Biologically active pyridine mono- and bis-phosphonates: efficient ligands for co-ordination of Cu^{2+} ions

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Potentiometric and spectroscopic studies on the co-ordination ability of biologically important pyridine mono- and bis-phosphonates have shown that when sterically possible these compounds undergo tridentate co-ordination with Cu^{2+} ions. The complexes obtained are very stable. When the position of the nitrogen donor is sterically unfavourable the major binding occurs at the bis(phosphonate) site.

Our recent works on the co-ordination abilities of phosphonic analogues of carboxylic amino acids have shown that substitution of the carboxylic function by the phosphonic group changes drastically the metal binding abilities of these molecules. Substitution of the planar, weakly basic, mono-negative carboxylate by the more bulky, tetrahedral, di-negative and more basic phosphonate has a considerable effect on the structure and stability of the metal complexes formed. It has also a critical impact on their biological activity. Some heterocyclic dicarboxylic acids, like quinolinic acid (2,3-pyridinedicarboxylic acid) and its derivatives, are excitatory when applied to neurons. These neuroactive agents resembling N-methyl-D-aspartate may act as agonists of neuronal receptors. The replacement of the carboxylic function by the phosphonic group in a neuroactive molecule leads usually to a substance with antagonistic activity.

In this work copper(n) complexes with 6-phosphonopyridine-2-carboxylic acid (H_L^1) and -3-carboxylic acid (H_L^2), potential neuroactive compounds, and (3-methyl-2-pyridyl-amino)methylene bisphosphonic acid (H_L^4), an efficient herbicide, were studied by potentiometric and spectroscopic (UV/VIS, EPR) methods. A minomethylene bisphosphonic acid (H_L^4), a detergent and anticalculus agent, was also studied for comparison with H_L^4.

Experimental
Phosphonic acid synthesis

The compounds H_L^1 and H_L^2 were synthesized as described earlier. The bisphosphonic acid (H_L)^2 was prepared as follows: a mixture of aminodiphenylmethane (9.2 g, 0.05 mol), triethyl orthoformate (7.5 g, 0.05 mol) and diethyl phosphonate (13.8 g, 0.1 mol) was heated at 150–165°C for 3–4 h. The volatile materials evolved were collected separately using a condenser. The remainder was treated with toluene (50 cm^3) and then refluxed for 4 h, cooled, and the separated organic layer was removed. The remaining aqueous layer was evaporated. The residue was dissolved in hot water (100 cm^3), decolorized with charcoal, filtered and evaporated again to give an oily product. The oil was dissolved in hot aqueous methanol (50 cm^3) and refrigerated. The white crystals of the final product were filtered off and dried. Yield 25–30%, m.p. 220°C (decomp.). N M R (D_2O): H_4, δ 2.91 [t, J (CH–P) = 13.2 H, δ 12.13] (Found: N, 7.7; P, 31.85. Calc. for CH_N O_P^2N, 7.35; P, 32.45%.

The compound H_L^4 was synthesized as described earlier.

Potentiometric measurements

The purities and the exact concentrations of the stock solutions of the phosphonic acids were determined pH-metrically by the Gran method. The well separated protonation steps of the phosphonate groups were used in Gran evaluation. The concentration of the copper(n) stock solution was measured gravimetrically via precipitation of the quinolin-8-olate. The stability constants of the proton and copper(n) complexes of the phosphonic acids were determined by pH-metric titration of 5.00 cm^3 samples. The experimental parameters were as follows: phosphonic acid concentrations 0.002–0.004 mol dm^{-3}; metal-ion concentrations 0.001–0.002 mol dm^{-3}; metal ion to acid molar ratios 0.1: 1, 1:1: 2 or 1:1; ionic strength adjusted to 0.2 mol dm^{-3} (KCl); pH range studied 2.0–11.0; duplicate pH titration calibrated in concentration; pK_w = 13.76; temperature 25 ± 1°C.

The pH was measured with a Radiometer pH M 64 instrument, G 2040B glass and K 4040 calomel electrodes, and a TTA titration unit. The concentration stability constants were calculated with the aid of the PSEQUAD computer program.

Spectroscopic measurements

Visible absorption spectra were recorded with a Beckman DU 650 spectrometer, EPR spectra on a Bruker ESP 300E spectrometer at 9.2 GHz at liquid-nitrogen temperature in ethylene glycol-water (1:2 v/v) solutions, H N M R spectra on a Bruker AMX 300 M Hz spectrometer in D_2O solutions and UV/VIS
spectra were deconvoluted by analysis with the multipeaks Gaussian function of the M (icroal ORIGIN program.19

Results and Discussion

6-Phosphonopyridinecarboxylic acids

Both HLL and HLL contain three dissociable protons within the measurable pH range (Table 1). One of the two phosphonic protons dissociates at pH < 1 and cannot be evaluated from the pH-metric data obtained here. The second acidic deprotonation site with pK of 1.0 for HLL and 1.93 for HLL seems to be localised at the carboxylic function. The pK values obtained for pyridine-2,6- or -2,5-dicarboxylic acid clearly indicate that while one of the carboxylates is strongly acidic (pK < 1 is not measurable in standard pH-metric measurements), the other is almost the same for both derivatives (pKCOOH = 2.1 and 2.17, respectively14). The presence of the mononegative PO3H− substituent on the pyridine ring seems to differentiate the carboxylates at positions 2 and 3, and their pK values differ by 0.8 log unit. The other two protonation constants correspond to the second phosphonate and pyridine nitrogen. An upfield shift of the signal of the proton in position 2 of the aromatic ring (HLL) by 0.23 ppm in the 1H NMR spectra, following a change from pH 2 to 5, as well as the lack of such a shift between pH 5 and 7 may indicate that the pK = 3.73 corresponds to deprotonation of the pyridine ring nitrogen.

The metal complex speciations derived from potentiometric calculations for HLL and HLL differ considerably (Figs. 1 and 2). Only two complexes, [CuL]2 and [CuL]3, are formed as major species in the case of HLL, while for HLL five species are present, including three protonated complexes, [Cu(HLL)]2 and [Cu(HLL)]3 (Table 1). The formation of a single equimolar [CuL] complex with HLL at pH < 2 strongly suggests tridentate co-ordination of the ligand. The sterically possible simultaneous binding of carboxylic oxygen, pyridine nitrogen and phosphonate oxygen of HLL is also reflected in a very high stability constant (log K = 10.47) of the [CuL] complex. Owing to this fact the proton-displacement reaction Cu2+ + HLL → [CuL]2 + 2H+ is shifted strongly to the right at the starting pH value even in an equimolar solution [Cu2+] = 10% of the total copper(II) concentration. This is reflected in the rather high uncertainties in the log β values (see Table 1), although the speciation model is quite simple. In the case of HLL the carboxylate group is separated from the chelating function and is unable to co-ordinate the metal ion simultaneously, due to steric reasons. Thus, in the complex [Cu(HLL)] the Cu2+ ion binds to the (N Py, PO3−2) chelate donor system and the protonated carboxylate is unbound. Deprotonation of this species leads to the formation of [CuL]2. The stability constant of [CuL] species with HLL is 2.6 log units lower than that

<table>
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<tr>
<th>Parameter</th>
<th>HLL-1</th>
<th>HLL-2</th>
<th>HLL-3</th>
<th>HLL-4</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(HLL)</td>
<td>7.69(1)</td>
<td>6.69(1)</td>
<td>10.43(1)</td>
<td>10.89(1)</td>
<td>10.81</td>
<td>10.11</td>
</tr>
<tr>
<td>K(HLL)</td>
<td>7.69(1)</td>
<td>6.69(1)</td>
<td>10.43(1)</td>
<td>10.89(1)</td>
<td>10.81</td>
<td>10.11</td>
</tr>
<tr>
<td>K(HLL)</td>
<td>=1.0</td>
<td>1.93(9)</td>
<td>5.18(6)</td>
<td>5.30(1)</td>
<td>2.47</td>
<td>1.0</td>
</tr>
<tr>
<td>K(HLL)</td>
<td>=1.0</td>
<td>1.93(9)</td>
<td>5.18(6)</td>
<td>5.30(1)</td>
<td>2.47</td>
<td>1.0</td>
</tr>
<tr>
<td>log β[Cu(HLL)]</td>
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<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>2.47</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Stability constants of the proton (log K) and copper(II) complexes (log β) of some phosphonates at 25 °C and I = 0.2 mol dm−3 (KCl)

![](image1)

Fig. 1 Species distribution curves for the complexes formed in the copper(II)-HLL system as a function of pH; cCu = 0.002 mol dm−3; cHLL = 0.006 mol dm−3

![](image2)

Fig. 2 Species distribution curves for the complexes formed in the copper(II)-HLL system as a function of pH; cCu = 0.002 mol dm−3; cHLL = 0.006 mol dm−3
of H₃L² (Table 1), confirming the tridentate co-ordination of the latter and the bidentate binding of the former. It is interesting that the log K value for the reaction [Cu(H₃L)] = [Cu⁺] + H₃L is much higher (3.26) than the carboxylate pK of the free H₃L² (1.93). The metal ion co-ordination to the {NₓPₓOᵧ}²⁻ donor set leads to complete deprotonation of both groups involved in metal binding and a strong increase in the negative charge on the pyridine ring. This may be the main cause for a distinct increase in the protonation constant of the 3-CO₂⁻ group in the [Cu(H₃L)] species. At first glance, the similar stability constants obtained for the bis complexes with both H₃L¹ and H₃L², log β = 14.55 and 14.23, respectively, could suggest a similar binding mode in these [Cu₃L₃]²⁻ species.

The spectroscopic data (both absorption and EPR) are, however, distinctly different, indicating different geometries and donor sets involved in metal ion co-ordination. The tridentate co-ordination of the {NₓPₓOᵧ}²⁻ donor set is possible only when one donor is bound to the metal ion axially. This may lead to a strongly distorted square-pyramidal rather than a tetragonal geometry with unusual EPR parameters (very low g₁ and relatively high g₂ (Table 2). Low A₁ values are observed for both H₃L¹ species, [CuL¹] and [CuL₃], indicating that also in the bis complex tridentate co-ordination of at least one ligand occurs (Table 2). The A₂ and g₁ values for the [CuL¹] and [Cu₃L₃]²⁻ complexes of H₃L² are similar to those for simple tetragonal systems with one or two nitrogens involved in the metal-ion binding.¹² The variations of the d-d transition energies also support this. The simple chelation of H₃L² by {NₓPₓOᵧ}²⁻ shifts the d-d transition band towards higher energies expected for 1N ([CuL¹]) or 2N ([Cu₃L₃]) co-ordination,¹²,¹⁷ while tridentate co-ordination changes d-d transitions only slightly when compared to those of Cu²⁺(aq) (=800 nm). The apical co-ordination diminishes the high-energy shift caused by the equatorial nitrogen binding in both the mono and bis complexes with H₃L². Thus, the similarity in stability constants of the bis complexes is rather accidental. The low value of log K₂ for H₃L¹ (log K₂ = 4.08) suggests a bidentate axial-equatorial co-ordination of the second ligand. For H₃L² this log K₂ value is much higher (6.36), as the bidentate chelation of the first ligand in [CuL¹] leaves enough coordination sites free in the equatorial plane for a much stronger equatorial chelation of the second ligand.

### Bis(phosphonic acids)

The compound H₄L₃ has five protonation sites (Table 1) with a relatively basic amino nitrogen (pK = 11.03). The high basicity of this donor stems from the high negative charge of the two deprotonated dinegative phosphonic groups. The protonation constants for both phosphonic functions correspond well to those found for other bis(phosphonic acids) by taking into account the electron-attracting effect of the nearby NH₂⁺ group, which slightly increases the acidity of these groups.³⁴

During the titration of the Cu²⁺-H₄L₃ system at a 1:1 metal ion to phosphonic acid ratio slight precipitation was observed in the acidic pH range. The precipitate is most likely a neutral phosphonato complex. In order to obtain reliable pH-metric data over as wide a pH range as possible, back titrations with 0.2 mol dm⁻³ HCl starting from pH 11 were also carried out. In this way the precipitation down to pH 3 could be avoided even under equilinor conditions, probably due to the formation of oversaturated solutions. The joint evaluation of the pH titration data obtained in this way indicates the formation of four major species, [Cu(H₄L)], [Cu(H₃L)], [CuL¹] and [Cu₃L₃]²⁻ and one minor complex [CuL(Η₂L)]²⁻ (Fig. 3). The complex [CuL¹]²⁻ dominating at pH > 8 is very likely to contain a tridentate co-ordinated ligand through the amino and the two phosphate donors. Both the d-d transition (686 nm) and the EPR parameters (Table 2) suggest tetragonal geometry around Cu²⁺ and 1N binding in the equatorial plane. The tridentate co-ordination of the ligand is clearly reflected in the much higher stability of the complex when compared to that of the bidentate [NH₂P₂O₄]⁻ co-ordinated ligand through the amino and two phosphonate groups having bidentate co-ordination. Accordingly, the equilibrium between the two isomers of [Cu(H₄L)] is only 0.006 mol dm⁻³, which indicates a continuous rearrangement of the tridentate (NH₂P₂O₄⁻, PO₄⁻) bonding mode to a mixture of linkage isomers having bidentate co-ordination. The complex can bind two protons with log K ([Cu(Η₂L)]²⁻) = 5.86 and log K ([CuL(Η₂L)]²⁻) = 3.79. These stepwise protonation processes are accompanied by a significant shift of the d-d transition to ~740 nm, which indicates a continuous rearrangement of the tridentate (NH₂P₂O₄⁻, PO₄⁻) bonding mode to a mixture of linkage isomers having bidentate co-ordination. This may be supported by the d-d transition at around 743 nm, while for a species with two monoprotonated phosphonic functions this should be shifted to around 800 nm. The formation of the [Cu₃L₃]²⁻ complex at pH > 8 leads to a distinct shift in the d-d transition to higher energies (from 686 to 657 nm). This indicates that two nitrogens are involved in co-ordination. The parameters of the EPR spectra suggest a tetragonal geometry around Cu²⁺ in the bis-co-ordinated H₄L₃ system as a function of pH; ε = 0.002 mol dm⁻³, c₉/O₄ = 0.006 mol dm⁻³.

### Table 2

Spectroscopic parameters for copper(II) complexes formed by some phosphonates

<table>
<thead>
<tr>
<th>Ligand Species</th>
<th>UV/VIS λ/ε (𝜇mumol⁻¹ cm⁻¹)</th>
<th>EPR</th>
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<tr>
<td>H₃L¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[CuL¹]</td>
<td>101</td>
<td>772(46)*</td>
</tr>
<tr>
<td>[CuL²]</td>
<td>104</td>
<td>781(48)*</td>
</tr>
<tr>
<td>[Cu(H₃L¹)]</td>
<td>149</td>
<td>705(69)*</td>
</tr>
<tr>
<td>[Cu(H₃L²)]</td>
<td>157</td>
<td>679(53)*</td>
</tr>
<tr>
<td>[Cu₃L₃]</td>
<td>166</td>
<td>287(1196)*</td>
</tr>
<tr>
<td>[CuL(Η₂L)]</td>
<td>211</td>
<td>743(84)*</td>
</tr>
<tr>
<td>[Cu(Η₂L)]</td>
<td></td>
<td>289(1883)*</td>
</tr>
<tr>
<td>[CuL¹]</td>
<td>866(45)*</td>
<td></td>
</tr>
<tr>
<td>[CuL²]</td>
<td>289(1883)*</td>
<td></td>
</tr>
<tr>
<td>[Cu₃L₃]</td>
<td>735(63)*</td>
<td></td>
</tr>
</tbody>
</table>

* d-d Transition. # Unresolved and very broad EPR spectrum. * Some precipitation was observed. * NH₃⁺ → Cu²⁺ charge-transfer transition. *

### Fig. 3

Species distribution curves for the complexes formed in the copper(II)-H₄L₃ system as a function of pH; c₉/O₄ = 0.002 mol dm⁻³, c₉/O₄ = 0.006 mol dm⁻³.
complex, and thus the binding mode is based on the formation of two effective \( \text{N} \text{H}_2, \text{PO}_2 \) chelate rings. Some apical interaction of the second phosphonate cannot be excluded. The compound \( \text{H}_4 \) also has five protonation sites, four of them at the two phosphonic groups and one at the pyridine nitrogen. The lack of an adjacent positively charged amino group results in high values of the first and second phosphonic protonation constants (log \( K([\text{H}L]^+) = 10.49 \) and log \( K([\text{H}L]^2) \approx 8.05 \)), while the other two (log \( K([\text{H}L]) \) and log \( K([\text{H}L]^+) \)) are similar to those observed for \( \text{H}_4 \) (Table 1). High protonation constants for the phosphonic groups are usually observed for bis(phosphonates).  

The set of complex species calculated from potentiometric titrations for \( \text{Cu}^{2+}-\text{H}_4 \) up to pH 8 is similar to that discussed above for \( \text{Cu}^{2+}-\text{H}_3 \) (Figs. 3 and 4). The stability constants of the \([\text{Cu(HL)}], [\text{Cu(H}_2L)]^+\) and \([\text{CuL}]^2\) complexes formed, are however distinctly lower than those for the corresponding \( \text{H}_3 \) complexes (Table 1). The log \( \beta \) values of the \([\text{CuL}]^2\) species of \( \text{H}_4 \) (14.32) and that of \( \text{H}_2L \) (11.59) indicate that the pyridine-N donor of \( \text{H}_4 \), if bound at all, is much less effective in metal binding than is the amino group of \( \text{H}_3 \). This is due partly to its lower basicity (Table 1) and its unfavourable position within the molecule. The lesser binding ability of \( \text{H}_4 \) compared to \( \text{H}_3 \) could also be attributed to steric hindrance by the phenyl moiety to amine N-coordination. Accordingly, in the case of the \( \text{H}_4 \) complexes the proposed equilibrium between isomers (see above) is shifted strongly to the purely phosphonate-chelated species, e.g. the d-d transition at 800 nm with low \( \epsilon \) value observed for \([\text{Cu(HHL)}]\) strongly suggests the binding of two protonated phosphonic functions (see above and ref. 14). Both the stability data and absorption spectra support this assumption also for other species (Table 2). The d-d transition energy of 740–750 nm for the \([\text{Cu(HHL)}]\) and \([\text{CuL}]^2\) complexes and very low \( \epsilon \) values when compared to nitrogen-bound complexes (see e.g. complexes with \( \text{H}_4 \) in Table 2) correspond to chelation of one or two deprotonated phosphonic groups. Further, the basicity-adjusted equilibrium constants (log \( K^* \), which take into account the differences in the basicity of the co-ordinating donors, and characteristic of the reaction \( \text{Cu}^{2+} + [\text{HL}]^- \rightarrow [\text{CuL}]^+ + 3\text{H}^+ \) for \( \text{H}_4 \) and 1-hydroxy-2-propyl-1,3-dithiphosphonic acid only able to participate in bis(phosphonate) chelation), agree fairly well with each other (log \( K^* \approx -8.05 \) for \( \text{H}_4 \) and −8.23 for the pentane derivative). The less effective binding properties of the pyridine \( N \) are reflected in the less favoured formation of the \([\text{CuL}]^2\) complex. As seen in Fig. 4, besides the bis complex a deprotonated 1:1 complex \([\text{CuHL}]^+\) is also formed in significant concentration in the \( \text{Cu}^{2+}-\text{H}_4 \) system. The deprotonation leading to this species at pH 9 corresponds to the ionisation of a metal-bound water molecule; log \( K \) of 9.93 is expected for this type of hydrolysis reaction. A corresponding, the much weaker co-ordination of the second \( \text{H}_4 \) molecule (log \( K([\text{CuL}]^2) \approx 3.53 \) vs. 4.74 for \( \text{H}_4 \)) will not prevent hydrolytic reactions, namely the formation of mixed-ligand hydroxocomplexes.

Acknowledgements

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References