Iron(III) Complexes of Lactobionic Acid: Equilibrium and Structural Studies in Aqueous Solution

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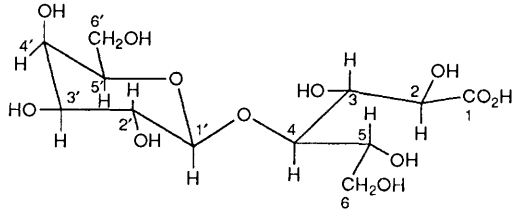
The equilibrium constants for the protonation of lactobionic acid (4-*O*-β-D-galactopyranosylgluconic acid) and its co-ordination with Fe^{III} were studied by potentiometric methods in aqueous solution, at 20.0 °C and $I = 0.100$ mol dm^{-3} ($NaNO_3$). The equilibrium data were processed with the Fortran computer program BEST. Correlations of chelate hydrolysis constants with those of the D-gluconic acid–iron(III) system were carried out. Co-ordination bonding sites and stereochemistry of metal–ligand interactions were inferred from UV and NMR spectroscopy.

The sugar acids play an important role in biological systems, both structurally and as carriers of metal ions.[1-3] Furthermore, their tendency to bind metal ions has promoted their use as sequestering agents in different areas.[4,5]

We have previously reported studies of the co-ordination chemistry of these saccharides with transition-metal ions in aqueous solution.[6-8] As part of this general programme and in order to obtain information on the complex-formation behaviour of related disaccharides, we decided to investigate the quantitative interaction between lactobionic acid (4-*O*-β-D-galactopyranosylgluconic acid) and iron(III) ion. This compound is a disaccharide constituted by D-galactose and D-gluconic acid. Since D-aldoses have a low tendency to bind Fe^{III},[9] the ability of lactobionic acid to form iron(III) complexes is expected to arise from the affinity of its D-gluconic acid portion for transition-metal ions. Owing to the lower flexibility of the sugar acid in the disaccharide molecule, the interaction of lactobionic acid with hard metal ions would be expected to be slightly lower than that of the monomeric moiety. The computer program selected to process the data allows us to provide an accurate description of the aqueous co-ordination chemistry of the system under investigation. Structural information is obtained by means of NMR spectroscopy. A comparative study between the behaviour of the iron(III)–lactobionic acid and –D-gluconic acid systems has also been carried out.



Lactobionic acid

Experimental

Reagents.—The sodium salts of lactobionic and D-gluconic acids were obtained from Sigma. Their purity was checked by elemental analysis and pH titration. The stability of lactobionic acid in acidic and basic media was confirmed by paper chromatography. A stock solution of Fe^{III} (0.020 mol dm^{-3}) was prepared by dissolving $Fe(NO_3)_3 \cdot 9H_2O$ (Merck) in 0.05 mol dm^{-3} nitric acid solution and its exact concentration was determined by potentiometric titration with ethylenediaminetetraacetic acid (H_4edta) solution.[10] The exact amount of excess of acid was evaluated both by a Gran's plot[11] and by the procedure described by Harris and Martell.[12] Stock solutions of NaOH and HNO_3 were standardized against potassium hydrogenphthalate and sodium carbonate respectively.

Potentiometric Titrations.—Lactobionic acid was dissolved in standard base both in the absence and presence of metal ion and a back titration was carried out by addition of standard acid in small increments, so that 50 or more experimental points were provided for each run. The pH range studied was 2–11. Both the protonation constant of this acid and its iron(III) co-ordination equilibria were determined by using a Corning 125 research pH meter equipped with glass and calomel reference electrodes calibrated with HNO_3 and acetic acid to read $-\log[H^+]$ rather than hydrogen-ion activity. Titrations were carried out under a nitrogen atmosphere. The temperature was maintained at 20.0 ± 0.2 °C and the ionic strength adjusted to 0.100 mol dm^{-3} by addition of $NaNO_3$. The solutions were diluted to a final volume of 100 cm^3 in order to obtain a final concentration of 2×10^{-3} mol dm^{-3} in both metal ion and ligand. In addition, solutions containing this same metal-ion concentration and 2 or 3 molar equivalents of ligand were investigated.

The potentiometric data were converted into stability constants with the use of the program BEST.[13] In contrast to the D-gluconic acid–iron(III) system, the complexes of lactobionic acid were not fully formed at low pH and the constants were determined by direct potentiometric titration.

Spectrophotometric Measurements.—The UV spectra were recorded on a Gilford Response II spectrophotometer, at 20.0 °C and $I = 0.100$ mol dm^{-3} ($NaNO_3$).

NMR. All spectra were acquired on a Bruker AC-200 E pulsed Fourier-transform instrument with a 4.3 T supercon magnet, at a carbon-13 resonance frequency of 50.1 MHz. All measurements were conducted at 22 °C. The samples were 10% w/v ligand solutions in distilled deionized water mixed with 10% D_2O to provide the deuterium lock. The iron(III) ion was introduced as the appropriate aliquot of 0.05 mol dm^{-3} Fe$(NO_3)_3$ solution. Adjustments of the pH were made by adding either 0.1 or 1 mol dm^{-3} NaOH. Since in the presence of a large excess of ligand in an alkaline medium the iron(III) undergoes reduction, the solutions were kept at a weakly basic pH.

Results and Discussion

Potentiometric Measurements.—Different metal–ligand ratios were potentiometrically investigated and only 1:1 complexes were found. The formation of 1:1 species was confirmed by spectrophotometric measurements, using Job's continuous-variation method.[14] The equilibrium potentiometric profiles of the iron(III)–lactobionic acid system showed the release of three protons from the complex, in addition to the carboxyl proton. Iron(III) hydroxide precipitation was not detected.

This information, together with that in the absence of Fe^{III}, was analysed by applying the computer program BEST, yielding the protonation and complex stability constants listed in Table 1. From these values, the species distribution curves were calculated (Fig. 1).

Although the complexes of the iron(III)–lactobionic acid (HL) and –gluconate are similar, the species distribution pattern is different.[6] Obviously, this is due to the different stabilities of the complexes formed. Fig. 1 reveals the interference of iron(III) ion hydrolysis ($\log K_{FeOH} = -2.56$ at $I = 0.1$ mol dm^{-3})[15] in the $[FeH_{-1}L]^+$ complex formation at low pH. Between pH 5.5 and 7.5, $[FeH_{-2}L]$ is the dominant species, and above pH ≈ 7 the most deprotonated $[FeH_{-3}L]^-$ complex is detected. Neither the normal $[FeL]^{2+}$ complex nor other hydrolytic iron(III) species were found to exist in appreciable amounts.

The release of non-carboxylic protons from the complex can be attributed either to the sugar hydroxyl groups or to the co-ordinated water molecules. Although the ability of Fe^{3+} to displace aliphatic hydroxyl protons is well established,[16] it is not possible to distinguish between the above processes on the basis of potentiometric results. Furthermore, it is not possible to decide which of the hydroxyl groups are involved. Therefore, spectroscopic methods were applied to the structural study of the species formed.

UV Measurements.—The UV spectra of iron(III) complexes of D-gluconic acid and lactobionic acid are similar at different pH but are not very informative. They consist of a family of closely spaced curves with a broad maximum at 302 nm. Although the absorptivities of the partially hydrolysed iron(III) ion at pH 3 are a little lower than those of the sugar complexes in the range of wavelengths investigated, the profiles are similar. This fact suggests that the co-ordination arrangements for the hydroxoaqua and for the sugar complexes are similar, with the metal ion octahedrally co-ordinated at the binding sites.

NMR Measurements.—The measurement of selective line broadening in the ^{13}C NMR spectra of paramagnetic complexes is a convenient means of gaining qualitative information about possible binding sites.[17,18] The ^{13}C NMR spectrum of lactobionic acid equilibrated in water in a weakly alkaline medium consists of 12 discrete peaks; the full assignment has not previously been described. Five signals appear between δ 73 and 75 and their assignments are thus difficult, although they are critical for the structural study described below. On the other hand, the ^1H NMR spectrum in D_2O shows a severe

Table 1 Protonation constants of lactobionic and D-gluconic acids and deprotonation constants for their iron(III) complex at $I = 0.100$ mol dm^{-3} ($NaNO_3$) and 20.0 °C

Quotient	Lactobionic acid	D-Gluconic acid *
$[HL]/[H^+][L^-]$	3.53 (0.01)	3.40
$[H^+][MH_{-1}L^+]/[M^{3+}][L^-]$	2.03 (0.03)	2.43
$[H^+][MH_{-2}L]/[MH_{-1}L^+]$	−3.89 (0.03)	−3.23
$[H^+][MH_{-3}L^-]/[MH_{-2}L]$	−9.93 (0.03)	−4.38

* Ref. 6.

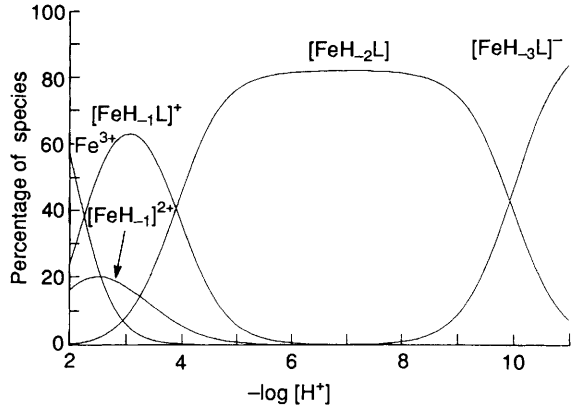


Fig. 1 Species distribution in iron(III)–lactobionic acid (HL) as a function of pH at 20.0 °C and $I = 0.100$ mol dm^{-3} ($NaNO_3$), $c_L = c_M = 1.896 \times 10^{-3}$ mol dm^{-3}. Hydrolytic iron(III) species showing percentages smaller than 15% were not included

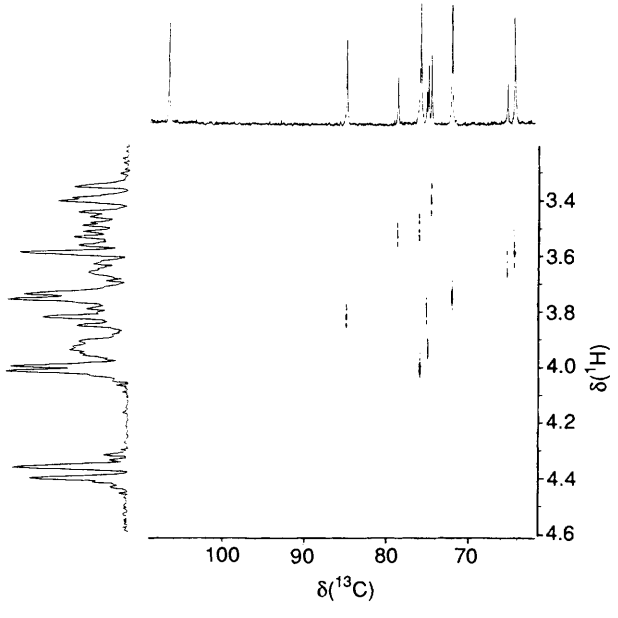


Fig. 2 The ^1H–^1H coupled two-dimensional ^1H–^{13}C chemical shift correlation NMR spectrum of lactobionic acid in water at pH 8

overlapping of signals within *ca.* 1 ppm. In order to assist in the assignment of the ^{13}C spectrum, a ^1H–^1H coupled two-dimensional ^1H–^{13}C chemical shift correlation NMR spectrum was recorded. Fig. 2 shows the two-dimensional spectrum and Fig. 3 presents selected one-dimensional proton slices of the former corresponding to the more controversial carbon assignments. Table 2 lists the chemical shifts for the carbon resonances and the multiplicity of the corresponding protons.

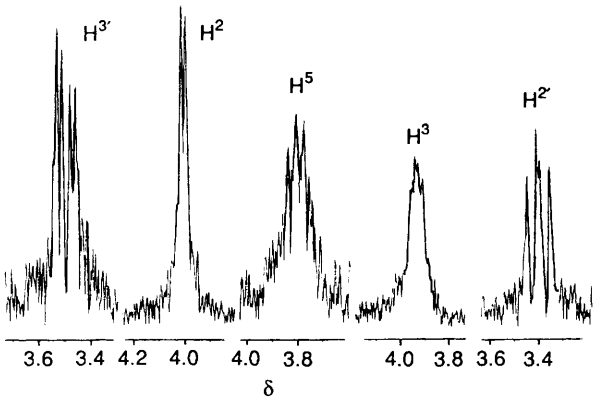


Fig. 3 Selected one-dimensional slices of the spectrum shown in Fig. 2

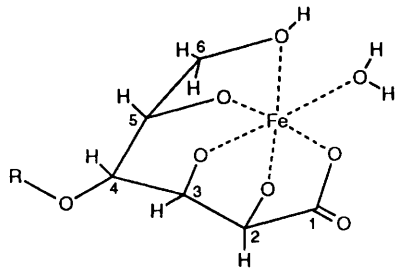
Table 2 Chemical shifts of carbons and chemical shifts and multiplicities of hydrogens. The latter were obtained from ^1H–^1H coupled two-dimensional ^1H–^{13}C correlation spectra

Atom	δ(C)	δ(H)	Multiplicity of H (J, $W_{\frac{1}{2}}$ in Hz)
$C^{1'}$	105.73	4.38	d (J = 7.8)
$C^{2'}$	73.51	3.39	dd (J = 8.0, 10.5)
$C^{3'}$	74.98	3.49	dd (J = 3.8, 10.3)
$C^{4'}$	71.05	3.75	d (J = 3.8)
$C^{5'}$	77.60	3.52	dd (J = 5.7, 8.9)
$C^{6'}$	63.45	3.59	m ($W_{\frac{1}{2}}$ = 22)
C^1	180.78		
C^2	74.82	4.00	d (J = 3.4)
C^3	73.89	3.93	dd (J = 2.4, 3.5)
C^4	83.94	3.82	t (J = 6.1)
C^5	74.10	3.78	m ($W_{\frac{1}{2}}$ = 23)
C^6	64.34	3.62	m ($W_{\frac{1}{2}}$ = 35)

Both the carboxylate carbon (C^1) and the anomeric one ($C^{1'}$) are easily identified by their chemical shifts at δ 180.8 and 105.7 respectively. Furthermore, the signal of $H^{1'}$ appears as a doublet with a coupling constant (J) of 7.8 Hz. This value is consistent with a vicinal coupling between the axial hydrogens $H^{1'}$ and $H^{2'}$.[19] The signals corresponding to $H^{2'}$ and $H^{3'}$ both appear as double doublets. The former shows large J values (8.0 and 10.5 Hz), in accord with the dihedral angles of 180° with respect to the adjacent protons $H^{1'}$ and $H^{3'}$ within a conformationally fixed structure.[19] The J values for $H^{3'}$ are 10.3 and 3.8 Hz, in agreement with a coupling with the axial $H^{2'}$ and equatorial $H^{4'}$ protons respectively. The signal from H^2 is, as expected, a doublet; deshielding of this proton with respect to other CHOH protons is explained as the result of its vicinity to the carboxylate group. The double doublet obtained for H^3, with two small J values (Table 2), is in good agreement with the coupling to two protons in a conformationally mobile structure. The peak of H^5 is a multiplet which reflects the interaction with H^4 and with the non-equivalent H^6 protons. Therefore, all five carbons appearing in the range δ 73–75 can be readily assigned from the above analysis of the H–H-coupled ^1H–^{13}C two-dimensional spectrum. Both the $H^{6'}$ and H^6 proton signals are very complex and the carbon assignments were therefore performed by comparison with the carbon chemical shifts for D-galactose and D-gluconic acid respectively.

The proton assignments and multiplicities discussed above were confirmed by a ^1H–^1H two-dimensional spectrum, using pyridine as solvent. All the remaining ^1H assignments were in agreement with the expected multiplicities for a six-membered D-galactose ring and a gluconic acid moiety.

Upon addition of iron(III) ion to the lactobionic acid solution, selective and significant peak broadening in the ^{13}C NMR spectrum was observed. This is due to a paramagnetic relaxation enhancement caused by a random variation of the



nuclear-electron spin–spin interaction,[20] and allows one to obtain qualitative information about the possible binding sites. Since an increase in the iron(III) ion concentration beyond 5×10^{-3} mol dm^{-3} causes the spectrum to become unresolved, only a small metal concentration range was evaluated. As the iron(III) ion concentration is raised the signals of C^2, C^3 and C^5 are the most affected. It should be noticed that this latter result can only be obtained after the extensive ^{13}C and ^1H NMR analysis discussed above, which allowed these resonances to be discerned in the severely crowded region at δ 73–75. The linewidths at half-height for both C^1 and C^6 were too broad, while the remaining peaks did not broaden significantly. These results confirm that the complex formation only involves the gluconic acid moiety of the disaccharide. Furthermore, they strongly suggest that the three deprotonations observed during the potentiometric study correspond to the three hydroxyl groups bonded to C^2, C^3 and C^5.

The co-ordination structure of the iron(III)–lactobionic acid complex consistent with the above data and the examination of Corey–Pauling–Koltun (CPK) molecular models can be represented as shown, with a '1,2,3,5' chelation of Fe^{III}.

In order to compare this mode of chelation of Fe^{III} with an analogous system, similar spectroscopic experiments were performed with D-gluconic acid. In the latter case all signals broaden in the presence of the metal ion, and thus any conclusion about the co-ordination sites would be solely speculative. Several different models have been proposed for this iron(III)–D-aldonic acid complex,[21] but the geometry of the '2,4,6' cavity seems to be particularly amenable towards metal complex formation.[6,16] In both the lactobionic and gluconic acid complexes, the C^2 hydroxyl group co-ordination is generally accepted, given the known co-ordination tendency of C^2 in α-hydroxy acids.[22] This co-ordination site is corroborated both by similar values of the first complex deprotonation constants (Table 1) and by the NMR results. However, the increasing differences in the subsequent deprotonation magnitudes suggest differences in the remaining binding sites. The glucosidic linkage in the disaccharide prevents the C^4 metal co-ordination and therefore C^3 and C^5 alkoxide formation in the lactobionic acid complex is allowed to occur. Further, although the binding to the C^6 alkoxide group could be proposed instead of C^5,[23] both the NMR spectroscopic evidence and the magnitude of the third deprotonation constant ($\log K = -9.93$) are in good accord with the former assignment. The low value of this deprotonation constant may be explained by the increased strain in the lactobionic complex with respect to the D-gluconic acid analogue. It is conceivable that the hydroxyl group of C^6 could be involved in the complex formation through the oxygen lone pairs.

In conclusion, the combination of potentiometric methods and spectroscopic techniques is shown to be a powerful tool to study both the structural and thermodynamic aspects of metal ion–sugar acid complex systems.

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