Besonders wichtig ist dabei das Ergebnis, daß die zwei krystallographisch selbständigen Moleküle, die in asymmetrischer Lage existieren, durch röntgenanalytische Untersuchungen eine identische Raumstruktur ergaben.

Wir danken Herrn Dr. Y. Sasada (Institut für Eiweißstoff der Universität Osaka) herzlich für die erfahrene Hilfe und freundliches Interesse.

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On the Structure of Tetrodotoxin*[1]

In a previous communication[1] we described the preparation of a crystalline tetrodaminotoxin*[2] (I) from 6,11-diacetylanhydrotetrodotoxin, anhydrotetrodotoxin and methoxytetrodotoxin on treatment with aqueous ammonia. The tetrodaminotoxin, pKa' 8.8, exhibits infrared absorption bands at 1679, 1623 (guanidine), and 1228 cm^{-1}, and shows no absorption maximum in ultraviolet region.

It could be converted into tetrodotoxin, anhydrotetrodotoxin, its diacetate and tetrodonic acid by the procedures shown in Chart 1.

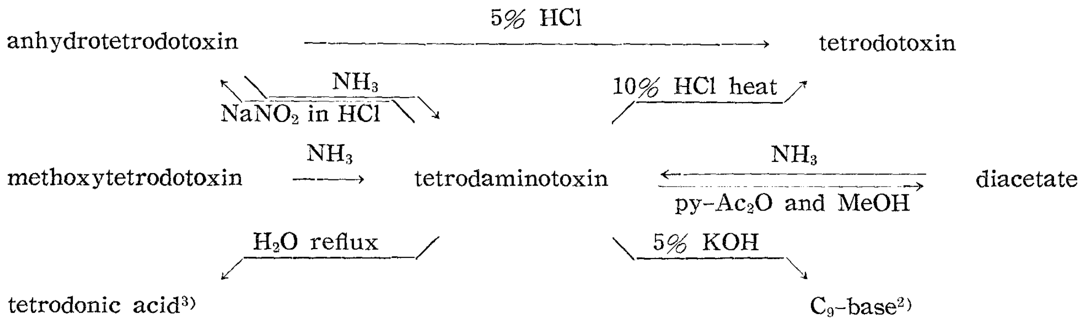

Chart 1.

*1 Presented at the 84th Annual Meeting of the Pharmaceutical Society of Japan, April 7, 1964 (Tokyo University) and at the IUPAC Symposium on the Chemistry of Natural Products, April 13, 1964 (Kyoto).

*2 It was found that the tetrodaminotoxin is dimorph, the second form shows in IR ν_{max}^{Nujol} cm^{-1}: 1667, 1614 (guanidine) 1228 bands.

1) K. Tsuda, *et al.*: This Bulletin, **12**, 634 (1964).
2) K. Tsuda, *et al.*: *Ibid.*, **10**, 245, 856, 865 (1962); T. Goto, *et al.*: Bull. Chem. Soc. Japan, **35**, 1045 (1962).
3) K. Tsuda, *et al.*: This Bulletin, **11**, 1473 (1963); T. Goto, *et al.*: Tetrahedron Letters, No, **30**, 2105 (1963).

The nuclear magnetic resonance spectrum (Table I), the infrared absorption spectrum and the chemical behavior of the tetrodaminotoxin are very similar to those of tetrodotoxin.

Table I. Nuclear Magnetic Resonance Spectra of Tetrodaminotoxin and Tetrodotoxin at 60 Mc. in D_2O containing CD_3COOD

Tetrodaminotoxin		Tetrodotoxin	
p.p.m.[a]	$I_{rel.}$	p.p.m.[a]	$I_{rel.}$
2.53 doublet (J=10 c.p.s.)	1	2.41 doublet (J=10 c.p.s.)	1
4.01		4.01	
4.10		4.07	
4.27	6	4.33	6
4.40		4.42	
5.32 doublet (J=10 c.p.s.)	1	5.54 doublet (J=10 c.p.s.)	1

a) Band position given as downfield displacement in p.p.m. from external Me_4Si.

Table II. Powder X-ray Diffraction Data of Tetrodotoxin and Tetrodaminotoxin

Tetrodotoxin		Tetrodaminotoxin	
d (Å)	I/I_1	d (Å)	I/I_1
7.314	7	7.255	8
6.281	100	6.237	100
5.985	22	5.985	18
5.680	26	5.718	18
5.539	36	5.539	18
5.215	28	5.155	11
4.927	3	4.874	1
4.745	36	4.720	32
4.647	7	4.647	2
4.418	11	4.396	7
3.850	1	3.834	2
3.678	6	3.619	5
3.520	8	3.590	5
3.351	14	3.351	7
3.267	57	3.267	13
3.132	3	3.132	3
3.028	36	3.058	20
2.978	3	2.950	3
2.921	4	2.940	4
2.849	1	2.896	1
2.763	10	2.755	3
2.730	7	2.714	2
2.600	4	2.622	1
2.557	3	2.557	1
2.411	1	2.455	1
2.380	4	2.417	3
2.350	11	2.398	8
2.270	4	2.304	5
2.146	4	2.166	3
2.127	1	2.127	1
2.058	1	2.090	1
2.040	1	2.058	1
1.981	3	1.981	1
1.965	4	1.957	2
1.734	1	1.765	2
1.664	2	1.664	1
1.561	2	1.561	1

Furthermore, the similarity of tetrodaminotoxin and tetrodotoxin were strongly supported by the interplanar spacings calculated from the X-ray diffraction angles; comparison of these data shows quite a good correspondence not only of the *d*-spacings but also of the intensities, indicating that the lattice constants and the atomic co-ordinates are similar (cf. Table II).

Therefore, both toxins should possess the very similar structures. The analytical values of tetrodaminotoxin were in better agreement with the formula $C_{22}H_{33}O_{14}N_7$ (*Anal*. Calcd.: C, 42.65; H, 5.33; N, 15.83; O, 36.19. Found: C, 42.41, 42.00, 41.99; H, 5.82, 5.84, 5.56; N, 16.27, 16.08, 16.20; O, 36.15, 35.75, 36.00) than with $C_{11}H_{18}O_7N_4H_2O$ (*Anal*. Calcd.: C, 39.29; H, 5.95; N, 16.67; O, 38.09), indicating that the compound (I) is formed from 1 mole of ammonia and 2 moles of anhydrotetrodotoxin. This was also supported by the quantitative determination of nitrogen in tetrodotoxin, its derivatives and in tetrodaminotoxin by the Kjeldahl method. In order to distinguish the newly introduced nitrogen from those of the guanidine moiety, 6,11-diacetylanhydrotetrodotoxin*[3] was treated with aqueous ammonia containing 68% excess of N^{15} to yield tetrodaminotoxin containing N^{15} (N^{15}-tetrodaminotoxin), in which the concentrations of N^{15} were measured by mass spectrometry. The results are summarized in Tables III and IV.

TABLE III.

Compound	Weight of compd. (mg.)	Nitrogen by titrat. (mg.)	Nitrogen 1 mole equiv. (mg.)
1) Tetrodonic acid $C_{11}H_{17}O_8N_3 \cdot H_2O$	119.1	5.06	14.33
2) Anhydrotetrodotoxin $C_{11}H_{15}O_7N_3$	105.6	5.33	15.20
3) Tetrodotoxin $(C_{11}H_{17}O_8N_3)_{1\text{ or }2}$	117.1	5.05	13.78 (or 27.56)
4) N^{15}-Tetrodotoxin $(C_{11}H_{17}O_8N_3)_{1\text{ or }2}$	100.4	4.86	15.45 (or 30.90)
5) N^{15}-Tetrodaminotoxin $C_{22}H_{33}O_{14}N_7$	122.7	8.62	43.55
6) N^{15}-Tetrodaminotoxin $C_{22}H_{33}O_{14}N_7$	180.2	12.51	43.05

Table III shows that the observed values for nitrogens of the guanidine moiety in tetrodotoxin and in its derivatives, correspond approximately to one nitrogen atom calculated for C_{11}-formulae or two nitrogens calculated for C_{22}-formulae and about three nitrogen atoms for tetrodaminotoxin. Table IV shows the results of N^{15} measurement

TABLE IV.

Compound	Weight of compd. (mg.)	N^{15}-Atom (%)	Weight of N^{15} (mg.)
4) N^{15}-Tetrodotoxin	100.4	0.47	0.024
5) N^{15}-Tetrodaminotoxin	122.7	25.1	2.277
(N^{15} Value calcd. from $C_{22}H_{33}O_{14}N_7$)			(2.019)
(N^{15} Value calcd. from $C_{11}H_{18}O_7N_4 \cdot H_2O$)			(3.715)
6) N^{15}-Tetrodaminotoxin	180.2	24.8	3.266
(N^{15} Value calcd. from $C_{22}H_{33}O_{14}N_7$)			(2.964)
(N^{15} Value calcd. from $C_{11}H_{18}O_7N_4 \cdot H_2O$)			(5.455)

*[3] Diacetate and other derivatives of tetrodotoxin were described in the previous communication: footnote 1.

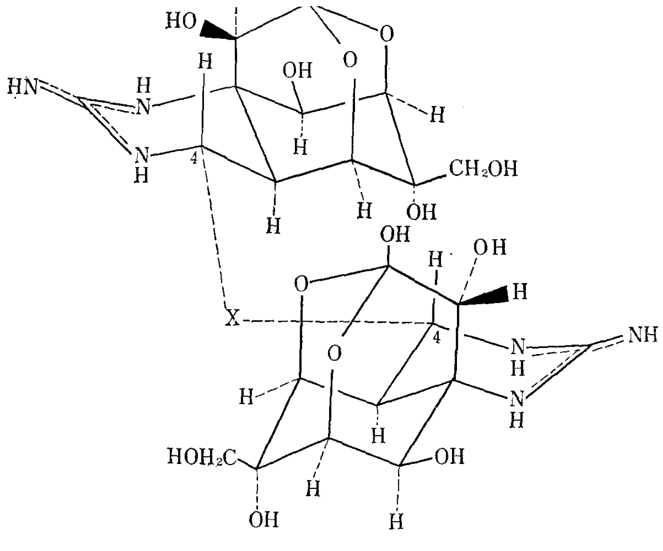

Fig. 1.

Ⅰ : X =-NH- Tetrodaminotoxin
Ⅱ : X =-O- Tetrodotoxin

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*4 The quantitative measurement of nitrogen with CORNWAY-apparatus also gave the similar results.