Acta Cryst. (1968). B**24**, 1147

The Crystal and Molecular Structure of an Anti-Leukemia Drug: Methylglyoxal Bisguanylhydrazone Dihydrochloride Monohydrate, $C_5N_8H_{12}.2HCl.H_2O$. Neutron and X-Ray Diffraction Studies*

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(*Received* 27 *September* 1967)

The compound methylglyoxal bisguanylhydrazone,

$$NH_2.C(NH).NH.N.C(CH_3).CH.N.NH.C(NH).NH_2,$$

which has previously been shown to have a marked activity toward human acute myelocytic leukemia, has been studied by X-ray and neutron diffraction in the form of the crystalline dihydrochloride monohydrate, $C_5H_{12}N_8.2HCl.H_2O$. The salt crystallizes in the orthorhombic space group $Pna2_1$ with four molecules in a unit cell of dimensions $a = 15{\cdot}64$ (2), $b = 12{\cdot}75$ (2), $c = 6{\cdot}29$ (1) Å. The structure consists of dipositive ions $C_5H_{14}N_8^{2+}$, Cl^- ions and free water molecules. The planar ion has the completely *trans* configuration of the chain. Hydrogen bonds of the type $N\text{–}H\cdots Cl^-$, $N\text{–}H\cdots O$, and $O\text{–}H\cdots Cl^-$ are formed. The dominant interaction determining the intermolecular packing is however a specific interaction involving π-orbitals on adjacent parallel molecules; the mean planes of the molecules are only 3·19 Å apart; the closest point of contact is between a $C=N$ double bond and the positively charged $C(NH_2)^+$ terminal portion of an adjacent molecule. The neutron and X-ray diffraction results are in good agreement with respect to bond lengths and thermal parameters of the non-hydrogen atoms. The hydrogen atoms were precisely located in the neutron diffraction study, and the average apparent N–H, O–H, and C–H bond lengths are 0·1 Å longer than those found in the X-ray study; this result is not surprising in view of the assumption of spherical atomic electron distributions in the X-ray refinement.

Introduction

Methylglyoxal bisguanylhydrazone (methyl GAG, Fig. 1) was shown by Freedlander & French (1958) to possess activity against L1210 mouse leukemia. It was later shown to be an effective agent against human acute myelocytic leukemia (Freireich, Frei & Karon, 1962), although its use is accompanied by undesirable side effects which render it of questionable clinical importance. A review of its application has been given by Carbone, Freireich, Frei, Rall, Karon & Brindley (1964).

When the methyl group or the hydrogen atom on the central two carbon atoms is replaced by another group, the compound loses its activity. This is in marked contrast to the usual behavior in oncolytic drugs, where replacement of side groups may lead to reduction but not to complete cessation of activity. It seemed worthwhile to investigate in detail the molecular structures of methyl GAG and related compounds to see whether there might be any clues to this behavior. This paper reports the structure of methyl $GAG.2HCl.H_2O$ as studied by X-ray and neutron diffraction. Future reports will be made of the structures of the free base methyl GAG and of substituted compounds such as dimethyl GAG. Any possible biological implications of the differences in structure will be reserved to such future reports.

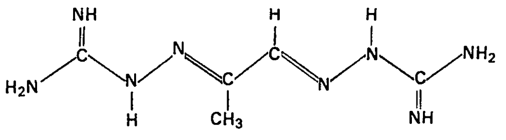

Fig. 1. Chemical formula of methylglyoxal bisguanylhydrazone.

Crystal data

Methylglyoxal bisguanylhydrazone dihydrochloride monohydrate, $C_5H_{16}ON_8Cl_2$. Orthorhombic. Probable space group $Pna2_1$. $a = 15{\cdot}64$ (2), $b = 12{\cdot}75$ (2), $c = 6{\cdot}29$ (1) Å. $V = 1254$ Å^3. Calculated density for four formula units per cell $= 1{\cdot}457$ g.cm^{-3}. The colorless crystals are chunky prismatic.

Experimental

X-ray study

Samples were obtained by recrystallization from water slightly acidified with HCl. A spherical crystal of diameter 0·19 (1) mm was obtained by grinding. All data were collected using Ni filtered Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å). Weissenberg and precession photographs revealed that the systematic extinctions were $0kl$, $k+l=2n+1$; $h0l$, $h=2n$. The possible space groups are $Pna2_1$ and $Pnam$. The former was adopted because of a positive piezoelectric effect. This was confirmed by the structure solution found and by the observation

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* Research carried out under the auspices of the United States Atomic Energy Commission.

of anomalous scattering effects which led to a determination of the absolute configuration.

Multiple-film equi-inclination integrated Weissenberg data were taken from a crystal mounted with the c axis parallel to the goniometer head axis. The intensities of the 1260 possible reflections with $2\theta \leq 70°$ were visually estimated by comparison with a standard film strip prepared by timed exposures of a spot equal in dimensions to that obtained from the crystal. Of these, 391 were too weak to be recorded. An absorption coefficient for a spherical crystal was applied using a value of $\mu r = 0{\cdot}442$ and the tabulated corrections (*International Tables for X-ray Crystallography*, 1959) linearly interpolated. The data from the several levels were placed on a common scale by comparison with a smaller set of data collected from the same crystal by use of a θ–2θ scan technique on a four-circle diffractometer. The diffractometer data were also used for the confirmation of the absolute configuration of the crystal studied. The absorption-corrected intensities were multiplied by the appropriate Lorentz–polarization factors to obtain F^2 values.

A Wilson (1942) plot was used to obtain an approximate scale factor K and overall temperature factor B. A sharpened origin-removed Patterson function with coefficients

$$F^2_{\text{modified}} = KF^2_{\text{obs}} \frac{\sum\limits_i Z_i^2}{\sum\limits_i f_i^2\,(\sin\theta)} \exp\,(2B\,\sin^2\theta/\lambda^2) - \sum_i Z_i^2\,,$$

where Z_i and f_i are the atomic number and scattering factor of the ith atom respectively, revealed the positions of the two chloride ions in the asymmetric unit. (All atoms were found to be in the general positions of $Pna2_1$: xyz; $\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}+z$; $\frac{1}{2}+x, \frac{1}{2}-y, z$; $-x, -y, \frac{1}{2}+z$.) The other atoms, excluding hydrogen, were found in difference electron density syntheses, and the structure was refined by a series of full-matrix least-squares refinements. Weights used in these refinements were inversely proportional to the estimated variance $\sigma^2(F)$ with $\sigma^2(F)$ defined as follows:

For the 819 film data used in the refinements

$$\sigma^2(F) = 3{\cdot}24 \quad \text{for } F < 18$$
$$\sigma^2(F) = 0{\cdot}01F^2 \quad \text{for } F \geq 18\,.$$

For the fifty reflections observed by counting, the counter values were used in preference to the film data. These were assigned a separate scale factor and weights based on

$$\sigma(F) = 0{\cdot}05F\,.$$

A final difference map revealed the positions of all hydrogen atoms along the backbone of the molecule. The two hydrogen atoms of the water molecule could not be readily identified. The neutron diffraction data discussed below became available at this point, and the positions of all hydrogen atoms as determined from the neutron data were taken as the initial positions for the final least-squares refinement. In this refinement, anisotropic thermal parameters for the heavy atoms, positional parameters for all atoms, and a single scale factor were varied. The thermal parameters for hydrogen atoms were kept fixed at the final values from the neutron diffraction study. In this final refinement, the spherical scattering factor proposed by Stewart, Davidson & Simpson (1965) (SDS) was used for the hydrogen atoms in order to account partially for the changes in electron density for a bonded hydrogen atom*. Scattering factors for C, N and Cl^- were Hartree–Fock scattering factors as tabulated in *International Tables for X-ray Crystallography* (1962, p.202). The parameters resulting from this refinement are presented in Table 1 and the observed and final calculated structure factors in Table 2. The final values of the agreement factors were

$$R = \frac{\Sigma\,||F_{\text{obs}}| - |F_{\text{calc}}||}{\Sigma\,|F_{\text{obs}}|} = 0{\cdot}0293$$

$$R' = \left\{\frac{\Sigma\, w||F_{\text{obs}}| - |F_{\text{calc}}||^2}{\Sigma\, w|F_{\text{obs}}|^2}\right\}^{1/2} = 0{\cdot}0797\dagger$$

Absolute configuration

The effects of anomalous scattering were correctly included in the structure factor calculations (Ibers & Hamilton, 1964). The anomalous scattering contributions were those tabulated (*International Tables for X-ray Crystallography*, 1962, p.214). A refinement of the structure with an absolute configuration opposite to that in Table 1 led to a value of the ratio of the two agreement factors $\mathscr{R} = R'(+)/R'(-) = 1{\cdot}006$. Use of the $\mathscr{R}$ test (Hamilton, 1965) indicates that the difference is significant and that the choice of handedness implied by the parameters in Table 1 with a right-handed coordinate system is correct. The results of the statistical test were confirmed by diffractometer measurement of several Friedel pairs of reflections which exhibited large anomalous scattering effects. The absolute configuration is of no molecular significance, and crystals of each chirality probably exist in any batch.

Neutron diffraction

A prismatic crystal whose bounding planes are given in Table 3 was mounted on a goniometer head with

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* Refinement was also carried out using the free atom scattering factor for hydrogen. There was no significant difference between the two refinements, although the X–H bond lengths were slightly greater on the average when the SDS scattering factor was used (see Table 4). Allowing for variation of isotropic thermal parameters for hydrogen with either scattering factor led to no useful information; in particular, the thermal parameters so obtained made no more sense in one case than in the other. We conclude that despite the relatively high precision of the X-ray data set it was not good enough to obtain meaningful information regarding hydrogen atom thermal motion; we consider this to be typical of photographic X-ray data.

† Because of the scaling factors used the counter data contribute more heavily to R than to R'. R' gives a more realistic feeling for the quality of the agreement.

Table 1. *Final fractional coordinates and thermal parameters for methyl GAG*.$2HCl.H_2O$

For each atom, the neutron result is given on the first line and the X-ray result on the second. The estimated standard deviations from the least-squares refinements are given in parentheses in units of the last significant figure in the parameter value itself. The *z* parameter of Cl(1) was not refined; this arbitrary choice fixes the origin in this space group. The Debye–Waller factor is defined as

$$\exp\{-2\pi^2 \sum_{i,j} h_i h_j a_i^* a_j^* U_{ij}\}\ .$$

	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Cl(1)	0·1045 (4)	0·4077 (6)	0·0	61 (5)	56 (4)	35 (3)	−2 (3)	14 (4)	6 (3)
	0·10440 (1·7)	0·40881 (2·0)	0·0	59·7 (1·5)	45·8 (1·3)	44·4 (1·9)	−5·2 (1·1)	21·3 (1·5)	−2·6 (1·3)
Cl(2)	0·0812 (5)	0·0307 (6)	−0·1648 (1·6)	54 (5)	44 (4)	49 (4)	10 (3)	3 (4)	7 (3)
	0·07990 (1·5)	0·03036 (1·8)	−0·16820 (6·4)	45·2 (1·2)	39·5 (1·2)	53·1 (2·1)	9·4 (1·7)	1·6 (1·4)	10·7 (1·8)
N(1)	−0·3032 (4)	0·2556 (5)	−0·3099 (16)	35 (4)	38 (3)	25 (3)	6 (3)	10 (3)	6 (3)
	−0·3036 (6)	0·2562 (7)	−0·3119 (16)	50 (5)	36 (4)	30 (5)	2 (4)	5 (4)	1 (4)
N(2)	−0·2254 (4)	0·1184 (5)	−0·4392 (14)	45 (4)	31 (3)	20 (3)	−7 (3)	9 (3)	2 (3)
	−0·2252 (5)	0·1167 (6)	−0·4432 (16)	48 (4)	26 (4)	25 (5)	−3 (3)	6 (4)	8 (3)
C(1)	0·2493 (5)	0·2867 (6)	−0·4584 (18)	31 (6)	19 (3)	28 (5)	9 (3)	4 (4)	−9 (3)
	0·2479 (5)	0·2845 (6)	−0·4555 (18)	32 (4)	28 (4)	29 (7)	3 (3)	8 (4)	4 (5)
N(3)	−0·2702 (4)	−0·2262 (5)	−0·1231 (16)	33 (4)	30 (3)	25 (3)	−5 (2)	−9 (3)	4 (2)
	−0·2702 (4)	−0·2263 (5)	−0·1238 (13)	37 (4)	25 (3)	16 (5)	−1 (3)	−5 (4)	−1 (3)
N(4)	0·1767 (4)	−0·2275 (4)	−0·2706 (15)	33 (4)	20 (3)	29 (3)	6 (2)	−5 (3)	−0 (2)
	0·1775 (4)	−0·2264 (5)	−0·2710 (14)	31 (4)	26 (3)	25 (5)	−3 (3)	−10 (4)	−4 (4)
C(2)	−0·1517 (5)	0·2845 (6)	0·0723 (19)	27 (5)	22 (3)	32 (5)	1 (3)	0 (4)	−6 (4)
	−0·1516 (5)	0·2840 (6)	0·0713 (15)	26 (4)	30 (4)	15 (6)	3 (3)	−3 (4)	10 (4)
C(3)	−0·1008 (5)	0·2246 (6)	−0·0771 (18)	25 (5)	30 (4)	21 (4)	6 (3)	5 (3)	8 (3)
	−0·0996 (5)	0·2256 (7)	−0·0794 (17)	26 (4)	32 (4)	25 (5)	2 (4)	−4 (4)	4 (4)
N(5)	−0·0714 (4)	0·2706 (5)	−0·2464 (15)	38 (4)	32 (3)	13 (3)	−1 (3)	4 (3)	5 (2)
	−0·0733 (5)	0·2720 (5)	−0·2477 (13)	36 (4)	27 (4)	23 (5)	1 (3)	1 (4)	−2 (3)
N(6)	−0·0287 (5)	0·2077 (5)	−0·3859 (15)	44 (4)	36 (3)	14 (3)	6 (3)	5 (3)	5 (2)
	−0·0297 (6)	0·2092 (6)	−0·3873 (16)	47 (4)	24 (4)	33 (6)	5 (3)	14 (4)	5 (4)
C(4)	−0·0090 (5)	−0·2563 (6)	−0·0562 (17)	17 (4)	34 (4)	23 (4)	7 (4)	−4 (4)	−4 (4)
	−0·0078 (6)	−0·2553 (6)	−0·0561 (16)	30 (4)	30 (4)	27 (6)	−6 (3)	0 (4)	1 (4)
N(7)	0·4947 (5)	−0·1421 (5)	−0·0758 (15)	56 (4)	25 (3)	32 (3)	0 (3)	−9 (3)	8 (2)
	0·4943 (6)	−0·1425 (6)	−0·0773 (16)	47 (4)	37 (4)	36 (5)	9 (3)	−10 (4)	1 (4)
N(8)	−0·0476 (5)	−0·1944 (5)	−0·1927 (17)	44 (4)	42 (3)	34 (3)	7 (3)	−14 (3)	2 (3)
	−0·0475 (5)	−0·1922 (7)	−0·1970 (17)	43 (4)	50 (5)	33 (5)	11 (4)	−6 (4)	1 (5)
(O)	0·2180 (9)	0·0130 (9)	−0·5313 (21)	57 (7)	41 (6)	36 (6)	4 (5)	6 (6)	11 (5)
	0·2195 (5)	0·0144 (6)	−0·5358 (15)	51 (4)	41 (4)	44 (5)	−5 (3)	7 (4)	4 (4)
C(5)	−0·1710 (7)	0·3966 (7)	0·0394 (18)	51 (6)	35 (4)	19 (4)	6 (4)	4 (4)	2 (3)
	−0·1708 (8)	0·3967 (7)	0·0384 (21)	69 (6)	22 (4)	32 (6)	16 (4)	12 (6)	4 (4)
H(1)	0·3999 (11)	−0·2090 (13)	0·3019 (26)	37 (9)	44 (8)	13 (7)	11 (7)	−17 (7)	−3 (6)
	0·311 (6)	−0·219 (13)	0·264 (13)						
H(2)	−0·1737 (14)	−0·1690 (19)	0·1704 (44)	58 (13)	65 (14)	78 (15)	−13 (10)	−39 (13)	25 (11)
	−0·158 (7)	−0·168 (9)	0·195 (21)						
H(3)	0·2358 (17)	−0·0741 (15)	0·1921 (31)	126 (19)	30 (8)	32 (10)	8 (10)	−9 (12)	−11 (8)
	0·243 (9)	−0·086 (8)	0·146 (18)						
H(4)	−0·1834 (15)	0·0909 (15)	0·4516 (29)	99 (16)	44 (10)	18 (8)	20 (10)	10 (9)	1 (7)
	−0·177 (7)	0·091 (8)	0·462 (16)						
H(5)	0·2545 (12)	0·1472 (13)	0·3802 (34)	41 (10)	31 (8)	50 (10)	−4 (7)	−6 (8)	−2 (7)
	0·242 (5)	0·152 (7)	0·419 (17)						
H(6)	−0·1489 (22)	0·4466 (20)	0·1681 (42)	152 (24)	56 (14)	39 (11)	−26 (15)	−21 (15)	−7 (9)
	−0·139 (10)	0·444 (9)	0·142 (20)						
H(7)	−0·1424 (21)	0·4233 (20)	−0·1001 (42)	137 (22)	53 (12)	52 (12)	−8(13)	55 (15)	13 (10)
	−0·153 (10)	0·404 (10)	−0·054 (22)						
H(8)	−0·2435 (22)	0·4082 (22)	0·0462 (64)	142 (25)	64 (14)	105 (21)	43 (17)	−3 (21)	29 (17)
	−0·223 (10)	0·409 (11)	0·020 (33)						
H(9)	0·0891 (11)	−0·1386 (14)	0·4587 (34)	41 (10)	40 (8)	52 (10)	5 (6)	−4 (9)	−11 (8)
	0·072 (5)	−0·147 (8)	0·480 (18)						
H(10)	0·0138 (17)	−0·1365 (25)	0·1542 (39)	93 (17)	88 (20)	38 (10)	4 (15)	−28 (12)	−19 (10)
	0·017 (9)	−0·164 (11)	0·136 (20)						
H(11)	0·0318 (16)	0·1208 (20)	0·3000 (46)	73 (16)	72 (13)	77 (15)	−26 (12)	36 (14)	−9 (12)
	0·016 (7)	0·115 (9)	0·285 (21)						
H(12)	0·0705 (12)	0·2291 (18)	0·1716 (32)	56 (12)	84 (14)	22 (8)	6 (10)	14 (9)	15 (9)
	0·070 (7)	0·230 (9)	0·169 (16)						
H(13)	−0·4664 (13)	0·1082 (16)	0·2957 (34)	59 (12)	54 (11)	43 (10)	−14 (10)	5 (10)	−26 (9)
	−0·465 (7)	0·111 (8)	0·323 (19)						
H(14)	−0·4851 (18)	−0·0977 (17)	0·0537 (45)	107 (17)	50 (11)	73 (14)	20 (13)	1 (12)	12 (11)
	−0·470 (8)	−0·099 (9)	0·035 (24)						
H(15)	−0·1742 (16)	−0·0051 (18)	0·0811 (38)	73 (14)	61 (11)	44 (11)	−17 (10)	−13 (11)	1 (10)
	−0·192 (8)	0·007 (9)	0·063 (21)						
H(16)	0·2698 (17)	−0·0269 (20)	−0·4732 (42)	79 (14)	73 (14)	50 (13)	20 (13)	1 (12)	12 (11)
	0·280 (8)	−0·004 (9)	−0·509 (21)						

Table 2. *Observed and calculated structure factor magnitudes based on the parameters of Table* 1 *for methyl GAG*.2HCl.H$_2$O

The X-ray values are in electrons per cell and include anomalous scattering contributions. The neutron values are in 10^{-13} cm per cell. The calculated $F(000)$ for neutrons is 152·48. The neutron scattering lengths were $\bar{b}_{\mathrm{C}} = +0{\cdot}66$, $\bar{b}_{\mathrm{N}} = +0{\cdot}94$, $\bar{b}_{\mathrm{O}} = +0{\cdot}58$, $\bar{b}_{\mathrm{Cl}} = +0{\cdot}98$, $\bar{b}_{\mathrm{H}} = -0{\cdot}378$.

H	K	XRAY OBS	XRAY CAL	NEUTRON OBS	NEUTRON CAL
L= 0					
0	0		582		
2	0	7	6	25	25
4	0	34	34	49	48
6	0	60	61	54	52
8	0	7	6	9	10
10	0	33	33	66	65
12	0	34	35		
14	0	9	9		
1	1	10	10		
2	1	33	33	73	84
3	1	12	11	10	5
4	1	41	38	44	40
5	1	15	15	8	3
6	1	10	9	23	26
7	1	64	69	78	74
8	1	9	9	22	20
9	1	13	13	14	18
10	1			38	36
11	1	12	11		
12	1	19	19		
13	1	20	22		
14	1	16	16	76	90
17	1	8	9		
0	2	99	98		
1	2	20	19	34	37
2	2			29	29
3	2	7	5	29	32
4	2	61	60	92	100
5	2	6	8	26	23
6	2	55	57	52	57
7	2	40	40	65	63
8	2	7	7		
9	2	9	7	51	54
10	2	17	19	27	29
11	2	30	30		
13	2	16	16	35	35
14	2	15	14	29	30
15	2	9	8		
16	2	9	9		
1	3	39	39	52	53
2	3	97	98	129	156
3	3	17	17	14	9
4	3	78	77	50	53
5	3	16	16	3	12
6	3	36	36	68	57
7	3	23	22		
8	3	39	40	33	40
9	3	16	17	44	46
10	3	12	10	43	43
11	3	18	17		
12	3	11	11		
13	3	31	31	87	90
14	3	19	20	31	70
15	3	9	10		
0	4	94	96		
2	4	17	18		
3	4	9	9	52	50
4	4	19	19	60	59
5	4	11	11	57	33
6	4	27	27		
7	4	15	16		
8	4	37	37	30	29
9	4	8	8		
11	4	9	8		
12	4	17	16	33	29
13	4	16	14		
14	4	16	15		
15	4	6	7		
17	4	12	12		
18	4	10	11		
1	5	83	95	88	95
2	5	15	15		
3	5	6	5		
4	5	12	12	65	54
5	5	26	28	21	0
6	5	9	7		
7	5	16	17		
8	5	32	33		
9	5			20	19
10	5	9	8		
11	5	26	24	60	63
13	5	19	17	90	84
14	5			30	32
15	5	11	11		
16	5	12	11		
0	6	69	70	104	101
1	6	30	30	66	64
2	6	20	18	62	67
3	6	34	35	33	25
4	6	31	30	44	42
5	6	31	32	70	80
6	6			17	9
7	6	24	24	46	46
8	6	12	10	16	21
9	6	24	22		
10	6	24	22	39	50
11	6	15	12		
12	6	9	7	63	58
13	6	17	16	82	82
14	6	16	16	24	26
15	6	19	21		
1	7	7	7	79	78
2	7	31	30	54	55
3	7	36	33	57	55
4	7	9	8	40	44
5	7	25	24	57	50
6	7	29	29	43	46
7	7	28	26	83	91
8	7	14	14		
9	7	16	13	19	32
10	7	23	21		
11	7	11	11	82	82
12	7	24	23	69	68
13	7	19	20	87	81
14	7	14	14	31	28
0	8	42	43	94	98
1	8	32	35	15	12
2	8	22	23	95	96
3	8	35	36		
4	8	24	23	20	26
5	8	20	19	49	49
7	8	31	32	75	67
9	8	19	21		
10	8	16	16	63	62
11	8	11	11	57	58
12	8	6	7	39	40
13	8	18	20	60	57
14	8	12	11	50	50
1	9	8	9	8	7
2	9	6	6		
5	9	22	20	68	57
6	9	22	21		
10	9	9	9		
11	9			19	23
12	9			36	36
13	9	10	9	55	55
14	9	5	8		
0	10	22	23		
1	10	17	16		
2	10	13	11		
3	10	26	25	32	38
4	10	11	10		
5	10	9	10		
6	10	7	10		
7	10	16	14		
9	10	21	22	40	38
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2	9	19	21	36	37
3	9	16	14	63	65
4	9	22	21	40	38
5	9	11	10	38	36
6	9	10	9	45	45
8	9	9	9		
10	9	13	15		
11	9	6	6		
0	10	25	26	65	65
1	10			22	23
3	10	23	24	71	68
4	10	13	11	42	38
5	10	9	7	37	38
6	10	14	15	40	37
9	10	10	10		
10	10	7	8		
3	11	11	11	53	50
4	11	12	13	31	34
0	12	22	25	71	73
1	12			23	26
2	12	8	8	24	28
3	12	16	18		
4	12	8	9		
5	12	6	6		
6	12	7	8		
1	13	8	8		
7	13			30	35
L= 5					
2	0	23	23	40	42
4	0	30	30		
6	0	36	38	145	137
8	0	31	35	94	97
10	0	11	11	33	32
12	0	9	10		
0	1	14	14		
1	1	21	21	83	82
2	1	12	12	65	61
3	1	9	7		
4	1	8	8		
5	1	14	13		
7	1	9	9		
8	1	8	8	37	38
9	1	15	14		
10	1	13	11	33	34
11	1	15	15		
1	2	17	17	54	48
2	2	20	21	46	47
3	2	7	6	31	26
4	2	6	7	53	49
5	2	15	14	76	69
6	2	16	15	76	78
7	2	13	10	39	37
8	2			35	34
9	2	15	15	26	29
11	2	11	9	9	11
0	3	27	29	84	84
1	3	8	8	13	11
2	3			13	17
3	3	22	22	50	49
4	3	23	24	67	57
5	3	13	12	46	39
6	3	27	28	83	86
7	3	9	9	33	33
9	3	10	11	38	39
10	3	15	15	31	31
1	4	10	9		
2	4	15	13	66	61
3	4	11	11	36	28
4	4	16	16	58	59
5	4	13	12	26	25
6	4	8	9	68	68
7	4	6	8	44	44
8	4	15	15	44	39
9	4			18	21
10	4	13	14	41	41
0	5	16	14	40	27
3	5	16	15		
6	5	11	10	78	75
7	5	29	30	104	103
9	5	14	12	33	30
10	5			38	39
1	6	21	21	61	59
2	6	11	9		
3	6			11	7
4	6	16	14	60	57
5	6			62	62
6	6	14	14	37	39
7	6	19	19	70	70
8	6			31	34
9	6			31	32
10	6	12	10		
11	6	10	9		
0	7	15	15	41	45
1	7	6	8		
2	7			16	19
3	7	17	17	44	45
4	7			21	10
5	7	10	8		
6	7	24	27	82	83
7	7			54	54
1	8	14	13		
2	8			28	28
3	8	13	11	34	35
4	8	14	13	66	64
5	8	22	23	53	56
6	8			34	33
7	8	13	13	48	50
8	8			42	46
9	8	11	11		
0	9	14	13	28	36
1	9	6	6	30	27
2	9	8	9	36	30
3	9	9	10	30	30
4	9	8	9	20	18
7	9			40	38
1	10	10	11	33	32
2	10	10	11		
5	10	8	8		
6	10	9	9		
7	10	11	12		
8	10			41	42
0	11	7	6		
1	11	5	5		
3	11	9	10		
4	11	10	10	40	37
7	11			48	51
4	12			19	23
6	12			16	19
7	12			12	35
L= 6					
0	0			108	103
6	0			28	29
8	0			25	27
1	1			19	12
2	1			91	90
4	1			30	25
6	1			21	25
0	2			107	119
1	2			31	32
2	2			128	137
3	2			32	28
4	2			36	35
5	2			32	38
6	2			26	25
1	3			22	21
2	3			43	42
3	3			31	31
4	3			24	21
0	4			37	36
1	4			17	11
2	4			64	63
4	4			52	55
6	4			22	26
1	5			52	53
2	5			61	64
3	5			33	31
5	5			31	31
7	5			33	32
0	6			66	66
2	6			65	65
4	6			16	16
1	7			34	36
2	7			25	27
4	7			36	37
0	8			47	48
2	8			31	34
1	10			23	27
L= 7					
8	0			21	17
6	2			13	30
7	2			24	25
2	3			77	81
1	4			31	28
2	4			42	42
3	4			36	27
8	4			14	21
0	5			52	52
2	5			52	48
7	5			10	34
2	6			41	42
2	7			33	31
5	8			30	35

the c axis of the crystal parallel to the axis of the goniometer head. The intensities of 722 reflections were measured by the θ–2θ scan technique on the HORROR automatic diffractometer (Tillinger, 1962) at the Brookhaven Graphite Research Reactor. The mean neutron wavelength was 1·067 Å. Structure amplitudes were obtained by correction of the intensities for background and absorption ($\mu=2{\cdot}23$). A structure factor calculation based on heavy atom positions from the X-ray study was followed by calculation of a difference map which clearly revealed the positions of all the hydrogen atoms. A series of least-squares refinements led to the parameters in Table 1. Each reflection was assigned a weight inversely proportional to the estimated variance of the observation:

$$W^{-1}=\sigma^2(F)=\sigma^2(F^2)/4F^2$$

and

$$\sigma^2(F^2)=\sigma^2{}_{\text{count}}(F^2)+k^2F^4$$

with k equal to 0·05 and $\sigma^2_{\text{count}}(F^2)$ being obtained from the Poisson counting statistics. The 95 positional parameters and 192 anisotropic thermal parameters were refined alternately in two blocks. The observed and calculated structure magnitudes are reported in Table 2. The final values of R and R' were 0·072 and 0·073 respectively.

Table 3. *Bounding planes for crystal used in neutron diffraction study*

$A_1x+A_2y+A_3z=A_4$ (cm).

A_1	A_2	A_3	A_4	Indices
	−1·0000		−0·0867	010
	+1·0000		−0·0800	$0\bar{1}0$
+0·8526	+0·5230		−0·0623	$\bar{2}\bar{1}0$
+0·8526	−0·5230		−0·0817	$\bar{2}10$
−0·8526	−0·5230		−0·0987	210
−0·8526	+0·5230		−0·0647	$2\bar{1}0$
		+1·000	−0·1500	$00\bar{1}$
−0·2430	+0·6400	−0·750	−0·0542	'cleaved' surface

Comparison of the neutron and X-ray studies

The X-ray study reported here is perhaps as good as it is possible to do with film data. The neutron study is of satisfactory quality but is not as good as we consider to be easily possible. The comparison between the two is of some interest.

First of all, a comparison of the difference maps shown in Fig. 2 shows clearly the superiority of the neutron method over the X-ray method for hydrogen atom location. Both maps are based on the phases from the heavy atoms alone. In the neutron case, use of the phases for all the atoms (hydrogen atoms included) did

Table 4. *Bond lengths in methyl GAG*.$2HCl.H_2O$

X-ray 1 is from refinement based on the SDS scattering factor for hydrogen. X-ray 2 is based on the free atom scattering factor for hydrogen. (The errors for the latter case are comparable to those for X-ray 1.) We do not consider the differences between neutron and X-ray values for the heavy atoms to be significant. The neutron values for the distances involving hydrogen are to be preferred. For the heavy atoms, the column labeled *Mean* is the mean of X-ray 1 and neutron.

Distance	X-ray 1	X-ray 2	Neutron	Mean
N(1)–C(1)	1·317 (13)	1·316	1·355 (11)	1·336
N(2)–C(1)	1·330 (11)	1·334	1·279 (11)	1·304
N(7)–C(4)	1·310 (12)	1·310	1·302 (10)	1·306
N(8)–C(4)	1·349 (12)	1·347	1·313 (11)	1·331
N(6)–C(4)	1·347 (12)	1·350	1·371 (10)	1·359
N(3)–C(1)	1·339 (12)	1·336	1·331 (10)	1·335
N(4)–C(2)	1·298 (11)	1·296	1·286 (11)	1·292
N(5)–C(3)	1·280 (12)	1·278	1·299 (10)	1·290
N(3)–N(4)	1·375 (9)	1·376	1·378 (8)	1·377
N(5)–N(6)	1·370 (10)	1·371	1·364 (9)	1·366
C(2)–C(3)	1·454 (12)	1·453	1·449 (11)	1·452
C(2)–C(5)	1·482 (11)	1·483	1·475 (11)	1·478
O——H(15)	0·81 (12)	0·75	0·99 (3)	
O——H(16)	0·98 (12)	0·94	1·02 (3)	
N(1)–H(1)	0·69 (9)	0·70	1·01 (2)	
N(1)–H(2)	1·14 (11)	1·04	1·03 (2)	
N(2)–H(3)	0·74 (12)	0·75	1·01 (2)	
N(2)–H(4)	1·02 (10)	0·90	1·01 (2)	
N(7)–H(13)	0·88 (11)	0·84	1·02 (2)	
N(7)–H(14)	1·06 (12)	1·04	1·04 (2)	
N(8)–H(11)	1·10 (11)	1·07	0·97 (3)	
N(8)–H(12)	1·03 (10)	0·91	1·03 (2)	
N(3)–H(5)	1·08 (9)	0·99	1·04 (2)	
N(6)–H(10)	0·63 (13)	0·65	0·97 (3)	
C(3)–H(9)	1·15 (9)	1·15	1·13 (2)	
C(5)–H(6)	1·01 (12)	0·94	1·09 (2)	
C(5)–H(7)	0·65 (12)	0·72	1·04 (2)	
C(5)–H(8)	0·84 (14)	0·87	1·15 (4)	

not lead to a map of appreciably better quality; this was a surprise to us in view of the large hydrogen atom contribution to the structure factors.

The reader may judge for himself the quality of the agreement between the parameters presented in Table 1. More interesting perhaps is the comparison between bond lengths in Table 4, bond angles in Fig. 3, and principal axes of the thermal motion in Table 5. The agreement for the heavy atoms is satisfactory for the most part. The agreement between the shapes of the thermal ellipsoids is best seen by an examination of Fig. 4. For most of the atoms, the calculated direction cosines of the principal axis directions agree within the standard deviations of the two measurements; there are however some exceptions.

Except for differences in intermolecular environment (particularly in the hydrogen bonding) the bonds N(1)–C(1), N(2)–C(1), N(7)–C(4), N(8)–C(4) are almost chemically equivalent. The agreement between the four distances determined by X-ray diffraction and the four determined by neutron diffraction is not so good as the standard deviations suggest it should be. Most of the discrepancy is involved in the neutron values for N(1)–C(1) and N(2)–C(1), which suggests that the position of C(1) is in error by more than the standard deviation would indicate. This underestimation of positional errors based on the least-squares standard deviations is probably not uncommon. We believe that no significant differences exist between these bond lengths and prefer to quote the results of this study in terms of the mean value for the two techniques and

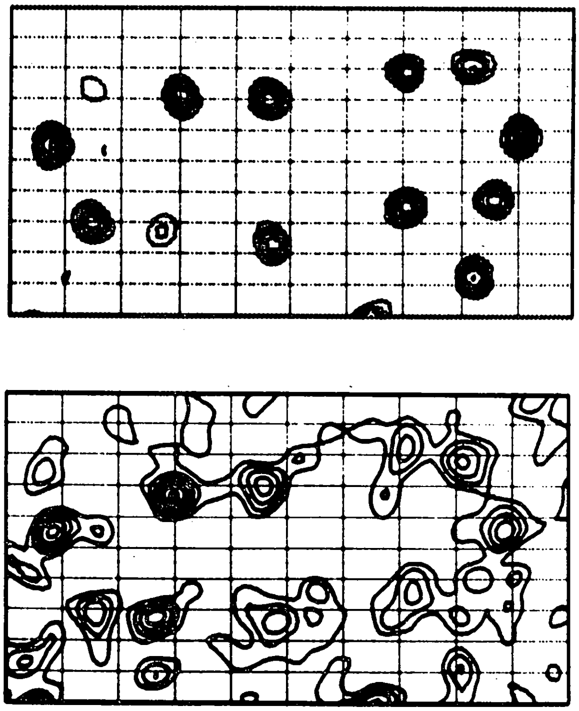
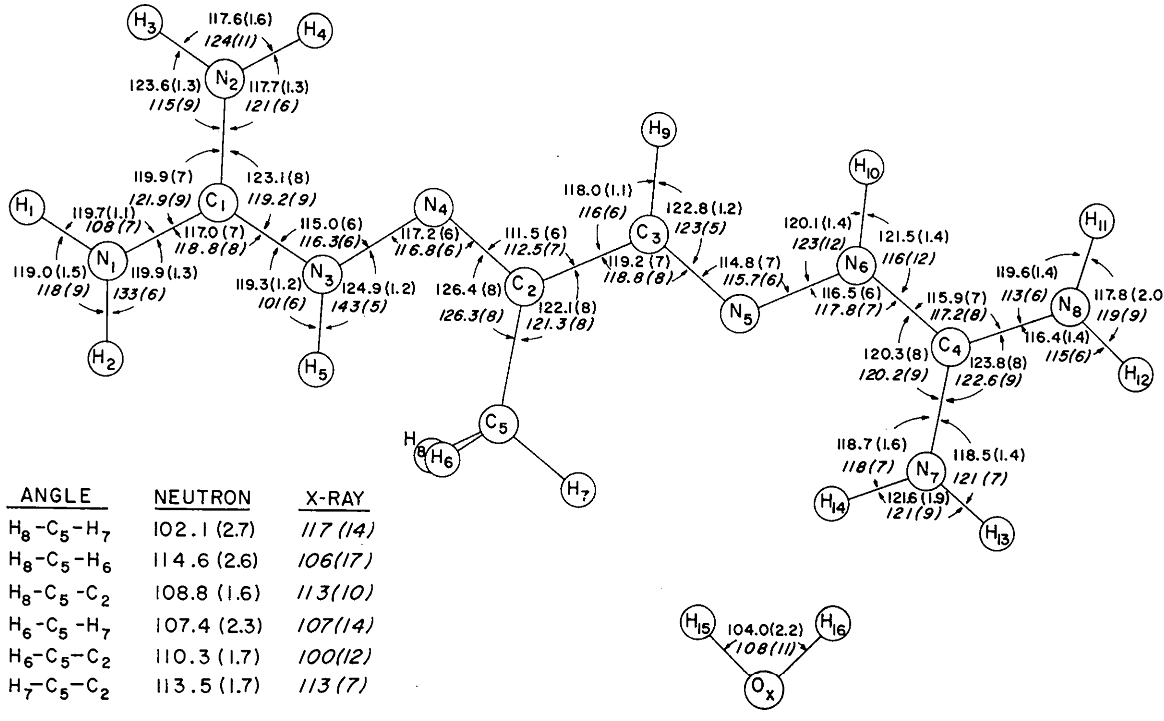


Fig. 2. Difference density syntheses in molecular plane of methyl GAG. Neutron above, X-ray below. Starting in the upper left-hand corner of the neutron map, the single-contour is H(11). In clockwise order around the molecule then appear H(10), H(9), H(4), H(3), H(1), H(2), H(5), H(7), H(14), H(13), H(12). The peak near the lower border is not part of this molecule.



ANGLE	NEUTRON	X-RAY
$H_8-C_5-H_7$	102.1 (2.7)	*117 (14)*
$H_8-C_5-H_6$	114.6 (2.6)	*106(17)*
$H_8-C_5-C_2$	108.8 (1.6)	*113(10)*
$H_6-C_5-H_7$	107.4 (2.3)	*107(14)*
$H_6-C_5-C_2$	110.3 (1.7)	*100(12)*
$H_7-C_5-C_2$	113.5 (1.7)	*113 (7)*

Fig. 3. Bond angles from X-ray and neutron studies of the methyl GAG molecule; X-ray values in italics.

four bonds as well as for the equivalent bond length pairs: [N(6)–C(4), N(3)–C(1)], [N(4)–C(2), N(5)–C(3)] and [N(3)–N(4), N(5)–N(6)]. The resulting idealized average structure is presented in Fig. 5.

The molecular structure

The crystal is ionic. The hydrogen atoms supplied by the acid have become attached to the imino groups of the base to form doubly positively-charged ions $[C_5H_{14}N_8]^{2+}$. The positive charge is probably spread nearly equally over the terminal ends of the molecule centered at C(1) and C(4). The bond orders and related formal charges derived from the bond lengths suggest that N(3) and N(6) are slightly more positively charged than the terminal nitrogens.

If we adopt 1·47 and 1·27 as the standard C–N single and double bond distances, we see that there is approximately 50% double bond character in the six C–N bonds surrounding C(1) and C(4). C(2)–N(4) and C(3)–N(5) are however almost pure double bonds. The central C–C bond and the C(2)-methyl bond have lengths that are not unusual for bonds to sp^2 carbon atoms. Few data are available on N–N bond lengths

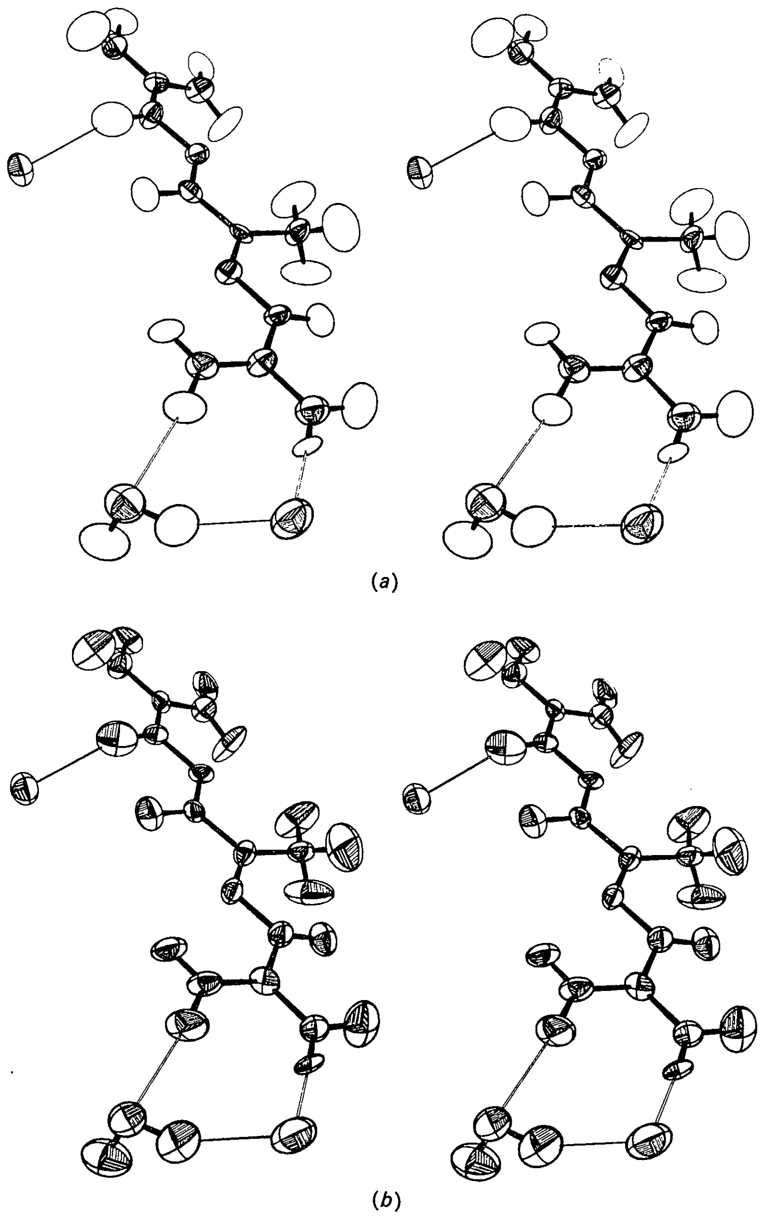


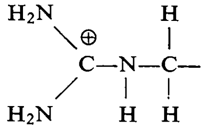
Fig. 4. Asymmetric unit of methyl GAG·2HCl·H_2O. Thermal vibrational ellipsoids are drawn at the 50% contour level. (*a*) Neutron results, (*b*) X-ray results; the boundary ellipsoids for the hydrogen atoms are taken from the neutron study. These are stereo pairs.

Table 5. *Comparison of heavy atom principal axes of thermal motion ellipsoids for methyl GAG*.2HCl.H$_2$O

The directions are most clearly seen by an examination of Fig. 4. The neutron values are on the first line in each case and the X-ray on the second. The estimated standard deviations are in parentheses. The units are 10^{-11} cm.

	r.m.s. 1	r.m.s. 2	r.m.s. 3
Cl(1)	167 (10)	239 (9)	260 (11)
	172 (5)	212 (3)	275 (4)
Cl(2)	190 (10)	220 (10)	251 (10)
	173 (4)	216 (4)	248 (4)
N(1)	136 (10)	176 (9)	219 (9)
	170 (15)	189 (11)	227 (10)
N(2)	127 (11)	174 (9)	224 (9)
	128 (16)	182 (12)	222 (10)
N(3)	141 (12)	164 (10)	205 (9)
	123 (19)	159 (11)	196 (10)
N(4)	133 (10)	163 (10)	193 (10)
	125 (16)	166 (12)	196 (12)
N(5)	104 (12)	182 (9)	196 (11)
	148 (16)	166 (12)	191 (11)
N(6)	110 (13)	181 (10)	221 (9)
	145 (14)	160 (14)	238 (12)
N(7)	136 (10)	186 (9)	243 (9)
	164 (14)	195 (12)	235 (11)
N(8)	150 (11)	204 (9)	235 (9)
	168 (15)	198 (13)	241 (11)
C(1)	097 (18)	180 (14)	190 (14)
	148 (19)	164 (13)	202 (14)
C(2)	138 (14)	163 (16)	188 (14)
	096 (27)	164 (13)	187 (13)
C(3)	129 (14)	144 (15)	197 (12)
	140 (17)	172 (14)	185 (13)
C(4)	116 (18)	149 (14)	196 (12)
	153 (14)	165 (19)	191 (12)
C(5)	134 (14)	181 (12)	231 (12)
	129 (15)	169 (17)	279 (13)
O	166 (16)	212 (15)	248 (16)
	185 (11)	215 (11)	236 (10)

in organic compounds. The value of 1·37 found here is considerably shorter than the value of 1·47 found in hydrazine (Giguère & Schomaker, 1943), but bond order considerations do not suggest large amounts of double bond character for this bond. It seems more likely that the distance in hydrazine is not a good value for comparison here and that a single N–N bond adjacent to a N–C double bond may have a normal length closer to 1·4 Å. The bond lengths at the terminal ends of the molecule may be compared with those in L-arginine hydrochloride (Donohue, Mez & Venkatesan, 1964) which have a similar grouping:



The C–NH$_2$ distances are 1·31 and 1·35 Å, and the C–N distance is 1·35 Å. The C–N distances are in satisfactory agreement with those found here if one takes the average of 1·33 for the C–NH$_2$ bonds in L-arginine.

The molecules are nearly planar: the mean plane through the heavy atoms (X-ray parameters) has the equation

$$13{\cdot}033x+2{\cdot}754y-3{\cdot}198z=2{\cdot}563\,,$$

where x, y, and z are the fractional coordinates of the atoms. The terminal nitrogen atoms lie as much as 0·20 ± 0·01 Å out of this plane in a way that suggests twisting around the N(6)–C(4) and N(3)–C(1) bonds; the CN$_3$ groups are however planar. The hydrogen atoms – except for two on the methyl group

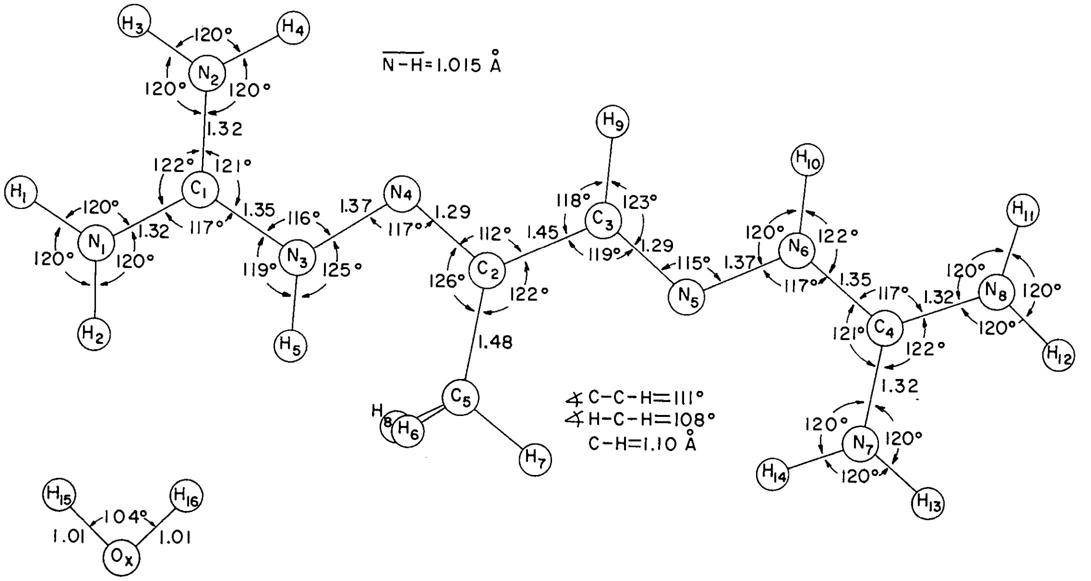


Fig. 5. Average idealized molecular structure from the neutron and X-ray results. (The structure is idealized in the sense that the bond lengths and angles have been averaged when there is reason to assume near chemical equivalence and when the present investigation has indicated no significant differences.)

– also lie approximately in the molecular plane. This suggests that the molecule has a true delocalized π-electron system rather than directed lone pair orbitals on the nitrogen atoms.

The crystal structure contains stacks of parallel planes of the dipositive ions. The distance between the mean planes is only 3·19 Å – a very short distance which is indicative of a strong, specific intermolecular interaction. Examination of the intermolecular distances and of Fig. 6, where part of the structure is shown, indicates that the interaction occurs between the positively charged terminal end of the molecule and the region of greatest π density in the molecule above – namely the C=N double bond. The shortest distance is in fact between C(1) of one molecule and N(5) of the molecule below – 3·15 Å. This strong interaction is indicative of a fairly substantial charge separation in the molecule and may well have some biological implications. Discussion of any such implications is reserved to a future paper. It is interesting to note that the interplanar spacing in the crystal structure of 6-mercaptopurine, also an anti-leukemia agent, is also short (Brown, 1967; Jensen, 1967).

Hydrogen positions

The large number of independently determined hydrogen atom positions make possible interesting comparisons between those determined by neutrons and those by the two X-ray refinements in which on the one hand the free atom scattering factor for hydrogen was used, and on the other hand the 'best spherical scattering factor' of Stewart, Davidson & Simpson (1965). The mean values for the various types of hydrogen in the crystal are presented in Table 6. (Since the hydrogen atoms are involved in hydrogen bonds, there may well be small differences in N–H bond lengths; we ignore these here.)

Table 6. *Bond lengths involving hydrogen for the three refinements*

	X(free)	X(SDS)	Neutron
8 N–H_{terminal}	0·91 (5)	0·97 (6)	1·016 (007)
2 N–H	0·82 (17)	0·85 (22)	1·003 (034)
2 O–H	0·84 (10)	0·88 (7)	1·006 (027)
3 C–H_{methyl}	0·94 (12)	0·94 (15)	1·090 (027)
1 C–H	0·87 (14)	0·84 (14)	1·145 (036)

Table 7. *Hydrogen bonds* A–H···B *in methyl GAG, in order of increasing strength*

A	H	B	A···B	A···H	H···B	∠A–H···B
N(3)	H(5)	O	2·90	1·04	1·89	163
N(2)	H(3)	O	3·07	1·01	2·08	163
O	H(16)	Cl^-(1)	3·09	1·02	2·14	153
O	H(15)	Cl^-(2)	3·15	0·99	2·19	166
N(7)	H(13)	Cl^-(1)	3·15	1·02	2·17	160
N(1)	H(1)	Cl^-(1)	3·20	1·01	2·20	170
N(6)	H(10)	Cl^-(2)	3·16	0·97	2·31	146
N(2)	H(4)	Cl^-(2)	3·27	1·01	2·34	152
N(1)	H(2)	Cl^-(2)	3·39	1·03	2·51	144
N(8)	H(12)	Cl^-(1)	3·48	1·03	2·58	143
N(8)	H(11)	Cl^-(2)	3·51	0·97	2·63	151
N(7)	H(14)	Cl^-(1)	3·48	1·04	2·82	122

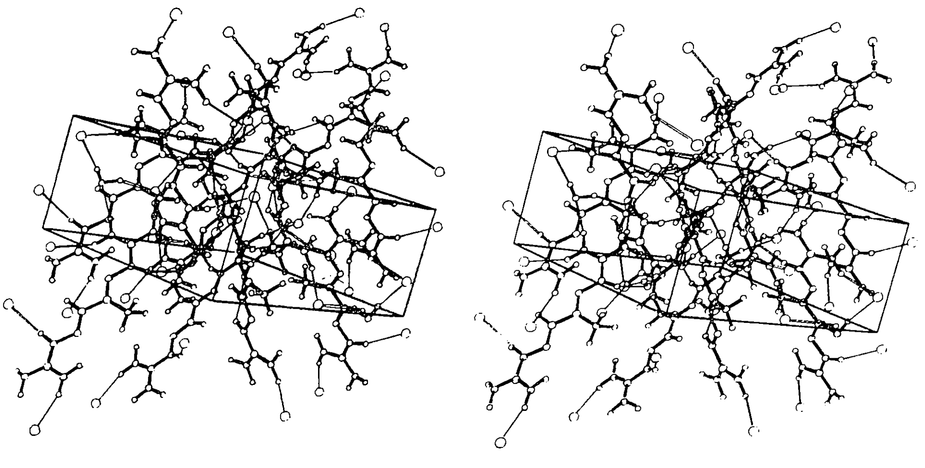


Fig. 6. Packing in methyl GAG.2HCl.H_2O. The structure consists of two stacks of nearly parallel planar molecules. These are tied together by hydrogen bonds through water and Cl^- ions as well as by a strong interaction perpendicular to the molecular planes.

The use of the SDS form factor for hydrogen has for the most part led to a greater average bond length, as one would hope. The remaining discrepancy between the X-ray and neutron values is less than 2σ in each case (for the mean values of several bond lengths, not for some of the individual values). However the fact that the differences are all in the same direction suggests that the SDS form factor is not adequate to correct fully for the short bond lengths and that it will be necessary to resort to non-spherical scattering factors for precise determination of hydrogen atom positions by X-rays. In the following section, we will use only the neutron diffraction values for hydrogen parameters.

Hydrogen bonding

Hydrogen bonds exist between the base and the chloride ion, between the base and the water molecule, and between the water molecule and chloride ions. The eight $N-H\cdots Cl^-$ hydrogen bonds represent the largest group of this type that has been studied. The interesting hydrogen bond parameters are given in Table 7. In examining this table, the reader should bear in mind that one useful criterion for hydrogen bonding is a hydrogen atom–heavy atom distance that is 0·2 Å or more shorter than the sum of the van der Waals radii (Hamilton & Ibers, 1967). For this purpose we may take the van der Waals radii of H, O, N, and Cl^- as 1·2, 1·4, 1·5, 1·8 Å. The variability in the $H\cdots B$ distances as well as in the $A-H\cdots B$ angles is worthy of note.

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On the Structure of Picrotoxin. II.
Direct Determination of the Crystal Structure of β-Bromopicrotoxinin

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(*Received* 26 *June* 1967 *and in revised form* 2 *November* 1967)

The crystal structure of β-bromopicrotoxinin, $C_{15}H_{15}O_6Br$, has been determined from three-dimensional, visually estimated X-ray data by a direct method. The parameters were refined anisotropically by the full-matrix least-squares method. The space group is $P4_3$, $a=b=7{\cdot}14$ Å, $c=28{\cdot}74$ Å. The four molecules in the unit cell are roughly spherical and are placed one on top of the other along the c axis. The molecular geometry is compared to that of the epimeric compound α_1-bromopicrotoxinin, a molecule of which the absolute configuration is already known from the X-ray crystallographic work of Craven [*Acta Cryst.* (1962), **15**, 387].

Introduction

Picrotoxin is a crystalline compound which has found use as a drug in the treatment of barbiturate poisoning. The substance is composed of two molecules, picrotoxinin, $C_{15}H_{16}O_6$, and picrotin, $C_{15}H_{18}O_7$, of which the former is the physiologically active principle. The interest of two of us (BJ & R-J) in the structure of picrotoxin dates back to about 1950, when the molecular structures of the components were still unknown.