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2-(Hydroxyimino)propanohydroxamic acid, a new effective ligand for aluminium

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The capacity of a new ligand, 2-(hydroxyimino)propanohydroxamic acid (HPH, an analog of alanine), to bind the Al^{3+} ion has been examined. A particular objective was to check whether the HPH oximic group could take part in aluminum co-ordination together with the two hydroxamic oxygens, a possibility that could make HPH an efficient low-molecular-mass ligand for detrimental Al^{3+} ions *in vivo*. Glass electrode potentiometry, NMR spectroscopy and Electrospray Mass Spectrometry were used to investigate Al^{3+}–HPH complex equilibria and their related structural aspects. The aluminium binding capacity of HPH was compared to that of other monohydroxamic acids. In spite of the lowest basicity of its hydroxamic moiety and the non-involvement of its oximic moiety in metal ion binding, HPH was found to be a very competitive ligand. Comparative quantum calculations performed on the systems involved suggest that electrostatic interactions induced by the ligand molecule are determining for complex stability.

Hydroxamic acids represent a very important family of chelating agents.[1,2] Siderophores synthesized by microorganisms to prevent the hydrolysis of the very acidic Fe^{3+} ions[3] contain hydroxamate or catecholate as basic chelating functions. A similar efficiency was found in a variety of model systems.[4–7] Simple hydroxamic acids also are quite effective ligands for trivalent "hard" metal ions,[1,7,8] even though the binding strength of monohydroxamates can be reduced by adjacent unbound functions, *e.g.* NH_3^+ in aminohydroxamates.[7] Basically, Fe^{3+} or Al^{3+} complexation by hydroxamates takes place *via* the two oxygens of the hydroxamic group through the formation of a stable 5-membered chelate ring. Hydrogen bonds involving protonated hydroxyl groups may also play a role, as is usually the case when hydroxamic nitrogen is the co-ordinating atom.[2]

An oxime function (=NOH) with a very easily anchoring acidic nitrogen is a very effective binding site for Cu^{2+} and Ni^{2+} ions:[9–12] when protonated the hydroxyl moiety of the oxime group may be involved in a very effective and specific hydrogen bond; when deprotonated it can act as a bridging donor between metal ions in oligomeric species. The insertion of oximic and hydroxamic functions into a given ligand, for example by modification of carboxylic and amino groups of a simple amino acid, results in a much more efficient chelating agent for these metals.[12,13]

2-(Hydroxyimino)propanohydroxamic acid (HPH, an analog of alanine) contains five potential donor atoms, three oxygens (one oximic and two hydroxamic) and two nitrogens (N_{ox} and N_{hydr}). This rather unusual donor arrangement in a very small molecule makes this type of ligand very versatile, capable of effective binding with various metal ions. In particular, its capacity to co-ordinate Cu^{2+} and Ni^{2+} ions into very stable complexes has clearly been established.[12,13] The main donor atoms involved in the major complexes are the two nitrogens, N_{ox} and N_{hydr}. In addition, oxygen donors also may be engaged in the formation of oligomeric species, *e.g.* with Cu^{2+},[13] or in the bonding of "hard" counter ions like Na^+ as was observed in the solid state.[12] When any of these oxygens is protonated, effective hydrogen bonds can be formed that impart additional stability on corresponding complexes.[12]

In this context the objective of the present work was to check whether the HPH oximic group could participate directly or indirectly in the complexation of the Al^{3+} ion together with the two hydroxamic oxygens. The possible involvement of this group in aluminum co-ordination, which is expected to enhance the stability of the resulting complexes, could make HPH an efficient low-molecular-mass ligand for detrimental Al^{3+} ions (see, *e.g.*, ref. 14 and references therein). Glass electrode potentiometry and NMR spectroscopy have been used to investigate Al^{3+}–HPH complex equilibria and their related structural aspects. Electrospray mass spectrometry was applied quantitatively to confirm the existence of species made predominant under particular conditions (ligand excess in the present case). Finally, quantum calculations were run to discriminate among interaction energy components at the origin of the complex stabilities obtained from potentiometric data.

Experimental

Reagents

2-(Hydroxyimino)propanohydroxamic acid (H_2L) was synthesized as reported in a recent work.[12] The compound is soluble in water, acetone and alcohols (Found: C, 30.39; H, 5.20; N, 23.93%. Calc. for $C_3H_6N_2O_3$: C, 30.51; H, 5.12; N, 23.72%). All other chemicals were of reagent grade used without further purification. As two series of potentiometric titrations were made in parallel in two different laboratories (see (i) and (ii) below), technical details relative to the reagents used are described separately.

(i) Regarding the first series, the aluminum stock solution was prepared by dissolving Alfa products 99.997% pure aluminium chloride in aqueous hydrochloric acid as previously described.[15] Its metal and proton contents were Gran-titrated before each

Table 1 Summary of titration data used for calculating formation constants[a]

System	C_{Al}	C_{HPH}	C_H	pH range used (investigated)	n
Proton–HPH (i)[b]		2.498	2.411	(2.61) 6.17–10.30 (11.12)	33
		4.984	4.894	(2.30) 5.07–11.13 (11.42)	44
		12.476	12.227	(1.99) 5.29–11.17 (11.38)	32
		4.984	—	5.99–10.68 (11.21)	37
		2.498	—	6.44–9.65 (10.98)	25
		12.476	—	5.29–10.24 (11.09)	37
Proton–HPH (ii)[b]		3.425	2.849	(2.56) 6.54–10.92 (11.04)	56
		3.458	2.854	(2.58) 6.37–10.81 (10.95)	63
		3.478	2.816	(2.57) 8.10–10.95	47
		3.481	2.812	(2.58) 7.96–10.87 (10.94)	54
		2.136	1.868	(2.74) 5.83–10.97	76
Al^{III}–HPH (i)[b]	1.068	12.483	13.097	1.88–9.43 (10.49)	60
	1.017	10.060	10.743	(1.86) 2.06–9.94 (10.06)	63
	1.017	9.054	9.763	2.01–9.94 (10.22)	65
	1.015	4.985	5.840	2.22–4.52 (9.02)	16
	1.015	3.997	3.878	2.40–4.35 (8.72)	16
	1.117	2.498	2.436	2.61–4.29 (4.73)	15
	5.079	9.991	9.619	2.01–4.61 (5.41)	37
	5.081	4.985	4.727	2.30–4.11 (8.04)	40
	5.080	2.498	4.726	2.39–4.04 (9.04)	20
Al^{III}–HPH (ii)[b]	0.207	2.097	1.847	2.70–9.95 (10.56)	78
	0.367	3.314	2.759	2.56–10.37 (10.58)	92
	0.537	3.316	2.693	(2.58) 2.81–10.53 (10.65)	93
	0.383	2.064	1.873	2.72–5.82 (10.68)	44
	0.802	3.235	2.674	(2.55) 2.60–4.76 (10.30)	52

[a] The initial total concentrations of metal (C_{Al}), ligand (C_{HPH}) and mineral acid (C_H) in the titrate are expressed in mmol dm^{-3}; the pH notation stands for $-\log$ [H] (see text); n represents the number of experimental observations in each titration. [b] Numbers between parentheses refer to the two series of experiments (see Experimental section).

series of measurements (see ref. 15). The ligand concentration was titrated during protonation experiments using a given standardized pipette for each delivered volume, the same pipette being then used to deliver the same volume in complexation experiments. Sodium chloride (Merck pro analysi reagent) was used as a background salt. Titration sodium hydroxide solutions were prepared by diluting the contents of BDH or Merck ampoules into fresh Millipore water, boiled and cooled under a purified nitrogen atmosphere. Both alkali titre and absence of carbonate were checked through Gran titrations against Merck *pro analysi* potassium hydrogenphthalate.

(ii) For the second series, the aluminium stock solution (0.0114 mol dm^{-3}) was prepared by dissolving Merck $Al(NO_3)_3 \cdot 9H_2O$ in 0.004 mol dm^{-3} aqueous nitric acid. Its metal content was determined by inductively coupled plasmal analysis (ICP) (vacuum spectrometer ICP-AES ARL 3410). Ligand stock solutions were 0.0035 and 0.00214 mol dm^{-3}. Their concentrations were internally titrated during protonation calculations. Sodium hydroxide solutions (Merck 0.1 mol dm^{-3}) were standardized against Merck *pro analysi* potassium hydrogenphthalate.

Potentiometric studies

The two different series of titrations performed involved distinct experimental protocols.

(i) The first series was under the direct control of the experimenter. The emf values were monitored with a Consort microcomputer pH-meter. The electrode system, which opposed a Beckman glass electrode to a saturated sodium chloride Ingold calomel electrode, was calibrated on the concentration scale.[16] It was fitted to an Ingold cell containing 20 cm^3 of initial solution into which NaOH was delivered by means of a Metrohm 715 burette. The reaction was thermostat-controlled at 37 ± 0.02 °C. The background electrolyte was 0.15 mol dm^{-3} NaCl. The ionic product of water for these conditions was determined as $10^{-13.27}$ mol^2 dm^{-6}.

Aluminium complex formation titrations were performed in accordance with the experimental protocol previously specified for Al^{3+} hydrolysis studies.[15] However, given the relatively strong binding strength of HPH with respect to Al^{3+} ions, the 2 min time interval imposed between successive sodium hydroxide additions was virtually sufficient for true equilibrium to be reached throughout all titrations. Table 1 reports the reactant concentrations and pH ranges used in the calculations. (The limits of investigated pH ranges that were not included in final calculations are indicated between parentheses.)

(ii) The second series of potentiometric experiments was carried out at constant temperature in a room thermostatted at 25 °C using a MOLSPIN automatic titration system, with a Russell microcombined glass-calomel electrode calibrated daily on the concentration scale using HNO_3.[17] The background electrolyte was 0.1 mol dm^{-3} KNO_3 and the ionic product of water for these conditions was $10^{-13.77}$ mol^2 dm^{-6}. Initial solutions of 2 cm^3 were titrated with sodium hydroxide delivered by a 0.25 cm^3 micrometer syringe previously calibrated by weight titrations and titrations of standard materials. The 2 min time interval between successive NaOH additions was assumed to be sufficient for true equilibrium in the solutions studied (see above). Corresponding titration data are summarized in Table 1.

Formation constant calculations

Distinct calculation procedures also were used to determine complex formation constants from the two above series of titrations.

(i) The selection of the best set of chemical species likely to account for the experimental data was performed with the usual two-step approach of one of the authors.[14,15] Possible stoichiometries were deduced from the shapes of protonation, formation and deprotonation curves.[18] The hydrolysis constants on which the calculations were based were those determined previously (see Table 2).[15] Complex formation constants of the species suggested from the above analysis were tested using both the optimizing module of the ESTA program library[19,20] and MINIQUAD.[21] However, as no allowance is made for precipitation in this program, it was checked in parallel to species selection that the pH values at which aluminium trihydroxide precipitation had experimentally been observed

Table 2 Stability constants determined for the Al^{III}–HPH system at 37 (i) and 25 °C (ii)[a]

System	Species	log β	SD	S	R	n	Ref.
Al^{III}–Hydroxide (i)[b]	MH_{-1}	−4.666					15
	MH_{-3}	−13.598					15
	MH_{-4}	−21.031	0.046				This work
	M_3H_{-11}	−54.694					15
	M_6H_{-15}	−49.398					15
	M_8H_{-22}	−76.425					15
Al^{III}–Hydroxide (ii)[b]	MH_{-1}	−5.409					24
	MH_{-2}	−9.982					24
	MH_{-3}	−15.692					24
	MH_{-4}	−23.455					24
	M_3H_{-4}	−13.694					24
	M_2H_{-2}	−7.7					24
Proton–HPH (i)[b]	HL	10.738	0.005	0.750E-6	0.004813	208	This work
	H_2L	18.694	0.006				
Proton–HPH (ii)[b]	HL	10.917	0.002	[c]	[c]	296	This work
	H_2L	19.079	0.004				
Al^{III}–HPH (i)[b]	MHL	17.370	0.007	0.893E-7	0.001068	332	This work
	MH_2L_2	34.461	0.007				
	MH_3L_3	50.130	0.030				
	MH_2L_3	43.968	0.029				
	MHL_3	36.176	0.038				
	ML_3	26.940	0.074				
	$M_3H_{-1}L_2$	27.322	0.035				
Al^{III}–HPH (ii)[b]	MHL	17.885	0.008	[c]	[c]	359	This work
	MH_2L_2	34.996	0.009				
	MH_3L_3	51.036	0.011				
	MH_2L_3	43.981	0.010				
	MHL_3	35.579	0.012				
	ML_3	26.471	0.019				

[a] SD = Standard deviation calculated with MINIQUAD for series (i) (those obtained with ESTA are about one-third smaller) or SUPERQUAD for series (ii) (see text); S = sum of squared residuals and R as defined in ref. 20 for series (i); n = number of experimental observations. [b] Numbers in parentheses refer to the two series of experiments (i) and (ii) (see Experimental section). [c] These parameters are not available in SUPERQUAD

coincided with the values obtained from simulations run with an adapted version of the SPE program.[22]

(ii) Formation constant determinations from the second series of data were carried out with SUPERQUAD.[23] The values of the hydrolysis constants corresponding to the experimental conditions used (25 °C and I = 0.1 mol dm^{-3}) were taken from ref. 24.

^{1}H NMR spectra

Proton NMR spectra were measured on a Bruker AMX (300 MHz) spectrometer in D_2O using TSP (sodium 2,2,3,3-tetradeuterio 3-trimethylsilylpropionate) as an internal standard, an HPH concentration of 0.01 mol dm^{-3} and a metal-to-ligand ratio of 1 : 4.

Mass spectrometry

ESI-MS spectra were obtained on a Finnigan TSQ 700 triple stage quadrupole mass spectrometer. Samples were continuously introduced through the electrospray interface (nitrogen) at a rate of 3 μl min^{-1} by biasing the electrospray probe at a voltage of 4.5 kV. The ions were detected by scanning the third quadrupole and the scans were monitored over 2 s in the range m/z 50–1000. The scans were averaged and the abundances of the ions are reported as relative intensity with respect to the base peak. Solutions were prepared in aqueous ethanol 50%/50% (v/v) with a ligand concentration of 0.01 mol dm^{-3} and metal-to-ligand ratios of 1 : 4 and 1 : 6.

$Al(HL)_3{\cdot}2H_2O$ solid state complex

Aluminium chloride hexahydrate (0.121 g, 0.5 mmol) dissolved in water (10 ml) was added to an aqueous solution (10 ml) of HPH (0.177 g, 1.5 mmol). Then lithium hydroxide (1.5 ml of 1 mol dm^{-3} aqueous solution) was added (pH of the resulting solution *ca.* 8), and the mixture obtained set aside for evaporation at room temperature. After 36 h the resulting oily residue was treated with warm ethanol (3 × 15 ml); the white solid formed was filtered off, washed with diethyl ether and dried in vacuum. Yield: 0.153 g (74%). Calc. $C_9H_{19}AlN_6O_{11}$: C, 26.09; H, 4.62; N, 20.29. Found: C, 26.30; H, 4.83; N, 20.06%. IR (cm^{-1}): 909 ($N–O_{hydroxamic}$); 1025 ($N–O_{oxime}$); 1541 ($C=N_{hydroxamic}$); 1595 (C=O, Amide I); 3233 (br), 3421 (br) (O–H). IR spectra were obtained on a Perkin-Elmer 983 G spectrometer (as KBr pellets) in the 400–4000 cm^{-1} range.

Quantum calculations

Quantum chemistry calculations were performed using the GAUSSIAN 94 set of programs.[25] Geometry optimizations of complexes and isolated ligands were performed at a Density Functional level with the hybrid B3LYP functional[26,27] and 6-31G** basis set.[28–33] Interaction energies were calculated both with 6-31G** and 6-31+G**[34] as differences between complex energies, and corresponding sums of energies for isolated ligand and Al^{3+} ion were corrected by the Basis Set Superposition Error (BSSE) in accordance with the Full Counterpoise (FCP) method proposed by Boys and Bernardi.[35] Interaction energy components were calculated using the Kitaura–Morokuma energy decomposition procedure implemented in the GAMESS program.[36] Atomic charges were obtained from Natural Bond Orbital (NBO) theory.[37–44]

Results and discussion

Protonation equilibria

Protonation constants and complex formation constants are collected in Table 2. Two protonation steps have been observed for HPH, whose corresponding constants slightly vary from one medium to the other. The protonations correspond to hydroxamic and oxime groups (pK_{a1} = 7.96–8.16 and pK_{a2} = 10.74–10.91), respectively.[12,13]

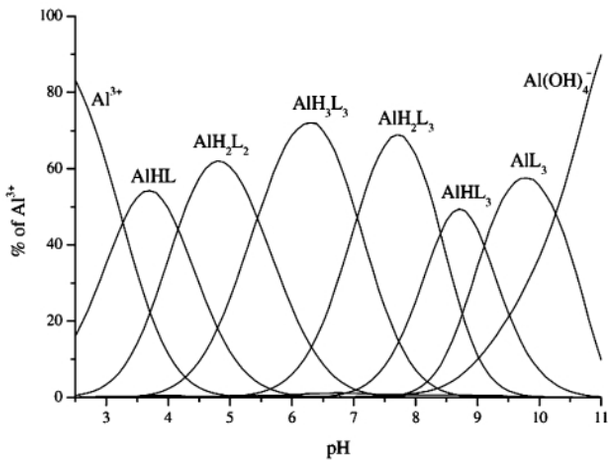

Fig. 1 Distribution profile of aluminium into its different Al^{3+}–HPH complexes at reactant concentrations used in NMR experiments: $C_{Al} = 2.5$ mmol dm^{-3}; $C_{HPH} = 10$ mmol dm^{-3}.

Al^{3+}–HPH complex equilibria

The "best" set of species likely to account for the experimental data obtained from series (i) can be seen in Table 2. On account of the complexity of the Al^{3+}–HPH system, the above-described approach required the examination of a large number of combinations of possible complexes. Once the composition of the "best" set was definitively established, a last refinement was run with the constants of aluminium hydroxides[15] mentioned in Table 2 being allowed to vary as recommended in the conclusion of the previous aluminium hydrolysis study.[15] A satisfactory agreement was found (−4.88 *vs.* −4.67 for AlH_{-1},† −13.44 *vs.* −13.60 for AlH_{-3}, −49.78 *vs.* −49.40 for Al_6H_{-15}) except for AlH_{-4} (−21.03 *vs.* −23.87). As the AlH_{-4} constant was not determined with accuracy in the Al^{3+} hydrolysis study because of the partial precipitation of $Al(OH)_3$ and the "kinetic" protocol used (the limit of the average hydrolysis number did not reach 4), the AlH_{-4} constant determined here is considered more realistic and therefore reported in Table 2.

Table 2 also reports the constants obtained from series (ii) at 25 °C. Two different sets of species (mostly of 1:2 and 1:3 metal-to-ligand stoichiometries in turn) were first characterized as best fitting with the experimental data. The set already identified in series (i) was finally selected, mineral acid concentrations being refined simultaneously to complex formation constants.

As can be seen in Table 2, the two series of data led to almost identical results as to species stoichiometries. The only discrepancy, which is relative to the finding of the $Al_3H_{-1}L_2$ minor species in series (i) only, is quite logical since series (ii) is exclusively composed of 1:4 up to 1:10 metal-to-ligand ratio experiments in which the polynuclear species is expected to be negligible. This perfect agreement tends to substantiate the reliability of the whole results.

The differences between the values of both log β and stepwise log K observed between experiment (i) and (ii) are typical for the data obtained for 25 and 37 °C.[45–47] Additional confirmation of the existence of the species characterized from potentiometric determinations was obtained from proton NMR spectra. The methyl group of HPH exhibits a well defined singlet at δ 2.0243 (reference TSP). In the presence of aluminium part of the ligand becomes co-ordinated to give rise to inert (on the NMR scale) Al^{3+} complexes. Besides the metal-free HPH signal, three other peaks are observed that can be assigned to some of the species formed. According to speciation calculations based on constants in Table 2 (Fig. 1), the two peaks observed at pH* (pH not corrected for the isotopic effect) around 4.2 (Fig. 2) correspond to AlHL and AlH_2L_2. Likewise, the only one proton singlet observed for the co-ordinated ligand above pH* 5.5 corresponds to 1:3 metal-to-ligand molar ratio complexes. Integrating proton NMR signals relative to bound and unbound ligand allows a quantitative evaluation of its corresponding fractions in solution at a given pH. The same information can be obtained from speciation calculations run for the metal and ligand concentrations used in NMR studies. A comparison of these results (Table 3) shows that: (a) bound ligand percentages evaluated from potentiometric and NMR data are close to one another; the small deviations observed may derive from difficulties correlating pH* and pH values and from the different concentration ranges used for potentiometric and NMR measurements; (b) the average co-ordination number evaluated from NMR spectra near pH* 8 (≈2.75) confirms the major species to be of 1:3 metal-to-ligand

† Formation constants are expressed according to the general formula $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$ in which the value of r is negative for hydroxo species. For example, the equilibrium $M^{2+} + L^{2-} + H_2O \rightleftharpoons ML(OH)^- + H^+$ is represented by $\beta_{11\text{-}1} = [ML(OH)][H]/[M][L]$, with $\log \beta_{11\text{-}1} = \log \beta_{ML(OH)} - pK_w$.

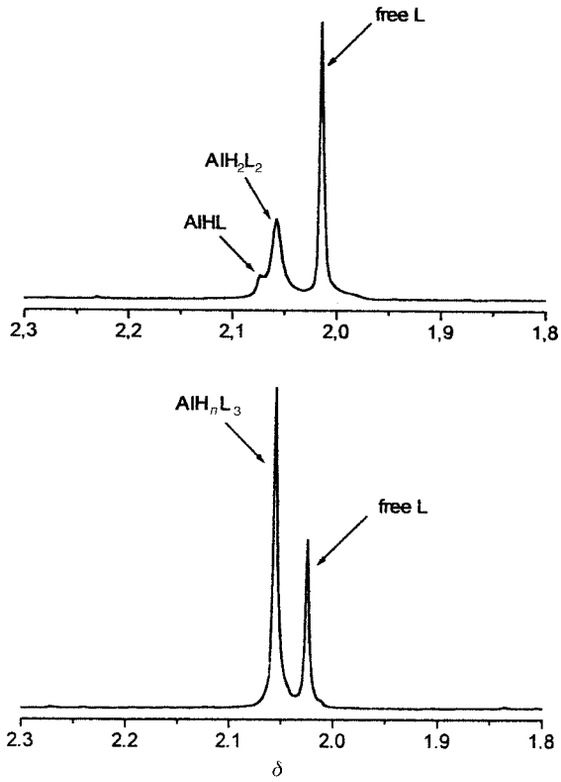

Fig. 2 ^{1}H NMR spectra for the Al^{3+}–HPH system in the 1:4 molar ratio (concentrations as in Fig. 1): pH* 4.2 (top) and 8.05 (bottom). Species assignments refer to the main complexes in the distribution profile in Fig. 1.

Table 3 Comparison between NMR and potentiometric results (Al^{III}-bound ligand percentages given as potentiometric results have been calculated from constants in Table 2 with reactant concentrations of NMR experiments as specified in the text)

	NMR		Potentiometry	
pH*	Al^{III}-bound ligand (%)	Average co-ordination number	Al^{III}-bound ligand (%)	Predominant species
4.20	46	1.83	45.25	MHL, MH_2L_2
5.43	51	2.05	60.00	MH_2L_2, MH_3L_3
6.64	54	2.17	72.00	MH_3L_3
8.05	69	2.75	73.25	MH_2L_3, MHL_3
9.16	61	2.45	64.25	MHL_3, ML_3
9.85	49	1.96	50.00	ML_3
10.95	11	0.45	11.25	ML_3, $Al(OH)_4^-$

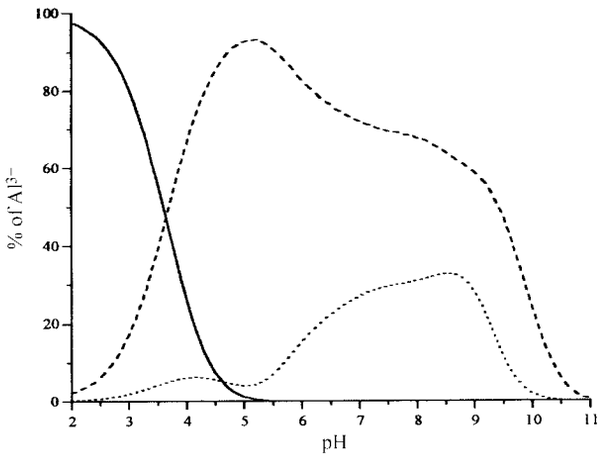

Fig. 3 Distribution profiles of free (solid line) and complexed fractions of aluminium in the presence of both HPH (dashed line) and α-Alaha (dotted line). The Al^{3+} : HPH : α-Alaha molar ratio was 1 : 8 : 8.

stoichiometry at the particular metal and ligand concentrations and concentration ratios used for NMR investigations; the distinct decrease in that number above pH* 9 is due to the progressively increasing importance of $Al(OH)_4^-$ (Fig. 1).

Electrospray mass spectrometry (ESMS) of the Al^{3+}–HPH system

ESMS was found to be a very useful technique to determine the solution stoichiometry and structure of hydroxamic acid complexes.[48–50] The ESMS spectra for the present system are rather complicated. Even so, two well defined peaks could be observed in the pH 6–8 range (*m*/*z* 417.1 and 379) that respectively correspond to $[Al(HL)_3 + K^+]^+$ and $[Al(HL)_3 + H^+]^+$, which again confirms the 1 : 3 metal-to-ligand stoichiometry as that of the main Al^{3+}–HPH complexes at the high ligand-to-metal concentration ratios used. The distinct peak observed at *m*/*z* 794.2 indicates the formation of an aggregate of the $[Al(HL)_3]_2K^+$ type. The former species represent the complexes preformed in solution while aggregates may be formed during the ionization process.[50] (The use of a ligand excess to avoid metal hydrolysis considerably complicated the spectra, making a more detailed ESMS data analysis very difficult.)

IR spectra of solid $Al(HL)_3 \cdot 2H_2O$

Elemental analysis and IR spectra (see above and ref. 12 for "free" ligand IR spectra) support the formation of $Al(HL)_3$ as the major species in the system studied. Mixing $AlCl_3 \cdot 3H_2O$ with HPH in the respective 1 : 3 molar ratio in aqueous solution in the presence of 3 equivalents of alkali effectively led to the isolation of a solid complex that was found to be $Al(HL)_3 \cdot 2H_2O$, a composition in accordance with potentiometric results that predict the predominance of this species around pH 8. The IR spectrum of this complex, in which the $N–O_{oxime}$ stretching mode band is only slightly shifted (5 cm^{-1}) with respect to that of the "free" ligand, clearly indicates that the oximic group does not interact with the Al^{3+} ion. In contrast, the significant shift (18 cm^{-1}) in the same spectrum of the $N–O_{hydroxamic}$ stretching band towards higher wavenumbers suggests that the hydroxamic group does contribute to aluminium co-ordination. In addition, the observation of a significant low frequency shift (35 cm^{-1}) of the amide I (C=O) band indicates that the amide oxygen also participates in co-ordination. Thus, the hydroxamic group is thought to chelate the Al^{3+} ion *via* the O,O mode whereas the oxime group is not involved in co-ordination.

Comparison of aluminium binding capacities of HPH and other monohydroxamates

Earlier investigations on simple α-alanine hydroxamic acid (α-Alaha) have shown that, within the concentration range used in the present studies, the major complexes formed were of 1 : 2 metal-to-ligand stoichiometry.[7] For amino acid derivatives with the amino group situated far away from the co-ordinated hydroxamic function, *e.g.* for β-alanine hydroxamic acid (β-Alaha) and the glutamic acid analog with a hydroxamic group at the γ position (Glu-γ-ha), the complexes formed are more resistant to hydrolysis but only bis complexes were suggested to be formed.[7] In order to visualize the comparison of the binding capacities of HPH and α-Alaha a competition plot for this pair of ligands was calculated. The stability constants for HPH were taken from Table 2 (25 °C), those for α-Alaha from ref. 7. The metal-to-ligand molar ratio was taken as 1 : 8 : 8 so that metal ion hydrolysis and precipitation were made negligible. The respective fractions of Al^{3+} bound to each of the two ligands opposed in such hypothetical solutions are presented in Fig. 3. HPH is considerably more effective in binding Al^{3+} ions than α-Alaha. The comparison with other amino acid derivatives including β-Alaha, β-Aspha and acetohydroxamic acid has shown that HPH has a comparable ability to bind Al^{3+} ions as other considered ligands. It is interesting that the competition with HPH of all the amino hydroxamic acids is more effective around pH 9 where their amino groups deprotonate. This observation is in line with the earlier suggestion[7] that the ammonium group may destabilize Al^{3+} monohydroxamate complexes, especially when it is in close proximity to the hydroxamic function (α-Alaha). Two main reasons for this destabilization were proposed:[7] an electron withdrawing effect, which makes the hydroxamic acid group more acidic, and an electrostatic repulsion between the NH_3^+ group and the co-ordinating M^{3+} ion. The acidity of HPH, however, is considerably higher than that of the above-mentioned ligands. Thus, the impact of the vicinal functions on the binding capacity of the hydroxamate moiety could be more complicated than that previously advanced.[7]

Quantum calculations

Recent theoretical calculations have shown[51] that it is possible to evaluate even low energy differences in the interactions of amino-hydroxamic groups with metal ions. Specific contributions of the above-discussed ligands to the stability of their respective aluminium(III) complexes have thus been determined similarly.

Five of the above ligands (with their hydroxamic oxygen being considered deprotonated) were subjected to B3LYP/6-31G** calculations. The most stable conformations of these ligands were determined by careful search of corresponding potential energy surfaces (Fig. 4). Of particular interest is the finding that the most stable structures of α-Alaha, β-Alaha and Glu-γ-ha imply proton transfer from NH_3^+ to the hydroxamic group.

The next calculation step addressed the structures of 1 : 1 metal-to-ligand complexes. In all of these the Al^{3+} ion was found to be attached to the deprotonated hydroxamic group, *i.e.* through its two electronegative centers. This means that, for α-Alaha, β-Alaha and Glu-γ-ha, aluminium(III) complexation cancels the proton transfer observed on the "free" ligand molecule, forcing the proton to move back onto the amino group.

All the complexes investigated are very strongly bonded, with interaction energies over 500 kcal mol^{-1}. However, two groups can be distinguished among these. The first includes ligands with −1 global electrical charge (acetohydroxamic acid, Aha, and HPH), the second neutral ligands (α-Alaha, β-Alaha and Glu-γ-ha). As can be seen in Table 4, complexes belonging to the first group are more stable than those of the second by more than 250 kcal mol^{-1}. Within the whole series, complex stability is observed in the order HPH > Aha > Glu-γ-ha ≈ β-Alaha > α-Alaha and HPH > Aha > β-Alaha ≈ Glu-γ-ha > α-Alaha

Table 4 DFT calculated interaction energies with their components (with 6-31+G** basis set) for all Al^{III}–ligand complexes investigated (expressed in kcal mol^{-1})

	Aha	HPH	α-Alaha	β-Alaha	Glu-γ-ha
$\Delta E_{CP\ (6\text{-}31G^{**})}$	−819.7	−833.5	−524.9	−549.9	−551.6
$\Delta E_{CP\ (6\text{-}31+G^{**})}$	−804.6	−817.4	−520.0	−545.2	−545.0
Rel. stability$_{(6\text{-}31G^{**})}$	13.8	**0.0**	308.6	283.6	281.9
Rel. stability$_{(6\text{-}31+G^{**})}$	12.8	**0.0**	297.4	272.2	272.4
E_{es}	−656.6	−622.6	−402.1	−448.5	−436.7
E_{ex}	114.2	86.5	103.1	107.3	102.4
E_{del} [a]	−262.8	−273.9	−266.6	−267.7	−271.8
E_{bind} [b]	−805.0	−810.5	−565.6	−608.9	−606.1

[a] The delocalization energy is equal to the sum of polarization, charge transfer and mixing contributions, *i.e.* $E_{del} = E_{PL} + E_{CT} + E_{MIX}$. [b] Note that the binding energy, E_{bind}, is calculated for monomers having the geometry in the complex. The difference between E_{bind} and ΔE_{CP} is the so-called distortion energy.

Table 5 B3LYP/6-31G** calculated bond lengths (*r*/*R*) of hydroxamic groups and their changes (*Δ*) due to complex formation (expressed in ångströms)

	Aha		HPH		α-Alaha		β-Alaha		Glu-γ-ha	
Bond	*r*/*R*	*Δ*	*r*/*R*	*Δ*	*r*/*R*	*Δ*	*r*/*R*	*Δ*	*r*/*R*	*Δ*
r(C=O)	1.333	0.081	1.306	0.049	1.330	−0.014	1.332	−0.009	1.325	−0.015
r(C–N)	1.327	−0.019	1.344	−0.005	1.315	0.008	1.317	0.006	1.316	0.004
r(N–H)	1.026	−0.001	1.026	0.002	1.031	−0.001	1.029	−0.002	1.027	−0.004
r(N–O)	1.388	0.061	1.341	0.030	1.387	0.108	1.389	0.108	1.383	0.104
R(Al···O=)	1.757		1.812		1.777		1.769		1.780	
R(Al···O–)	1.753		1.817		1.750		1.748		1.756	

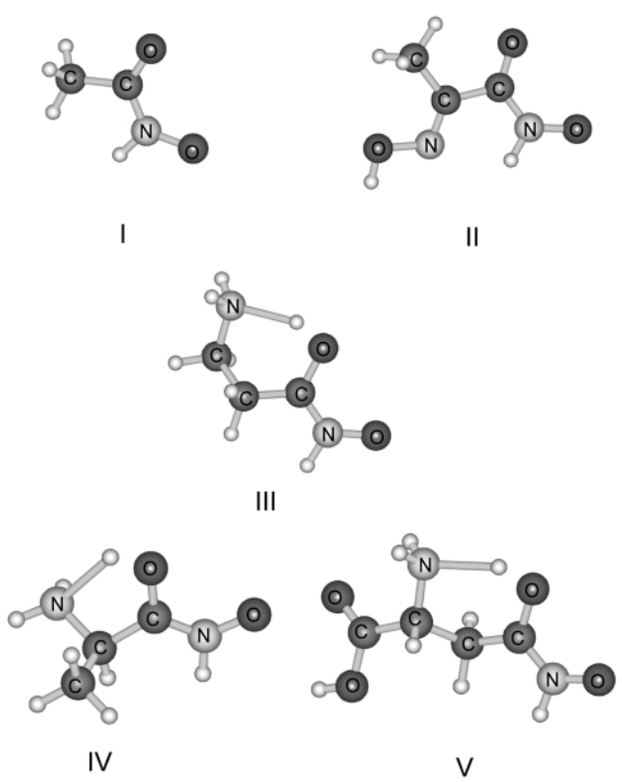

Fig. 4 The most stable ligand structures (I, Aha; II, HPH; III, β-Alaha; IV, α-Alaha; V, Glu-γ-ha) obtained from B3LYP/6-31G** calculations.

(same order as binding energies) with respect to the 6-31G** and 6-31+G** basis sets, which is in good accordance with the order of equimolar complex stabilities derived from potentiometric data.

In order to describe the nature of the interactions occurring in the above systems, the Kitaura–Morokuma procedure with the 6-31G** basic set was applied, the total interaction energy being decomposed into electrostatic, E_{es}, exchange-repulsion, E_{ex}, and delocalization, E_{del}, contributions. As may be expected from "hard"–"hard" interactions, the most important role was found to be played by electrostatic interactions (Table 4), exchange-repulsion terms being notably less important. As the delocalization term implies about the same additional stabilization for all complexes (except for the slightly larger stability of the Al–HPH complex), complex stability as a whole appears to be mainly determined by electrostatic interactions between metal ion and ligand. These in particular account for the above discrimination between the two groups of complexes. Incidentally, the effect of the proton back transfer from the hydroxamic group to NH_2 in α-Alaha, β-Alaha and Glu-γ-ha complexes induces a respective destabilization of 40–50 kcal mol^{-1} and is the reason for the huge distortion energy difference between complexes with and without proton transfer to the NH_2 group. The main influence on the interaction energy stems from the global electrical charge of the ligand.

Deformations of ligand geometrical parameters due to complex formation have been noted. For the sake of brevity, Table 5 reports the geometrical parameters of ligand atoms that are directly involved in the interaction with the Al^{3+} ion. Especially sensitive to complex formation are the C=O and N–O bonds of the hydroxamic group. Indeed, it is worth noting that the Al···O metal–ligand distance remains unchanged from one group of complexes to the other.

As mentioned above, the delocalization term, E_{del}, provides additional stability to the complexes formed. The results are corroborated by atomic charges calculated by the NBO method (Table 6). First, a charge transfer of about 0.5e is noted from the ligand to the metal ion and the decrease in the cation charge is almost proportional to the interaction energy of the complex. Moreover, the largest negative charge is localized on both oxygen atoms of the hydroxamic group.

Conclusion

As a preliminary step in the search for new molecules likely to be used as aluminium-sequestering agents, a new ligand, 2-(hydroxyimino)propanohydroxamic acid (HPH, an analog of alanine), has been synthesized and tested for its capacity to

Table 6 B3LYP/6-31G** calculated atomic charges (q) and changes in atomic charges (Δq) due to complex formation between Al^{III} and the hydroxamic group (expressed in e)

	Aha		HPH		α-Alaha		β-Alaha		Glu-γ-ha	
Atom	q	Δq	q	Δq	q	Δq	q	Δq	q	Δq
O=	−0.96	−0.12	−0.94	−0.11	−0.97	−0.21	−0.97	−0.19	−0.97	−0.19
C	0.88	0.13	0.81	0.15	0.83	0.21	0.86	0.23	0.87	0.24
N	−0.30	0.03	−0.26	0.01	−0.26	−0.13	−0.28	−0.13	−0.27	−0.14
H	0.53	0.19	0.53	0.15	0.55	0.16	0.54	0.15	0.54	0.15
O–	−0.79	0.01	−0.75	0.02	−0.78	−0.11	−0.78	−0.11	−0.79	−0.12
Al	2.46		2.34		2.52		2.51		2.51	

bind the Al^{3+} ion. A particular objective of this work was to examine the possibility for the HPH oximic group to take part in aluminium co-ordination together with the two hydroxamic oxygens.

The investigation of the Al^{3+}–HPH system by glass electrode potentiometry, within the largest reactant concentrations, concentration ratios and pH ranges possible excluding $Al(OH)_3$ precipitation, led to the characterization of seven complexes, four of which were of 1:3 metal-to-ligand stoichiometry with protonation levels from 0 to 3. The 1:3 metal-to-ligand stoichiometry of the predominant Al^{3+}-HPH complexes formed under ligand excess conditions was confirmed by proton NMR and electrospray mass spectrometries. From IR spectra of the neutral $Al(HL)_3$ species isolated in the solid state, it was inferred that HPH chelates the Al^{3+} ion through its two hydroxamic oxygens whereas its oximic group remains unaffected.

A comparison of the aluminum binding capacity of HPH with monohydroxamic acids revealed that HPH was a very competitive ligand. The analysis based on quantum calculations confirmed HPH to display high affinity for the Al^{3+} ion. As expected from "hard"–"hard" interactions, the Al^{3+}–HPH complex stability was shown mainly to stem from metal–ligand electrostatic interactions, the prime factor influencing complexation being therefore the global electrical charge of the ligand.

As to the possible therapeutic application of ligands of the HPH type, blood plasma simulations run with formation constants in Table 2 revealed that meeting this objective, though somewhat premature, is not out of reach: the aluminium mobilizing power of HPH in plasma is lower than that of ligands in clinical use[14] by two to three orders of magnitude only. Research on more efficient ligands is currently in progress in our laboratories.

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