

X-ray Studies of Crystalline Complexes Involving Amino Acids. II. The Crystal Structure of L-Arginine L-Glutamate*

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Arginine glutamate monohydrate, $C_6H_{15}N_4O_2 \cdot C_5H_8NO_4 \cdot H_2O$, a crystalline complex of two amino acids, belongs to the orthorhombic space group $P2_12_12_1$ with four formula units in a unit cell of dimensions $a = 5.047$ (8), $b = 9.849$ (8), $c = 32.500$ (12) Å. The structure was solved by the symbolic addition procedure and refined to an R value of 0.083 for 1007 photographically observed reflexions. The conformation of the arginine molecule is different from those reported so far, whereas the conformation of the glutamate ion is the same as that observed in the structure of glutamic acid hydrochloride. An analysis of the conformations of the two molecules found in crystal structures containing them is also presented. The crystal structure of the complex is stabilized by ionic interactions and hydrogen bonds. The crystal structure consists of alternating layers, one layer consisting of cationic arginine molecules and the other containing negatively charged glutamate ions. The adjacent layers are interconnected principally through a specific ion-pair interaction between the guanidyl group of arginine and the γ -carboxyl group of the glutamate, and a water bridge. The former, which involves the electrostatic attraction between the positively charged guanidyl group and the negatively charged carboxylate group as well as two nearly parallel $N-H \cdots O$ hydrogen bonds, is the first such specific side chain–side chain interaction to be observed at atomic resolution.

Introduction

A programme of X-ray structure analyses of crystalline complexes involving amino acids and short peptides has been undertaken in order to enhance our understanding of the atomic details of the non-covalent interactions which are important in the structure, assembly and function of proteins. The crystal structure of L-lysine L-aspartate has already been determined as part of this programme (Bhat & Vijayan, 1976). Here we report the results of the X-ray analysis of another such crystalline complex, namely, L-arginine L-glutamate monohydrate. A preliminary account of the analysis has been published (Bhat & Vijayan, 1975).

Experimental

A few tiny crystals of the complex could be grown, after carefully controlled and repeated attempts, by the slow diffusion of acetone into an aqueous solution of the compound (obtained commercially). The unit-cell dimensions and the space group were determined from oscillation and Weissenberg photographs. The density

was measured by flotation in a mixture of benzene and carbon tetrachloride.

Crystal data

L-Arginine L-glutamate monohydrate, $C_6H_{15}N_4O_2 \cdot C_5H_8NO_4 \cdot H_2O$; orthorhombic, $P2_12_12_1$; $a = 5.047$ (8), $b = 9.849$ (8), $c = 32.500$ (12) Å; $D_m = 1.400$ (7), $D_c = 1.395$ g cm⁻³; $Z = 4$, $\mu(\text{Cu } K\alpha) = 10.05$ cm⁻¹.

The diffraction data were collected on multiple-film equi-inclination Weissenberg photographs with Cu $K\alpha$ radiation about the a axis for reciprocal levels Hkl , $H = 0$ through 4, from a crystal of approximate dimensions $0.3 \times 0.2 \times 0.12$ mm. $h0l$ data were also recorded for scaling purposes from a crystal of approximate dimensions $0.2 \times 0.14 \times 0.1$ mm. The intensities were estimated visually and corrected for Lorentz and polarization factors and for spot shape. Of the 2178 independent reflexions in the Cu sphere, 1907 were recorded, of which 1007 were in the measurable range. The $h0l$ data were used to put the intensities on a common relative scale. The initial scale factor and overall temperature factor were determined from Wilson's statistics.

The crystal structure was solved by the noncentrosymmetric symbolic addition procedure (Karle & Karle, 1966). The structure was refined, first isotropically and then anisotropically, to an R value of 0.114 with the full-matrix structure-factor least-

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Table 1. *Final positional coordinates ($\times 10^4$) and anisotropic thermal parameters ($\times 10^4$) of the nonhydrogen atoms*

The standard deviations are given in parentheses.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>b</i> ₁₁	<i>b</i> ₂₂	<i>b</i> ₃₃	<i>b</i> ₁₂	<i>b</i> ₁₃	<i>b</i> ₂₃
N(1)	3052 (15)	5267 (7)	2652 (2)	108 (39)	40 (8)	5 (1)	-17 (29)	3 (10)	-1 (4)
C(2)	3784 (21)	6673 (9)	2530 (3)	151 (49)	41 (10)	4 (1)	12 (37)	3 (11)	-1 (5)
C(1)	1831 (21)	7729 (10)	2727 (3)	108 (49)	60 (12)	4 (1)	-17 (39)	3 (12)	-6 (5)
O(1)	24 (15)	7289 (7)	2942 (2)	160 (38)	104 (9)	7 (1)	-4 (32)	21 (9)	-4 (4)
O(2)	2349 (16)	8942 (7)	2653 (2)	234 (38)	61 (9)	7 (1)	7 (30)	11 (9)	-1 (4)
C(3)	3627 (20)	6783 (11)	2054 (3)	63 (51)	91 (13)	4 (1)	-2 (41)	5 (10)	-4 (6)
C(4)	878 (23)	6789 (9)	1881 (3)	201 (53)	42 (10)	6 (1)	43 (39)	-10 (12)	0 (5)
C(5)	621 (21)	6114 (10)	1465 (3)	153 (48)	74 (12)	4 (1)	-4 (42)	1 (12)	-2 (5)
N(6)	1307 (17)	4677 (8)	1492 (2)	179 (41)	46 (9)	6 (1)	-5 (33)	10 (10)	-1 (4)
C(7)	3308 (19)	4052 (9)	1302 (2)	11 (41)	50 (10)	3 (1)	-2 (32)	0 (10)	-2 (4)
N(8)	4869 (19)	4695 (9)	1033 (2)	187 (46)	80 (10)	6 (1)	2 (39)	14 (10)	3 (5)
N(9)	3802 (19)	2742 (8)	1385 (2)	302 (47)	44 (9)	6 (1)	11 (36)	13 (11)	5 (5)
N(11)	5691 (17)	4339 (7)	5080 (2)	104 (34)	51 (8)	4 (1)	24 (30)	7 (9)	-1 (4)
C(12)	5550 (20)	4342 (9)	4617 (2)	68 (43)	38 (9)	3 (1)	-22 (33)	0 (10)	7 (4)
C(11)	5232 (20)	2902 (10)	4464 (3)	84 (46)	50 (10)	5 (1)	2 (36)	-2 (11)	-2 (5)
O(11)	5742 (15)	1932 (6)	4709 (2)	211 (33)	38 (7)	7 (1)	-4 (28)	-7 (8)	2 (4)
O(12)	4524 (19)	2741 (7)	4096 (2)	502 (48)	68 (8)	6 (1)	-36 (38)	-13 (11)	-1 (4)
C(13)	7965 (20)	5020 (9)	4428 (3)	130 (45)	29 (9)	5 (1)	10 (35)	11 (12)	0 (4)
C(14)	8434 (21)	6467 (3)	4571 (3)	133 (50)	57 (11)	6 (1)	-14 (37)	7 (12)	-2 (5)
C(15)	10610 (20)	7226 (9)	4343 (2)	123 (43)	30 (9)	3 (1)	6 (36)	0 (10)	5 (5)
O(16)	11908 (16)	6610 (7)	4070 (2)	291 (38)	68 (8)	7 (1)	-46 (31)	27 (9)	-6 (4)
O(17)	11130 (15)	8427 (6)	4442 (2)	314 (38)	40 (7)	5 (1)	-43 (27)	10 (8)	-3 (4)
W(1)	2896 (18)	4660 (7)	3496 (2)	609 (50)	71 (8)	5 (1)	49 (39)	5 (10)	2 (4)

squares program *LALS*, originally written by Gantzel, Sparks and Trueblood and modified by one of us (TNB) for the IBM 360/44 system with 128 K bytes memory. The anisotropic temperature factors were of the form $\exp[-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + 2b_{12}hk + 2b_{23}kl + 2b_{13}hl)]$. The limited memory of the computer constrained us to refine the two molecules in the structure in alternate cycles. H atoms, fixed from geometrical considerations and a difference Fourier map, were included at this stage in the structure factor calculations, but their positions and temperature factors (assumed to be the same as the isotropic temperature factors of the heavy atoms to which they are attached) were not refined. Further refinement of the positional and thermal parameters of the nonhydrogen atoms reduced *R* to 0.083 for 1007 observed reflexions in the final cycle. All the shifts in this cycle were much less than the corresponding estimated standard deviations. The weighting functions used in the above calculations had the form $1/(a + b|F_o| + c|F_o|^2)$, where $a = 1.555$, $b = -0.019$ and $c = 0.0007$. The atomic form factors for nonhydrogen atoms were taken from Cromer & Waber (1965) and those for H atoms from Stewart, Davidson & Simpson (1965). The final positional and thermal parameters of the nonhydrogen atoms and their standard deviations are given in Table 1.*

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32322 (6 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Discussion

Bond lengths and angles

The α -amino and the guanidyl groups are protonated and hence positively charged, whereas the α -carboxyl group is deprotonated and hence negatively charged in the cationic arginine molecule. The glutamate ion, with two deprotonated carboxyl groups and a protonated amino group, carries a net negative charge. The bond lengths and angles (Fig. 1) are comparable, within experimental error, to the corresponding values observed in other crystal structures containing arginine or glutamic acid (or the glutamate ion).

Conformation of arginine

The conformation of the arginine molecule is shown in Fig. 2(a). The torsional angles (IUPAC-IUB Commission on Biochemical Nomenclature, 1970) which define the conformation of the arginine molecule are listed in Table 2. The conformational angles observed in other crystal structures containing arginine are also given for comparison. As can be seen from the table, the conformation of the molecule in the present structure is different from those observed in the other crystal structures containing arginine. In fact, arginine glutamate is the only structure analysed so far in which χ^3 has a value in the neighbourhood of 60° .

Of the torsional angles that define the conformation of the arginine side chain, the values of χ^{51} and χ^{52} are

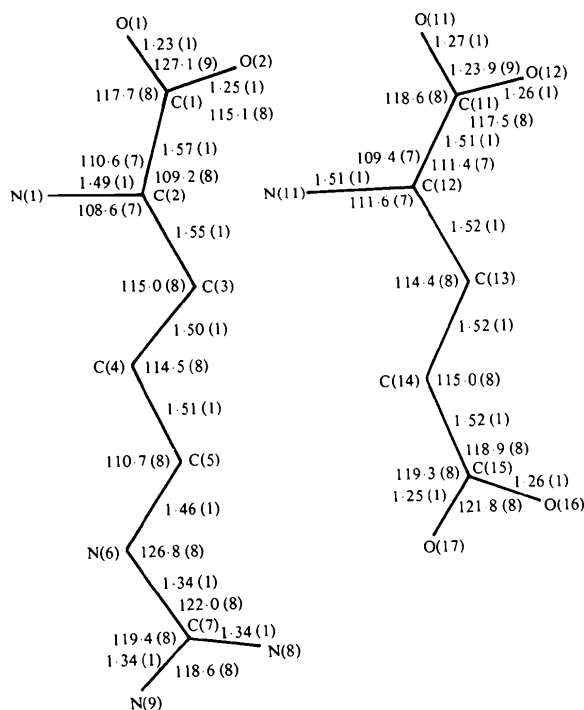


Fig. 1. Bond lengths (Å) and angles ($^{\circ}$) in the structure. The standard deviations are given in parentheses.

restricted to regions around 0 and 180° respectively on account of the planarity of the guanidyl group. Hence, the conformational variety of the side chain results from the different values which the other four dihedral angles can assume. Steric considerations and energy

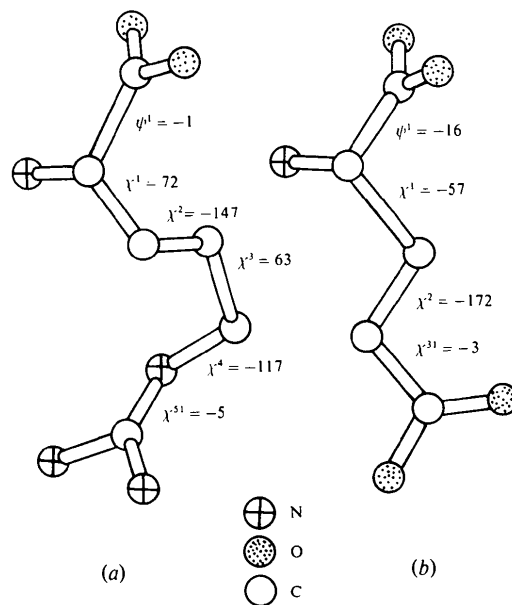


Fig. 2. Perspective views of (a) the arginine and (b) the glutamate ions in the structure. The conformational angles ($^{\circ}$) are indicated.

calculations indicate that the non-bonded interactions dependent on χ^{51} and χ^{52} restrict the value of χ^4 to regions around 90 , 180 and -90° (Ponnuswamy, Lakshminarayanan & Sasisekharan, 1971). Taken individually, ethane-like staggered conformations are preferred about $C^{\alpha}-C^{\beta}$, $C^{\beta}-C^{\gamma}$ and $C^{\gamma}-C^{\delta}$ bonds, corresponding to preferred values of 60 , 180 or -60° for each of the three torsional angles χ^1 , χ^2 and χ^3 . Thus it would appear at first sight that 81 different sterically

Table 2. Conformational angles ($^{\circ}$) of arginine in arginine glutamate monohydrate and other crystal structures

	ψ^1	ψ^2	χ^1	χ^2	χ^3	χ^4	χ^{51}	χ^{52}	Idealized values of $\chi^1, \chi^2, \chi^3, \chi^4$	Reference
Arginine glutamate. H_2O	-1	178	72	-147	63	-117	-5	175	60, 180, 60, 90	(a)
Arginine. $2H_2O$	-13	168	64	151	175	163	-10	173	60, 180, 180, 180	(b)
Arginine. HCl										
Molecule I	-51	134	171	-173	172	-172	-7	175	180, 180, 180, 180	(c)
Molecule II	-41	138	168	166	174	169	6	-179	180, 180, 180, 180	(c)
Arginine. HCl. H_2O										
Molecule I	-28	155	-54	174	-175	97	-14	166	-60, 180, 180, 90	(d)
Molecule II	-6	174	-59	-164	179	-84	11	-170	-60, 180, 180, -90	(d)
Arginine. HBr. H_2O										
Molecule I	-29	156	-55	168	-173	101	-17	165	-60, 180, 180, 90	(e)
Molecule II	-6	177	-61	-163	-179	-85	9	-167	-60, 180, 180, -90	(e)
Arginine phosphate. H_2O	-34	148	-164	175	-64	113	5	-176	180, 180, -60, 90	(f)
Arginine diethyl phosphate	-18	170	75	-177	-174	91	9	-175	60, 180, 180, 90	(g)
Dinitrobis(L-arginato)-cobalt(III) nitrate. $2H_2O$										
Molecule I	9	-172	77	-167	-173	-173	0	176	60, 180, 180, 180	(h)
Molecule II	27	-156	-70	-172	-176	-170	0	-179	-60, 180, 180, 180	(h)

(a) Present study. (b) Lehmann, Verbist, Hamilton & Koetzle, 1973. (c) Mazumdar, Venkatesan, Mez & Donohue, 1969. (d) Dow, Jensen, Mazumdar, Srinivasan & Ramachandran, 1970. (e) Mazumdar & Srinivasan, 1966. (f) Aoki, Nagano & Iitaka, 1971. (g) Furberg & Solbakk, 1973. (h) Watson, Johnson, Celap & Kamberi, 1972.

favoured conformations are possible for the arginine side chain. However, it can be shown that some combinations of torsional angles become energetically unfavourable when more than one torsional angle is considered simultaneously. For example, Ponnuswamy & Sasisekharan (1971) have shown that of the nine possible combinations of χ^1 and χ^2 , only five are energetically favourable. On the other hand, all the nine possible combinations of χ^3 and χ^4 are found to be permissible on the basis of energy calculations (Ponnuswamy, Lakshminarayanan & Sasisekharan, 1971). The actual number of different energetically allowed conformations can be obtained only after such calculations have been carried out for all the possible combinations of the four torsional angles χ^1, χ^2, χ^3 and χ^4 , keeping χ^{51} and χ^{52} at 0 and 180° respectively.

In the light of the above discussion, the observed structures can be classified into different groups on the basis of the expected idealized values of the torsional angles. These idealized values for each structure are also given in Table 2. It is readily seen that even though twelve sets of torsional angles are listed, they represent only eight independent observed conformations. Several more independent conformations are likely to be possible in view of the length and the flexibility of the arginine side chain. However, in the crystal structures containing arginine analysed so far, it is interesting to note that though χ^1 occurs with nearly equal frequency in the regions around $60, 180$ and -60° , there is a greater concentration around 180° for χ^2 and χ^3 , as noted earlier by Ramachandran, Mazumdar, Venkatesan & Lakshminarayanan (1966). In fact, χ^2 has values in the region of 180° in all twelve cases, whereas χ^3 falls in this region in ten out of the twelve cases. Values of 180° for χ^2 and χ^3 imply an all-*trans* conformation for the linear chain segment between C^α and N^ϵ .

Conformation of the glutamate ion

Fig. 2(b) illustrates the conformation of the glutamate ion. The torsional angles are listed in Table 3 along

with those in glutamic acid, its hydrochloride salt and four metal complexes, the structures of which are presently available. The glutamate ion in the present structure exists in the extended conformation found earlier in the structure of glutamic acid hydrochloride.

As can be seen from Table 3, the torsional angles χ^{31} and χ^{32} lie within comparatively narrow limits, χ^{31} varying from -3 to 55° and χ^{32} from 180 to -123° . Hence, conformational differences among side chains in different compounds essentially arise from differing values of χ^1 and χ^2 . In glutamic acid, χ^1 and χ^2 represent rotations about $C(sp^3)-C(sp^3)$ bonds and, hence, each is likely to have values in the neighbourhood of $60, 180$ or -60° .

Five distinct conformations corresponding to χ^1, χ^2 values in the regions of $(60, 180), (60, -60), (180, 180), (-60, 180)$ and $(-60, -60^\circ)$ are observed in the structures listed in Table 2. Of these, the combination $(-60, 180^\circ)$ leads to a fully extended conformation with an all-*trans* side chain *trans* to the α -carboxyl group. The combination $(180, 180^\circ)$ corresponds to a less extended conformation with an all-*trans* side chain *trans* to the amino N atom but *gauche* to the α -carboxyl group. In the combination $(60, 180^\circ)$, the side chain is all-*trans*, but the γ -atom is *gauche* to the α -amino and the α -carboxyl groups. The other two combinations, namely $(-60, -60^\circ)$ and $(60, -60^\circ)$ give rise to highly folded molecular geometries.

According to the classical energy calculations by Ponnuswamy & Sasisekharan (1971), the energetically favourable side-chain conformations in glutamic acid should correspond to χ^1, χ^2 combinations of $(60, 180), (180, 60), (180, 180), (-60, 180)$ and $(-60, -60^\circ)$. As can be seen from Table 2, all these combinations except $(180, 60^\circ)$ have been observed in the crystal structures containing glutamic acid. However, the combination $(60, -60^\circ)$ observed in L-glutamatois(ethylenediamine)cobalt(III) perchlorate occurs in a region of the χ^1, χ^2 map predicted to be energetically unfavourable by these workers. Admittedly, the calculations were empirical and approximate, and were carried out

Table 3. Conformational angles ($^\circ$) of glutamic acid (or the glutamate ion) in arginine glutamate monohydrate and other crystal structures

	ψ^1	ψ^2	χ^1	χ^2	χ^{31}	χ^{32}	Idealized values of χ^1, χ^2	Reference
Arginine glutamate. H_2O	-16	165	-57	-172	-3	180	-60, 180	(a)
Glutamic acid	-42	141	-52	-73	19	-161	-60, -60	(b)
Glutamic acid.HCl	-21	161	-69	-173	15	-167	-60, 180	(c)
Cu glutamate. H_2O	-4	179	68	-177	7	-172	60, 180	(d)
Zn glutamate. H_2O	0	180	67	-177	8	-170	60, 180	(e)
Ca glutamate. $3H_2O$	-18	164	179	175	12	-169	180, 180	(f)
Glutamatois(ethylenediamine)cobalt(III) perchlorate	12	-168	71	-92	55	-123	60, -60	(g)

(a) Present study. (b) Lehmann, Koetzle & Hamilton, 1972. (c) Sequeira, Rajagopal & Chidambaram, 1972. (d) Gramaccioli & Marsh, 1966a. (e) Gramaccioli & Marsh, 1966b. (f) Einspahr & Charles, 1974. (g) Gillard, Payne & Robertson, 1970.

for an isolated molecule. Therefore, the occurrence of a conformation predicted to be unfavourable by these calculations in a metal complex such as L-glutamato-bis(ethylenediamine)cobalt(III) perchlorate is perhaps not surprising.

Crystal structure and intermolecular interactions

The crystal structure of arginine glutamate monohydrate (Fig. 3) is stabilized by ionic interactions and hydrogen bonds involving amino, guanidyl and carboxylate groups and water molecules. Table 4 lists the parameters of what appear to be hydrogen bonds from geometrical considerations.

The crystal structure consists of alternating layers parallel to the *ab* plane, one layer consisting of arginine molecules and the other of glutamate ions. The former are centred on $z = \frac{1}{4}$ and $\frac{3}{4}$ and the latter on $z = 0$ and $\frac{1}{2}$. It may be recalled that the same structural feature, namely the existence of alternating layers, one layer consisting of the basic amino acid molecules and the other consisting of the acidic amino acid molecules, was also found in the structure of lysine aspartate. Thus it would appear that, when complex formation takes place in these crystals, like molecules aggregate first into independent layers and then these layers further aggregate (with each layer sandwiched between layers of unlike molecules) to form the crystal nucleus.

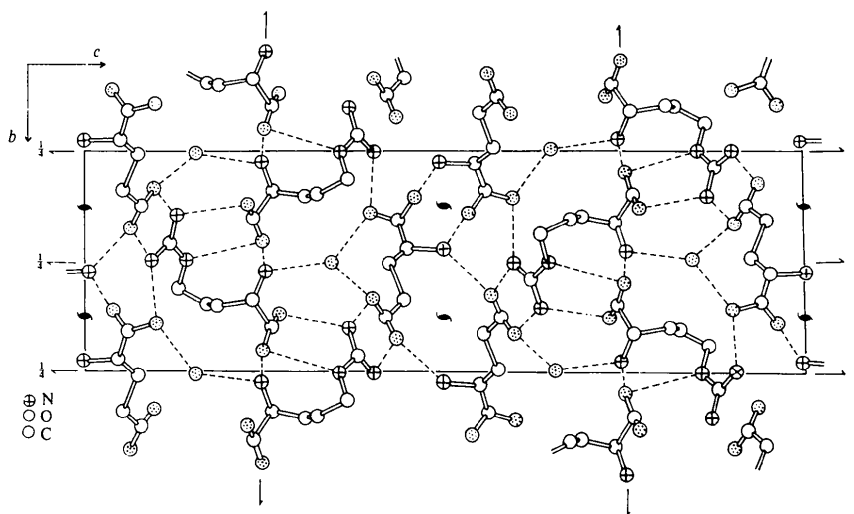


Fig. 3. The crystal structure as viewed along *a*. The broken lines indicate probable hydrogen bonds.

Table 4. *Hydrogen-atom parameters*

Unrefined positions of the hydrogen atoms were used to calculate the parameters involving them.

<i>A</i> -H... <i>B</i> - <i>C</i>	<i>A</i> ... <i>B</i>	H... <i>B</i>	H- <i>A</i> ... <i>B</i>	H... <i>B</i> - <i>C</i>
N(1)-H(11)...O(2)-C(1) ^{iv}	2.84 Å	1.9 Å	16°	124°
N(1)-H(12)...W(1) ^j	2.81	1.8	13	—
N(1)-H(13)...O(2)-C(1) ⁱⁱⁱ	3.18	2.2	16	109
N(6)-H(6)...O(1)-C(1) ⁱⁱⁱ	3.06	2.2	24	94
N(8)-H(81)...O(12)-C(11) ^{vi}	3.04	2.1	16	99
N(8)-H(82)...O(17)-C(15) ^v	2.83	1.8	7	113
N(9)-H(91)...O(1)-C(1) ⁱⁱⁱ	2.95	2.0	21	158
N(9)-H(92)...O(16)-C(15) ^v	2.85	1.9	6	128
N(11)-H(111)...O(11)-C(11) ^{vii}	2.92	1.9	5	105
N(11)-H(112)...O(17)-C(15) ^{ix}	2.70	1.7	17	144
N(11)-H(113)...O(11)-C(11) ^{viii}	2.88	2.1	10	127
W(1)-WH(1)...O(16)-C(15) ⁱⁱ	2.72	2.1	43	138
W(1)-WH(2)...O(12)-C(11) ^j	2.84	2.0	24	135

Symmetry code: (i) x, y, z ; (ii) $x - 1, y, z$; (iii) $-x, y + \frac{1}{2}, -z + \frac{1}{2}$; (iv) $-x + 1, y + \frac{1}{2}, -z + \frac{1}{2}$; (v) $-x + 2, y + \frac{1}{2}, -z + \frac{1}{2}$; (vi) $-x + 1, y - \frac{1}{2}, -z + \frac{1}{2}$; (vii) $x + \frac{1}{2}, -y + \frac{3}{2}, -z + 1$; (viii) $x - \frac{1}{2}, -y + \frac{3}{2}, -z + 1$; (ix) $x - \frac{1}{2}, -y + \frac{1}{2}, -z + 1$.

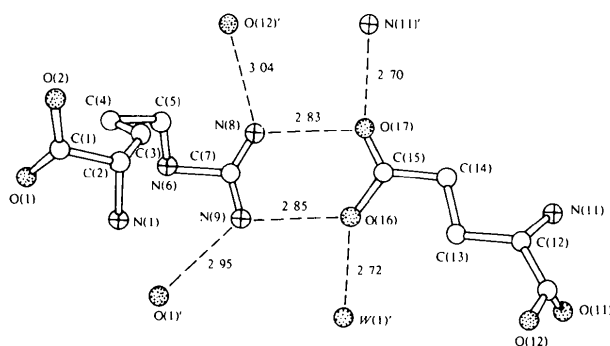


Fig. 4. Disposition of the arginine and the glutamate ions involved in the specific ion-pair interaction between the guanidyl group and the γ -carboxylate group, as viewed along the normal to the plane containing the interacting groups. The broken lines indicate hydrogen bonds. The hydrogen-bonded contact distances are also given. The symbols representing atoms in the neighbouring molecules are primed.

The adjacent layers in the structure are connected to each other principally through a specific ion-pair interaction (salt bridge) between the guanidyl group of arginine and the γ -carboxylate group of the glutamate, and a water bridge. This specific interaction between the arginine and the glutamate ions, which we consider as the most important feature of the structure, is illustrated in Fig. 4. It involves the electrostatic attraction between the positively charged guanidyl group and the negatively charged carboxylate group, and also two nearly parallel N—H...O hydrogen bonds. It may be mentioned that this is the first time such a specific side chain—side chain interaction has been observed at atomic resolution.

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