BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 3332—3341 (1970)

The Crystal and Molecular Structure of Tetrodotoxin Hydrobromide

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(Received February 26, 1970)

The crystal and molecular structure of the hydrobromide of tetrodotoxin itself, $C_{11}H_{17}O_8N_3\cdot HBr$, have been studied by means of three-dimensional X-ray analysis. The crystals are triclinic, with two formula units in a unit cell of the dimensions; $a=8.79$, $b=9.02$, $c=9.51$ A, $\alpha=106°51'$, $\beta=88°1'$, and $\gamma=96°23'$; the space group is $P1$. The intensities of reflections were recorded on equi-inclination Weissenberg photographs taken with Cu$K\alpha$ radiation around the *b* and *c* axes, and were measured visually with a calibrated intensity scale. The structure was elucidated by means of the Fourier and least-squares methods, and was refined by the block-diagonal-matrix least-squares method, the anisotropic thermal motion being assumed only for bromine atoms. The stereostructures of the two crystallographically-independent molecules of tetrodotoxin thus obtained are almost identical and correspond to that deduced by Tsuda *et al.*, by Hirata *et al.*, and by Woodward *et al.*, all in 1964. It is noteworthy that, in both of the two molecules, the C–O distance for the free hydroxyl group in the ortho-ester part (1.36 and 1.39 Å) is somewhat smaller than the ordinary aliphatic C–O distance, 1.43±0.01 Å, and that it is rather closer to the phenolic C–O distance, 1.36±0.01 Å.

The molecular structure of tetrodotoxin, one of the most poisonous natural substances, had remained unknown for a long time in spite of extensive organic chemical investigations, until it was, in 1964, at last elucidated independently by Tsuda *et al.*, by Hirata *et al.*, and by Woodward *et al.*[1–3] All of these three groups derived the same structure for the poison from those structures determined by X-ray crystal analyses for its different derivatives.[3–6] The X-ray determination of one of the derivatives, bromoanhydrotetrodoic lactone hydrobromide, was carried out in our laboratory in cooperation with Hirata *et al.* of Nagoya University.[6,7] Since Professor Yoshimasa Hirata kindly provided us also with single crystals of the hydrobromide of tetrodotoxin itself, a three-dimensional X-ray crystal analysis was later undertaken in order to confirm directly the molecular structure deduced from those of the derivatives.

Experimental

The crystal data were obtained from oscillation and Weissenberg photographs taken with Cu$K\alpha$ radiation around the three principal axes; the results are summarized in Table 1. The intensities were recorded on equi-inclination Weissenberg photographs taken with Cu$K\alpha$ radiation for 0—8 layers around the *b* axis and for 0—3 layers around the *c* axis, and were measured visually with a calibrated intensity scale. No absorption correction was applied, though the

TABLE 1. CRYSTAL DATA OF TETRODOTOXIN HYDROBROMIDE

Formula	$C_{11}H_{17}O_8N_3\cdot HBr$
Triclinic	
	$a=8.79\pm0.04$ Å
	$b=9.02\pm0.04$
	$c=9.51\pm0.04$
	$\alpha=106°51'\pm15'$
	$\beta=88°1'\pm15'$
	$\gamma=96°23'\pm15'$
Space Group	$P1$
Z	2
D_x	1.86 g/cm³

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[1] K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, K. Sakai, C. Tamura and O. Amakasu, *Chem. Pharm. Bull.*, **12**, 1357 (1964).

[2] T. Goto, Y. Kishi, S. Takahashi and Y. Hirata, *Tetrahedron Lett.*, **1964**, 779.

[3] R. B. Woodward, "Special Lectures presented at the Third International Symposium on the Chemistry of Natural Products," ed. by IUPAC (1964), p. 49.

[4] K. Tsuda, C. Tamura, R. Tachikawa, K. Sakai, O. Amakasu, M. Kawamura and S. Ikuma, *Chem. Pharm. Bull.*, **11**, 1473 (1963); C. Tamura, O. Amakasu, Y. Sasada and K. Tsuda, *Acta Cryst.*, **21**, 219 (1966).

[5] K. Tsuda, C. Tamura, R. Tachikawa, K. Sakai, O. Amakasu, M. Kawamura and S. Ikuma, *Chem. Pharm. Bull.*, **12**, 643 (1964); C. Tamura, O. Amakasu, Y. Sasada and K. Tsuda, *Acta Cryst.*, **21**, 226 (1966).

[6] Y. Tomiie, A. Furusaki, K. Kasami, N. Yasuoka, K. Miyake, M. Haisa and I. Nitta, *Tetrahedron Lett.*, **1963**, 2102.

[7] A. Furusaki, Y. Tomiie and I. Nitta, The preceding paper.

samples used were not sufficiently small (about 0.5 mm in diameter). After the Lorentz and polarization correction, these data were put on an absolute scale by means of Wilson's method. The overall temperature factor obtained at this stage was 2.08 Å^2. Thus, the absolute values of the structure factors of 2839 independent reflections were derived.

All the photographs used for the present study were taken in the Crystallography Laboratory, University of Pittsburg, when one of the authors (Y. T.) was there (1963).

Structure Determination

A Patterson function, $P(u, v, w)$, was calculated at intervals of 1/30 for u, for v and for w. The resulting map showed that if one of two bromine atoms in a unit cell, Br(a), was placed at the point of origin (0, 0, 0), the other Br(b) should be situated close to the (1/2, 1/2, 1/2) position. In order to refine the latter position, the diagonal-matrix least-squares method was applied. After three cycles, the R factor dropped from the initial value of 0.554 to 0.345, and the bromine position became (0.5180, 0.5276, 0.4900). On the other hand, we tried to estimate the positions of light atoms from the Patterson map on the basis of the proposed molecular structure of tetrodotoxin. In this way, out of the positions of forty-four independent light atoms present in an asymmetric unit, twenty were found. Using the two bromine positions and these positions, and assuming all the light atoms to be carbon, the first Fourier synthesis was carried out; the calculated R factor was 0.312. The map thus obtained had many maxima; out of these forty-eight higher maxima were taken as temporary atomic positions and were tested for the divergence of temperature factors.[8] After three cycles of the least-squares method, nine positions were found to be inadequate. Then, using the thirty-nine remaining positions, the second Fourier map was calculated. From the map drawn, forty-seven maxima were chosen again as positions of the light atoms and were tested again by the least-squares method. The results after three cycles are given in Table 2, which shows that, out of the forty-seven positions, the three marked with asterisks may be inadequate. However, from a comparison of these positions with the structure determined on the basis of the chemical evidence, it turned out that not all the forty-four light-atom positions which seemed adequate before, in view of the results of the least-squares test, were actually cor-

Table 2. The result of the second structure test

No.	Atom	x/a	y/b	z/c	B(Å^2)	No.	Atom	x/a	y/b	z/c	B(Å^2)
1	Br(a)	0.000	0.000	0.000	2.19	24	Br(b)	0.518	0.528	0.490	2.32
2	O(A1)	0.260	0.513	1.137	0.07	25	O(B1)	0.773	0.030	0.640	0.66
3	O(A2)	0.520	0.613	1.147	−0.14	26	O(B2)	1.033	0.183	0.653	0.75
4	O(A3)	0.360	0.727	1.327	1.13	27	O(B3)	0.863	0.227	0.820	0.92
5	O(A4)	0.080	0.553	0.917	2.38	28	O(B4)	0.577	0.060	0.413	0.69
6	O(A5)	0.443	0.283	0.803	0.98	29	O(B5)	0.977	−0.167	0.313	0.49
7	O(A6)	0.783	0.693	0.820	0.38	30	O(B7)	0.887	0.440	0.643	0.37
8	O(A7)	0.433	0.897	1.113	0.41	31	O(B8)	1.143	−0.213	0.543	1.63
9	O(A8)	0.377	0.147	1.020	0.73	32	N(B1)	1.040	0.393	0.317	0.67
10	N(A1)	0.583	0.853	0.817	1.53	33	N(B2)	0.787	0.317	0.350	0.36
11	N(A2)	0.333	0.770	0.813	1.38	34	N(B3)	0.830	0.497	0.240	1.51
12	N(A3)	0.430	0.953	0.683	0.80	35	C(B1)	0.873	0.413	0.300	1.14
13	C(A1)	0.450	0.863	0.780	2.95	36	C(B2)	0.847	0.203	0.430	1.58
14	C(A2)	0.350	0.700	0.940	1.59	37	C(B3)	0.727	0.037	0.380	1.88
15	C(A3)	0.233	0.530	0.890	1.17	38	C(B4)	0.787	−0.047	0.483	1.28
16	C(A4)	0.267	0.423	0.983	2.14	39	C(B5)	0.953	−0.097	0.447	2.68
17	C(A5)	0.447	0.380	0.960	1.22	40	C(B6)	1.047	0.080	0.497	1.12
18	C(A6)	0.537	0.550	0.997	3.06	41	C(B7)	1.000	0.187	0.403	1.16
19	C(A7)	0.513	0.640	0.897	1.33	42	C(B8)	1.110	0.323	0.400	1.54
20	C(A8)	0.627	0.773	0.897	2.74	43	C(B9)	0.807	0.293	0.607	1.32
21	C(A9)	0.337	0.763	1.083	0.44	44	C(B10)	0.867	0.167	0.673	1.70
22	C(A10)	0.373	0.657	1.177	1.16	45	**	0.270	0.267	0.160	2.01
23	C(A11)	0.473	0.277	1.043	1.62	46	**	0.420	0.933	0.603	1.24
						47	*	0.060	0.663	0.713	11.30
						48	*	0.907	0.673	0.667	8.22
						49	*	0.173	0.747	0.663	10.30

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[8] M. Nishikawa, K. Kamiya, M. Tomita, Y. Okamoto, T. Kikuchi, K.Osaki, Y. Tomiie, I. Nitta and K. Coto, *J. Chem. Soc., B*, **1968**, 652.

rect for the two of them marked with a double asterisk were inadequate.

The reason why the temperature factors corresponding to the two positions do not diverge remains unknown, but this fact seems to warn against the formal use, without criticism, of the divergence of the temperature factors as a structure test.

The two remaining atomic positions were obtained from the third Fourier map. The coordinates of the forty-six atoms thus obtained were refined by the block-diagonal-matrix least-squares method using isotropic temperature factors for all the atoms. After four cycles, the *R* factor dropped to 0.150. Next, the atomic coordinates were further refined by the above-mentioned method, using anisotropic temperature factors only for the bromine atoms; the final *R* factor was 0.130. The atomic parameters thus obtained and the electron density distribution calculated with these parameters are given in Table 3 and Fig. 1 respectively. Tables of the observed and calculated structure factors are preserved by the Chemical Society of Japan.[*1]

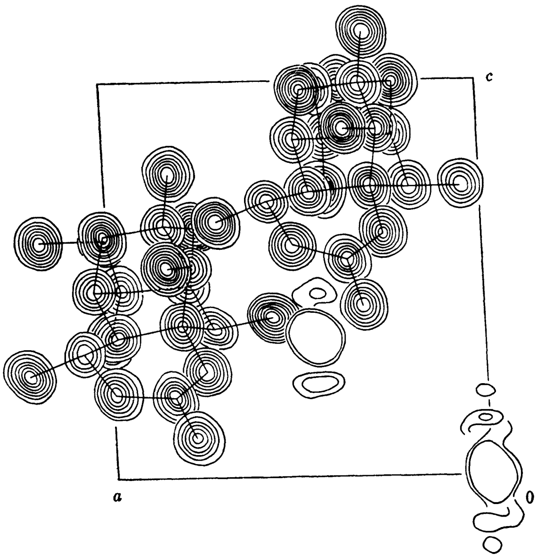

Fig. 1. The final electron density distribution. Contours are at intervals of 2.0 e/Å^3 for the oxygen, nitrogen, and carbon atoms, beginning at the 2.0 e/Å^3 level.

TABLE 3. THE FINAL ATOMIC PARAMETERS

Atom	x/a	y/b	z/c	B(Å^2)	Atom	x/a	y/b	z/c	B(Å^2)
Br(a)	−0.0020	0.0025	0.0000	*	Br(b)	0.5163	0.5271	0.4900	*
O(A1)	0.2708	0.5256	1.1370	1.038	O(B1)	0.7710	0.0284	0.6364	1.316
O(A2)	0.5337	0.6112	1.1424	1.075	O(B2)	1.0232	0.1551	0.6494	1.113
O(A3)	0.3761	0.7299	1.3196	1.501	O(B3)	0.8536	0.2354	0.8321	1.834
O(A4)	0.0913	0.5792	0.9029	1.587	O(B4)	0.5693	0.0677	0.4169	1.269
O(A5)	0.4500	0.2931	0.8076	1.579	O(B5)	0.9775	−0.1688	0.3057	1.546
O(A6)	0.7856	0.6889	0.8314	1.072	O(B6)	1.2454	0.2650	0.3298	1.838
O(A7)	0.4532	0.9060	1.1256	1.206	O(B7)	0.8947	0.4328	0.6432	1.384
O(A8)	0.3712	0.1355	1.0229	1.684	O(B8)	1.1523	−0.2069	0.5400	2.013
N(A1)	0.6033	0.8575	0.8159	0.946	N(B1)	1.0401	0.4066	0.3192	1.406
N(A2)	0.3412	0.7754	0.8219	0.826	N(B2)	0.7852	0.3071	0.3486	0.991
N(A3)	0.4264	0.9448	0.6843	1.308	N(B3)	0.8320	0.5014	0.2345	2.217
C(A1)	0.4561	0.8578	0.7766	0.788	C(B1)	0.8852	0.4018	0.3052	0.833
C(A2)	0.3629	0.6742	0.9162	0.531	C(B2)	0.8297	0.2064	0.4340	0.927
C(A3)	0.2459	0.5316	0.8792	1.025	C(B3)	0.7306	0.0505	0.3881	0.646
C(A4)	0.2734	0.4350	0.9849	1.226	C(B4)	0.7835	−0.0518	0.4787	1.176
C(A5)	0.4385	0.3722	0.9575	1.115	C(B5)	0.9559	−0.0842	0.4558	1.057
C(A6)	0.5481	0.5234	0.9925	1.000	C(B6)	1.0459	0.0839	0.4966	1.121
C(A7)	0.5235	0.6229	0.8862	0.413	C(B7)	0.9979	0.1722	0.3961	0.772
C(A8)	0.6475	0.7524	0.8965	0.819	C(B8)	1.1076	0.3198	0.4028	1.453
C(A9)	0.3504	0.7663	1.0814	0.726	C(B9)	0.8139	0.2836	0.5999	1.046
C(A10)	0.3801	0.6611	1.1720	0.934	C(B10)	0.8626	0.1766	0.6807	1.095
C(A11)	0.4716	0.2771	1.0590	1.315	C(B11)	0.9906	−0.1801	0.5506	1.401

*	B_{11}	B_{22}	B_{33}	B_{12}	B_{23}	B_{31}
Br(a)	0.00532	0.00971	0.00966	−0.00279	0.00690	0.00400
Br(b)	0.00541	0.00744	0.00774	0.00625	0.00618	0.00557

[*1] The complete data of the F_o-F_c table are kept as Document No. 7007 at the office of the bulletin of the Chemical Society of Japan. A copy may be secured by citing the doqument number and by remitting, in advance, ¥1200 for photoprints. Pay by check or money order, payable to: The Chemical Society of Japan.

Results and Discussion

Molecular Structure. The bond lengths and angles of the two crystallographically-independent tetrodotoxin molecules, A and B, are listed in Tables 4 and 5, together with the arithmetical means of the corresponding values of the two. The average standard deviations of the atomic distances are estimated to be 0.033 Å for C–C, 0.031 Å for C–N, and 0.029 Å for C–O. If these deviations are taken into consideration, the dimensions of the two independent molecules are in good agreement with each other. Thus, from the present results, it may be concluded that the stereostructures of the two tetrodotoxin molecules, A and B, are almost identical and that they correspond to that derived from the organic chemical investigations.[1–3)] In Figs. 2 and 3, the framework of one of the two independent molecules and the corresponding structural formula are, respectively, drawn with reference to the absolute configuration based on that of the derivative of the poison, bromoanhydrotetrodoic lactone hydrobromide.[7)]

The molecules are characterized by an ortho-ester system rarely found in natural products. This system makes a part of an adamantane-like cage structure and might have some close connection with the powerful toxicity. It has been reported by Tamura *et al.* that the remarkable system in the molecule is still preserved in the derivative, diacetylanhydrotetrodotoxin hydroiodide.[5)] The geometry of the ortho-ester part found in the present molecule is shown in Fig. 4, as is that in the derivative.*2

Table 4. The bond distances (Å)

Bond	Mol. A	Mol. B	Mean
C (2)-C (3)	1.52	1.52	1.52
C (3)-C (4)	1.55	1.55	1.55
C (4)-C (5)	1.60	1.57	1.59
C (5)-C (6)	1.54	1.58	1.56
C (5)-C (11)	1.52	1.48	1.50
C (6)-C (7)	1.57	1.51	1.54
C (7)-C (8)	1.49	1.54	1.52
C (2)-C (7)	1.53	1.55	1.54
C (2)-C (9)	1.56	1.54	1.55
C (9)-C (10)	1.50	1.50	1.50
C (1)-N (1)	1.36	1.37	1.36
C (1)-N (2)	1.32	1.30	1.31
C (1)-N (3)	1.38	1.39	1.39
C (2)-N (2)	1.48	1.47	1.48
C (8)-N (1)	1.47	1.45	1.46
C (3)-O (4)	1.46	1.45	1.46
C (4)-O (1)	1.44	1.47	1.46
C (5)-O (5)	1.40	1.43	1.41
C (6)-O (2)	1.42	1.43	1.42
C (8)-O (6)	1.44	1.44	1.44
C (9)-O (7)	1.43	1.40	1.42
C (10)-O (1)	1.43	1.44	1.44
C (10)-O (2)	1.46	1.46	1.46
C (10)-O (3)	1.36	1.39	1.37
C (11)-O (8)	1.43	1.46	1.45

Table 5. The bond angles (°)

Angle	Mol. A	Mol. B	Mean
N (1)-C (1)-N (2)	122	124	123
N (1)-C (1)-N (3)	119	117	118
N (2)-C (1)-N (3)	119	118	119
N (2)-C (2)-C (3)	111	109	110
N (2)-C (2)-C (7)	107	109	108
N (2)-C (2)-C (9)	110	111	111
C (3)-C (2)-C (7)	109	107	108
C (3)-C (2)-C (9)	110	110	110
C (7)-C (2)-C (9)	109	110	109
C (2)-C (3)-C (4)	107	107	107
C (2)-C (3)-O (4)	110	112	111
C (4)-C (3)-O (4)	109	109	109
C (3)-C (4)-C (5)	111	115	113
C (3)-C (4)-O (1)	112	110	111
C (5)-C (4)-O (1)	106	104	105
C (4)-C (5)-C (6)	103	104	103
C (4)-C (5)-O (5)	108	108	108
C (4)-C (5)-C (11)	113	109	111
C (6)-C (5)-O (5)	109	112	111
C (6)-C (5)-C (11)	110	114	112
C (11)-C (5)-O (5)	114	109	112
C (5)-C (6)-C (7)	112	110	111
C (5)-C (6)-O (2)	111	108	109
C (7)-C (6)-O (2)	111	115	113
C (6)-C (7)-C (8)	113	113	113
C (2)-C (7)-C (6)	107	108	107
C (2)-C (7)-C (8)	115	113	114
C (7)-C (8)-N (1)	110	108	109
C (7)-C (8)-O (6)	109	106	108
N (1)-C (8)-O (6)	109	109	109
C (2)-C (9)-C (10)	108	109	109
C (2)-C (9)-O (7)	114	112	113
C (10)-C (9)-O (7)	109	113	111
C (9)-C (10)-O (1)	110	110	110
C (9)-C (10)-O (2)	110	109	110
C (9)-C (10)-O (3)	114	114	114
O (1)-C (10)-O (2)	109	110	109
O (1)-C (10)-O (3)	109	108	109
O (2)-C (10)-O (3)	105	105	105
C (5)-C (11)-O (8)	109	111	110
C (1)-N (1)-C (8)	122	122	122
C (1)-N (2)-C (2)	123	122	122
C (4)-O (1)-C (10)	114	113	113
C (6)-O (2)-C (10)	111	111	111

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*2 Since, for both the present substance and the derivative, the crystals contain two crystallographically-independent molecules, the respective mean values are given in Fig. 4.

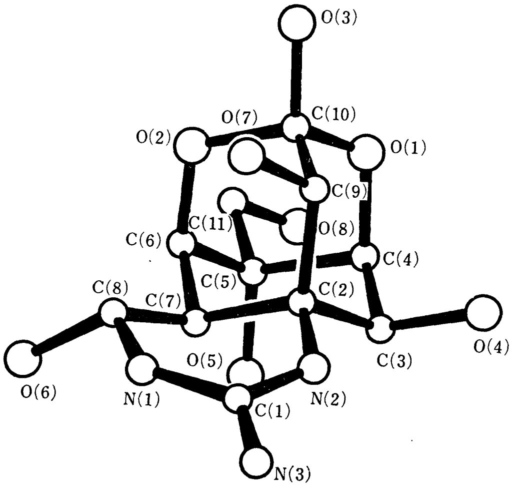

Fig. 2. The framework of the tetrodotoxin ion (A).

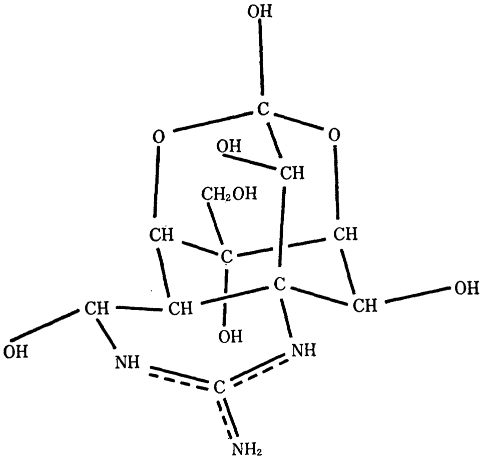

Fig. 3. The structural formula corresponding to Fig. 2.

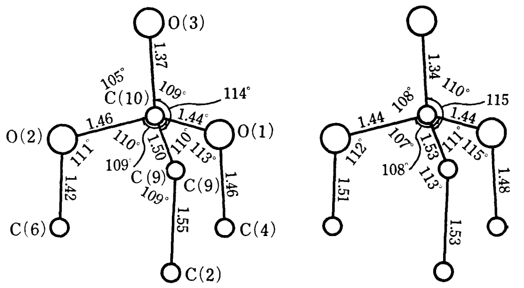

Fig. 4. The geometry of the ortho-ester parts.

II: in tetrodotoxin.

II: in diacetylanhydrotetrodotoxin.

Though neigher of the analyses has a sufficiently high accuracy for a detailed discussion, the two results show some common characteristics. For example, the bond distance between the free hydroxyl oxygen atom and the central carbon atom of the ortho-ester is 1.37 Å in the present molecule and 1.34 Å in the derivative; both of these values are considerably smaller than the ordinary aliphatic C–O distance, 1.43±0.01 Å, and rather closer to the phenolic C–O distance, 1.36±0.01 Å.[9]

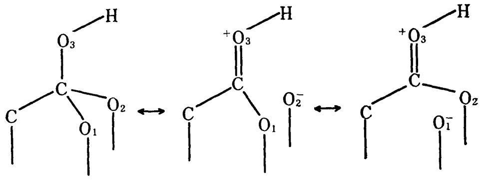

Fig. 5. The resonance of the ortho-ester.

This shortening of the C–O bond may be explained by the resonance, shown in Fig. 5. In this resonance, in addition to the ortho-ester structure, I, two ionic structures of the ordinary ester type, II and III, take part, and both of these contribute to the shortening of the C–O bond under consideration. In an acidic solution where the free hydroxyl oxygen O(3) has a proton, the structures

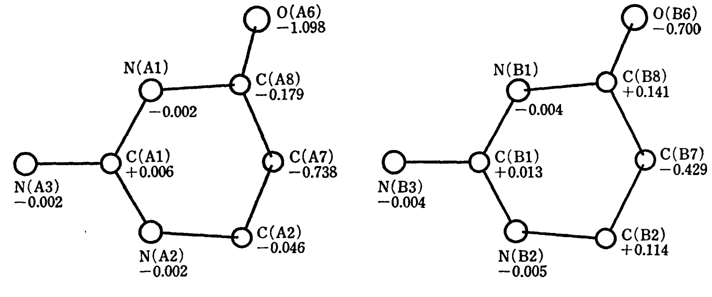

Fig. 6. The deviations from the guanidinium planes.

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[9] "Tables of Interatomic Distances and Configuration in Molecules and Ions," ed. by L. E. Sutton *et al.*, Burlington House, London (1958).

II and III may contribute only a little to the resonance. However, in an alkaline solution where O(3) loses a proton and where it becomes easy to feed electrons to C(10), the contribution of structures corresponding to II and III would increase and, consequently, the molecules would become more unstable with respect to C(10)–O(1) and C(10)–O(2). As another of the common characteristics, it is further found that the bond angle C(9)–C(10)–O(3) and the corresponding angle in the derivative are 114° and 115° respectively, both larger than the tetrahedral angle, 109.5°.

It is of interest to compare the two independent molecules of tetrodotoxin with each other. Though almost identical, strictly speaking, these two may be conformational isomers since they appear, so far as the present results are concerned, to be different only in conformation and different only in part. Thus, appreciable conformational differences are found in two parts of the molecules; one of them is the hydroxymethyl group, and the other is the six-membered ring containing a guanidinium group. The former part is different in respect to the conformation of the hydroxyl oxygen O(8) around C(5)–C(11); in one of the molecules, A, O(8) is *trans* to C(6), while, in the other, B, it is *trans* to C(4). Since the potential energy curve for the internal rotation of O(8) around the C(5)–C(11) bond seems to be approximately symmetrical with respect to the plane through C(5), C(11), and O(5), the two observed conformations which are almost related to each other by the mirror plane may not be appreciably different in energy. Accordingly, which of the two conformations the molecule takes seems to

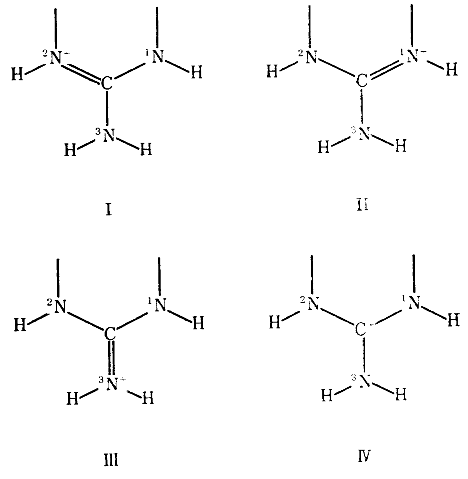

Fig. 7. The four predominant formulae for the resonance of the guanidinium group.

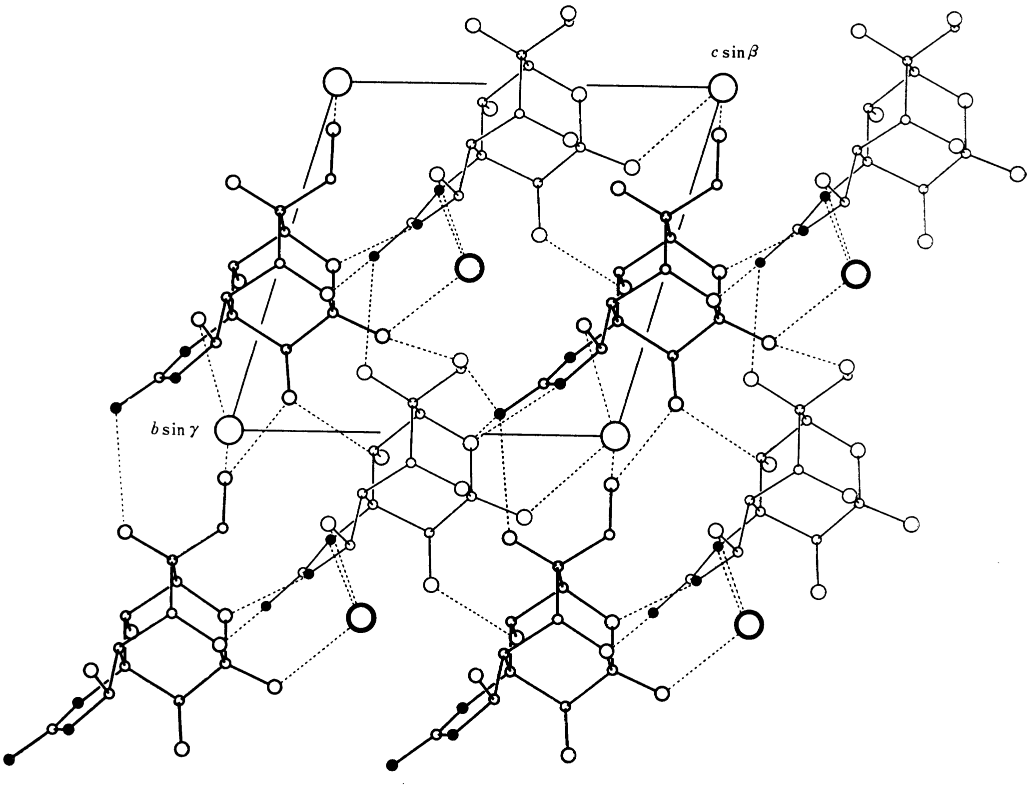

Fig. 8. The crystal structure viewed along the *a* axis.

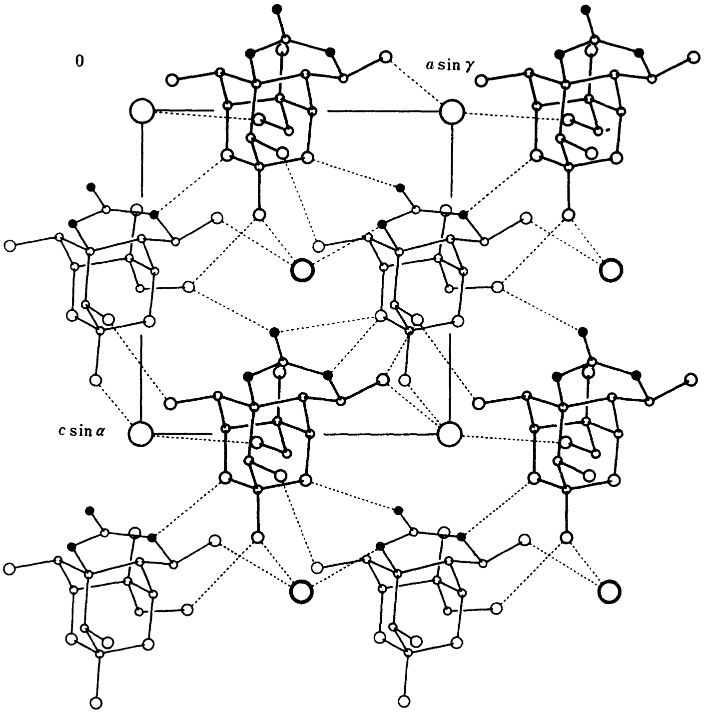

Fig. 9. The crystal structure viewed along the *b* axis.

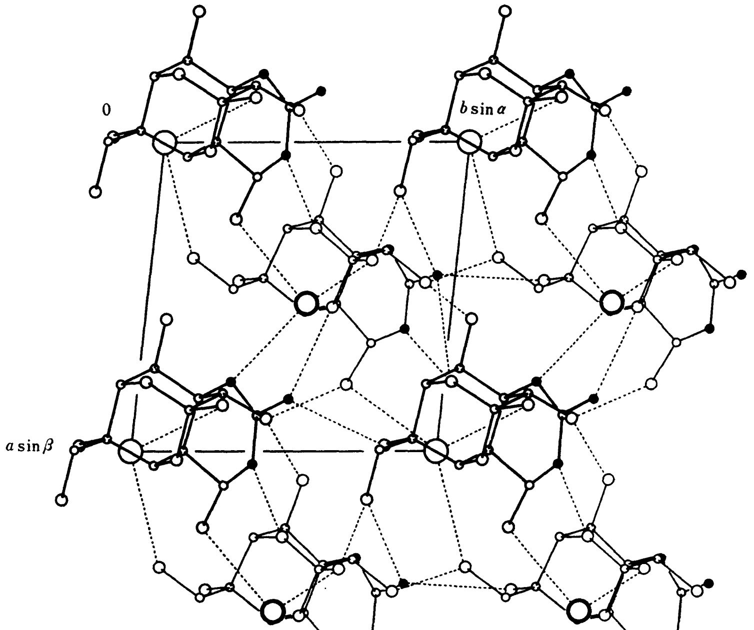

Fig. 10. The crystal structure viewed along the *c* axis.

depend on the intermolecular rather than on the intramolecular interactions. On the other hand, the six-membered rings with a guanidinium group are different in overall form; in A, the ring is in rather the boat form, while, in B, it is in the chair form, though, in either case, the five atoms, C(2), N(2), C(1), N(1), and C(8), lie roughly on a plane. The guanidinium group itself is almost planar in both of the molecules. The deviations of the atoms from the mean plane of the group are shown in Fig. 6 for the respective molecules. In A, C(2) and C(8) deviate from the guanidinium plane in the same sense as C(7), while, in B, the two atoms are on the opposite side of the plane to C(7). This shows that the two corresponding rings take different conformations, as has already been mentioned.

Considering the resonance of the guanidinium group, the two molecules have parallel characteristics, as may be seen from the bond distances in Table 4. To this resonance, the four formulae given in Fig. 7 seem to contribute mainly. In view of the bond distances, in both molecules, out of the three formulae containing a double bond, I would seem to be the most predominant, and III, the least. That I is superior to II is consistent with the fact that, in both molecules, C(8) deviates more considerably from the guanidinium plane than does C(2); in other words, the twisting of the bond C(1)–N(1) is larger than that of the C(1)–N(2) bond.

Crystal Structure. The projections of the crystal structure viewed along the *a*, *b*, and *c* axes are shown in Figs. 8, 9, and 10 respectively. Further, in Table 6, possible hydrogen bonds are listed, together with the distances, in consideration of the correspondence between the two independent molecules. Since the present molecules have many proton donors and acceptors, the crystal takes quite a complicated three-dimensional hydrogen-bonded structure; for A molecules, each makes fourteen hydrogen bonds with adjacent molecules and bromide ions, while, B molecules make thirteen.

Both of the bromide ions, Br(a) and Br(b), form three hydrogen bonds with their adjoining tetrodotoxin molecules. For the former bromide ion, the three hydrogen bonds are all of the O–H···Br^- type; the distances are 3.23 Å for O(A6)–H···Br(a) and 3.36 Å for O(B3)–H···Br(a) and O(A8)–H···Br(a). On the other hand, for the latter, out of the three hydrogen bonds, two are O–H···Br^- and the other is N–H···Br^-; the distances are 3.14 Å for O(A3)–H···Br(b), 3.24 Å for O(B6)–H···Br(b), and 3.26 Å for N(B2)–H···Br(b). So far as only the atomic distances smaller than 4.0 Å are concerned, Br(b) is surrounded merely by the three molecules which make hydrogen bonds with it, while Br(a) is approached by two additional molecules as well.

In some of the hydrogen bonds found in the present crystal, the proton seems to be, to a considerable degree, off the line joining the proton donor and acceptor. Since, for the four protons of the guanidinium grouping, their approximate positions can be estimated on reasonable assumptions,[*3] the deviations of the N–H bond directions from the respective donor-acceptor lines have been calculated; the results are listed in Table 7. The maximum deviation is found for N(B2)–H···Br(b), the angle being no less than about 47°. Nevertheless, the approach between N(B2) and Br(b) may be taken as hydrogen-bonded because the proton at N(B2) is separated from Br-

Table 6. Possible hydrogen bonds

O(A3)-H···Br(b)	3.14 Å	O(B3)-H···Br(a)	3.35 Å
O(A6)-H···Br(a)	3.23	O(B6)-H···Br(b)	3.24
O(A8)-H···Br(a)	3.36	——	
——		N(B2)-H···Br(b)	3.26
O(A4)-H···O(B7)	2.95	O(B4)-H···O(A7)	2.89
O(A7)-H···O(A8)	2.70	——	
——		O(B7)-H···O(A6)	2.73
——		O(B8)-H···O(A3)	2.80
O(A5)-H···N(A3)	3.00	——	
N(A1)-H···O(B1)	2.88	N(B1)-H···O(A1)	2.94
N(A3)-H···O(B8)	2.82	——	
——		N(B3)-H···O(A2)	3.14
N(A3)-H···O(B1)	3.09	——	
——		N(B3)-H···O(B5)	3.00

[*3] The proton positions were estimated on the following assumptions: the N–H bond distances are 1.01 Å; for H at N(1), ∠C(8)–N(1)–H=∠C(1)–N(1)–H and these four atoms are coplanar; for H at N(2), ∠C(2)–N(2)–H=∠C(1)–N(2)–H and these four atoms lie on a plane; for the two H atoms at N(3), ∠C(1)–N(3)–H=120° and the H atoms are coplanar with, respectively, N(1), C(1), and N(3), and N(2), C(1), and N(3).

Table 7. The deviations of the guanidinium protons from the hydrogen bond directions

N-H···X	N···X (Å)	H···X (Å)	∠H-N···X (°)	N-H···X	N···X (Å)	H···X (Å)	∠H-N···X (°)
N(A1)-H···O(B1)	2.88	1.98	22	N(B2)-H···Br(b)	3.26	2.68	47
N(A3)-H···O(B1)	3.09	2.26	29	N(B3)-H···O(A2)	3.14	2.25	24
N(A3)-H···O(B8)	2.82	1.99	28	N(B3)-H···O(B5)	3.00	2.28	37
N(B1)-H···O(A1)	2.94	1.97	14				

Table 8. Intermolecular contacts except hydrogen-bonded

Br(a)	N(A1)	I	3.86 Å	O(A7)	C(A11)	V	3.57 Å
	C(A8)	I	3.60		O(B6)	XI	3.88
	O(B2)	V	3.95		C(B3)	VIII	3.42
	O(A4)	IV	3.83	O(A8)	N(A1)	III	3.51
	N(A2)	IV	3.90		N(A2)	III	3.24
	O(B5)	VIII	3.66		N(A3)	III	3.23
	O(B6)	VIII	3.90		C(A1)	III	3.04
	C(B7)	VIII	3.63		C(A2)	III	3.98
Br(b)	O(A2)	VII	3.59		C(A9)	III	3.52
	O(A6)	XII	3.91		O(B6)	IX	3.03
	N(A1)	XII	3.66	N(A1)	O(B3)	V	3.81
	N(A2)	XII	3.70		C(B4)	V	3.79
	N(A3)	XII	3.84		C(B10)	V	3.95
	C(A1)	XII	3.48	N(A2)	O(B8)	X	3.25
	C(A7)	XII	3.61	N(A3)	C(A5)	V	3.97
	C(A8)	XII	3.95		C(A11)	V	3.93
	C(A10)	VII	3.84		O(B4)	V	3.22
	O(B7)	XII	3.95		C(B4)	V	3.64
	N(B3)	XII	3.61	C(A1)	C(A11)	V	3.94
	C(B1)	XII	3.76		O(B1)	V	3.43
	C(B8)	VIII	3.86		O(B8)	X	3.44
	C(B9)	XII	3.94	C(A3)	O(B2)	II	3.83
O(A1)	O(B5)	XI	3.94		O(B7)	II	3.75
	O(B6)	IX	3.36	C(A4)	O(B6)	IX	3.99
	N(B3)	IX	3.93		N(B1)	IX	3.78
	C(B1)	IX	3.89	C(A6)	O(B3)	I	3.91
	C(B8)	IX	3.71		N(B3)	VI	3.51
O(A3)	N(A3)	VI	3.46	C(A8)	O(B7)	I	3.98
	O(B4)	VIII	3.22	C(A9)	O(B4)	VIII	3.94
	O(B5)	XI	3.73		O(B5)	XI	3.85
	N(B1)	IX	3.91	C(A10)	O(B4)	VIII	3.95
	C(B3)	VIII	3.94		O(B8)	XI	3.91
O(A4)	O(A6)	II	3.13	C(A11)	O(B6)	IX	3.22
	O(B2)	II	3.88		N(B2)	VI	3.90
	O(B3)	II	3.45		N(B3)	VI	3.73
	C(B9)	II	3.99	O(B3)	N(B3)	VI	3.89
	C(B10)	II	3.99	O(B4)	O(B6)	II	3.76
O(A5)	C(A1)	III	3.86	O(B5)	N(B1)	III	3.97
	O(B1)	I	3.90		C(B1)	III	3.87
	O(B3)	I	3.67	O(B7)	C(B11)	V	3.84
	O(B4)	I	3.86	O(B8)	N(B1)	III	3.56
	C(B9)	I	3.70	N(B1)	C(B11)	V	3.80
	C(B10)	I	3.94	N(B3)	C(B5)	V	3.78
O(A6)	C(B9)	I	3.71		C(B11)	V	3.69
	C(B11)	V	3.58	C(B1)	C(B11)	V	3.85

I: x/a, y/b, z/c (given in Table 3); II: $-1+x/a$, y/b, z/c; III: x/a, $-1+y/b$, z/c; IV: $1+x/a$, y/b, z/c; V: x/a, $1+y/b$, z/c; VI: x/a, y/b, $1+z/c$; VII: $1+x/a$, $1+y/b$, z/c; VIII: x/a, $1+y/b$, $1+z/c$; IX: $-1+x/a, y/b$, $1+z/c$; X: $-1+x/a$, $1+y/b, z/c$; XI: $-1+x/a$, $1+y/b$, $1+z/c$; XII: $1+x/a$, $1+y/b$, $1+z/c$.

(b) by only 2.68 Å; this distance is much smaller than the sum of the van der Waals radii, 3.15 Å. That such large deviations of the hydrogen bond directions are found in the present crystal is probably related with the fact that the molecules make extremely numerous hydrogen bonds.

As has been found for Br(a) and Br(b), for the two independent molecules also the hydrogen bond natures are different in some respects. The most interesting of these differences is that, in the O(5)···N(3) hydrogen bond, for the A molecule the proton seems to be donated by the former atom, while, for the B, it seems to be donated by the latter. As for the A molecule, the estimated positions of the two protons at N(A3) are separated from O(B1) and O(B8) respectively by only 2.26 and 1.99 Å, and hence the two approaches to N(A3) are considered hydrogen-bonded. On the other hand, since O(A5) is sufficiently separated from both of the protons (2.70 and 3.17 Å), these two protons have little relation with the close approach between O(A5) and N(A3). Considering the facts that the O(A5)···N(A3) direction makes a relatively small angle of about 33° with the normal of the guanidinium plane and that the π-electrons are localized to some extent at N(A3), since, out of the three important formulae for the resonance of the guanidinium group, III is the least predominant, as has been mentioned above, O(A5) and N(A3) seem to make a hydrogen bond of the O–H···N type. On the other hand, as for the B molecules, since O(B5) is only 2.28 Å apart from the proton at N(B3), the O(B5)···N(B3) approach would seem to be due to a hydrogen bond of the N–H···O type.

All the small intermolecular atomic distances except the hydrogen-bonded ones are listed in Table 8. These distances nearly correspond to the van der Waals contacts, though some of them are somewhat smaller than the ordinary values because of the intermolecular approach due to hydrogen bonding and other forces.

In conclusion, the authors wish to express their thanks to Professor Yoshimasa Hirata, Nagoya University, for providing them with the samples. The authors would also like to express their thanks to the staff of the X-ray group, University of Pittsburgh, for their kind help in the collection of the intensity data.