Crystal and Molecular Structure of Glyoxal Bis(amidinohydrazone) Dihydrochloride; Biochemical Aspects

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The crystal and molecular structure of the title compound has been determined by single-crystal *X*-ray analysis and refined to an *R* value of 0.027. The crystal is monoclinic, space group $P2_1/c$ with a = 5.476(3), b = 14.540(7), c = 6.915(3) Å, β = 99.88(4)°, and Z = 2. The structure consists of layers of dipositive cations $[C_4H_{12}N_8]^{2+}$ and Cl^- ions. The planar cation has the *trans*-configuration of the chain. Hydrogen bonds of the type N–H ··· Cl^- are formed within the layers, but not between them. The molecules in the crystal are held together through stacking and *via* the delocalized π-electron system. The mean planes of the cations are approximately 3.51 Å apart. The crystal structure is compared with that of other bis(amidinohydrazone) derivatives and the relationship between crystal structure and biochemical properties is discussed.

Recently glyoxal bis(amidinohydrazone) (GBA) and methylglyoxal bis(amidinohydrazone) (MGBA) were studied with respect to their effectiveness in inhibiting the growth of mouse leukemia L 1210 cells.[1,2] In this connection the question arose as to whether the differences in biological activity of bis(amidinohydrazones) are due to differences in structure or composition.

MGBA hydrochloride monohydrate is known to exist in the solid state in the *trans*-configuration.[3] We now report the crystal and molecular structure of GBA dihydrochloride.

Experimental

Synthesis of GBA Dihydrochloride.—To aminoguanidinium hydrogen carbonate (EGA-Chemie) (27.3 g, 0.2 mol) dissolved in 0.7M-HCl (280 ml) and warmed to 60 °C, aqueous 30% glyoxal (EGA-Chemie) (19.15 ml, 0.1 mol) was added slowly with stirring. The mixture was set aside overnight at room temperature, then evaporated at reduced pressure; the product was dissolved in a mixture of methanol (250 ml) and 0.2M-HCl (50 ml) under reflux, and methanol (several hundred ml) and diethyl ether (130 ml) were added. The yellow solution was cooled in an ice-salt bath: a white precipitate separated. Addition of diethyl ether (350 ml) caused further precipitation. The precipitates were filtered off, combined, and dried *in vacuo*; yield 16.5 g (68%). On rapid heating the product melted at 278—280 °C with decomposition (lit.,[4] 277 °C). The white powder was dissolved in water and the solution allowed to evaporate to dryness at room temperature in the dark; 2—6 mm long, slightly brownish, transparent needles crystallized. These were used for the crystal-structure determination.

Crystal Data.—$C_4H_{12}Cl_2N_8$, M = 243.1, monoclinic, space group $P2_1/c$, a = 5.476(3), b = 14.540(7), c = 6.915(3) Å, β = 99.88(4)°, Z = 2, U = 542.4(5) Å^3, D_m = 1.49, D_c = 1.49 Mg m^{-3}, μ(Mo-K_α) = 5.8 cm^{-1}, F(000) = 252.

Crystallographic Measurements.—The crystal selected had the dimensions 1.0 × 0.9 × 0.45 mm. The unit-cell parameters were determined by least-squares treatment of the adjusted angular settings of 20 reflections (8° ⩽ 2θ ⩽ 27°) measured with a Nicolet P3F diffractometer. The intensity measurements were carried out at room temperature (18 °C) with graphite-monochromatized Mo-K_α radiation and the ω—2θ scan technique. The scan rate varied from 1.5 to 29.3° min^{-1}, depending on the number of counts in a fast preliminary scan through the peak. A set of 1 245 unique reflections was obtained from 1 418 reflections measured over the range 3° ⩽ 2θ ⩽ 55° (max.). 1 086 reflections with $I > 3\sigma(I)$ were considered as observed and used for refinement. Three strong reflections monitored periodically exhibited no significant variation. The intensities were corrected for Lorentz and polarization effects. Absorption corrections were made from φ-scan data.

Systematic absences ($h0l$, $l = 2n + 1$; $0k0$, $k = 2n + 1$; hkl no restrictions) established the space group as monoclinic $P2_1/c$ (no. 14). The density was measured by flotation in toluene–CCl_4.

Structure Determination.—The structure was solved by Patterson and Fourier methods. Only half the glyoxal bis(amidinohydrazone) molecule and one Cl^- ion had to be determined, because of the molecular symmetry. Full-matrix least-squares refinement with all non-hydrogen atoms anisotropic and hydrogen atoms with isotropic thermal parameters led to $R = \Sigma||F_o| - |F_c||/|F_o| = 0.027$ and $R_w = [\Sigma\omega(|F_o| - |F_c|)^2/\Sigma\omega|F_o|^2]^{1/2} = 0.031$, where $\omega = 1/\sigma(F_o)^2$. After the last cycle the average shift/error was 0.061 and the maximum shift/error 0.392. A final difference map was essentially featureless; general background ±0.07 e Å^{-3}, maximum peaks in the range ±0.15—0.21 e Å^{-3}.

Scattering factors were taken from ref. 5 and anomalous dispersion corrections and extinction corrections were applied. All calculations were carried out on an UNIVAC 1100/61 E1 computer with the program X-RAY 76,[6] and for planes also with the program MPLN.[7]

Anisotropic thermal parameters are given in Supplementary Publication No. SUP 56419 (3 pp.).†

Results and Discussion

Description of the Structure.—An ORTEP drawing[8] of the molecular cation and the numbering scheme are shown in Figure 1. The packing of the molecules is shown in Figure 2. The atomic co-ordinates and the equivalent values of the anisotropic temperature factor coefficients[9] are listed in Table 1, and bond lengths and angles in Table 2. The least-squares planes through the GBA cation and the distances of various atoms from the

† For details of the Supplementary Publication Scheme see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 2*, 1986, Issue 1. Structure factor tables are available on request from the editorial office.

Table 1. Fractional atomic co-ordinates ($\times 10^4$ for non-hydrogen atoms; $\times 10^3$ for H) and U_{eq} ($\times 10^4$ Å^2)[a] with standard deviations in parentheses

Atom	x	y	z	U_{eq}/Å^2
Cl	2 056(1)	1 632(1)	1 175(1)	412(2)
N(1)	7 010(3)	2 183(1)	8 090(3)	465(8)
N(2)	7 129(3)	630(1)	8 699(2)	412(8)
C(1)	6 031(3)	1 351(1)	7 808(2)	325(7)
N(3)	3 896(3)	1 271(1)	8 546(2)	360(7)
N(4)	2 759(2)	434(1)	6 304(1)	336(7)
C(2)	655(3)	431(1)	5 177(2)	337(8)
H(1)	620(4)	267(2)	756(3)	
H(2)	846(5)	222(2)	879(3)	
H(3)	656(4)	11(2)	842(3)	
H(4)	845(4)	70(1)	954(3)	
H(5)	325(4)	172(1)	611(3)	
H(6)	−4(3)	97(1)	457(3)	

[a] $U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \vec{a}_i \vec{a}_j$.[9]

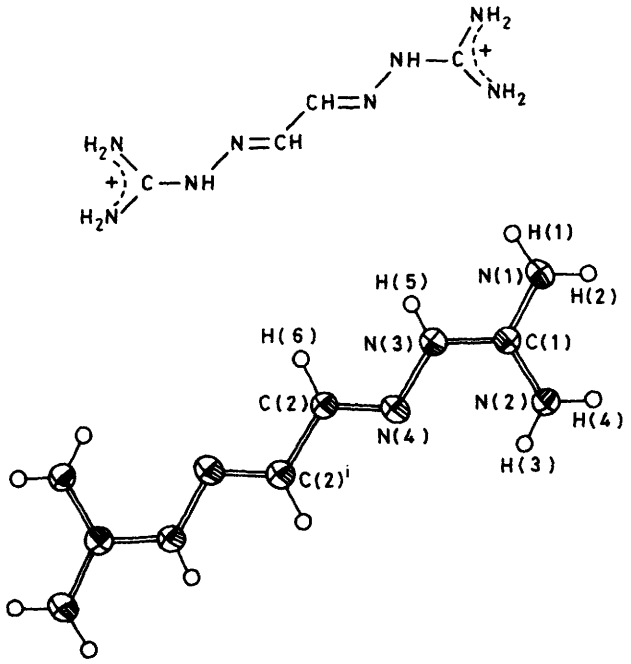

Figure 1. An ORTEP drawing of the glyoxal bis(amidinohydrazone) dication (with 291 K parameters) showing the molecular geometry and atom numbering scheme. Thermal ellipsoids are drawn at the 50% probability level for the non-hydrogen atoms

Table 2. Interatomic distances (Å) and angles (°) of the GBA cation

(a) Heavy atoms

N(1)–C(1)	1.324(2)	N(1)–C(1)–N(2)	121.4(1)
N(2)–C(1)	1.309(2)	N(1)–C(1)–N(3)	117.5(1)
C(1)–N(3)	1.339(2)	N(2)–C(1)–N(3)	121.1(1)
N(3)–N(4)	1.364(2)	C(1)–N(3)–N(4)	119.1(1)
N(4)–C(2)	1.275(2)	N(3)–N(4)–C(2)	115.1(1)
C(2)–C(2)^i	1.444(2)	N(4)–C(2)–C(2)^i	118.5(1)

(b) Hydrogen atoms

N(1)–H(1)	0.89(2)	H(1)–N(1)–C(1)	121(1)
N(1)–H(2)	0.86(2)	H(1)–N(1)–H(2)	122(2)
N(2)–H(3)	0.83(2)	H(2)–N(1)–C(1)	117(2)
N(2)–H(4)	0.85(2)	H(3)–N(2)–C(1)	120(1)
N(3)–H(5)	0.78(2)	H(3)–N(2)–H(4)	121(2)
C(2)–H(6)	0.94(2)	H(4)–N(2)–C(1)	120(1)
		H(5)–N(3)–C(1)	118(1)
		H(5)–N(3)–N(4)	122(1)
		H(6)–C(2)–N(4)	121(1)
		H(6)–C(2)–C(2)^i	120(1)

Symmetry position i: $-x, -y, 1-z$.

Table 3. Least-squares planes for the GBA cation. The equations are expressed in direct space. Asterisks denote atoms defining the plane. Displacements from the planes are in Å and estimated standard deviations are given in parentheses

(I): $-3.372\,28x + 2.245\,47y + 5.993\,95z = 2.949\,30$

N(1)*	0.026(2)	N(2)*	0.002(2)	C(1)*	0.001(1)		
N(3)*	−0.054(1)	N(4)*	−0.004(1)	C(2)*	0.030(2)		
H(1)	0.09(2)	H(2)	−0.04(2)	H(3)	−0.09(2)		
H(4)	0.08(2)	H(5)	0.01(2)	H(6)	0.02(2)	Cl	−2.572(2)

(II): $-3.519\,21x + 2.044\,25y + 5.893\,25z = 2.748\,74$

N(1)*	−0.002(2)	N(2)*	−0.002(2)	C(1)*	0.006(1)	N(3)*	−0.002(1)
H(1)	0.07(2)	H(2)	−0.09(2)	H(3)	−0.07(2)	H(4)	0.04(2)
H(5)	0.06(2)	N(4)	0.084(2)				

Table 4. Distances and angles in interactions of the type D–H ··· A

D	H	A	D–H (Å)	D ··· A (Å)	H ··· A (Å)	D–H ··· A (°)
N(1)	H(2)	Cl^a	0.86(2)	3.287(2)	2.50(2)	153
N(2)	H(4)	Cl^a	0.85(2)	3.278(2)	2.50(2)	152
N(2)	H(3)	Cl^b	0.83(2)	3.318(2)	2.64(2)	139
N(1)	H(1)	Cl^c	0.89(2)	3.294(2)	2.52(2)	146
N(3)	H(5)	Cl^c	0.78(2)	3.208(2)	2.49(2)	154

Symmetry position of atom A: a $x+1, y, z+1$; b $-x+1, -y, -z+1$; c $x, -y+1/2, z+1/2$.

planes are given in Table 3, and the hydrogen-bond distances and angles in Table 4.

Molecular Structure and Arrangement, and Hydrogen-bond System.—The hydrogen atoms of the acid bind to the imino groups of the base to form the ion $[C_4H_{12}N_8]^{2+}$. The positive charge is spread over the terminal ends of the molecule. The bond lengths (Table 2) suggest that the three C–N bonds around C(1) have approximately the same double-bond character and that C(2)–N(4) is nearly a double bond.[3] The N(3)–N(4) bond length, 1.364(2) Å, fits well with similar bond lengths in related compounds[3] and points also to some degree of double-bond character.

The U_{eq} values (Table 1) of the nitrogen atoms decrease on going from N(1) to N(4). The results in Table 3 show the grouping N(1)N(2)N(3)C(1) to be almost planar (very slightly pyramidal) [C(1) deviates 0.008(2) Å from the plane N(1)N(2)N(3)]. The group plane (II) is at an angle of 2.06° to the molecular cation plane (I) (Table 3).

The molecule is nearly planar, the largest deviation from the least-squares plane through the molecule being that of N(3) [−0.054(1) Å] (Table 3). The hydrogen atoms also lie approximately in the molecular plane, suggesting that the molecule has a delocalized π-electron system.

The crystal structure consists of parallel layers of $[C_4H_{12}N_8]^{2+}$ ions held together by an extensive hydrogen-bond network through the Cl^- ions (Figure 2; Table 4). The terminal

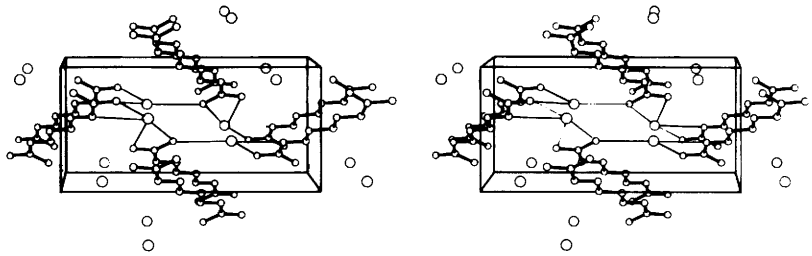

Figure 2. Stereoarrangement of the molecules in a unit cell viewed down the *a* axis. The hydrogen contacts are shown

imino nitrogen atoms N(1) and N(2) form two hydrogen bonds with Cl^- ions, and N(3) forms one hydrogen bond with a Cl^- ion. The details of the hydrogen bonds are given in Table 4. The hydrogen bonds are weak; their total lengths vary from 3.21 to 3.32 Å and the angles are large (139—154°) (Table 4).

The molecular planes are tilted by 51.99 and 61.70° to the *a*-axis and the *bc*-plane, respectively (Table 3; Figure 2). There are no hydrogen bonds between successive layers; thus the crystal structure is held together through van der Waals forces. The distance between successive layers is approximately 3.51 Å.

Comparisons and Biochemical Considerations.—Both the crystal structure of GBA dihydrochloride and that of MGBA dihydrochloride monohydrate[3] have a *trans*-configuration of the chain[3] (Figure 1). The corresponding heavy-atom bond distances[3] in the chains are the same within about 0.01 Å (Table 2). The corresponding angles of the chains are equal within 2°, with the exception that in GBA (Table 3) the angles C(1)–N(3)–N(4) and N(4)–C(2)–C(2)^i are 2.8 and 6.0° larger, respectively, and the angle N(3)–N(4)–C(2) is 1.5° smaller than in MGBA.[3] The CH_3 group in MGBA at C(2) is responsible for these effects.

The doubly charged molecular ions are approximately planar in both cases[3] (Table 3). The hydrogen bonds (Table 4) in GBA are approximately equally long and of the same order as the mean length of N–H · · · Cl bonds in MGBA.[3] In place of N(2)–H(3) · · · Cl^b and N(3)–H(5) · · · Cl^c bonds in GBA dihydrochloride, there are N(2)–H(3) · · · O and N(3)–H(5) · · · O bonds, respectively,[5] in MGBA dihydrochloride monohydrate. In addition, there are two O–H · · · Cl bonds due to the H_2O molecules in the latter structure.[3]

The distances between two successive layers in the crystal structures are 3.19 and 3.51 Å in MGBA[3] and GBA, respectively. In GBA this is the distance between the mean plane (Table 3) of the molecular cation $C_4H_{12}N_8^{2+}$ and the atom C(1)^c. The different layer distances have no biological significance.

The antiproliferative activity of bis(amidinohydrazones) against L 1210 decreases with increasing alkylation above the monomethyl stage.[1,2] This depends on the fact that GBA and MGBA are better retained in cells than the higher alkyl-substituted derivatives.[1,2]

On the other hand the inhibition of the eukaryotic *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) function increases and hence the biosynthesis of spermidine and spermine decreases with increasing alkylation.[1,2] It is possible that alkyl substitution of GBA at C(2) and C(2)^i, which increases the hydrophobicity of the molecule, leads to better binding at the active site of the enzyme and causes stronger inhibition. Furthermore, GBA strongly inhibits ornithine decarboxylase (EC 4.1.1.17) activity *in vivo*.[1,2] GBA resembles spermidine[10] more than do alkyl-substituted GBA derivatives, and can replace it as a repressor of ornithine decarboxylase formation.[1,2]

Recently we have determined the crystal structure of PGBA[11] [propylglyoxal bis(amidinohydrazone)] which also has a *trans*-configuration. Therefore, it is to be expected that EGBA [ethylglyoxal bis(amidinohydrazone)] and M_2GBA [dimethylglyoxal bis(amidinohydrazone)] have a similar *trans*-structure.

On the basis of these results we conclude that the differences in biological effects between GBA and its alkyl derivatives do not depend on their crystal structures in the solid state but only on the presence of alkyl substituents.

However, this does not exclude the possibility that the compounds (from GBA to M_2GBA) could also exist in the *cis*-configuration with respect to the C(2)–C(2)^i bond in cytoplasm. This might be the reason for the biological activity in general.

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