# Complexes of Aminophosphonates. Part 3.† Copper(II) Complexes of Several Monophosphono and Diphosphono Dipeptides

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The stoicheiometries and stability constants of the proton and copper(II) complexes of several monophosphono dipeptides (where the C-terminal carboxylate group is replaced by a phosphonate moiety) and diphosphono dipeptides (where both the C-terminal carboxylate and the N-terminal amino groups are replaced by phosphonate moieties) have been determined pH-metrically at 25 °C and at an ionic strength of 0.2 mol $dm^{-3}$ (KCl). From the stability data and spectral parameters of the complexes, it has been established that the $PO_3^{2-}/CO_2^-$ and $PO_3^{2-}/NH_2$ substitutions do not significantly change the basic bonding modes of the simple dipeptides. However, the copper(II)-induced deprotonation and co-ordination of the peptide-amide group becomes less favoured, although, similarly to the $NH_2$ group, the terminal $PO_3^{2-}$ group can act as an anchor to bind the metal ion and thus induce deprotonation of amide NH.

Peptides of aminoalkylphosphonic acids are of considerable interest because of their biological activity. For instance, many phosphonic acid analogues of dipeptides show significant antibacterial properties, act as effective enzyme regulators, or display promising antitumour activity.[1–4]

In earlier publications[5,6] we have characterized the complex-forming abilities of some phosphonic acid analogues of simple bidentate and potentially tridentate amino carboxylic acids, such as alanine, phenylalanine, tyrosine, aspartic acid, and glutamic acid, with several 3*d* transition-metal ions. The effects of $PO_3^{2-}/CO_2^-$ substitution on the complex-forming properties were explained in terms of the differences in basicity, charge, and size of the phosphonate and carboxylate groups.

In the present work those studies have been extended to various monophosphono dipeptides, alanyl- or leucyl-aminoethylphosphonic acids, $XNHCH(R)PO_3H_2$, where the terminal carboxylate is substituted by a phosphonate group, 1-(*N*-L-alanylamino)-2-isopropylethylphosphonic acid [Ala-Leu-(*P*)], 1-(*N*-L-leucylamino)ethanephosphonic acid [Leu-Ala-(*P*)], 2-isopropyl-1-(*N*-L-leucylamino)ethanephosphonic acid [Leu-Leu-(*P*)], and (*N*-L-alanylamino)cyclopropylmethylphosphonic acid [Ala-cp-Gly-(*P*)], and to diphosphono dipeptides [*N*-(phosphonoacetyl)aminophosphonic acids, $H_2O_3PCH_2CONHCH(R)PO_3H_2$], where both the terminal carboxylate and the terminal amino groups are substituted by phosphonate groups 2-isopropyl-1-[*N*-(phosphonoacetyl)amino]ethylphosphonic acid [(*P*)-Mal-Leu-(*P*)] and cyclopropyl[*N*-(phosphonoacetyl)amino]methylphosphonic acid [(*P*)-Mal-cp-Gly-(*P*)].

The principal bonding modes of simple dipeptides (HA) to copper(II) ions are well established.[7] In the initial step of complex formation the metal ion co-ordinates to the terminal amino and the peptide-carbonyl groups and a species $[CuA]^+$ is formed. At higher pH (≈ 4—5) copper(II) ion-induced deprotonation and co-ordination of the peptide amide take place, to yield the species $[CuAH_{-1}]$ involving tridentate N,N,O co-ordination. At pH 9—10 the equatorial water molecule ionizes and a species $[CuAH_{-1}(OH)]^-$ is formed. Besides these 1:1 complexes, formation of bis complexes can also be detected: mainly $[CuA(AH_{-1})]^-$ with a five-co-ordinate structure and a polynuclear species $[Cu_2(AH_{-1})_2(OH)]^-$ containing a monohydroxy bridge.

The aim of the present work was to study the influence of the substitution of one or both terminal binding sites of dipeptides by phosphonate groups on the metal-ion co-ordination properties of simple dipeptides with non-co-ordinating side-chains. For this purpose, the stoicheiometries, stability constants, and bonding modes of the species formed in systems containing copper(II) and the above phosphono peptides were determined by pH-metric, spectrophotometric, and e.s.r. techniques.

## Experimental

The phosphono peptides were obtained by the methods described in refs. 8—10. The purities and the exact concentrations of the solutions of the ligands were determined by the method of Gran.[11] The concentration of the copper(II) chloride stock solution was determined gravimetrically *via* precipitation of the quinolin-8-olate.

The stability constants of the proton and metal complexes of the ligands were determined by pH-metric titration of 5-$cm^3$ samples. The ligand concentration in the various samples was $4 \times 10^{-3}$ mol $dm^{-3}$ and the metal ion:ligand ratio was 1:1, 1:2, 1:4, or 1:6. The ionic strength was adjusted to 0.2 mol $dm^{-3}$ with KCl in each case. Titrations were performed over the range pH 3—11 with a KOH solution of known concentration (*ca.* 0.2 mol $dm^{-3}$).

The pH was measured with a Radiometer pHM64 instrument with G2040 B and K4040 calomel electrodes, using a TTA 80 titration unit. The electrode system was calibrated by the method of Irving *et al.*,[12] so that the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

To establish the bonding modes in copper(II) complexes formed, visible spectra were recorded with a Beckman UV 5240 recording spectrophotometer. E.s.r. spectra were obtained on a JEOL JMN-3X spectrometer at 9.15 GHz and 120 K in methanol–water (1:1) mixtures.

† Part 2 is ref. 6.

**Table 1.** Dissociation constants of the ligands at 25 °C and $I$ = 0.2 mol $dm^{-3}$ (KCl)

| Dipeptide | $pK_{CO_2H}$ | $pK_{NH_3^+}$ | |
|---|---|---|---|
| Ala-Leu* | 3.15 | 8.32 | |
| Leu-Leu* | 3.20 | 8.34 | |
| Phosphono dipeptide | $pK_{PO_3H_2}$ | $pK_{PO_3H^-}$ | $pK_{NH_3^+}$ |
| Ala-Leu-(*P*) | 1.5 ± 0.1 | 6.69 ± 0.02 | 8.35 ± 0.01 |
| Leu-Ala-(*P*) | 1.6 ± 0.1 | 6.49 ± 0.02 | 8.23 ± 0.02 |
| Leu-Leu-(*P*) | 1.6 ± 0.1 | 6.67 ± 0.02 | 8.27 ± 0.01 |
| Ala-cp-Gly-(*P*) | 1.8 ± 0.1 | 6.61 ± 0.02 | 8.24 ± 0.01 |
| Diphosphono dipeptides | $pK_{PO_3H_2}$ | $pK_1$ | $pK_2$ |
| (*P*)-Mal-Leu-(*P*) | 2.04 ± 0.04 | 6.21 ± 0.02 | 7.96 ± 0.02 |
| (*P*)-Mal-cp-Gly-(*P*) | 2.05 ± 0.03 | 6.30 ± 0.01 | 7.80 ± 0.01 |

* Ref. 17, 20 °C.

The concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[13] Depending on the type of ligands, the fully deprotonated forms have different charges, *i.e.* $A^-$ refers to simple dipeptides, $A^{2-}$ to monophosphono dipeptides, and $A^{4-}$ to diphosphono dipeptides. Hence species with the same stoicheiometric composition may have different charges. For this reason the charges of the complexes are generally omitted.

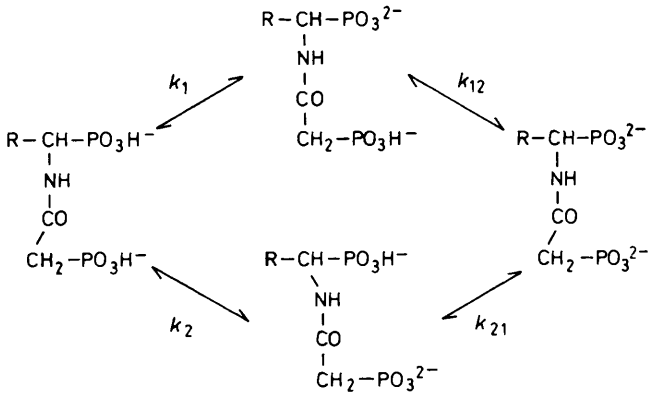
## Results and Discussion

The acid dissociation constants of the ligands together with those of some simple dipeptides (Ala-Leu and Leu-Leu), are given in Table 1.

The monophosphono dipeptides contain three dissociable protons, while the diphosphono dipeptides contain four. These are the protons of the $-PO_3H_2$ and the $-NH_3^+$ groups for the former ligands and the four protons of the two $-PO_3H_2$ groups for the latter ligands. The first proton of $PO_3H_2$ is very acidic, however[14,15] ($pK \approx 1.0$ for aminophosphonic acids), and thus it is fully deprotonated in the pH range studied (2—11). If the acidities of a $-CO_2H$ group in an amino acid and in a dipeptide molecule are compared a decrease in acidity (a 0.8—0.9 log unit increase in $pK$) is observed.[16] A similar effect is found in the case of the phosphonic acid analogues, with higher $pK$ values for the $PO_3H_2$ groups of the phosphono peptides. The $pK$ values characteristic of the dissociation of the $PO_3H_2$ and $PO_3H^-$ groups of the ligands studied are therefore generally higher than those of the simple aminophosphonic acids [*e.g.* $pK_{PO_3H_2} \approx 1.0$ and $pK_{PO_3H^-} = 5.55$ for Ala-(*P*)[5]]. The $PO_3H_2/CO_2H$ substitution has hardly any effect on the acidity of the terminal $-NH_3^+$ group (see Table 1) because of the large distance between the acidic groups ($pK_{NH_3^+} = 8.32$ and 8.34 for Ala-Leu and Leu-Leu, respectively[17]).

In the diphosphono dipeptides, the first measured proton dissociation can be ascribed to one of the $PO_3H_2$ groups (the other is even more acidic and is fully deprotonated at pH > 2). The values of $pK_2$ and $pK_3$, however, cannot be ascribed unambiguously to the dissociation of one or the other terminal $PO_3H^-$ group, since only a little if any difference in acidity of the two $PO_3H^-$ groups can be expected and thus the two processes overlap one another considerably. If it is assumed that the acidities of the C-terminal $PO_3H^-$ groups of the corresponding monophosphono and diphosphono dipeptides [Ala-Leu-(*P*) and (*P*)-Mal-Leu-(*P*), and Ala-cp-Gly-(*P*) and (*P*)-Mal-cp-Gly-(*P*)] are the same ($pK$ = 6.69 and 6.61, respectively), then the microconstants characteristic of the

**Table 2.** Dissociation microconstants * of the diphosphono dipeptides at 25 °C and at 0.2 mol $dm^{-3}$ (KCl)




| Ligand | $pk_1$ | $pk_2$ | $pk_{12}$ | $pk_{21}$ |
|---|---|---|---|---|
| (*P*)-Mal-Leu-(*P*) | 6.69 | 6.38 | 7.48 | 7.79 |
| (*P*)-Mal-cp-Gly-(*P*) | 6.61 | 6.59 | 7.49 | 7.51 |

* See text.

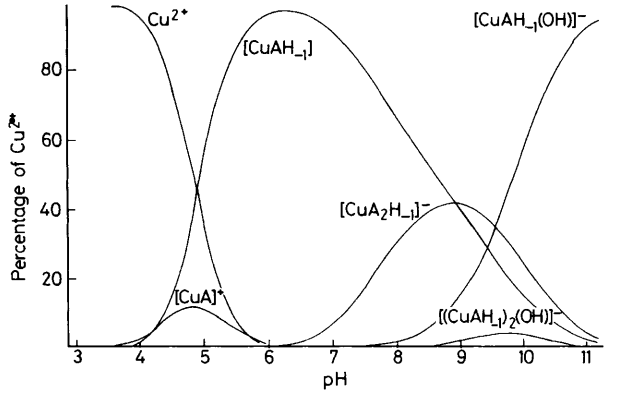



**Figure 1.** Concentration distribution of the complexes formed in the copper(II)–Ala-Leu system as a function of pH. Calculated with the data given in ref. 19; $c_{Cu}$ = 0.004, $c_{ligand}$ = 0.008 mol $dm^{-3}$

acidities of the individual groups can be calculated[18] (see Table 2). It can be seen that, in accordance with expectations, the actual acidities of the two terminal $PO_3H^-$ groups do not differ significantly ($pk_1 \approx pk_2$) and the considerable hindrance of the dissociation of the second proton is due mainly to electrostatic reasons.

These results are in accordance with the $pK$ values obtained for the methylenediphosphonic acid ($pK_1 \approx 1.0$, $pK_2$ = 2.49, $pK_3$ = 6.87, and $pK_4$ = 10.54),[19] where the two acidic groups are much nearer to each other and the electrostatic repulsion between them is enhanced.

The titration curves for the copper(II)–ligand systems were evaluated by assuming complexes formed with simple dipeptides; because of the higher basicity of $-PO_3^{2-}$ as compared to that of $-CO_2^-$, the formation of protonated species was also taken into account. The best fits between the measured and calculated titration curves were obtained by assuming the species given in Table 3. For comparability, the Table contains literature data[20] relating to some corresponding simple aliphatic dipeptides, such as Ala-Leu and Leu-Leu. For a more exact understanding of the role of $PO_3^{2-}/CO_2^-$ substitution in

**Table 3.** Copper(II) complex formation constants of the ligands at 25 °C and at $I$ = 0.2 mol dm$^{-3}$ (KCl)

| Dipeptides | Diphosphono dipeptides | Ala-Leu* | Leu-Leu* | (*P*)-Mal-Leu-(*P*) | (*P*)-Mal-cp-Gly-(*P*) |
|---|---|---|---|---|---|
| — | $[CuAH]^-$ | — | — | 12.18 ± 0.03 | 12.30 ± 0.02 |
| $[CuA]^+$ | $[CuA]^{2-}$ | 5.47 | 4.99 | 6.49 ± 0.02 | 6.41 ± 0.02 |
| $[CuAH_{-1}]$ | $[CuAH_{-1}]^{3-}$ | 1.14 | 0.88 | −1.35 ± 0.04 | −1.26 ± 0.03 |
| $[CuAH_{-1}(OH)]^-$ | $[CuAH_{-1}(OH)]^{4-}$ | −8.26 | −8.67 | −11.24 ± 0.05 | −11.19 ± 0.09 |
| — | $[CuA_2]^{6-}$ | — | — | 9.35 ± 0.1 | 8.98 ± 0.09 |
| $[CuA_2H_{-1}]^-$ | — | 4.18 | 4.24 | — | — |
| — | $[Cu_2(AH_{-1})_2(OH)]^{7-}$ | — | — | −9.73 ± 0.09 | −9.89 ± 0.2 |
| $[CuAH]^- \rightleftharpoons [CuA]^{2-} + H^+$ | | — | — | −5.69 | −5.89 |
| $[CuA] \rightleftharpoons [CuAH_{-1}] + H^+$ | | −4.32 | −4.11 | −7.84 | −7.67 |
| $[CuAH_{-1}] \rightleftharpoons [CuAH_{-1}(OH)] + H^+$ | | −9.40 | −9.53 | −9.89 | −9.93 |
| $[CuA]^{2-} + A^{4-} \rightleftharpoons [CuA_2]^{6-}$ | | — | — | 2.86 | 2.57 |
| $[CuAH_{-1}] + A^- \rightleftharpoons [CuA_2H_{-1}]^-$ | | 3.04 | 3.36 | — | — |

| Monophosphono dipeptides | Ala-Leu-(*P*) | Leu-Leu-(*P*) | Leu-Ala-(*P*) | Ala-cp-Gly-(*P*) |
|---|---|---|---|---|
| $[CuAH]^+$ | 12.34 ± 0.03 | 12.01 ± 0.06 | 11.50 ± 0.14 | 12.26 ± 0.04 |
| $[CuA]$ | 6.69 ± 0.03 | 6.72 ± 0.03 | 6.88 ± 0.03 | 6.80 ± 0.03 |
| $[CuAH_{-1}]^-$ | 1.11 ± 0.03 | 0.98 ± 0.02 | 1.65 ± 0.02 | 1.45 ± 0.02 |
| $[CuAH_{-1}(OH)]^{2-}$ | −8.27 ± 0.03 | −8.56 ± 0.02 | −8.02 ± 0.02 | −8.00 ± 0.02 |
| $[CuA_2]^{2-}$ | 12.11 ± 0.08 | 11.87 ± 0.09 | 11.95 ± 0.09 | 12.55 ± 0.05 |
| $[CuA_2H_{-1}]^{3-}$ | 3.75 ± 0.10 | 2.83 ± 0.12 | 3.03 ± 0.10 | 4.13 ± 0.07 |
| $Cu^{2+} + HA^- \rightleftharpoons [CuAH]^+$ | 5.65 | 5.34 | 5.01 | 5.65 |
| $[CuAH]^+ \rightleftharpoons [CuA] + H^+$ | −5.65 | −5.29 | −4.62 | −5.46 |
| $[CuA] \rightleftharpoons [CuAH_{-1}]^- + H^+$ | −5.58 | −5.74 | −5.23 | −5.35 |
| $[CuAH_{-1}]^- \rightleftharpoons [CuAH_{-1}(OH)]^{2-} + H^+$ | −9.38 | −9.54 | −9.67 | −9.45 |
| $[CuA] + A^{2-} \rightleftharpoons [CuA_2]^{2-}$ | 5.42 | 5.15 | 5.07 | 5.75 |
| $[CuAH_{-1}]^- + HA^- \rightleftharpoons [CuA_2]^{2-}$ | 4.31 | 4.17 | 3.62 | 4.30 |
| $[CuAH_{-1}]^- + A^{2-} \rightleftharpoons [CuA_2H_{-1}]^{3-}$ | 2.64 | 1.85 | 1.38 | 2.68 |
| $[CuA_2]^{2-} \rightleftharpoons [CuA_2H_{-1}]^{3-} + H^+$ | −8.36 | −9.04 | −8.92 | −8.42 |

* See ref. 20, 20 °C.

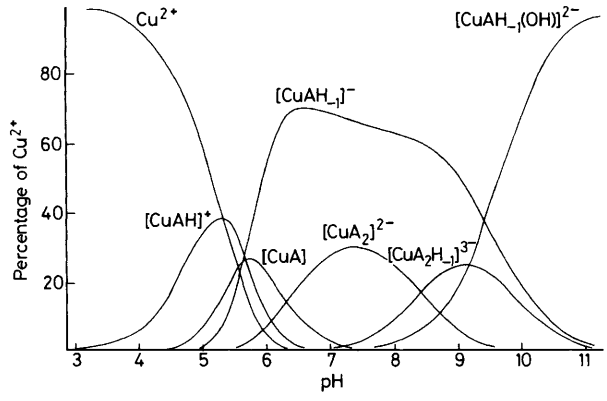



**Figure 2.** Concentration distribution of the complexes formed in the copper(II)–Ala-Leu-(*P*) system as a function of pH; $c_{Cu}$ = 0.004, $c_{ligand}$ = 0.008 mol dm$^{-3}$

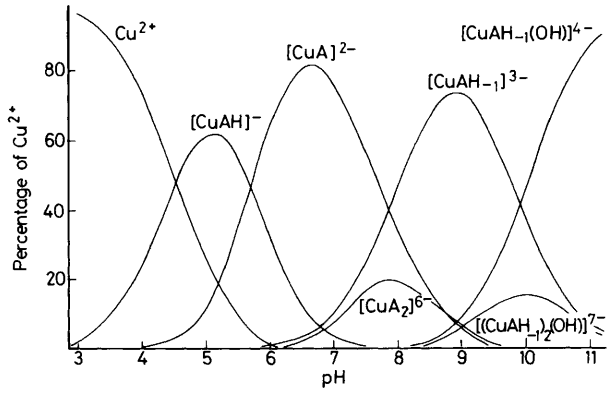



**Figure 3.** Concentration distribution of the complexes formed in the copper(II)–(*P*)-Mal-Leu-(*P*) system as a function of pH; $c_{Cu}$ = 0.004, $c_{ligand}$ = 0.008 mol dm$^{-3}$

the complex-forming abilities of the ligands, equilibrium data for the individual complex-formation steps, obtained from the overall stability data, are also listed in Table 3.

As an illustration, the concentration-distribution curves for the complexes formed in the copper(II)–Ala-Leu, –Ala-Leu(*P*), and –(*P*)-Mal-Leu-(*P*) systems are depicted in Figures 1—3.

To clarify the bonding modes of the complexes formed, visible and e.s.r. spectral measurements were performed. The results are given in Table 4.

1:1 *Complexes.*—At low pH, a species [CuAH] is formed in the systems studied; this is not formed with the amino acid dipeptides because of the lower basicity of the carboxylate group. In these complexes, similarly to those of simple dipeptides, the monophosphono dipeptides are co-ordinated to the metal ion in a bidentate way, *via* the terminal amino and the peptide carbonyl groups, forming a five-membered chelate ring; the phosphonate group is still protonated. A similar bonding mode can be assumed for the diphosphono dipeptides, but the amino group in the co-ordination sphere is replaced by a phosphonate group and thus a six-membered chelate ring is formed. The $PO_3^{2-}/CO_2^-$ substitution in the C-terminal part of the dipeptide has practically no effect on the metal ion-binding ability of the ligands; this is reflected in the good

**Table 4.** Spectral data for the copper(II) complexes[a]

| System | pH | $g_{\|}$ | $A_{\|}$ | $\lambda_{max.}$ | ε |
|---|---|---|---|---|---|
| $Cu^{II}$-Gly-Phe, 1:1[b] | 5.9 | 2.245 | 177 | 622 | 84 |
| | 11.0 | 2.243 | 156 | 625 | 82 |
| $Cu^{II}$-Ala-Leu-(*P*), 1:1 | 5.0 | 2.332 | 145 | 650 | 21 |
| | 7.0 | 2.249 | 178 | 617 | 55 |
| | 10.5 | 2.234 | 162 | 615 | 55 |
| $Cu^{II}$-Leu-Leu-(*P*), 1:1 | 5.0 | 2.335 | 150 | 660 | 20 |
| | 7.0 | 2.251 | 175 | 617 | 55 |
| | 10.5 | 2.231 | 161 | 615 | 51 |
| $Cu^{II}$-Leu-Ala-(*P*), 1:1 | 5.0 | 2.328 | 150 | 632 | 26 |
| | 7.0 | 2.252 | 173 | 618 | 53 |
| | 10.5 | 2.230 | 164 | 615 | 57 |
| $Cu^{II}$-Ala-cp-Gly-(*P*), 1:1 | 5.0 | 2.331 | 147 | 635 | 30 |
| | 7.5 | 2.252 | 175 | 618 | 48 |
| | 10.5 | 2.231 | 163 | 615 | 48 |
| $Cu^{II}$-(*P*)-Mal-Leu-(*P*), 1:1 | 6.0 | 2.324 | 143 | 800 | 15 |
| | 9.0 | 2.319 | 148 | 680 | 30 |

[a] The *A* values are in G ($10^{-4}$ T), $\lambda_{max.}$ in nm, and ε in $dm^3\ mol^{-1}\ cm^{-1}$. [b] See T. Kiss and Z. Szücs, *J. Chem. Soc., Dalton Trans.*, 1986, 2443.

agreement of the derived equilibrium data (log $K_{Cu(AH)}$, referring to the process $Cu^{2+} + HA^- \rightleftharpoons [CuAH]^+$) of the monophosphono peptides with the log $\beta_{CuA}$ values of the simple dipeptides (see Table 3), both being characteristic of the N,O bonding mode. The $PO_3^{2-}/NH_2$ substitution at the other end of the molecule, however, favours formation of the species [CuAH] even more (*cf.* Figures 2 and 3); this is presumably due to charge neutralization between the $Cu^{2+}$ ion and the $PO_3^{2-}$ donor group.

As the pH is raised, the complex [CuAH] undergoes three stepwise deprotonation processes, resulting finally in formation of the species $[CuAH_{-2}]$. For both monophosphono and diphosphono dipeptides the loss of the first two protons can presumably be ascribed to the terminal $PO_3H^-$ and the peptide amide groups. For the former type of ligands these processes overlap each other considerably, while for the latter they are fairly well separated (see $pK_{CuAH}$ and $pK_{CuA}$ values in Table 3). The deprotonation and the simultaneous co-ordination of the peptide-amide group are accompanied by an appreciable spectral change. Figure 4 shows the changes in energy of the *d–d* transition as a function of pH for several of the copper(II)–ligand systems studied.

It can be seen that until pH ≈ 6 there is no spectral change in the copper(II)–diphosphono dipeptide systems; thus, proton loss occurs from the terminal $PO_3H^-$ group in the range pH 5—6. Since the $pK_{CuAH}$ values are considerably lower than the corresponding dissociation microconstants of the free ligand (see Table 2), the co-ordination of the second $PO_3^{2-}$ with the formation of a 6 + 7-membered joined chelate system may be assumed. The purely O,O,O co-ordination of the ligand is also indicated by the e.s.r. parameters (see Table 4). In the case of the monophosphono peptides, because of the very close overlap of the two deprotonation processes, a correct assignment of the two p*K* values cannot be achieved. It is most probable that parallel deprotonation of the terminal $PO_3H^-$ and the peptide amide takes place. Thus, an equilibrium of two different micro-species, one with bidentate N,N bonding and the other with tridentate N,O,O co-ordination (5 + 7-membered joined chelates), occurs in solution.

In the complex $[CuAH_{-1}]$ both the monophosphono and the diphosphono dipeptides co-ordinate in a tridentate manner, with N,N,O and O,N,O bonding modes, respectively. Besides the considerable increase in energy of the *d–d* transition ($\lambda_{max.}$ ≈ 615 nm for the monophosphono and ≈ 650 nm for the diphosphono derivatives) the e.s.r. parameters ($g_{\|}$ = 2.25, $A_{\|}$ = 175; and $g_{\|}$ = 2.319, $A_{\|}$ = 147 G, respectively) also indicate 2 N co-ordination in the first case and 1 N co-ordination in the second case (see Table 4).

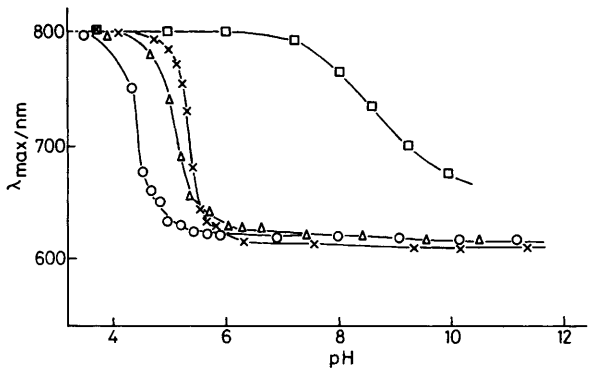



**Figure 4.** Changes of the *d–d* absorption in the 1:1 copper(II)–ligand systems as a function of pH: (×) Ala-Leu-(*P*), (△) Leu-Leu-(*P*), (○) Leu-Ala-(*P*), and (□) (*P*)-Mal-Leu-(*P*) (1:5 ratio)

These results show that $PO_3^{2-}/CO_2^-$ substitution makes the copper(II) ion-induced deprotonation and simultaneous co-ordination of the peptide amide somewhat disfavoured. (The p*K* characteristic of this process is about 1 log unit higher for the monophosphono peptides than for the simple dipeptides.) This is again very probably due to electrostatic reasons, since [CuA] is neutral for the simple dipeptides, while it has a negative charge in the monophosphono complexes.

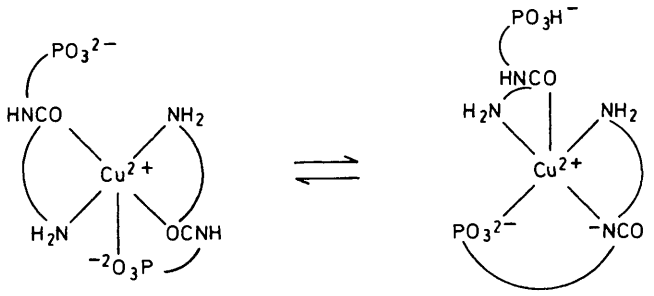
The $PO_3^{2-}/NH_2$ substitution further decreases the co-ordination ability of the peptide amide, although the results show that the $PO_3^{2-}$ group can also serve as an anchor first to bind metal ions, which can then induce deprotonation of the peptide NH, but only at much higher pH (p*K* ≈ 7.7—7.8). Besides the electrostatic explanation ([CuA] has three negative charges in the case of the diphosphono peptides), the lower basicity of the $PO_3^{2-}$ group plays a role in this behaviour.

The loss of a further proton leads to the formation of a species $[CuAH_{-2}]$. This proton loss, as in simple amino acid dipeptides, occurs from the co-ordinated water molecule in the fourth equatorial site. In accordance with expectations,[20] this process is accompanied by almost no change in the *d–d* transition and by small decreases in the $A_{\|}$ values. It is noteworthy, however, that the different charges of the complexes [CuA] with the three different types of ligands exert only a slight effect on the ionization of the co-ordinated water molecule ($pK_{CuAH_{-1}}$ = 9.4, 9.5, and 9.8 for the dipeptides, monophosphono and diphosphono derivatives, respectively).

At a metal ion:ligand ratio of 1:1 a dimeric species $[Cu_2(AH_{-1})_2(OH)]$ (probably involving a monohydroxy bridge) is also formed in measurable concentration (≈ 20% of the $Cu^{II}$) for the diphosphono derivatives, but in negligible concentration (≈ 3% of the $Cu^{II}$) for the monophosphono dipeptides.

1:2 *Complexes.*—It is noteworthy that in the presence of excess of ligand a species with the composition $[CuA_2]$ is also formed. The formation of such species was assumed for the amino acid dipeptides too, but they could not be detected because this species and $[CuAH_{-1}]$ could substitute each other pH-metrically.[21] For these phosphonic derivatives, peptide-amide deprotonation is less favoured; it takes place only at higher pH, and thus the formation of $[CuAH_{-1}]$ and that of $[CuA_2]$ are better separated and both species can be detected.

For both monophosphono and diphosphono dipeptides the



Scheme.

most probable bonding mode is equatorial bidentate co-ordination of two ligand molecules through the terminal $PO_3^{2-}$ and the peptide carbonyl groups. Thus, the co-ordination of the second molecule displaces the weakly co-ordinating phosphonate group in the equatorial plane of the species [CuA].[11] However, this group can easily co-ordinate in the apical position (the larger chelate ring is more favoured for axial–equatorial than for equatorial–equatorial co-ordination).[22] This bidentate–tridentate N,N,$O_{ax}$; N,O co-ordination is reflected in the rather high values of the stepwise stability constants characteristic of the process $[CuA] + A^{2-} \rightleftharpoons [CuA_2]^{2-}$ for the complexes of monophosphono dipeptides. At the same time, apical co-ordination of the terminal $PO_3^{2-}$ is not likely with the diphosphono dipeptides because of the very large electrostatic repulsion from the two phosphonate groups. This assumption of bidentate O,O; O,O co-ordination of the ligands, together with the stepwise formation constants given in Table 3, is in accordance with the stability data published for copper(II) complexes of phosphonoacetic acid (log $K_{CuA} = 7.14$ and log $K_{CuA_2} = 3.85$), which exhibit the same bonding mode.[23]

For the monophosphono dipeptides it is not possible to exclude N,N,O; N,$O_{ax}$ co-ordination, when the second ligand molecule co-ordinates to the species $[CuAH_{-1}]$ in an equatorial–axial way *via* the amino and the peptide carbonyl groups and the phosphonate group remains protonated (see Scheme). The equilibrium constants characteristic of the process $[CuAH_{-1}]^- + AH^- \rightleftharpoons [CuA_2]^{2-}$ (see Table 3) correspond fairly well to equatorial–axial N,O co-ordination.[21]

The species $[CuA_2H_{-1}]^{3-}$, which is formed with monophosphono peptides, similarly to simple dipeptides, is likely to involve the tridentate–bidentate N,N,O; N,$O_{ax}$ bonding mode (see Scheme) with the deprotonated non-co-ordinating $PO_3^{2-}$ group of the second ligand molecule. The equilibrium constants for the process $[CuAH_{-1}]^- + A^{2-} \rightleftharpoons [CuA_2H_{-1}]^{3-}$ are smaller than the corresponding values for the amino acid dipeptides, which again is probably due to the larger electrostatic-hindrance of the non-co-ordinating $PO_3^{2-}$ than that of the $CO_2^-$ group. The deprotonation constants of the complexes $[CuA_2]^{2-}$ are about two orders of magnitude higher than those of the $PO_3^{2-}$ group of the free ligands, which indicates that this process is accompanied by a rearrangement of the co-ordination sphere. Therefore, the equilibrium in the Scheme must be strongly shifted in the direction of the lower arrow. The stability constants obtained for the 1:2 complexes of the monophosphono peptides confirm the earlier findings for simple dipeptides that co-ordination of a second ligand is generally less favoured if the molecule contains a non- or weakly co-ordinating side-chain donor atom in the N-terminal amino acid moiety.[21] Here, the 1:2 complexes of the leucyl peptides, containing a large aliphatic side-chain in the N-terminal moiety, are less stable than those of the corresponding alanyl peptides.

## Conclusions

The differences in basicity, charge, electron-releasing effect, and size of the phosphonate, carboxylate, and amino groups result in the following main differences in the complex-forming properties of the dipeptides and their mono- and di-phosphonic analogues.

(1) Because of the more basic character of the phosphonate group, $PO_3^{2-}/CO_2^-$ substitution leads to the formation of various protonated 1:1 and 1:2 complexes.

(2) The extent of the copper(II) ion-induced deprotonation and simultaneous co-ordination of the peptide-amide group decreases in the following sequence: dipeptides > monophosphono dipeptides > diphosphono dipeptides. It is noteworthy, however, that, similarly to the $NH_2$ group, the less basic $PO_3^{2-}$ can also act as an anchor donor group and can bind copper(II) strongly enough to induce deprotonation of the peptide amide.

(3) The less favoured the peptide-amide deprotonation, the more favoured is the co-ordination of a second ligand molecule, and thus the extent of the formation of 1:2 complexes follows just the opposite sequence.

(4) There is a possibility of ambidentate co-ordination of the ligands, which results in an equilibrium between species with the same stoicheiometric composition but different bonding modes.

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