Crystal and Molecular Structure of Aminoguanidine Sulphate, an Important Enzyme Inhibitor and Starting Material of Drug Syntheses

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Aminoguanidine is not only an agent with a variety of pharmacological effects but also an important starting material of amidinohydrazone-type drugs and enzyme inhibitors. Therefore, we have now synthesized aminoguanidine sulphate $CN_4H_8^{2+}.SO_4^{2-}$ and determined its structure by single-crystal X-ray diffraction. The doubly protonated (dication) form of aminoguanidine that is present in the sulphate could, in principle, exist in the form of several different tautomers. The crystal studied consisted exclusively of one tautomer: one of the nitrogens of the hydrazine moiety bears three hydrogen atoms while the other one (the one bound to the carbon) bears one hydrogen. The other two nitrogens are bound to two hydrogens each. The predominance of this tautomer can be explained by the very strong resonance in it. The dication of aminoguanidine is remarkably planar. The hydrogens of the hydrazine moeity are, however, clearly out of the plane of the other atoms. There is a strong hydrogen bond between the proton of the monoprotonated nitrogen and one sulphate oxygen. This bond obviously causes the deviation of the hydrogen from the plane. The bonds between the carbon atom and the adjecnt nitrogens are essentially equally long, indicating that each bond has approximately the same amount of double bond character. One of the positive charges of the dication is thus delocalized, being shared by all of the atoms of the CN_3 moiety. In this respect, the structure is similar to that of all bis(amidinohydrazones) whose structures have been determined. The other positive charge of aminoguanidine dication is localized at the nitrogen bearing three hydrogens.

Introduction

Aminoguanidine has many important applications in pharmacology as well as in synthetic organic chemistry (for structural formula, see Fig. 1). Its various pharmacological effects are currently subject to intensive studies. For example, it inhibits nitric oxide synthase activity and may be of value in the prevention of diabetic vascular dysfunction [1–3]. It has also been reported to promote significantly the healing of stress gastric lesions [4] and to prevent some pathophysiologic changes typical of streptozocin diabetes [5, 6]. Further, it has been suggested that aminoguanidine might be efficacious in prevention or treatment of human diabetic polyneuropathy [4, 5, 7]. Aminoguanidine also inhibits advanced glycation end-product formation [1, 7].

A well-known and important property of aminoguanidine is constituted by its ability to inhibit diamine oxidase, an important polyamine-degrading enzyme [8]. It is also a widely used starting material in the syntheses of bis(amidinohydrazones) [bis(guanylhydrazones)], many of which are highly potent specific inhibitors of adenosylmethionine decarboxylase, one of the two rate-limiting enzymes of polyamine biosynthesis [8–19]. Its synthetic importance is further increased by the fact that some of the bis(amidinohydrazones) and related compounds prepared from it are also potent antileukemic agents, the best known examples being glyoxal bis(amidinohydrazone) and methylglyoxal bis(amidinohydrazone), commonly known as GBG and MGBG, respectively [8, 10–11, 13]. Also many other types of compounds prepared from aminoguanidine have important pharmacological and other applications [10–11]. An excellent detailed review has recently appeared on the chemistry and pharmacology of

Abbreviations: GBG, glyoxal bis(guanylhydrazone); MGBG, methylglyoxal bis(guanylhydrazone).

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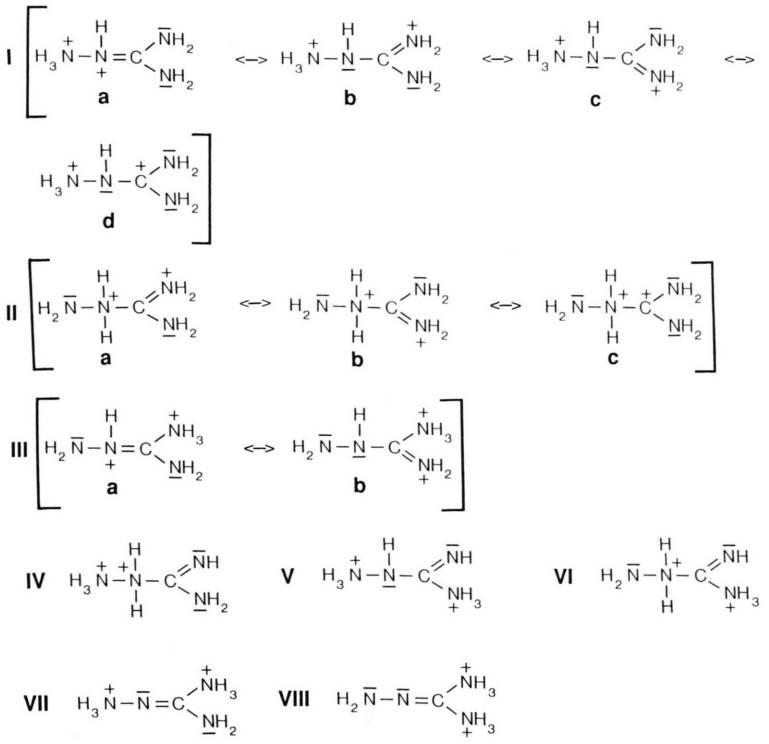

Fig. 1. Some possible tautomers of aminoguanidine dication. Some further resonance forms can also be envisioned but they obviously cannot have any real significance.

bis(amidinohydrazones) and related compounds [10–11].

Detailed knowledge of the structure and chemical properties of aminoguanidine are needed for a better appreciation of the biochemical and pharmacological properties of this important compound. The crystal structures, solution equilibria and spectral properties of many derivatives of aminoguanidine have been reported recently [8, 17–29], and also this has created an interest in the structure of aminoguanidine itself. We have therefore now synthesized aminoguanidine sulphate, $CN_4H_8^{2+}.SO_4^{2-}$, and determined its structure by single-crystal X-ray diffraction.

Materials and Methods

Synthesis of aminoguanidine sulphate

Aminoguanidine sulphate, a doubly protonated form of free aminoguanidine, was prepared from aminoguanidine bicarbonate (Aldrich Chemie, Steinheim, Germany) by treating the latter with 1 M aqueous sulphuric acid (1.0 mol : 1.1 mol) at 60 °C for 30 min. The colourless solution obtained was allowed to evaporate at room temperature, and yielded colourless single crystals (plates).

Crystal data

$CN_4H_8^{2+}.SO_4^{2-}$, M = 172.2, triclinic, space group $P\bar{1}$, a = 6.0510(10), b = 7.3970(10), c = 7.732(2) Å, α = 105.97(3)°, β = 100.80(3)°, γ = 94.43(3)°, Z = 1, V = 323.77(16) Å^3, D_c = 1.77 gcm^{-3}, μ(MoKα) = 0.468 mm^{-1}, F(000) = 180.

Crystallographic measurements

The crystal of dimensions 0.45×0.40×0.20 mm was sealed in a Lindeman capillary and used for X-ray measurements. Crystal data were measured at room temperature (296 K) on a Nicolet $P2_1/F$ single-crystal diffractometer using graphite monochromatized MoKα radiation (λ = 0.71073 Å). The cell parameters were determined by least-square treatment of the adjusted angular settings of 24 reflections ($15° < 2\theta < 22°$). The intensity measurements were carried out by the ω-2θ scan technique. The scan rate varied from 2.5 to 29.3° min^{-1} depending on the number of counts measured in a fast preliminary scan. A set of 1489 reflections were measured over the range $3° < 2\theta < 53°$ resulting in 1355 independent reflections, of which 1191 were considered as observed [$I_o > 3\sigma(I_o)$]. Two standard reflections measured every 50 reflections showed the intensity variation to be random and within 1% with respect to the mean. The in-

tensities were corrected for Lorentz, polarization and extinction effects and absorption (transmission 1.000–0.780).

The structures were solved by direct methods. Hydrogen atoms were detected from the difference map and refined using a riding model with fixed isotropic temperature factor (0.08 Å^2). Successive calculations and full-matrix least-squares refinement with non-hydrogen atoms anisotropic and hydrogen atoms isotropic led to $R = 0.065$ and $R_w = 0.118$ ($R_w = [\Sigma w(|F_o| - |F_c|)^2 / \Sigma w|F_o|]^{1/2}$ with $w = 1/(\sigma|F_o|)^2$). After the last cycle, $s/\Delta_{av} = 0.011$ and $s/\Delta_{max} = 0.937$. In the final difference map the largest difference peak was $+0.84 e Å^{-3}$ and the minimum $-1.14 e Å^{-3}$. All calculations were performed by SHELXTL PLUS (PC version packet) [30].

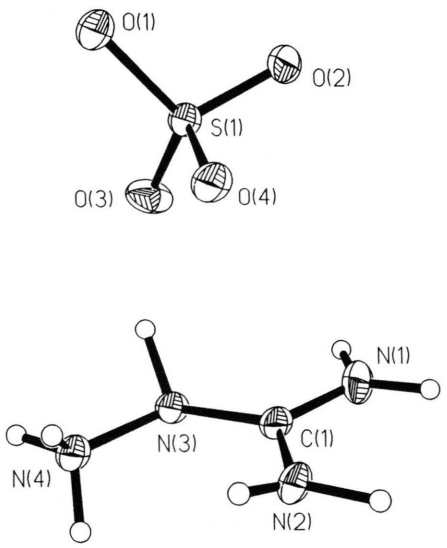

Fig. 2. A drawing of $CN_4H_8^{2+}.SO_4^{2-}$.

Results and Discussion

In principle, the doubly protonated (dication) form of aminoguanidine could exist in the form of several different tautomers (see Fig. 1). In the present study, it was found that the crystal studied consisted exclusively of tautomer **I.** A drawing of the compound is shown in Fig. 2. Stereoarrangement of the ions in a unit cell is shown in Fig. 3. The atomic parameters are listed in Table I, interatomic distances in Table II, interatomic angles in Table III, anisotropic displacement coefficients of non-hydrogen atoms in Table IV, and coordinates and isotropic displacement coefficients of hydrogen atoms in Table V. Further details of the structure determinations can be obtained from the Fachinformationszentrum Karlsruhe GmbH, D-76344 Eggenstein-Leopoldshafen, referring to the deposition number CSD 57944, the names of the authors, and the literature citation.

The dication of aminoguanidine was found to be remarkably planar. The hydrogens bound to N(3) and N(4), however, are clearly out of the plane of the non-hydrogen atoms and the hydrogens of N(1) and N(2). There is a strong hydrogen

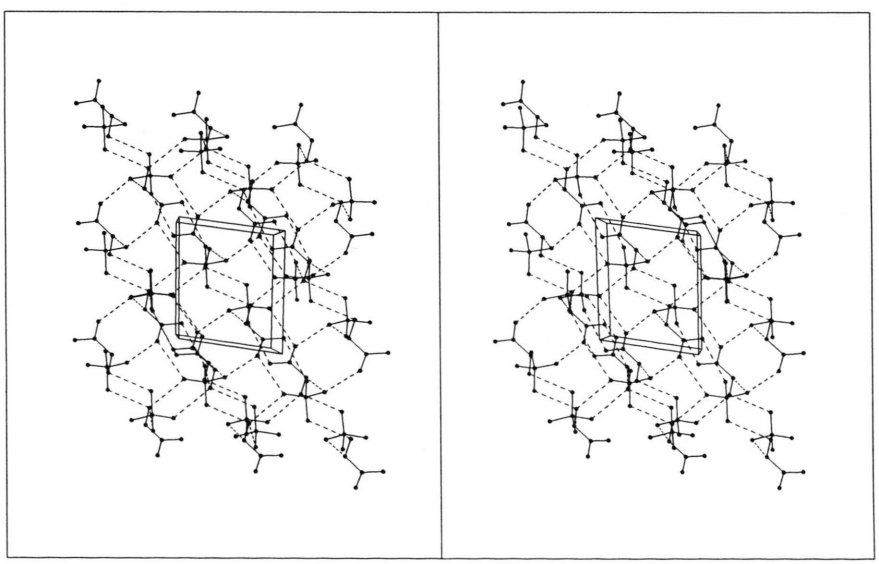

Fig. 3. Stereoarrangement of ions in a unit cell of aminoguanidine sulphate viewed along b.

Table I. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement coefficients ($Å^2 \times 10^3$) of aminoguanidine sulphate.

Atom	*x*	*y*	*z*	U(eq)*
S(1)	2685(1)	6529(1)	2819(1)	36(1)
O(1)	2768(3)	4652(3)	1528(3)	46(1)
O(2)	2593(3)	7950(3)	1785(3)	47(1)
O(3)	4747(3)	7099(3)	4304(2)	50(1)
O(4)	644(3)	6444(3)	3599(3)	44(1)
N(1)	2076(4)	10574(3)	6887(3)	48(1)
N(2)	−321(4)	8712(3)	7909(3)	43(1)
C(1)	1638(4)	9097(4)	7451(3)	38(1)
N(3)	3330(3)	7981(3)	7579(3)	39(1)
N(4)	2942(3)	6372(3)	8212(3)	39(1)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table II. Bond lengths (Å) of aminoguanidine sulphate.

S(1)−O(1)	1.482(2)	S(1)−O(2)	1.484(2)
S(1)−O(3)	1.472(2)	S(1)−O(4)	1.477(2)
N(1)−C(1)	1.310(4)	N(2)−C(1)	1.331(4)
C(1)−N(3)	1.370(4)	N(3)−N(4)	1.426(4)

Table III. Bond angles (°) of aminoguanidine sulphate.

O(1)−S(1)−O(2)	108.4(1)	O(1)−S(1)−O(3)	110.4(1)
O(2)−S(1)−O(3)	108.2(1)	O(1)−S(1)−O(4)	109.6(1)
O(2)−S(1)−O(4)	109.9(1)	O(3)−S(1)−O(4)	110.3(1)
N(1)−C(1)−N(2)	122.2(3)	N(1)−C(1)−N(3)	116.2(2)
N(2)−C(1)−N(3)	121.6(3)	C(1)−N(3)−N(4)	118.2(2)

Table IV. Anisotropic displacement coefficients of non-hydrogen atoms ($Å^2 \times 10^3$) of aminoguanidine sulphate.

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S(1)	37(1)	50(1)	28(1)	31(1)	12(1)	12(1)
O(1)	50(1)	54(1)	40(1)	36(1)	18(1)	11(1)
O(2)	56(1)	57(1)	37(1)	37(1)	18(1)	18(1)
O(3)	38(1)	85(1)	32(1)	27(1)	8(1)	19(1)
O(4)	40(1)	59(1)	38(1)	30(1)	16(1)	13(1)
N(1)	47(1)	58(1)	51(1)	36(1)	16(1)	25(1)
N(2)	43(1)	53(1)	41(1)	35(1)	17(1)	16(1)
C(1)	39(1)	52(1)	25(1)	31(1)	7(1)	10(1)
N(3)	41(1)	57(1)	28(1)	36(1)	13(1)	17(1)
N(4)	41(1)	50(1)	33(1)	32(1)	12(1)	14(1)

Table V. Hydrogen atom coordinates ($\times 10^4$) and isotropic displacement coefficients ($Å \times 10^3$) of aminoguanidine sulphate.

Atom	*x*	*y*	*z*	U
H(1A)	3424	10805	6601	80
H(1B)	1023	11355	6779	80
H(4A)	2831	6819	9395	80
H(4B)	4208	5809	8220	80
H(3A)	3933	7384	6428	80
H(4A)	1891	5551	7710	80
H(2A)	−763	7638	7943	80
H(2B)	−1244	9713	7953	80

bond between the proton bound to N(3) and one of the oxygens of the sulphate ion [O(3)]. This bond obviously causes the deviation of the hydrogen of N(3) from the plane. The bonds between C(1) and the three adjacent nitrogen atoms are essentially equally long, indicating that each one of these bonds has approximately the same amount of double-bond character. A localized double bond cannot be found. One of the positive charges of the dication is thus delocalized, being shared by all of the nitrogen atoms and probably also by the carbon atom of the terminal CN_3 moiety and the structure of the CN_3 moiety can therefore be considered to be a resonance hybrid of canonical forms **Ia, Ib, Ic** and **Id.** In this respect, the structure is similar to that of all bis(amidinohydrazone) dications whose structures have been determined [8]. The other positive charge of aminoguanidine dication is localized at the quaternary N(4) atom.

The results obtained strongly suggest that aminoguanidine dication has an intrinsic tendency to exist only in the form of tautomer **I** at least in the solid state and probably also in solution. The predominance of this tautomer is easily explained with the very strong resonance in **I,** in which one of the positive charges is delocalized on three different nitrogen atoms and, to some extent, probably also on the carbon atom. Notably, two of the resonance forms are identical having thus exactly the same energy. The double bond is also delocalized in this tautomer. Since the guanidino group is highly stabilized by the resonance and since this stabilization would be weakened on further protonation of the group, it is understandable that the other proton obtained on formation of the dication resides on the terminal nitrogen N(4). Further protonation of the guanidino group is hindered also by the positive charge already held by this group.

Tautomer **II** that could not be detected is indeed less probable than **I** because in it the resonance is restricted to the amidino group (the CN_2 moiety) only and does not involve N(3). In this structure,

the two positive charges also reside close to each other, increasing the energy of **II.**

The experimental proof for the existence of **I** and not **II** has an important synthetic implication. If **II** were the predominant tautomer, the optimum pH for performing the syntheses of amidinohydrazones from aminoguanidine and aldehydes or ketones would be lower than in the case of the predominance of **I,** since in the former case, the concentration of the active nucleophile [N(4) with an unshared electron pair] would be nearly maximal even when aminoguanidine exists in the dication form (i.e. as a divalent salt). Since, however, **I** is in reality the predominant form, it is advisable not to use the dication form for syntheses, even though the reactions are acid catalyzed.

In tautomer **III,** some delocalization would still be possible, albeit far less prominent than in **I** and **II.** In **IV–VII,** any delocalization would require charge separation and obviously would have no real importance. In **VIII,** both charges also localized and reside in the vicinity of each other. Thus, tautomers **III–VIII** are indeed far less favoured on energetic grounds, as compared to **I** or even **II.**

Considering the structure of **I,** it is easily appreciated that further protonation of the dication form of aminoguanidine would obviously require a great deal of energy, because the further proton(s) would have to be accepted by the guanidino group since N(4) already bears a maximum number of bonds and, in addition, is already positively charged. Protonation of the guanidino group would, however, weaken or destroy the resonance stabilization. The positive charge of this group also repels protons.

The striking planarity of aminoguanidine dication is easily explained on the basis of the resonance in tautomer **I.** The carbon atom and nitrogens 1, 2 and 3 obviously must have sp^2-hybridization.

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