Complexes of Aminophosphonates. Part 6.† Copper(II) Complexes of Some Catecholaminophosphonic Acids

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The stoicheiometries and stability constants of the proton and copper(II) complexes of L-1-amino-2-(3',4'-dihydroxyphenyl)ethylphosphonic acid (L-adep), 1-amino-1-(3',4'-dihydroxyphenyl)methylphosphonic acid (3,4-admp), and 1-amino-1-(2',3'-dihydroxyphenyl)methylphosphonic acid (2,3-admp) 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 the spectral (visible and e.s.r.) parameters of the complexes, it has been established that similarly to the aminocarboxylate analogues the ligands show marked ambidentate character. At lower pH aminophosphonate-type complexes while at higher pH catecholate-type complexes are formed. In the intermediate pH range various monomeric and dimeric species with the participation of all binding sites are produced. In the case of 2,3-admp, where the chelate-forming donor-group pairs are not fully separated from each other, the tendency to dimer formation is more favoured.

3-(3',4'-Dihydroxyphenyl)-L-alanine (L-dopa) has been applied for about 25 years in the treatment of Parkinson disease. For therapeutic use it must be administered in a high daily dose, as a considerable proportion is decarboxylated in the peripheral regions, and accordingly does not reach the brain, where its action is to be exerted. To avoid this extracerebral decarboxylation, L-dopa is generally used in combination with peripheral L-dopa decarboxylase inhibitors. Rajan *et al.*[1,2] suggested and investigated an alternative possibility, the application of metal complexes of L-dopa, in which the metal ion co-ordinated at the amino acid side-chain at physiological pH can prevent decarboxylation of the drug. It has recently been found that phosphonic acid analogues of various aminocarboxylates are potent inhibitors of (metallo)enzymes which have amino acid substrates.[3] The differences in size, shape, basicity, and charge of the carboxylate and phosphonate groups may play important roles in the differences in the enzyme–substrate interactions. Hence, an investigation of the interaction of the phosphonic acid analogue of L-dopa [L-1-amino-2-(3',4'-dihydroxyphenyl)ethylphosphonic acid, L-adep] with metal ions may provide useful information on the potential use of L-adep in the combined treatment of Parkinsonism.

The metal-binding ability of L-dopa, which contains two separate chelate-forming donor-group pairs, has been extensively studied by different techniques such as pH-metry, spectrometry (u.v.–visible, c.d., and e.s.r.), *etc.*[4] In the acidic pH range it binds metal ions at the amino acid side-chain forming N,O co-ordinated complexes; at higher pH the catecholate type O,O co-ordination is favoured, while in the intermediate pH interval mixed bonding predominates where the metal ion co-ordinates to both separate binding sites, forming various monomeric and dimeric species.

We have recently reported results on metal complexes of simple aminophosphonic acids.[5] It has been established that, although the PO_3^{2-}/CO_2^- substitution results in higher stability of transition metal(II)–aminophosphonate complexes, arising from the higher basicity of the PO_3^{2-} group, steric hindrance and electrostatic repulsion due to the larger space requirement and higher charge of the PO_3^{2-} group lead to a significant decrease in the basicity-adjusted stability of the complexes.

The aim of the present work was to obtain information on the complex-forming ability of L-adep and some other catecholaminophosphonates, 1-amino-1-(3',4'-dihydroxyphenyl)-methylphosphonic acid (3,4-admp) and 1-amino-1-(2',3'-dihydroxyphenyl)methylphosphonic acid (2,3-admp), by establishing the compositions and stabilities of the species formed with copper(II) ion. Spectral studies (u.v.–visible and e.s.r.) helped to clarify the bonding modes in the complexes of these ambidentate ligands.

Experimental

The catecholaminophosphonic acids were obtained by the method described in ref. 6. The purities and the exact concentrations of the solutions of the ligands were determined pH-metrically by the method of Gran.[7] The concentration of the copper(II) chloride stock solution was measured gravimetrically *via* precipitation of the quinolin-8-olates.

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

The pH was measured with a Radiometer pHM 64 instrument with G2040B glass and K4040 calomel electrodes. Since the solutions of the ligand are oxygen-sensitive, measurements were made in a TTA 80 titration unit in an argon atmosphere

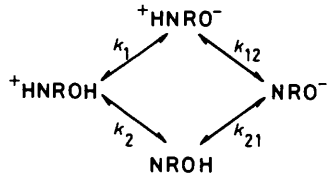
† Part 5, J. Balla, M. Jezowska-Bojczuk, T. Kiss, H. Kozlowski, B. Lejczak, and E. Matczak-Jon, *J. Inorg. Biochem.*, in the press.

Table 1. Dissociation constants of some catecholaminophosphonic acids and their aminocarboxylic analogues at 25 °C and I = 0.20 mol dm^{-3} (KCl)

	L-adep	L-dopa[a]	3,4-admp	dopg[b]	2,3-admp
pK_1	5.37 ± 0.01	2.22	5.64 ± 0.01	1.99	5.06 ± 0.01
pK_2	9.08 ± 0.01	8.80	9.00 ± 0.01	8.56	9.44 ± 0.01
pK_3	10.50 ± 0.02	9.83	10.28 ± 0.02	9.75	12.42 ± 0.14
pK_4	13.4 ± 0.2	13.40	13.6 ± 0.2	13.64	> 14.0[c]

[a] See ref. 14. [b] See ref. 19. [c] Non-dissociable group in the measurable pH range.

Table 2. Dissociation microconstants of some catecholaminophosphonic acids and their aminocarboxylic analogues at 25 °C and I = 0.20 mol dm^{-3} (KCl)



	L-adep	L-dopa*	3,4-admp
pk_1	9.16	8.98	9.11
pk_2	9.85	9.19	9.66
pk_{21}	9.73	9.42	9.62
pk_{12}	10.42	9.63	10.17
k_1/k_2	4.9	1.6	3.6

* See ref. 14.

with the complete exclusion of air. The electrode system was calibrated by the method of Irving *et al.*,[8] so that the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

For determination of the proton complex-formation microconstants of the ligands, pH-spectrophotometric titrations were performed as described previously.[9] In order to establish the metal-ion binding sites in the copper(II) complexes, spectral studies were made in the visible and u.v. wavelength ranges, similarly with the complete exclusion of air. Beckman ACTA MIV and UV 5240 recording spectrophotometers were used. E.s.r. spectra were obtained on a JEOL JMN-3X spectrometer at 9.15 GHz and 120 K in water–ethylene glycol (1:2) solvent.

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.[10] Depending on the type of ligands the fully deprotonated forms have different charges, *i.e.* A^{3-} refers to the aminocarboxylate derivatives, L-dopa and 3,4-dihydroxyphenylglycine (dopg), A^{4-} to the aminophosphonate derivatives, L-adep and 3,4-admp, and A^{3-} to 2,3-admp which contains a proton on the phenolic hydroxy group in position 3 non-dissociating in the measurable pH range. Hence, species with the same stoicheiometry may have different charges.

Results and Discussion

The pH-metrically determined dissociation constants of the ligands are given in Table 1. (The pK characteristic of the dissociation of the very acidic PO_3H_2 is < 1.0;[11] it is fully deprotonated in the pH range studied, and thus is not included in Table 1.)

The dissociation constants (pK_4) of the very weakly acidic second phenolic hydroxy groups of the ligands were determined at high ligand concentration by the pH-metric method described earlier.[12] The pK_1 values of the aminophosphonic acid derivatives can be ascribed without any doubt to the dissociation of the PO_3H^- group. The values of pK_2 and pK_3, however, cannot be ascribed unambiguously to the dissociation of the first phenolic hydroxy group and the amino group of the ligand as they overlap one another considerably; thus, they arise as the superposition of microconstants. The dissociation microconstants characteristic of the acidity of the individual groups were determined by selective monitoring of the dissociation of the phenolic hydroxy group *via* the u.v. band of the phenolate.[9] The values obtained, together with those for L-dopa,[13] are listed in Table 2. Microconstants could not be obtained for the dissociation processes of 2,3-admp because the ligand underwent irreversible oxidation during the pH-spectrophotometric titration. Further, the possible formation of an intramolecular hydrogen bond between the side-chain amino/ammonium group and the phenolic OH/phenolate group in position 2 after the dissociation of one proton from one of the groups makes the two microspecies indistinguishable; thus, the assumption of microscopic dissociation processes is not necessary. The significantly larger pK_3 for 2,3-admp confirms the hydrogen-bond formation. A similar observation was made with *o*-tyrosine (2-hydroxyphenylalanine).[9]

It can be seen from Table 2 that PO_3^{2-}/CO_2^- substitution does not change the acidity sequence of the two groups, *i.e.* the phenolic hydroxy group remains the more acidic. The difference in acidity of the phenolic OH and the ammonium groups, however, significantly increases (see k_1/k_2 values). This can be attributed to the stronger electron-releasing effect of the PO_3^{2-} group than that of CO_2^-, and therefore the acidity of NH_3^+ decreases more significantly for the phosphonic acid derivatives. A similar change in acidity of the ammonium group has been found for other aminophosphonic acids.[5]

Copper(II) Complexes of the 3,4-Dihydroxy Derivatives.—On the basis of earlier experience with the metal complexes of L-dopa,[14] the pH-metric titration data for the copper(II)–ligand systems were evaluated by the assumption of complex formation *via* both the aminophosphonate side-chain and the *ortho* phenolic hydroxy groups. Thus the formation of various NH_2,PO_3^{2-}; O^-,O^-, and mixed co-ordinated monomeric and dimeric/tetrameric species was assumed. The stability constants obtained together with some derived equilibrium constants are given in Tables 3 and 4.

The stability data in Table 3 clearly show that analogous species with similar stabilities are formed in the copper(II)–L-adep and 3,4-admp systems. Hence, as an illustration, only the pH dependence of the concentration of the complexes formed in the copper(II)–L-adep systems is presented in Figure 1, together with that of the aminocarboxylate analogue L-dopa system for the sake of comparison.

Both the derived equilibrium data given in Table 3 and the spectral parameters (visible and e.s.r.) given in Table 5 suggest that, as for the aminocarboxylate analogues L-dopa and dopg, the complex formation starts at the aminophosphonate side-chain and NH_2, PO_3^{2-} co-ordinated 1:1 and 1:2 complexes $[Cu(H_2A)]$ and $[Cu(H_2A)_2]^{2-}$ are formed. The spectral data are in good agreement with those of simple copper(II)–aminophosphonate complexes,[5] indicating 1N or 2N donors in the co-ordination sphere. A comparison of the derived

Table 3. Stability and derived equilibrium constants of the copper(II) complexes[a] of L-dopa and dopg and their phosphonic acid analogues at 25 °C and I = 0.20 mol dm^{-3} (KCl)

	L-adep	L-dopa[b]	3,4-admp	dopg[c]
$[Cu(H_2A)]$	32.74 ± 0.03	30.75	31.38 ± 0.05	
$[Cu(H_2A)_2]^{2-}$	64.36 ± 0.05	60.61	61.32 ± 0.09	
$[CuA_2H_3]^{3-}$	57.27 ± 0.07	53.81	53.66 ± 0.09	
$[CuA_2H_2]^{4-}$	48.82 ± 0.09	45.33	44.89 ± 0.09	
$[CuA_2H]^{5-}$	38.56 ± 0.09	35.83	34.79 ± 0.11	
$[CuA_2]^{6-}$	27.68 ± 0.08	25.47	24.03 ± 0.08	
$[Cu_2A_2H_2]^{2-}$	55.57 ± 0.20	53.35	54.17 ± 0.09	
$[Cu_2A_2H]^{3-}$	48.64 ± 0.14	—	46.22 ± 0.10	
$[Cu_2A_2]^{4-}$ or	39.36 ± 0.09	41.90	37.23 ± 0.09	
$[Cu_4A_4]^{8-}$	81.66 ± 0.11	—	77.72 ± 0.12	
[d] $Cu^{2+} + H_2A^{2-} \rightleftharpoons [Cu(H_2A)]$	8.92	7.92	7.61	6.96
[d] $[Cu(H_2A)] + H_2A^{2-} \rightleftharpoons [Cu(H_2A)_2]^{2-}$	7.80	7.03	6.17	5.94
$[Cu(H_2A)_2]^{2-} \rightleftharpoons [CuA_2H_3]^{3-} + H^+$	−7.09	−6.80	−7.66	
$[CuA_2H_3]^{3-} \rightleftharpoons [CuA_2H_2]^{4-} + H^+$	−8.45	−8.47	−8.77	
$[CuA_2H_2]^{4-} \rightleftharpoons [CuA_2H]^{5-} + H^+$	−10.26	−9.51	−10.10	
$[CuA_2H]^{5-} \rightleftharpoons [CuA_2]^{6-} + H^+$	−10.88	−10.35	−10.76	
[e] $Cu^{2+} + 2H_2A^{2-} \rightleftharpoons [CuA_2]^{6-} + 4H^+$	−19.96	−20.19	−23.51	

[a] Charges of the complexes refer only to the aminophosphonate analogues (see text). [b] See ref. 14. [c] See ref. 19. [d] Complex formation *via* the aminophosphonate side-chain; the complex-forming species is H_2A^{2-} protonated at the catechol moiety (log $K_{Cu(H_2A)} - pK_4 - pk_{12}$ and log $K_{Cu(H_2A)_2} - pK_4 - pk_{12}$). [e] Calculated from the overall stability constants by taking into account the pK values of the phenolic hydroxy groups (log $\beta_{CuA_2} - 2pK_4 - 2pk_{12}$).

Table 4. Stability and derived equilibrium constants of the copper(II) complexes of 2,3-admp at 25 °C and I = 0.20 mol dm^{-3}

$[Cu(HA)]$	21.35 ± 0.05	$[Cu_2A_2]^{2-}$	34.35 ± 0.08
$[CuA_2H]^{3-}$	32.36 ± 0.09	$[Cu_2A_2H_{-1}]^{3-}$	24.92 ± 0.13
$[CuA_2]^{4-}$	21.46 ± 0.13	$[Cu_2A_2H_{-2}]^{4-}$	13.68 ± 0.25
$[CuA_2H_{-1}]^{5-}$	9.37 ± 0.32		

$Cu^{2+} + HA^{2-} \rightleftharpoons [Cu(HA)]$	8.93
$[CuA_2H]^{3-} \rightleftharpoons [CuA_2]^{4-} + H^+$	−10.90
$[CuA_2]^{4-} \rightleftharpoons [CuA_2H_{-1}]^{5-} + H^+$	−12.09
$[Cu_2A_2]^{2-} \rightleftharpoons [Cu_2A_2H_{-1}]^{3-} + H^+$	−9.43
$[Cu_2A_2H_{-1}]^{3-} \rightleftharpoons [Cu_2A_2H_{-2}]^{4-} + H^+$	−11.24
* $Cu^{2+} + 2H_2A^- \rightleftharpoons [CuA_2]^{4-} + 4H^+$	−22.26

* Calculated from the overall stability constants by taking into account the pK values of the phenolic hydroxy and the ammonium groups (log $\beta_{CuA_2} - 2pK_2 - 2pk_{12}$).

equilibrium constants characteristic of the formation of the aminophosphonate type complexes of L-adep and 3,4-admp strengthens the earlier finding[5] that PO_3^{2-}/CO_2^- substitution results in a stability increase due to the higher basicity of the phosphonate group. However, this effect is overcompensated by the steric and electrostatic hindrance due to the larger space requirement and higher charge of the phosphonate group. It is reflected only in the basicity-adjusted stability constants, which take into account the differences in basicity of the co-ordinating donor groups.

At higher pH the stepwise deprotonation of $[Cu(H_2A)_2]^{2-}$ to $[CuA_2]^{6-}$ takes place, which is accompanied by a structural rearrangement from the NH_2,PO_3^{2-} bonding mode to the purely O^-,O^- bonding mode through the mixed NH_2,PO_3^{2-}; O^-,O^- bonding mode, where one ligand is co-ordinated in an aminophosphonate-like and the other in a catechol-like manner. The appearance of a phenolate to copper(II) charge-transfer band at *ca.* 400 nm clearly indicates the involvement of the catecholate moiety in the co-ordination. This structural rearrangement and the deprotonation of the non-co-ordinated side-chain donor groups of the ligands take place in overlapping processes, and thus these deprotonation processes can be described only by a rather complicated microscopic dissociation

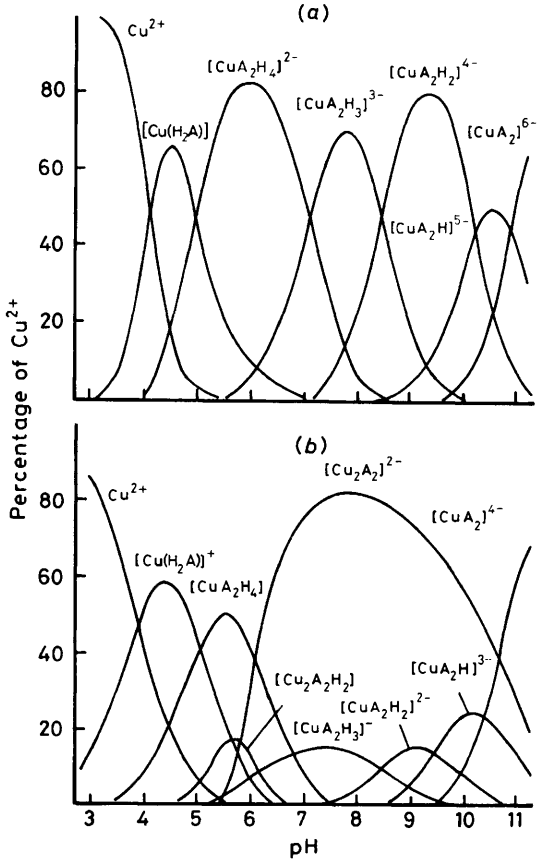


Figure 1. Concentration distribution of the complexes formed in the copper(II)–L-adep (*a*) and L-dopa (*b*) systems as a function of pH. c_{Cu} = 0.002, c_{ligand} = 0.004 mol dm^{-3}

scheme.[15] Although such a 'continuous' rearrangement cannot be excluded in the case of the aminophosphonate derivatives either, the spectral data (see Table 5) suggest that the rearrangement to a purely O^-,O^- bonding mode is completed

Table 5. Spectroscopic data for the copper(II) complexes of some catecholaminophosphonic acids[a]

Species	pH	E.s.r. $g_{\|}$	E.s.r. $A_{\|}$	Absorption, λ (ε) b	Absorption, λ (ε) c	Ligand bonding mode
L-adep						
$[Cu(H_2A)]$	4.5	2.325	154	705(40)		NH_2,PO_3^{2-}
$[Cu(H_2A)_2]^{2-}$	6.0	2.272	161	645(61)		$2(NH_2,PO_3^{2-})$
$[CuA_2H_3]^{3-}$	7.8	2.275	166	650(43)	424(96)	NH_2,PO_3^{2-}; O^-,O^-
$[CuA_2H_2]^{4-}$	9.2					
$[CuA_2H]^{5-}$	10.2	2.281	174	638(40)	406(270)	$2(O^-,O^-)$
$[CuA_2]^{6-}$	11.3	2.254	196			
$[Cu_4A_4]^{8-}$	10.0	Isotropic band		652(52)	413(150)	$4(NH_2,PO_3^{2-}; O^-,O^-)$ Cyclic arrangement
3,4-admp						
$[Cu(H_2A)]$	4.3	2.328	155	710(40)		NH_2,PO_3^{2-}
$[Cu(H_2A)_2]^{2-}$	6.0	2.270	160	650(67)		$2(NH_2,PO_3^{2-})$
$[CuA_2H_3]^{3-}$	7.8	2.268	167	658(53)	400(280) 350(700)	NH_2,PO_3^{2-}; O^-,O^-
$[CuA_2H_2]^{4-}$	9.0					
$[CuA_2H]^{5-}$	10.1	2.281	178	638(45)	400(450) 354(1 050)	$2(O^-,O^-)$
$[CuA_2]^{6-}$	11.3	2.254	196			
$[Cu_4A_4]^{8-}$	10.0	Isotropic band		655(70)	400(170) 349(650)	$4(NH_2,PO_3^{2-}; O^-,O^-)$ Cyclic arrangement
2,3-admp						
$[Cu(HA)]$	4.8	2.334	153	710(50)		NH_2,PO_3^{2-}
$[CuA_2H]^{3-}$	9.2	2.260	181	641(74)	400(240)	NH_2,PO_3^{2-}; NH_2,O^-
$[CuA_2]^{4-}$	11.0	2.255	182	630(95)	430(450)	$2(NH_2,O^-)$
$[CuA_2H_{-1}]^{5-}$	11.6	2.252	189	—	420(600)	NH_2,O^-; O^-,O^-
$[Cu_2A_2]^{4-}$	6.8	Significantly decreased intensity		660(90)	434(400)	Phenolate-bridged dimers
$[Cu_2A_2H_{-1}]^{5-}$	10.2					
$[Cu_2A_2H_{-2}]^{6-}$	11.2					

[a] Values of ε (in $dm^3\ mol^{-1}\ cm^{-1}$) were calculated for the total copper(II) concentration for the pH at which the respective species is a major complex; the A values are in G (10^{-4} T) and λ in nm. [b] *d–d* Transition. [c] Phenolate oxygen-to-copper charge-transfer transition.

by pH 8 and no further changes are subsequently observed in the spectral parameters. It is unusual that complexes containing a $Cu(O^-,O^-)_2$ chromophore have *d–d* transitions at lower wavelengths (*ca.* 640 nm) than those containing a $Cu(NH_2, PO_3^{2-})_2$ bonding mode (*ca.* 645 nm); generally the former occurs at much higher wavelengths (*ca.* 680 nm).[14] It has to be mentioned, however, that the high-intensity charge-transfer bands in the wavelength range 400—410 nm, being near to the *d–d* transitions, may distort the spectra and cause a shift of the absorption maxima to lower wavelengths. Similar observations were made on the copper(II) complexes of other catechol derivatives.[16,17] The relatively less favoured formation of the complexes involving the mixed bonding mode is confirmed by the results obtained for the copper(II)–catechol–α-Ala-P (1 aminoethanephosphonic acid) mixed-ligand system, where the potential donor groups of L-adep occur in two different ligands. The stabilization constant $\Delta\log\beta_{CuAB}$ characterizing the stability enhancement, which is the difference between the measured stability constant and that calculated on the basis of statistical considerations [$\Delta\log\beta_{CuAB} = \log\beta_{CuAB}^{measd.} - \frac{1}{2}(\log\beta_{MA_2} + \log\beta_{MB_2} + \log 4)$], is 0.21, *i.e.* somewhat smaller than that obtained for the copper(II)–catechol–α-Ala mixed-ligand system ($\Delta\log\beta_{CuAB} = 0.53$).[14] Thus, the mixed binding mode can be assigned to the complex $[CuA_2H_3]^{3-}$, since in its formation pH range only a mixed NH_2,PO_3^{2-}; O^-,O^- co-ordination can contain three dissociable protons. Then, after a more or less complete structural rearrangement, the purely catechol-type co-ordination can to a good approximation be assigned to the complexes $[CuA_2H_2]^{4-}$, $[CuA_2H]^{5-}$, and $[CuA_2]^{6-}$. As further evidence of this, the stepwise deprotonation constants of $[CuA_2H_2]^{4-}$ correspond fairly well to the microconstant pk_{12} characteristic of the dissociation of the side-chain ammonium group (see Table 2), and the difference between them is *ca.* 0.6 log unit, which corresponds to the statistical case of the dissociation of two separate acidic groups of equal acidity.

The PO_3^{2-}/CO_2^- substitution has practically no effect on the co-ordinating ability of the catecholate moiety of L-dopa and L-adep, as reflected in the good agreement of the equilibrium constants for the process $Cu^{2+} + 2H_2A^{2-} \rightleftharpoons [CuA_2]^{6-} + 4H^+$ characteristic of the relative stability of the O,O co-ordinated 1:2 species. This can be explained by the relatively large distance of the phosphonate group from the phenolate donor atom.

It can be seen from Figure 1 that the most important difference due to PO_3^{2-}/CO_2^- substitution in the complex-forming behaviour of L-dopa and L-adep (and similarly dopg and 3,4-admp) is the negligible formation of dimeric species with the aminophosphonate analogues at a ligand excess. The ligand L-dopa readily forms open-chain dimers $[Cu_2A_2H_2]$ and $[Cu_2A_2H]^-$, in which a second copper(II) is co-ordinated to a mixed binding-type 1:2 complex at the free aminocarboxylate or catecholate moiety, and a cyclic dimer, formed *via* ring closure of the open-chain dimers.[5,18] Gorton and Jameson[19] assumed the formation of a cyclic tetramer $[Cu_4A_4]^{4-}$ in the copper(II)–dopg system and explained this difference from the chelation properties of L-dopa in terms of the larger steric hindrance due to the shorter side-chain of the dopg molecule.

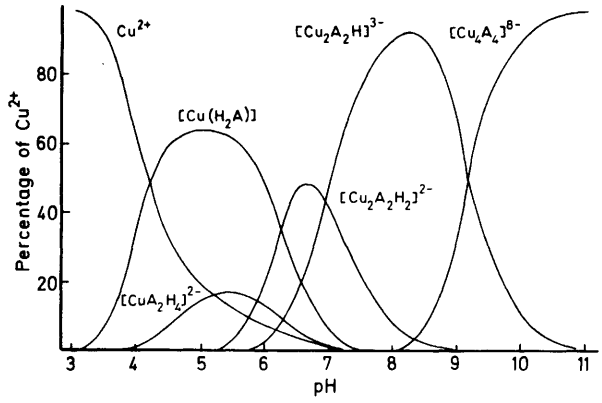


Figure 2. Concentration distribution of the complexes formed in the copper(II)–L-adep system as a function of pH. $c_{Cu} = c_{ligand} = 0.004$ mol dm^{-3}

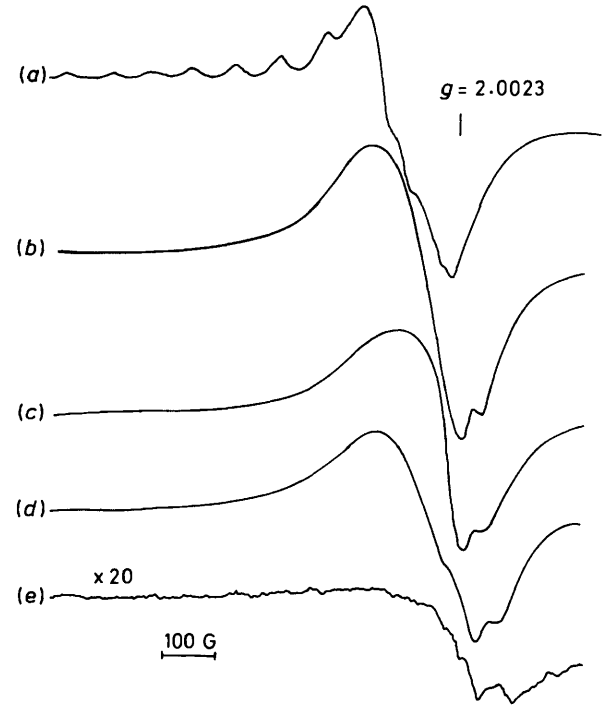


Figure 3. E.s.r. spectra of the copper(II)–L-dopa (*a*), -adrenaline (*b*), -L-adep (*c*), -3,4-admp (*d*), and (*e*) -2,3-admp systems at a 1:1 metal ion:ligand ratio, pH *ca.* 7.0, and 77 K

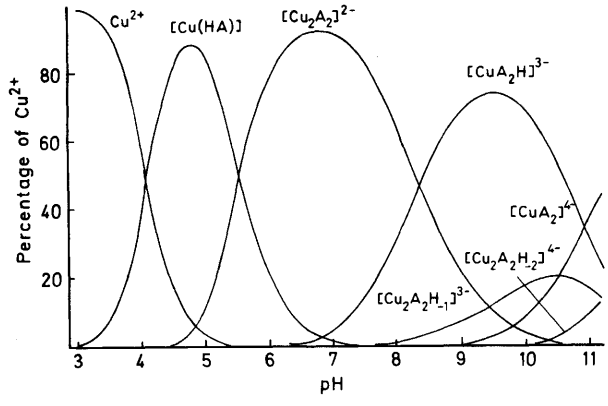


Figure 4. Concentration distribution of the complexes formed in the copper(II)–2,3-admp system as a function of pH. $c_{Cu} = 0.002$, $c_{ligand} = 0.004$ mol dm^{-3}

Polymer formation occurs with the aminophosphonate analogues of L-dopa and dopg too (see Figure 2), although the e.s.r. parameters (see Figure 3) suggest tetramer formation for both adep and 3,4-admp. In a cyclic dimer, as in the copper(II)–L-dopa system, the magnetic coupling between the copper(II) centres is strong enough to cause a seven-line splitting of the e.s.r. signal,[5] while in a cyclic tetramer, as found in the copper(II)–adrenaline {4-[1′-hydroxy-2′-(methylamino)ethyl]-benzene-1,2-diol} system,[20] the weaker copper(II)–copper(II) interaction results in a broad, poorly resolved signal.

The e.s.r. spectra recorded at a 1:1 metal ion:ligand ratio in the copper(II)–aminophosphonate systems, together with those obtained earlier for the copper(II)–L-dopa and –adrenaline systems are presented in Figure 3.

Although the pH-metric titration data can be fitted identically well by the assumption of a cyclic dimeric or a tetrameric species (the two species cannot be distinguished unambiguously by pH-metry, because the pH effects of their formation are very similar), the similarity of the e.s.r. behaviour to that observed in the copper(II)–adrenaline system suggests tetramer formation.

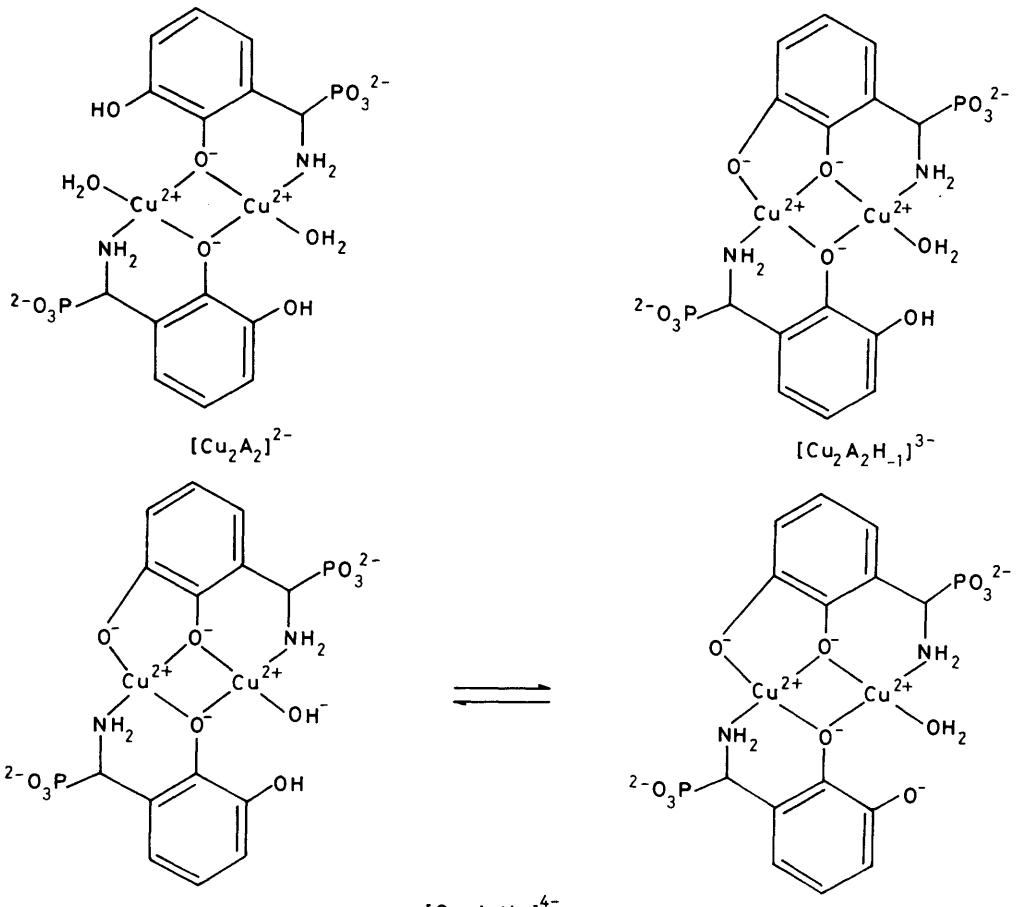
The smaller extent of formation of polymeric species in the case of the aminophosphonate analogues as compared to that of the catecholaminocarboxylates L-dopa and dopg can be explained by the less favoured formation of the mixed-binding mode with the former ligands (see above).

Copper(II) Complexes of the 2,3-Dihydroxy Derivative.—The four metal binding sites, the phosphonate, the amino, and the two phenolic hydroxy groups, are not greatly separated from one another in the 2,3-admp molecule. Thus, it can be expected that its complex-forming properties will differ from those of the 3,4-dihydroxy derivatives, as it can bind metal ions *via* the aminophosphonate side-chain, *via* the catechol moiety, and in an *o*-tyrosine manner *via* the side-chain amino group and the phenolic hydroxy group in position 2.

The concentration distribution of the complexes formed in the copper(II)–2,3-admp system as a function of pH is depicted in Figure 4.

The derived equilibrium data (see Table 4) and the spectral parameters (see Table 5) clearly indicate NH_2,PO_3^{2-} co-ordination in the complex [Cu(HA)], which is the dominant species at low pH. As the pH is raised the appearance of a charge-transfer band strongly suggests the involvement of the phenolic hydroxy group(s) in the co-ordination. Thus, the most probable bonding mode in the species $[CuA_2H]^{3-}$ is a mixed aminophosphonate (NH_2,PO_3^{2-})–*o*-tyrosinate (NH_2,O^-) co-ordination. The two stepwise deprotonation processes of this complex are certainly accompanied by structural rearrangement. In the first step the rearrangement to purely *o*-tyrosinate $2(NH_2,O^-)$ co-ordination takes place, which is confirmed by the very good agreement of the equilibrium constant for the process $Cu^{2+} + 2H_2A \rightleftharpoons [CuA_2]^{4-} + 4H^+$, characteristic of the relative stability of *o*-tyrosine type co-ordination, with that obtained for *o*-tyrosine itself, $\log K = -22.17$.[21] In the next step the structural rearrangement goes further to a mixed NH_2,O^-; O^-,O^- bonding mode, which is suggested by the larger parameter $A_{\parallel}$ and the continuous changes in the wavelength and molar absorptivity of the charge-transfer band characteristic of the copper(II)–phenolate interaction.

It can be seen from Figure 4 that the possibility of formation of polynuclear species is more favoured in the copper(II)–2,3-admp system than for the 3,4-dihydroxy derivatives (*cf.* Figure 1). The dimeric species $[Cu_2A_2]^{2-}$, $[Cu_2A_2H_{-1}]^{3-}$, and $[Cu_2A_2H_{-2}]^{4-}$, however, should involve an entirely different bonding mode: as can be seen in Figure 3, these species are e.s.r. inactive. (The spectra show little change in the range pH 7—11.) The most probable structure of these complexes is a phenolate-



Scheme.

bridged dimer, in which the antiferromagnetically coupled copper(II) centres result in e.s.r.-inactive complexes (see Scheme). Similar phenolate-bridged dimers have been found and extensively studied as models for copper proteins of type 3.[22] The first pK of the species $[Cu_2A_2]^{2-}$, which is about 3 log units lower than pK_3 of the free ligand, can presumably be ascribed to the deprotonation and the simultaneous co-ordination of the phenolic OH in position 3, while the next pK, which is about 2 log units higher than the previous one, is probably characteristic of simple deprotonation of the other phenolic OH or dissociation of the water molecule bound in the fourth equatorial position of one of the copper(II) ions. As the molecular models show, the tridentate co-ordination of both ligand molecules is sterically hindered in the dimeric species.

Acknowledgements

This work was supported by the Hungarian Ministry of Education (Project 40/86) and by the Polish Academy of Sciences (Project CPBP 01.12).

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Received 15*th November* 1989; *Paper* 9/04887A