Structures of Lanthanoid Complexes of Glyceric Acid, Gluconic Acid, and Lactobionic Acid from the Lanthanoid-Induced ^{1}H NMR Shifts: pH Dependence of the Lanthanoid-Substrate Equilibria

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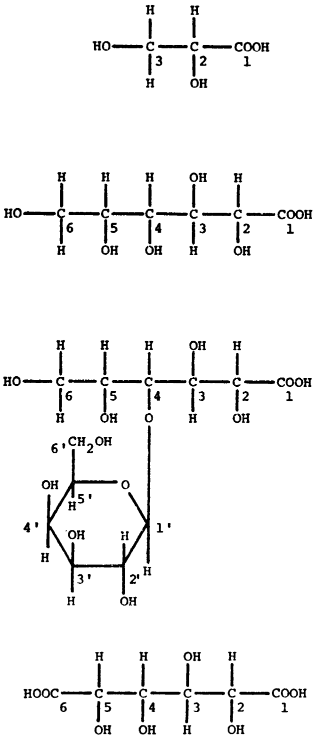
Lanthanoid-induced ^{1}H NMR shifts of glyceric acid, gluconic acid, and lactobionic acid were observed in D_2O. Stoichiometric analysis using the shift data at various pH values indicated that the concentration of a 1: 1 complex between substrates and lanthanoid ions was higher than that of a 2: 1 complex at a low pH. The structure of an axially symmetric 1: 1 complex was obtained on the assumption of a predominant pseudo-contact interaction between a europium ion and the ligand nuclei. All the title glycosidic acids were found to chelate the cation with α-carboxy, α-hydroxy, and β-hydroxy oxygens. The relative position of β-hydroxy oxygen is particularly important for the complex formation between β-hydroxy carboxylic acids and metal ions.

In previous papers[1,2] dealing with calcium binding to polyhydroxy dicarboxylic acid, DL-glucaric acid, we have reported that the α-carboxyl group chelates the cation with α-carboxy, α-hydroxy, and β-hydroxy oxygens in an aqueous solution, while the δ-carboxyl group is not bound to the cation either in solution or in the crystalline state. The configuration about the carbon chain of glyceric acid, **1**, gluconic acid, **2**, and lactobionic acid, **3**, is the same as that of glucaric acid, **4**, with respect to the α-carboxyl part (Fig. 1). We were, therefore, interested in the chelating geometry of Compounds **1**, **2**, and **3** to a lanthanoid ion in relation to that of glucaric acid, **4**. It has been known that acidic and neutral carbohydrates occasionally well chelate a metal ion with hydroxyls.[3] The chelating stability depends on the steric arrangement of hydroxyls in the substrate and on the size of the cation. For instance, an axial-equatorial-axial hydroxyl arrangement about a six-membered ring of inositol or pyranose sugar is suitable for the chelation to an alkaline-earth ion, as has been pointed out by Angyal.[4] X-Ray and neutron diffraction studies[5–8] have revealed that **1** and **2** have two different conformations in the crystalline states, and that Compounds **1**, **2**, and **3** have a large flexibility. However, it is possible for them to have a conformation suitable for the stable complexing with a lanthanoid ion in solution. Thus, we studied the structures of the lanthanoid complexes of these compounds in D_2O by the lanthanoid probe method of ^{1}H NMR.



(1)

(2)

(3)

(4)

Fig. 1. Structural formulas of D-glyceric acid (**1**), D-gluconic acid (**2**), D-lactobionic acid (**3**), and D-glucaric acid (**4**).

Since the "fast exchange" assumption for complexing between the substrate and the lanthanoid ion in D_2O may be correct, and since complexes with three or more substrates may be negligible, the paramagnetic lanthanoid-induced shift of the *i*th nucleus in the substrate should conform to the following equation:[9]

$$\Delta\delta_i = \frac{1}{S_0}(x\Delta_{1i} + 2y\Delta_{2i}), \tag{1}$$

where Δ_{1i} and Δ_{2i} are the shifts depending intrinsically on 1: 1 and 2: 1 complexes respectively, where x and y are the concentrations of the corresponding complexes, and where S_0 is the total concentration of substrates in solution. The Δ_{1i} and Δ_{2i} shifts have usually been derived from chemical shifts observed by changing the molar ratio, ρ, between lanthanoid ions and substrates or by changing the total concentration, S_0, at a constant ρ.[10–12] However, the present complexes are precipitated in an aqueous alkaline solution, and the chemical shift below pK_a depends largely on the pH of the solution. Hence, the evaluation of Δ_{1i} and Δ_{2i} from the data given by the pH change of a solution is desirable.

This paper will describe a treatment of the pH dependence of the lanthanoid-induced shift and will show an application of this method for the cases of three carboxylate complexes. The pseudo-contact shift for an axially symmetric cmoplex is given by the McConnell-Robertson equation:[13]

$$\Delta\nu_i/\nu_0 = A\cdot(3\cos^2\theta_i - 1)/r_i^3, \tag{2}$$

where A is a constant for a lanthanoid ion at a given temperature, where r_i is the distance between the paramagnetic ion and the ^1H nucleus, and where θ_i is the angle between the principal magnetic axis of the complex and the vector, r_i. As has been reported by many authors,[14,15)] the application of Eq. 2 in establishing the ligand conformation in a lanthanoid complex has been successful. The structure can be determined by minimizing the square errors between observed and calculated shifts for trial complex models.

Experimental

The ^1H NMR spectra were recorded on a VARIAN HA-100D spectrometer in the frequency-sweep mode at 100 MHz, using HDO or tetramethylsilane sealed in a capillary tube as an internal lock signal. The probe temperature was kept at 30 °C. The chemical shifts were measured from the acetone peak as an internal standard. The D_2O stock solution of 0.5 M glyceric acid was prepared from *ca.* 70% DL-glyceric acid, whose exact concentration was determined by the titration of 1 M NaOH. The D_2O solutions of sodium gluconate and lactobionic acid were prepared from commercial reagents supplied by the Tokyo Kasei Company. Europium nitrate, $Eu(NO_3)_3 \cdot 6H_2O$, and praseodymium nitrate, $Pr(NO_3)_3 \cdot 6H_2O$, were used as paramagnetic shift reagents, while lanthanum nitrate, $La(NO_3)_3 \cdot 6H_2O$, was used as a diamagnetic shift reagent. The pH of the solution was adjusted by adding concd DCl and NaOD, using a CE-150A glass electrode (Toko Chemical Laboratory Co. LTD) connected with a Hitachi-Horiba M-7E pH meter.

Calculations of the stoichiometry and structures of lanthanoid-substrates complexes were performed on a NOVA 1200 computer operating in connection with a DIABLO-31 disk drive, using the FORTRAN IV programs written by the present authors.

Results and Discussion

Stoichiometry of Lanthanoid-Substrate Complexes. Figures 2, 3, and 4 show the pH dependence of Eu(III)-induced shifts for Compounds **1**, **2**, and **3** respectively in D_2O solutions. Since diamagnetic effects may be included in the Eu(III)-induced shifts,[16,17)] the effect was corrected by means of La(III)-induced shifts observed under the same experimental conditions, on the assumption that Eu(III) and La(III) form complexes with similar structures in solution. All the protons bonded to the carbons in **1** and **2**, and three protons of H(2), H(3), and H(1′) in **3** were assigned to the observed signals. The other protons of **3** could not be assigned because of the heavy overlapping of the peaks. When the pH value increases, the peaks of H(2) and H(3) for all compounds were shifted upfield, while the other peaks were shifted downfield. The *J* values given in Table 1 do not change greatly in this experimental pH range.

The pH dependence of the concentrations, *x* and *y*, corresponding respectively to the 1 : 1 and 2 : 1 complexes

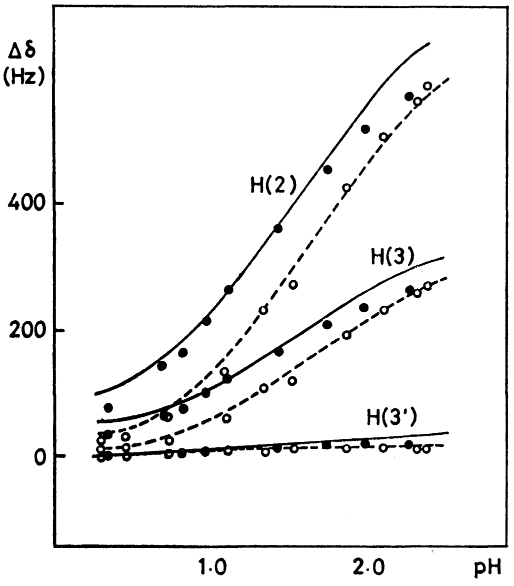


Fig. 2. Eu(III)-induced shifts ($\Delta\delta_{obsd}$) of DL-glyceric acid observed by successive pH changes. ●, $\rho=0.5$, $S_0=0.5$ M; ○, $\rho=2.0$, $S_0=0.05$ M. The curves were obtained by theoretical fitting (see text).

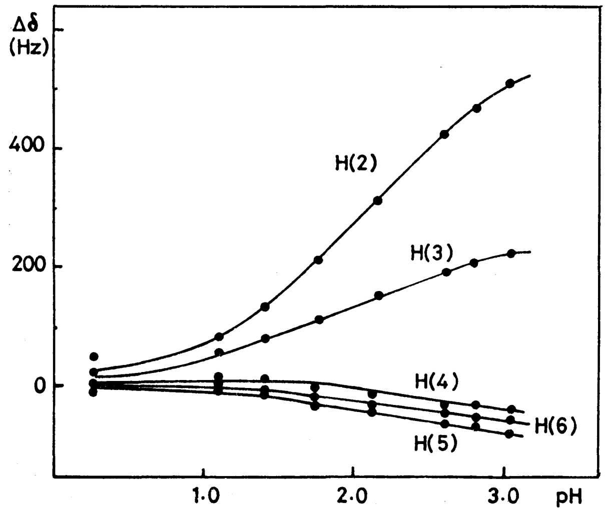


Fig. 3. Eu(III)-induced shifts ($\Delta\delta_{obsd}$) of D-gluconic acid observed by successive pH changes. ○, $\rho=0.5$, $S_0=0.1$ M. The curves were obtained by theoretical fitting (see text).

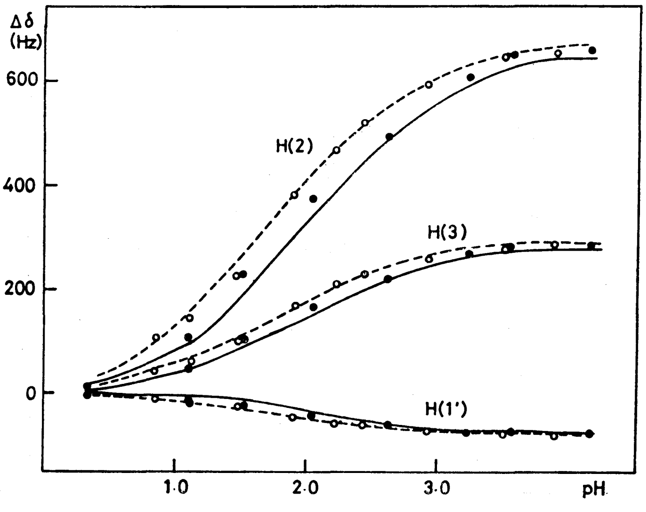


Fig. 4. Eu(III)-induced shifts ($\Delta\delta_{obsd}$) of D-lactobionic acid observed by successive pH changes. ●, $\rho=0.5$, $S_0=0.1$ M; ○, $\rho=0.5$, $S_0=0.2$ M. The curves were obtained by theoretical fitting (see text).

TABLE 1. COUPLING CONSTANTS (± 0.2 Hz) OBSERVED FOR LANTHANOID COMPLEXES

Glycerate		Gluconate		Lactobionate	
$J_{2\text{-}3}$	4.1	$J_{2\text{-}3}$	3.5	$J_{2\text{-}3}$	2.7
$J_{2\text{-}3'}$	3.0	$J_{3\text{-}4}$	3.0	$J_{3\text{-}4}$	3.7
$J_{3\text{-}3'}$	12.1	$J_{4\text{-}5}$	7.6	$J_{1'\text{-}2'}$	9.5
		$J_{5\text{-}6}$	3.0		

can be derived from the following stoichiometric consideration. If we denote the lanthanoid ion as L, the protonated carboxylic acid as SH, and the unprotonated carboxylate anion as S, the following equilibria may exist in solution:

$$\mathrm{SH} \overset{K_a}{\rightleftarrows} \mathrm{S} + \mathrm{H}, \tag{3}$$

$$\mathrm{L} + \mathrm{S} \overset{K_s}{\rightleftarrows} \mathrm{LS}, \tag{4}$$

$$\mathrm{L} + \mathrm{SH} \overset{K_{sh}}{\rightleftarrows} \mathrm{LSH}, \tag{5}$$

$$\mathrm{LS} + \mathrm{S} \overset{K_{ss}}{\rightleftarrows} \mathrm{LSS}, \tag{6}$$

$$\mathrm{LSH} + \mathrm{S} \overset{K_{shs}}{\rightleftarrows} \mathrm{LSHS}, \tag{7}$$

$$\mathrm{LS} + \mathrm{SH} \overset{K_{ssh}}{\rightleftarrows} \mathrm{LSSH}, \tag{8}$$

$$\mathrm{LSH} + \mathrm{SH} \overset{K_{shsh}}{\rightleftarrows} \mathrm{LSHSH}, \tag{9}$$

where K_a is a dissociation constant of carboxylic acid given as

$$K_a = [\mathrm{S}]\cdot[\mathrm{H}]/[\mathrm{SH}], \tag{10}$$

and where K_s, K_{sh}, K_{ss}, K_{shs}, K_{ssh}, and K_{shsh} are association constants corresponding to each complex. Since the total concentration of lanthanoid ions, $L_0=[\mathrm{L}]+x+y$, and that of substrates, $S_0=[S]+[\mathrm{SH}]+x+2y$, the concentrations for each complex are expressed in terms of x and y by using Eq. 10 as follows:

$$[\mathrm{LS}] = K_s(L_0 - x - y)(S_0 - x - 2y)/(1 + h), \tag{11}$$

$$[\mathrm{LSH}] = K_{sh}(L_0 - x - y) \times (S_0 - x - 2y)h/(1 + h), \tag{12}$$

$$[\mathrm{LSS}] = K_{ss}K_s(L_0 - x - y) \times (S_0 - x - 2y)^2/(1 + h)^2, \tag{13}$$

$$[\mathrm{LSHS}] = K_{shs}K_{sh}(L_0 - x - y) \times (S_0 - x - 2y)^2h/(1 + h)^2, \tag{14}$$

$$[\mathrm{LSSH}] = K_{ssh}K_s(L_0 - x - y) \times (S_0 - x - 2y)^2h/(1 - h)^2, \tag{15}$$

$$[\mathrm{LSHSH}] = K_{shsh}K_{sh}(L_0 - x - y) \times (S_0 - x - 2y)^2h^2/(1 + h)^2, \tag{16}$$

where $$h = [\mathrm{H}]/K_a = 10^{(\mathrm{p}K_a - \mathrm{pH})}. \tag{17}$$

The following quadratic equations are obtained from Eqs. 11—16 by using the $x=[\mathrm{LS}]+[\mathrm{LSH}]$ and $y=[\mathrm{LSS}]+[\mathrm{LSHS}]+[\mathrm{LSSH}]+[\mathrm{LSHSH}]$ relations:

$$x = \eta(L_0 - x - y)(S_0 - x - 2y), \tag{18}$$

$$y = \xi x(S_0 - x)/(\eta + 2\xi x), \tag{19}$$

where $$\eta = (K_s + hK_{sh})/(1 + h), \tag{20}$$

$$\xi = \{(K_{ss} + hK_{ssh})K_s + (K_{shs} + hK_{shsh})hK_{sh}\}/(1 + h)^2. \tag{21}$$

From Eqs. 18 and 19,

$$x^3 + Ax^2 + Bx + C = 0, \tag{22}$$

where $$A = \{-2\rho S_0 + \eta/\xi - 4/\eta\}/\{1 - 4\xi/\eta^2\}, \tag{23}$$

$$B = \{(2\rho - 1)S_0^2 - (\rho + 1) \times (\eta/\xi)S_0 - 1/\xi\}/\{1 - 4\xi/\eta^2\}, \tag{24}$$

$$C = \{\rho(\eta/\xi)S_0^2\}/\{1 - 4\xi/\eta^2\}. \tag{25}$$

Equations 22—25, which are similar to the Shapiro-Johnston equations,[9] are a general expression including the pH dependence for the "two-step mechanism." If the pK_a of the carboxylate in D_2O is known, and if a suitable value of the association constant is assumed, the concentration, x, at a given pH can be obtained by solving Eq. 22, while y can be given by Eq. 19. If the induced shifts of the proton signals of the LS and LSH complexes are the same, and if those of the LSS, LSSH, LSHS, and LSHSH complexes are also the same, (the total lanthanoid-induced shift of the ith nucleus can be calculated by means of Eq. 1. The best fit parameters, Δ_{1i} and Δ_{2i}, can be obtained by the least-squares method, minimizing the sum of errors between the observed and calculated induced shifts.[9]

TABLE 2. ASSOCIATION CONSTANTS ASSIGNED FOR 1 : 1 AND 2 : 1 COMPLEXES, AND THE pK_a OF EACH ACID

		Glyceric acid	Gluconic acid	Lactobionic acid
K_s	(mol^{-1})	980	1180	600
K_{sh}	(mol^{-1})	0.001	0.001	0.001
K_{ss}	(mol^{-2})	180	185	100
K_{shs}	(mol^{-2})	0.0	0.0	0.0
K_{ss}	(mol^{-2})	0.0	0.0	0.0
K_{shsh}	(mol^{-2})	0.0	0.0	0.0
pK_a		3.553	3.60	3.60

The full and dotted lines in Figs. 2, 3, and 4 are the best-fit curves obtained by means of the least-squares calculation. The association constants used for the calculations are listed in Table 2, while the parameters, Δ_1 and Δ_2, determined through the procedure are given in Table 3. The agreement factors defined by $\Sigma(||\Delta\delta_{obsd}| - |\Delta\delta_{calcd}||)/\Sigma|\Delta\delta_{obsd}|$ are 0.082, 0.068,

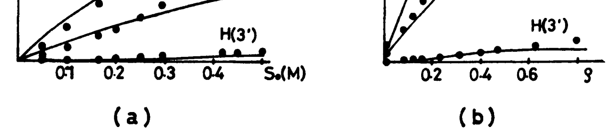


Fig. 5. Eu(III)-induced sflifts ($\Delta\delta_{obsd}$) of DL-glyceric acid observed by successive S_0 changes at $\rho=2.0$, pH$=$0.45 (a), and by successive ρ changes at $S_0=0.42$ M, pH$=$2.0 (b).

Table 3. Lanthanoid-induced shifts, Δ_1 and Δ_2 (± 0.05 ppm)[a] depending intrinsically on 1 : 1 and 2 : 1 complexes

	Glycerate			Gluconate			Lactobionate	
	Δ_1	Δ_2		Δ_1	Δ_2		Δ_1	Δ_2
H(2)	6.41	9.91	H(2)	5.07	7.76	H(2)	10.43	7.58
H(3)	2.96	4.73	H(3)	3.44	2.91	H(3)	4.69	3.31
H(3′)	0.21	0.41	H(4)	0.77	−1.09	H(1′)	−1.20	−0.82
			H(5)	−0.51	−1.24			
			H(6)	−0.15	−0.97			

a) A minus sign denotes a downfield shift.

and 0.042 for the cases of Compounds **1**, **2**, and **3** respectively. Figure 5 shows the ρ and S_0 dependences of the Eu(III)-induced shifts in the case of glyceric acid, **1**. The full curves were theoretically calculated from the association constants assumed in the case of the pH dependence. Since the observed points lie approximately on the curves, the data obtained by analysis based on the pH dependence will be consistent with those obtained on the basis of the ρ and S_0 dependences. Figure 6 shows the pH dependence of the concentrations of 1 : 1 and 2 : 1 complexes. For all compounds, the concentration of the 1 : 1 complex is higher than that of the 2:1 complex at low pH's. When the pH value increases to pK_a, the concentration of the 2 : 1 complex increases markedly. A large amount of lanthanoid ions in solution results in a high concentration of the 1 : 1 complex, as is indicated in the case of glyceric acid (Fig. 6a). The concentrations, [LSH], [LSHS], [LSSH] and [LSHSH], calculated by means of Eqs. 12 and 14—16 are negligible for all cases. Only an unprotonated carboxyl substrate is bound to the lanthanoid ion; they form a stable complex.

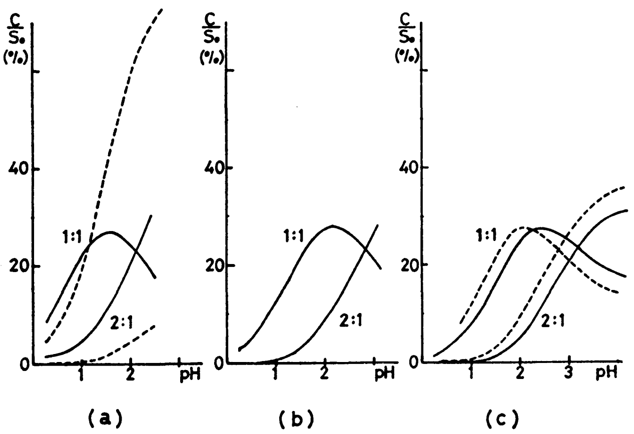


Fig. 6. PH dependence of concentrations of 1 : 1 and 2 : 1 complexes in D_2O solutions; (a) for glyceric acid, where the full curves are for ρ=0.5 and S_0=0.5 M, and the dotted curves are for ρ=2.0 and S_0=0.05 M, (b) for gluconic acid, where the full curves are for ρ=0.5 and S_0=0.1 M, and (c) for lactobionic acid, where the full curves are for ρ=0.5 and S_0=0.1 M, and the dotted curves are for ρ=0.5 and S_0=0.2 M.

Structure of the 1 : 1 Complex. The Δ_1 and Δ_2 shifts generally include two different interactions, contact and pseudo-contact. The contact interaction is usually strong at the nucleus, which is only a few bonds away from the complexing sites, and the shift value depends on the kind of paramagnetic ion.[18] When praseodymium nitrate was added to a glyceric acid solution, ^{1}H peaks were shifted in the direction opposite to that in the case of europium nitrate. The shift ratios of each signal observed in both cases agree within an error of 10%. Thus, the effect of europium ions on the present ^{1}H signals should be attributed mainly to the pseudo-contact interaction.[16] Thus, Eq. 2 can be used in the structure determination of axially symmetiric complexes. Figure 7 shows the structures of 1 : 1 complexes obtained by the application of Eq. 2, and Table 4 lists the observed and calculated shifts for each complex; the agreement factors are 0.009, 0.090, and 0.092 for **1**, **2**, and **3** respectively. The carboxyl parts of the ligand anions have similar conformations for all com-

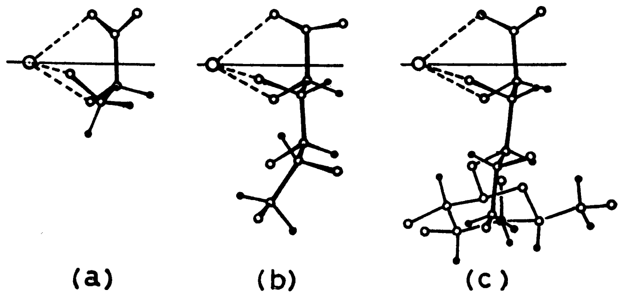


Fig. 7. Structures of 1 : 1 complexes between Eu(III) and substrates; (a) for glyceric acid, (b) for gluconic acid, and (c) for lactobionic acid.

Table 4. Observed and calculated induced-shifts (± 0.05 ppm)[a] of ^{1}H peaks of 1 : 1 complexes

	Glycerate			Gluconate			Lactobionate	
	obsd	calcd		obsd	calcd		obsd	calcd
H(2)	6.41	6.41	H(2)	5.07	4.95	H(2)	10.43	10.00
H(3)	2.96	2.95	H(3)	3.44	3.39	H(3)	4.69	5.50
H(3′)	0.21	0.28	H(4)	0.77	1.28	H(1′)	−1.20	−1.15
			H(5)	−0.51	−0.62			

a) A minus sign denotes a downfield shift.

TABLE 5. Eu···O DISTANCES IN THE 1 : 1 COMPLEX BETWEEN β-HYDROXY CARBOXYLIC ACID AND THE EUROPIUM ION (±0.05 Å)

	Glycerate	Gluconate	Lactobionate
Eu···O(1)	2.53	2.55	2.54
Eu···O(2)	2.42	2.39	2.43
Eu···O(3)	2.33	2.35	2.36

plexes, α-hydroxy oxygen being approximately on the same plane as the four carboxy atoms and β-hydroxy oxygen being *gauche-gauche* to the α-substituents. The anions chelate a europium ion through three oxygens, α-carboxy, α-hydroxy, and β-hydroxy oxygens. The Eu–O distances are given in Table 5. The magnetic axis of the complex is assumed as the mean direction of the three Eu–O vectors. Two β-protons of glyceric acid **1**, were nonequivalent in the experimental pH range, and the glycerate anion may have two or more stable conformations in D_2O.19) Two favorable conformations, of **1** have been found in the crystal structure of calcium glycerate dihydrate.5) The β-protons in one conformer are *gauche-gauche* to the α-proton, while those in other conformer are *trans-gauche*. The ligand conformation of the 1 : 1 complex established here (Fig. 7a) is similar to the former. The C(1)–C(2)–C(3)–O(3) torsion angle is 30°, compared with that of 48° in the crystal structure. Two stable conformations of the gluconate anion, **2**, have also been found as dimorphic crystals in the potassium salts.6) One has a straight chain conformation stabilized by the intramolecular hydrogen bond between the two *syn* axially related hydroxyls, while the second has a bent chain conformation. The present ^1H NMR study indicates that the gluconate anion in the 1 : 1 complex (Fig. 7b) has the latter bent conformation. The torsion angles are −60 and −144° for H(3)–C(3)–C(4)–H(4) and H(4)–C(4)–C(5)–H(5) respectively. The gluconic acid moiety of lactobionic acid **3** also has a bent conformation in solution (Fig. 7c). Not only is the conformation of the lactobionate anion similar to that observed in the crystal structure8) as a whole, but also the chelating structure to the cation is similar to that in the crystal, the cation being bound to the gluconic acid moiety rather than to the neutral galactoside moiety. The torsion angles about the glycosidic bond in the complex are 133 and −167° for C(3)–C(4)–O(1′)–(1′) and C(4)–O(1′)–C(1′)–C(2′) respectively.

As has previously been described, the three carboxylates have the same type of "three O atoms" chelation. The chelating structure is similar to that of glucaric acid, **4**, reported previously.2) The difference in calcium affinities between the two carboxyl groups in glucaric acid, **4**, may be due to the configurations of hydroxyls about the C–C bonds located near each carboxyl group. The configuration given in Fig. 8a, corresponding to the cases of the present three compounds and the α-carboxyl part of glucaric acid, is favored for the complex formation with a cation, while the configuration given in Fig. 8b, corresponding to the case of the δ-carboxyl part of glucaric acid, is not favored for the chelation because of repulsion between parallel C(1)–O(1′) and C(3)–C(4) bonds. Hence, the relative arrangement of hydroxyl groups, particularly that of β-hydroxyl, is important for a stable complex formation. Generally, the carboxylic acid of acyclic polyol, which has the Fig. 8a configuration, forms a stable chelating complex with a metal ion.

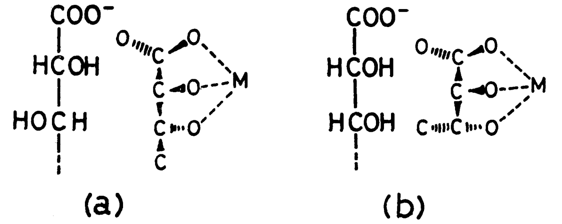


Fig. 8. Two types of configurations and possible 'three O atoms' chelations to a metal ion M.

In conclusion, the data analysis based on the pH dependence of lanthanoid-induced NMR shifts is useful for the structural study of lanthanoid–carboxylates complexes in D_2O. The application of this method to the three carboxylates showed that glycosidic acids of acyclic polyol form a stable complex with a lanthanoid ion in the "three O atoms" chelation including the β-hydroxy oxygen.

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