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Coordination Properties of N-protected Amino-acids: Simple and Mixed (N-benzoyl-DL-leucinate)copper(II) Complexes

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One green compound of the type $Cu(BzLeu)_2\cdot 2H_2O$ and mixed complexes with amines of the type $Cu(BzLeu)_2\cdot B_n\cdot mH_2O$ ($n = 2$, $m = 0$ and B = pyridine, 3- and 4-picoline and morpholine; $n = 1$, $m = 2$ and B = 1,10-phenanthroline, 2,2'-bipyridyl and piperazine) were prepared and characterized in the solid state by means of low- and room-temperature magnetic measurements, room-temperature electronic, infrared and e.p.r. spectra. For the green compound, which displays physical characteristics similar to those of copper(II) acetate monohydrate and similar dimeric complexes, the complicated magnetic properties, discussed in detail, demonstrate the presence of a great amount (~36%) of mononuclear copper(II) impurities, although the complex is analytically pure. For all the mixed complexes with amines, the physical measurements are consistent with a tetragonally distorted configuration of the copper(II) ion with the presence of a CuO_4N_2 chromophore. In all the complexes the amino acid coordinates only through the carboxylate group, acting as a 'simple' carboxylic acid.

Introduction

The major interest in the interaction of transition metal ions with amino acids and peptides has been focused on the simplest amino acids, glycine and alanine, and the biologically important compounds containing histidine and related species. Our recent studies deal with the interaction of transition metal ions with N-protected amino acids, in which the protecting group is an acetyl or benzoyl group. This is because the introduction of a substituent directly on the amino group, by reducing the ligand field of the in-plane donor, diminishes the affinity of the amino group for the metal ion. This gives the metal ions a greater reactivity with a consequent ease of formation of mixed complexes [1–4]. Further interest in these N-protected amino acids derives from the fact that they are the most simple systems in which only a peptide group is present.

In this paper we have studied the coordination properties of the N-benzoyl-DL-leucine (hereafter abbreviated as BzLeuH) with the copper(II) ion and their mixed complexes with saturated and unsaturated heterocyclic amines in order to investigate the perturbing effect of axial ligands on the amino acid coordination.

Experimental

All chemicals were reagent grade and used as received.

Preparation of the Complexes

$Cu(BzLeu)_2\cdot 2H_2O$

The compound was prepared by adding a copper(II) perchlorate hexahydrate (1 mmol) solution in anhydrous ethanol to an ethanolic N-benzoyl-DL-leucine (2 mmol) solution, neutralized with a stoichiometric amount of potassium hydroxide. The precipitated potassium perchlorate was filtered off and by evaporating the solution a green compound was separated. This compound was used as starting material for the adduct preparations. *Anal.* Calcd. for $C_{26}H_{36}N_2O_8Cu$: C, 54.94; H, 6.39; N, 4.93. Found: C, 54.43; H, 6.54; N, 4.93. M.p. 202–6 °C dec.

$Cu(BzLeu)_2(py)_2$

A blue compound separated by mixing the $Cu(BzLeu)_2\cdot 2H_2O$ with an amine excess and by cooling at 4–5 °C for some hours. *Anal.* Calcd. for $C_{36}H_{42}N_4O_6Cu$: C, 62.61; H, 6.14; N, 8.12. Found: C, 62.85; H, 6.13; N, 8.17. M.p. 130–5 °C.

$Cu(BzLeu)_2(npic)_2$ ($n = 3, 4$)

A blue compound separated by evaporating slowly a methanolic solution containing the $Cu(BzLeu)_2\cdot 2H_2O$ and an amine excess. $Cu(BzLeu)_2(3pic)_2$: *Anal.* Calcd. for $C_{38}H_{46}N_4O_6Cu$: C, 63.51; H, 6.46; N, 7.80. Found: C, 63.22; H, 6.58; N, 7.68. M.p. 131–5 °C. $Cu(BzLeu)_2(4pic)_2$: *Anal.* Calcd. for $C_{38}H_{46}N_4O_6Cu$: C, 63.51; H, 6.46; N, 7.80. Found: C, 63.88; H, 6.44; N, 7.84. M.p. 121–5 °C.

$Cu(BzLeu)_2B \cdot 2H_2O$ (B = bipy, o-phen)

Blue compounds separated by cooling a solution prepared as reported for py complex. $Cu(BzLeu)_2 \cdot$ bipy$\cdot 2H_2O$: *Anal.* Calcd. for $C_{36}H_{44}N_4O_8Cu$: C, 59.67; H, 6.13; N, 7.74. Found: C, 59.88; H, 6.38; N, 7.71. M.p. 115–9 °C.

$Cu(BzLeu)_2 \cdot$ o-phen$\cdot 2H_2O$

Anal. Calcd. for $C_{38}H_{44}N_4O_8Cu$: C, 60.97; H, 5.93; N, 7.49. Found: C, 60.92; H, 5.84; N, 7.51. M.p. 150–5 °C.

$Cu(BzLeu)_2 \cdot$ pipz$\cdot 2H_2O$

A violet compound precipitated by cooling a solution prepared as reported for the py complex. *Anal.* Calcd. for $C_{30}H_{46}N_4O_8Cu$: C, 55.05; H, 7.09; N, 8.57. Found: C, 55.79; H, 7.12; N, 8.51. M.p. 214–8 °C dec.

$Cu(BzLeu)_2(morph)_2$

A blue compound separated by adding diethyl ether and cooling a solution prepared as reported for the py complex. *Anal.* Calcd. for $C_{34}H_{50}N_4O_8Cu$: C, 57.79; H, 7.14; N, 7.94. Found: C, 57.69; H, 7.32; N, 8.01. M.p. 152–6 °C.

Physical Measurements

The i.r. spectra of the solid compounds in KBr pellets or nujol mulls on KBr pellets as support were recorded with a Perkin Elmer 180 spectrophotometer in the 4000–250 cm^{-1} spectral range. The room-temperature electronic spectra of the solid compounds were recorded as mull transmission spectra with a Shimadzu MPS 50L spectrophotometer. The low- and room-temperature magnetic moments were measured with the Gouy method, using $HgCo(SCN)_4$ as calibrant and correcting for diamagnetism with the appropriate Pascal constants. The e.p.r. spectra were recorded on a JEOL PE-3X spectrometer; quartz sample tubes were employed for polycrystalline samples. Spectra were calibrated with diphenylpicrylhydrazyl (DPPH, g = 2.0036) as a field marker.

Analyses

Nitrogen, carbon and hydrogen were determined with a C. Erba Elemental Analyzer Mod. 1106 by Mr. G. Pistoni.

Results and Discussion

All the complexes are stable in air, although they appear hygroscopic, particularly the amine adducts.

The direct interaction between the copper(II) ion and the amino acid gives rise to the formation of one dihydrate green complex. This resembles the general behavior of many bis(N-protected amino acidate)-copper(II) complexes [1, 5, 6].

Many attempts were made using different precipitation techniques and non-aqueous solvents to obtain macrocrystalline materials, but without success.

The room-temperature e.p.r. spectrum (Fig. 1) and the e.p.r. parameters (Table I) show clear evidence for the presence of dinuclear species with large antiferromagnetic exchange [6–9], but also for a large

TABLE I. Room-temperature Electronic and E.p.r. Spectra and Magnetic Moments of the Solid Mixed Complexes [a]

	d–d bands kK		μ_{eff} BM	$g_\parallel$	$g_\perp$	g_0		
$Cu(BzLeu)_2(py)_2$	15.3		1.84	2.272	2.050	2.124		
$Cu(BzLeu)_2(3pic)_2$	14.8		1.87	2.329	2.050	2.143		
$Cu(BzLeu)_2(4pic)_2$	15.1		1.95	2.219	2.050	2.106		
$Cu(BzLeu)_2 \cdot ophen \cdot 2H_2O$	14.5		1.83	2.190	2.037	2.088		
$Cu(BzLeu)_2 \cdot pipz \cdot 2H_2O$	14.1sh	16.7	1.95	2.249	2.057	2.121		
$Cu(BzLeu)_2(morph)_2$	15.1		1.97	2.190	2.050	2.106		
				g_3	g_2	g_1	g_0	
$Cu(BzLeu)_2 \cdot bipy \cdot 2H_2O$[b]	15.6		1.81	2.317	2.253	2.018	2 196	
				Monomer[c]		Dimer		D
				$g_\parallel$	$g_\perp$	g_z	$g_\perp$	cm^{-1}
$Cu(BzLeu)_2 \cdot 2H_2O$	14.1		1.68	2.358	2.056	2.40	2.12	0.37

[a] Abbreviations: BzLeu = N-benzoyl-DL-leucinate ion; py = pyridine; 3- or 4-pic = 3- or 4-methylpyridine, bipy = 2,2'-bipyridyl, ophen = 1,10-phenanthroline; pipz = piperazine, morph = morpholine. [b] $A_3(\times 10^4\ cm^{-1}) = 162$; $A_2(\times 10^4\ cm^{-1}) = 158$. [c] $A_\parallel(\times 10^4\ cm^{-1}) = 154$.

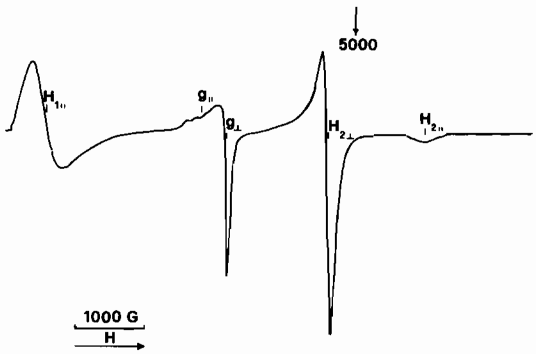

Fig. 1. Room-temperature e.p.r. spectra of the $Cu(BzLeu)_2 \cdot 2H_2O$ complex in the solid state.

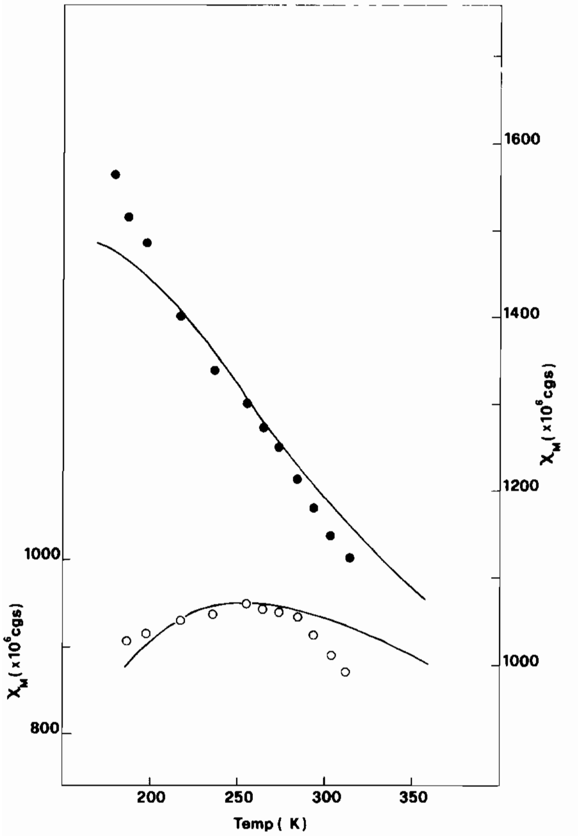

Fig. 2. Variation of the magnetic susceptibility with temperature of the $Cu(BzLeu)_2 \cdot 2H_2O$ complex. Top: ● experimental values, black line calculated from eqn. 1 of ref. 10; bottom: experimental (○) and calculated (black line) values corrected for the presence of monomeric impurities from eqn. 4 of ref. 10.

amount of mononuclear impurities of spin $S = \frac{1}{2}$ in the 3000 G region. On the other hand the magnetic properties are very complicated and do not vary with preparation. In fact the room-temperature magnetic moment (performed on amorphous samples) of 1.68 B.M. is intermediate between that expected for a pure dimer like copper(II) acetate monohydrate and that of a monomeric complex. Consequently in the temperature *vs.* corrected molar susceptibilities (χ_M^c) plot (Fig. 2) a shoulder appears and not a sharp maximum as expected for dimeric copper(II) carboxylate complexes. Furthermore the presence of mononuclear impurities, not uncommon for dinuclear or polynuclear copper(II) complexes [10–14] has been found to contribute significantly to the total susceptibility [10–14]. Therefore the results for $Cu(BzLeu)_2 \cdot 2H_2O$ could not be satisfactorily fitted to the simple Bleaney–Bowers equation [15], but to one modified for the presence of mononuclear impurities [10]. By using the experimental $\bar{g}$ value of 2.20 and an N_α value of 60 X 10^{-6} c.g.s. units a $-2J$ value of 284 (±10) cm^{-1}, a T_N value of 245 °K and a mol fraction of monomeric impurity of 0.36, which is the higher reported so far in the literature [10–14], are found. The results of the experimental magnetic measurements (corrected for monomeric impurities by comparison with standard samples) for diamagnetism (–321.27 X 10^{-6} c.g.s./mol) and T.I.P., and the calculated magnetic measurements from equation 4 in ref. 10 are all presented in Table II and Fig. 2.

TABLE II. Experimental and Calculated Corrected Molar Susceptibilities and Magnetic Moments for the $Cu(BzLeu)_2 \cdot 2H_2O$ Complex[a].

Temp (K)	χ_M X 10^6	μ_{eff} (BM)	χ_M X 10^6	μ_{eff} (BM)
	Exptl		Calcd	
187	911	1.17	877	1.15
197	920	1.20	901	1.19
217	930	1.27	933	1.28
237	933	1.33	949	1.34
255	950	1.39	953	1.40
264	946	1.41	952	1.42
274	944	1.44	949	1.44
284	934	1.46	945	1.47
293	914	1.46	940	1.49
303	890	1.47	933	1.51
312	877	1.48	927	1.52
340			903	1.57
350			894	1.58

[a] The molar susceptibility (χ_M) is corrected for the diamagnetism of the complex (–321.27 X 10^{-6} cgs/mol), for T.I.P. (60 X 10^{-6} cgs/mol) and for the mol fraction of monomeric impurities (Y = 0.36). The magnetic moment is calculated as $\mu_{eff} = 2.828 (\chi_M \cdot T)^{1/2}$.

The $-2J$ value for the $Cu(BzLeu)_2 \cdot 2H_2O$, which agrees with those found for similar dimeric N-protected amino acids [3, 4, 6, 9, 16–18] (Table III) and carboxylate [7, 8, 19] copper(II) complexes thus

TABLE III. Comparison of −2J Values for Dimeric N-protected Amino Acid Copper(II) Complexes.

	−2J	Ref.
Cu(Hip)$_2$	327	9
Cu(Hip)$_2$·Me$_2$SO	292	6
Cu(Hip)$_2$·DMF^a	313	6
Cu(Acgly)$_2$·H$_2$O	272	17
Cu(Acgly)$_2$	292	17
Cu(Ac-β-ala)$_2$·2H$_2$O	324	16
Cu(AcVal)$_2$·H$_2$O	351	4
Cu(Actrp)$_2$·H$_2$O	351	3
Cu(BzLeu)$_2$·2H$_2$O	284^b	this work
	176^c	this work

^{a}Mol fraction of monomeric impurities (Y) = 0.05 ^{b}Y = 0.36. ^{c}−2J value calculated assuming Y = 0.

indicating a relatively strong magnetic interaction between two copper ions, demonstrates the correctness of such a procedure. Table III also shows the −2J value uncorrected for the mononuclear impurities.

A superexchange mechanism via the bridging carboxylate groups, compatible with these results, indicates that this N-protected amino acid coordinates only through the carboxylic group, the peptide group being probably involved in hydrogen bonding in the packing of the molecules.

The electronic spectrum of the green compound is consistent with these conclusions, although it shows only one band, assignable to d–d transition (Band I) near 14.1–14.4 kK, which may be considered to be the 'ordinary copper(II) band' [8]. The more characteristic band, indicated as Band II [8] which should appear near 27 kK, and which is considered to be diagnostic of dimeric structure, is not observed.

Studies in the i.r. spectral region performed by comparing the spectra of the amino acid, its alkali salts, the complex and their deuteriated analogues, agree with the above suggestions. The band at 3420 cm^{-1} in the dihydrate green complex originates from the water molecules, while the band at 3340 (found at higher energies than in the free amino acid and at similar energies as the alkali salts) may be assigned to uncoordinated N–H stretching vibration of the peptide group.

The positions of the bands assignable to antisymmetric and symmetric carboxylate stretching vibrations at 1640–1615 and 1415 cm^{-1} respectively are characteristic of the bridging carboxylate group [3]. A similar behavior is also well established for all the N-protected amino acids previously examined, where the protecting group is an acetyl or a benzoyl group [1–5, 16], which act as 'simple' carboxylic acids.

In agreement with the suggestion of Sharrock, Thibaudeau and Caille [6] these systems may be considered as model complexes for the e.p.r.-non-detectable copper in enzymes.

The dihydrate green complex easily reacts with amines separating blue mixed-ligand complexes, the physical measurements of which (Table I) suggest the presence of mononuclear units. In these complexes the copper(II) ion appears to be involved in a distorted tetragonal coordination. In fact the presence of tetragonal elongated structure may be suggested from their 'normal' magnetic moments and their e.p.r. spectra, which are of the axial type [20]. In particular the two g values of the mixed complexes, indicating a predominantly $d_{x^2-y^2}$ ground state and an essentially square-planar arrangement around the copper(II) ion [20, 21], lower than those of complexes containing oxygen donors [21, 22] suggest the presence of mixed oxygen and nitrogen donors as a consequence of the amine coordination [21, 23, 24]. The presence of heteroatomic N base moiety and O donors enhances the complex stability [25].

Also the room-temperature electronic spectra, which show a band envelope in the 14–16 kK spectral range, indicate tetragonally distorted ligand fields consistent with the presence of an essentially CuO_4N_2 chromophore [23, 24]. In particular the d–d band maximum order in the pyridines, different from their basicity order, may be attributed to the steric interference of the methyl group prevailing on the inductive effects.

The coordination of the amino acid in the mixed complexes through the carboxylate group is also confirmed by the i.r. spectra. The stretching vibrations of the peptide group are found at energies similar to those observed for the alkali salts and the green complexes, excluding the participation of this group in the metal coordination. In particular, the position of the symmetric carboxylate stretching vibration in the spectral range of 1368–1392 cm^{-1}, lower than that of 1400 cm^{-1} observed for the alkali salts, may be considered strongly indicative of 'asymmetric' bidentate carboxylate coordination [3, 26], the band being directly connected with oxygen atom linked to the copper(II) ion.

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