Evaluation of 3,4-Hydroxypyridinecarboxylic Acids as Possible Bidentate Chelating Agents for Aluminium(III): Synthesis and Metal−Ligand Solution Chemistry

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In view of a possible application to aluminium(III) chelation therapy, 3-hydroxy-4-pyridinecarboxylic acid (3H4P) and 4-hydroxy-3-pyridinecarboxylic acid (4H3P) were synthesised, and their chemical interactions with the metal ion were investigated in aqueous 0.6 m (Na)Cl at 25 °C by means of potentiometric titrations. Only mononuclear complexes of the type AlL_lH_h (l = 1, 2 and 3; h = 0, 1, ..., l) were formed. The qualitative and quantitative results obtained were confirmed by UV spectrophotometry and ^{1}H NMR spectroscopy. The efficiencies of the ligands to chelate aluminium(III) were evaluated at physiological pH and ionic strength (in vitro), together with the n-octanol/water distribution coefficients of the free ligands and of the ligand–metal complexes. The results suggest that 3H4P and 4H3P lack sufficient chelation efficacy to be proposed as chelation therapy agents for aluminium(III). However, the results do suggest a strategy for the synthesis of more promising compounds.

Introduction

The therapy for metal overload pathologies usually involves the administration of suitable chelating agents to selectively remove the metal from the body. Regarding aluminium(III) and iron(III), medical research continuously emphasises the need for new safe, efficient and orally effective chelators.[1−3] The existing chelating agents, desferrioxamine and deferiprone, have a number of drawbacks, including oral inefficacy and high cost for the former,[4−6] and several toxic side effects and controversial efficiency for the latter.[5−8] An attempt to address this need is the chemical modification of existing chelating agents, to improve their in vivo properties.[9−11] Another important approach is the synthesis of new hexadentate ligands.[12−16] However, although there is some evidence that hexadentate ligands may be more efficient than bidentate and tridentate ligands, they are more expensive and thought to be less absorbed orally.[5,17,18] There are very few attempts to synthesise new tridentate ligands[19] and, to the best of our knowledge, no attempts to synthesise new bidentate ones other than deferiprone analogues.

Hydroxypyridinecarboxylic acids (HPs) represent a class of compounds that have not been considered for metal chelation therapy. The limited data in biomedical literature seems to demonstrate their lack of toxicity. Some have been proposed as drugs.[20−23] Their structure suggests a good ligating capability towards aluminium(III) and iron(III). The interactions between 2-hydroxy-3-pyridinecarboxylic acid (2H3P) and 3-hydroxy-2-pyridinecarboxylic acid (3H2P) with aluminium(III) have been investigated.[24] Although complexes with reasonably high stability constants form in solution, their stability is much lower than those of the corresponding desferrioxamine and deferiprone complexes. Therefore, neither 2H3P nor 3H2P appear to be good candidates for chelation therapy.

It is known, for hydroxypyridinones, that the 3,4 derivatives form significantly more stable complexes with aluminium(III) and iron(III), than the 2,3 derivatives.[25] The same behaviour is expected for HPs, which are structurally very similar to hydroxypyridinones. Therefore, an investigation of the stability of the metal complexes of 3,4-HPs is advisable.

In this work, the formation of complexes of aluminium(III) and 3-hydroxy-4-pyridinecarboxylic acid (3H4P),

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and 4-hydroxy-3-pyridinecarboxylic acid (4H3P) (Figure 1) have been studied in aqueous solutions by means of potentiometric titrations. The speciation data obtained has been integrated and confirmed by UV/Vis spectrophotometry, NMR spectroscopy and elemental analysis. The efficiency of the ligands to chelate aluminium(III) at physiological pH and ionic strength in vitro, and the octanol/water distribution of both the free ligands and the metal−ligand complexes, have been determined according to a method developed by Yokel et al.[26] For comparison, the efficiency and the octanol/water distribution of 2H3P and 3H2P have also been analysed.

Figure 1. 3-Hydroxy-4-pyridinecarboxylic acid (3H4P, left) and 4-hydroxy-3-pyridinecarboxylic acid (4H3P, right), shown as the H_2L forms

Results And Discussion

Synthesis of the Ligands

Among the methods proposed for the synthesis of 3H4P,[27,28−31] we selected the three-step procedure starting from 4-methylpyridine described by Dejarden.[27] The advantage of this pathway lies in the bromination of the starting material, which gives the precursor 3-monobromo derivative of 3H4P as the main reaction product as a result of the deactivation caused by the bromine atom itself. In all cases the yields were lower than those reported; for the hydrolysis of the bromo acid of 3H4P, no product was obtained under the literature conditions;[27] the use of a water/methanol mixture (6:4, v:v) for 12−24 h yielded over 40% of pure 3H4P (ref.[27] 80%).

The synthesis of 4H3P was accomplished via butadienedicarbonitriles according to the method of Mittelbach,[32] that produces the desired isomeric compound. This pathway, although requiring five steps, is quite straightforward and has proven to be more convenient (17% overall yield) than others.[33−36]

Thermodynamic Study

Acidity Constants of the Ligands

The pK_a values for H_2L and HL (here and in the following, charges are omitted for simplicity), corresponding to the deprotonation of the pyridine nitrogen atom and the phenol oxygen atom, respectively, were computed for the potentiometric titration data of the free ligands (Table 1).[24,37]

Table 1. Acidic properties of 3H4P and 4H3P, at 25 °C in aqueous (Na)Cl 0.6 m

Species	3H4P pK_a	n[a]	4H3P pK_a	n[a]	Source
H_3L	0.64 ± 0.03	4	−0.06 ± 0.04	3	UV
H_2L	4.713 ± 0.001	30	5.955 ± 0.002	24	pot.
HL	10.300 ± 0.004	15	10.85 ± 0.01	12	pot.

[a] n is the number of experiments from which the data were obtained; the reported uncertainty is the standard deviation of the mean.

Accurate potentiometric measurements at pH values lower than ca. 1.5 cannot be executed, because under these conditions the pH modification induced by the acid-base equilibria is negligible. Therefore, the more acidic pH region has been explored by means of UV spectrophotometric measurements, which allowed for the detection of H_3L and the calculation of its pK_a value (Table 1). This deprotonation has been assigned to the carboxylic group.[37] It may be noted that for the carboxylic group in nicotinic acid, $pK_{a1} \approx 2$;[38] as the *ortho*-phenol group can be expected to produce a significant increase in the acidity (for example, the carboxylic pK_a of salicylic acid is ca. 1.2 units lower than that of benzoic acid[38]), the values obtained for H_3L appear to be reasonable.

Metal−Ligand Complexes − Potentiometric Results

The potentiometric titrations of solutions containing both the metal ion and the ligand allowed for the identification of a large number of complexes, 9 in the case of 3H4P and 8 in the case of 4H3P; their stoichiometries are reported in Table 2, together with the values of their stability constants. The distribution diagrams for the two systems, containing an excess ligand concentration, are reported in Figure 2. It is worth noting that the precipitation of aluminium hydroxide was not observed in any systems in the presence of an excess ligand concentration (at least three-

Table 2. Stability constants for Al−L complexes, at 25 °C in aqueous (Na)Cl 0.6 m (reactions: $m\,\mathrm{Al} + l\,\mathrm{L} + h\,\mathrm{H} \rightleftarrows \mathrm{Al}_m\mathrm{L}_l\mathrm{H}_h$; pK_a values refer to the equilibrium $\mathrm{Al}_m\mathrm{L}_l\mathrm{H}_h \rightleftarrows \mathrm{Al}_m\mathrm{L}_l\mathrm{H}_{h-1} + \mathrm{H}$)

	3H4P			4H3P		
m, l, h	log β	pK_a	n[a]	log β	pK_a	n[a]
1, 1, 1	15.97 ± 0.05	5.13	5	18.19 ± 0.09		8
1, 1, 0	10.84 ± 0.06		4	−		
1, 2, 2	30.78 ± 0.02	5.31	20	35.15 ± 0.03	6.4	16
1, 2, 1	25.47 ± 0.04	5.97	4	28.7 ± 0.1	8.2	6
1, 2, 0	19.50 ± 0.03		10	20.5 ± 0.1		3
1, 3, 3	44.05 ± 0.03	5.36	16	50.76 ± 0.06	7.84	16
1, 3, 2	38.69 ± 0.07	6.02	10	42.92 ± 0.07	8.15	7
1, 3, 1	32.67 ± 0.06	6.58	10	34.77 ± 0.05	9.01	7
1, 3, 0	26.09 ± 0.07		6	25.76 ± 0.09		7

[a] n is the number of experiments from which the data were obtained; the reported uncertainty is the standard deviation of the mean.

fold), due to the high affinity of the ligands towards the metal ion.

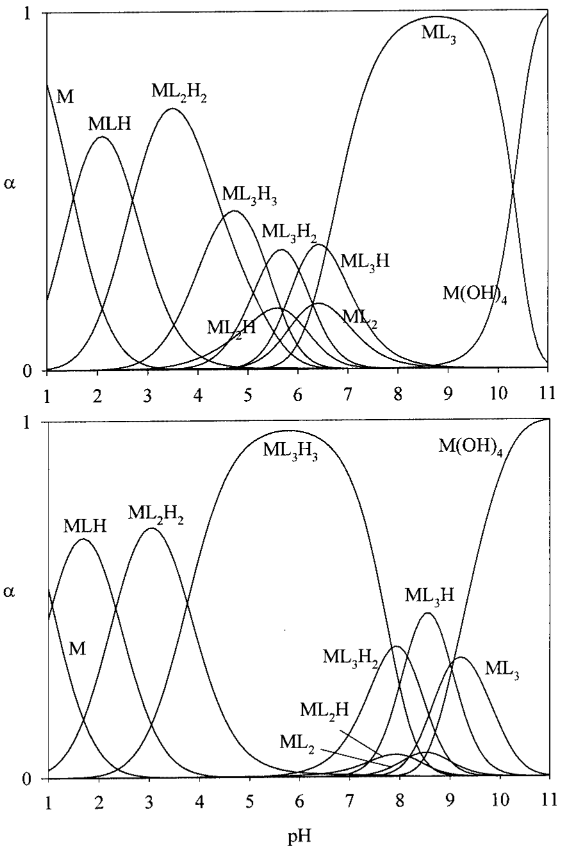

Figure 2. Distribution diagrams of the most important aluminium species in the presence of 3H4P (above) and 4H3P (below) in aqueous (Na)Cl 0.6 m, $T = 25$ °C; $[Al]_0 = 5 \cdot 10^{-4}$ m, $[ligand]_0 = 4 \cdot 10^{-3}$ m

Owing to the large number of species, the simultaneous optimisation of the metal−ligand complexation constants led to strong correlations between the various values. Therefore, the data has been elaborated in a sequential manner, considering the titrations with large metal/ligand ratios (1:1, 1:2) separately from the others. In this way, some of the complexes could be neglected in the former titrations and optimised only in the latter, and most of the correlations could be avoided. Of course the consistency of the above procedure needed to be verified a posteriori: distribution diagrams like those reported in Figure 2 have been drawn for the actual metal and ligand concentrations adopted for the former titrations in order to verify that the species rejected were actually negligible.

In some of the titrations of the system Al^{III}/3H4P, the precipitation of a white powder was observed (for $[Al]_0 >$ ca. $8 \cdot 10^{-4}$ m, $[Al]_0/[L]_0 \leq 1:3$ and pH > ca. 5−6). The solid was examined by elemental analysis (C = 37.9, H = 3.1, Al = 6.92, N = 7.1, Na = 4.22). The relative mol ratios between aluminium, carbon and nitrogen were calculated. The values obtained, 1:12.3:1.96, are in close agreement with the theoretical ratios 1:12:2 expected for an AlL_2 species (= $C_{12}H_8AlN_2O_6$). The 1:2 metal/ligand stoichiometry is also suggested by the observation that in all the titrations, the precipitation occurred when the concentration of the neutral species AlL_2H (as calculated from the stability constants reported in Table 2) reached a value of about $3 \cdot 10^{-4}$ m. The solid obtained is likely to be coordinated with water molecules (elemental analysis suggests two) and with NaCl (elemental analysis suggests Na/Al = 0.7). Unfortunately, the stoichiometry and the structure of this solid cannot be defined exactly because no crystals suitable for X-ray analysis could be obtained.

The distribution curves in Figure 2 show that the speciation in the two aluminium−ligand systems is very similar at acidic pH values, where only the free metal ion and the complexes AlLH and AlL_2H_2 exist in solution. At pH > 4, 3H4P forms a large number of complexes, which include AlL_3H_3 and the deprotonation products of the 1:2 and 1:3 metal/ligand species; AlL_3 and $Al(OH)_4$ are the only existing metal species at pH values larger than ca. 8. On the contrary, 4H3P forms only one main species, AlL_3H_3, in the pH range 4−6, and the deprotonation products only appear at higher pH values; among these, AlL_3 is not as predominant as in 3H4P. The different behaviour of 3H4P and 4H3P is due to the different acidity of the pyridine hydrogen atom of the two ligands (pK_{a2}, Table 1), the pK_a value of 3H4P is 1.2 log units lower. This acidity difference is further increased in the metal complexes (pK_a values, Table 2), and therefore, the deprotonation products of the Al/3H4P species are more stable than those of Al/4H3P. For the same reason, the species AlL is not present in a significant amount in the Al/4H3P system.

Two interesting properties regarding the acidity of the complexes in both aluminium/ligand systems should be noted. (i) For the Al/3H4P system, the acidity decreases systematically (i.e. pK_a increases) in the order $H_2L > AlLH > AlL_2H_2 > AlL_3H_3$ (Table 2). For the Al/4H3P system, no pK_a value for AlLH is available, but the acidity order for the other species is the same. The decrease in the acidities is less marked for 4H3P than for 3H4P. (ii) The increase in the pK_a values of the complexes having the same number of ligands (namely AlL_2H_2 and AlL_2H; AlL_3H_3, AlL_3H_2 and AlL_3H) by decreasing the number of acidic protons is close to that expected on statistical grounds (for example, the K_a ratio of the 3H4P complexes AlL_2H_2/AlL_2H is 4.6, the statistical value is 4). All these results are very similar to those obtained for the complexes formed by 2H3P and 3H2P with aluminium(III)[24] and iron(III).[39] In ref.[24] an explanation is given for both the effects.

In order to confirm the potentiometric results with some independent techniques, UV spectrophotometric quantitative data and NMR spectroscopic qualitative and semiquantitative data have also been obtained.

Metal−Ligand Complexes − UV Spectrophotometric Results

UV spectra for the Al/4H3P system at several pH values are reported in Figure 3. The following value was obtained for log β (AlLH): log $\beta_{1,1,1}$ = 18.03 ± 0.10 (fitting of 5 spectra, values at three λs). This value compares well (*t*-test) with the corresponding potentiometric value (18.19 ± 0.09). The agreement between these results suggests the absence of any bias on final results.

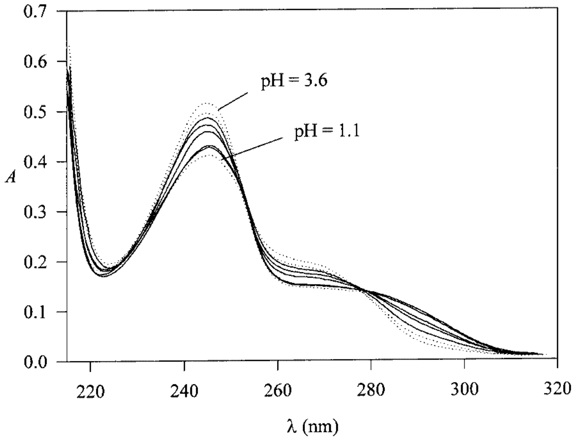

Figure 3. UV spectrophotometric study for Al/4H3P; see Table 6 for experimental details; dashed curves were excluded for the log β calculation

Metal−Ligand Complexes − NMR Spectroscopic Results

^{1}H NMR spectra were collected for both 4H3P and 3H4P systems at various pD values in D_2O solutions containing only the free ligand or the ligand with the metal ion. As an example, the spectra obtained for the 4H3P system are shown in Figure 4.

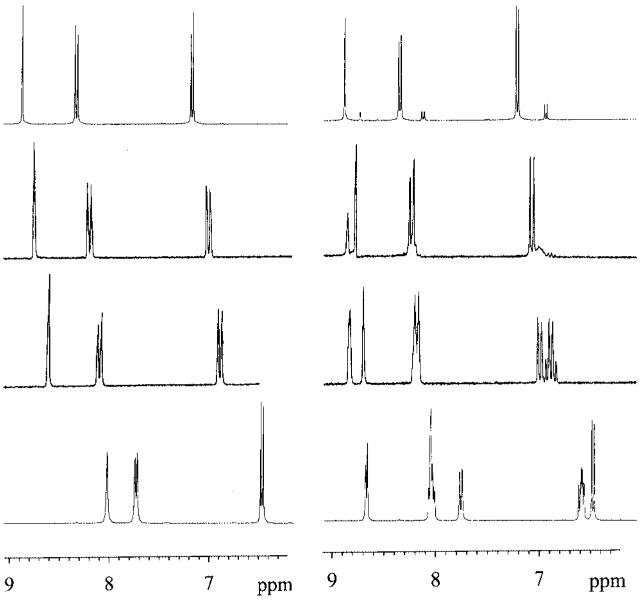

Figure 4. Left side: ^{1}H NMR spectra of 4H3P alone (0.050 m); right side: ^{1}H NMR spectra of Al^{III} (0.010 m) + 4H3P (0.050 m); from top to bottom: pD = 0.11, 1.73, 5.20, 8.58

On complexation with aluminium, three new groups of peaks in addition to those of the free ligand are observed. At pD = 0.11 the multiplicity and the shape of the "new" peaks are almost identical to those of the free ligand; according to the potentiometric data, these signals can be assigned to the AlLH complex. On increasing the pH, the peaks of the complexes become more complicated, since under these conditions different species coexist in solution (AlL_2H_x and AlL_3H_x). Furthermore, as the ligands are not symmetric and may chelate the metal ion in different spatial configurations, AlL_2H_x and AlL_3H_x can form with 4 and 2 different diastereoisomers, respectively, each with a slightly different NMR response. At pD = 5.20 and 8.58, the patterns of the signals become more simple, because, according to potentiometric data, only the AlL_3H_x species (and therefore fewer diastereoisomers) exist in solution.

The dependence of the chemical shift of the different signals on the pD can be explained by the acid-base properties of the ligand and the complexes: deprotonation of the pyridine nitrogen atom causes a shift of the ligand signals towards lower ppm values; this shift is maximal at pD = pK_a. However, for a correct interpretation it is necessary to take into account the difference in the pK_a values of the acid in D_2O and in H_2O due to isotopic effects. In order to estimate the pK_a difference, the empirical equation p$K_a(D_2O)$ = 0.41 + 1.02·p$K_a(H_2O)$ has been proposed[40] (for example, the pK_a values for 4H3P in heavy water are: pK_{a1} ≈ 0.3, pK_{a2} ≈ 6.5 and pK_{a3} ≈ 11.5). Considering the NMR spectra in the light of the above, the lowest-field signal at pD = 0.11 is due to the free ligand, whereas the signals at pD = 1.73 and 5.20 are assigned to the complexes since the free ligand deprotonates between pD = 0.11 and 1.73 ($H_3L \rightarrow H_2L$) and the complex species do not [for example, for AlL_2H_2 p$K_a(D_2O)$ ≈ 6.9]. For a similar reason, at pD = 8.58 the chemical shift difference between the same lowest-field signals of the complexes and of the free ligands become significantly larger than in the spectrum at pD = 5.20. This is because in this pD range the free ligand further deprotonates ($H_2L \rightarrow HL$), whereas the complexes deprotonate only partially [for example, for AlL_3H_3 p$K_a(D_2O)$ ≈ 8.4]. These semi-quantitative data are in good agreement with the potentiometric results.

The integration of the signals of the free ligand and of the complexes gives the relative amount of free and complexed ligand; the values obtained are reported in Table 3, together with the corresponding values calculated from the stability constants reported in Table 1 and Table 2. The agreement between the two sets of data is good, in view of the uncertainties of the NMR integration values, and of the presence of isotopic and solvent effects introduced by the change in solvent from H_2O to D_2O.

n-Octanol/Water Distribution and Chelation Efficacy Study

The results obtained for each ligand are summarised in Table 4. 2H3P and 3H2P have also been analysed for comparison.

A significant increase in efficiency can be observed for the 3,4-HPs, and especially for 4H3P, with respect to the

Table 3. Percentages of free and complexed ligand (Al/4H3P spectra)

pD	from NMR spectroscopic data		from potentiometric/UV results	
0.11	free ligand	88.8	$H_3L + H_2L$	90.4
	complexed ligand	11.2	$AlLH + 2\ AlL_2H_2$	9.6
1.73	free ligand	61.5	$H_3L + H_2L$	64.2
	complexed ligand	38.5	$AlLH + 2\ AlL_2H_2 + 3\ AlL_3H_3$	35.8
5.20	free ligand	42.7	$H_2L + HL$	40.1
	complexed ligand	57.3	$2\ AlL_2H_2 + 3\ AlL_3H_3 + 3\ AlL_3H_2$	59.9
8.58	free ligand	44.2	$H_2L + HL + L$	40.3
	complexed ligand	55.8	$3\ AlL_3H_3 + 3\ AlL_3H_2 + 3\ AlL_3H + 3\ AlL_3$	59.7

Table 4. Octanol/water distribution coefficients, and Al^{III} chelation efficiencies

Ligand	$D_{o/w}$ free ligand	$D_{o/w}$ Al complexes	Chelation efficiency (%)
3H4P	0.014 ± 0.001	0.005	20 ± 2
4H3P	0.004 ± 0.001	0.002	43 ± 6
2H3P	0.0006 ± 0.0001	–	4 ± 1
3H2P	0.006 ± 0.001	–	13 ± 2

2,3-HPs. This is in agreement with the thermodynamic data. The very low lipophilicity of all the ligands and the metal−ligand complexes at physiological pH values should be noted; in fact, from thermodynamic data it is known that at pH = 7.4 both the free ligand and the metal−ligand complexes are predominantly in the charged form (HL^- and deprotonated $AlL_3H_x{}^{(3-x)-}$ species, respectively). Actually, the octanol/water distribution of the Al ligand complexes could not accurately be calculated from its distribution in the presence and the absence of Al borate, because a significant (if not predominant) percentage of the ligand was not complexed with Al, as evidenced by the generally low efficiency obtained. The values obtained for 3H4P and 4H3P suggest that the metal−ligand complexes are slightly more hydrophilic than the free ligand.

Conclusions

Both 3H4P and 4H3P form strong complexes in solution with aluminium(III) such that no metal hydroxide precipitates at neutral pH in the presence of a threefold (or higher) ligand excess. The speciation patterns of the two ligands with aluminium(III) are similar, and a number of 1:1, 1:2 and 1:3 metal/ligand species are formed in solution.

In order to compare the affinities of the various HPs (3H4P, 4H3P, 3H2P and 2H3P) for the metal, the direct analysis of the data reported in Table 2 (and in the corresponding Table of ref.[24]) is not useful since the competition arising from the proton (i.e. the basicity of the ligand) cannot be taken into account. The comparison could be accomplished by computing the conditional stability constants of the complexes, however, in the literature the calculation of pAl (= $-\log [Al^{3+}]$) vs. pH at a given metal and ligand concentration is much more common. Generally,[41] the pAl is computed at a metal concentration of 10^{-6} m and at a ligand concentration of $5{\cdot}10^{-5}$ m. For different systems, the higher the pAl, the stronger the complexes. In Figure 5, pAl plots for the four HPs are shown, together with that of a deferiprone-like molecule (2-methyl-3-hydroxy-4-pyridinone, 2m3h4p, data taken from ref.[42]). It is evident that 4H3P forms significantly more stable complexes with aluminium than the other HPs, especially at slightly acidic and neutral pH values. This result is as expected (see Introduction) and confirms that 3,4-HPs are more promising chelating agents than 2,3-HPs. Surprisingly, the pAl of 3H4P is quite low, similar to those of the 2,3-HPs; this latter data cannot be explained as yet. However, it is important to note from Figure 5 that the pAl of 4H3P is significantly lower than that of the deferiprone-like molecule at all pH values.

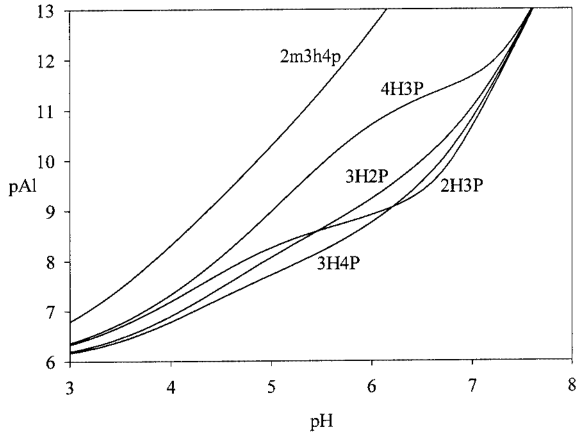

Figure 5. pAl for several ligands (see text); $[Al]_0 = 10^{-6}$ m, $[ligand]_0 = 5{\cdot}10^{-5}$ m

The chelation efficiencies give an indication of the ability of the ligands to solubilise aluminium(III) at physiological pH. The data obtained correlate well with the thermodynamic results, and a reasonably high efficiency, 43%, was obtained for only 4H3P.

Table 5. Potentiometric titrations (titrant: NaOH)

Titrations	Conditions $[Al]_0/10^{-3}$ m	$[L]_0/10^{-3}$ m	$[Al]_0/[L]_0$	pH
1.				
3H4P + HCl	–	0.51–1.27	–	2.7–10.5
4H3P + HCl	–	0.36–2.98	–	2.1–10.5
2.				
Al + HCl	0.50–2.88	–	–	2.4–3.5
	1.01–3.39	–	–	
3.				
Al + 3H4P + HCl	0.50–3.81	1.88–4.01	1:1;1:2;1:4;1:8	1.7–10.0
Al + 4H3P + HCl	0.31–3.01	1.58–4.95		

The octanol/water distribution coefficients $D_{o/w}$ indicate that all HPs and their aluminium complexes show a marked hydrophilic behaviour. Porter[43] has suggested that an orally effective Fe (or Al) chelator should probably have a $D_{o/w}$ of 0.2 or greater to be absorbed. However, in our evaluation of a series of 3,4-hydroxypyridinones we found that some compounds with a $D_{o/w}$ much lower were among the most orally bioavailable and effective chelators of Al.[44–46]

In conclusion, the thermodynamic data and the chelation efficiencies suggest that the HPs considered in this work cannot be regarded as possible alternatives to desferrioxamine and deferiprone in the chelation therapy of aluminium(III). The synthesis of more lipophilic HPs with higher stability constants towards the metal ion is advisable. For deferiprone-like molecules, both of these characteristics have been obtained by introducing electron-donating, lipophilic groups into the pyridinic ring. The synthesis of 1- and 2-methyl HP derivatives is therefore now in progress.

Experimental Section

Synthesis of the Ligands: The ligands 3H4P and 4H3P (which are not commercially available) were prepared acording to reported multi-step methods,[27,32] with some modifications. All chemicals and solvents were obtained from Aldrich Chimica or Janssen Chimica (now Acros). The identity and purity of all intermediates and final compounds were checked by melting point (m.p.) measurements (Gallenkamp MFB 595 010 M/B capillary m.p. apparatus), ^{1}H NMR spectra (Bruker 300 MHz), TLC (Merck silica gel 60 F-254 glass plates), mass spectrometry (Mat 112 Varian Mat Bremen mass spectrometer) and elemental analyses (Perkin–Elmer Elemental Analyzer Model 240B).

3H4P: White needles. Yield 24% (4 g). M.p. 321 °C (ref.[32] 316–319 °C). ^{1}H NMR (in [D$_6$]DMSO), $\delta = 7.83$ (d, $J_{5,6} = 5.2$ Hz, 1 H, 5-H), 8.10 (d, $J_{6,5} = 5.2$ Hz, 1 H, 6-H), 8.39 (s, 1 H, 2-H) ppm. $C_6H_5NO_3$ (139.11): calcd. C 51.80, H 3.62, N 10.07; found C 51.72, H 3.65, N 9.97.

4H3P: White needles. Yield 17% (4 g). ^{1}H NMR ([D$_6$]DMSO): $\delta =$ 6.73 (d, $J_{5,6} = 5$ Hz, 1 H, 5-H), 8.07 (d, $J_{6,5} = 5$ Hz, 1 H, 6-H), 8.61 (s, 1 H, 2-H), 12.99 (s, 1 H, OH), 15.70 (s, 1 H, COOH) ppm. EI MS (70 eV): *m/z* (%) = 139 (100) [M$^+$], 122 (80.66) [M$^+$ − OH], 94 (63.94) [M$^+$ − COOH]. $C_6H_5NO_3$ (139.11): calcd. C 51.80, H 3.62, N 10.07, found C 51.74, H 3.59, N 10.00.

Thermodynamic Study: The experimental apparatus, reagents and measurement methods were almost the same as those reported previously.[47] In the following, a summary will be given; details are reported only when different from those described previously.

Apparatus: All potentiometric measurements were performed using a Radiometer ABU93 Triburette apparatus. UV spectra were recorded using Perkin–Elmer Lambda 20 and Lambda 5 instruments, ^{1}H NMR spectra using Varian Gemini 200 MHz and Bruker 300 MHz instruments. The elemental analyses were performed using an Iris Intrepid ICP-AES for Al and Na, and a Perkin–Elmer Elemental Analyzer Model 240B for C, N and H.

Reagents: All analyte concentrations were expressed using the molality scale (mol/kg of water). For potentiometric and UV spectrophotometric measurements, working solutions of HCl (0.1 m), NaOH (0.1 m) and $AlCl_3$ (0.05 m, containing HCl 0.01 m) were prepared and standardised as described previously;[47] 3H4P and 4H3P were used as synthesised to prepare 0.004 m and 0.05 m working solutions, respectively, (the concentrations correspond to about 80% of their water solubility). The ionic strength of all the solutions was adjusted to 0.6 m (≈ 0.594 M) (Na)Cl. Solutions for ^{1}H NMR spectroscopic measurements were prepared by dissolving weighted amounts of ligand, $AlCl_3$ (Fluka, ≥ 99%) and NaCl in D_2O.

Potentiometric Measurements: Titrations were carried out at 25 ± 0.1 °C; duplicate potentiometric measurements were performed using two different glass electrodes (Radiometer pHG201 and BDH 309/1015/02) and an Ag/AgCl/0.6 m NaCl reference electrode,[48,49] with a J-shaped junction; its acidic liquid junction potential has been determined previously[39] and can be represented by the equation[49] $E_j = -S\cdot\log(1 + J\cdot[H^+])$, with $J = 4.251 \pm 0.003$. Besides the calibration and the base standardisation experiments,[47] the titrations reported in Table 5 were performed. All stability constants were calculated using the computer program PITMAP.[50] The hydroxometal species reported by Öhman[51] have been considered and the corresponding log β values were kept constant during the processing of the data. After the addition of the metal ion and either of the two ligands to an acidic solution (pH < 3), the measured e.m.f. drifted and reached a constant value only after ca. 1 h, suggesting a low complex formation rate. The kinetics of the aluminium–ligand reaction became "normal" (equilibration after ca. 1 min) at pH values > 3. These findings are in agreement with previous results with 2,3-HPs.[24,52] Experimental details regarding the treatment of the slow kinetics during the titrations are reported.[24]

UV Spectrophotometric Measurements: The measurements reported in Table 6 were performed. The pH of the solutions was computed

Table 6. UV spectrophotometric measurements

System			Conditions		
	$[Al]_0/10^{-4}$ m	$[L]_0/10^{-4}$ m	pH	λ [nm] for calculation	Cell length [cm]
3H4P	–	6.00	0.30−2.90	215; 245; 322; 346	0.1
4H3P	–	6.61	0.23−1.80	226; 248; 283	0.1
Al + 4H3P	0.806	1.16	1.10−3.60	245; 262; 290	1

from the stoichiometric concentration of HCl,[53] because the changes in $[H^+]$ produced by the other species were always negligible under the chosen conditions. The pK_a for the equilibrium $H_3L^+ \rightleftarrows H_2L + H^+$ (for both ligands) and the log β for the equilibrium $H_2L + Al^{3+} \rightleftarrows AlLH^{2+} + H^+$ (for 4H3P only) were computed using a described method.[24,47]

^{1}H NMR Spectroscopic Measurements: Spectra were obtained in D_2O for solutions containing only the ligand (0.0041 m in the case of 3H4P and 0.050 m in the case of 4H3P) and for solutions also containing aluminium (0.010 m and 0.0012 m, respectively, with the same ligand concentration as before) at various pH values, in the presence of (Na)Cl 0.6 m. The pH was adjusted with small additions of concentrated HCl or NaOH solutions (prepared in D_2O), and was measured with a glass electrode BDH 309/1025/02 previously calibrated in aqueous solutions containing (Na)Cl 0.6 m and known concentrations of HCl. 0.41 pH units were added to the pH-meter readings to correct for isotopic and solvent effects,[54] i.e. "pD" rather than "pH" values were measured.

n-Octanol/Water Distribution and Chelation Efficacy Studies

Materials: NaCl, KCl, $NaHCO_3$, H_3BO_3 and *n*-octanol were obtained from EM, Fisher, or Sigma; Al borate from Pfaltz and Bauer (Waterbury, CT) and an Al atomic absorption standard from Sigma. Polypropylene test tubes (10 mL) with polyethylene caps were purchased from VWR.

Method: The octanol/water distribution of the ligands (3H4P, 4H3P, 2H3P and 3H2P), their ability to solubilise Al as an indicator of chelation potential, and the octanol/water distribution of the aluminium−ligand complex were assessed in an octanol/aqueous system, as described previously;[26] 4 mL of *n*-octanol and 4 mL of an aqueous buffer were added to 10-mL test tubes. The tubes also contained Al borate, ligand or Al borate + ligand. Al borate (7−8 mg) was added as a source of Al, which is quite insoluble, but not kinetically inert. The ligand was introduced in the aqueous phase at $1 \cdot 10^{-3}$ M. The aqueous phase contained 125 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl and 100 mM H_3BO_3 at pH = 7.4. The tubes were shaken overnight at room temperature in order to achieve equilibrium. Al was quantitated in the aqueous and octanol phases of tubes that contained Al borate, using a Perkin−Elmer 4100 ZL atomic absorption spectrophotometer, S-70 sampler and DEC workstation. All samples were analysed at least twice at λ = 309.3 nm. The concentration of the ligand, both in the presence and absence of Al borate, was determined in the aqueous and octanol phases using a Shimadzu UV2501PC recording spectrophotometer. Samples were compared, at the isosbestic point of absorbance of the ligand (λ = 306.0, 298.3, 257.0 and 305.4 nm for 3H4P, 4H3P, 2H3P and 3H2P, respectively), to aqueous standards containing the ligand in the absence of Al borate. The amount of Al in each phase in the absence of ligand was subtracted from the amount of Al in the presence of ligand, as a measure of the Al solubilised by the chelator. Since 1:3 complexes form for all compounds (see thermodynamic results above and ref.[24]), the efficiency of the test compound to solubilise (chelate) Al was calculated as threefold the sum of the increase in Al (in mol) in the two phases in the presence of ligand, divided by the molar concentration of the ligand introduced into the aqueous phase ($1 \cdot 10^{-3}$ M). The octanol/water distribution of each ligand was determined from its concentration in the octanol/aqueous phase (in the absence of Al borate). The octanol/water distribution of the Al−ligand complex was calculated from the total ligand concentration in each phase (determined by UV spectrophotometry in the presence of Al borate) minus the calculated free ligand concentration, as described.[55] The total free ligand concentration was calculated from (1-% efficiency/100) × the total chelator concentration. The free ligand concentration in each phase was calculated from the octanol/water distribution of the ligand. The results are the mean of three or four independent experiments containing duplicate observations for each aluminium−ligand system.

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