrodotoxin, $C_{11}H_{17}O_8N_3$, which was obtained from ovaries of swellfish (Spheroides rubripes), was elucidated with the assistance of the X-ray crystalography (1) of a derivative, bromoanhydrotetrodoic lactone hydrobromide (III).

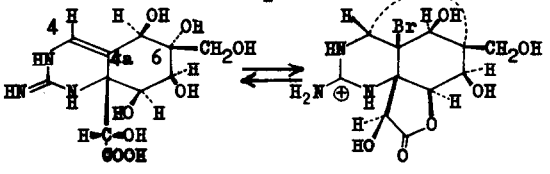
Treatment of tetrodotoxin (I) with 5% barium hydroxide solution at room temperature under nitrogen atmosphere, followed by neutralizarion with carbon dioxide, afforded anhydrotetrodoic acid (II), $C_{11}H_{17}O_8N_3 \cdot 2.5H_2O$ (Found: C, 36.02, 36.63, 36.48; H, 6.12, 6.40, 6.24; N, 11.65, 11.59, 11.24, 11.31. Calcd.: C, 36.26; H, 6.09; N, 11.54%), which was obtained as plates in 95% yield. The acid (II) darkened above 240° without melt and exhibited $\lambda_{max}^{H_2O}$ 261 mμ (ε 5930), $\lambda_{max}^{0.1nNaOH}$ 290 (6420), $\lambda_{max}^{0.1nHCl}$ 257 (6050); $\nu_{max}^{KBr}$ 1710 (guanidine), 1580 $cm^{-1}$ ($COO^-$); pKa's 2.5 (COOH) and 10.9 (guanidine) as a zwitterion.

Anhydrotetrodoic acid (II) consumed 1 mole of bromine in water and afforded, in almost quantitative yield, bromoanhydrotetrodoic lactone hydrobromide (III), elementary analysis of which indicated the empirical formula $C_{11}H_{15}O_7N_3Br_2$ (Found: C, 28.69, 28.74; H, 3.17, 3.33; N, 8.93, 8.85; O, 25.05; Br, 33.62, 33.66. Calcd.: C, 28.65; H, 3.28; N, 9.11; O, 24.29; Br, 34.66). It decomposes at ca. 190°, shows no

UV absorption maximum ($\varepsilon^{H_2O}_{215\ m\mu}$ 2250); $\nu^{KBr}_{max}$ 1800 (γ-lactone), 1665 and 1608 $cm^{-1}$ (guanidine); pKa's 6.7 and 8.5?; and is stable to acids but extremely unstable to bases. It has a γ-lactone ring (IR 1800 $cm^{-1}$), which can be opened by neutralization to pH 7.5 (the band at 1800 is displaced to 1605 $cm^{-1}$) and reclosed by acidification. The pKa (6.7) corresponds to this lactone. The structure of the lactone (III) was established as (IIIa) by the X-ray analysis (1) and, hence, the structure (IIa) can be assigned for anhydrotetrodoic acid (II) without ambiguity. Thus, electrophilic attack of bromonium ion at the $C_{4a}$ was accompanied by addition of the $C_6$-hydroxyl group at the other end of the double bond, and simultaneously the formation of the lactone ring occured by the assistance of the acidity of hydrogen bromide formed. That the free hydroxyl group is present at the $C_6$-position in (II) is evident from the facts that the toxin (I) affords formaldehyde by treatment with 1 mole of periodic acid and that no change of the group can be expected during the formation of (II). Incidentally, the lactone (III) (as the nitrate) does not consume periodic acid and (as the bromide) can be converted to (II) by hydrogenation with Pd-C in the presence of calcium carbonate.



(IIIa)



(IIa) (IIIa)

The NMR spectrum of (II) (Fig. 1) is also consistent with the formula (IIa). A singlet at 0 ppm (form external benzene) corresponds to $H_4$ and no signal is present above 2.6 ppm.

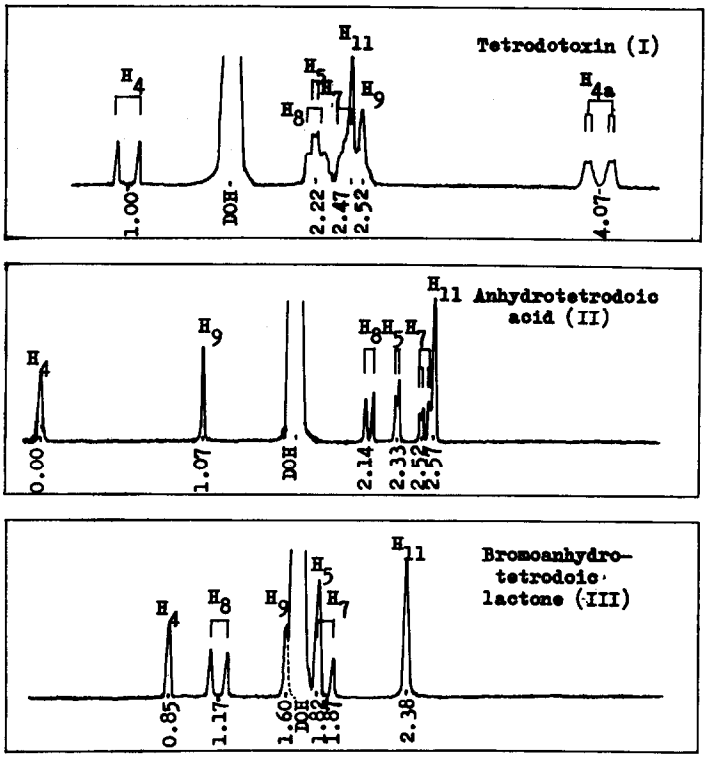
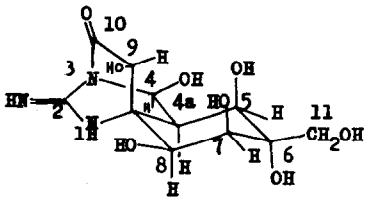


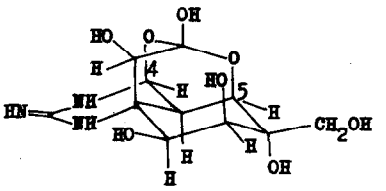

**FIG. 1. NMR spectra at 60 Mc in $D_2O$ containing HCl (Ppm from external $C_6H_6$)**

Since tetrodotoxin (I)* has no acidic function and shows no UV absorption, the free carbonyl group and the double bond, both of which are present in the acid (II), must not have been in the toxin(I). That tetrodotoxin (I) has a proton at $C_{4a}$ is evident from its NMR spectrum. Thus, it shows signals (doublet of doublets) at 4.07 ppm, which is absent in the spectrum of (II). The coupling constants (9 and ca. 2 cps) suggest that the dihedral angle between $C_4$-H and $C_{4a}$-H bonds would be ca. 0° or 180°, and that $H_{4a}$ and $H_5$ are cis to each other. Therefore, only four possible formuals, (Ia)~(Id), can be written for the toxin (I).

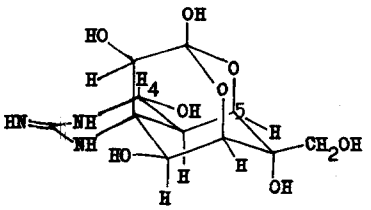
* Tetrodotoxin (I): end absorption $\varepsilon_{220\ m\mu}^{0.1nHCl}$ 75; monoacidic base (pKa' 8.3; $\nu_{max}^{KBr}$ 1670, 1613, 1075 $cm^{-1}$. For more detailed properties, see preceding paper.



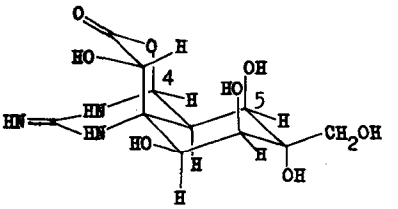
(Ia)



(Ib)



(Ic)



(Id)

Among them, the lactone (Id) is ruled out since the toxin exhibits no IR band between 1700 and 1800 $cm^{-1}$. The ortho-ester type formulas, (Ib) and (Ic), can also be eliminated since they, especially (Ic), can not account for the low pKa value (8.3) and since the conformations of $H_4$ and $H_{4a}$ in (Ib) are not consistent with the large coupling constant (9 cps). Thus, tetrodotoxin (I) must have the formula (Ia). Though the IR spectrum of the toxin (I) has no band between 1700 and 1800 $cm^{-1}$, an amorphous sulfate of the toxin (I) exhibits a carbonyl band at 1730 $cm^{-1}$. The positive charge of the guanidinium group displaces the carbonyl band from 1670 (overlapped with a guanidine band) to 1730 $cm^{-1}$.

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