Binary and Ternary Copper(II) Complexes of N^{τ}- and N^{π}-Methyl-L-histidine in Aqueous Solution

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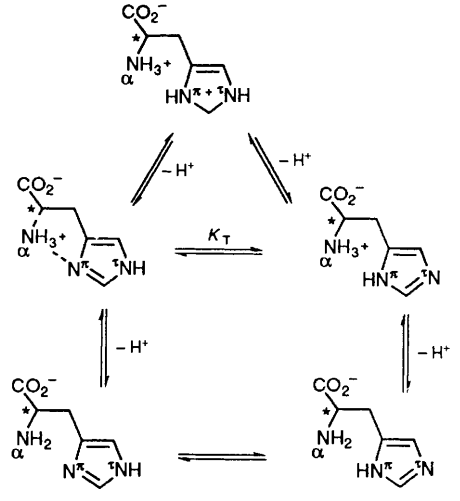
Protonation, binary and ternary copper(II) complex formation constants of N^{τ}-methyl-L-histidine, His(τMe) and N^{π}-methyl-L-histidine, His(πMe), have been measured through potentiometric titrations. The UV/VIS spectra have been recorded for both the binary systems and the Cu^{II}–His(πMe) complexes have been investigated by means of fast atom bombardment mass spectroscopy. The data on the protonation equilibria of the histidine derivatives show the existence of a difference in basicity between the respective π- and τ-nitrogen atoms, thus giving information on the tautomerism involving the imidazole residue of L-histidine (L-His) in the physiological pH range. The complexation behaviour of His(τMe) is quite similar to that of underivatized L-His: the same species are formed and their log β values are very similar. On the contrary, the presence of a methyl substituent on the N^{π} atom deeply affects the complexing properties of the amino acid; His(πMe) cannot act as a tridentate ligand for steric reasons and tends to form polynuclear complexes.

The imidazole residue of histidine (His) plays an important role in protein and enzyme activity[1] due to its unique pK_a value ($\approx$6). In fact, it can behave both as a proton donor and proton acceptor in the biological pH range. In its unprotonated form, imidazole contains two nitrogen atoms of different types: (*a*) α (pyrrole like), bonded to three different atoms which are very close to a plane; it supplies two electrons to the π-orbital delocalized system of the heteroaromatic ring; (*b*) β (pyridine like), bonded only to two other atoms; it supplies only one electron to the π-delocalized system and possesses a lone electron pair. Therefore, besides all its 4-substituted derivatives, imidazole can then exist in two tautomeric forms; they are indistinguishable for imidazole itself but distinguishable for His (see Scheme 1). A lot of work has been done to clarify the distribution of these forms at different pH values, both for His and for histidine residues in some proteins. These investigations, carried out through ^1H,[1-3] ^{13}C,[4-6] ^{14}N[7-9] and ^{15}N NMR[10-13] spectroscopy, unambiguously demonstrated that the $N^{\tau}H$ tautomeric form is the most abundant, especially in the pH region around neutrality. Results in agreement with this have been obtained in the solid state[13] where only the $N^{\tau}H$ tautomer was found. Besides the asymmetric effect of the alanyl residue on the N^{π} and N^{τ} atoms, for the zwitterionic form of His, the formation of a hydrogen bond between the protonated amino group and the unprotonated π-nitrogen has been suggested to explain the spectroscopic results.[6,10,11] However, thermodynamic parameters obtained through potentiometric measurements of pK_a values of His and a number of its derivatives, at different temperatures, did not support this hypothesis.[14] An ion–dipole interaction between the imidazole ring and the positively charged amino group was instead suggested.[14]

The strong affinity of His for copper(II) ion has long been recognized. Histidine is a potentially tridentate ligand and can form copper(II) complexes with different stoichiometries. These have, in most cases, a distorted-octahedral structure.[15] Histidine can co-ordinate to Cu^{II} in two main ways, depending on which atoms are bound in the equatorial plane: glycine like (α-amino nitrogen and carboxylic oxygen) and histamine like (α-amino and π-imidazole nitrogens). An additional interaction, in the axial position, can be exerted by the third donor atom; the simultaneous co-ordination of all three donor atoms in the equatorial plane is sterically hindered. Binary copper(II) complexes of His have been extensively investigated both in solution[16-30] and in the solid state.[31-33] The complex-formation constants of His with several divalent cations have been critically reviewed.[34]



Scheme 1 Tautomeric and protonation equilibria of histidine

Significant thermodynamic stereoselectivity has been found in ternary copper(II) complex formation of L- or D-His with various L-amino acids.[30,35-38] This stereoselectivity has been attributed both to intramolecular non-bonding interactions between the side chains of the two ligands and to the tridentate behaviour of His.

Moreover, the importance of the hystidyl residue of proteins in the binding of Cu^{II} has been emphasized[15] and copper(II) complexes with many simple histidine-containing oligopeptides have been investigated.[39-47] Some of these complexes have been found to catalyse superoxide dismutation.[48,49] Finally, suitable histidine derivatives have been employed in resolving enantiomeric mixtures of some chiral compounds (*e.g.* amino acids and their derivatives) through ligand exchange chromatography (LEC), both in TLC[50] and in HPLC.[51-54]

In the present study the affinity of two derivatives, N^{π}-methyl-

Table 1 Experimental details of the potentiometric titrations at 25 °C, I = 0.1 mol dm^{-3} (KNO_3); titrant 0.1 mol dm^{-3} KOH

Reagent	Analytical concentration (mmol dm^{-3}) Cu^{II}	Amino acid	His	Number of titrations*	pH Range
L-His	—	—	4.5–15.0	27	2.2–9.0
Cu,L-His	2.5–6.0	—	6.6–10	12	3.0–8.0
His(πMe)	—	—	4.0–12	21	2.0–9.0
Cu,His(πMe)	1.0–6.0	—	3.0–10.0	20	2.5–8.0
Cu,His(πMe),L-Phe	3.0–5.0	6.0–8.0	3.0–6.0	12	2.5–8.0
Cu,His(πMe),D-Phe	3.0–5.0	6.0–8.0	3.0–6.0	10	2.5–8.0
Cu,His(πMe),L-Trp	3.0–5.0	5.0–8.0	3.0–6.0	12	3.0–8.0
Cu,His(πMe),D-Trp	3.0–5.0	5.0–8.0	3.0–6.0	12	3.0–8.0
His(τMe)	—		5.6–16.5	24	2.1–9.0
Cu,His(τMe)	3.0–6.0	—	6.6–10.0	12	3.0–8.0
Cu,His(τMe),L-Phe	4.0–7.0	6.0–7.5	5.0–7.0	8	3.0–8.0
Cu,His(τMe),D,Phe	4.0–7.0	6.0–7.5	5.0–7.0	8	3.0–8.0
Cu,His(τMe),L-Trp	4.0–7.0	6.0–7.5	5.0–7.0	8	3.0–8.0
Cu,His(τMe),D-Trp	4.0–7.0	6.0–7.5	5.0–7.0	9	3.0–8.0

* 40–70 Points for each titration curve.

L-histidine, His(πMe), and N^{τ}-methyl-L-histidine, His(τMe),* for Cu^{II} has been examined with different experimental techniques. These two ligands are of special interest for different reasons. The N^{τ} derivative is a lighter, water-soluble homologue of N^{τ}-*n*-decyl-L-histidine, His(τDec), which was recently used as a chiral selector in LEC.[50,54] In addition, both forms occur in nature: they are present in some dipeptides and proteins contained in skeletal muscles.[56,57] The concentration of His(τMe) in serum and urine is an index of skeletal-muscle-protein degradation and various analytical methods have been developed for its determination.[57–59] The study of the complexation of such ligands with Cu^{II}, a life-essential oligoelement,[60] is of great interest in the biomedical field, and can be the basis for the development of new analytical procedures for their determination.

Experimental

Materials.—L-Histidine (L-His), L/D-tryptophan (L/D-Trp), L/D-phenylalanine (L/D-Phe) and L-alanine (L-Ala) were obtained from Aldrich, N^{π}-methyl-L-histidine and N^{τ}-methyl-L-histidine from Sigma. They were all high-purity products used without further treatment. Copper(II) nitrate was an extra pure Merck product. The concentration of a stock solution of this salt was determined by ethylenediaminetetraacetate titration. Carbonate-free stock solutions of KOH were prepared by dilution of a saturated KOH (Aldrich, semiconductor grade) solution and then standardized potentiometrically by titrating with potassium hydrogenphthalate. A HNO_3 stock solution was prepared by diluting concentrated HNO_3 (Merck, Suprapur) and was then standardized with KOH. All sample solutions were prepared with CO_2-free freshly distilled (four-fold) water. The ionic strength was adjusted to 0.1 mol dm^{-3} by adding KNO_3 (Merck, Suprapur). Grade A glassware was employed throughout.

Potentiometric Measurements.—Potentiometric titrations were performed with the following equipment: an Orion EA 940 pH meter (resolution 0.1 mV, accuracy 0.2 mV) equipped with a combined glass electrode (Metrohm); a Hamilton MicroLab M motor burette (resolution 0.1 μl, accuracy 0.2 μl) equipped with a Hamilton syringe (delivery volume 0.5 cm^3). The potentiometer and burette were interfaced with an IBM PS/2 model 30 computer with which the titrations were performed automatically. Constant-speed magnetic stirring was applied throughout. The temperature of the titration cell was kept at 25 ± 0.02 °C by means of a Haake F3C circulation thermostat. Nitrogen (UPP grade) previously saturated with water (0.1 mol dm^{-3} KNO_3, 25 °C) was blown onto the test solution in order to maintain an inert atmosphere. The electrode couple was standardized on the pH = $-\log c_{H^+}$ scale by titrating HNO_3 (0.01 mol dm^{-3}) with standard KOH (0.1 mol dm^{-3} in 0.1 mol dm^{-3} KNO_3) at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Aliquots (2 cm^3) containing suitable amounts of Cu^{II}, of HNO_3 and of the amino acids were titrated with standard KOH until a precipitate or opalescence was just observed in the titration cell. Experimental details are in Table 1.

Spectrophotometric Measurements.—The UV/VIS spectra were recorded with a Uvicon 931 (Kontron) spectrophotometer, with a quartz cell of 1 cm path length, at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Aqueous KNO_3 (0.1 mol dm^{-3}) was used in the reference cell. The solutions being examined were 10^{-3} mol dm^{-3} in $Cu(NO_3)_2$ and amino acid (1 : 2) for the UV readings and 10^{-2} mol dm^{-3} for the spectra in the visible region. They had an initial pH value of about 2.5; suitable amounts of standard KOH (≈0.1 mol dm^{-3} in 0.1 mol dm^{-3} KNO_3) were added in order to get the desired pH value.

Fast Atom Bombardment (FAB) Mass Spectrometry Measurements.—The FAB mass spectra were obtained with a ZAB 2F BE (VG ANALYTICAL, UK) spectrometer. The kinetic energy of the beam (Xe) was 8 keV (*ca.* 1.28 × 10^{-15} J). The samples were obtained by evaporation of an equimolar solution (10^{-2} mol dm^{-3}) of His(πMe) plus one of the salts $Cu(NO_3)_2$, $Cu(O_2CMe)_2$ or $CuCl_2$. The sample solution pH was previously adjusted to neutrality by addition of a suitable amount of standard KOH. The solid, a non-crystalline powder, was then dissolved in glycerol and analysed.

Calculations.—The calculations concerning the $E°$ of the electrode system were performed by the least-square computer program ESAB,[61] which refines the parameters of acid–base titration, and BEATRIX,[62] based on the Gran method. The formation constants of the copper(II) complexes were calculated by means of the least-squares computer program SUPERQUAD.[63] To obtain the species distribution within the pH ranges explored the computer program DISDI[64] was used.

A pK_w value of 13.74[65] was employed in the calculations; the hydrolytic constants of Cu^{2+} have been taken from Arena *et al.*;[66] protonation and binary complex formation constants for Phe and Trp were literature values.[38,65]

* To eliminate the ambiguity in the nomenclature of the *N*-methylhistidines, due to conflicting numbering systems, used by chemists and biochemists, for the imidazole ring, the imidazole nitrogens have been named *pros* (π) and *tele* (τ).[55]

Table 2 Protonation constants of L-histidine, N^{τ}-methyl-L-histidine and N^{π}-methyl-L-histidine at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). The precision reported as the standard deviation on the last figure is given in parentheses

Reaction	log K_a (this work)	log K Ref. 14, *a*	Ref. 3, *b*
L-HisO$^-$ + H$^+$ ⇌ L-His	9.06(1)	9.20	9.04
L-His + H$^+$ ⇌ L-HHis$^+$	6.02(1)	6.14	6.03
L-H$^+$His + H$^+$ ⇌ L-H$_2$His^{2+}	1.69(2)	1.80	1.78
His(τMe)O$^-$ + H$^+$ ⇌ His(τMe)	9.16(1)	9.27	9.20
His(τMe) + H$^+$ ⇌ HHis(τMe)$^+$	5.87(1)	5.99	5.91
HHis(τMe)$^+$ + H$^+$ ⇌ H$_2$His(τMe)$^{2+}$	1.70(4)	1.87	1.84
His(πMe)O$^-$ + H$^+$ ⇌ His(πMe)	8.61(1)	8.73	8.64
His(πMe) + H$^+$ ⇌ HHis(πMe)$^+$	6.46(2)	6.61	6.52
HHis(πMe)$^+$ + H$^+$ ⇌ H$_2$His(πMe)$^{2+}$	1.64(4)	1.78	1.76

^{a} I = 0.15 mol dm^{-3}, potentiometry. ^{b} 37 °C, ^{1}H NMR spectroscopy.

Table 3 Overall formation constants of binary copper(II) complexes of L-histidine, N^{τ}-methyl-L-histidine and N^{π}-methyl-L-histidine at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). The precision reported as the standard deviation on the last figure is given in parentheses

Reaction	log β
Cu^{2+} + HisO$^-$ + H$^+$ ⇌ [Cu(HisO)H]$^{2+}$	14.14(2)
Cu^{2+} + HisO$^-$ ⇌ [Cu(HisO)]$^+$	10.15(2)
Cu^{2+} + 2HisO$^-$ + 2H$^+$ ⇌ [Cu(HisO)$_2$H$_2$]$^{2+}$	27.27(4)
Cu^{2+} + 2HisO$^-$ + H$^+$ ⇌ [Cu(HisO)$_2$H]$^+$	23.83(3)
Cu^{2+} + 2HisO$^-$ ⇌ [Cu(HisO)$_2$]	18.03(5)
Cu^{2+} + His(τMe)O$^-$ + H$^+$ ⇌ [Cu{His(τMe)O}H]$^{2+}$	14.07(3)
Cu^{2+} + His(τMe)O$^-$ ⇌ [Cu{His(τMe)O}]$^+$	10.22(3)
Cu^{2+} + 2His(τMe)O$^-$ + 2H$^+$ ⇌ [Cu{His(τMe)O}$_2$H$_2$]$^{2+}$	27.1(11)
Cu^{2+} + 2His(τMe)O$^-$ + H$^+$ ⇌ [Cu{His(τMe)O}$_2$H]$^+$	23.87(2)
Cu^{2+} + 2His(τMe)O$^-$ ⇌ [Cu{His(τMe)O}$_2$]	18.38(4)
Cu^{2+} + His(πMe)O$^-$ + H$^+$ ⇌ [Cu{His(πMe)O}H]$^{2+}$	14.04(2)
Cu^{2+} + His(πMe)O$^-$ ⇌ [Cu{His(πMe)O}]$^+$	9.3(2)
Cu^{2+} + 2His(πMe)O$^-$ + 2H$^+$ ⇌ [Cu{His(πMe)O}$_2$H$_2$]$^{2+}$	27.0(1)
Cu^{2+} + 2His(πMe)O$^-$ + H$^+$ ⇌ [Cu{His(πMe)O}$_2$H]$^+$	20.9(1)
2Cu^{2+} + 2His(πMe)O$^-$ + H$^+$ ⇌ [Cu$_2${His(πMe)O}$_2$H]$^{3+}$	25.5(1)
2Cu^{2+} + His(πMe)O$^-$ ⇌ [Cu$_2${His(πMe)O}$_2$]$^{2+}$	21.4(1)
2Cu^{2+} + 2His(πMe)O$^-$ ⇌ [Cu$_2${His(πMe)O}$_2$H$_{-1}$]$^+$ + H$^+$	16.5(5)

Throughout, errors are expressed as the standard deviation of the mean on the results of the individual titrations, which corresponds approximately to five times the precision given by SUPERQUAD, in the cumulative calculations.

Results and Discussion

Protonation.—Protonation constants of L-His, His(πMe) and His(τMe) are reported in Table 2, along with some literature data. The agreement is very good considering the differences in experimental conditions.

The protonation constant of the carboxyl group is roughly the same for all three amino acids, within experimental precision. Only a weak electrostatic interaction may occur between the carboxylate group and the protonated imidazole residue.[2,10] This interaction does not seem to be affected by the presence of the methyl substituent.

The N^{π}-hydrogen of His(τMe) proved to be more acidic than the N^{τ}-hydrogen of His(πMe). The same is probably true also for L-His even though it is not possible to measure this difference potentiometrically. The tautomeric constant K_T (see Scheme 1) can be estimated from the ratio of the protonation constants of the two corresponding methylated derivatives.[3,67-69] This calculation gives K_T = 3.9, in good agreement with previous results.[4,5,10,11,13,15,69] The formation of the N$^{\pi}$···H–N$^{\alpha}$ hydrogen bond is impossible for His(πMe) but it is likely for His(τMe) in the range pH 5–8; thermodynamic data[14] support this suggestion.

The presence of the methyl substituent has a significant effect on the amino-group basicity as well, especially in the case of

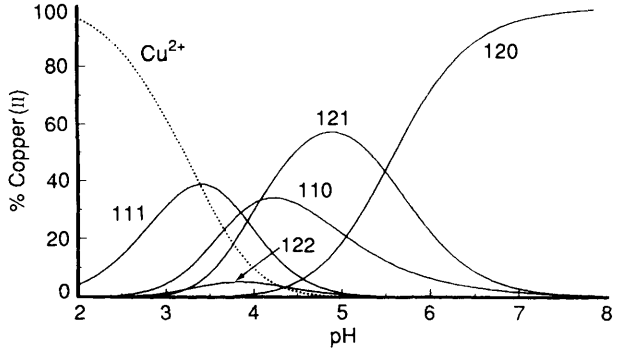


Fig. 1 Species distribution in the Cu^{II}–His(τMe) system. Labels refer to the complex stoichiometry in the following order: metal, ligand, proton. [Cu^{II}]$_{tot}$ = 0.003 mol dm^{-3}, [His(τMe)]$_{tot}$ = 0.006 mol dm^{-3}, 25 °C, I = 0.1 mol dm^{-3} (KNO_3)

His(πMe). The amino-group pK_a values are similar for L-His and His(τMe), while that of His(πMe) is much lower. The breakdown of a hydrogen bond N$^{\pi}$···H–N$^{\alpha}$ in the case of L-His and His(τMe) may explain this difference.[6,10,11]

Binary Complexes.—Table 3 shows the results of the potentiometric investigation on the Cu^{II}–His(τMe) binary system; the corresponding data obtained for the L-His system are also reported. The distribution diagram for the Cu^{II}–His(τMe) binary complexes is shown in Fig. 1. In the investigated pH range (2.5–8.0) the same complexes are formed

by both L-His and His(τMe) and the corresponding formation constants are very similar especially for the protonated species. A significant difference between the corresponding log β values is present only for the neutral $[CuL_2]$ (120) complexes, possibly due to the difference between the amino-group protonation constants. A smaller difference, but in the same direction, is also present between the stability constants of the $[CuL]^+$ (110) complexes. These results suggest that the Cu^{II}–His(τMe) binary complexes are very similar in structure to the corresponding L-His complexes. The presence of the methyl substituent on the N^τ atom does not significantly modify the co-ordination properties of the ligand. These conclusions are in excellent agreement with those already deduced by Casella and Gullotti who studied the Cu^{II}–L-His[28] and –His(τMe)[29] and related systems through electronic and CD spectroscopy.

The situation is somewhat more complicated in the case of the Cu^{II}–His(πMe) for which it was more difficult to determine a set of complex-formation constants, capable of unambiguously representing the system under all the reagent concentration conditions employed. In the acidic range (up to pH 5) the main species is the $[Cu(HL)]^{2+}$ (111) complex; its formation constant ($\log \beta_{111} = 14.04$) is equal to those for L-His and N^τ-MH. The less important, but well defined, $[Cu(HL)_2]^{2+}$ (122) complex is also formed ($\log \beta_{122} = 27.0$) in approximately the same pH range. In these two complexes the protonated His(πMe) binds the Cu^{II} in glycine-like fashion, as L-His and His(τMe) do. Once again, the presence of the methyl substituent on the imidazole ring has a negligible effect. With increasing pH the imidazole τ-nitrogen becomes available for complexation; however, the simultaneous co-ordination of both N^α and N^τ atoms with the same copper(II) ion is improbable as it would lead to the formation of a seven-atom ring. Thus, His(πMe) does not act as tridentate ligand. Species like 110, 121 and 120, which, in the Cu^{II}–L-His and –His(τMe) systems, are present in high percentages in the pH range closest to neutrality, become less important, while binuclear (and also polynuclear) complexes appear.

Similar observations had been made by Letter and Jordan[70] in a kinetic study on the Ni^{II}–His(πMe) complexes. They suggested the formation of a 210 complex containing a deprotonated His(πMe) ligand bridging two nickel(II) ions. They stated that 'the ligand acts essentially as two independent units, the imidazole part and the glycine part' due to 'its inability to chelate with the two nitrogen atoms'. These authors worked with a large molar excess of Ni^{II} in relation to the ligand; however, the same behaviour is followed by His(πMe) in the present investigation, even though the metal-to-ligand ratios employed here range from 1:1 to 1:4.

The binuclear complexes which fit the potentiometric data are 221, 220 and 22 – 1. The existence of the 210 complex cannot be ruled out with certainty. In particular, the 22 – 1 species largely prevails in the pH range around neutrality (see Fig. 2), even at low metal-to-ligand ratio (1:4) or at low copper(II) concentration (1 mmol dm^{-3}). Although good fittings for each individual titration can be obtained, the formation constant values of the binuclear complexes were rather scattered and undergo some variation with reagent concentrations. Averaged log β values are reported in Table 3.

Electronic spectra of copper(II) complexes with amino acids show two distinct absorption maxima.[21,71] The first one, in the UV region at about 250 nm, is rather intense ($\varepsilon \approx 3000$ dm^3 mol^{-1} cm^{-1}); it is due to a ligand-to-metal electron-transfer transition, involving both the carboxylate and the amino groups bound to copper.[71] The second is a weaker band ($\varepsilon \approx 30$–100 dm^3 mol^{-1} cm^{-1}), occurring in the VIS region at about 600–730 nm, due to a d–d transition.

The UV/VIS electronic spectra for the binary systems Cu^{II}–L-Ala, –L-His, –His(πMe) and –His(τMe) were recorded at different pH values. In separate experiments copper(II) nitrate absorption spectra were also recorded at the same pH values (up to 4.5). These were then substracted from the complex spectra, taking into account the correct free copper(II) ion concentration, computed by means of the DISDI[64] program. Molar absorption coefficients reported in Tables 4 and 5 are then referred to the amount of Cu^{II} really involved in complex formation and not to the total (stoichiometric) concentration, as is usually reported.[21,28,29] The spectra obtained after this correction are more significant in view of the complex-structure investigation, especially at low pH, where free copper(II) ion is present in considerable amount. An example is shown in Fig. 3.

The UV spectra (data in Table 4)* do not seem to show

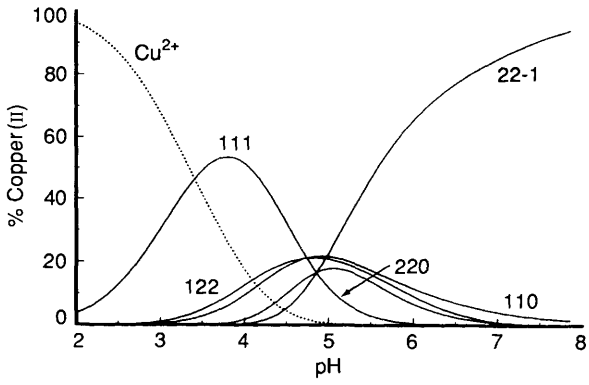


Fig. 2 Species distribution in the Cu^{II}–His(πMe) system. Labels and experimental conditions as in Fig. 1

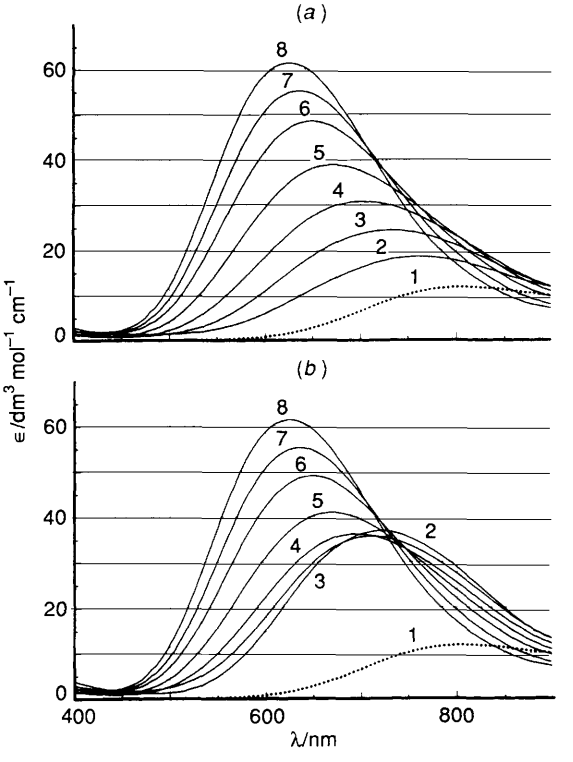


Fig. 3 Visible absorption spectra for the Cu^{II}–His(πMe) system (10^{-2} mol dm^{-3}, 1:2, 25 °C): (*a*) experimental spectra, (*b*) spectra obtained after subtraction of the free copper(II) contribution (see text) at pH 2.5 (2), 3.0 (3), 3.5 (4), 4.0 (5), 4.5 (6), 5.0 (7) and 7.5 (8). Curve 1 shows the spectrum of $Cu(NO_3)_2$ at pH 3.5

* It is worth noting that data shown in Table 4 are rather different from those reported by Martin and co-workers[21] and Casella and Gullotti,[28,29] especially as far as the absorption intensities are concerned. However, it should be underlined that, in the former studies, the ionic strength was unadjusted and the charge-transfer band occurred as a shoulder on a larger peak in the UV region. Conversely, in the present case, the resulting peak can be entirely ascribed to the charge-transfer band of complexed Cu^{II}.

Table 4 The UV electronic spectral data for $Cu(NO_3)_2$ and binary copper complexes, at different pH values

System	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	System	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}
$Cu(NO_3)_2$	2.5	247	28	Cu^{II}–His(πMe)	3.1	248	2417
	3.0	247	28		3.5	248	2274
	3.5	247	29		4.0	249	2252
	4.0	247	30		4.5	251	2419
	4.4	247	31		5.0	252	2678
Cu^{II}–L-Ala	3.1	247	2679		5.5	253	2876
	3.5	247	2231		6.0	254	2978
	4.0	248	2229		6.6	254	3026
	4.5	249	2196		7.5	254	3076
	5.5	251	2446		8.5	255	3133
	6.5	253	2915		9.5	254	3118
	8.4	256	3195		10.5	254	2988
	10.4	255	3162	Cu^{II}–His(τMe)	3.0	248	1830
Cu^{II}–L-His	3.0	248	2434		3.5	248	1911
	3.5	248	2332		4.0	250	2116
	4.0	250	2272		4.5	252	2408
	4.5	252	2459		5.0	253	2728
	5.0	253	2683		5.5	254	2940
	5.5	254	2895		6.0	255	3064
	6.5	255	3110		6.6	256	3138
	8.2	255	3181		7.4	256	3181
	9.4	256	3187		8.5	256	3202
	10.5	255	3219		9.4	256	3212
	11.5	255	3585		10.4	256	3220
					11.5	255	3259

Table 5 The VIS electronic spectra of $Cu(NO_3)_2$ and binary copper complexes, at different pH values

System	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	System	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}
$Cu(NO_3)_2$	2.5	810	12	Cu^{II}–His(πMe)	2.5	720	40
	3.0	809	12		3.0	711	36
	3.5	809	12		3.5	694	36
	4.0	809	12		4.0	670	41
	4.5	808	12		4.5	649	49
					5.0	636	55
Cu^{II}–L-Ala	2.5	731	41		5.6	628	59
	3.0	722	38		6.0	626	60
	3.5	717	37		6.5	625	61
	4.0	708	36		7.5	625	61
	4.5	689	36		8.5	626	61
	5.5	640	43		9.5	628	62
	6.5	624	52		10.5	633	66
	8.6	619	57		11.5	629	75
	10.5	620	56	Cu^{II}–His(τMe)	2.5	719	35
					3.0	702	35
Cu^{II}–L-His	2.4	723	35		3.5	665	37
	3.0	707	36		3.9	639	42
	3.5	673	36		4.3	626	49
	4.0	638	42		4.7	624	55
	4.5	624	49		5.1	626	63
	5.0	621	56		5.5	631	72
	5.5	625	64		6.0	638	83
	6.5	638	81		6.5	642	89
	7.5	641	87		7.1	643	92
	8.5	641	88		7.6	644	94
	9.5	641	88		8.5	644	94
	10.5	639	89		9.4	644	94
	11.5	626	104		10.4	644	94
					11.5	642	89

differences among the various complex systems. The appearance of an intense charge-transfer band in presence of an amino acid ligand can be observed; however, the wavelength of maximum absorption shows nearly the same behaviour in all the systems, as the pH varies. The corresponding ε values at neutral pH tend to be slightly lower for L-Ala and His(πMe) than for L-His and His(τMe).

Most significant for the investigation on the complexes formed in solution are the results in the VIS region, reported in Table 5. First it can be observed that the spectra of Cu^{II}–L-His and –His(τMe) are very similar: the wavelength of maximum absorption progressively decreases with increasing pH, until approximately pH 5, *i.e.* as long as histidine exhibits glycine-like co-ordination. The same behaviour is observed in the Cu^{II}–L-Ala system, investigated for the sake of comparison, taking into account that, in this system, bis complexation occurs at higher

pH (≈6.5). When approaching neutrality, both L-His and His(τMe) prefer a histamine-like co-ordination in the equatorial plane and become tridentate; as a consequence, the maximum wavelength shifts back to higher values. This behaviour is in agreement with previous investigations[28,29,37,72] and with calculations made by the Billo[73] equation.

A similar behaviour is followed by the Cu^{II}–His(πMe) system, but only at the lowest pH values, where the complexation behaviour of His(πMe) is the same as that of the other two histidine ligands (see above). On the contrary, as the pH is raised, the wavelength of maximum absorption remains roughly the same up to pH ≈9.5 where hydroxylated complexes most likely start to form. This behaviour is very similar to that observed in the case of L-Ala. Also the ε values, in such a pH range, are significantly lower than those exhibited by L-His and His(τMe) and similar to those obtained with L-Ala. This is in agreement with the consideration that His(πMe) cannot bind Cu^{II} in the histamine-like fashion. The spectra obtained at neutral pH can be almost entirely ascribed to the binuclear 22 − 1 complex, as shown in Fig. 3. The position of the absorption band suggests that this complex contains one copper centre in which the two ligands are bound in the glycine-like manner. The second Cu^{II} will consequently be bound to one of the unprotonated τ-nitrogen atoms of the side imidazole rings, giving a lower contribution to the global spectrum.

The Cu^{II}–His(πMe) system was further investigated by means of FAB mass spectroscopy. This technique is able to give information on the molecular weight and the stoichiometry of ionic or ionizable species present in the matrix,[74] and has been successfully applied to organometallic and co-ordination chemistry.[75–77] The spectra contain a number of peak clusters deriving from all the ionic species either existing in the matrix or formed during the 'sputtering' step. Most of the information is contained in the quasi-molecular peak cluster. In all the examined cases the cluster with the highest detectable *m/z* value can be attributed to a complex $[(Cu^{2+})_2\{His(\pi Me)\text{-}O^-\}_2]^{2+}A^-$, where A^- is the counter ion (NO_3^-, $MeCO_2^-$ or Cl^-). This assignment is based on both the *m/z* values of the individual peaks of the cluster and their relative intensities; they were compared with the corresponding theoretical values computed from the known isotopic distributions of the atoms involved in the complex. An example is shown in Fig. 4.

A deeper investigation, *e.g.* by means of ESR and/or CD spectroscopy, is required further to clarify the solution structure of these binuclear complexes. This will be the subject of future work.

Ternary Complexes.—The potentiometric study of the ternary systems containing Cu^{II}, His(τMe) and a second amino acid (L- or D-Phe, L- or D-Trp) revealed the formation of two ternary complexes: the neutral species 1110 and the positively charged protonated complex 1111. Experimental log β values are reported in Table 6; the corresponding $\Delta\log K_{1110}$ values[78]

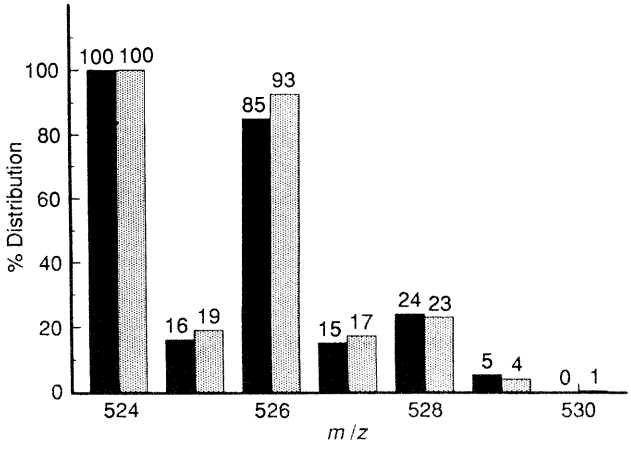


Fig. 4 Observed (solid bar) and calculated (shaded bar) isotopic distribution for the FAB mass spectrum of the complex $[Cu_2\{His(\pi Me)O\}_2(NO_3)]^+$

Table 6 Overall formation constants of ternary copper(II) complexes of L- or D-histidine, N^{τ}-methyl-L-histidine or N^{π}-methyl-L-histidine with phenylalanine or tryptophan; 25 °C, I = 0.1 mol dm^{-3} (KNO_3). The precision reported as the standard deviation on the last figure is given in parentheses

Reaction	log β	Δ log *K*
Cu^{2+} + L-$HisO^-$ + L-$PheO^-$ + H^+ ⇌ $[Cu(L\text{-}HisO)(L\text{-}PheO)H]^+$	21.52(3)*	
Cu^{2+} + L-$HisO^-$ + L-$PheO^-$ ⇌ $[Cu(L\text{-}HisO)(L\text{-}PheO)]$	17.53(1)*	−0.41
Cu^{2+} + D-$HisO^-$ + L-$PheO^-$ + H^+ ⇌ $[Cu(D\text{-}HisO)(L\text{-}PheO)H]^+$	21.55(6)*	
Cu^{2+} + D-$HisO^-$ + L-$PheO^-$ ⇌ $[Cu(D\text{-}HisO)(L\text{-}PheO)]$	17.70(1)*	−0.24
Cu^{2+} + L-$HisO^-$ + L-$TrpO^-$ + H^+ ⇌ $[Cu(L\text{-}HisO)(L\text{-}TrpO)H]^+$	21.83(6)*	
Cu^{2+} + L-$HisO^-$ + L-$TrpO^-$ ⇌ $[Cu(L\text{-}HisO)(L\text{-}TrpO)]$	18.29(1)*	−0.17
Cu^{2+} + D-$HisO^-$ + L-$TrpO^-$ + H^+ ⇌ $[Cu(D\text{-}HisO)(L\text{-}TrpO)H]^+$	21.88(9)*	
Cu^{2+} + D-$HisO^-$ + L-$TrpO^-$ ⇌ $[Cu(D\text{-}HisO)(L\text{-}TrpO)]$	18.75(1)*	0.29
Cu^{2+} + His(τMe)O^- + L-$PheO^-$ + H^+ ⇌ $[Cu\{His(\tau Me)\}(L\text{-}PheO)H]^+$	21.40(2)	
Cu^{2+} + His(τMe)O^- + L-$PheO^-$ ⇌ $[Cu\{His(\tau Me)\}(L\text{-}PheO)]$	17.67(1)	−0.39
Cu^{2+} + His(τMe)O^- + D-$PheO^-$ + H^+ ⇌ $[Cu\{His(\tau Me)\}(D\text{-}PheO)H]^+$	21.42(3)	
Cu^{2+} + His(τMe)O^- + D-$PheO^-$ ⇌ $[Cu\{His(\tau Me)\}(D\text{-}PheO)]$	17.87(1)	−0.19
Cu^{2+} + His(τMe)O^- + L-$TrpO^-$ + H^+ ⇌ $[Cu\{His(\tau Me)\}(L\text{-}TrpO)H]^+$	22.07(4)	
Cu^{2+} + His(τMe)O^- + L-$TrpO^-$ ⇌ $[Cu\{His(\tau Me)\}(L\text{-}TrpO)]$	18.57(2)	0.11
Cu^{2+} + His(τMe)O^- + D-$TrpO^-$ + H^+ ⇌ $[Cu\{His(\tau Me)\}(D\text{-}TrpO)H]^+$	22.01(3)	
Cu^{2+} + His(τMe)O^- + D-$TrpO^-$ ⇌ $[Cu\{His(\tau Me)\}(D\text{-}TrpO)]$	18.99(1)	0.53
Cu^{2+} + His(πMe)O^- + L-$PheO^-$ + H^+ ⇌ $[Cu\{His(\pi Me)O\}(L\text{-}PheO)H]^+$	21.21(3)	
Cu^{2+} + His(πMe)O^- + L-$PheO^-$ ⇌ $[Cu\{His(\pi Me)O\}(L\text{-}PheO)]$	15.0(1)	−2.2
$2Cu^{2+}$ + 2His(πMe)O^- + L-$PheO^-$ ⇌ $[Cu_2\{His(\pi Me)O\}_2(L\text{-}PheO)]^+$	28.76(3)	
Cu^{2+} + His(πMe)O^- + D-$PheO^-$ + H^+ ⇌ $[Cu\{His(\pi Me)O\}(D\text{-}PheO)H]^+$	21.24(3)	
Cu^{2+} + His(πMe)O^- + D-$PheO^-$ ⇌ $[Cu\{His(\pi Me)O\}(D\text{-}PheO)]$	14.9(1)	−2.3
$2Cu^{2+}$ + 2His(πMe)O^- + D-$PheO^-$ ⇌ $[Cu_2\{His(\pi Me)O\}_2(D\text{-}PheO)]^+$	28.70(3)	
Cu^{2+} + His(πMe)O^- + L-$TrpO^-$ + H^+ ⇌ $[Cu\{His(\pi Me)O\}(L\text{-}TrpO)H]^+$	21.94(2)	
Cu^{2+} + His(πMe)O^- + L-$TrpO^-$ ⇌ $[Cu\{His(\pi Me)O\}(L\text{-}TrpO)]$	15.6(1)	−2.0
$2Cu^{2+}$ + 2His(πMe)O^- + L-$TrpO^-$ ⇌ $[Cu_2\{His(\pi Me)O\}_2(L\text{-}TrpO)]^+$	29.77(3)	
Cu^{2+} + His(πMe)O^- + D-$TrpO^-$ + H^+ ⇌ $[Cu\{His(\pi Me)O\}(D\text{-}TrpO)H]^+$	21.97(3)	
Cu^{2+} + His(πMe)O^- + D-$TrpO^-$ ⇌ $[Cu\{His(\pi Me)O\}(D\text{-}TrpO)]$	15.5(3)	−2.1
$2Cu^{2+}$ + 2His(πMe)O^- + D-$TrpO^-$ ⇌ $[Cu_2\{His(\pi Me)O\}_2(D\text{-}TrpO)]^+$	29.71(4)	

* Ref. 38.

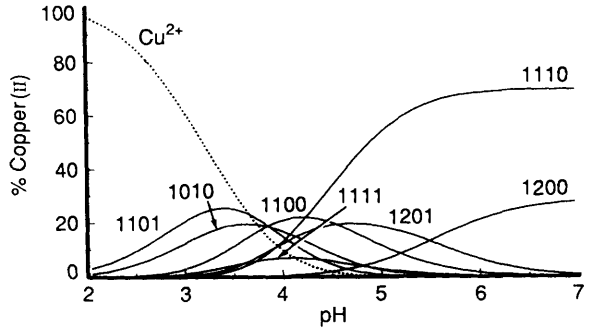


Fig. 5 Species distribution in the Cu^{II}–His(τMe)–L-Trp system. Labels refer to the complex stoichiometry in the following order: Cu^{II}, His(τMe), L-Trp, H^+. $[Cu^{II}]_{tot}$ = 0.003, $[His(\tau Me)]_{tot}$ = 0.004, $[L\text{-}Trp]_{tot}$ = 0.004 mol dm^{-3}; 25 °C, I = 0.1 mol dm^{-3} (KNO_3)

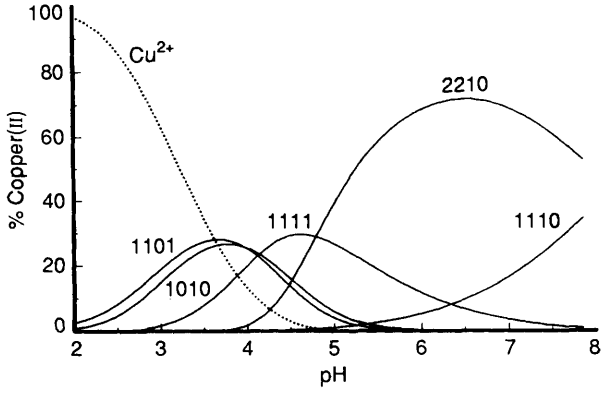


Fig. 6 Species distribution in the Cu^{II}–His(πMe)–L-Trp system. Labels and experimental conditions as in Fig. 5

are also shown. The theoretical value of $\Delta\log K_{1110}$, based on statistical considerations and referred to octahedral copper(II) complexes, is −0.9;[78] the experimental values are always higher and, in the case of Trp complexes, they even become positive. This means that, in these systems, the ternary complex formation is particularly favoured compared with purely statistical contributions.

A distribution diagram of a Cu^{II}-His(τMe) ternary system is shown in Fig. 5. The 1111 complex is present in the acidic pH range, at low concentrations; the 1110 complex is the main species at pH around neutrality. A significant stereoselective effect, quantified by the difference between the log β values of the corresponding homo- and hetero-chiral 1110 complexes is present in both systems, especially when the second ligand is Trp. This behaviour was found previously in ternary Cu^{II}–His complexes with the same amino acids.[36,38] The higher stability of the hetero-chiral ternary copper(II) complexes of His with a second amino acid bearing an aromatic side chain, with respect to their homo-chiral homologues, has been essentially ascribed to the tridentate behaviour of His. In the former complexes the aromatic side chain of Phe or Trp lies on the opposite side of the equatorial plane of the complex with respect to the histidine carboxyl group which is co-ordinated in distorted axial position. This allows for a weak interaction between this aromatic group with either the copper ion or the imidazolyl residue of His (stacking). Such an interaction, sterically hindered in the homo-chiral complexes, gives rise to an extra stability contribution to the complex. The same behaviour can be envisaged for the ternary complexes of His(τMe); the experimental $\Delta\log \beta_{1110}$ values are very similar to those already reported for His (see Table 6). As in the case of binary complexes, the presence of the N^{τ}-methyl substituent does not have a significant effect on histidine complexation. These results are of particular interest also for the mechanism of resolution of underivatized amino acid enantiomers in LEC, when the chiral selector His(τDec) is used (see Introduction). The complexation behaviour of the His residue of His(τDec) should be very close to that of His(τMe) (and hence His).

The picture is rather different in the case of His(πMe), as illustrated by the data reported in Table 6 and the distribution diagram of Fig. 6. At acidic pH the protonated 1111 complex is still formed in appreciable amounts. Its log β value is only slightly different from that of the corresponding ternary complexes of His(τMe) or L-His with either Phe or Trp. As the pH rises and the imidazole ring is deprotonated the neutral species 1110 appears. Its log β value is much lower than those of the other two histidines; in fact, His(πMe) can not bind Cu^{2+} in a tridentate fashion. The experimental $\Delta\log K_{1110}$ values are more negative than the reference statistical value; in this case the 1110 complex formation is disfavoured compared with the formation of the corresponding binary 1:1 complexes. As a consequence, the 1110 complex is not so important in this ternary system and is not the main species at pH around neutrality. Instead, a binuclear ternary complex (2210) is dominant in this pH range. It is most likely that this complex is only the most relevant of the binuclear mixed-ligand complexes formed in the system. Other binuclear ternary species, like 2120, 2211 and 221 − 1, seem to form in low percentages; however, their presence cannot be confirmed or ruled out with certainty. Finally, no stereoselective effect was found in the formation of any of the ternary complexes of His(πMe).

Conclusion

The presence of a methyl substituent on the N^{τ} or the N^{π} atom of the imidazole ring of His has different effects on both the protonation and affinity of this amino acid towards the copper(II) ion.

The protonation constants of both the imidazole and amine nitrogens of the methylated histidines are different from those of His. The methyl substituent eliminates the tautomeric equilibrium, blocking the imidazole residue (when not fully protonated) in one of the two forms. Moreover, the $N^{\pi}\cdots H\text{–}N^{\alpha}$ intramolecular hydrogen bond, previously suggested for His and His(τMe) in the range pH 5–9, cannot be formed by His(πMe).

The complexing behaviour of His(τMe) towards Cu^{II} is very similar to that of His. It can co-ordinate in both a glycine- and a histamine-like fashion, as His does. The same complex species are formed in the same pH range, both in binary and in ternary systems. Moreover, the corresponding log β values are very similar and the same stereoselective effects are observed in the corresponding ternary complexes. These results confirm that the N^{τ} atom of His is not involved in copper(II) complexation.

Conversely, His(πMe) behaves like L-His only in its protonated form (acidic pH range) when both ligands bind Cu^{2+} in the glycine-like manner. The presence of the methyl substituent on the N^{π} atom hinders the histamine-like binding mode and thus His(πMe) cannot co-ordinate Cu^{II} in a tridentate way. As a consequence, it behaves as two independent units giving rise to polynuclear complexes, both binary and ternary systems. No stereoselective effect was observed in the presence of an aromatic amino acid as the second ligand.

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