Interactions of Calcium Ions with Carbohydrates: X-Ray Diffraction and NMR Spectroscopic Studies on the Potassium Salt and the Calcium Salt of D-Glucaric Acid

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The crystal structure of potassium D-glucarate was determined by X-ray analysis. The conformation of the glucaric anion in the potassium salt differs from that of the dianion in the crystal of the calcium salt. The proton magnetic resonance spectra of the solutions of the potassium salt and the calcium salt show that the glucarate solution is an equilibrium mixture of the two conformers observed in the crystals. Studies on the induced shifts by adding europium nitrate to the acidic solution indicate that the europium ions are selectively bound to the potassium type conformer. Calcium sequestant by the glucaric acid in alkaline solutions should occur when calcium ions bind the calcium type conformers in a dianionic state.

D-Glucaric acid is known to have high calcium sequestering effect in alkaline solutions. The calcium affinity of the glucaric acid was supposed to be due to the increased ionization of the hydrogen of the hydroxyl groups in the glucaric acid in aqueous alkaline solution, the calcium–glucaric acid complex in chelating form being expected in the solution.[1] In relation to this subject, a report was given on the crystal structure of calcium D-glucarate tetrahydrate.[2] In the crystal structure, a stable chelating structure of the glucaric dianions binding to the calcium ion was observed. However, the structural evidence for such a calcium binding or a calcium–sugar complex in the solution is still not known. We have therefore studied the crystal structure of potassium D-glucarate and the NMR spectra of several glucarate salt solutions. The relationship between the structures of the crystals and the solutions was discussed. We were particularly interested in the structure of the metal ion–glucaric acid complex, which is elucidated by NMR spectroscopy using the shift reagent.

Experimental

X-Ray Analysis of the Potassium D-Glucarate. The single crystals of potassium D-glucarate, K^+ $C_6H_9O_8^-$, were obtained by the evaporation method. Preliminary X-ray diffraction experiments showed that the crystals are monoclinic, space group $P2_1$, $Z=2$, $D_x=1.807$ g/cm^3, with cell dimensions $a=8.55$, $b=10.9$, $c=4.85$ Å and $\beta=90.0°$. The intensity data of 675 independent reflections were collected from the equi-inclination Weissenberg photographs taken by rotating the crystals about the b- and c-axes, using Cu $K\alpha$ radiation. The intensities on the films were measured on a SYNTEX AD-1 densitometer controlled by a NOVA 1200 computer. Absorption correction for a cylindrical crystal was applied ($\mu R=2.0$). The structure was solved by the direct method using the program MULTAN,[3] and refined by the block diagonal least-squares method. The hydrogens bonded to the carbons were located from the difference Fourier maps and included in the structure factor calculations. Successive cycles of the anisotropic least-squares refinement gave an R-value of 0.123. Scattering factors for all atoms were obtained from the International Table for X-Ray Crystallography IV.[4] The final structural parameters are given in Table 1.

NMR Studies on the Solution. The 100 MHz proton magnetic resonance spectra of the solutions were measured on a VARIAN HA-100D spectrometer, using an internal lock signal of tetramethylsilane sealed in a capillary tube. The deuterium oxide solutions of 0.1 mol potassium glucarate and 0.1 mol calcium glucarate were prepared and adjusted to predominantly diprotonated (LH_2), monoanionic (LH^-)

TABLE 1. ATOMIC PARAMETERS AND THEIR STANDARD DEVIATIONS IN POTASSIUM D-GLUCARATE

The values have been multiplied by 10^4. Temperature factors are in the form, $T^{-1}=\exp(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)$.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
K	2155 (6)	0	3430 (14)	67	32	577	25	30	95
C (1)	1316 (21)	7284 (17)	7661 (47)	27	15	296	6	−41	20
C (2)	2051 (20)	6342 (15)	9653 (42)	26	4	206	4	−39	4
C (3)	3008 (21)	5435 (16)	7964 (43)	22	11	197	11	−2	3
C (4)	3826 (20)	4451 (16)	9672 (44)	17	11	211	13	−11	−3
C (5)	2719 (22)	3549 (17)	11050 (43)	47	20	126	−1	−14	−1
C (6)	1890 (21)	2705 (17)	9018 (47)	23	17	284	−5	−34	−23
O (1)	1790 (17)	8394 (12)	7658 (36)	76	13	380	−17	−17	17
O (1′)	217 (15)	6840 (12)	6103 (32)	45	12	291	1	−37	25
O (2)	3002 (16)	6893 (13)	11744 (31)	65	27	211	−7	−35	11
O (3)	4266 (15)	6088 (12)	6584 (31)	29	21	273	−15	−6	2
O (4)	4935 (15)	3735 (13)	8002 (32)	34	25	252	16	−10	−11
O (5)	3549 (16)	2863 (12)	13147 (31)	66	24	156	10	−28	15
O (6)	2295 (17)	1559 (12)	8826 (34)	88	10	324	2	−19	0
O (6′)	804 (16)	3216 (12)	7491 (32)	40	21	256	−4	−46	5

and dianionic (L^{2-}) states by adding an equimolar amount of KOH and DCl. The pH values corresponding to each state were approximately 1.5, 3.8, and 9.8 in the pH-meter readings for the potassium salts, and 0.7, 1.4, and 6.1 for the calcium salts, respectively. The concentration of calcium glucarate in the solution at pH 6.1 was extremely low, since the calcium salt was insoluble in neutral deuterium oxide. The induced shifts of protons H(2)—H(5) by europium ions were measured by adding europium nitrate to the 0.1 mol potassium salt solution at pH 1.5. The shift ratios of H(2)/H(4), H(3)/H(4) and H(5)/H(4) at null concentration of the europium ions were derived from the induced shifts observed in various ionic strengths of the solutions. The water in the commercial $Eu(NO_3)_3 6H_2O$ was removed by drying the sample solved in the deuterium oxide. All the experiments were performed at room temperature (23 °C).

Calculations of the Europium–Glucarate Complex Model. The shift ratios of ligand protons for an axially symmetric complex can be calculated by the following McConnell-Robertson equation:

$$\Delta\nu/\nu_0 = K\cdot(3\cos^2\theta - 1)/r^3,$$

where K is the constant related to the magnetic field, r is the distance between the europium ion and the proton, and θ is the angle between the principal magnetic axis of the complex and the vector $\boldsymbol{r}$.[5] A number of europium–glucarate complex models were generated on a FACOM 230-75 high-speed computer in the Data Processing Center, Kyoto University. The most reasonable model was selected by minimizing the sum of the square errors between the calculated and observed shift ratios.[6-8] In consideration of several stereochemical situations of the present complex, the following assumptions were made. (i) The complex has a symmetric axis which coincides with the principal magnetic axis; (ii) the symmetric axis is coincident with one of the principal axes of the inertia tensor of the complex in the time average; (iii) the ligand has the rigid conformation observed in the crystals; (iv) there are no abnormally short contacts between the europium ion and the ligand oxygens, nor between the neighboring ligand atoms, and (v) each ligand has at least two oxygens bonded to the europium ion. The stoichiometric component of the europium–glucarate complex was not determined by the experiments. However, the europium ion observed in the crystal structure determined hitherto[9-12] is usually bonded to seven to nine oxygens, a similar coordination number being expected for the europium ions in the solution. A certain number of water molecules can be inculuded in this coordination. For the stable complex formation, two or three oxygens of each ligand should be bonded to the europium ion (assumption v). Hence, the stoichiometric component of the present complex was limited in the three cases of 1:1, 1:2, and 1:3. Since the principal axis of the inertia tensor of the complex coincides with the symmetric axis (assumption ii) and all the ligand anions are limited in the D-isomer, two-fold and three-fold axes were assigned to the symmetric axes of the 1:2 and 1:3 complexes, respectively. If such an axial symmetry of the complex is assumed and the internal freedom of the ligand is forbidden by fixing the atomic parameters of the ligand to those of the crystal structures (assumption iii), the structure of the complex can be determined by the three positional parameters of the ligand relative to the europium ion and by the three orientational parameters of the symmetric axis through the central europium ion. In the actual calculation, the positional parameters in the first place changed step by step. The atomic distance between the europium ion and ligand atoms was inspected at each position, from the criterion that an abnormally short distance less than 2.2 Å is unallowable and that at least two oxygens have Eu–O proximity in the range 2.2—3.4 Å. After fixing the position of the ligand, the directions of the principal axes of the inertia tensor and the mean direction of the Eu–O bonds were calculated, one of them being selected as the direction of the magnetic axis of the complex. The symmetric axes of the 1:2 and 1:3 complexes were determined according to the following procedure. The minimum of the sum of the square errors between the calculated and observed shift ratios was found by changing three Eulerian angles of the axis at suitable intervals. The second and third ligands corresponding to the 1:2 and 1:3 complexes were generated by the symmetric operation around this axis, and the atomic contacts between the adjacent ligands were checked. The two cases of the ligand forms, TGG and GTT, observed in the crystal state were examined by iterative application of the procedure described above. The 1:2 complex composed of the TGG conformers gave the best fit between the calculated and observed shift ratios.

Although the complex model derived here is based upon the assumption that the ligand has the fixed conformation observed in the crystals, the glucaric acid in the actual solution might have a flexibility and many different types of ligand conformers might interact with the europium ions. The observed shifts would result from an averaging of these various conformations. Thus, the complex models including further different conformers should be taken into account for the model calculations, and the shift ratios calculated as a mixture of these complex models. However, the large conformational change of the ligand by the complex formation was unexpected, since the J values did not change much on adding the europium ions. The mixture model of the TGG and GTT conformers also seemed impossible since the GTT conformer complex models gave a very large discrepancy between the calculated and observed shift in any assignment of the ligand positions and of the directions of the magnetic axis. The TGG conformer complex proposed here is, therefore, the most probable form in spite of the large flexibility of the ligand, and should be the main component in various complexes in the equilibrium solution.

Results and Discussion

The Crystal Structure of Potassium D-*Glucarate.* Figure 1 shows a perspective view of the crystal structure of potassium glucarate. The crystal structure is stabilized by the attractive forces between the potassium ions and the oxygen atoms of the glucaric anions. The potassium ions are surrounded by four carboxyl oxygens and four hydroxyl oxygens in a distorted cubic-prism arrangement, and one cation binds six ligand anions. The K–O distances range from 2.43 to 3.32 Å, the average value being 2.97 Å. This chelating structure differs from that of the calcium ion observed in the crystal of calcium glucarate tetrahydrate, in which the cations are surrounded by two carboxyl oxygens, three hydroxygens and three water molecules, and the central cation binds only two ligand dianions.[2] The hydrogen bonds also stabilize the crystal structure of the potassium salt. The hydrogen bond linkage –O(3)–O(2)–O(4)–O(5)– binds four hydroxyl groups of the adjacent glucaric anions. Another O(1′)–O(6′) hydrogen bond binds the carboxyl groups of the adjacent glucaric anions at the head and the tail along the molecular axis. Although the positions of the hydrogen in the O(1′)–O(6′) hydrogen bond are of interest in relation to the

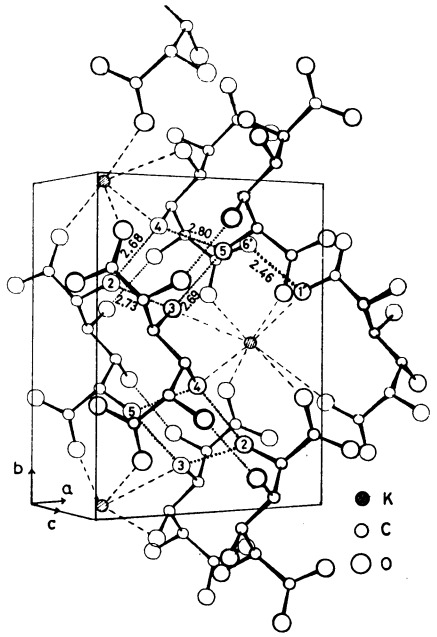
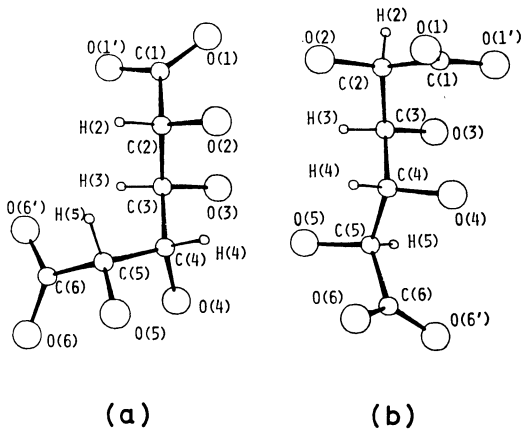


Fig. 1. A perspective view of the crystal structure of potassium glucarate. The dotted lines show the hydrogen bonds. Their distances are given in Å unit.



(a) (b)

Fig. 2. Conformations of the glucaric acid, (a) in the crystal of the potassium salt and (b) in the crystal of the calcium salt.

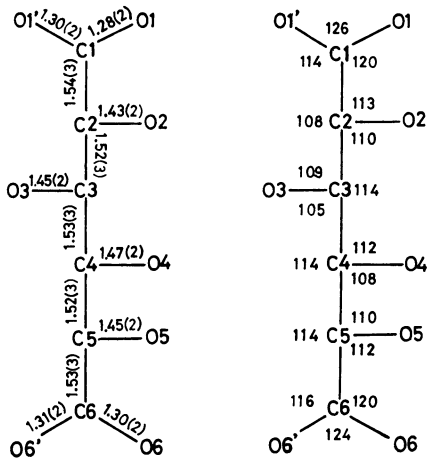


Fig. 3. Bond lengths (Å) and angles (°) of the glucaric acid in the crystal of the potassium glucarate.

dissociation state of the protons of the carboxyl groups at C(1) and C(6), they were not determined accurately because of the poor resolution of the difference Fourier maps.

Figure 2(a) shows a perspective view of the glucaric anion in the crystal of the potassium salt. The bond lengths and angles are given in Fig. 3. The carbon chain has a bent conformation. The structure differs from that of the calcium salt given in Fig. 2(b). The torsional angles about the C–C bonds in each case are given in Table 2. In the case of the potassium salt, C(1) and C(4) are located in trans positions with respective to the C(2)–C(3) bond. C(2) and C(5) are gauche for C(3)–C(4), and C(3) and C(6) are gauche for C(4)–C(5). Thus, the structure for the potassium salt can be represented in terms of "TGG form," while that for the calcium salt will be of "GTT form." In these structures, unfavorable parallel interaction between the C(2)–O(2) and C(4)–O(4) bonds,[13,14] as would occur in a fully extended structure, is avoided, both structures being more stable than other possible structures.

Table 2. Torsional angles ϕ (°) in D-glucaric acid

Bond	Crystal		Solution	
	K salt	Ca salt	$0 \leqq \phi \leqq 90$	$90 \leqq \phi \leqq 180$
H(2)–C(2)–C(3)–H(3)	57.6	−67.6	62	122
H(3)–C(3)–C(4)–H(4)	−168.2	−71.3	51	136
H(4)–C(4)–C(5)–H(5)	68.3	167.8	54	133
O(1)–C(1)–C(2)–C(3)	−110.5	−124.2		
O(1')–C(1)–C(2)–C(3)	70.1	56.1		
C(1)–C(2)–C(3)–C(4)	179.3	55.7		
C(2)–C(3)–C(4)–C(5)	67.4	162.4		
C(3)–C(4)–C(5)–C(6)	68.7	168.0		
C(4)–C(5)–C(6)–O(6)	106.1	67.3		
C(4)–C(5)–C(6)–O(6')	−71.5	−109.9		

Solutions of the Potassium Salt and the Calcium Salt. The 100 MHz NMR spectra of the potassium salt and the calcium salt consist of twelve peaks in the most separated case and each peak can be assigned to four protons H(2), H(3), H(4), and H(5) in the glucaric acid (Fig. 4). The assignments agree with those given for the sodium salt by Sawyer and Brannan.[15] When the pH value of the solution decreases, the H(2) and H(5) peaks shift lowfield to a greater extent than the H(3) and H(4) peaks. There is no significant difference between the chemical shifts in the potassium salt and the calcium salt (Table 3). The coupling constants J for both salts are also similar, the J values for each pair of protons remaining almost the same even when the dissociation states of the glucaric acid change (Table 4). The H–C–C–H torsional angles calculated from the average J values for each pair of protons by application of the modified Karplus equation[16] of Lemieux and Lown[17] are also given in Table 2. When the J values were calculated from the torsional angles observed in the two crystal structures, their arithmetic average values are given as 3.0, 5.9, and 6.2 Hz for $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$, respectively. These values are close to the

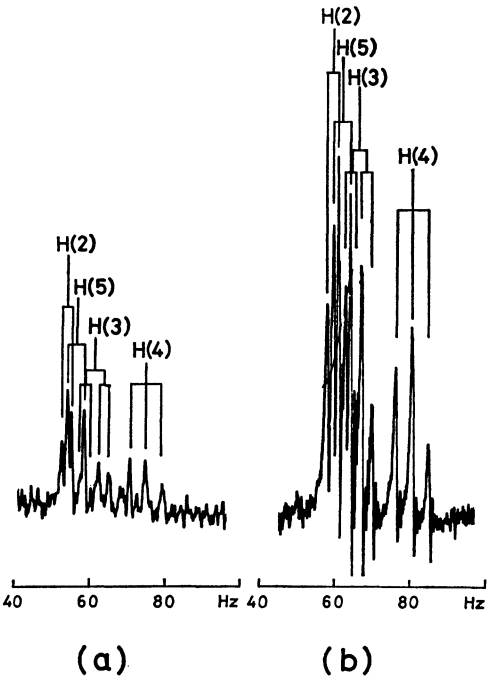


Fig. 4. The 100 MHz proton magnetic resonance spectra of D-glucaric acid. (a) For the saturated solution of the calcium salt at pH 6.1. (b) For the solution of 0.1 mol potassium salt at pH 9.8. The chemical shifts (Hz) are measured from internal HDO in upfield.

TABLE 3. CHEMICAL SHIFTS (Hz) OF D-GLUCARIC ACID UPFIELD FROM INTERNAL HDO

Proton	LH_2	LH^-	L_2^-
		Potassium salt	
H(2)	23	43	59
H(3)	56	62	66
H(4)	75	78	80
H(5)	34	49	61
		Calcium salt	
H(2)	26		55
H(3)	59		61
H(4)	77		75
H(5)	37		57

TABLE 4. PROTON COUPLING CONSTANTS (Hz) OF D-GLUCARIC ACID FOR K, Ca, AND Eu SALTS

Coupling constants	K salt			Ca salt		Eu salt
	LH_2	LH^-	L^{2-}	LH_2,LH^-	L^{2-}	LH_2
$J_{2,3}$	3.0	3.0	2.8	3.0	2.5	3.0
$J_{3,4}$	5.5	5.0	4.3	5.5	4.3	5.5
$J_{4,5}$	4.8	4.8	4.5	5.0	4.5	4.8

corresponding observed values of 3.0, 5.5, and 5.0 Hz. The glucaric acid in the solution is thus supposed to be an equilibrium mixture of the TGG and GTT conformers found in the crystals, although other conformers may be more or less mixed.

The Europium–Glucarate Complex in the Solution. When europium nitrate is added to an acidic solution of potassium glucarate, the H(2), H(3), and H(5) peaks shift highfield and the H(4) peak shifts lowfield (Fig. 5). The shift ratios, H(2)/H(4), H(3)/H(4), and H(5)/H(4), induced by the europium ion are linear for the relative concentration of the europium ions [Eu]/[S], and the values of the shift ratios extrapolated to the null concentration of europium ions are −2.89, −1.56, and

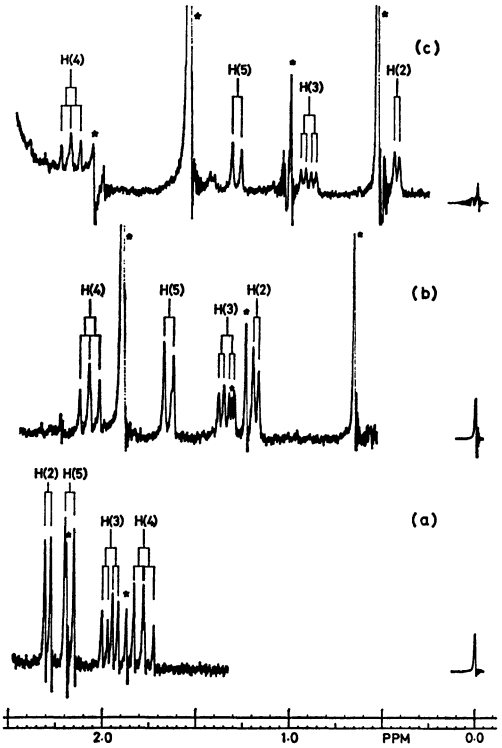


Fig. 5. Change of the 100 MHz proton magnetic resonance spectra of D-glucaric acid by adding europium nitrate to the solution of 0.1 mol potassium salt at pH 1.5.
(a) Eu was not included. (b) [Eu]: [S]=2: 3. (c) [Eu]: [S]=4: 3.
The chemical shifts were measured in relative values to the internal acetone. The peaks noted by asterisks (*) indicate the side band by the sample spinning.

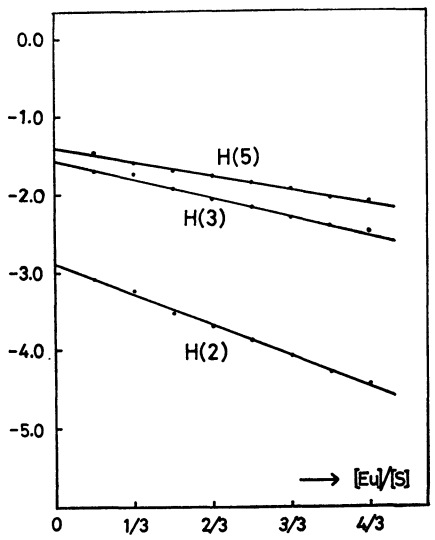


Fig. 6. Shift ratios of the protons of the glucaric acid *vs.* ionic strength [Eu]/[S]. The shift of proton H(4) was chosen as the reference.

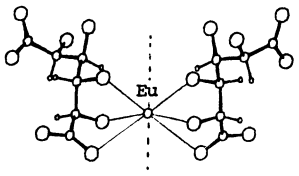


Fig. 7. Structure of Eu-glucaric acid complex. The assumed direction of the magnetic axis is indicated by the broken line through the center of the Eu ion.

−1.40, respectively (Fig. 6). The observed values were compared with the calculated shift ratios, and the best fit was obtained in the case of the 1 : 2 complex composed of the TGG conformers (see Experimental). The calculated shift ratios were −2.90, −1.57, and −1.10 for H(2)/H(4), H(3)/H(4), and H(5)/H(4), respectively. Figure 7 shows a perspective view of the obtained complex model. The three oxygens of the glucaric acid bind to the europium ion. The Eu–O distances are 3.17 Å for Eu–O(1′), 2.21 Å for Eu–O(2) and 2.64 Å for Eu–O(3). The europium–glucarate binding feature agrees with the calcium chelating form of gluconic acid found in lactobionic acid CaBr crystal, in which the gluconic acid moiety has the same TGG conformation.[18]

The Calcium Sequestering Effect by Glucaric Acid in Alkaline Solutions. Quite a large amount of the calcium salt of glucaric acid is precipitated in alkaline solutions. This can be explained as follows. The ionic radius of the calcium ion (0.99 Å) is close to that of the europium ion (1.11 Å), and in spite of their different charges, both ions show a similar behavior in glucaric acid solutions. From this analogy between the calcium and europium ions, the calcium ion should form a complex with the TGG conformers of glucaric acid as well as the europium ion did, the complex being stable in the solution over a wide pH range. The assumption of the calcium–glucarate complex is also supported by the fact that the calcium salt of glucaric acid is more easily dissolved in a high concentrated solution of calcium chloride than pure water, since the increased solubility of glucaric acid may be due to the complex formation between calcium ions and glucaric acid. Although the TGG conformers form such a calcium complex, the GTT conformers in an equilibrium state weakly interact with the calcium ions, not forming the calcium complex in the acidic solution. However, the calcium affinity of the glucaric acid is increased in the alkaline solution since the hydrogens of the carboxyl groups and probably of the hydroxyl groups in the glucaric acid are ionized in the aqueous alkaline solution. In this state, the calcium ions will bind not only to the TGG conformers but also to the GTT conformers. Once the GTT conformers bind to the calcium ions, they will form insoluble calcium salt and will be precipitated. Since the equilibrium ratio between the TGG and GTT conformers in the solution is unchangeable during the course of precipitation, the GTT conformers lost by precipitation should be supplied by the conformational change of the TGG conformers to the GTT form. Precipitation of the calcium salt would proceed until the solution attains an equilibrium state of low limited concentration of the glucaric acid in the last stage.

In conclusion, glucaric acid has two stable conformations found in the two salt crystals. The glucarate solutions are an equilibrium mixture of these two conformers. Their equilibrium ratio is not affected by the ionization states of glucaric acid or by the kind of cation in the solution. The TGG conformers form the 1 : 2 calcium complex in a wide pH range. The precipitation of the calcium salt occurs when the GTT conformers in the dianionic state bind to the calcium ions.

All the computations were performed on a FACOM 230-75 in the Data Processing Center, Kyoto University. The program system KPAX for the X-ray crystallography was used for the structure analysis of potassium glucarate crystal. The FORTRAN programs for the computations of complex models and of the chemical shifts were written by one of the authors (T.T).

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