

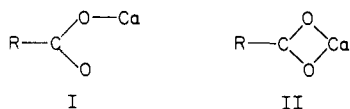
Calcium and Magnesium Binding to γ -Carboxyglutamate and β -Carboxyaspartate Residues: Structures of Calcium and Magnesium Complexes of Methylmalonic Acid

Maria E. Curry,^{1a} Drake S. Eggleston,^{*1b} and Derek J. Hodgson^{*1a}

Contribution from the Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27514, and Smith, Kline, & French Laboratories, Philadelphia, Pennsylvania 19101. Received July 15, 1985

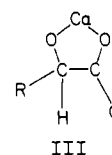
Abstract: The syntheses and molecular structures of the calcium and magnesium complexes of α -methylmalonate ion are described. The calcium complex, $\text{Ca}_3(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$, crystallizes in the monoclinic space group $C2/c$ with four formula units in a cell of dimensions $a = 16.869(7) \text{ \AA}$, $b = 18.951(7) \text{ \AA}$, $c = 6.635(7) \text{ \AA}$, $\beta = 90.80(7)^\circ$, and its structure has been refined to a final R -factor of 0.077 based on 812 independent observed intensities. The magnesium complex, $\text{Mg}(\text{C}_4\text{H}_4\text{O}_4)(\text{H}_2\text{O})_4$, crystallizes in space group $Pnma$ of the orthorhombic system with four molecules in a cell of dimensions $a = 7.455(2) \text{ \AA}$, $b = 7.985(2) \text{ \AA}$, and $c = 14.279(2) \text{ \AA}$; the structure has been refined to a final R -factor of 0.035 based in 848 independent observed intensities. In the calcium complex there are two inequivalent calcium centers, Ca(1) and Ca(2). The geometry at Ca(1) is seven-coordinate, and the methylmalonate ions interacting with this calcium site exhibit all three possible binding modes: unidentate, bidentate, and "malonate" mode (i.e., a bidentate mode involving O atoms from the two different carboxylate groups of a single methylmalonate ion). The geometry at Ca(2), which lies on a twofold axis, is eight-coordinate, and there is no malonate binding at this site. The magnesium complex exhibits nearly regular octahedral geometry, the only interaction with the anion being of the malonate type. The role of these various binding modes and of the observed geometric preferences of Ca(II) and Mg(II) in determining the affinities of Ca(II) and Mg(II) for the various metal binding sites in proteins containing γ -carboxyglutamate (Gla) or β -carboxyaspartate (Asa) residues is discussed in the light of the observed structures.

The presence of both γ -carboxyglutamic (Gla) and β -carboxyaspartic (Asa) acid residues in a variety of proteins is well-established,²⁻⁸ although the precise reason for the occurrence of the post-translational⁹ carboxylation of glutamate (Glu) and aspartate (Asp) residues to Gla and Asa is still a matter of some speculation. Several authors, however, have noted that a primary role of Gla may be in discriminating between calcium and magnesium ions.¹⁰ It is noteworthy in this regard that the presence of calcium is essential to the functioning of prothrombin and that substitution of calcium by magnesium renders the enzyme incapable of binding appreciably to phospholipids.^{11,12} The significant modes of binding of calcium (and other metal ions) to carboxylate groups have been examined in detail by Einspahr and Bugg,¹³ who note two common modes of calcium carboxylate interaction (I, II). In addition,

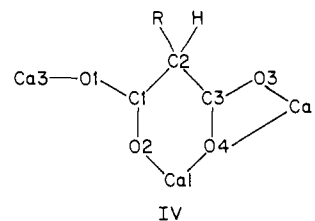


these authors note that the presence of a suitable ligand atom (presumably another oxygen atom) on the carbon atom α to the

carboxyl carbon atom leads to a third mode of binding (III), which they refer to as the α -mode.¹³



In a protein containing only Glu and/or Asp carboxylate side chains, modes I and II would be available but mode III would be unavailable. The substitution of Glu by Gla or of Asp by Asa, however, leads to the possibility of three separate modes of metal-carboxylate interaction, all of which are depicted in IV;



the binding mode involving Ca1, O2, and O4 in IV is analogous to that depicted in III and involves intramolecular binding to two oxygen atoms from two different carboxylate moieties; this mode of binding is sometimes referred to as the "malonate mode" since it is unavailable in a monocarboxylic system but available in a dicarboxylate such as malonate ion.

Since the only crystallographic study of a metal complex of Gla or Asa which has appeared is a preliminary communication published in 1977,¹⁴ the precise modes of calcium and magnesium binding to these species may be best probed by the use of suitable models. All three binding modes depicted in IV are available to malonate ion and its derivatives, so the use of substituted malonates as models for Gla and Asa in such studies is indicated. Metal complexes of malonate ion itself have been extensively studied,¹⁵⁻²⁰

(1) (a) Department of Chemistry, University of North Carolina. (b) Smith, Kline and French Laboratories.

(2) Stenflo, J.; Fernlund, P.; Egan, W.; Roepstorff, P. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *73*, 2730-2733.

(3) Jackson, C. M.; Nemerson, Y. *Annu. Rev. Biochem.* **1980**, *49*, 765-811.

(4) Hauschka, P. V.; Lian, J. B.; Gallop, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3925-3929.

(5) Price, P. A.; Otsuka, A. S.; Poser, J. W.; Kristaponis, J.; Raman, N. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1447-1451.

(6) Linde, A.; Bhowm, M.; Butler, W. T. *J. Biol. Chem.* **1980**, *255*, 5931-5942.

(7) Christy, M. R.; Barkley, R. M.; Koch, T. H.; Van Buskirk, J. J.; Kirsch, W. M. *J. Am. Chem. Soc.* **1981**, *103*, 3935-3937.

(8) Christy, M. R.; Koch, T. H. *J. Am. Chem. Soc.* **1982**, *104*, 1771-1772.

(9) Esmon, C. T.; Sadowski, J. A.; Suttie, J. W. *J. Biol. Chem.* **1975**, *250*, 4744-4748.

(10) Williams, R. J. P. In "Calcium Binding Proteins and Calcium Function"; Wasserman, R. H. et al., Eds.; Elsevier: New York, 1977; pp 1-12.

(11) Esmon, C. T.; Suttie, J. W.; Jackson, C. M. *J. Biol. Chem.* **1975**, *250*, 4095-4099.

(12) Lewis, M. R.; Deerfield, D. W.; Hoke, R. A.; Koehler, K. A.; Pedersen, L. G.; Hiskey, R. G., submitted to *J. Org. Chem.*

(13) Einspahr, H.; Bugg, C. E. *Acta Crystallogr., Sect. B: Struct. Sci.* **1981**, *B37*, 1044-1052.

(14) Satyshur, K.; Rao, S. T. Abstracts American Crystallographic Association Summer Meeting, 1977, p 74.

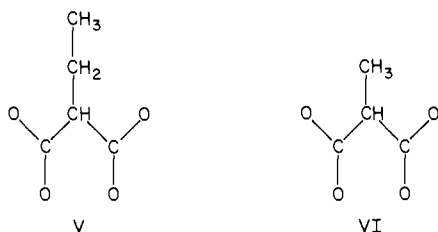
(15) Briggman, B.; Oskarsson, A. *Acta Crystallogr., Sect. B: Struct. Sci.* **1977**, *B33*, 1900-1906.

Table I. Crystallographic and Data Collection Parameters

parameters	Ca ₃ (C ₄ H ₄ O ₄) ₃ ·4H ₂ O	Mg(C ₄ H ₄ O ₄)·4H ₂ O
space group	C2/c	<i>Pnma</i>
<i>a</i> (Å)	16.869 (7)	7.455 (2)
<i>b</i> (Å)	18.951 (7)	7.985 (2)
<i>c</i> (Å)	6.635 (7)	14.279 (2)
β (deg)	90.80 (7)	90
<i>V</i> (Å ³)	2121	850
NO[I > 3σ(I)]	812	848
<i>D</i> ₀	1.71	1.65
<i>Z</i>	4	4
<i>D</i> _c	1.692	1.660
μ (cm ⁻¹)	8.2	2.1
data range	2° ≤ θ(Mo) ≤ 25°	1° ≤ θ(Mo) ≤ 28°
<i>R</i> ₁	0.077	0.035
<i>R</i> ₂	0.079	0.049

and the malonate form of interaction is frequently observed. In the case of calcium, however, there are two crystal forms of Ca(mal)·2H₂O, one of which exhibits the malonate mode¹⁷ and one which does not.^{15,16} In the case of magnesium, the cell constants and space group of Mg(mal)·2H₂O have been deduced from powder data,²¹ but no molecular structure has been reported; in the acid form, Mg(C₃H₃O₂)₂·2H₂O, the malonate mode is observed.¹⁸

Malonate ion itself is a less satisfactory model than some of its derivatives for Asa and Gla because of the absence of a substituent at C(2); α-ethylmalonate ion V and α-methylmalonate ion VI are, however, excellent structural analogues for the Gla and Asa side chains, respectively.



Very recently, Zell, Einspahr, and Bugg have reported the crystal structure of a calcium complex of α-ethylmalonate, in which they observe all three of the binding modes shown in IV.²² Moreover, on the basis of this structural study, they suggest that the malonate binding mode is significant in the biochemistry of Gla residues and may, therefore, be a functional role of the post-translational carboxylation process.²²

Our own studies in this area have concentrated on α-methylmalonate ion VI and its interactions with calcium and magnesium.²³ We here report the structures of hydrated calcium and magnesium complexes of α-methylmalonate ion, Ca₃(memal)₃·4H₂O, and Mg(memal)·4H₂O.

Experimental Section

Synthesis. Ca₃(memal)₃·4H₂O. A solution of 0.0185 g (2.5 × 10⁻⁴ m) of calcium hydroxide in 10 mL of warm (60 °C) water was added dropwise with vigorous stirring to a solution of 0.0295 g (2.5 × 10⁻⁴ m) of α-methylmalonic acid in 2 mL of methanol. The mixture was lyophilized, and the resultant white powder was recrystallized from water.

(16) Karipides, A.; Ault, J.; Reed, A. T. *Inorg. Chem.* **1977**, *16*, 3299–3302 and references therein. Marsh, R. E.; Schomaker, V. *Inorg. Chem.* **1979**, *18*, 2331–2336.

(17) Albertsson, J.; Oskarsson, A.; Svensson, C. *Acta Crystallogr. Sect. B: Struct. Sci.* **1978**, *B34*, 2737–2743.

(18) Briggman, B.; Oskarsson, A. *Acta Crystallogr., Sect. B: Struct. Sci.* **1978**, *B34*, 3357–3359.

(19) Soriano-Garcia, M.; Rao, S. N. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1983**, *C39*, 850–852.

(20) Hansson, E. *Acta Chem. Scand.* **1973**, *27*, 2813–2826, 2827–2840, 2841–2851.

(21) Walter-Levy, L.; Perrotey, J.; Visser, J. W. *Bull. Soc. Chim. Fr.* **1973**, 2596–2602.

(22) Zell, A.; Einspahr, H.; Bugg, C. E. *Biochemistry* **1985**, *24*, 533–537.

(23) Curry, M. E.; Hodgson, D. J.; Eggleston, D. S. *Rev. Port. Quim.* **1985**, *27*, 344.

Table II. Atomic Positional Parameters for Ca₃(C₄H₄O₄)₃·4H₂O

atom	x	y	z
Ca(1)	0.2015 (1)	0.2041 (1)	-0.2304 (4)
Ca(2)	0.0000 (0)	-0.0584 (2)	0.2500 (0)
O(1)	0.1978 (5)	0.1716 (5)	0.433 (1)
O(2)	0.2335 (5)	0.1917 (4)	0.119 (1)
O(3)	0.0757 (5)	0.0209 (5)	0.059 (1)
O(4)	0.1363 (5)	0.1004 (4)	-0.126 (1)
O(5)	0.3823 (5)	0.2010 (6)	0.379 (1)
O(6)	0.4186 (5)	0.2339 (5)	0.076 (1)
OW(1)	0.1661 (5)	0.3390 (5)	0.251 (1)
OW(2)	0.4715 (6)	0.3452 (5)	0.479 (2)
C(1)	0.2067 (7)	0.1519 (6)	0.256 (2)
C(2)	0.1934 (7)	0.0749 (6)	0.206 (2)
C(3)	0.1303 (7)	0.0658 (7)	0.033 (2)
C(4)	0.2690 (9)	0.0395 (7)	0.146 (2)
C(5)	0.4264 (9)	0.1969 (7)	0.236 (2)
C(6)	0.5000 (0)	0.1472 (9)	0.250 (0)
C(7)	0.5027 (16)	0.0947 (16)	0.420 (5)

Table III. Atomic Positional Parameters for Mg(C₄H₄O₄)(OH)₂

atom	x	y	z
Mg	0.3428 (1)	0.250	0.57873 (5)
O1W	0.4473 (2)	0.0634 (2)	0.66309 (8)
O1	0.2461 (2)	0.0689 (2)	0.48888 (8)
O2W	0.1099 (3)	0.250	0.6561 (1)
O2	0.1717 (2)	-0.0026 (2)	0.34532 (9)
O3W	0.5806 (3)	0.250	0.5066 (1)
C1	0.1621 (2)	0.0940 (2)	0.4137 (1)
C2	0.0427 (3)	0.250	0.4083 (2)
C3	-0.0828 (4)	0.250	0.3254 (2)
HC2	-0.014 (4)	0.250	0.459 (2)
HO2W	0.029 (4)	0.330 (3)	0.659 (2)
HO3W	0.655 (3)	0.335 (2)	0.503 (1)
H1O1W	0.414 (3)	0.042 (3)	0.718 (2)
H1C3	-0.167 (3)	0.139 (3)	0.327 (1)
H2O1W	0.560 (4)	0.040 (3)	0.659 (2)
H2C3	-0.008 (4)	0.250	0.264 (2)

Anal. Calcd for Ca₃(C₄H₄O₄)₃·4H₂O: C, 26.67; H, 3.73. Found: C, 26.80; H, 3.65.

Mg(memal)·4H₂O. A solution of 0.175 g (3 × 10⁻³ m) of magnesium hydroxide in 10 mL of warm water was added dropwise to a solution of 0.3543 g (3 × 10⁻³ m) of α-methylmalonic acid in 5 mL of methanol. The mixture was allowed to stand for several days, after which time colorless crystals were removed by filtration. Anal. Calcd for Mg(C₄H₄O₄)·4H₂O: C, 22.61; H, 5.69. Found: C, 22.81; H, 5.60.

X-ray Data Collection. Ca₃(memal)₃·4H₂O. A colorless prismatic crystal was mounted on an Enraf-Nonius CAD-4 diffractometer; preliminary analysis demonstrated that the complex belongs to the monoclinic system, the space group being either C2/c (no. 15) or Cc (no. 9). The crystal quality, as judged from examination of the ω-scans, was very poor, but no superior sample could be found. A weighting scheme of the type described by Ibers and co-workers²⁴ was applied, the weighting factor *p* being set to 0.02. The data were corrected for Lorentz-polarization effects but not for absorption. Cell constants and other experimental data are collected in Table I.

Mg(memal)·4H₂O. Preliminary observations indicated the orthorhombic system, space group *Pnma* (no. 62) or *Pna*₂ (no. 33). Data were collected as above, but the value of *p* was set to 0.05. Other data are assembled in Table I.

Solution and Refinement of the Structures. (a) Ca₃(memal)₃·4H₂O. The structure was solved in the centrosymmetric space group C2/c. The positions of two independent calcium atoms, one in a general position and one on the twofold axis, were deduced from a three-dimensional Patterson function. The remaining non-hydrogen atoms were located in subsequent difference Fourier summations, and isotropic refinement gave values of the conventional agreement factors *R*₁ = ∑|F_o| - |F_c| / ∑|F_o| and *R*₂ = [∑w(|F_o| - |F_c|)² / ∑w(F_o)²]^{1/2} of 0.113 and 0.141, respectively. Hydrogen atoms could not be found in a difference Fourier map, but those associated with the ligands were included in calculated positions. In C2/c one of the ligands must sit on the twofold axis, which leads to disorder of the α-methyl group. Attempts to refine the model in the noncentrosymmetric space group *Cc* were unsuccessful, leading to a model with no

(24) Corfield, P. W. R.; Doedens, R. J.; Ibers, J. A. *Inorg. Chem.* **1967**, *6*, 197–204.

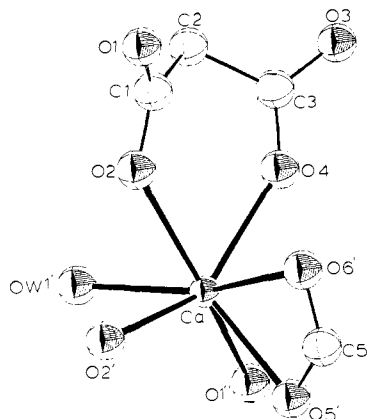


Figure 1. View of the coordination around Ca(1) in $\text{Ca}_3(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$; extraneous atoms are omitted for clarity. Primed and doubly primed atoms are related to their unprimed equivalents by the symmetry operations $1/2 - x, 1/2 - y, -z$ and $x, y, z - 1$, respectively.

improvement in R_2 and with significantly different bond lengths for chemically equivalent bonds. The disordered model was chosen, therefore, the final values of R_1 and R_2 being 0.077 and 0.079 based on 812 observed [$I > 3\sigma(I)$] data. The refined atomic positional parameters are collected in Table II. Hydrogen atom coordinates, anisotropic thermal parameters, and listings of observed and calculated structure amplitudes are available as supplementary material.

(b) **Mg(memal)·4H₂O.** The structure was solved in an analogous manner in space group $Pnma$. In this space group the magnesium atom and atoms C(2) and C(3) are constrained to lie on a mirror plane; it transpired that two water molecules also lie on this plane. The final values of R_1 and R_2 are 0.035 and 0.049 based on 848 observations with $I \geq 3\sigma(I)$. A final difference Fourier map was featureless, with no peak in excess of $0.36 \text{ e } \text{Å}^{-3}$. The refined positional parameters are collected in Table III; anisotropic thermal parameters and listings of $|F_o|$ and $|F_c|$ are available as supplementary material.

Description of the Structures. Ca₃(memal)₃·4H₂O. The structure consists of two independent Ca(memal) complexes and of water molecules. The structure is polymeric, as is invariably the case in calcium complexes of malonate and its derivatives.¹³⁻¹⁸ The geometry around the calcium in the general position [Ca(1)] is depicted in Figure 1. The coordination polyhedron is seven-coordinate, and while it does not approximate well to any of the three idealized polyhedra,²⁵ it is best described as a capped octahedron in which the trigonal planes are comprised of O(2), O(4), O(6) and O(2'), O(5'), OW(1)', respectively, and O(1)' is the cap. As was the case for the α -ethylmalonate complex, the coordination at Ca(1) exhibits all three binding modes seen in IV: (a) the unidentate coordination through O(1)' and O(2)', (b) the bidentate coordination through O(5)' and O(6)', and (c) the malonate coordination through O(2) and O(4).

The Ca(1)–O distances are in the range 2.319 (7)–2.568 (7) Å, which is consistent with values reported for other calcium carboxylate structures.^{13-18,22} The Ca–O(1) bond of 2.319 (7) Å is suggestive of a very strong interaction, although several unidentate Ca–O bonds have been documented in which the distance is shorter than this value.¹³ The malonate coordination is essentially symmetric, with Ca–O(2) and Ca–O(4) distances of 2.387 (6) and 2.363 (6) Å, respectively; these distances are comparable to those of 2.341 (2) and 2.421 (2) Å in the ethylmalonate complex²² and to those of 2.361 (1) and 2.575 (1) Å in the gel form of calcium malonate dihydrate. As is usually found,¹³ the Ca–O bonds to the bidentate carboxylate group are longer, at 2.483 (7) and 2.568 (7) Å. All of these distances can be considered normal. The Ca–O–C angles to the unidentate oxygen atoms are 116.1 and 171.3°, those to the bidentate oxygen atoms are 89.5 and 95.1°, and those to the malonate oxygen atoms are 132.6 and 137.0°; all of these angles are within their expected ranges.^{13,22} The bond angles at C(2) are normal, suggesting that the six-membered ring is free from any internal strain; significant deviations from tetrahedral geometry have been observed in some metal malonate complexes which exhibit the malonate coordination mode.^{18,26-28}

(25) Beauchamp, A. L.; Belanger-Gariepy, F.; Arabi, S. *Inorg. Chem.* **1985**, *24*, 1860–1863 and references therein.

(26) Duc, G.; Faure, R.; Loiseleur, H. *Acta Crystallogr., Sect. B: Struct. Sci.* **1978**, *B34*, 2115–2118.

(27) Soriano-García, M.; Parthasarathy, R. *J. Chem. Soc., Perkin Trans. 2* **1978**, 668–670.

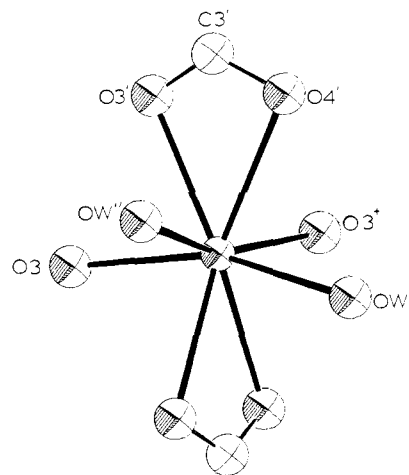


Figure 2. View of the coordination around Ca(2) in $\text{Ca}_3(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$. Drawn as in Figure 1. Primed atoms are related by the operations $-x, -y, -z$. Atom OW'' is related to OW(2) by the operation $x - 1/2, y - 1/2, z$. Atoms shown with a superscript + and unlabeled atoms are related to labeled atoms by the twofold rotation.

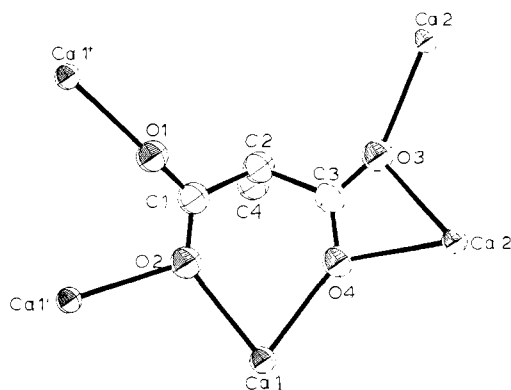


Figure 3. Calcium interactions with methylmalonate ion (1). Atom Ca(1)' is related to Ca(1) by the operation $1/2 - x, 1/2 - y, -z$; Ca(2)'' is related to Ca(2) by $-x, -y, -z$; Ca(1)'' is related to Ca(1) by $x, y, 1 + z$. The "malonate" coordination involves atoms O(2) and O(4).

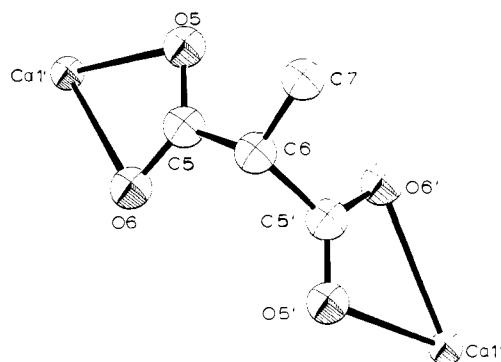


Figure 4. Calcium interactions with methylmalonate ion (2); only one of two disordered positions for methyl carbon atom C(7) is shown. Atoms Ca(1)' and Ca(1)'' are related to Ca(1) by the operations $1/2 - x, 1/2 - y, -z$ and $1/2 + x, 1/2 - y, 1/2 + z$, respectively.

The geometry around Ca(2) is shown in Figure 2. The coordination number is eight, the geometry being best described as an octahedron in which the bidentate pairs [O(5), O(6)] are viewed as occupying a single site. The Ca–O bond lengths, which are in the range 2.355 (7)–2.562 (6) Å, are again normal, the bonds to the bidentate groups again being slightly longer than those to unidentate donors. The two independent malonate ions are shown in Figures 3 and 4. Inspection of Figure 3 suggests that the reason for the very short Ca(1)–O(1) bond noted above

(28) Pajunen, A.; Pajunen, S. *Acta Crystallogr., Sect. B: Struct. Sci.* **1980**, *B36*, 2425–2428.

Table IV. Bond Lengths (Å) in $\text{Ca}(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$

atoms ^a	distance	atoms	distance
Ca(1)-O(1) ⁱ	2.316 (7)	C(1)-O(1)	1.243 (10)
Ca(1)-O(2)	2.386 (6)	C(1)-O(2)	1.270 (10)
Ca(1)-O(2) ⁱⁱ	2.371 (6)	C(1)-C(2)	1.512 (12)
Ca(1)-O(4)	2.362 (6)	C(2)-C(3)	1.562 (13)
Ca(1)-O(5) ⁱⁱ	2.481 (7)	C(2)-C(4)	1.500 (14)
Ca(1)-O(6) ⁱⁱ	2.566 (6)	C(3)-O(3)	1.267 (9)
Ca(1)-OW(1) ⁱⁱ	2.384 (6)	C(3)-O(4)	1.245 (9)
Ca(2)-O(3)	2.355 (6)	C(5)-O(5)	1.215 (11)
Ca(2)-O(3) ⁱⁱⁱ	2.503 (6)	C(5)-O(6)	1.279 (11)
Ca(2)-O(4) ⁱⁱⁱ	2.559 (5)	C(5)-C(6)	1.560 (14)
Ca(2)-OW(2) ^{iv}	2.431 (7)	C(6)-C(7)	1.503 (24)

^aSymmetry operations are given as superscripts: i = x, y, -z; ii = $1/2 - x, 1/2 - y, -z$; iii = -x, -y, -z; iv = $x - 1/2, y - 1/2, z$.

may be that O(1) participates in only this interaction while all other unidentate interactions involve oxygen atoms which also bind to a second calcium center. The C(5)-C(6)-C(5)' angle is much larger than the C(1)-C(2)-C(3) angle, despite the absence of malonate coordination involving O(5) and O(6) which might have been expected to lead to an opening of this angle. The bond lengths and angles in the complex are collected in Tables IV and V, respectively.

Mg(memal)·4H₂O. The structure of the magnesium complex is shown in Figure 5. The geometry at magnesium is octahedral, with cis bond angles in the range 88.10 (6)-92.23 (6)° and trans angles from 177.03 (6)-177.69 (8)°. The Mg-O distances fall in the narrow range 2.050 (2)-2.069 (1) Å. Thus, as expected, the distortions from octahedral geometry are very small. The geometry observed here can be compared with that in the hydrogen malonate complex $\text{Mg}(\text{C}_3\text{H}_3\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$,¹⁸ in which the bond lengths are in the range 2.045 (1)-2.063 (1) Å, the cis angles are in the range 85.9-94.1°, and the trans angles are all 180°. The only interaction between magnesium and the ligand is of the malonate

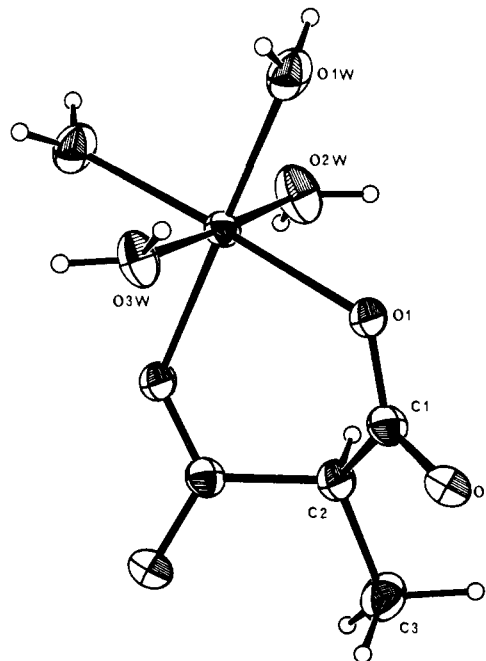


Figure 5. View of the complex $\text{Mg}(\text{C}_4\text{H}_4\text{O}_4)(\text{OH}_2)_4$. Unlabeled atoms are related to labeled atoms by the mirror plane passing through Mg, O(2)W, O(3)W, C(2), and C(3).

type, as is seen in Figure 5. The bond lengths and angles in the complex are listed in Table VI.

Table V. Bond Angles (deg) in $\text{Ca}_3(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$

atoms ^a	angle	atoms ^a	angle
O(1) ⁱ -Ca(1)-O(2)	155.9 (2)	O(1)-C(1)-O(2)	123.2 (8)
O(1) ⁱ -Ca(1)-O(2) ⁱⁱ	121.8 (2)	O(1)-C(1)-C(2)	118.6 (8)
O(1) ⁱ -Ca(1)-O(4)	93.2 (2)	O(2)-C(1)-C(2)	117.9 (8)
O(1) ⁱ -Ca(1)-O(5) ⁱⁱ	78.6 (2)	C(1)-C(2)-C(3)	111.5 (7)
O(1) ⁱ -Ca(1)-O(6) ⁱⁱ	119.8 (2)	C(1)-C(2)-C(4)	111.4 (7)
O(1) ⁱ -Ca(1)-OW(1) ⁱⁱ	82.3 (2)	C(3)-C(2)-C(4)	109.2 (7)
O(2)-Ca(1)-O(2) ⁱⁱ	71.5 (2)	O(3)-C(3)-O(4)	122.5 (7)
O(2)-Ca(1)-O(4)	74.4 (2)	O(3)-C(3)-C(2)	117.5 (7)
O(2)-Ca(1)-O(5) ⁱⁱ	125.3 (2)	O(4)-C(3)-C(2)	119.9 (7)
O(2)-Ca(1)-O(6) ⁱⁱ	80.0 (2)	O(5)-C(5)-O(6)	123.7 (9)
O(2)-Ca(1)-OW(1) ⁱⁱ	79.7 (2)	O(5)-C(5)-C(6)	119.1 (8)
O(2) ⁱⁱ -Ca(1)-O(4)	144.8 (2)	O(6)-C(5)-C(6)	117.1 (8)
O(2) ⁱⁱ -Ca(1)-O(5) ⁱⁱ	77.2 (2)	C(5)-C(6)-C(5) ^{viii}	105 (1)
O(2) ⁱⁱ -Ca(1)-O(6) ⁱⁱ	81.9 (2)	C(5)-C(6)-C(7)	117 (1)
O(2) ⁱⁱ -Ca(1)-OW(1) ⁱⁱ	82.7 (2)	C(5) ^{viii} -C(6)-C(7)	109.8 (8)
O(4)-Ca(1)-O(5) ⁱⁱ	117.1 (2)	Ca(1) ^{ix} -O(1)-C(1)	171.3 (6)
O(4)-Ca(1)-O(6) ⁱⁱ	83.7 (2)	Ca(1)-O(2)-C(1)	132.6 (6)
O(4)-Ca(1)-OW(1) ⁱⁱ	99.9 (2)	Ca(1) ⁱⁱ -O(2)-C(1)	116.1 (6)
O(5) ⁱⁱ -Ca(1)-O(6) ⁱⁱ	51.6 (2)	Ca(2)-O(3)-C(3)	154.6 (6)
O(5) ⁱⁱ -Ca(1)-OW(1) ⁱⁱ	139.0 (2)	Ca(2) ⁱⁱⁱ -O(3)-C(3)	93.5 (4)
O(6) ⁱⁱ -Ca(1)-OW(1) ⁱⁱ	157.6 (2)	Ca(1)-O(4)-C(3)	137.0 (5)
O(3)-Ca(2)-O(3) ⁱⁱⁱ	69.7 (2)	Ca(2) ⁱⁱⁱ -O(4)-C(3)	91.4 (5)
O(3)-Ca(2)-O(3) ^v	89.2 (1)	Ca(1) ⁱⁱ -O(5)-C(5)	95.1 (6)
O(3)-Ca(2)-O(3) ^{vi}	100.7 (3)	Ca(1) ⁱⁱ -O(6)-C(5)	89.5 (6)
O(3)-Ca(2)-O(4) ⁱⁱⁱ	121.1 (2)		
O(3)-Ca(2)-O(4) ^v	83.1 (1)		
O(3)-Ca(2)-OW(2) ^{iv}	158.6 (2)		
O(3)-Ca(2)-OW(2) ^{vii}	91.7 (2)		
O(3) ⁱⁱⁱ -Ca(2)-O(3) ^v	147.0 (3)		
O(3) ⁱⁱⁱ -Ca(2)-O(4) ⁱⁱⁱ	51.6 (2)		
O(3) ⁱⁱⁱ -Ca(2)-O(4) ^v	143.0 (2)		
O(3) ⁱⁱⁱ -Ca(2)-OW(2) ^{iv}	128.5 (2)		
O(3) ⁱⁱⁱ -Ca(2)-OW(2) ^{vii}	78.7 (2)		
O(4) ⁱⁱⁱ -Ca(2)-O(4) ^v	143.7 (2)		
O(4) ⁱⁱⁱ -Ca(2)-OW(2) ^{iv}	77.5 (2)		
O(4) ⁱⁱⁱ -Ca(2)-OW(2) ^{vii}	75.5 (2)		
OW(2) ^{iv} -Ca(2)-OW(2) ^{vii}	82.5 (3)		

^aSymmetry operations as in Table IV, plus: v = x, -y, $1/2 + z$; vi = -x, y, $1/2 - z$; vii = $1/2 - x, y - 1/2, 1/2 - z$; viii = $1 - x, y, 1/2 - z$.

Table VI. Bond Lengths (Å) and Bond Angles (deg) in $\text{Mg}(\text{C}_4\text{H}_4\text{O}_4)(\text{OH}_2)_4$

Bond Lengths			
atoms	distance	atoms	distance
Mg-O(1)	2.063 (1)	C(1)-O(1)	1.260 (2)
Mg-O(1)W	2.069 (1)	C(1)-O(2)	1.246 (2)
Mg-O(2)W	2.057 (2)	C(1)-C(2)	1.533 (2)
Mg-O(3)W	2.050 (2)	C(2)-C(3)	1.509 (3)
Bond Angles			
atoms	angle	atoms	angle
O(1)-Mg-O(1)'	89.00 (6)	O(2)W-Mg-O(3)W	177.69 (8)
O(1)-Mg-O(1)W	89.35 (5)	Mg-O(1)-C(1)	126.3 (1)
O(1)-Mg-O(1)W'	177.03 (6)	O(1)-C(1)-O(2)	122.7 (2)
O(1)-Mg-O(2)W	92.23 (6)	O(1)-C(1)-C(2)	117.5 (1)
O(1)-Mg-O(3)W	89.42 (5)	O(2)-C(1)-C(2)	119.8 (1)
O(1)W-Mg-O(1)W'	92.19 (8)	C(1)-C(2)-C(1)'	108.7 (2)
O(1)W-Mg-O(2)W	90.30 (6)	C(1)-C(2)-C(3)	113.5 (1)
O(1)W-Mg-O(3)W	88.10 (6)		

Discussion

As is apparent in Figure 2, the coordination environment at Ca(2) does not include the malonate coordination mode but is restricted to unidentate and bidentate modes. Consequently, it would appear that while the malonate binding mode is evidently possible for a calcium complex of a malonate derivative, and (by implication) for a calcium complex of Asa or Gla, it is not more probable than these other modes. A similar conclusion would be drawn from the structures of the two forms of calcium malonate dihydrate, one of which contains the malonate binding mode while the other does not.¹⁵⁻¹⁷

Similarly, examination of Figure 5 and of the structure of magnesium hydrogen malonate dihydrate¹⁸ leads to the conclusion that the malonate coordination mode *is* the only form observed in magnesium complexes of this type, and presumably this would

obtain in magnesium complexes of Gla and Asa also.

The other major distinction between the magnesium and the calcium complex is in their geometries. The magnesium complexes,¹⁸ along with all known magnesium complexes of polyethers or peptides,²⁹⁻³² are six-coordinate, very nearly octahedral, and monomeric. The calcium complexes are seven- and eight-coordinate, highly distorted, and invariably polymeric. Thus, it appears probable that the ability of Gla and Asa to contribute additional carboxylate moieties to these polymeric arrays is as important as their ability to form malonate type complexes in the calcium proteins. Moreover, the present structural results suggest that Mg^{2+} will not bind preferentially at a high affinity Ca^{2+} site in a protein, since the stereochemical requirements for the magnesium binding site are evidently very explicit. This conclusion is entirely consistent with the recent results of Hiskey and co-workers,^{33,34} who have demonstrated that the tight binding Ca^{2+} sites in bovine prothrombin are calcium specific and are not involved in magnesium binding³³ but that there are other sites in the protein which have a lower affinity for calcium than for magnesium.³⁴

Supplementary Material Available: Tables of positional and thermal parameters and observed and calculated structure amplitudes for $\text{Ca}_3(\text{C}_4\text{H}_4\text{O}_4)_3 \cdot 4\text{H}_2\text{O}$ and $\text{Mg}(\text{C}_4\text{H}_4\text{O}_4)(\text{OH}_2)_4$ (18 pages). Ordering information is given on any current masthead page.

(29) Neuman, M. A.; Steiner, E. C.; van Remoortere, F. P.; Boer, F. P. *Inorg. Chem.* **1975**, *14*, 734-740.

(30) Karle, I. L.; Karle, J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 681-685.

(31) Kartha, G.; Varughese, K. I.; Aimoto, S. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4519-4522.

(32) Karle, I. L. *Int. J. Pept. Protein. Res.* **1984**, *23*, 32-38.

(33) Deerfield, D. W.; Berkowitz, P.; Olson, D. L.; Wells, S.; Hoke, R. A.; Koehler, K. A.; Pedersen, L. G.; Hiskey, R. G., submitted to *J. Biol. Chem.*

(34) Marsh, H. C.; Robertson, P., Jr.; Scott, M. E.; Koehler, K. A.; Hiskey, R. G. *J. Biol. Chem.* **1979**, *254*, 10268-10275.