Proton and Zinc(II) Complexes of 2-(Polyhydroxyalkyl)thiazolidine-4-carboxylic acid Derivatives

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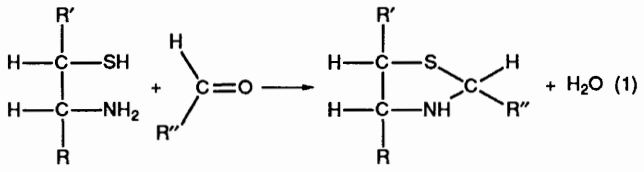
The protonation and zinc-ion co-ordination equilibria of 2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acid derivatives have been studied by potentiometric titration in the range pH 1.5—8. In most cases the formation of complexes with a metal-to-ligand ratio of 1 : 2 was demonstrated, but at above pH 6 mixed-ligand complexes involving hydroxide-ion co-ordination were also observed. The protonation and complex-formation constants were shown to depend on the structure of the polyhydroxy chains. In the case of the protonation constants this is due to the rearrangement of the intramolecular hydrogen-bonding network, while the complex-formation constants depend on the conformation of the OH groups on the first carbon atoms of the polyol chains.

The reactions of aldehyde compounds with β-aminothiols, and in particular cysteine, lead to the formation of a thiazolidine ring [equation (1)]. Such reactions have been the subject of



numerous previous studies[1] because of their relevance to the binding of carbonyl compounds to proteins containing sulphydryl and amino groups in close proximity.[2] The condensation of sulphydryl-containing amino acids with naturally occurring monosaccharides[3–6] takes place under mild conditions. Lote and co-workers isolated a similar type of oligopeptide from human urine[7] and the erythrocyte membrane.[8] In these oligopeptides, galactose[7] and glucose[8] are linked to cysteine as the N-terminal amino acid. Gosálvez and co-workers reported that thiazolidine-4-carboxylic acid is capable of inducing reverse transformation in tumour cells,[9] and this compound was selected for chelation of a metal from a protein complex in the plasma membrane.[10–12] It should be noted that such compounds do not poison cells. Fazakerley *et al.*[13] have studied thiaproline–transition metal(II) ion systems. Recently, 2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acids were tested as protective agents against acetaminophen (*N*-4-hydroxyphenylacetamide)-induced hepatoxicity in a mouse model.[14] The results discussed above suggest that a study of the complex formation of these compounds is warranted. The complex formation of six such derivatives was studied by Weitzel *et al.*,[6] but only in the range pH 2—6. They did not find any correlation between the protonation and complex-formation constants and the structure of the polyol chain.

The present paper reports a protonation equilibrium study of ten 2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acid derivatives and of 2-propylthiazolidine-4-carboxylic acid. The co-ordination of these molecules by Zn^{2+} was also investigated by pH-metry in the range pH 2—8.

Experimental

Materials.—2-Deoxy-D-glucose and zinc(II) perchlorate were Fluka products; all other reagents were Reanal products. The $Zn(ClO_4)_2$ stock solution was standardized complexometrically.

Synthesis.—In the cases of pentoses, (D-lyxose, D-xylose, D-arabinose, D-ribose, L-arabinose, L-rhamnose, and D-deoxyglucose) the ligands were prepared by the method of Bognár et al.[3] as follows. Generally equimolar mixtures of monosaccharides and cysteine hydrochloride were allowed to react in water (20—40 cm^3) in the presence of pyridine. The mixtures were allowed to stand for approximately 12 h, and ethanol (200 cm^3) was then added. The resulting precipitate was filtered off, recrystallized several times from a water–ethanol mixture, and dried in a vacuum desiccator. In the cases of hexoses, (D-mannose, D-glucose, and D-galactose) the ligands were prepared according to Weitzel *et al.*[6] The latter procedure is similar to the method presented above, but methanol was used as solvent instead of water, and the mixture was refluxed for 1—2 h. After the recrystallizations (at neutral pH), racemic S(*S*) and 2(*R*) mixtures were formed in a 1:1 ratio, as revealed by our n.m.r. investigations and also reported previously,[15] but the proportion of the 2(*S*) and 2(*R*) compounds was shown[16] to depend on the pH of the solution. The analytical data for the compounds obtained are given in Table 1. The structure of the ligands are depicted in Figure 1.

pH-*Metric Measurements.*—Both the protonation and zinc(II) co-ordination equilibria were investigated by potentiometric titration at 25.0 ± 0.1 °C, under a nitrogen atmosphere, in aqueous solutions of constant ionic strength (0.1 mol dm^{-3} $NaClO_4$). Changes in pH were followed by using a G222B Radiometer glass electrode and an OP-2801 Radelkis silver–silver chloride reference electrode.

The titrations were performed with a computer-controlled on-line automatic titration apparatus constructed in our laboratory.[17] For the quantitative evaluation of the data, the correlation (2) was used between the experimental e.m.f. values

$$E = E_0 + \frac{RT}{F}\log[H^+] + j_H[H^+] + j_{OH}[H^+]^{-1}K_w \quad (2)$$

(E) and the equilibrium hydrogen-ion concentrations $[H^+]$, where j_H and j_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction and to possible alkaline and acidic errors of the glass electrode; K_w is the autoprotolysis constant of water, $10^{-13.75}$.[18] The protonation constants were determined from the

Table 1. Analytical results for the ligands, with calculated values in parentheses

Ligand	Analysis (%) C	H	N	M*
L^1	37.8	6.05	5.40	253.0
	(37.9)	(5.90)	(5.50)	(253.3)
L^2	35.6	6.50	4.60	302.0
	(35.9)	(6.30)	(4.65)	(301.3)
L^3	37.8	6.15	5.35	250.7
	(37.9)	(5.90)	(5.50)	(253.3)
L^4	38.3	5.90	4.85	280.6
	(38.1)	(6.00)	(4.95)	(283.3)
L^5	37.7	6.10	5.35	253.4
	(37.9)	(5.90)	(5.50)	(253.3)
L^6	37.7	6.00	5.40	260.2
	(37.9)	(5.90)	(5.50)	(263.3)
L^7	37.7	5.95	5.40	260.8
	(37.9)	(5.90)	(5.50)	(263.3)
L^8	35.6	6.45	4.60	303.2
	(35.9)	(6.30)	(4.65)	(301.3)
L^9	40.3	6.60	5.10	267.0
	(40.3)	(6.70)	(5.20)	(268.3)
L^{10}	40.2	6.50	5.10	267.9
	(40.3)	(6.70)	(5.20)	(268.3)
L^{11}	47.0	7.40	8.20	173.5
	(48.0)	(7.50)	(8.00)	(175.3)

* From acid–base titration.

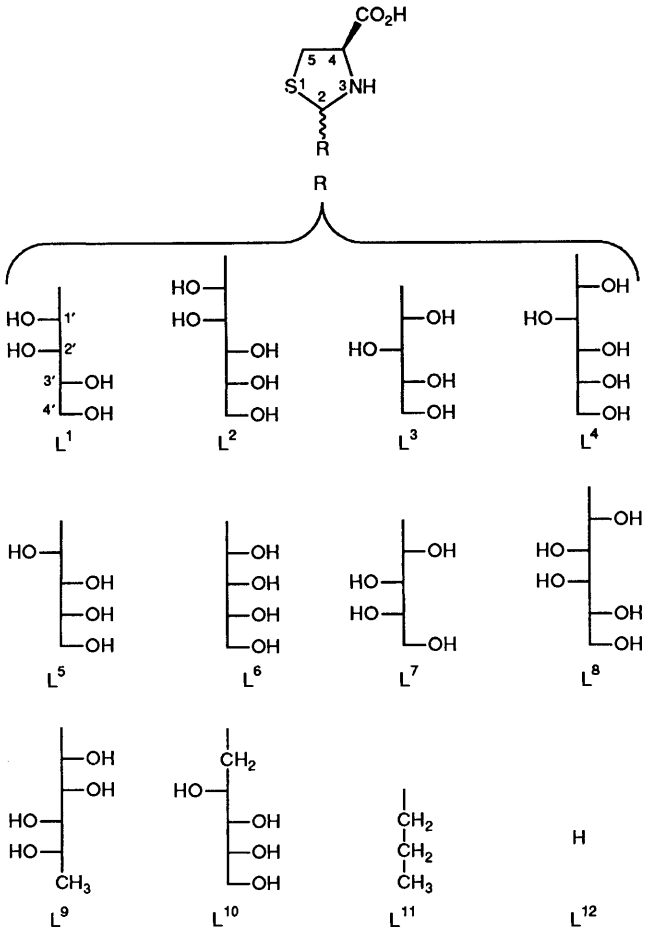


Figure 1. Schematic structure and numbering of ligands studied

data from six independent titrations. In the zinc complex-formation studies the titrations were carried out in systems involving six different metal-to-ligand ratios, varying from 1:5 to 1:15. For L^2 and L^5, eight independent measurements were made at metal-to-ligand ratios from 1:2 to 1:15.

Calorimetric Measurements.—Calorimetric measurements were carried out with an LKB-2107 batch calorimeter. For the determination of proton dissociation heats, three measurements were carried out. The dilution heat of NaOH was determined in separate experiments in 0.1 mol dm^{-3} $NaClO_4$. For the ionization heat of water (0.1 mol dm^{-3} KCl) a value of −54.9 kJ mol^{-1} was used.[19]

N.M.R. Measurements.—Proton and ^{13}C n.m.r. spectra were recorded on a Varian VFT-100 spectrometer at room temperature, with acetonitrile in D_2O as internal standard.

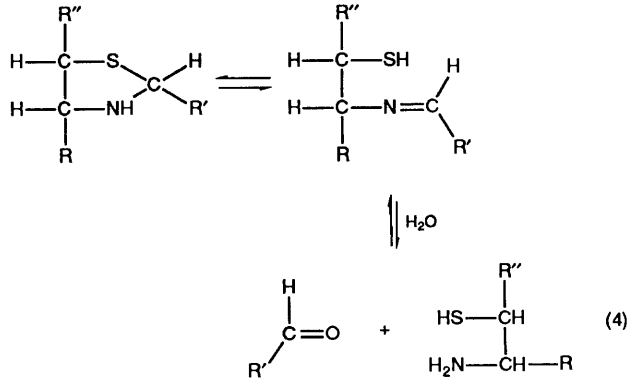
Calculations.—The species of various compositions formed in the systems studied can be characterized by the general equilibrium process (3) (charges omitted). The formation

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \qquad (3)$$

constant for this generalized reaction is β_{pqr}. The protonation constants and the zinc(II) complex-formation constants defined by equation (3) were evaluated from the pH-metric titration data with the PSEQUAD computer program.[20]

Results and Discussion

Several earlier results[2,21] have shown that the thiazolidine ring is in equilibrium with an acyclic thiol form or, in aqueous media, with the β-aminothiol and carbonyl compound from which it is formed [equation (4)].



The state of the equilibrium strongly depends on the chemical nature of the substituent at position C(2) of the ring. The acyclic form is the favoured structure when the double bond is conjugated with aromatic or unsaturated substituents (R'). In our case, when the carbonyl compounds are aldose monosaccharides, the thiazolidine rings are stable and do not open under mild conditions.[6] The present ^1H and ^{13}C n.m.r. results confirmed this. The acyclic form gives a characteristic ^1H n.m.r. signal, due to the hydrogen on the C=N double bond. Decomposition into two molecules also results in characteristic ^{13}C n.m.r. signals, *e.g.* that assigned to the carbonyl group of aldose. In the pH range applied in this study, the latter signals did not appear in the n.m.r. spectra of the ligands, either in the presence or in the absence of zinc(II) ions. Consequently, the thiazolidine rings do not open upon complex formation. The

Table 2. Protonation constants of ligands (log values)

	Ligand	This work β_{011}	This work β_{012}	Literature data β_{011}	Literature data β_{012}	Literature data β_{013}	Ref.
Group I	L^1	5.10 ± 0.02	6.56 ± 0.05				
	L^2	5.14 ± 0.02	6.64 ± 0.06	5.23	7.38		6
Group II	L^3	5.32 ± 0.02	6.78 ± 0.05	5.43	7.66		6
	L^4	5.31 ± 0.02	6.77 ± 0.06	5.37	7.52		6
	L^5	5.37 ± 0.02	6.84 ± 0.05				
	L^6	5.43 ± 0.03	6.94 ± 0.05	5.51	7.57		6
Group III	L^7	5.50 ± 0.02	6.93 ± 0.06	5.66	7.92		6
	L^8	5.53 ± 0.02	6.96 ± 0.05	5.67	7.85		6
	L^9	5.51 ± 0.02	6.99 ± 0.06				
Group IV	L^{10}	5.53 ± 0.02	7.08 ± 0.06				
	L^{11}	6.25 ± 0.02	7.84 ± 0.05				
	L^{12}			6.109	7.616[a]		13
				6.104	7.829[b]		10
	Cysteine			10.29	18.445	20.36	22*b*
	Glycine			9.56	11.92		22*b*

[a] 298 K. [b] 310 K.

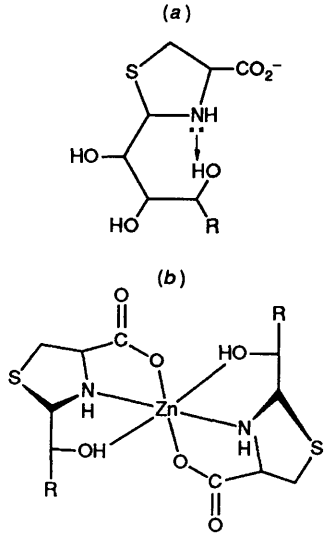


Figure 2. (*a*) Structure of hydrogen-bonded ring. (*b*) Suggested structure of zinc(II) complexes

stability of the ring in the absence of metal ions was recently confirmed by Radomski and Temeriusz.[15]

Protonation Constants.—The pH-metrically determined protonation constants of the ligands are listed in Table 2 together with some literature data; log β_{011} is assigned to protonation of the amino group (log β_{011} = 5.1—5.5), and log $\beta_{012} \approx 6.8$ (log $K_{CO_2H} \approx 1.5$) to that of the carboxylate. The log K_{CO_2H} values agree within the experimental error. The differences in log β_{012} values determined by us and 30 years ago[6] are probably due to acidity errors in the latter measurements.

A comparison of the protonation constants obtained for cysteine or other amino acids with the constants measured here demonstrated that the thiazolidine-4-carboxylic acid derivatives display a very weak basic character. This may be attributed to the inductive effect of the sulphur atom, which is separated from the amino group, by one carbon atom in the present ligands and by two carbon atoms in cysteine. Similarly, the sulphur atom in the thiazolidine ring gives rise to an increased acidity of the carboxyl group. The same conclusion has been made for the protonation behaviour of thiaproline.[13]

On the other hand, a comparison of the log β_{011} values for compounds L^1—L^{10} with those for L^{11} and L^{12} reveals that the inductive effects of the polyhydroxy chains further decrease the basicity of the secondary nitrogens. However, this effect does not influence the acidity of the carboxylate groups.

The protonation constants of the amino groups in compounds L^1—L^{10}, containing a polyol chain, exhibit small, but significant differences. On the basis of these data the ligands can be classified into four groups.

It is obvious that the stability differences are due to the differences in the structures of the polyalcohol moiety of the ligand. From a comparison of the log β_{011} values of compounds having the same OH group configurations in positions C(1′), C(2′), and C(3′) (*e.g.* L^1 and L^2, L^3 and L^4, L^7 and L^8, respectively) (Figure 1), it can be seen that the corresponding values agree with each other very well (Table 2). This is probably due to the nature of the intramolecular hydrogen bonding between the amino group and the OH group on C(3′). In this way a six-membered hydrogen-bonded chelate ring can form [Figure 2(*a*)].

For compounds L^1 and L^2 (group I) the hydrogen-bonded chelate rings form without hindrance, and therefore these ligands have the lowest β_{011} values. For compounds L^3—L^6 (group II), the configuration of the OH group on C(1′) or C(2′) is sterically not favourable for formation of the hydrogen-bonded chelate ring. Consequently, the log β_{011} values here are higher than those in the previous group. In the case of group III the configuration of the OH group on C(3′) does not permit the formation of hydrogen bonds with the amino group at all, resulting in the largest of the log β_{011} values for compounds L^1—L^9. In the case of compound L^{10}, which has no OH group on C(1′), the log β_{011} value is large because of the smaller inductive effect of the polyhydroxy chain, but it is closer to the log β_{011} values for compounds L^1—L^9 than to that for compound L^{12}. This fact supports the assumption of the effect of the hydrogen-bonded ring. The log β_{011} value for compound L^{11}, which is slightly larger than that for compound L^{12}, reveals the steric inhibition and the electron-donating effect of the alkyl chain in the former compound.

Calorimetric measurements on the protonation of the amino groups have been performed to verify the existence of the

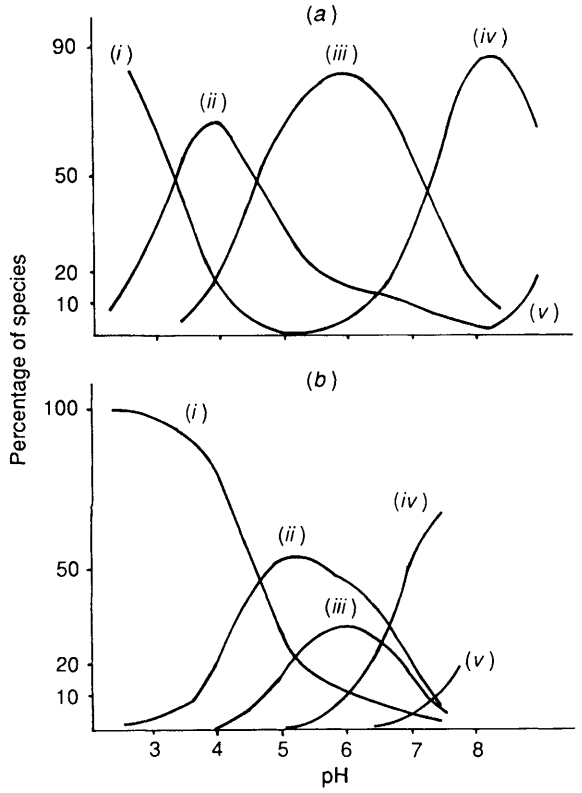


Figure 3. Percentage distributions of total zinc(II) concentration in different complexes plotted as a function of pH. $[Zn^{II}] = 1.4 \times 10^{-3}$ mol dm^{-3}. (*a*) Zn–L^7, $[L^7] = 1.5 \times 10^{-2}$ mol dm^{-3}; (*b*) Zn–L^5, $[L^5] = 1.48 \times 10^{-2}$ mol dm^{-3}. Curve: (*i*) Zn^{2+}, (*ii*) $[ZnL]^+$, (*iii*) $[ZnL_2]$, (*iv*) $[ZnL_2(OH)]^-$, and (*v*) $[ZnL_2(OH)_2]^{2-}$

Table 3. Thermodynamic data relating to the first protonation processes of the ligands

Ligand	log β_{011}	ΔG^0/ kJ mol^{-1}	ΔH^0/ kJ mol^{-1}	ΔS^0/J K^{-1} mol^{-1}
L^1 (Group I)	5.10	−29.14	−20.0 ± 0.4	31.1 ± 1.2
L^5 (Group II)	5.37	−30.65	−25.2 ± 0.5	18.7 ± 1.6
L^7 (Group III)	5.50	−31.41	−27.8 ± 0.4	12.4 ± 1.4
L^9 (Group III)	5.51	−31.49	−27.5 ± 0.5	13.6 ± 1.6
L^{10} (Group IV)	5.53	−31.53	−32.3 ± 0.5	−2.3 ± 2.0

hydrogen-bonded chelate ring in some members of the above four groups. The thermodynamic quantities determined are given in Table 3.

The hydrogen-bonded chelate rings open during protonation of the ligands studied. Consequently, for a stable hydrogen-bonded ring the energy liberated during protonation is smaller than for a less-stable one. On the other hand, the protonated species have nearly identical structures. Before protonation, the ligand with the most stable hydrogen chelate ring has the smallest entropy content. Therefore, the entropy change will be the greatest in this case. Accordingly, the smallest ΔH^0 and the largest ΔS^0 values were found for compound L^1 (Table 3), and the largest ΔH^0 and the smallest ΔS^0 values for compounds L^7 and L^8. The ΔH^0 and ΔS^0 values for compound L^{10} are different from the previous ones, because of the different structure and basicity.

Formation Constants of Zinc(II) *Complexes.*—In every system investigated, except Zn–L^{10}, the $\bar{n}$ *vs.* pH formation curves, where $\bar{n}$ is the average number of protons released by one metal ion in the complex-formation process, did not show a zinc concentration dependence, which indicates that only mononuclear complexes were formed under the conditions applied. The data indicate that the complex-formation reaction is not complete until pH 6 (Weitzel *et al.*[6] measured the same system only up to this point) and further complexes are formed in the interval pH 6—8. No precipitation occurs in the pH range studied; the complexes are water-soluble. The observed formation curves may be due to the simultaneous formation of mononuclear parent and mixed-ligand complexes (hydroxide ion co-ordination) in the system, but further deprotonation of the ligand should also be considered.

Earlier studies of the co-ordination of thiaproline with bivalent transition-metal ions have shown that the co-ordination centres are the amino N and carboxyl O atoms.[10–13] It is obvious that analogous co-ordination occurs in the present case.

Our equilibrium studies revealed that in slightly acidic solution ligands L^1—L^9 form 1:1 and 1:2 parent complexes, whereas ligands L^{10} and L^{11} yield only 1:1 complexes. The overall formation constants (log β values) of the zinc(II) mono and bis parent and mixed-ligand complexes, together with the corresponding stepwise stability constants (log K_1 and log K_2) of the mononuclear parent complexes (ZnL^+ and ZnL_2), are presented in Table 4.

The log *K* values for the zinc(II) complexes differ from each other significantly and show no relationship with the protonation values log β_{011}. Consequently, the OH group on C(3′) has no influence on the magnitude of the stability of the zinc(II) complexes. On the basis of their equilibrium constants, the complexes can be divided into four groups (Table 4), which is of help in the explanation of the stability differences in terms of the different structures of the polyhydroxy chain linked to the thiazolidine ring. With regard to the structure of the sugar moiety in the compounds and the magnitude of the stability constants measured, it is obvious that the conformation of the OH group on C(1′) has a great influence on the stability. It seems that both the carboxylate and amino groups of the ligands are co-ordinated to the zinc(II) ion, forming a five-membered chelate ring.[12] When the OH group on C(1′) is *cis* relative to the amino group, the former can also be bound to the zinc(II) ion. In this way, two different five-membered chelate rings can form [Figure 2(*b*)], resulting in an enhancement of the stability of the complexes. This phenomenon occurs for the ligands in group A. The ligands in group C have a C(1′) OH group *trans* to the amino group, and this is too far from the central zinc(II) atom (already bound by the amino and carboxylato groups) to be co-ordinated. Formation of the second chelate ring is therefore not possible. This is the reason for the lower stability of the zinc(II) complexes in group C than in group A. Finally, in group B, the OH groups on C(2′) and C(3′) sterically hinder the formation of two five-membered chelate rings, and therefore the stability constants in this group are between those for groups A and C.

A comparison of the log *K* values for zinc(II)–thiaproline (L^{12}) with the corresponding values for the complexes formed with ligands L^1—L^9 likewise confirms the existence of the two five-membered chelate rings discussed above. Both the log K_1 and log K_2 values for the group A complexes are larger than the corresponding values for the zinc(II)–thiaproline system, which is probably due to the double chelate effect in the former systems. For group B, the log K_1 values are slightly larger than 3.1 (Table 4) [the first stability constant of zinc(II)–thiaproline] indicating that the first ligand in the group B complexes is co-ordinated to the zinc(II) in a tridentate manner. Finally, the compounds in group C cannot bind in a tridentate manner, and the steric effect of the long polyol chain decreases the stability of the zinc complex. Figure 1 shows that compounds L^{10} and L^{11}

Table 4. Formation constants (log values) for complexes $(Zn^{2+})_p(ligand)_q(proton)_r$

	Ligand	β_{110}	β_{120}	β_{12-1} (log K_3)	β_{12-2} (log K_4)	log K_1	log K_2	n
Group A	L^5	4.19 ± 0.06	7.13 ± 0.10	−0.18 ± 0.16 (6.44)	−9.28 ± 0.21 (4.64)	4.19 4.10[a]	2.94 2.5[a]	330
	L^3	4.05 ± 0.05	6.86 ± 0.11	−0.44 ± 0.15 (6.45)	−9.31 ± 0.22 (4.88)	4.05 3.80[a]	2.81 2.14[a]	340
	L^8	4.01 ± 0.05	6.93 ± 0.10	−0.94 ± 0.16 (5.88)	−9.20 ± 0.24 (5.49)	4.01 3.90[a]	2.92 2.10[a]	320
	L^4	3.75 ± 0.06	6.51 ± 0.12	−0.95 ± 0.17 (6.29)	−8.24 ± 0.25 (5.86)	3.71	2.76	300
Group B	L^6	3.45 ± 0.07	6.1 ± 0.13	−1.23 ± 0.17 (6.42)	−9.67 ± 0.26 (5.31)	3.45 3.40[a]	2.65 2.25[a]	280
	L^9	3.23 ± 0.07	5.57 ± 0.14	−1.9 ± 0.18 (6.28)	−9.93 ± 0.28 (5.72)	3.23	2.34	290
Group C	L^5	2.9 ± 0.08	5.02 ± 0.15	−1.46 ± 0.18 (7.28)	−9.27 ± 0.26 (5.93)	2.9	2.12	320
	L^1	2.9 ± 0.08	4.9 ± 0.14	−1.44 ± 0.19 (7.41)	−9.44 ± 0.25 (5.75)	2.9	2.00	280
	L^2	2.69 ± 0.09	4.98 ± 0.16	−2.14 ± 0.20 (6.83)	−9.99 ± 0.28 (5.7)	2.9 2.67[a]	2.29 2.30[a]	280
		β_{110}	β_{11-1} (log K_2)	β_{11-2} (log K_3)	β_{22-5}	log K_1	n	
Group D	L^{10}	2.81 ± 0.10	−3.72 ± 0.18 (7.22)	−11.07 ± 0.25 (6.4)	−27.00 ± 0.3	2.81	270	
	L^{11}	3.06 ± 0.08	−2.40 ± 0.17 (8.29)	−9.28 ± 0.23 (6.87)		3.06	250	
	L^{12}	3.103[b]	5.629[b]					

n = The number of experimental points.

[a] Ref. 6. [b] Ref. 13.

(group D) have no OH group on C(1′). The log K_1 value for the Zn–L^{10} complex (2.81) agrees well with the log K_1 values for the group C compounds.

Various models have been used for evaluation of the equilibrium data on Zn–L^{10} from potentiometric titration curves. The assumption of the species given in Table 4 gave the best agreement between the experimental and calculated values of the titration curves. Consequently, our results show that at higher pH a dimer complex is also formed, with composition $[Zn_2L_2(OH)_5]^{3-}$. A hydroxy-bridged zinc(II)-containing dimer has been observed among the hydrolysis products of zinc(II).[22]

For ligand L^{11}, besides the mixed-ligand complexes, only one 1:1 parent complex is formed, in contrast with the 1:1 and 1:2 parent complexes observed in the zinc(II)–thiaproline system. This phenomenon is probably due to the steric effect of the hydrophobic propyl chain.

It has already been pointed out that the zinc(II) ion is co-ordinated by two thiazolidine-4-carboxylate ligands in a bidentate fashion, through the amino N and carboxyl O atoms, forming a five-membered chelated ring. In the crystals of the zinc(II) complex prepared from aqueous solution the octahedral co-ordination is completed by two water molecules in *cis* positions.[12]

In contrast, the complexes precipitated from methanolic solution were found by extended *X*-ray absorption fine structure (EXAFS) measurements[23] to be four-co-ordinated (the Zn–L^9 system was an exception). The Zn–O,N bond distances are 204 pm, in good agreement with those observed for four-co-ordinated complexes.[12] The average Zn···C and Zn···S distances are 293 and 390 pm, respectively. The carbon atoms are located in the second, and the sulphur atoms in the third, co-ordination shells. Under neutral and slightly acidic solutions the OH groups on C(1′) of the sugar moiety could also be co-ordinated to a smaller or larger extent to the central zinc(II) ion, forming an elongated octahedral structure. The formation curves calculated from measurements on slightly alkaline solutions can be explained on the following basis: (1) deprotonation of the OH group on C(1′) of the polyol; (2) the substitution of the sugar OH group by a hydroxide ion, resulting in the formation of mixed-ligand complexes.

The carbohydrates and their derivatives are very weak acids; the first protonation constants lie in the range log K = 12—14.[24] In the presence of metal ions, sugars become more acidic. For instance, ribose protons are ordinarily neutralized at around pH ≈ 12—14, but on chelation with copper(II) their deprotonations shifted by 2 pH units.[25] Our results indicate that the formation of new species starts around pH 5—7, probably by the second mechanism discussed above. The five-membered chelate ring containing the alcoholic OH group opens, due to attack by OH^- ions, and one or (at higher pH) two OH^- ions co-ordinate to the central zinc(II) atom, completing the octahedral co-ordination sphere. The energy required by this substitution reaction depends on the bonding energy of the OH groups of the polyols. The distribution curves show that the formation of mixed-ligand complexes of compounds in group A and B starts at higher pH than for groups C and D. In the former case, the OH on C(1′) are strongly co-ordinated to the central zinc(II) ion. Consequently, a higher OH^- concentration is needed for substitution of the OH group than in the latter case, where the formation of mixed-ligand complexes starts at lower pH values [Figures 3(*a*) and (*b*), curves (*iv*) and (*v*)] according to equations (5) and (6). The

$$[ZnL_2] + OH^- \overset{K_3}{\rightleftharpoons} [ZnL_2(OH)]^- \quad (5)$$

$$[ZnL_2(OH)]^- + OH^- \overset{K_4}{\rightleftharpoons} [ZnL_2(OH)_2]^{2-} \quad (6)$$

complexes [ZnL(OH)] appear only at very low ligand excess (Zn:L = 1:2 or 1:3). The formation of such complexes was demonstrated for compounds L^2 and L^4, with stability constants of log $\beta_{11-1} = -4.90 \pm 0.07$ and -3.21 ± 0.06, respectively.

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