Preparation and Structure of Magnesium Bis(hydrogen β-glutamate) Hexahydrate

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The dissociation equilibria of aqueous solutions of β-glutamic acid were studied by potentiometric titration and the three p*K* values determined under standard conditions. The hydrogen β-glutamate anion β-GluH$^-$ was found to be the dominating species in the physiologically relevant pH range 4.0–9.4. Neutralization of β-glutamic acid by magnesium oxide affords magnesium bis(hydrogen β-glutamate) Mg (β-GluH)$_2$, which crystallizes as the hexahydrate from dilute aqueous solution. A single-crystal X-ray study showed that the β-GluH$^-$ anions are not part of the coordination sphere of the magnesium ion. Instead hexahydrated dications $[Mg(H_2O)_6]^{2+}$ are intimately associated with free β-GluH$^-$ anions through a three-dimensional network of H-bonds. This study provides the first structural and conformational reference data for the β-GluH$^-$ anion.

Introduction. – Magnesium salts play a central role as cofactors for most enzymes that participate in the biochemistry of nucleic acids [1–3]. The outstanding stability of the hexahydrate dication $[Mg(H_2O)_6]^{2+}$, as compared to hydrates of the other three most common metal ions in biological systems (sodium, potassium, and calcium ions), is responsible for the superior template function of magnesium dications in aqueous solution under physiological conditions. In many cases, the mode of action of Mg^{2+} on the molecular level is not perfectly understood, and few model complexes are available where structural information gives a clue as to the details of the binding sites of the substrates.

However, recent structural work on magnesium complexes of amino acids has revealed the chelation modes of interaction for L-aspartic and α-L-glutamic acids which are known to be the prominent binding sites for magnesium ions in proteins [4][5]. This information may also be of key importance for an understanding of the mechanism of many biomineralization processes [6]. Complementary studies also focused on complexes with citrate (=2-hydroxypropane-1,2,3-tricarboxylate) and orotate (=1,2,3,6-tetrahydro-2,6-dioxopyrimidine-4-carboxylate) ligands [7–9].

Magnesium salts of these four acids (L-aspartic, α-L-glutamic, citric, and orotic acid) are among the most common preparations for the treatment of magnesium deficiency. Depending on the deficiency syndroms and the general conditions, the bis(L-aspartate/L-glutamate) or mixed L-asparte/L-glutamate chloride salts are administered. However, magnesium citrate was found to have a complicated structure in the solid state, with only part of the magnesium cations present as anionic chelates, complemented by magnesium hexahydrate cations: $[Mg(H_2O)_6]^{2+}$ $[Mg(Citr)(H_2O)_2]^-{}_2(H_2O)_2$ [9]. The orotate is a genuine hexaaquo complex $[Mg(H_2O)_6]^{2+}$ $2(OrotH)^-$ $(H_2O)_2$ with the orotate anions excluded from the coordination sphere of the metal dication [7][8].

With the advent of β-amino acids as new building blocks for nonnatural proteins or other derivatives with a new and fascinating structural chemistry [10–13], the area of metal complexes of these units is of growing interest. To date, only very few complexes

of β-amino acids have been prepared, and structural information is very limited, and this is true also for β-glutamic acid (= 3-aminopentanedioic acid) and its anions [14].

In the present study, we therefore investigated the interaction of β-glutamic acid with magnesium salts in aqueous solution under physiologically relevant conditions. The main question to be answered concerned the ligand strength of β-glutamate anions in competition with H_2O molecules.

In previous studies, magnesium α-L-glutamate has been shown to be a chelate complex in the solid state, which undergoes partial dissociation in aqueous solution depending on pH, concentration, and temperature [7][15]. The crystals obtained upon careful concentration of alkaline aqueous solutions (pH 10) at room temperature consist of a molecular tetrahydrate complex of the composition $[Mg(H_2O)_4$ (α-L-Glu)]. The α-L-Glu^{2-} ligand has two deprotonated carboxylate groups, one of which is chelating the magnesium dication together with the amino group in *cis*-positions of an octahedron to form a five-membered ring (*Fig. 1*) [7][15]. It is, thus, the carboxylate group closer to the amino group that is involved in chelation, while the more distant γ-carboxylate unit is dangling free and is engaged only in H-bonding with H_2O molecules of neighboring complexes. The structures of a magnesium α-L-glutamate dihydrate $Mg(H_2O)_2$ (α-L-Glu) (also obtained from alkaline solutions) and of magnesium bis(hydrogen α-L-glutamate) hydrates $Mg(\alpha$-L-$GluH)_2(H_2O)_n$ (from neutral solutions) are still unknown.

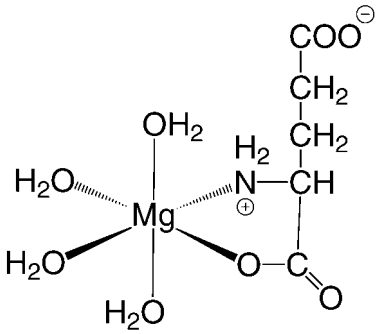


Fig. 1. *Schematical view of the molecular structure of [Mg(H_2O)$_4$ (α-L-Glu)]* [7]

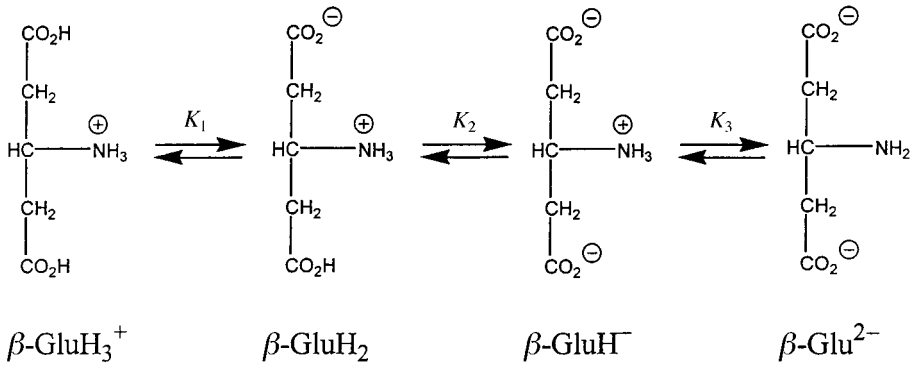
Results and Discussion. – *Acid-Base Characteristics of β-Glutamic Acid* (β-$GluH_2$). β-Glutamic acid has only recently become commercially available, and knowledge of its physical data is still limited. To obtain information on the protonation/deprotonation equilibria (*Scheme*) in aqueous solution, we, therefore, determined the pK_s values under standard conditions. Conventional potentiometric titration gave a titration curve (*Fig. 2*) from which the following parameters could be extracted:

$$pK_1 = 2.30,\ pK_2 = 4.00,\ pK_3 = 9.42$$

The results are to be compared with the data for α-glutamic acid, which were obtained in control experiments under strictly comparable conditions and found to agree with literature data [16][17]:

$$pK_1 = 2.00,\ pK_2 = 4.20,\ pK_3 = 9.14$$

Scheme



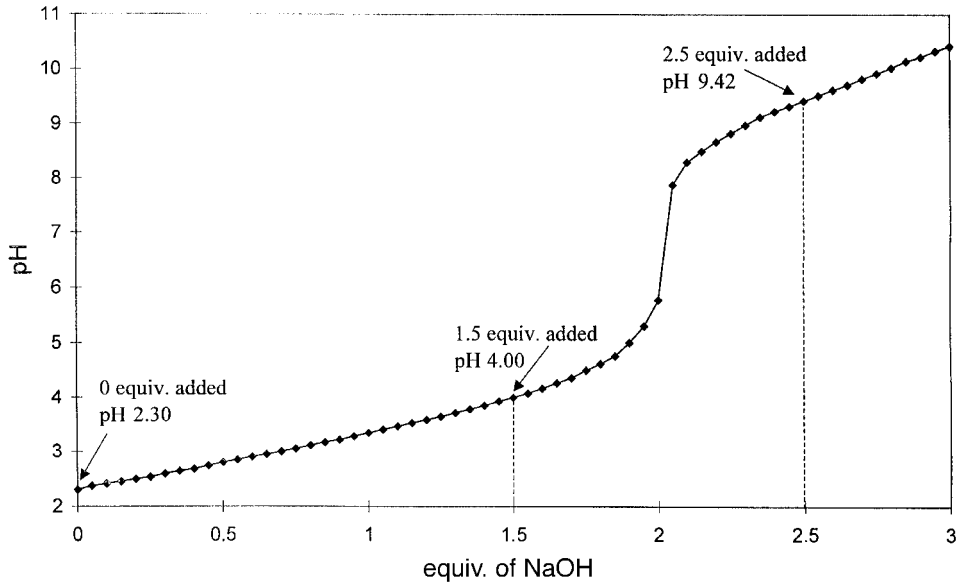


Fig. 2. *Titration curve for the determination of the* p**K** *values of β-glutamic acid*

It appears that pK_1 and pK_3 are lower (by *ca.* 0.3 p*K* units) for α-glutamic acid, while pK_2 is higher (by 0.20 p*K* units). Since pK_2 represents the protonation equilibrium for the distant (γ) carboxylate group, this difference (lowering) is to be expected. The increase of the other two parameters (pK_1 and pK_3) has many parallels in other pairs of α- and β-amino acids [18].

With the pK_s values given above, the distribution of the four species involved as a function of pH can be calculated. The result is shown in *Fig. 3*. The diagram demonstrates that β-GluH$^-$ is the dominating species in the broad range between pH 4.00 and pH 9.42, which is also the physiologically most relevant range. At pH 6.71, it is even the only stable species. At pH 3.15, the concentration of the (zwitterionic) β-glutamic acid β-$GluH_2$ is at a maximum (78%), coexisting with 11% of each β-$GluH_3^+$

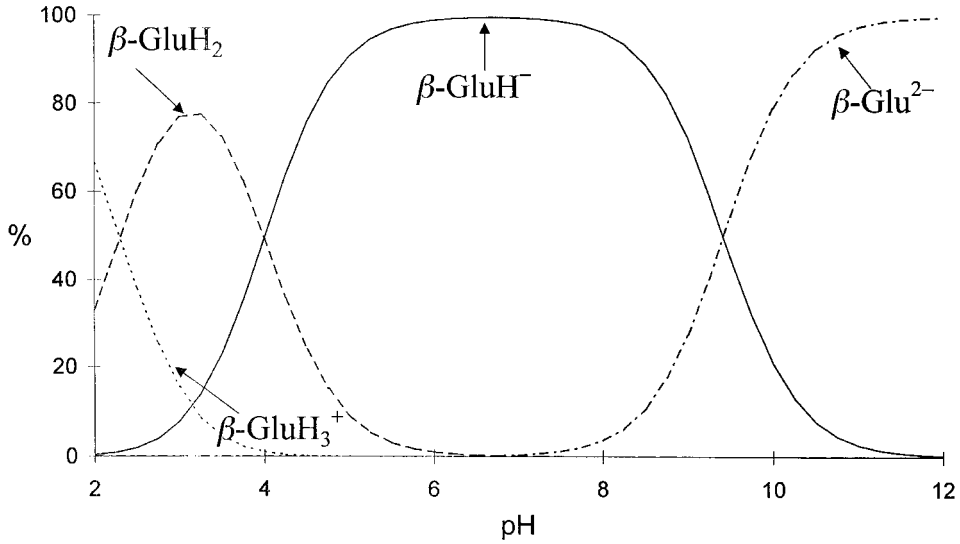


Fig. 3. *Distribution of the species involved in the protonation/deprotonation equilibria of β-glutamic acid as a function of pH*

and β-GluH$^-$. At pH 9.42, β-GluH$^-$ and β-Glu^{2-} have the same concentration (50%), and above pH 11, β-Glu^{2-} is the dominant species.

Also the ^{1}H- and ^{13}C{^{1}H}-NMR spectra of aqueous solutions of β-glutamic acid were recorded and assigned. In the ^{1}H-NMR spectrum, the $CH_2-CH-CH_2$ skeleton gives rise to an *AA′BB′C* spin system, its *multiplet* resembling the patterns observed and analyzed, *e.g.*, for glycerol [19]. Note that the protons of each CH_2 group are diastereotopic. Detailed data are given in the *Exper. Part*.

Magnesium Bis(hydrogen β-glutamate) Hexahydrate. Neutralization of magnesium oxide or hydroxide with two equivalents of β-glutamic acid in water suspension affords in a slow reaction an almost neutral aqueous solution ($c = 0.15$M) from which the title compound precipitates as a white microcrystalline powder. Larger, transparent single crystals can be grown in low yield after prolonged standing (2 months) of a less concentrated solution ($c = 0.05$M) at room temperature. The samples loose water upon prolonged exposure to the laboratory atmosphere or in a vacuum.

The IR spectrum is inconclusive regarding the structure of the material. Broad bands in the region 3550–2500 cm^{-1} are indicative of strong and extensive H-bonding, and equally broad absorptions between 1600 and 1350 cm^{-1} show the strong involvement of the carboxylate groups in H-bonding, but there is also some overlap with $-NH_3^+$ deformations in this region [20][21]. When compared with reference data for free and complexed carboxylate groups, the results suggest that, in the β-GluH$^-$ ion, the amino group is protonated ($-NH_3^+$) and the carboxylic acid groups are deprotonated ($-CO_2^-$) and most likely not engaged in coordinative bonding with magnesium dications.

The mass spectrum (FAB) shows a series of peaks in the regions around *m*/*z* 220 and 228, which can all be assigned to condensation products of β-glutamic acid and their

fragments without magnesium atoms being incorporated. This result also indicates that the metal dications are not directly coordinated to the β-GluH$^-$ anions.

These tentative conclusions were confirmed by a single-crystal X-ray-structure analysis. The compound crystallizes in the monoclinic space group $P2_1/c$ with $Z=2$ formula units in the unit cell. The lattice is built of independent hexaaquomagnesium dications $[Mg(H_2O)_6]^{2+}$ and two symmetry-related hydrogen β-glutamate anions β-GluH$^-$, but contains no further interstitial H_2O molecules. The magnesium dication resides on a center of inversion, and all H_2O molecules in *trans*-positions are symmetry-related (*Fig. 4*).

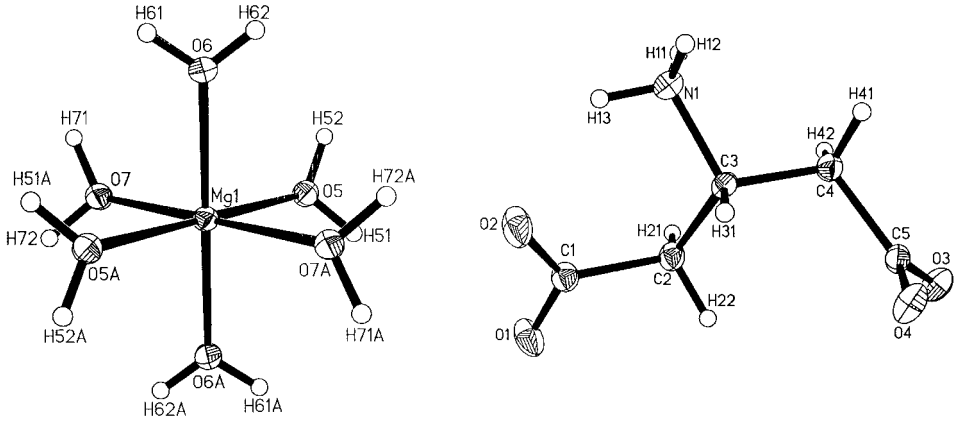


Fig. 4. *Structure of the dication and of one of the two anions of [Mg(H$_2$O)$_6$](β-GluH)$_2$.* ORTEP drawing with 50% probability ellipsoids. The Mg-atom resides on a center of inversion, and the second anion is related to the first one by inversion. Selected bond lengths [Å] and angles [°]: Mg(1)–O(5) 2.091(1), Mg(1)–O(6) 2.000(1), Mg(1)–O(7) 2.075(1), C(1)–O(1) 1.254(2), C(1)–O(2) 1.249(2), C(1)–C(2) 1.530(2), C(2)–C(3) 1.523(2), C(3)–C(4) 1.523(2), C(3)–N(1) 1.503(2), C(4)–C(5) 1.528(2), C(5)–O(3) 1.252(2), C(5)–O(4) 1.257(2); O(5)–Mg(1)–O(6) 95.09(5), O(5)–Mg(1)–O(7) 90.40(4), O(6)–Mg(1)–O(7) 94.17(5), O(5)–Mg(1)–O(6A) 84.91(5), O(5)–Mg(1)–O(7A) 89.70(4),O(6)–Mg(1)–O(7A) 85.83(5), O(1)–C(1)–O(2) 125.0(1), O(1)–C(1)–C(2) 116.4(1), O(2)–C(1)–C(2) 118.6(1), O(3)–C(5)–O(4) 125.0(1), O(3)–C(5)–C(4) 118.6(1), O(4)–C(5)–C(4) 116.3(1). For dihedral angles, see text.

As proposed on the basis of the IR data, the N-atom is an ammonium center $-NH_3^+$, and the two carboxylate groups are not protonated and show virtually equidistant C–O bond lengths (C(1)–O(1) 1.254(2), C(1)–O(2) 1.249(2), C(5)–O(3) 1.252(2), C(5)–O(4) 1.257(2) Å). However, all four carboxylate O-atoms (O(1) to O(4)) are engaged in H-bonding with H_2O molecules of the aquo complex (O(5) to O(7) and O(5A) to O(7A)). The protons involved were positively located and their positions refined isotropically. The O–H$\cdots$O H-bonds are generally short and quasi-linear indicating strong interactions (*Table*).

The H-atoms of the $-NH_3^+$ group can also be considered as H-bonded with carboxylate and/or H_2O O-atoms (one of these intra-anionic: N(1)–H(13)$\cdots$O(2)), but the connections are generally longer and more strongly bent, suggesting weaker interactions (*Table*). One of the N-bound H-atoms (N(1)–H(11)) appears to be triply connected to the O-atoms of three H_2O molecules, which are in facial positions of the MgO_6 octahedron (O(5) to O(7)). By symmetry, the same situation is found at the

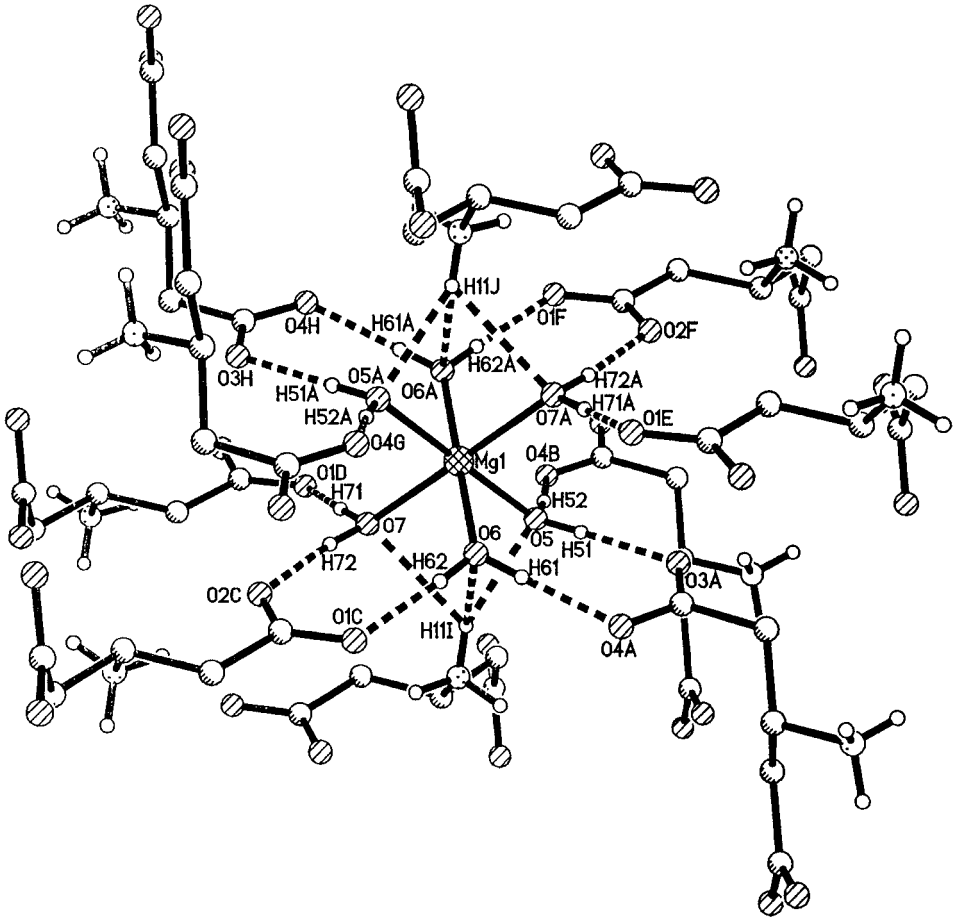


Fig. 5. *Hydrogen-bonding network in [Mg(H_2O)$_6$](β-GluH)$_2$.* The corresponding bond lengths and angles are given in the *Table*.

trans-'face' O(5A)–O(7A) of the octahedron. The structure of the title compound is, thus, to be described as an intimately H-bonded three-dimensional network (*Fig. 5*).

The conformation of the hydrogen β-glutamate anion β-GluH$^-$ shows only minor distortions from standard staggered arrangements. The C-chain C(1)–C(2)–C(3)–C(4) has a dihedral angle of 179.3(1)°, which makes these four atoms essentially coplanar. The dihedral angles O(1)/O(2)–C(1)–C(2)–C(3) of $-$ 172.6(1)/8.1(2)° are consistent with the location of the involved O-atoms in this reference plane too, *i.e.*, the carboxylate plane defined by C(1), O(1), and O(2) is roughly bisecting the angle H(21)–C(2)–H(22) of the neighboring CH_2 group. The atom sequence N(1)–C(3)–C(4)–C(5) has a dihedral angle of 166.4(1)° which shows that there is a larger deviation (13.6°) from planarity for this part of the molecular skeleton. Note that the overall conformation of the heavy-atom skeleton allows for the intra-anionic H-bond N(1)–H(13)$\cdots$O(2).

Table. *Hydrogen-Bond Lengths* [Å] *and Angles* [°] *for [Mg(H$_2$O)$_6$](β-GluH)$_2$*

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
O(5)–H(52)···O(4)[a])	0.88(3)	1.81(3)	2.6776(15)	171(2)
O(5)–H(51)···O(3)[b])	0.89(3)	1.86(3)	2.7477(15)	178(2)
O(6)–H(61)···O(4)[c])	0.86(3)	1.82(3)	2.6690(15)	173(3)
O(6)–H(62)···O(1)[d])	0.84(3)	1.81(3)	2.6416(15)	172(3)
O(7)–H(71)···O(1)[e])	0.93(3)	1.73(3)	2.6478(16)	171(2)
O(7)–H(72)···O(2)[f])	0.91(3)	1.79(3)	2.6953(15)	175(3)
N(1)–H(11)···O(5)[g])	0.89(2)	2.23(2)	3.0510(16)	153(2)
N(1)–H(11)···O(7)[g])	0.89(2)	2.45(2)	3.0749(16)	127(2)
N(1)–H(11)···O(6)[h])	0.89(2)	2.63(2)	3.2203(17)	124(2)
N(1)–H(12)···O(3)[i])	0.91(2)	1.98(2)	2.7939(16)	149(2)
N(1)–H(13)···O(2)	0.93(2)	1.98(2)	2.7086(17)	134(2)

[a]) –[i]) Symmetry transformations used to generate equivalent atoms.
[a]) $-x, -y+1, -z+1$.
[b]) $-x+1, -y+1, -z+1$.
[c]) $x, y, z-1$.
[d]) $-x, y+1/2, -z+3/2$.
[e]) $x, -y+1/2, z-3/2$.
[f]) $x+1, -y+1/2, z-3/2$.
[g]) $-x, y-1/2, -z+1/2$.
[h]) $x-1, -y+1/2, z+1/2$.
[i]) $x-1, y, z$.

The maximum attainable symmetry of the hydrogen β-glutamate anion β-GluH$^-$ is point group C_s with a mirror plane through the N-atom, the central C-atom (C(3)) and its single H-atom (H(31)). However, in the conformation found in the crystal, this anion is chiral (*Fig. 4*), and the two pairs of anions in the unit cell are pairs of enantiomers related by the symmetry operations of space group $P2_1/c$, including inversion symmetry (C_i).

To the best of our knowledge, the present investigation is the first structure determination of β-glutamic acid or of any of its salts. Regarding the potential role of magnesium β-glutamates in pharmacology, it is important to note that β-glutamate anions appear to be poor ligands for Mg^{2+} in aqueous solution, being unable to compete with H_2O molecules for a position in the coordination sphere of the metal ion. Magnesium bis(hydrogen β-glutamate) thus resembles magnesium orotate and (in part) citrate, which were also found to be aquo complexes of magnesium. By contrast, both aspartate and α-glutamate (as the pure enatiomers or as racemates) are known to become part of the coordination sphere of Mg^{2+} in aqueous solution and in crystalline phases [7][15][22–24]. This difference is probably highly significant for the pharmacological action of the amino-carboxylates (see *Introduction*).

Experimental Part

General. β-Glutamic acid (= 3-aminopentanedioic acid) was purchased from *Sigma Chemical Company* and used as received. The electrochemical investigations were carried out with a pH meter *CG 818* (*Schott*) equipped with a glass electrode and an integrated microprocessor. IR: *Prospect* spectrometer (*Midac*); in cm^{-1}. NMR: δ in ppm, *J* in Hz. FAB-MS: *Varian MAT 50*; *p*-nitrobenzyl alcohol (NBA); in *m*/*z* (rel. %).

NMR Data (see also [25]). *β-GluH$_2$:* A sat. soln. in D_2O at 25° was used. Data were referenced against a $CHCl_3$ capillary and converted to ref. $SiMe_4$. ^{1}H-NMR: *AA′BB′C* spin system at 3.72 (CH, 1 H), and 2.47 and 2.55 (CH_2, 4 H); $J_{AB} = 11.00$, $J_{AC} = 5.64$, $J_{BC} = 6.70$. ^{13}C-NMR: 175.61 (COO); 45.72 (CH); 36.93 (CH_2).

Mg (β-GluH)$_2$: A sat. soln. in D_2O at 25° was used. ^{1}H-NMR: 3.85 (CH, 1 H); 2.63, 2.71 (CH_2, 4 H); J_{AB} = 10.86, J_{AC} = 5.67, J_{BC} = 6.53. ^{13}C-NMR: 177.59 (COO); 46.81 (CH); 38.25 (CH_2).

Titration. β-Glutamic acid (29.4 mg) was dissolved in bidistilled H_2O (18 ml) and 0.1M HCl (2 ml) (ionic strength I = 0.01M). In the subsequent calculations, concentrations instead of activitites could be used [26]. Titration with 0.1M NaOH was carried out at 25° (*Fig. 2*). pK_1 was calculated from the initial pH value, pK_2 and pK_3 from the pH value after addition of 1.5 and 2.5 equiv. of NaOH, resp.

Magnesium Bis(hydrogen β-glutamate) Hexahydrate (= *Magnesium Bis(3-ammoniopentanedioate) Hexahydrate = Hexaaquomagnesium(2+) Bis(hydrogen 3-aminopentanedioate)*). Anh. magnesium oxide (27.4 mg, 0.68 mmol) is added to a soln. of β-glutamic acid (200 mg, 1.36 mmol) in bidistilled H_2O (4.5 ml). The mixture is first stirred at r.t. for 2 h and then heated to reflux for 30 min. On cooling, the product separates as a white microcrystalline powder, which looses water on standing.

For crystal growth, the mixture obtained after the reflux period (see above) is diluted with bidistilled H_2O (10 ml) and kept at r.t. for two months: 19.6 mg (6.8%) of transparent colorless crystals. IR (KBr): 3114, 2500, 2100, 1557, 1404, 1024, 714. FAB-MS: 220.4 (8.3), 201.3 (7.6), 149.5 (100), 132.2 (12.8), 114.2 (63.6). Anal. calc. for the tetrahydrate $Mg(GluH)_2(H_2O)_4$ (388.6): C 30.91, H 6.20, N 7.20; found (air-dried sample): C 32.00, H 7.15, N 6.42.

Single-Crystal X-Ray-Diffraction Analysis. The crystalline sample was placed in inert oil, mounted on a glass pin, and transferred to the cold gas stream of the diffractometer. Crystal data were collected and integrated with an *Enraf-Nonius DIP-2020* image plate system (*Silicon-Graphics 02* workstation) with monochromated MoK_α (λ 0.71073 Å) radiation at −130°. The structure was solved by direct methods by using SHELXS-97 and refined by full-matrix least-squares calculations on F^2 with SHELXL-97. Non-H-atoms were refined with anisotropic thermal parameters. H-Atoms were located and refined with isotropic contributions. Selected bond lengths and angles are given in *Fig. 4* and the *Table*, and thermal parameters and tables of interatomic distances and angles have been deposited with the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK. The data are available on request on quoting CCDC-181703.

Crystal Data for $C_{10}H_{28}MgN_2O_{14}$. M 424.65; monoclinic; a = 6.0172(1), b = 16.8298(4), c = 9.2175(2) Å; β = 102.625(1)°; space group $P2_1/c$, Z = 2; U = 910.87(3) Å^3; μ(MoK_α) 1.74 cm^{-1}; 39451 measured and 2862 unique reflections (R_{int} = 0.057); wR_2 = 0.1003, R = 0.0456 ($I \geq 2\sigma(I)$) for 180 parameters.

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REFERENCES

[1] J. A. Cowan, *Chem. Rev.* **1998**, *98*, 1067.
[2] 'Biological Chemistry of Magnesium', Ed. J. A. Cowan, VCH, New York, 1995.
[3] D. E. Wilcox, *Chem. Rev.* **1996**, *96*, 2435.
[4] G. Krampitz, G. Graser, *Angew. Chem.* **1998**, *100*, 1181; *Angew. Chem., Int. Ed.* **1998**, *27*, 1145.
[5] H. D. Jakubke, H. Jeschkat, 'Aminosäuren, Peptide, Proteine', Verlag Chemie, Weinheim, 1982.
[6] 'Biomineralization', Eds. S. Mann, J. Webb, and R. J. P. Williams, VCH, Weinheim, 1989.
[7] H. Schmidbaur, H. G. Classen, J. Helbig, *Angew. Chem.* **1990**, *102*, 1122; *Angew. Chem., Int. Ed.* **1990**, *29*, 1090.
[8] I. Bach, O. Kumberger, H. Schmidbaur, *Chem. Ber.* **1990**, *123*, 2267.
[9] C. K. Johnson, *Acta Crystallogr.* **1965**, *18*, 1004.
[10] D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, *111*, 1700; *Angew. Chem., Int. Ed.* **1999**, *38*, 1595.
[11] D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015.
[12] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martioni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.
[13] S. Poenaru, J. R. Lamas, G. Folkers, J. A. Lopez de Castro, D. Seebach, D. Rognan, *J. Med. Chem.* **1999**, *42*, 2318.
[14] A. Böhm, D. Seebach, *Helv. Chim. Acta* **2000**, *83*, 3262.
[15] H. Schmidbaur, I. Bach, D. L. Wilkinson, G. Müller, *Chem. Ber.* **1989**, *122*, 1433.
[16] J. I. Partanen, P. M. Juusola, P. O. Minkkinen, *Acta Chem. Scand.* **1998**, *52*, 198.
[17] M. M. Taqui Khan, A. Hussain, *Indian J. Chem., Sect. A* **1980**, *19*, 44.
[18] R.-S. Tsai, B. Testa, N. El Tayar, P.-A. Carrupt, *J. Chem. Soc., Perkin Trans.* **1991**, 1797.

[19] E. C. Garrett, A. S. Serianni, *Carbohydr. Res.* **1990**, *208*, 23.
[20] G. Socrates, 'Infrared Characteristic Group Frequencies', 2nd edn., Wiley, New York, 1994.
[21] J. Weidlein, U. Müller, K. Dehnicke, 'Schwingungsfrequenzen', Vol. 1, ' Hauptgruppenelemente', 1st edn., Thieme, Stuttgart, 1981, p. 125.
[22] H. Schmidbaur, I. Bach, D. L. Wilkinson, G. Müller, *Chem. Ber.* **1989**, *122*, 1445.
[23] H. Schmidbaur, G. Müller, J. Riede, G. Manninger, J. Helbig, *Angew. Chem*. **1986**, *98*, 1014; *Angew. Chem., Int. Ed.* **1986**, *25*, 1013.
[24] H. Schmidbaur, D. L. Wilkinson, A. Schier, *New. J. Chem.* **1994**, *18*, 507.
[25] D. E. Robertson, S. Lesage, M. F. Roberts, *Biochim. Biophys. Acta* **1998**, *992*, 320.
[26] H. Galster, 'pH-Messung: Grundlagen, Methoden, Anwendungen, Geräte, VCH, Weinheim, 1990.

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