symmetry, while the N(3′)···N(6) bond of length 2·915 (4) Å, and N(3′)–H···N(6) angle of 178·0 (3)°, links the molecules forming chains along **c**. The other distances for intermolecular contacts correspond to normal van der Waals interactions.

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References

CLOSS, G. L. (1959). *J. Amer. Chem. Soc.* **81**, 5456–5461.
DUAX, W. L. & NORTON, D. A. (1975). *Atlas of Steroid Structure*. New York: Plenum.
FODOR, G., TOTH, J. & VINCZE, I. (1955). *J. Chem. Soc.* pp. 3504–3510.
GONZALEZ TRIGO, J., AVENDAÑO, C. & MARTÍNEZ MORENO, M. (1974). *Pharm. Mediterr.* **10**, 639–653.
International Tables for X-ray Crystallography (1974). Vol. IV, pp. 72–98. Birmingham: Kynoch Press.
MACGILLAVRY, C. H. & FODOR, C. (1964). *J. Chem. Soc.* pp. 597–600.
MAIN, P., WOOLFSON, M. M., LESSINGER, L., GERMAIN, G. & DECLERCQ, J.-P. (1974). *MULTAN 74. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Univs. York, England, and Louvain, Belgium.
MOOTZ, D. (1965). *Acta Cryst.* **19**, 726–734.
SCHENK, H., MACGILLAVRY, C. H., SKOLNIK, S. & LAAND, J. (1967). *Acta Cryst.* **23**, 423–426.
STEWART, J. M., KUNDELL, F. A. & BALDWIN, J. C. (1970). The XRAY 70 system. Computer Science Center, Univ. of Maryland, College Park, Maryland.
VISSER, J. W., MANASSEN, J. & DE VRIES, J. L. (1954). *Acta Cryst.* **7**, 288–291.
VOOREN, M., SCHENK, H. & MACGILLAVRY, C. H. (1970). *Acta Cryst.* B**26**, 1483–1487.
WALKER, L. A., FOLTING, K. & MERRITT, L. L. JR (1969). *Acta Cryst.* **19**, 726–734.

Acta Cryst. (1977). B**33**, 3385–3390

Calcium Binding to α-Amino Acids: The Crystal Structure of Calcium L-Glutamate Chloride Monohydrate

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Crystals of calcium L-glutamate chloride monohydrate [$CaCl(C_5H_8NO_4).H_2O$] are orthorhombic, space group $P2_12_12_1$, with $a = 5·0731$ (5), $b = 8·2168$ (5), $c = 23·067$ (2) Å and four formula weights per unit cell. A trial structure was obtained by Patterson and Fourier techniques and refined by full-matrix least-squares calculations. The final R index, at convergence, is 0·045 for 2463 absorption-corrected, Mo $K\alpha$, diffractometer data. The Ca ion is coordinated by carboxyl O atoms from five different glutamate anions. The sixfold coordination polyhedron is completed by the water molecule and is a distorted octahedron. Ca···O distances range from 2·319 to 2·396 Å. The glutamate carboxyl groups do not chelate Ca ions but serve only as unidentate ligands in the Ca coordination shell.

Introduction

The biological functions of the Ca ion are numerous and varied. Processes in which Ca plays a role include biological mineralization, muscle contraction, and nerve transmission. In many of these processes, protein–calcium complexes have been implicated.

Crystal-structure studies of a number of proteins that have calcium-binding sites have been reported in recent years (Stroud, Kay & Dickerson, 1971; Matthews, Colman, Jansonius, Titani, Walsh & Neurath, 1972; Edelman, Cunningham, Reeke, Becker, Waxdal & Wang, 1972; Cotton, Bier, Day, Hazen & Larsen, 1972; Kretsinger & Nockolds, 1973; Bode & Schwager, 1975). In most of these structure determinations, resolution was sufficient to establish the identity and spatial distribution of the residues that form the binding sites, and the results confirm that aspartate and glutamate residues are the major components of these sites.

To obtain information about structural factors that are likely to influence Ca interactions with proteins, we are currently investigating the crystal structures of several calcium–glutamate and –aspartate complexes. In this paper we describe the crystal structure of calcium L-glutamate chloride monohydrate and compare it with the previously reported crystal structure of calcium L-glutamate trihydrate (Einspahr & Bugg, 1974; also referred to herein as the trihydrate).

Experimental

Colorless, transparent crystals of the monohydrate complex were obtained by evaporation of an aqueous solution of a commercial product that contained Ca and L-glutamate ions* to which had been added an excess of $CaCl_2$. The solution was adjusted to pH 7·5 by adding HCl prior to evaporation. Weissenberg, oscillation, and precession photographs indicate an orthorhombic space group, $P2_12_12_1$ (systematic absences: $h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$).

The crystal chosen for data collection approximated an irregular prism of dimensions 0·1 × 0·1 × 0·2 mm. It was mounted on a Picker FACS-1 diffractometer with its a axis (corresponding to the long dimension of the crystal) slightly inclined to the φ axis of the goniostat. A scintillation counter, Nb-filtered Mo $K\alpha$ radiation, and a θ–2θ scanning procedure were employed to collect intensity data for the 2463 unique reflections with $2\theta \leq 70°$. A scan speed of 0·5° min^{-1} was used, and the background was sampled for 40 s at each terminus of the scans.

The intensities of three standard reflections (0,0,14, 051, 2,3,10), which were monitored periodically during the data-collection procedure, varied by no more than 4% from average values, and no systematic decay was observed. However, the intensities of the standards appeared to fluctuate in a manner that suggested slight, long-period instability in the X-ray generator or slow variations in temperature. The data were scaled to minimize fluctuations in the intensity of the 0,0,14 reflection. Remeasurement of the 20 most intense reflections, with tube current reduced by 50%, indicated that coincidence losses had not affected data collection. Intensities were assigned variances, $\sigma^2(I)$, according to counting statistics plus an additional term, $(0{\cdot}03S)^2$, S being the scan count. Reflections with scan counts below background levels were assigned negative intensity values and retained in subsequent calculations; 242 reflections, about 10% of the data, have intensities that are less than corresponding estimated standard deviations. Intensities and variances were corrected for Lorentz and polarization effects; program *ORABS* (Wehe, Busing & Levy, 1962) was used to make absorption corrections. Transmission factors range between 0·900 and 0·925. The data were placed on absolute scale by means of a Wilson (1942) plot.

Table 1. *Crystal data*

Calcium glutamate chloride monohydrate
$CaCl(C_5H_8NO_4).H_2O$
$P2_12_12_1$
a 5·0731 (5) Å
b = 8·2168 (5)
c = 23·067 (2)
V = 961·54 Å^3

FW 239·7
ρ_o* = 1·63 (1) g cm^{-3}
ρ_c = 1·66
Z = 4
μ(Mo $K\alpha$) = 8·96 cm^{-1}

* The density was measured by flotation in a mixture of carbon tetrachloride and ethylene bromide.

Approximate cell parameters for use in data collection were calculated by a least-squares analysis of the angular settings for 12 medium-angle reflections. Immediately after data collection, more precise cell parameters were obtained by a least-squares analysis of the 2θ values of 18 reflections (with $2\theta > 35°$) measured with Mo $K\alpha_1$ radiation (λ = 0·70930 Å) at room temperature (24 ± 2°C). These latter values are not significantly different from those obtained prior to intensity measurements. Table 1 lists these values together with other crystal data.

Structure determination and refinement

Coordinates for the Ca ion were determined from a three-dimensional Patterson map. Coordinates for the Cl ion were obtained from a three-dimensional, sum-function superposition of sharpened Patterson maps translated to the Ca positions. All remaining non-hydrogen atoms were identified in an electron density map calculated with phases given by the Ca and Cl ion contributions. Seven of the ten H atoms were found in a difference map calculated after eight cycles of block-diagonal least-squares refinement of the heavy-atom parameters, the last two cycles of which included anisotropic thermal parameters for all atoms. The remaining hydrogens, one on the amino group and two on the water molecule, were located in a difference map calculated after four cycles of block-diagonal refinement in which the positional and isotropic thermal parameters of the H atoms were allowed to vary. Six additional cycles of least-squares refinement of the completed model produced convergence. The final value of the R index $(\Sigma||F_o| - |F_c||/\Sigma|F_o|)$ is 0·045; and the goodness-of-fit $\{[\Sigma w\Delta^2/(m-s)]^{1/2}$, where m is the number of observations and s is the number of parameters$\}$ is 1·14. A modified version of the full-maxtrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962; Busing, 1971) was used for the last four cycles. The quantity minimized was $\Sigma w(F_o^2 - F_c^2/k^2)^2$, where k is the scale factor and weights w are equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms (Ca^{2+},Cl^-,O,N,C) were taken from *International Tables for X-ray Crystallography* (1962); anomalous scattering factors for these atoms were taken from

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* This same commercial product (labeled L Glutamic Acid Di Calcium Monohydrate, Nutritional Biochemicals Corporation, Lot 8244) was also used to prepare the trihydrate complex, and was referred to in the report of that structure (Einspahr & Bugg, 1974) as calcium diglutamate.

Cromer & Liberman (1970). The atomic scattering factors of Stewart, Davidson & Simpson (1965) were used for H atoms. No attempt was made to refine a D-isomer model to corroborate the choice of the L-enantiomer; structure analysis of the trihydrate complex confirmed that the starting material contains the L-glutamate ion and the quality of the L-isomer refinement does not suggest otherwise.

An attempt to refine a secondary extinction parameter [*g* of Zachariasen (1967) as formulated by Coppens & Hamilton (1970)] in the first two cycles of full-matrix refinement was unsuccessful, as evidenced by convergence to a negative value. This parameter was removed for the last two cycles of refinement. In the final structure factor calculation only 2 of the 20 strongest reflections had $|\Delta/\sigma|$ terms greater than 3·0, whereas 17 had $|\Delta/\sigma| < 2$; however, 15 of the 20 had $\Delta/\sigma > 0$. Of the 25 reflections with $|\Delta/\sigma| > 3{\cdot}0$, 17 had $\Delta/\sigma > 0$; the 3 with $|\Delta/\sigma| > 5{\cdot}0$ all had $F_o^2 > F_c^2$. The only truly aberrant reflection was 002, one of the twenty strongest, which had $\Delta/\sigma = 7{\cdot}4$. The low-angle background count for this reflection is approximately two thirds as much as that on the high-angle side because the Nb *K* absorption edge is within the scan range (0·8° plus dispersion correction) for this reflection. It is the only reflection in the data set so affected. This reflection was not removed from the data set, and no attempt was made to refine an extinction parameter with the 002 reflection given zero weight.

Table 2. *Positional and isotropic thermal parameters*

(*a*) Nonhydrogen atoms. *z* parameters of all atoms and *x* and *y* parameters of Ca and Cl are multiplied by 10^5; all other parameters are multiplied by 10^4.

	x	*y*	*z*
Ca	79387 (9)	73145 (5)	43786 (2)
Cl	15429 (16)	61415 (7)	24550 (2)
O(1)	4058 (4)	6129 (2)	40076 (8)
O(2)	47 (3)	5032 (2)	39612 (7)
O(3)	5352 (4)	−470 (2)	46319 (9)
O(4)	1162 (4)	−1000 (2)	47809 (7)
W	8841 (7)	8269 (3)	34353 (9)
N(1)	5917 (5)	4141 (3)	31777 (9)
C(1)	2498 (4)	5049 (3)	38572 (8)
C(2)	3587 (4)	3579 (2)	35247 (9)
C(3)	4458 (5)	2200 (3)	39291 (11)
C(4)	2212 (5)	1409 (3)	42453 (12)
C(5)	2985 (5)	−135 (2)	45663 (9)

(*b*) Hydrogen atoms. All parameters are multiplied by 10^3. The isotropic thermal parameter is the coefficient in the expression $T = \exp(-8\pi^2 U \sin^2 \theta/\lambda^2)$.

	x	*y*	*z*	*U*
H(1)	548 (7)	497 (4)	295 (2)	54 (10)
H(2)	660 (6)	330 (3)	297 (1)	32 (8)
H(3)	716 (8)	445 (4)	344 (2)	56 (11)
H(4)	219 (6)	320 (3)	323 (1)	25 (7)
H(5)	520 (6)	134 (4)	370 (1)	31 (8)
H(6)	562 (7)	251 (4)	420 (2)	51 (10)
H(7)	56 (8)	121 (5)	402 (2)	64 (11)
H(8)	137 (10)	223 (5)	457 (2)	99 (15)
H(9)	853 (10)	898 (5)	327 (2)	77 (15)
H(10)	951 (9)	767 (6)	323 (2)	84 (17)

The final difference Fourier map has no peaks or troughs of magnitude greater than 0·48 e Å^{-3}. The major features occur near the Ca and Cl ions, or in positions intermediate between atoms joined by covalent bonds, or in the vicinity of carboxylate O atoms. Disregarding these features, the noise level of the map might be estimated at about 0·35 e Å^{-3}.

Results

Table 2 lists the final atomic parameters and estimated standard deviations.* Fig. 1 shows the coordination geometry of the Ca ion. Coordination is sixfold and the ligand atoms are arranged in a distorted octahedron. Five of the ligand atoms are O atoms from the α and γ-carboxyl groups of the glutamate anion. The sixth is the O atom of the water molecule. The carboxyl groups serve only as unidentate ligands in the coordination polyhedron; carboxylate chelation of Ca does not occur in this crystal structure. Ca--O distances range from 2·319 to 2·396 Å; no other calcium-oxygen distances are less than 3·0 Å. The shortest oxygen-oxygen distance within the polyhedron is 2·993 Å.

The Ca environment is presented in Fig. 2, wherein it

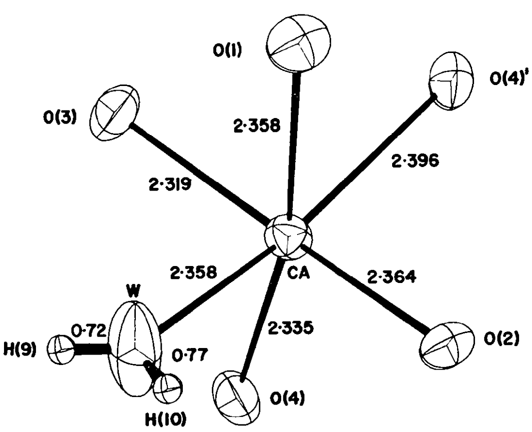

Fig. 1. The Ca coordination shell. The positions of O(2), O(3), O(4), and O(4)′ differ from those in Table 2 by operations $1 + x, y, z$; $x, 1 + y, z$; $1 + x, 1 + y, z$; and $\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$; respectively. The estimated standard deviation of a Ca--O distance is 0·002 Å; of an O–H distance, 0·05 Å. Thermal ellipsoids are drawn at the 50% probability level. [This drawing, as well as those of Figs. 2–5, was prepared with the aid of the program *ORTEP* (Johnson, 1965).]

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* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32695 (17 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

may be seen that four of the ligand O atoms are the four different carboxylate O atoms from glutamate residues related by unit-cell translations. A fifth ligand is O(4) from the γ-carboxylate of a symmetry-related glutamate. Atom O(4) serves to bridge Ca ions and link coordination polyhedra. The distance between Ca ions in adjacent polyhedra is 3·840 Å.

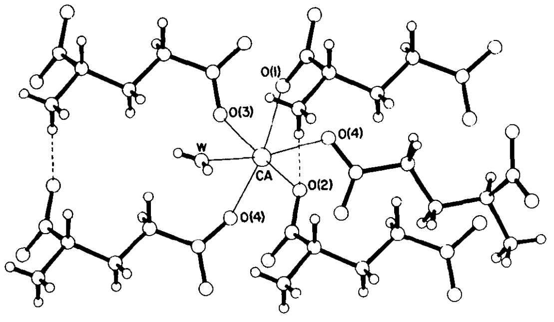

Fig. 2. A representation of the Ca ion environment. Hydrogen bonds are shown as dashed lines; Ca··O interactions are shown as thin lines.

Table 3. *Hydrogen-bond distances and angles*

D–H···A	D···A	H···A	∠D–H···A	∠H–D···A
N(1)–H(1)···Cl	3·226 Å	2·49 Å	140°	30°
N(1)–H(2)···Cl^{i}	3·141	2·23	174	4
N(1)–H(3)···O(2^{ii})	2·862	1·96	175	3
W–H(10)···Cl^{ii}	3·170	2·41	171	7
W–H(9)···Cl^{iii}	3·134	2·44	164	12

Symmetry code

(i) $1-x, y-\frac{1}{2}, \frac{1}{2}-z$; (ii) $1+x, y, z$; (iii) $1-x, \frac{1}{2}+y, \frac{1}{2}-z$

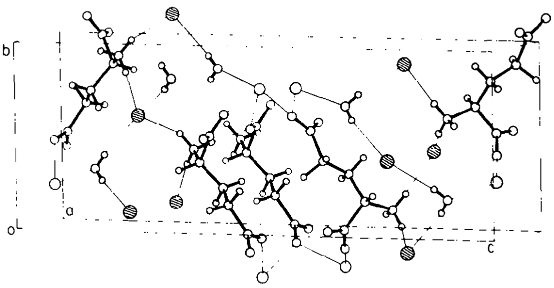

Fig. 3. A representation of the unit-cell contents. Ca and Cl ions are shown as circles of larger diameter and those of Cl are hatched. Hydrogen bonds and Ca··O interactions are shown as thin lines.

Table 4. *Valence angles*

E.s.d.'s of angles formed exclusively by nonhydrogen atoms are 0·2°, those involving H atoms are 2–3°.

O(1)–C(1)–O(2)	125·7°	C(2)–C(3)–C(4)	113·4°
O(1)–C(1)–C(2)	118·3	C(2)–C(3)–H(5)	108
O(2)–C(1)–C(2)	116·1	C(2)–C(3)–H(6)	114
C(1)–C(2)–C(3)	112·5	C(4)–C(3)–H(5)	105
C(1)–C(2)–N(1)	107·9	C(4)–C(3)–H(6)	106
N(1)–C(2)–C(3)	109·0	H(5)–C(3)–H(6)	110
C(1)–C(2)–H(4)	109	C(3)–C(4)–C(5)	113·7
N(1)–C(2)–H(4)	106	C(3)–C(4)–H(7)	117
C(3)–C(2)–H(4)	112	C(3)–C(4)–H(8)	111
C(2)–N(1)–H(1)	111	C(5)–C(4)–H(7)	109
C(2)–N(1)–H(2)	110	C(5)–C(4)–H(8)	106
C(2)–N(1)–H(3)	111	H(7)–C(4)–H(8)	98
H(1)–N(1)–H(2)	111	C(4)–C(5)–O(3)	119·6
H(1)–N(1)–H(3)	111	C(4)–C(5)–O(4)	118·0
H(2)–N(1)–H(3)	107	O(3)–C(5)–O(4)	122·3
H(9)–W–H(10)	107°		

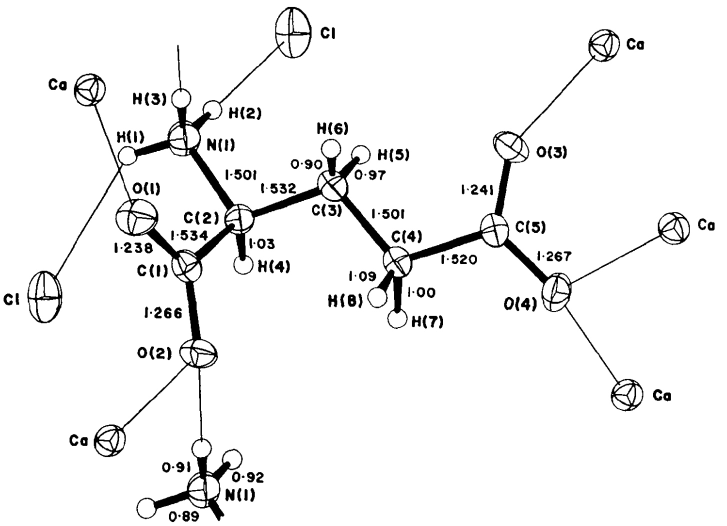

Fig. 4. The environment of the glutamate anion. Thermal ellipsoids are drawn at the 50% probability level. H atoms are drawn as spheres of radius 0·09 Å. Distances are in Å. E.s.d.'s for lengths of bonds between nonhydrogen atoms are 0·004 Å; those for distances involving an H atom are 0·04 Å.

Fig. 2 also shows the hydrogen bond, N(1)–H(3)···O(2), which links glutamates separated by a unit-cell translation in **a**. Table 3 lists the hydrogen bonds found in this structure together with pertinent distances and angles. All H atoms available for hydrogen-bond formation are so used. The Cl ion is the acceptor in four of the five bonds, two from the amino group and two from the water molecule. The water molecule thus serves as an intermediary between the Ca and Cl ions, and there are no calcium-chlorine distances less than 4 Å.

Fig. 3 is a representation of the unit-cell contents and shows the packing plus all hydrogen bonds. The main stabilizing factors of the structure are (1) a Ca-coordination system that links glutamate ions and is roughly localized in planes at $z = 0, \frac{1}{2}$ and (2) the hydrogen bonds to Cl ions that lie roughly in planes at $z = \frac{1}{4}, \frac{3}{4}$.

The glutamate ion and its environment are shown in Fig. 4. Included are bond distances. Valence angles for the glutamate are listed in Table 4. Table 5 compares the torsion angles for this ion with those found in the trihydrate structure.

Discussion

The pertinent features of this crystal structure are best recognized by comparing the structures of the mono- and tri-hydrate complexes. Bond distances and angles of the glutamate ion correspond closely in the two structures, and, as indicated in Table 5, there are only minor differences in the conformations of anions. Conformations of the *R* groups [atoms C(3) and C(4), and the γ-carboxyl group] are essentially the same, the maximum difference in corresponding torsion angles being some 9°. The chief conformational difference in the two glutamate residues is found in the orientation of the α-carboxyl groups. This variability in α-carboxyl group orientation appears to be a general feature of amino acid crystal structures (Marsh & Donohue, 1967). The α-carboxyl plane in the monohydrate is twisted farther out of the C(1)–C(2)–N(1) plane than in the trihydrate. In the latter structure, the amino group is not protonated, and it acts in concert with the α-carboxyl group to chelate a Ca ion; this bridging interaction of the Ca ion between the α-carboxyl and the amino groups probably accounts for the more nearly planar arrangement around the α-C atom in the trihydrate structure. In the copper– and zinc–glutamate dihydrate structures (Gramaccioli & Marsh, 1966; Gramaccioli, 1966) where this type of chelation is also observed, α-carboxyl groups are within 4° of coplanarity with α-C and N atoms.

Table 5. *Torsion angles for the glutamate anions in calcium glutamate chloride monohydrate* (I) *and calcium glutamate trihydrate* (II) (*Einspahr & Bugg,* 1974)

E.s.d.'s are 0·3° in both studies. Signs of the angles reflect the convention of Klyne & Prelog (1960).

	(I)	(II)
O(1)–C(1)–C(2)–N(1)	−30·7°	−18·2°
O(2)–C(1)–C(2)–N(1)	150·4	163·7
O(1)–C(1)–C(2)–C(3)	89·5	101·8
O(2)–C(1)–C(2)–C(3)	−89·4	−76·2
C(1)–C(2)–C(3)–C(4)	67·4	58·2
N(1)–C(2)–C(3)–C(4)	−173·0	179·4
C(2)–C(3)–C(4)–C(5)	170·2	174·6
C(3)–C(4)–C(5)–O(3)	12·3	12·3
C(3)–C(4)–C(5)–O(4)	−170·9	−169·1

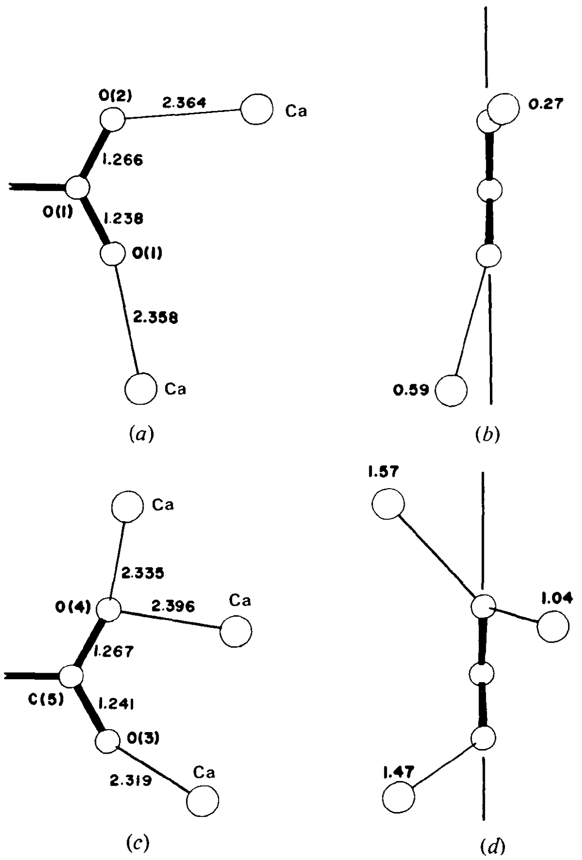

Fig. 5. The calcium coordination geometries of the glutamate carboxyl groups in the monohydrate complex: (*a*) and (*b*), views of the α-group; (*c*) and (*d*), views of the γ-group. Deviations (Å) of Ca ions from carboxyl planes are shown in (*b*) and (*d*). E.s.d.'s of out-of-plane distances are approximately 0·01 Å.

A major difference between the monohydrate and trihydrate structures is the coordination number of the Ca ions. The sixfold Ca coordination in the monohydrate structure contrasts with the eightfold coordination found in the trihydrate. As might be expected for these coordination numbers [see, for example, Shannon (1976), Fig. 2*b*], the Ca--O distances within the coordination polyhedra are somewhat shorter in the monohydrate than in the trihydrate; the average Ca--O distances are 2·35 and 2·48 Å, respectively.

Fig. 5 depicts the geometries found for the interactions between Ca ions and the O atoms of the α- and γ-carboxyl groups in the monohydrate structure. Although the Ca--O distances cover a rather narrow range, between 2·32 and 2·40 Å, there is no consistent pattern of coordination. The C–O--Ca angles cover a wide range of values, and the Ca displacements from the carboxyl planes range from 0·3 to 1·6 Å. This is in contrast to the findings in the trihydrate structure wherein both the α- and γ-carboxyl--Ca interactions are bidentate and show remarkably similar geometries. As might be expected, bidentate chelation appears to impose considerable geometrical constraints on carboxyl--Ca interactions relative to the unidentate mode which shows appreciable structural flexibility.

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References

Bode, W. & Schwager, P. (1975). *J. Mol. Biol.* **98**, 693–717.

Busing, W. R. (1971). *Acta Cryst.* A**27**, 683–684.

Busing, W. R., Martin, K. O. & Levy, H. A. (1962). *ORFLS*. Report ORNL-TM-305. Oak Ridge National Laboratory, Tennessee.

Coppens, P. & Hamilton, W. C. (1970). *Acta Cryst.* A**26**, 71–83.

Cotton, F. A., Bier, C. J., Day, V. W., Hazen, E. E. Jr & Larsen, S. (1972). *Cold Spring Harb. Symp. quant. Biol.* **36**, 243–249.

Cromer, D. T. & Liberman, D. (1970). *J. Chem. Phys.* **53**, 1891–1898.

Edelman, G. M., Cunningham, B. A., Reeke, G. N. Jr, Becker, J. W., Waxdal, M. J. & Wang, J. L. (1972). *Proc. Natl. Acad. Sci. US*, **69**, 2580–2584.

Einspahr, H. & Bugg, C. E. (1974). *Acta Cryst.* B**30**, 1037–1043.

Gramaccioli, C. M. (1966). *Acta Cryst.* **21**, 600–605.

Gramaccioli, C. M. & Marsh, R. E. (1966). *Acta Cryst.* **21**, 594–600.

International Tables for X-ray Crystallography (1962). Vol. III, pp. 202–209. Birmingham: Kynoch Press.

Johnson, C. K. (1965). *ORTEP*. Report ORNL-3794, revised. Oak Ridge National Laboratory, Tennessee.

Klyne, W. & Prelog, V. (1960). *Experientia*, **16**, 521–523.

Kretsinger, R. H. & Nockolds, C. E. (1973). *J. Biol. Chem.* **248**, 3313–3326.

Marsh, R. E. & Donohue, J. (1967). *Advanc. Protein Chem.* **22**, 235–255.

Matthews, B. W., Colman, P. M., Jansonius, J. N., Titani, K., Walsh, K. A. & Neurath, H. (1972). *Nature New Biol.* **238**, 41–44.

Shannon, R. D. (1976). *Acta Cryst.* A**32**, 751–767.

Stewart, R. F., Davidson, E. R. & Simpson, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

Stroud, R. M., Kay, L. M. & Dickerson, R. E. (1971). *Cold Spring Harb. Symp. quant. Biol.* **36**, 125–140.

Wehe, D. J., Busing, W. R. & Levy, H. A. (1962). *ORABS*. Report ORNL-TM-229. Oak Ridge National Laboratory, Tennessee.

Wilson, A. J. C. (1942). *Nature, Lond.* **150**, 151–152.

Zachariasen, W. H. (1967). *Acta Cryst.* **23**, 558–564.