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The Crystal Structures of Derivatives of Tetrodotoxin. I. Tetrodonic Acid Hydrobromide

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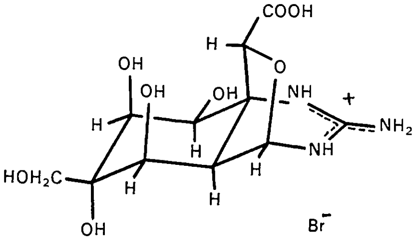
The structure of tetrodonic acid, one of the derivatives of the toxic substance tetrodotoxin, has been determined by X-ray analysis of the hydrobromide. The crystals are orthorhombic with four molecules in a unit cell of dimensions $a=20\cdot231$, $b=10\cdot590$, $c=6\cdot813$ Å, with space group $P2_12_12_1$. The position of the bromine atom was determined by three-dimensional Patterson methods.

After four cycles of three-dimensional Fourier refinement, the structure of the molecule was found to be



Atomic coordinates and anisotropic temperature factors were further refined by the block-diagonal matrix least-squares method. The final R value decreased to 0·129. Intra- and intermolecular bond lengths are within the range of normal values.

Introduction

Tetrodotoxin, one of the most toxic substances of low molecular weight, was found in the ovaries and livers of pufferfish (Tahara, 1909). Tsuda & Kawamura (1952) obtained it in crystalline form after chromatographic purification. This poisonous compound has attracted the attention of chemists and biologists, and many investigations on it have been carried out by several authors (Yokoo, 1950; Tsuda & Kawamura, 1953; Kakisawa, Okumura & Hirata, 1959).

Tsuda, Ikuma, Kawamura, Tachikawa, Miyadera & Baba (1962) proved by total chemical synthesis that an alkaline degradation compound from tetrodotoxin is 2-amino-6-hydroxymethyl-8-hydroxyquinazoline. It was impossible, however, to deduce uniquely the structure of tetrodotoxin from this result because some migration might occur in the course of the vigorous reaction. X-ray crystallographic analysis, therefore, was attempted for the determination of the molecular structure. The crystals of tetrodotoxin itself were not obtained in a suitable form for X-ray work. Several derivatives from it were examined and it was found that tetrodonic acid hydrobromide formed crystals of adequate size. Tetrodonic acid was derived easily from tetrodotoxin on refluxing with water, and this fact suggested that the chemical relationship between them would be close. The present paper describes an X-ray determination of the crystal structure of tetrodonic acid hydrobromide. On the basis of the structure of this compound, some chemical work was carried out to determine a possible structure of tetrodotoxin (Tsuda, Tamura, Tachikawa, Sakai, Amakasu, Kawamura & Ikuma, 1963). A full chemical report has been already published by Tsuda, Ikuma, Kawamura, Tachikawa, Sakai, Tamura & Amakasu (1964), with a brief account of the present X-ray study.

Experimental

Crystals of tetrodonic acid hydrobromide were obtained in the form of colourless thin needles by adding ether to an alcoholic solution containing hydrobromic acid. The space group is determined to be $P2_12_12_1$, and the unit-cell dimensions calibrated with standard sodium chloride powder diffraction on the same film are:

$$a=20\cdot231, \quad b=10\cdot590, \quad c=6\cdot813\ \text{Å}\,.$$

The volume of the unit cell is 1460 Å^3. The density of the crystal was measured by the flotation method in a mixture of carbon tetrachloride and tetrabromoethane. The observed density is $1\cdot821$ g.cm^{-3}. Because no reliable chemical formula was known at that stage, the molecular weight was calculated from the density by assuming that the unit cell contains four molecules. The calculated value, 400·3, suggests that the chemical formula is $C_{11}H_{17}O_8N_3HBr$ (M.W. 400·2).

Equi-inclination Weissenberg photographs were taken for the zero to 6th layers about the c axis with Cu $K\alpha$ radiation. The crystal used in the experiment had the dimensions $0\cdot4 \times 0\cdot3 \times 1\cdot5$ mm. To obtain scaling factors among the various l layers, the photographs were also taken around the b axis. Intensities were measured by visual comparison with a standard intensity scale. Strong and weak reflexions were correlated by the multiple-film technique. The maximum value of $\sin\theta$ observed was 0·995, and the intensity data for 1504 reflexions were collected out of 1689 accessible hkl's. Corrections for Lorentz and polarization factors were made in the usual way but those for absorption were neglected.

Structure determination

Because an attempt to derive the structure from the projection was not successful, three-dimensional analysis was proceeded with. The approximate coordinates of the bromine atom were determined from the Patterson function modified by the factors proposed by Donohue & Trueblood (1952),

$$M(\sin\theta)=(\sin\theta/\lambda)^n \exp\{-\alpha^2(\sin\theta/\lambda)^2\}\,,$$

where $n=4$ and $\alpha=2\cdot16$.

The first three-dimensional Fourier synthesis, shown in Fig. 1, was computed with the phases based on the bromine atom only. Some ten resolved peaks in the electron density distribution were chosen as atomic sites without any regard to chemical considerations. The discrepancy factor was 0·42 for these ten light atoms and bromine. Successive Fourier refinements were then carried out. Table 1 shows the number of atoms taken into the structure factor calculation and the R value for each cycle.

Table 1. *Number of light atoms included and R value for each cycle*

Refinement cycle	1	2	3	4
Number of light atoms included	10	16	19	22
R (%)	42	30	25	18

The clues for selecting the peaks in the course of these refinements were: (i) peak heights and (ii) reasonable spatial arrangement of two or more neighbouring

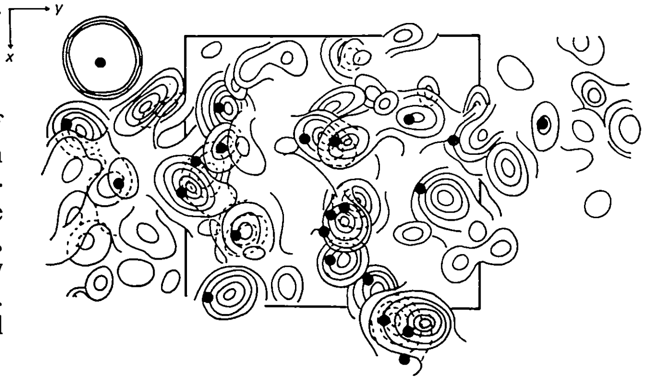


Fig. 1. The composite Fourier map projected along the c axis. Phases are based on the bromine atom only. Contour interval is arbitrary. Black circlets indicate the final atomic positions.

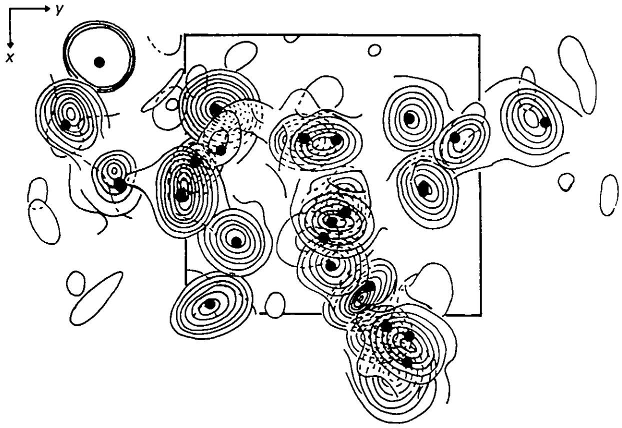


Fig. 2. The composite Fourier map projected along the c axis. Phases are based on the bromine and nineteen light atoms. Contour interval is arbitrary. Black circlets indicate the final atomic positions.

Table 2. *Observed and calculated structure factors* (× 10)

h	Fo	Fc
	0	0
2	19	17
4	23	7
6	105	89
8	137	139
10	63	66
12	167	156
14	51	39
16	97	95
18	109	117
20	70	87
22	40	57
24	77	90
26	53	79
	1	0
1	72	53
2	250	202
3	212	178
4	259	221
5	0	21
6	209	182
7	124	132
8	123	118
9	223	203
10	242	245
11	81	77
12	109	112
13	107	107
14	62	71
15	0	24
16	157	183
17	112	117
18	78	98
19	55	61
20	0	29
21	26	42
22	0	3
23	33	25
24	0	3
25	14	2
	2	0
0	132	152
1	296	278
2	30	13
3	322	304
4	151	144
5	86	80
6	132	132
7	407	379
8	113	97
9	23	15
10	61	52
11	0	10
12	88	80
13	42	30
14	73	64
15	0	3
16	104	103
17	92	97
18	0	19
19	69	87
20	27	25
21	56	80
22	44	42
23	29	51
24	0	4
25	53	66
	3	0
1	226	215
2	103	94
3	284	257
4	243	220
5	57	61
6	119	123
7	251	244
8	51	62
9	59	71
10	94	97
11	189	210
12	67	69
13	198	210
14	103	111
15	114	133
16	0	11
17	73	86
18	19	36
19	46	49
20	62	63
21	26	34
22	19	18
23	25	28
24	28	33
25	23	26
	4	0
0	174	159
1	267	256
2	175	172
3	110	106
4	350	328
5	109	113
6	52	49
7	96	82
8	77	79
9	26	39
10	87	79
11	0	5
12	24	19
13	47	51
14	69	61
15	61	56
16	0	6
17	0	4
18	77	88
19	71	82
20	65	86
21	66	75
22	19	41
23	69	72
24	48	60
	5	0
1	120	96
2	348	335
3	173	131
4	0	10
5	55	27
6	148	151
7	46	49
8	108	102
9	54	51
10	147	163
11	0	11
12	140	156
13	62	58
14	135	176
15	55	60
16	49	68
17	0	23
18	63	74
19	31	34
20	53	66
21	64	57
22	64	60
23	32	36
	6	0
0	42	38
1	134	137
2	147	165
3	153	160
4	176	187
5	0	6
6	67	80
7	133	131
8	163	177
9	30	31
10	25	12
11	61	51
12	72	65
13	0	9
14	27	30
15	34	27
16	38	26
17	27	19
18	59	68
19	33	41
20	67	64
21	30	39
22	42	46
	7	0
1	0	1
2	39	41
3	66	66
4	0	1
5	66	39
6	49	30
7	123	127
8	138	140
9	205	232
10	74	71
11	97	123
12	67	72
13	33	53
14	0	26
15	97	128
16	26	36
17	73	84
18	0	11
19	45	50
20	0	8
21	25	32
22	36	41
	8	0
0	111	114
1	116	131
2	0	8
3	120	133
4	0	4
5	103	120
6	153	160
7	33	35
8	19	20
9	39	50
10	47	53
11	65	78
12	0	14
13	46	47
14	50	45
15	45	52
16	54	7
17	58	63
18	47	15
19	23	42
20	0	2
21	43	46
	9	0
1	38	47
2	19	20
3	0	29
4	0	19
5	119	120
6	61	66
7	45	46
8	74	91
9	19	18
10	101	129
11	32	36
12	0	37
13	54	53
14	56	57
15	56	66
16	71	81
17	0	5
18	65	74
	10	0
0	75	94
1	34	41
2	57	81
3	0	20
4	96	114
5	47	38
6	72	94
7	0	18
8	45	50
9	18	16
10	25	32
11	59	64
12	0	17
13	67	55
14	74	74
15	16	19
16	20	24
	11	0
1	18	13
2	26	31
3	0	9
4	60	79
5	43	65
6	17	29
7	18	3
8	54	54
9	36	43
10	69	76
11	0	30
12	62	65
13	30	49
14	45	50
15	36	36
	12	0
0	68	31
1	36	56
2	0	2
3	37	52
4	0	12
5	73	79
6	63	57
7	75	78
8	23	16
9	31	40
10	31	27
	0	1
1	0	49
2	0	27
3	340	354
4	269	236
5	405	379
6	17	8
7	53	51
8	224	218
9	0	8
10	65	58
11	28	27
12	271	263
13	65	62
14	121	119
15	0	8
16	148	157
17	28	15
18	80	60
19	72	60
20	102	100
21	101	101
22	31	25
23	55	62
24	32	36
25	0	13
	1	1
0	52	39
1	178	159
2	174	135
3	153	146
4	310	283
5	284	247
6	220	200
7	222	199
8	172	143
9	180	149
10	22	19
11	194	172
12	55	58
13	85	80
14	95	90
15	88	99
16	75	70
17	63	61
18	65	67
19	0	12
20	73	69
21	76	72
22	38	35
23	26	32
24	56	70
25	40	51
26	7	28
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1	191	164
2	271	225
3	15	15
4	218	195
5	264	229
6	82	57
7	185	163
8	153	130
9	124	117
10	95	83
11	126	123
12	93	91
13	138	140
14	65	64
15	82	89
16	67	62
17	90	93
18	0	18
19	108	105
20	79	79
21	47	45
22	23	26
23	30	19
24	45	55
25	26	33
	3	1
0	249	217
1	175	167
2	141	116
3	217	178
4	77	38
5	239	212
6	50	46
7	36	36
8	223	199
9	217	188
10	167	151
11	79	73
12	98	100
13	40	36
14	82	85
15	99	104
16	79	73
17	46	44
18	56	62
19	51	53
20	39	40
21	54	52
22	39	46
23	79	84
24	0	4
25	18	36
	4	1
0	112	105
1	274	227
2	127	115
3	39	43
4	242	208
5	173	141
6	137	135
7	162	142
8	109	94
9	144	134
10	31	35
11	136	135
12	47	121
13	55	55
14	70	73
15	46	48
16	87	97
17	67	62
18	117	117
19	0	7
20	70	62
21	11	32
22	0	14
23	53	59
24	38	43
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1	71	52
2	219	214
3	70	53
4	127	116
5	118	98
6	166	171
7	171	153
8	63	62
9	114	100
10	133	117
11	83	81
12	45	40
13	68	78
14	49	50
15	66	69
16	110	104
17	104	91
18	49	46
19	79	77
20	36	33
21	26	20
22	46	55
23	22	19
24	36	54
	6	1
0	49	40
1	116	121
2	117	121
3	76	72
4	34	38
5	127	123
6	116	106
7	65	68
8	163	153
9	52	63
10	75	84
11	38	48
12	79	80
13	82	72
14	52	49
15	109	105
16	73	73
17	67	66
18	55	57
19	67	69
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22	26	30
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1	155	173
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4	111	106
5	95	99
6	77	82
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9	49	47
10	89	82
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12	43	129
13	104	89
14	92	92
15	0	11
16	47	42
17	42	31
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6	77	82
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8	73	67
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13	64	61
14	0	12
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18	0	25
19	40	62
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0	0	21
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5	19	23
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9	22	13
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14	58	69
15	0	21
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4	29	39
	0	2
1	34	6
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5	87	94
6	51	49
7	210	208
8	29	14
9	254	274
10	80	65
11	156	160
12	109	107
16	174	178
14	0	12
15	110	118
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17	85	87
18	0	12
19	97	112
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1	92	265
2	153	148
3	201	220
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9	163	147
10	140	121
11	37	38
12	76	66
13	55	46
14	87	83
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24	0	18
25	47	66
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6	38	35
7	138	133
8	104	104
9	41	31
10	116	124
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14	42	27
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19	42	25
20	41	49
21	32	36
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2	53	50
3	30	16
4	87	85
5	76	71
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7	58	66
8	101	110
9	38	34
10	108	124
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	9	2
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3	83	101
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5	88	97
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7	59	64
8	54	69
9	54	60
10	49	44
11	49	46
12	36	18
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14	50	44
15	36	24
16	66	56
17	57	55
18	46	48
	10	2
0	30	30
1	36	30
2	49	40
3	41	38
4	37	24
5	37	29
6	47	28
7	58	72
8	30	19
9	84	101
10	31	10
11	54	63
12	30	19
13	81	98
14	36	30
15	62	73
16	23	14
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2	49	38
3	57	68
4	48	45
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6	50	55
7	47	41
8	69	67
9	31	21
10	45	40
11	0	8
12	37	9
13	36	45
14	27	26
	12	2
0	29	15
1	43	25
2	39	38
3	37	32
4	33	28
5	27	3
6	39	44
7	39	38
8	43	53
9	40	39
10	55	78
	13	2
0	0	24
1	30	48
2	51	70
	0	3
1	152	210
2	104	99
3	213	218
4	52	50
5	124	128
6	132	108
7	169	165
8	20	23
9	34	14
10	139	115
11	97	80
12	116	99
13	42	26
14	58	59
15	101	100
16	44	48
17	0	3
18	59	67
19	46	38
20	0	9
21	41	46
22	0	12
23	66	80
24	24	22
	1	3
0	141	168
1	193	184
2	182	164
3	38	11
4	129	113
5	140	103
6	196	176
7	147	135
8	67	59
9	158	165
10	35	23
11	154	154
12	159	150
13	98	91
14	69	75
15	49	53
16	84	84
17	89	95
18	52	44
19	69	61
20	94	89
21	47	57
22	45	43
23	0	21
24	22	30
	2	3
0	218	210
1	112	88
2	82	74
3	92	59
4	266	254
5	105	84
6	212	189
7	139	130
8	143	138
9	146	135
10	61	55
11	131	124
12	58	44
13	71	63
14	23	18
15	92	96
16	65	59
17	42	30
18	45	49
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22	49	54
23	27	25
24	21	44
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0	80	75
1	161	144
2	92	69
3	112	89
4	52	36
5	48	47
6	101	82
7	40	41
8	155	147
9	108	102
10	164	170
11	77	62
12	123	129
13	127	125
14	82	84
15	49	35
16	105	113
17	65	61
18	67	64
19	55	43
20	49	54
21	39	39
22	25	21
23	27	34
24	7	29
	4	3
0	68	69
1	154	150
2	131	118
3	158	146
4	72	69
5	123	101
6	96	93
7	149	142
8	94	83
9	108	94
10	64	57
11	35	26
12	62	58
13	0	19
14	71	82
15	37	39
16	52	39
17	65	62
18	0	16
19	81	74
20	52	44
21	35	28
22	53	53
23	33	48
	5	3
0	122	106
1	23	16
2	136	133
3	156	146
4	101	97
5	59	52
6	73	71
7	125	130
8	64	59
9	108	106
10	52	46
11	49	51
12	60	59
13	117	132
14	43	36
15	100	106
16	35	25
17	69	69
18	67	60
19	63	66
20	56	51
21	44	42
22	26	32
	6	3
0	137	141
1	33	39
2	149	148
3	143	143
4	82	70
5	141	139
6	29	31
7	58	47
8	83	74
9	111	115
10	147	143
11	41	49
12	40	38
13	30	23
14	72	75
15	73	52
16	56	53
17	47	33
18	12	14
19	56	50
20	0	15
21	35	47
	7	3
0	61	52
1	87	88
2	0	10
3	87	85
4	86	81
5	48	51
6	120	126
7	47	43
8	104	98
9	79	72
10	88	96
11	30	28
12	72	86
13	29	31
14	83	92
15	29	2
16	71	76
17	0	17
18	55	56
19	44	40
20	39	51
	8	3
0	66	84
1	65	59
2	105	119
3	102	107
4	85	84
5	53	50
6	43	57
7	70	80
8	39	48
9	31	28
10	56	63
11	74	73
12	0	4
13	80	77
14	51	43
15	61	61
16	40	35
17	39	34
18	40	48
	9	3
0	0	28
1	67	62
2	58	53
3	83	84
4	80	75
5	75	91
6	43	41
7	0	7
8	87	95
9	47	49
10	0	15
11	57	64
12	46	41
13	64	71
14	0	23
15	58	66
16	38	49
17	38	65
	10	3
0	0	33
1	97	114
2	0	9
3	54	62
4	0	28
5	41	41
6	46	51
7	45	48
8	45	47
9	0	13
10	41	44
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12	32	39
13	28	29
14	28	24
	11	3
0	0	34
1	46	47
2	56	50
3	39	33
4	0	26
5	65	70
6	0	22
7	33	26
8	36	45
9	34	43
10	36	46
11	35	49
	12	3
0	0	28
1	0	17
2	51	67
3	0	17
4	23	33
5	22	35
6	66	86
	0	4
1	28	32
2	119	178
3	28	25
4	229	229
5	53	42
6	85	84
7	29	7
8	124	127
9	0	1
10	0	1
11	65	83
12	0	4
13	90	91
14	0	14
15	37	42
16	0	36
17	0	0
18	69	71
19	51	36
20	63	69
21	0	6
22	57	74
23	22	5
	1	4
0	51	58
1	83	98
2	109	118
3	39	32
4	118	113
5	65	67
6	60	61
7	109	112
8	143	144
9	106	117
10	60	60
11	53	54
12	112	124
13	69	61
14	142	166
15	93	91
16	21	34
17	86	90
18	76	71
19	72	53
20	51	13
21	0	30
22	0	28
23	33	23
	2	4
0	33	20
1	155	155
2	50	48
3	171	172
4	144	132
5	190	178
6	40	46
7	118	119
8	69	71
9	78	94
10	46	40
11	70	68
12	91	93
13	72	72
14	29	13
15	55	52
16	56	54
17	59	58
18	59	48
19	31	15
20	49	45
21	50	49
22	48	46
23	24	57
	3	4
0	173	128
1	93	77
2	75	65
3	99	77
4	59	54
5	123	112
6	51	45
7	122	121
8	43	26
9	73	74
10	51	40
11	129	151
12	64	59
13	112	125
14	0	30
15	74	95
16	74	67
17	67	54
18	53	48
19	56	56
20	45	18
21	55	52
22	39	9
	4	4
0	114	92
1	118	103
2	168	148
3	111	102
4	120	112
5	65	65
6	84	79
7	57	45
8	76	77
9	57	49
10	62	57
11	52	47
12	73	74
13	21	13
14	67	73
15	69	63
16	46	51
17	69	51
18	56	25
19	67	67
20	56	58
21	51	56
	5	4
0	93	83
1	57	50
2	64	50
3	43	16
4	93	88
5	73	67
6	167	158
7	54	38
8	77	88
9	72	70
10	114	132
11	0	20
12	75	80
13	0	4
14	89	92
15	54	37
16	88	97
17	0	12
18	70	79
19	36	21
20	51	49
21	0	30
	6	4
0	183	183
1	54	47
2	91	90
3	108	108
4	60	47
5	129	132
6	99	94
7	63	67
8	69	73
9	41	26
10	65	71
11	29	35
12	44	38
13	32	7
14	71	59
15	62	26
16	55	49
17	44	42
18	55	48
19	30	15
20	26	39
	7	4
0	0	4
1	48	32
2	70	66
3	69	71
4	64	55
5	60	49

Table 2 (*cont.*)

h	Fo	Fc
6	60	52
7	74	85
8	55	41
9	63	68
10	85	91
11	102	109
12	0	15
13	81	92
14	60	23
15	69	79
16	62	50
17	54	56
18	35	18
19	0	33
20	0	34
	8	4
0	0	12
1	121	131
2	41	38
3	74	91
4	51	37
5	0	34
6	53	39
7	74	70
8	72	56
9	66	42
10	47	38
11	0	18
12	0	9
13	51	8
14	57	56
15	39	26
16	34	19
17	47	59
	9	4
0	0	16
1	0	10
2	53	46
3	69	69
4	64	58
5	60	50
6	47	33
7	73	64
8	74	89
9	66	55
10	54	53
11	54	48
12	48	54
13	51	50
14	53	66
15	39	16
	10	4
0	76	88
1	0	14
2	0	59
3	0	19
4	102	104
5	0	21
6	0	37
7	0	36
8	34	34
9	63	52
10	0	26
11	31	26
	11	4
0	53	36
1	0	14
2	0	30
3	0	32
4	0	25
5	0	40
6	49	37
7	0	39
8	39	56
9	40	14
	0	5
1	34	54
2	42	55
3	65	91
4	56	41
5	50	31
6	56	51
7	75	66
8	210	227
9	61	54
10	113	127
11	30	25
12	94	113
13	31	12
14	23	37
15	25	29
16	120	128
17	25	27
18	52	38
19	42	42
20	45	49
21	0	16
22	0	24
	1	5
0	79	102
1	86	105
2	139	181
3	59	65
4	82	88
5	100	97
6	36	29
7	94	94
8	88	79
9	71	60
10	94	84
11	35	13
12	71	59
13	0	31
14	34	39
15	0	35
16	37	35
17	35	25
18	40	41
19	66	59
20	0	29
21	0	26
	2	5
0	88	77
1	75	68
2	88	86
3	90	79
4	0	17
5	97	85
6	111	104
7	48	57
8	65	68
9	144	164
10	30	16
11	106	102
12	0	18
13	78	89
14	0	26
15	88	89
16	51	14
17	80	77
18	79	72
19	53	48
20	56	48
21	0	43
	3	5
0	110	92
1	146	132
2	66	48
3	138	137
4	68	62
5	126	123
6	91	79
7	34	40
8	44	38
9	70	75
10	46	32
11	0	11
12	63	42
13	0	10
14	67	56
15	45	38
16	55	51
17	33	10
18	37	29
19	64	67
	4	5
0	61	47
1	50	39
2	58	39
3	0	26
4	87	84
5	61	56
6	55	43
7	70	74
8	71	75
9	59	42
10	75	85
11	70	75
12	112	119
13	81	72
14	79	74
15	61	52
16	71	65
17	44	15
18	48	45
19	47	13
	5	5
0	116	114
1	20	22
2	124	119
3	46	30
4	78	78
5	54	37
6	141	155
7	49	29
8	31	39
9	80	79
10	59	46
11	80	69
12	50	18
13	63	45
14	35	34
15	24	24
16	31	39
17	0	22
18	48	53
	6	5
0	69	69
1	73	69
2	37	21
3	38	18
4	93	93
5	61	49
6	44	32
7	52	54
8	76	66
9	77	70
10	76	75
11	80	73
12	76	64
13	59	47
14	68	68
15	56	46
16	66	53
17	49	49
18	32	30
	7	5
0	50	43
1	129	147
2	37	24
3	70	71
4	41	38
5	80	72
6	58	48
7	76	70
8	75	60
9	68	50
10	50	41
11	34	12
12	33	34
13	56	49
14	0	22
15	51	32
16	44	32
17	15	45
	8	5
0	0	1
1	0	27
2	62	44
3	36	32
4	85	67
5	84	76
6	48	36
7	50	50
8	39	41
9	33	52
10	45	23
11	68	67
12	0	16
13	71	81
	9	5
0	73	65
1	0	15
2	83	81
3	0	49
4	0	40
5	0	39
6	74	62
7	0	31
8	62	58
9	0	10
10	53	43
	10	5
0	0	24
1	0	14
2	0	21
3	0	36
4	0	34
5	0	25
6	53	60
	0	6
1	23	44
2	0	6
3	24	18
4	83	103
5	98	127
6	15	12
7	78	83
8	70	74
9	46	57
10	0	2
11	92	101
12	15	13
13	108	115
14	47	27
15	38	37
16	0	2
17	59	56
18	11	9
19	30	41
	1	6
0	59	81
1	61	87
2	58	81
3	81	109
4	53	61
5	96	114
6	56	60
7	88	97
8	12	14
9	21	15
10	46	40
11	0	8
12	67	53
13	62	49
14	49	44
15	47	36
16	49	45
17	59	65
18	31	31
	2	6
0	28	31
1	32	27
2	29	23
3	82	87
4	61	66
5	64	59
6	95	94
7	84	86
8	51	58
9	58	61
10	95	101
11	67	64
12	67	63
13	32	29
14	82	94
15	27	12
16	40	42
17	29	24
18	52	64
	3	6
0	122	115
1	75	64
2	103	101
3	82	82
4	72	72
5	29	19
6	97	100
7	60	68
8	63	65
9	64	52
10	52	45
11	38	38
12	21	10
13	51	50
14	25	20
15	45	45
16	50	48
17	35	31
18	34	41
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0	76	59
1	75	80
2	33	19
3	42	38
4	84	85
5	12	6
6	68	62
7	87	92
8	63	65
9	58	56
10	80	86
11	64	64
12	42	38
13	69	62
14	46	37
15	52	55
16	33	29
17	34	41
	5	6
0	42	38
1	81	77
2	42	32
3	131	135
4	37	34
5	68	65
6	63	51
7	51	47
8	27	20
9	22	24
10	49	49
11	45	28
12	24	12
13	38	29
14	49	51
15	17	12
16	42	45
	6	6
0	63	69
1	69	54
2	49	40
3	60	54
4	48	46
5	46	44
6	46	34
7	84	76
8	15	31
9	75	66
10	0	14
11	64	61
12	42	41
13	43	56
14	53	65
15	48	67
	7	6
0	37	32
1	45	36
2	78	75
3	51	43
4	122	114
5	33	22
6	47	41
7	56	60
8	45	47
9	52	54
10	34	29
11	39	46
12	23	11
13	37	30
	8	6
0	31	17
1	49	52
2	54	40
3	45	37
4	47	43
5	30	28
6	70	73
7	23	29
8	65	76
9	0	9
10	49	60
	9	6
0	38	40
1	66	82
2	61	59
3	30	26
4	27	25
5	37	43
6	28	34

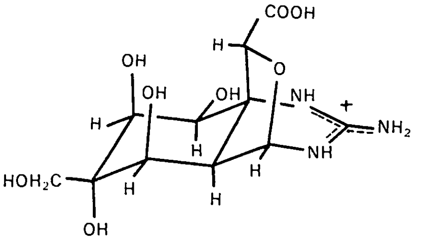
Table 3. *The final atomic coordinates and anisotropic temperature factors* (× 10⁵)

Temperature factor = exp $\{-(h^2B_{11}+k^2B_{22}+l^2B_{33}+hkB_{12}+klB_{23}+lhB_{31})\}$

Atom	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{23}	B_{31}
Br	0·0195	−0·1491	0·6122	127	531	916	−301	−439	191
N(1)	0·1386	0·4185	0·2055	76	278	1525	−53	365	75
C(2)	0·0986	0·4667	0·3615	103	246	1332	26	−38	334
N(3)	0·0764	0·3894	0·4893	86	189	1347	53	22	63
C(4)	0·0995	0·2555	0·4981	38	201	874	−25	−322	79
C(5)	0·1010	0·0607	0·2528	63	432	523	−103	331	−26
C(6)	0·1223	0·0229	0·0531	71	277	−1206	100	302	−72
C(7)	0·1833	0·0932	−0·0304	116	475	1391	64	−93	239
C(8)	0·1764	0·2421	−0·0007	105	226	992	−114	167	−152
C(9)	0·1552	0·2753	0·2054	52	247	1295	62	370	12
C(10)	0·0962	0·2065	0·2828	55	340	462	−39	−193	254
N(11)	0·0831	0·5922	0·3572	194	215	1598	59	207	−47
O(12)	0·1409	−0·0051	0·3976	99	336	550	−46	32	15
O(13)	0·0689	0·0616	−0·0764	69	419	828	79	−3	−238
C(14)	0·1368	−0·1208	0·0214	147	466	1900	61	−23	−221
O(15)	0·0798	−0·1998	0·0669	117	384	1339	15	82	104
O(16)	0·2401	0·0494	0·0800	100	417	1063	199	−65	71
O(17)	0·2328	0·3094	−0·0566	88	433	759	−91	−364	106
O(18)	0·1666	0·2503	0·5494	59	333	986	−54	−66	−18
C(19)	0·2059	0·2520	0·3747	70	244	320	41	350	−45
C(20)	0·2615	0·3456	0·4123	112	316	597	55	−307	−209
O(21)	0·2965	0·3813	0·2583	73	591	1393	−95	277	16
O(22)	0·2742	0·3874	0·5677	105	331	734	−115	−124	119

peaks, with probable interatomic distances, suggesting a possible functional group. No other chemical information was used in interpreting the Fourier map. At these stages, all light atoms were treated as nitrogen. The temperature factors were assumed to be isotropic with $B=2·7$ Å^2 for the light atoms and $B=2·3$ Å^2 for the bromine.

From the fourth Fourier map shown in Fig. 2, several possible models for the molecule were built up and examined to ascertain whether these would be compatible with the chemical information. Oxygen and carbon atoms were tentatively distinguished by their peak heights, because carbon atoms showed 70–80% peak values of those for oxygen. The only reasonable model was



On the basis of this molecular model, the R value without non-observed reflexions decreased to 0·18.

The structure was refined by the block-diagonal matrix least-squares method. In order to avoid disturbance from structure factors of low accuracy, about 900 reflexions in the range of sin θ between 0·011 and 0·485 were included. The temperature factors were taken as isotropic for the light atoms and anisotropic for the bromine. After two cycles of least-squares refinement, the $(F_o - F_c)$ synthesis was made with these reflexions. On the resulting difference map, ten hydrogen atoms were found as resolved maxima. Although contributions from the hydrogen atoms were included in the structure factors, their coordinates and temperature factors were held constant throughout the remaining calculations. The R value dropped to 0·129 after three more cycles of refinement including all reflexions, with anisotropic temperature factors for all atoms. The final observed and calculated structure factors are listed in Table 2. The final atomic coordinates and temperature factors are given in Table 3. Computations were done on an IBM 7090 computer with programs written by the authors.

Discussion

Fig. 3 shows the superimposed sections of the final electron density distribution cut through the atomic centres in tetrodonic acid hydrobromide. It is considered that the general structure of the molecule is now established except its absolute configuration. In the figures in the present paper, except Figs. 1, 2 and 3, the molecules have been shown with the correct absolute configuration, since Tomiie, Furusaki, Kasami, Yasuoka, Miyake, Haisa & Nitta (1964) have determined the absolute configuration of bromoanhydrotetrodoic lactone hydrobromide, another derivative of tetrodotoxin.

The cyclohexane ring in the perhydroquinazoline skeleton is of a chair form. As to the conformation of substituent atoms on the cyclohexane ring, O(12), O(13) and O(16) are in axial position and O(17) and C(14) in equatorial. The guanidine group is almost planar. A perhydrofuran ring is formed through the bridge made by C(4)–O(18)–C(19)–C(9) and has a steric effect on the perhydroquinazoline ring, leading to some deformation of the latter. Consequently, C(4)–H makes

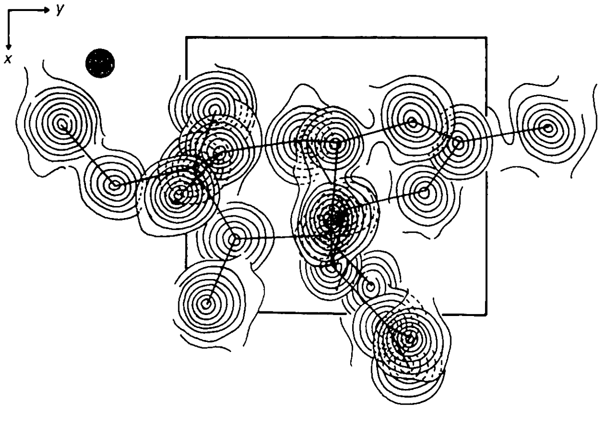


Fig. 3. The final composite Fourier map projected along the c axis. Contours are drawn at intervals of 1 e.Å^{-3}. Contours for the bromine atom are omitted and its position is shown by a black circle.

Table 4. *Bond angles*

C(2)—N(1)–C(9)	118·1°	C(7)—C(8)—C(9)	111·9°
N(1) –C(2)–N(3)	118·5	C(7)—C(8)—O(17)	113·4
N(1) –C(2)–N(11)	117·5	C(9)—C(8)—O(17)	111·5
N(3) –C(2)–N(11)	123·9	N(1) –C(9)—C(8)	106·9
C(2)—N(3)–C(4)	121·7	N(1) –C(9)—C(10)	107·6
N(3) –C(4)–C(10)	105·4	N(1) –C(9)—C(19)	107·2
N(3) –C(4)–O(18)	110·5	C(8)—C(9)—C(10)	116·2
C(10)–C(4)–O(18)	105·3	C(8)—C(9)—C(19)	117·6
C(6)—C(5)–C(10)	113·9	C(10)–C(9)—C(19)	100·8
C(6)—C(5)–O(12)	109·4	C(4)—C(10)–C(5)	116·8
C(10)–C(5)–O(12)	114·8	C(4)—C(10)–C(9)	97·8
C(5)—C(6)–C(7)	116·0	C(5)—C(10)–C(9)	112·7
C(5)—C(6)–O(13)	105·4	C(6)—C(14)–O(15)	112·4
C(5)—C(6)–C(14)	116·4	C(4)—O(18)–C(19)	109·3
C(7)—C(6)–O(13)	103·5	C(9)—C(19)–O(18)	104·5
C(7)—C(6)–C(14)	105·5	C(9)—C(19)–C(20)	120·4
O(13)–C(6)–C(14)	109·3	O(18)–C(19)–C(20)	106·2
C(6)—C(7)–C(8)	111·0	C(19)–C(20)–O(21)	116·7
C(6)—C(7)–O(16)	106·7	C(19)–C(20)–O(22)	124·0
C(8)—C(7)–O(16)	108·6	O(21)–C(20)–O(22)	119·3

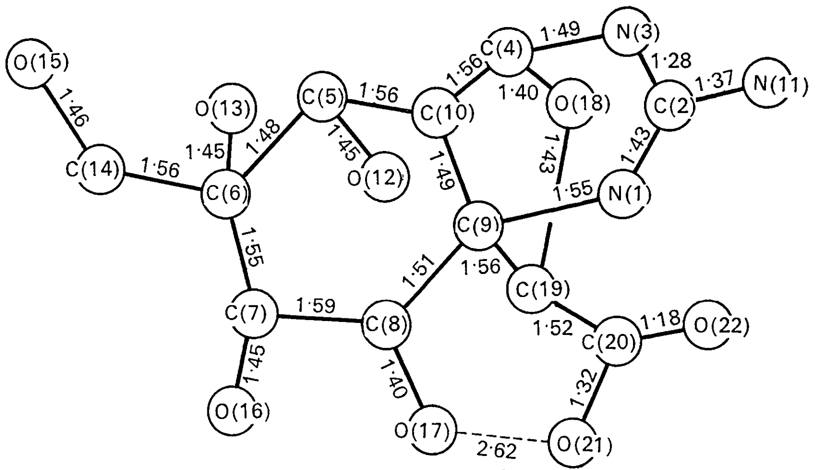


Fig. 4. Bond lengths (Å).

an angle of about 90° with C(10)–H around C(4)–C(10). Such a conformation can well explain the nuclear magnetic resonance spectra of this compound.

The intramolecular bond lengths are shown in Fig. 4 and the angles in Table 4. The estimated standard deviations in the atomic coordinates are approximately 0·005 Å for the bromine atom and 0·04 Å for the light atoms. Hence, the standard deviation of the bond length between the light atoms is about 0·06 Å and that of the bond angle is about 4°. None of the bond lengths and angles do deviate significantly from their normal values. The averages of the C–C and C–O bond lengths are 1·538 and 1·434 Å respectively. The dimensions of the carbonyl group are 1·18 Å for C(20)–O(22) and 1·32 Å for C(20)–O(21). The bond C(2)–N(3), 1·28 Å, in the nearly planar guanidine group, might possess considerable double bond character. The bond of C(9)–N(1), 1·55 Å, seems to be somewhat longer than the normal C–N bond length of 1·48 Å. An intramolecular hydrogen bond of length 2·62 Å is found between O(21) and O(17).

It is interesting that the configuration at C(19) is the antipode of that in bromoanhydrotetrodoic lactone hydrobromide (Tomiie, Furusaki, Kasami, Yasuoka, Miyake, Haisa & Nitta, 1963). A reasonable explanation for the apparent inconsistency has been given by considering the mechanism of chemical reaction in which these derivatives are produced from tetrodotoxin (Tsuda, Tamura, Tachikawa, Sakai, Amakasu, Kawamura & Ikuma, 1964; Tsuda, Ikuma, Kawamura, Tachikawa, Sakai, Tamura & Amakasu, 1964; Goto, Kishi, Takahashi & Hirata, 1964; Mosher, Fuhrman, Buchwald & Fisher, 1964; Woodward, 1964).

The crystal structure viewed along the *c* axis is illustrated in Fig. 5, where anticipated hydrogen bonds, except intramolecular ones, are shown by the broken lines. Hydrogen bond lengths and short intermolecular contacts are listed in Table 5. The donors of the hydrogen bonds in Table 5 are the atoms at *x*, *y*, *z*. There are two independent intermolecular NH···O and two OH···O hydrogen bonds, and these hydrogen bonds connect the molecules to form an infinite double-layered sheet parallel to the (100) plane. The bromine atoms are interposed between such double-layered sheets. Short contacts are found for Br···N(3), Br···O(13), Br···O(12), Br···O(15) and Br···N(11). Hydrogen atoms bonded with these oxygen and nitrogen atoms might be placed toward the bromine atom. Thus all

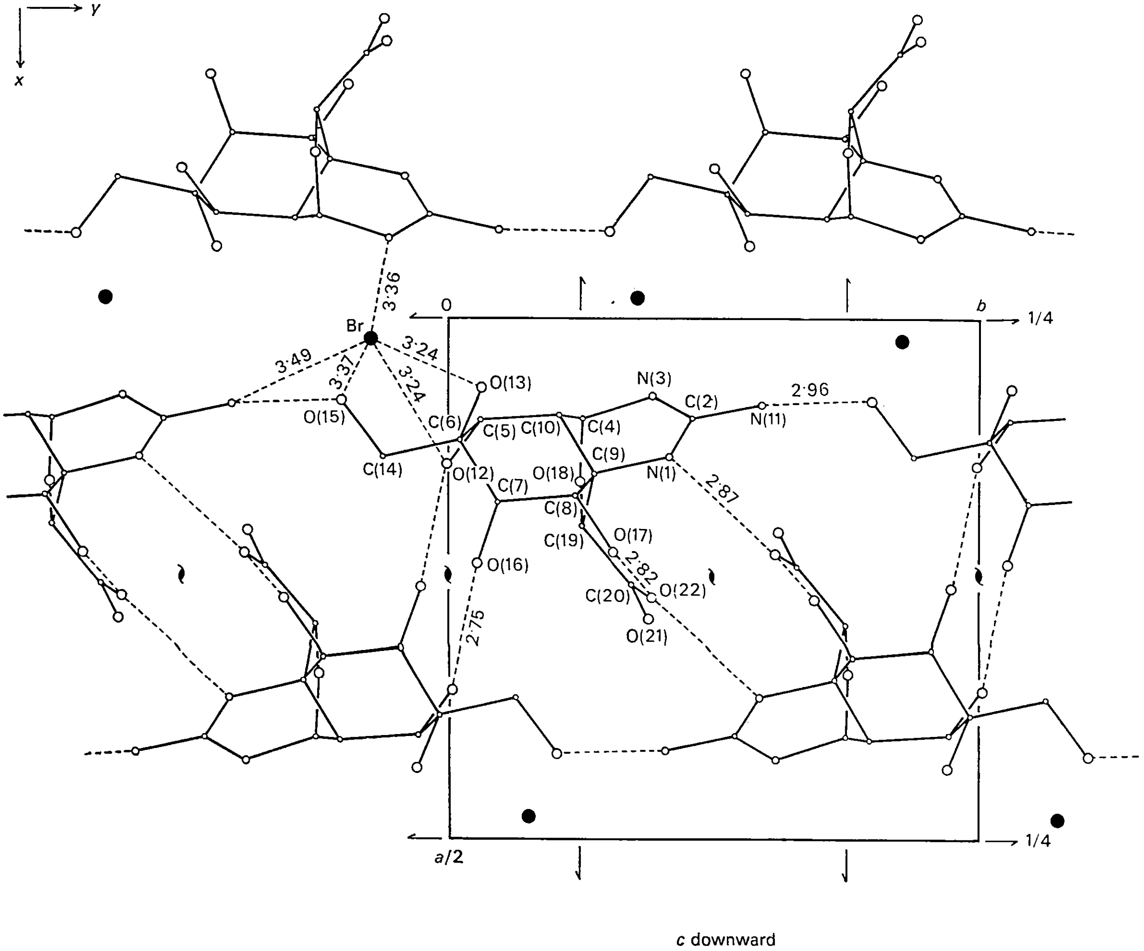


Fig. 5. Crystal structure viewed along the *c* axis. Intramolecular hydrogen bond O(21)---O(17) is not indicated here.

Table 5. *Hydrogen bonds and short intermolecular distances*

From atom at x, y, z	To atom	At position	Distance
	(*a*) Hydrogen bonds		
O(17)	O(22)	3	2·82 Å
N(11)	O(15)	4	2·96
O(16)	O(12)	6	2·75
N(1)	O(22)	7	2·87
	(*b*) Short intermolecular contacts		
Br	C(5)	1	3·70
Br	O(12)	1	3·24
Br	O(13)	2	3·24
Br	C(14)	2	3·67
Br	O(15)	2	3·37
Br	N(11)	5	3·49
Br	C(10)	8	3·88
Br	O(13)	8	3·56
Br	N(3)	9	3·36
Br	C(4)	9	3·72
C(4)	C(8)	2	3·76
O(18)	C(7)	2	3·33
O(18)	C(8)	2	3·07
O(18)	O(17)	2	3·06
C(7)	O(16)	6	3·42
C(14)	C(19)	6	3·61
C(14)	C(20)	6	3·23
C(14)	O(21)	6	3·56
C(14)	O(22)	6	3·36
O(15)	O(22)	6	3·56
O(16)	O(16)	6	3·59
O(21)	O(22)	7	3·12
N(11)	O(22)	7	3·50
O(17)	O(21)	7	3·56
O(17)	O(22)	7	3·32
O(13)	N(3)	8	3·51
O(13)	N(11)	8	3·43
O(15)	N(3)	8	3·32

Position 1	x	y	z	6	$\frac{1}{2}-x$	$-y$	$-\frac{1}{2}+z$
2	x	y	$1+z$	7	$\frac{1}{2}-x$	$1-y$	$-\frac{1}{2}+z$
3	x	y	$-1+z$	8	$-x$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$
4	x	$1+y$	z	9	$-x$	$-\frac{1}{2}+y$	$\frac{3}{2}-z$
5	x	$-1+y$	z				

All distances less than 3·9 Å between the reference molecule at x, y, z and the neighbouring molecules were calculated. Only those less than 3·9 Å for C · · · C and C · · · Br pairs and those less than 3·6 Å for the other pairs are listed.

the functional hydrogen atoms take part in the hydrogen bonding or interact with the bromine atom.

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