Equilibrium and Spectroscopic Studies of Transition Metal Complexes of Sugar-containing Thiazolidine Derivatives

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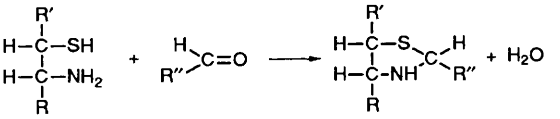
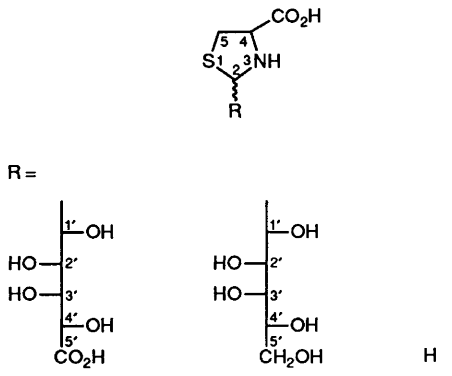
Transition-metal co-ordination equilibria of 2(*RS*)-D-galactothiazolidine-4(*R*)-carboxylic acid were studied by potentiometric titrations in the range pH 1.5–8.5. In most cases, (1,1,1), (1,2,0) and (1,2,0) parent complexes $M_pL_qH_r$ were formed, but in the cases of oxovanadium(IV), manganese(II) and zinc(II) at >pH 6 mixed-ligand complexes involving hydroxide-ion co-ordination or further deprotonation of alcoholic hydroxy groups were also observed. EPR measurements indicated that the manganese(II) complex is dimeric, while that of VO^{2+} is monomeric.

In recent years, research concerning the synthesis of glycopeptides has been directed towards the development of highly receptor-selective and specific derivatives for clinical use. The reactions of aldehydes with β-aminothiols, and in particular with cysteine, leads 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 sulfydryl and amino groups in close proximity.[2] The condensation of sulfydryl-containing amino acids with naturally occurring monosaccharides takes place under mild conditions.[3–6] Gosálvez and co-workers[7] reported that 1,3-thiazolidine-4-carboxylic acid is capable of inducing reverse transformation in tumour cells, and this compound was selected for chelation of a metal from a protein complex in the plasma membrane.[8–10] Fazakerley *et al.*[11] have studied 1,3-thiazolidine-4-carboxylic acid–transition metal(II) systems. The 2-(polyhydroxyalkyl)-thiazolidine-4-carboxylic acids were tested as protective agents against acetaminophen (*N*-4-hydroxyphenylacetamide)-induced hepatoxicity in a mouse model.[12] The complex formation behaviour 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. Recently, we studied the proton and zinc(II) complexes of a large number of such compounds.[13] It was found that both protonation and complex-formation constants depend on the structure of the sugar moiety. 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.



(1)

The present paper reports on equilibrium and spectroscopic studies of transition metal(II) and calcium(II) complexes of 2(*RS*)-D-galactothiazolidine-4(*R*)-carboxylic acid, H_2L^1 (galacto = 5-carboxy-1,2,3,4-tetrahydroxypentyl). Some of the results were compared with those obtained for the corresponding 2(*RS*)-1,2,3,4,5-pentahydroxypentylthiazolidine-4(*R*)-carboxylic acid (HL^2) and 1,3-thiazolidine-4-carboxylic acid (HL^3) systems.



H_2L^1 HL^2 HL^3

Experimental

Materials.—The metal perchlorates and D-galacturonic acid were Fluka products; all other reagents were obtained from Reanal. The metal-ion stock solutions were standardized complexometrically and gravimetrically.

Synthesis.—The ligands were prepared according to published methods[3,13] (Found: C, 36.25; H, 6.65; N, 4.70. Calc. for H_2L^1: C, 36.40; H, 6.60; N, 4.80%. Found: C, 35.60; H, 6.45; N, 4.60. Calc. for HL^2: C, 35.90; H, 6.30; N, 4.65%).

pH-*Metric Measurements.*—The 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.[14] For the quantitative evaluation of the data, the correlation (2) was used between the experimental electromotive

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

force values (E) and the equilibrium hydrogen-ion concentrations $[H^+]$, where j_H and j_{OH} are fitting parameters in acidic

Table 1 Protonation and complex-formation constants (log values) for complexes $(M^{2+})_p(\text{ligand})_q(\text{proton})_r$. The standard deviation is given in parentheses

	H_2L^1							HL^2	
β_{pqr}	H^+	Ca^{II}	VO^{2+}	Mn^{II}	Co^{II}	Ni^{II}	Zn^{II}	Zn^{II}	Mn^{II}
β_{011}	5.67 (0.02)	—	—	—	—	—	—	—	—
β_{012}	9.06 (0.05)	—	—	—	—	—	—	—	—
β_{013}	10.56 (0.07)	—	—	—	—	—	—	—	—
β_{111}	—	—	8.33 (0.10)	—	7.12 (0.10)	8.70 (0.06)	7.32 (0.10)	—	—
β_{110}	—	1.61 (0.10)	5.73 (0.05)	2.27 (0.06)	4.10 (0.07)	5.41 (0.07)	4.08 (0.04)	4.01 (0.05)	2.31 (0.06)
β_{120}	—	—	9.88 (0.10)	—	6.65 (0.10)	9.09 (0.10)	5.83 (0.07)	—	—
β_{11-1}	—	—	2.14 (0.10)	—	—	—	−3.75 (0.10)	—	—
β_{11-2}	—	—	—	—	—	—	12.51 (0.20)	—	—
β_{12-1}	—	—	4.34 (0.12)	—	—	—	−1.56 (0.12)	—	—
β_{12-2}	—	—	−2.40 (0.12)	—	—	—	−10.75 (0.18)	—	—
β_{12-3}	—	—	−11.89 (0.15)	—	—	—	—	—	—
β_{210}	—	—	—	—	—	—	—	6.93 (0.10)	—
β_{21-1}	—	—	—	—	—	—	—	−0.94 (0.16)	—
β_{21-2}	—	—	—	—	—	—	—	−9.20 (0.24)	—
β_{22-1}	—	—	—	0.77 (0.15)	—	—	—	—	0.66 (0.08)
β_{22-2}	—	—	—	−10.43 (0.18)	—	—	—	—	−10.30 (0.15)
β_{22-3}	—	—	1.46 (0.10)	−19.31 (0.20)	—	—	—	—	—
β_{22-4}	—	—	−4.96 (0.12)	−30.19 (0.22)	—	—	—	—	−30.40 (0.22)
β_{22-5}	—	—	−13.68 (0.15)	—	—	—	—	—	—
β_{22-6}	—	—	−23.88 (0.20)	—	—	—	—	—	—

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.[15] The protonation constants were determined from the data from six independent titrations. The titrations involving complex formation were carried out with six different metal-to-ligand ratios, varying from 1 : 1 to 1 : 14.

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

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

omitted). The formation 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.[16]

EPR Measurements.—The EPR investigations were made with ERS220 ZWG equipment. The microwave power was set at 10 mW, and the 100 kHz modulation amplitude was 2×10^{-4} T. The measured spectra were simulated by an IBM AT microcomputer. The EPR spectra of the VO^{2+}–H_2L^1 complex were measured in water and in D_2O at different temperatures.

Results and Discussion

The pH-metrically determined complex-formation constants are listed in Table 1. It must be emphasized that the deprotonation occurring after the co-ordination of the second organic ligand to the central metal ion may be due either to the formation of mixed-ligand complexes containing hydroxide ions besides the organic ligand or to the deprotonation of alcoholic hydroxy groups of the latter. On the basis of pH-metric equilibrium measurements alone one cannot distinguish between these possibilities. The equilibrium constants are therefore presented in the general form (4), β_{011}, β_{012} and β_{013}

$$\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r \qquad (4)$$

representing the protonation constants of the ligand, β_{110} and β_{120} the metal complex-formation constants of the parent complexes, β_{111} the formation constants of the protonated complex, and β_{11-1}, β_{11-2}, β_{12-1}, β_{12-2} and β_{12-3} the formation constants taking into consideration the deprotonation processes in general. The percentage distribution of the total metal-ion concentration in the different complexes as a function of pH is depicted in Fig. 1.

The ligand H_2L^1 has dissociation constants pK_1 (ring CO_2H) = 1.50, pK_2 (chain CO_2H) = 3.38 and pK_3 (NH_2^+) = 5.67. These values are similar to those reported earlier for

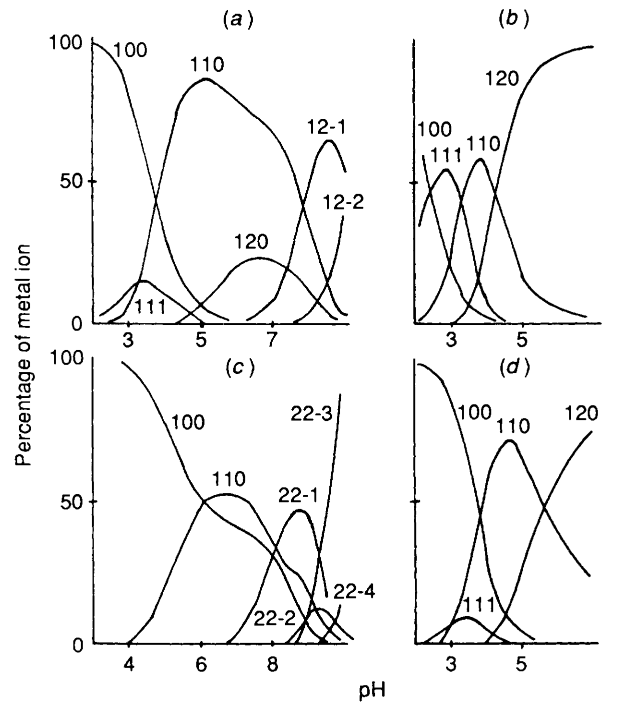


Fig. 1 Percentage distributions of total zinc(II) (*a*), nickel(II) (*b*), manganese(II) (*c*) and cobalt(II) (*d*) in different complexes as a function of pH. $[M^{2+}] = 1.4 \times 10^{-3}$ mol dm^{-3}, $[H_2L^1] = 7 \times 10^{-3}$ mol dm^{-3}, 298 K, $I = 0.1$ mol dm^{-3} $NaClO_4$

Table 2 Selected stability constants of metal(II)–HL^3 complexes[8,11]

	Ni^{II}	Co^{II}	Zn^{II}	Mn^{II}	Ca^{II}
log β_{110}	3.93	3.03	3.10	1.91	1.66
log β_{120}	7.21	5.35	5.63	—	—
log β_{130}	8.83	—	—	—	—

2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acids[13] or D-galacturonic acid.[17]

A comparison of the formation constants obtained for the calcium(II)–HL^3 system with the constants measured here demonstrated that, within experimental error, they are practically equal, indicating that the polyhydroxy chain does not participate in calcium(II) complex formation. In both cases only the 1:1 complex was observed. Similar behaviour was observed for the mononuclear manganese(II)-systems with H_2L^1 and HL^2, where at pH 2–6 only the 1:1 complexes are formed.

The EPR spectra of an aqueous solution containing 3×10^{-3} mol dm^{-3} manganese(II) and 1.5×10^{-2} mol dm^{-3} H_2L^1 or HL^2 (pH < 6) contained six lines of almost the same intensity, corresponding to the $\frac{5}{2}$ nuclear spin of manganese. On further increase of the pH the intensity of the six-line pattern decreased and it finally disappeared. This change in the EPR signal indicates that both manganese(II) complexes are dimerized and in accordance with the equilibrium measurements $Mn_2L_2(OH)_n$ complexes are formed (Table 1), resulting in a decrease in the number of unpaired electrons. This behaviour is analogous to that observed for the managanese(II)–saccharose,[18] -maltitol,[19] and -gluconate[20] systems.

The difference between the manganese(II)–H_2L^1 and –HL^2 system is the formation of $Mn_2L_2(OH)_3$ in the former case, but not in the latter.

The log β_{110} values of both manganese(II) complexes are 0.4 units higher than the corresponding value of the manganese(II)–HL^3 complex (Table 2). This is probably due to the weak co-ordination of the OH group on C(1′), as for the zinc(II) complexes.[13] All the log β values of the present manganese(II) complexes are practically the same, showing that the 5′-carboxylic group of H_2L^1 does not participate in the co-ordination.

The situation is different for the zinc(II)–H_2L^1 system. The co-ordination of the zinc(II) ion is relatively strong, and consequently complex formation starts at acidic pH, before the 5′-carboxylic group has dissociated completely. Owing to this, $Zn(HL^1)$ is formed (Fig. 1). The log β_{110} values of the zinc(II) complexes with the present ligands are practically the same, but are one order of magnitude higher than the corresponding value for zinc(II)–thiaproline, indicating co-ordination of the OH group on C(1′). In this way, two different five-membered chelate rings can be formed, resulting in an enhancement of the stability of the complexes.[13]

The ratio log β_{120}/log β_{110} = 0.43:1 for the zinc(II)–H_2L^1 complex is considerably smaller than the corresponding ratio for the HL^2 and HL^3 complexes. In the case of H_3L^1, the formation of the (1,2,0) complex starts above pH 4, where the 5′-carboxylic group is almost completely deprotonated. These negatively charged carboxylic groups are electrostatically repulsive to each other, resulting in a decrease in the stability of the (1,2,0) complex. In the zinc(II)–H_2L^1 and –HL^2 complexes further deprotonation indicating either the formation of hydroxo mixed-ligand complexes or deprotonation of the alcoholic OH group, was observed between pH 5.5 and 8.5. The log β_{12-1} and log β_{12-2} values of the zinc(II)–H_2L^1 complex are slightly smaller than the corresponding values of the HL^2 complex, demonstrating the greater electrostatic repulsion found in the former system.

The cobalt(II)–, nickel(II)– and zinc(II)–H_2L^1 systems are similar to each other, but the stability of the nickel complex is the highest. All three metal ions form protonated complexes $M(HL^1)$ in the range pH 2–4.5 (Fig. 1). The measured stability constants are higher than the corresponding values for HL^3. The enhancement of the stability of these complexes is probably due to the co-ordination of the OH group on C(1′). The ratios log β_{120}/log β_{110} [0.62 and 0.68:1 for cobalt(II) and nickel(II) respectively] are smaller than the values characteristic for amino acids (0.83:1). The explanation is the same as discussed above.

When the formation of the (1,1,1), (1,1,0) and (1,2,0) complexes is complete, the formation of new species starts at pH > 8.5. Calculation of the stability constants for these complexes was not possible because, as several earlier results showed,[2,21] the thiazolidine ring is in equilibrium with an acyclic thiol form at high pH (> 10).

Investigation of the copper(II) systems was prevented by redox reactions between the ligand and copper(II) ion.

Few equilibrium data have been reported on VO^{2+}–amino acid[22] and –hydroxycarboxylic acid systems. Investigations of the latter have shown[23] that the stabilities of the complexes depend strongly on the steric arrangement and conformation of the OH groups. Among the VO^{2+} complexes of tartaric acid H_2L, one dimeric complex $[(VO)_2L_2H_{-4}]$ was detected. Study of this complex in the solid state[24] has shown that both OH groups of tartaric acid are deprotonated and the ligand forms a bridge between two VO^{2+} ions. Fábián and Nagypál[25] investigated transition metal–glycine systems. Among the metal ions studied, only copper(II) formed more stable complexes than VO^{2+}. Similar results were obtained by Pessoa *et al.*[26] In the VO^{2+}–serine system, hydroxo mixed-ligand complexes are formed in acidic media, while at pH 6–7 the OH group of serine is deprotonated. Micera and co-workers[17,27,28] studied the VO^{2+}–D-galacturonic acid system and found that D-galacturonic acid is a good complexing agent for VO^{2+}, even at a 1:1 metal:ligand ratio. The high stability of the latter complex was suggested to be due to the co-ordination of the deprotonated alcoholic OH group. In alkaline media, four deprotonated OH groups are presumed to be co-ordinated to one central VO^{2+} ion. Similarly to the VO^{2+}–tartaric acid

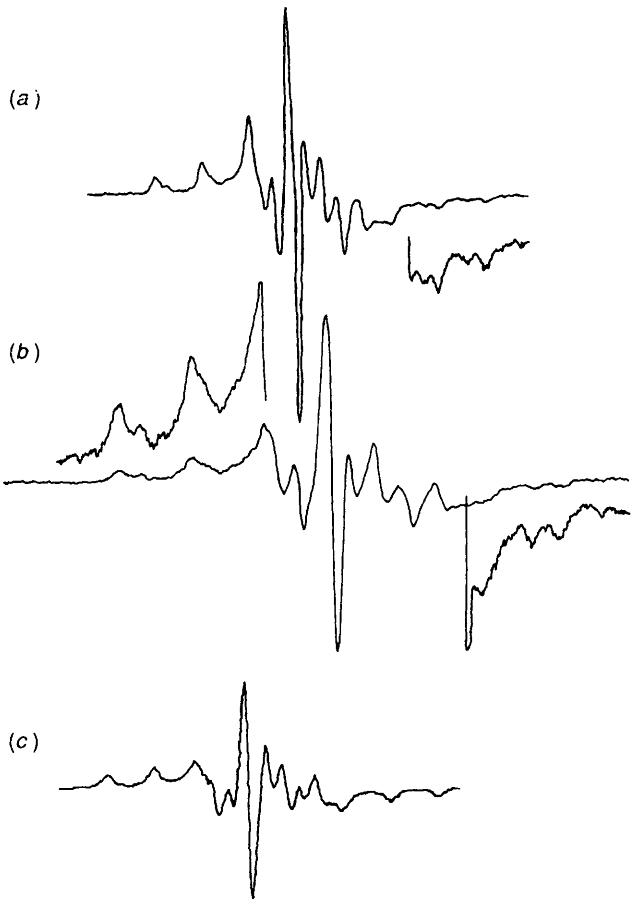


Fig. 2 Experimental and simulated (*c*) EPR spectra of the vanadyl–H_2L^1 complex VOL_2 in frozen solution in water (*a*) and in D_2O (*b*)

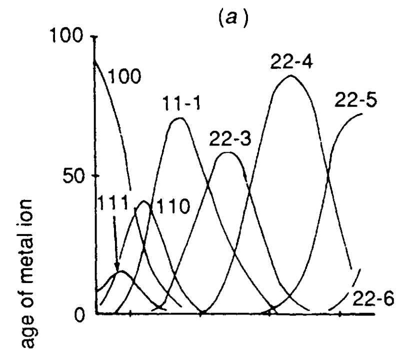


Fig. 3 Species distribution in the VO^{2+}–H_2L^1 system as a function of pH. $[VO^{2+}] = 2 \times 10^{-3}$ mol dm^{-3}, 298 K, $I = 0.1$ mol dm^{-3} $NaClO_4$. $VO^{2+}:H_2L^1 = 1:1$ (*a*) and 1:5 (*b*)

system, a dimeric complex of composition $(VO)_2L_2H_{-4}$ is also formed.

Our measurements on the VO^{2+}–H_2L^1 system revealed that complexes VOL^1 and $VOL^1{}_2$ of surprisingly high stability are formed (Table 1). Protonated species $VO(HL^1)$ are also detectable. Thus, the co-ordination modes of H_2L^1 and D-galacturonic acid are different. This may indicate the tridentate nature of H_2L^1. The 1′-OH group is assumed to participate in complex formation.

The EPR technique is sensitive to the co-ordination mode of oxovanadium(IV). However, the spectroscopic parameters do not distinguish between successively formed complexes. Their evaluation is possible only for solutions containing one of the species in great excess. The EPR spectrum recorded at room temperature for such a solution contained eight lines, according to the nuclear spin $\frac{7}{2}$ of the vanadium. The room-temperature spectrum is not informative because of line broadening. The spectrum recorded at liquid-nitrogen temperature is well developed, with even a fine structure [Fig. 2(*a*)].

For evaluation of the latter spectrum, first the reason for the splitting of the lines was studied. Repetition of the measurements in D_2O solution did not change the two-line splitting, excluding the possibility that interaction between vanadium(IV) and protons caused the splitting [Fig. 2(*b*)]. Simulation of the EPR spectra of the frozen samples, with the assumption that one mononuclear vanadyl complex is the dominant species and that rotational diffusion in the frozen solution is negligible, led to curves exhibiting a good fit to the experimental spectra [Fig. 2(*c*)].

The latter results indicated that under these conditions the fine structure of the spectrum is due to the different orientation of the molecules in the frozen system and not to the presence of two vanadium(IV) centres in different surroundings or to the interaction between equivalent vanadium centres.

From the equilibrium data, it could be concluded that $VOL^1{}_2$ is the dominant species in the frozen solution; according to the simulation, this has axial symmetry with the EPR parameters $g_{\parallel} = 1.950$, $g_{\perp} = 1.983$, $A_{\parallel} = 0.0388$ cm^{-1} and $A_{\perp} = 0.0140$ cm^{-1}.

The equilibrium measurements reflected stepwise complex formation in the vanadyl-containing systems. The distribution curves depicted in Fig. 3 show the effect of pH on the concentrations of the different species in solution. For both VO^{2+}–H_2L^1 and –D-galacturonic acid,[17] the major species in solution at around pH 7 is VOL_2H_{-2}. This complex is presumed to be formed by co-ordination of the deprotonated 1′-hydroxy group. Further increase of the pH resulted in a significant difference between the VO^{2+}–H_2L^1 and –D-galacturonic acid complexes. In the latter case, the formation of VOL_2H_{-3} and VOL_2H_{-4} started at pH 7 and 8, respectively,[17] while in the former case $VOL^1{}_2H_{-4}$ is not detectable below pH 9.5. Above pH 9.5 further deprotonation was observed.

The situation is slightly different in a solution containing VO^{2+} and H_2L^1 in 1:1 molar ratio. The experimental data indicate that complex formation begins at pH 2.5–4, and at >4.5 dimeric species in different protonation states (2,2, −3; 2,2, −4; 2,2, −5; and 2,2, −6) are formed.

Acknowledgements

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