218. Lanthanide-Ion Complexation by D-Glucuronic and D-Galacturonic Acids

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To assess the potential of naturally occurring substances in the treatment of heavy-metal intoxication, the interaction between D-galacturonic and D-glucuronic acids with several trivalent lanthanide ions has been studied in aqueous solutions by means of a spectrophotometric method (27°; 0.1M $NaClO_4$; pH 4.0). Values for the overall stability constants for [LnL] and [LnL_2] (Ln = La, Ce, Pr, Nd, Gd, or Lu) complexes are presented and discussed. The interpretation of the data shows that, similarly to acetates, the COOH group coordinates metal ions in both [LnL] and [LnL_2] complexes. The 1:1 complexation is supplemented by the ring C(5)–*O*-atom. Moreover, the C(4)–*O*-atom seems to play an important role in the steric hindrance of the chelating ligand molecules.

Introduction. – Lanthanide ions are found in living organisms in trace amounts only, and they are considered to play no biological role [1]. They interact, however, with biological materials in specific ways, which along with their unique magnetic and spectroscopic properties make them very informative substitution probes for Ca-, Mg- or Zn-containing biological materials [2]. Because of their chemical similarity, lanthanides may also act as substitution probes for the actinides, especially for those radioactive elements available only in submicroquantities.

Foreign chemicals entering into the organism are usually converted into compounds that can be excreted. For heavy metals deposited into the organism, there is no physiological process that will eliminate them rapidly from the body. Their excretion rate is so slow that, in a normal life span, only a fraction of the deposited metals can be removed from the body. Events such as the Chernobyl fallout have stimulated studies leading to the development of new therapeutic methods for the removal of immobilized radioactive materials. The strategy for this removal has been mainly based on the use of high-affinity chelating agents which migrate preferentially to certain organs *in vivo*. A toxic metal bound to a constituent of living organisms (usually a protein) is transformed into a metal chelate which is readily excreted. It has long been known that the increase in stability constant of a metal complex parallels an increase in excretion rate [3–5].

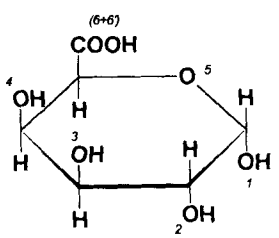
The majority of artificial chelating agents that are commerically available have been developed for purposes other than the removal of toxic metal ions from mammalian body. The most popular chelating agents EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid) have several COOH groups which are ionized under physiological conditions. Therefore, they cannot pass through cell membranes to any significant extent [6] [7]. As a result, EDTA and DTPA are very effective in

removing many metal ions from the serum, but relatively ineffective in removing metals from intracellular sites [8–11]. During recent years, the syntheses of artificial chelating agents with large stability constants and the delineation of the other factors which determine the effectiveness of these chelates as toxic metal decontaminents have remarkably improved. Several new artificial chelates which are significantly more effective or easier to administer have been proposed. A prolonged treatment cannot, however, be prescribed routinely, because the chelates may cause unwanted side reactions, resulting for example in kidney injury [12].

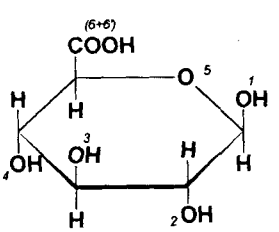
On the other hand, clinical tests have shown that pectines (partially methoxylated polygalacturonic acids) or alginic acids (straight-chain, hydrophilic, polyuronic acids comprised of anhydro D-β-mannuronic acid and L-guluronic acid, probably in the α-configuration) used as prophylactic or medication drugs in the case of heavy-metal poisoning induce an increased excretion of Pb [13–15] or Hg [16]. In addition, several workers have reported that sodium alginate incorporated into the diet of mices induces an increase of ^{90}Sr, ^{133}Ba, and ^{226}Ra concentrations in blood, as well as a decrease of their deposition in the skeleton [15].

In this context, we have launched a research program to assess the potential of naturally occurring chelating agents in the treatment of heavy-metal intoxication. We report here, as a first step towards this goal, the stability constants of the complexes formed by several lanthanide ions with two pyranuronic acids: (+)-D-galacturonic and D-glucuronic acids.

To our knowledge, only one paper reports data for the stability constants of complexes formed by one lanthanide ion, Eu^{3+}, with simple uronic acids, *i.e.* with glucuronic and galacturonic acids [16].



(+)-D-Galacturonic acid[a])



D-Glucuronic acid[a])

[a]) The numbering refers to the O-atoms.

Experimental. – *Chemicals.* Lanthanides (La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Gd^{3+} and Lu^{3+}) were purchased as their oxides from *Research Chemicals* or *Glucydur* in 99.99% purity and converted into the hydrated perchlorates by repeated treatment with conc. $HClO_4$ (*Merck*; *purum, p.a.*). $NaClO_4$ (*purum, p.a.*), D-glucuronic acid (*purum*), and (+)-D-galacturonic-acid monohydrate (*purum*) were from *Fluka*, while murexide (ammonium purpurate > 98%) was from *Merck*. The water used was deionized and doubly distilled in a quartz apparatus.

Murexide solns. were prepared just before the experiments. Constant ionic strength of the solns. was adjusted by 0.1M $NaClO_4$ and the acidity was set to pH 4.0. The murexide concentration was determined spectrophotometrically using the published molar absorption coefficient of 13550 l $mol^{-1}cm^{-1}$ at 520 nm [17]. The solns. were

regularly checked because of the limited stability of murexide [18]. The lanthanide solns. were standardized volumetrically using EDTA (*Tritriplex III*; *Merck*) in presence of urotropine and xylenol orange [19].

Spectrophotometric Measurements. We have chosen the spectrophotometric procedure recently proposed by *Ohyoshi* and *Kohata* [20]. It is based on the competition between a metallochromic indicator, murexide, and the investigated chelating agent. VIS Spectra were recorded on a *Perkin Elmer Lambda 7* UV/VIS spectrophotometer using 1-cm cells (3.5 ml) at 27 ± 0.2°. Aliquots (10 µl) of lanthanide or uronic-acid stock solns. (of *ca.* 0.05M and 0.5M concentrations, respectively) were added to the spectrophotometric cell containing the murexide soln. After each addition, the absorbance was measured in the 500–430-nm range with a 0.1M $NaClO_4$ solution in the reference cell. The overall procedure used consisted of two steps:

1) Determination of the conditional stability constants, $K_{mur} = [Ln(mur)]/[Ln]\cdot[mur']$ where $[mur'] = [mur] + [Hmur] = C_{mur} - [Ln(mur)]$ (the charges are omitted for simplicity), at constant ionic strength and acidity, in the absence of the investigated ligand.

2) Determination of a series of conditional stability constants, $K'_{mur} = [Ln(mur)]/[Ln']\cdot[mur']$ where $[Ln'] = [Ln] + [Ln(lig)] + [Ln(lig)_2] + \ldots + [Ln(lig)_n] = C_{Ln} - [Ln(mur)]$ and $[mur'] = [mur] + [Hmur] = C_{mur} - [Ln(mur)]$, under the same experimental conditions, but in presence of the investigated uronic acid (lig) in various concentrations ($C_{lig} \gg C_{Ln} \gg C_{mur}$).

Since murexide has a pK close to 0 [18], purpuric acid exists only in the presence of strong mineral acids. Moreover, it is very unstable and decomposes immediatly. We have, therefore, assumed that $[mur'] \approx [mur]$ under the experimental conditions used (pH 4.0).

The recorded absorption spectra of the murexide solns. had two maxima corresponding to the free murexide at 518 nm and to the murexide complex (486 nm to 463 nm depending upon the Ln^{3+} ion). *Fig. 1* shows the variation

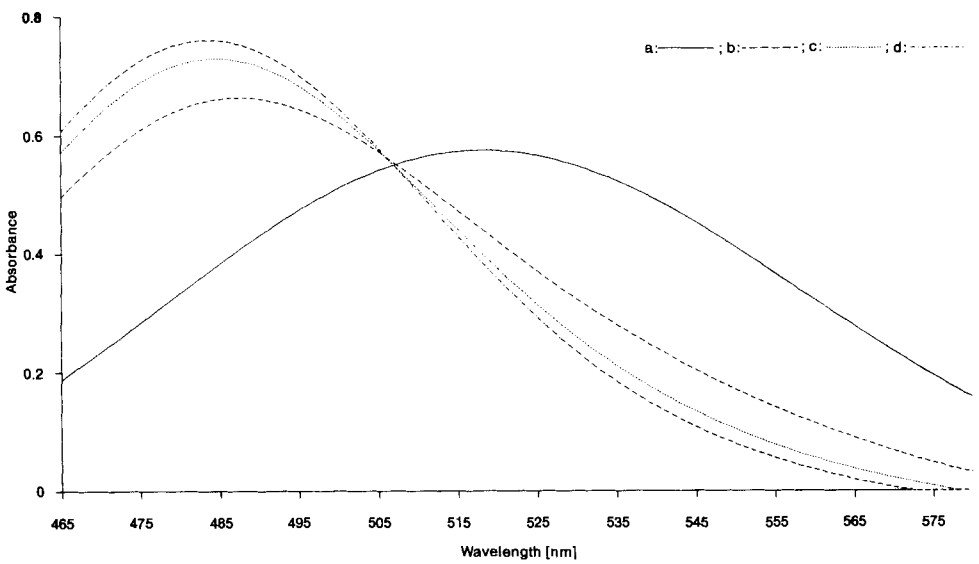


Fig. 1. *Spectral changes of* $4\cdot10^{-5}$ M *murexide solutions upon addition of lanthanum* (μ = 0.1M ($NaClO_4$), t = 27°, pH 4.0). *a*) No La^{3+} added; b)–d) $[La^{3+}] = 3.2\cdot10^{-5}$ M, $3.7\cdot10^{-5}$ M, and $4\cdot10^{-5}$ M, respectively.

in the absorption spectra of the La^{3+}/murexide binary system *vs.* the total La^{3+} concentration. *Fig. 2* presents the spectra for the La^{3+}/murexide/glucuronic acid ternary system with constant La^{3+} and murexide concentrations and varying glucuronic-acid concentration.

The experimental data obtained in absence of uronic acid show satisfactory linear relationship between $C_{Ln}C_{mur}/D$ and $C_{Ln} + C_{mur} - (D/\varepsilon^0_{Ln\text{-}mur})$, where D denotes the absorbance of the Ln/murexide complex. Initial values of $\varepsilon^0_{Ln\text{-}mur}$ were assumed to be D_{max}/C_{mur}, (D_{max} being the maximum absorbance of the Ln/murexide complex

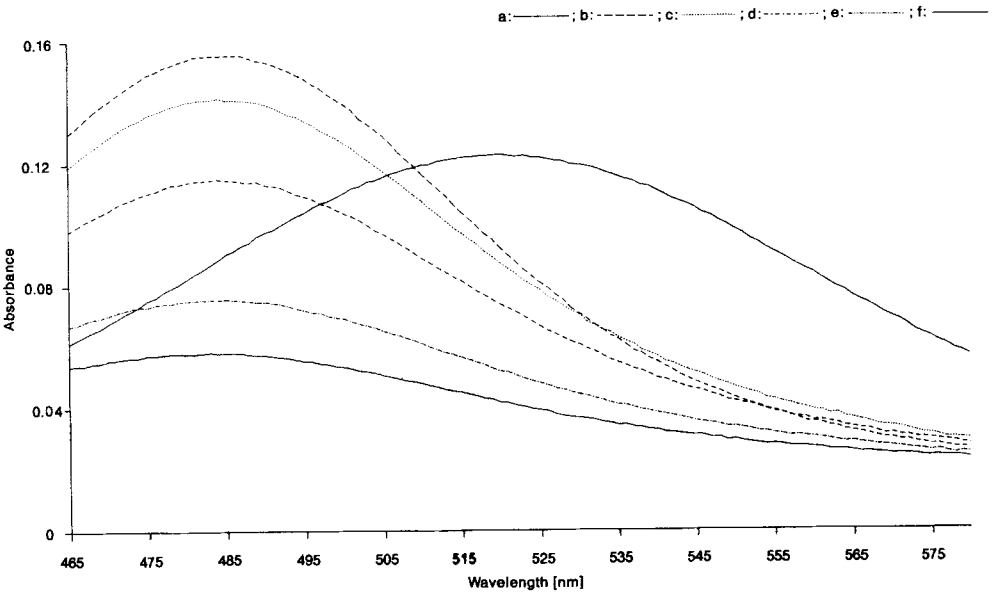


Fig. 2. *Spectral changes of solutions of the La/murexide complex in presence of uronic acid* (μ = 0.1M ($NaClO_4$), t = 27°, pH 4.0). a) [murexide] = $9 \cdot 10^{-6}$ M; b) [La^{3+}] = $5 \cdot 10^{-6}$ M; *c*)–*f*) [glucuronic acid] = $5 \cdot 10^{-4}$ M, $1.1 \cdot 10^{-3}$ M, $2.1 \cdot 10^{-3}$ M, and $2.7 \cdot 10^{-3}$ M, respectively.

in a solution with $C_{Ln} \gg C_{mur}$. The reciprocal of the slope of the line yields the corrected value of $\varepsilon_{Ln\text{-}mur}$ which was used in further calculations. The slope/intercept ratio, in turn, gave the K_{mur} value.

Calculations were performed on an *IBM-PC 486DX* computer using the commercial curve-fitting program PS-Plot (*Polysoft*) as well as some specially written subroutines. The quantitative results given below are, as a rule, averages of three measurements performed on independently prepared solutions.

Results and Discussion. – *Determination of the Stability Constants of Ln/Murexide Complexes.* The results obtained for the conditional stability constants of the studied Ln^{3+}/murexide 1:1 complexes are listed in *Table 1*. *Schwarzenbach* and *Gysling* have reported the murexide-acid dissociation constants to be equal to $pK_1 \approx 0$, $pK_2 = 9.2$, and $pK_3 = 10.9$ [17]. From these values, as well as from the linear relationship between

Table 1. *Conditional Stability Constants of 1:1 Ln/Murexide Complexes.* μ = 0.1M ($NaClO_4$), t = 27°, pH 4.0

Rare earth	[nm]	$\varepsilon_{Ln\text{-}mur}$ [1 mol^{-1} cm^{-1}]	log (K_{mur}) ± σ	log (K_{mur}) (literature values)
La	486	14,100 ± 500	3.85 ± 0.10	3.43[a]), 4.49[b])
Ce	482	13,230 ± 430	3.92 ± 0.04	3.65[a])
Pr	481	14,030 ± 680	4.22 ± 0.09	3.78[a])
Nd	478	15,600 ± 490	4.24 ± 0.05	4.04[a])
Gd	471	17,960 ± 530	4.26 ± 0.08	4.08[a]), 4.90[b])
Lu	463	19,220 ± 560	3.52 ± 0.03	3.45[a])

[a]) μ = 0.1M (KNO_3); t = 12°; pH 4.0 [21].
[b]) μ = 0.1M (KCl); t = 25°; pH 4.0; acetate buffer (0.1M) [22].

$C_{Ln}C_{mur}/D$ and $C_{Ln} + C_{mur}$, we conclude that only 1:1 lanthanide complexes are formed with murexide existing in the H_4R^- form. The values of the stability constants reported in the present paper are in good agreement with those of *Geier* [21], but are significantly lower than the values obtained by *Balaji et al.* [22]. The latter values, however, are corrected for metal-acetate buffer interactions. So, they should be accounted rather as the effective equilibrium constants in the lanthanide/acetate/murexide ternary system. The same comment holds for the data recently published by *Jain* and *Gupta-Bhaya*: $\log K(Eu) = 5.20$ at pH 5.0; $t = 25°$; $\mu = 0.1$M (KCl) [23]. We have not performed any correction, since we did not use buffer, and since ClO_4^- anions are non-complexing agents in aqueous solutions.

Determination of the Conditional Stability Constants of Ln/Uronic-Acid Complexes. In the ternary La^{3+}/murexide/uronic acid system the absorbance of the murexide complex, D, decreases with increasing concentration of the acid (*Fig. 2*). According to the *Ohyoshi* procedure [20], the conditional stability constants of La^{3+}/murexide complexes were calculated for each galacturonic and glucuronic acid concentration (C_{lig}). We have found that $1/K'_{mur}$ values increase with increasing acid concentration (*Fig. 3*). Subsequently, a model was fitted to these data in order to calculate the lanthanide – uronic acid cumulative stability constants. The estimation procedure applied to the experimental data was the least-squares minimalization technique with two independent kernel iteration algorithms: *Powell*'s [24] and *Marquardt-Levenburg* [25].

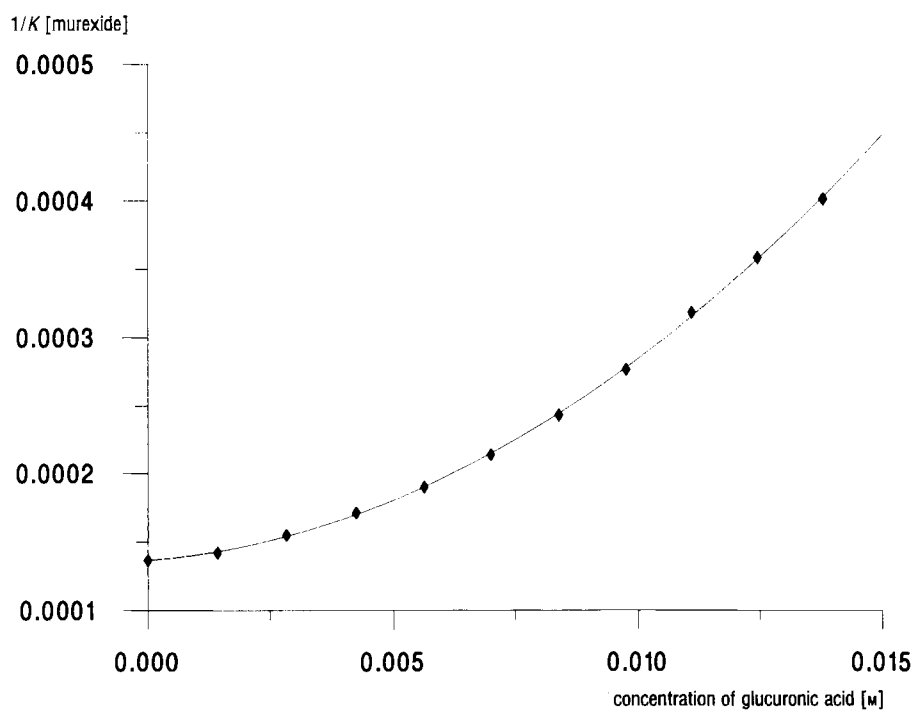


Fig. 3. *Plot of the reciprocal conditional stability constants for La/murexide complexation in solutions containing increasing concentration of glucuronic acid*

Table 2. *Testing Different Models for La^{3+} Complexation by Galacturonic and Glucuronic Acids.* $\mu = 0.1$ M ($NaClO_4$), $t = 27°$; pH 4.0.

$\log \beta_1$	$\log \beta_2$	$\log \beta_3$	Fit[a])
D-galacturonic acid			
1.99			2.7503
1.48	4.01		7.0390
1.55	3.77	5.19	6.8826
D-glucuronic acid			
2.07			2.7399
1.32	3.84		8.0476
1.56	3.21	5.48	5.5553

[a]) *Akaike* criteron for comparing the final least-squares fittings that two competing models produce for the same observed data set [27].

For La^{3+}, we tested three models assuming the existence of one ([LaL]), two ([LaL] and [LaL_2]) or three ([LaL], [LaL_2], and [LaL_3]) complexes in solution. Results of the overall stability-constant calculations are given in *Table 2*. The computer calculations suggest that in both investigated systems the model with two complexes fits best the experimental data. This conclusion differs seriously from the data published by *Makridou et al.* [26], who for both Eu^{3+}/glucuronic and Eu^{3+}/galacturonic acid systems take into account only the 1:1 complex formation. It is consistent, however, with the ^{13}C-NMR spectra analysis for the Yb^{3+}/glucuronate system. *De Bolster* and coworkers [28] have indicated that one lanthanide ion binds preferentially two glucuronic-acid molecules in a symmetrical way. On the other hand, similarly to what was reported by *Makridou et al.* [26], we find that D-galacturonic acid forms slightly stronger complexes than glucuronic acid. This fact is consistent with the trend in the free acid proton dissociation constants ($pK_1 = 3.17$–3.28 for D-galacturonic acid as compared to 2.95–3.06 for D-glucuronic acid [26] [29]) and seems to indicate that an 'acetate-like' complexation is preferentially realized. It means that the carboxylate group is the main coordinating site of both uronic acids.

The model with two complexes, [LnL] and [LnL_2], has been applied to all the investigated ternary systems (*Table 3*). For both ligands, β_1 increases with increasing atomic number up to neodymium, and then decreases with decreasing ionic radii. The stability constant β_2 is more constant along the series, although it follows the same trend as β_1. As

Table 3. *Stability Constants for Ln/Uronic-acid Complexes.* $\mu = 0.1$ M ($NaClO_4$); $t = 27°$; pH 4.0.

Lanthanide	Glucuronic acid		Galacturonic acid	
	$\log(\beta_1) \pm \sigma$	$\log(\beta_2) \pm \sigma$	$\log(\beta_1) \pm \sigma$	$\log(\beta_2) \pm \sigma$
La	1.32 ± 0.02	3.86 ● 0.07	1.41 ± 0.05	3.94 ± 0.07
Ce	1.51 ± 0.02	3.69 ± 0.06	1.48 ± 0.02	3.73 ± 0.07
Pr	1.52 ± 0.03	3.92 ± 0.08	1.63 ± 0.06	3.94 ± 0.07
Nd	1.56 ± 0.04	3.61 ± 0.06	1.83 ± 0.07	3.95 ± 0.07
Gd	1.47 ± 0.03	3.66 ± 0.03	1.54 ● 0.02	3.52 ± 0.02
Lu	1.13 ± 0.01	3.68 ± 0.05	1.39 ± 0.05	3.61 ± 0.04

far as the variation of the complex stability constants along the lanthanide series is concerned, the uronic-acid systems show a striking similarity with the acetates, meaning that the complexation *via* the COO^- group plays a major role. With respect to both β_1 and β_2 values, the rare-earth series can be divided into three subgroups [30]: for the light as well as for the heavy rare-earths (La–Sm and Er–Lu, respectively), stability of the complexes increases slightly with atomic number. Furthermore, the stability constants for heavy rare-earths (in our case for Lu) are smaller than those for lighter lanthanides. Finally, the Eu–Ho series forms an intermediate class, for which the stability constants decrease along the series.

These complicated trends reflect the interplay between the electrostatic and the steric effects which both increase along the series. Regarding the coordination mode of the ligand in the 1:1 complex, we can assess that apart the COOH group, the ring C(5)–*O*-atom is involved in binding. This has been demonstrated on the basis of ^{13}C-NMR spectra analysis for D-galacturonic acid by *Izumi* [31] and for D-glucuronic acid by *de Bolster* and coworkers [28]. The second dissociation step of these uronic acids in acidic solutions should have a negligible effect on the equilibria. Indeed, even in strong alkaline solutions pK_2 values are found to be as small as 12.24 ± 0.03 [26] or *ca.* 12.5 [29] for D-galacturonic acid and 12.04 ± 0.03 [26] for D-glucuronic acid. Such small values of pK_2 preclude the existence of $H_{-1}L^{2-}$ species, followed by the complexation *via* the C(4)–*O*-atom. Never-

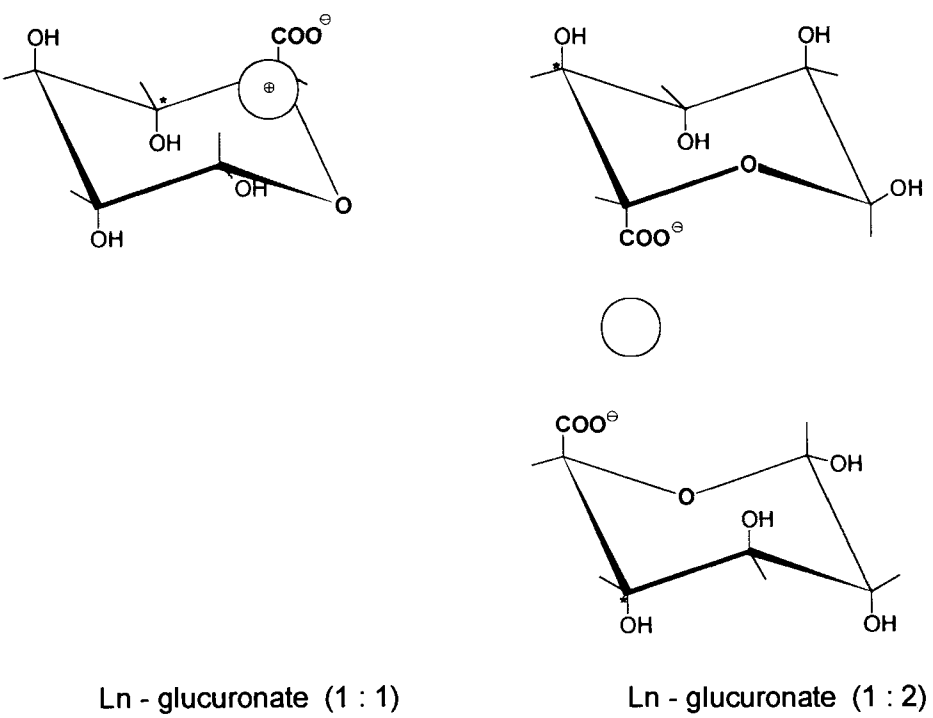


Fig. 4. *Structures proposed for the [LnL] and [LnL$_2$] species of the lanthanide/*D*-glucuronic-acid system*

theless, analyzing the effect of adding Eu^{3+} ions to glucuronate or galacturonate solutions on the chemical shift of the ^{1}H-NMR spectra, *Anthonsen et al.* have suggested that the complexation by the COOH group and the ring C(5)–*O*-atom is partially supplemented by interaction with the C(4)–*OH* group [32] [33]. If so, the equatorial C(4)–*O*-atom of D-galacturonic acid is more suitable for the complexation, contrary to the axial OH group in D-glucuronic acid (*cf.* left part of *Fig. 4*).

For 1:2 complexes, a ^{13}C-NMR analysis of the Yb^{3+}/D-glucuronate system point to the COO^- group being the only one involved in coordination [28] (*cf.* right part of *Fig. 4*). If so, taking into account the lanthanide contraction, the coordination requirements of the Ln^{3+} ions can be met more easily for D-glucuronic acid, for which steric hindrance of the axial C(4)–*OH* group may be smaller than that of equatorial groups in D-galacturonic acid. It results in the observed inversion of the pattern of the β_2 values for the small Gd^{3+} and Lu^{3+} ions complexed by galacturonic and glucuronic acids, respectively.

Conclusions. – The trends observed in the $\log(\beta_1)$ and $\log(\beta_2)$ values, together with the previously published NMR data [28] lead us to formulate the following conclusions on the structure of the complexes between lanthanide ions and simple uronic acids: *a*) the strong intermolecular sugar H-bonding network is rearranged upon the complex formation. *b*) The differences in stability between D-galacturonate and D-glucuronate complexes, and within in Ln series, can be explained in terms of the steric hindrance of the C(4)–*OH* groups. *c*) The binding in 1:1 complexes results from the interaction by both the C(6), C(6′) and the ring C(5)–*O*-atoms. It is complemented to a certain degree by an interaction with the C(4)–*OH* group. The 1:2 complexes are formed by two fully ionized acid anions bonded through the C(6) and C(6′) carboxylic O-atoms only.

The data reported in this work demonstrate that in spite of the fact that pectin or alginic acid induce an increased heavy metal excretion, simple uronic acids (*i.e.* D-glucuronic and D-galacturonic acids) do not form reasonably stable complexes with lanthanide ions. As such, they cannot be used for the treatment of heavy-metal intoxication and our investigation will turn to similar compounds having potentially a stronger coordination ability.

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