N-*p*-Amino- and *N*-*p*-Nitro-phenylsulfonyl Derivatives of Dipeptides, a New Family of Ligands for Copper(II). Potentiometric and Spectroscopic Studies

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The co-ordination ability of four dipeptide analogues substituted on the N-terminal amino group with *p*-nitrophenylsulfonyl (nps-Ala-Ala and nps-Ala-His) and *p*-aminophenylsulfonyl (aps-Ala-Ala and aps-Ala-His) groups was studied by potentiometric and spectroscopic (UV/VIS absorption, CD and EPR) techniques. The N-terminal sulfonyl substituent drastically changes the acidity of the sulfonamide proton making nitrogen very efficient in binding to Cu^{II}. The sulfonamide nitrogen having p*K* between 9 and 11 does not need any anchoring binding group to form complexes with Cu^{II}. The *para* substituent on the phenyl ring (amino or nitro) influences very strongly the acidity of the sulfonamide proton. The nps or aps moieties change the co-ordination equilibria considerably when compared to the parent dipeptide Ala-His. Both groups enforce the formation of dimeric complexes, whereas in the case of the parent dipeptide the major species are only monomeric.

The usefulness of the sulfonamides was considerably limited by the introduction of more efficient antibiotics. However, recent developments in the application of their metal complexes in burn therapy[1] has revived interest in sulfanilamides and their metal complexes.[2–5] The amide hydrogen of sulfonamides is considerably more acidic than a carbonyl amide hydrogen, changing drastically the co-ordination ability of this donor.[6,7] The involvement of sulfonamide nitrogen in metal-ion binding has mostly been studied in *N*-tolyl-*p*-sulfonyl-substituted amino acids.[8–14] Also sulfonylurea ligands appear to involve a sulfonamide type of nitrogen in metal-ion binding.[15] Our recent studies on *N*-*p*-aminophenylsulfonyl derivatives of amino acids[16] have shown that copper(II) ions are able to deprotonate and bind to sulfonamide nitrogen below pH 5 to form stable mono- and bis-$\{N^-,CO_2^-\}$ chelates. The *p*-phenyl substituent may have a distinct impact on acidity of the sulfonamide nitrogen. The formation of the chelate ring involves two donors almost simultaneously and it is unlikely that carboxylate acts as an anchor site for Cu^{II} as suggested earlier.[8]

Much more effective chelating agents for Cu^{II} are peptides, especially those containing the histidyl (His) residue.[7] Thus, in our studies on sulfonamide nitrogen co-ordination we have selected two *N*-*p*-aminophenylsulfonyl derivatives of dipeptides, containing Ala-Ala and Ala-His subunits (aps-Ala-Ala and -Ala-His). In order to establish the possible influence of the *para* substituent we have also studied *p*-nitrophenylsulfonyl derivatives of both dipeptides (nps-Ala-Ala and -Ala-His) and that of alanine (nps-Ala).

Experimental

Potentiometric Measurements.—Stability constants for H^+ and copper(II) complexes were calculated from titrations carried out at 25 °C using a total volume of 2.0 cm^3. Alkali was added from a 0.1 cm^3 micrometer syringe which was calibrated by both weight titration and the titration of standard materials. Experimental details: ligand concentration 2×10^{-3} mol dm^{-3}; metal to ligand ratios 1:2 and 1:3; ionic strength 0.1 mol dm^{-3} KNO_3; method, pH-metric titration on a MOLSPIN pH-meter system using a micro combined glass–calomel electrode, calibrated in concentration using HNO_3;[17] number of titrations 4; method of calculation SUPERQUAD.[18] The samples were titrated from pH 2.5 to 10.5.

Standard deviations (σ values) quoted were computed by SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of the particular species involved in the equilibria.

Spectroscopic Measurements.—The EPR spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120 K using Mn^{II} in MgO and diphenyl picrylhydrazyl (dpph) as the standards for *g*-value calculations, CD spectra on a JASCO J-600 spectropolarimeter in the range 200–750. The latter results are expressed in $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$, where $\Delta\varepsilon$ is evaluated at the maximum concentration of the particular species as obtained from potentiometric data. Absorption spectra were recorded on a Beckman DU650 spectrophotometer. The metal ion to ligand molar ratio was 1:2, and the metal-ion concentration was 5×10^{-3} mol dm^{-3}. The ^1H NMR spectra were recorded on a Bruker AMX spectrometer operating in the quadrature mode at 300 MHz for solutions in D_2O with $SiMe_4$ as internal standard.

Ligand Synthesis.—Amino acid esters were prepared using standard procedures. *N*-(4-Nitrophenylsulfonyl)alanine (nps-Ala) was obtained as described in ref. 16. Esters of the *N*-(4-nitrophenylsulfonyl)dipeptides were synthesised by dicyclohexylcarbodiimide–2-hydroxybenzotriazole coupling of the nps-Ala and Ala-OBu^t or His-OMe. After deprotection of the carboxylic acid groups the compounds nps-Ala-Ala and nps-Ala-His were obtained. Catalytic hydrogenolysis of the 4-nitro groups of the pns peptides gave the compounds aps-Ala-Ala and aps-Ala-His. Elemental analysis performed for C, N and H on a Perkin-Elmer analyser gave agreement with calculated values better than 0.4% (Found: C, 42.10; H, 4.30; N, 11.85. $C_{12}H_{15}N_3O_7S$, nps-Ala-Ala requires C, 41.75; H, 4.40; N, 2.15%. Found: C, 46.05; H, 5.40; N, 12.75. $C_{12}H_{17}N_3O_5S$, aps-Ala-Ala requires C, 45.70; H, 5.45; N, 13.30%. Found: C, 43.40; H, 4.05; N, 17.15. $C_{15}H_{17}N_5O_7S$, nps-Ala-His requires C, 43.75; H, 4.15; N, 17.00%. Found: C, 46.85; H, 4.85; N,

Table 1 Protonation constants for amino acids and dipeptides at 298 K, I = 0.1 mol dm^{-3} (KNO_3)

	log β			log K			
	HL	H_2L	H_3L	CO_2^-	NH_2	SO_2NH	Imidazole
nps-Ala	11.07 ±0.01	14.37 ±0.01		3.30		11.07	
aps-Ala[a]	3.53	5.57		3.53	2.04[b]		
Ala[c]	9.69	11.99		2.30	9.69		
nps-Ala-Ala	9.01 ±0.01	12.48 ±0.01		3.47		9.01	
aps-Ala-Ala	10.65 ±0.01	14.27 ±0.01		3.62		10.65	
Ala-Ala[d]	8.19	11.50		3.31	8.19		
nps-Ala-His	9.14 ±0.01	16.09 ±0.01	19.04 ±0.01	2.95		9.14	6.95
aps-Ala-His	10.41 ±0.01	17.37 ±0.01	20.23 ±0.01	2.86		10.41	6.96
His[e]	9.09	15.11	16.81	1.70	9.09		6.02
Gly-His[f]	8.22	14.99	17.50	2.51	8.22		6.77
Ala-His[g]	8.08	14.84	17.57	2.73	8.08		6.76
β-Ala-His[f]	9.30	16.14	18.67	2.53	9.30		6.84

[a] Ref. 16. [b] Protonation constant of *p*-aminophenyl group. [c] Ref. 20. [d] Ref. 21. [e] Ref. 22. [f] Ref. 19. [g] Ref. 23.

18.15. $C_{15}H_{19}N_5O_5S$, aps-Ala-His requires C, 47.25; H, 5.00; N, 18.35%). The purity was also checked by ^{1}H NMR and potentiometric titrations. It was satisfactory for the potentiometric measurements.

^{1}H *NMR data.* nps-Ala, δ 1.11 (d, 3 H, J = 6.99, CH_3), 3.54 (q, 1 H, J = 6.99 Hz, α-CH of Ala), 7.90 (d, 2 H, J = 8.79, aryl) and 8.26 (d, 2 H, J = 8.9, aryl); nps-Ala-Ala, δ 1.19 (d, 3 H, J = 7.3, CH_3), 1.20 (d, 3 H, J = 7.08, CH_3), 3.87 (q, 1 H, J = 7.3, α-CH of Ala), 3.91 (q, 1 H, J = 7.08, α-CH of Ala), 7.99 (d, 2 H, J = 8.8, aryl) and 8.35 (d, 2 H, J = 8.8, aryl); aps-Ala-Ala, δ 1.08 (d, 3 H, J = 7.26, CH_3), 1.27 (d, 3 H, J = 6.99, CH_3), 3.61 (q, 1 H, J = 7.26, α-CH of Ala), 3.73 (q, 1 H, J = 6.99, α-CH of Ala), 6.76 (d, 2 H, J = 8.67 Hz, aryl) and 7.49 (d, 2 H, J = 8.73 Hz, aryl); nps-Ala-His, δ 1.11 (d, 3 H, J = 7.11, CH_3), 2.86–3.11 (m, 2 H, CH_2 of His), 3.84 (q, 1 H, J = 7.11, α-CH of Ala), 4.18 (q, 1 H, α-CH of His), 7.13 (s, 1 H, His), 7.94 (d, 2 H, J = 9.0, aryl), 8.30 (d, 2 H, J = 8.8, aryl) and 8.41 (s, 1 H, His); aps-Ala-His, δ 1.10 (d, 3 H, J = 7.20, CH_3), 2.86–3.10 (m, 2 H, CH_2 of His), 3.69 (q, 1 H, J = 7.2, α-CH of ala), 4.18 (q, 1 H, α-CH of His), 6.74 (d, 2 H, J = 8.58, aryl), 7.13 (s, 1 H, His), 7.48 (d, 2 H, J = 8.6 Hz, aryl) and 8.50 (s, 1 H, His).

Results and Discussion

Proton Complexes.—The amino acid derivatives of *p*-aminophenylsulfonyl which do not have dissociable protons on the side chains, *e.g.* aps-Ala, dissociate two protons at low pH (H_2L^+), with log K values of about 3.5 and 2.2 (Table 1).[16] While these protonations overlap somewhat the major contributor to the first (log K = 3.5) would be the carboxyl group and the more acidic centre would be the *p*-aminophenylsulfonyl nitrogen. The proton on the sulfonamide nitrogen would be expected to ionise at high pH with log K around 12.[6,24] Deprotonation above pH 11 has also detected during titration of aps-Ala but the accuracy of the measurement was too low to give reliable values. The electron-withdrawing nitro-substituent in nps-Ala changes distinctly the acidity of the sulfonamide proton and it lowers pK to 11.07 (Table 1), while the pK of the carboxyl group decreases only slightly.

Two protonation constants are found for nps-Ala-Ala and aps-Ala-Ala (Table 1). The lower pK around 3.5 can be attributed to the carboxylic group while the higher value corresponds to proton dissociation from the sulfonamide nitrogen. The considerable difference in pK of the proton dissociating from the sulfonamide nitrogen in the two analogues, 1.64, clearly indicates the differences in electron-withdrawing ability of the *para* substituents (NH_2 and NO_2). The third protonation constants observed for nps-Ala-His and aps-Ala-His (≈6.95) derive from an imidazole nitrogen of the His side-chain. Also in the case of these two compounds the influence of the NO_2 group on the protonation constant of the amide nitrogen is clearly seen (Table 1). It is noteworthy that the pK of the sulfonamide proton in nps-Ala-His is quite similar to that of the amine group.

Copper(II) *Complexes with nps-Ala.*—According to the potentiometric data as well as the spectroscopic measurements, copper(II) ions readily form two complex species with nps-Ala, CuL and CuL_2. The co-ordination mode, one or two $\{N, CO_2^-\}$, is the same as in amino acids alone or *p*-amino derivatives.[16] The stability constants, however, are higher than those observed for amino acids due, most likely, to the more basic nitrogen donor of nps-Ala (Tables 1 and 2). They are also higher when compared to those of Cu^{II}–aps-Ala. The copper(II) complexes with aps-Ala and nps-Ala can be compared when both ligands are assumed to be monobasic acids HL (Table 2).

The co-ordination pattern is confirmed by the spectroscopic data. The formation of the equimolar CuL species results in d–d transitions centred at around 750 nm, both in the absorption and CD spectra. The corresponding energy is characteristic of 1N co-ordination.[7,16] The EPR parameters are also characteristic for such species observed in copper(II)–peptide systems.[7,25] The strong shift of the d–d bands towards higher energies upon co-ordination of the second nitrogen donor to Cu^{II} to give the CuL_2 species, was also observed for other systems[16] (Table 3). The formation of the CuL_2 complex is also accompanied by a band at around 379 nm in the CD and absorption spectra (Table 3). This can be assigned to a charge-transfer transition from N^- to Cu^{II} and is strong evidence for the involvement of nitrogen in the metal-ion co-ordination.[16]

Copper(II) *Complexes with aps-Ala-Ala and nps-Ala-Ala.*—Both ligands behave as dipeptide molecules with a free N-terminal amino group. The $CuH_{-1}L$ complex is readily formed at pH > 4 for the nps and > 5 for the aps derivative, respectively [Fig. 1(*a*)]. The distinct difference in co-ordination ability for the two ligands derives from the effect of the *para*-substituent on the pK of the sulfonamide proton (Table 1). The more basic nitrogen of aps-Ala-Ala leads to the formation of the stronger $CuH_{-1}L$ complex when compared to that of the

Table 2 Equilibrium data (log β) for copper(II) complexes of amino acids and dipeptides at T = 298 K, I = 0.1 mol dm^{-3} (KNO_3)

	Species							
L	CuHL	CuL	CuL_2	$CuH_{-1}L$	$CuH_{-2}L_2$	$CuH_{-1}L$	$CuH_{-2}L$	$Cu_2H_{-2}L_2$
nps-Ala		8.84 ± 0.01	15.81 ± 0.01					
				−2.23 ± 0.01[a]	−6.33 ± 0.01[a]			
aps-Ala[b]				−3.03	−7.54			
Ala[c]		8.13 ± 0.05	14.92 ± 0.1					
nps-Ala-Ala						0.81 ± 0.01	−8.82 ± 0.01	
aps-Ala-Ala						1.38 ± 0.01	−8.18 ± 0.01	
Ala-Ala[d]		5.54				1.82		
nps-Ala-His		8.28 ± 0.01					−7.03 ± 0.01	9.14 ± 0.01
aps-Ala-His		9.21 ± 0.01					−6.32 ± 0.01	10.32 ± 0.01
β-Ala-His[e]	13.26	8.25					−8.90	8.18
Gly-His[e,f]	12.45	9.06	15.96			4.91		

[a] Log β = log β_{CuL} − log β_{HL} for $CuH_{-1}L_1$ and log β = log β_{CuL_2} − 2log β_{HL} for $CuH_{-2}L_2$. [b] Ref. 16. [c] Ref. 20. [d] Ref. 21. [e] Ref. 19. [f] log β 8.02 for $CuH_{-1}L_2$.

Table 3 Spectroscopic data for copper(II) complexes with nps and aps derivatives of amino acids and peptides

	UV/VIS		CD		EPR	
Species	γ/nm	ε/dm^3 mol^{-1} cm^{-1}	γ/nm	Δε/dm^3 mol^{-1} cm^{-1}	$A_{\|}$	$g_{\|}$
nps-Ala						
CuL	749[a]	61	750[a]	−0.414	152	2.324
110			606[a]	+0.052		
CuL_2	674[a]	91	681[a]	−0.580	159	2.284
120			547[a]	+0.287		
			379[b]	−0.135		
nps-Ala-Ala						
$CuH_{-1}L$	655[a]	153	654[a]	−1.617	172	2.247
11 − 1			337[b]	+1.587		
$CuH_{-2}L$	652[a]	148	671[a]	−1.623	175	2.249
11 − 2			331[b]	+1.327		
aps-Ala-Ala						
$CuH_{-1}L$	653[a]	138	648[a]	−1.551	173	2.247
11 − 1			340[b]	+0.804		
			297[c]	−1.052		
$CuH_{-2}L$	647[a]	133	668[a]	−1.609	171	2.244
11 − 2			333[b]	+0.592		
			297[c]	−0.822		
nps-Ala-His						
CuL	665[a]	65				
110						
$Cu_2H_{-2}L_2$	655[a]	116	572[a]	−1.812	Dimer spectrum	
22 − 2	556 (sh)[a]	98	347v br[b]	+1.283	D = 0.0082 cm^{-1}	
			305[c]	−1.181	$g_{\|}$ = 2.393	
			295[c]	−1.084	A_{av} = 83 × 10^{-4} cm^{-1}	
$CuH_{-2}L$	588[a]	84	583[a]	−1.042	176	2.226
11 − 2			339v br[b]	+0.283		
			296[c]	−0.279		
			287	−0.392		
aps-Ala-His						
CuL	671[a]	28	600[a]	−0.223		
110	370 (sh)[b]	95	344[b]	+0.155		
			297[c]	−0.635		
$Cu_2H_{-2}L_2$	660[a]	115	571[a]	−2.102	Dimer spectrum	
22 − 2	575 (sh)[a]	105	345[b]	+0.740	D = 0.0081 cm^{-1}	
	371 (sh)[b]	379	301[c]	−1.163	$g_{\|}$ = 2.385	
					A_{av} = 84 × 10^{-4} cm^{-1}	
$CuH_{-2}L$	597[a]	93	576[a]	−1.258	184	2.220
11 − 2			336[b]	+1.067		
			293[c]	−1.233		

[a] d–d Transition. [b] N^-_{sulf}→Cu^{II} and N (imidazole)→Cu^{II} charge-transfer transitions. [c] N^-_{pep}→Cu^{II} charge-transfer transition.

nps derivative (Table 2). At pH > 8 the $CuH_{-2}L$ species is formed as the consequence of deprotonation of a bound water molecule. The pK of this reaction (≈9.5) is consistent with those of other such deprotonations.[6] The formation of the $CuH_{-1}L$ complex is clearly seen in the absorption, CD and EPR spectra. The d–d transition at around 650 nm corresponds to two nitrogen (2N) co-ordination,[7] {$2N^-$,CO_2^-}, and the band observed in the CD spectra about 340 nm indicates the involvement of the sulfonamide nitrogen, while that around 300 nm shows the co-ordination of the other amide nitrogen (Table

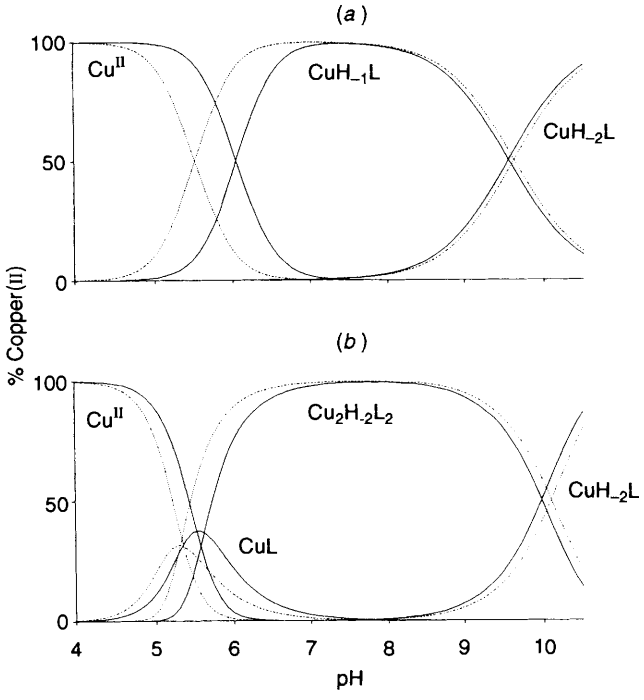

Fig. 1 Species distribution curves for (*a*) Cu^{II}–nps-Ala-Ala (. . .) and Cu^{II}–aps-Ala-Ala (—) and (*b*) Cu^{II}–nps-Ala-His (. . .) and Cu^{II}–aps-Ala-His (—)

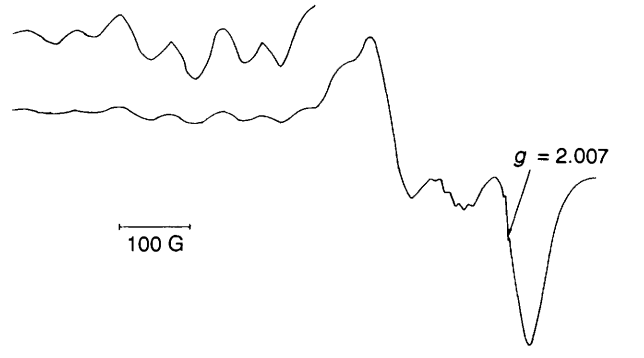

Fig. 2 The EPR spectrum of a Cu^{II}–aps-Ala-His solution at pH 7.4, at 120 K, 1:1.5 metal to peptide molar ratio. Estimated $D = 0.08$ cm^{-1}, $A_{av} = 84 \times 10^{-4}$ cm^{-1}, $g_{||} = 2.385$ (assuming D as mainly dipolar in origin;[26] estimated $Cu^{II} \cdots Cu^{II}$ distance in dimeric species is 7.71 Å)

3).[7,16] The EPR parameters are also in accord with those observed for this type of co-ordination in copper(II)–dipeptide systems.[7,25]

Copper(II) Complexes with nps-Ala-His and aps-Ala-His.—The presence of the imidazole nitrogen donor changes drastically the binding ability of both sulfonamide derivatives when compared to that of X-Ala-Ala (X = nps or aps) discussed above. The sulfonamide protection at the terminal nitrogen of Ala-His also cause both compounds to differ from the simple dipeptide Gly-His. At pH > 5 the EPR spectrum is typical for a dimeric species (triplet-type spectrum, Fig. 2) with small zero-field splitting parameter D (Table 3 and legend to Fig. 2). This EPR spectrum was observed in the range pH 5–10 and transforms into the corresponding spectrum of the monomeric species above pH 9 (Table 3). The potentiometric data support the formation of the dimeric species $Cu_2H_{-2}L_2$ in the same pH range as that for the EPR spectra [Fig. 1(*b*)]. According to the potentiometric data two monomeric complexes are formed in both systems. At pH 4–6 the complex CuL is found in a concentration too small to be recorded by the well resolved spectra and the other, $CuH_{-2}L$, species, is formed at pH > 9 [Fig. 1(*b*)].

At pH 5–10 the absorption spectrum exhibits d–d transitions around 660 nm with a clear shoulder at 560–575 nm (Table 3). In the CD spectra there is one negative d–d band at about 570 nm. The UV/VIS spectra may suggest two different co-ordination patterns around the two copper ions in a dimeric species. The charge-transfer region exhibits two or three $N \rightarrow Cu^{II}$ transitions (one or two close to 300 nm and one very broad centred at 350 nm). The very broad band may consist of two charge-transfer bands derived from N^- (sulfonamide) $\rightarrow Cu^{II}$[16] and N (imidazole)$\rightarrow Cu^{II}$[27–29] transitions. The other transition(s) corresponds to N^- (amide)$\rightarrow Cu^{II}$ charge transfer. Since both the nps and aps derivatives form very similar dimeric species the bridging unit between the two metal ions is certainly the imidazole of the His residue. The absorption spectra suggest that the two copper(II) ions are not equivalent (see above). Thus, for the $Cu_2H_{-2}L_2$ species obtained from potentiometric data one has to assume 3N co-ordination of one X-Ala-His molecule to one Cu^{II} *via* a $\{N^-_{sulf}, N^-_{amide}, N_{im}\}$ donor system and 2N binding of the second X-Ala-His to the second Cu^{II} *via* a $\{N^-_{sulf}, N^-_{amide}\}$ donor set. The imidazole nitrogen of the His residue bridges the two metal ions forming a dimeric species with 2N and 4N co-ordination respectively. This different number of bound nitrogens leads to two distinct d–d transitions at 660 (2N) and 560 nm (4N). The splitting into two bands assigned to $N^- \rightarrow Cu^{II}$ charge-transfer transitions is also consistent with the different binding modes around the two metal ions in the $Cu_2H_{-2}L_2$ complex. The formation of dinuclear complexes in Cu^{II}–X-Ala-His systems may be enforced by the bulky nps or aps moiety preventing among others the formation of CuL_2 species. Dinuclear complexes have also been observed with β-Ala-His (carnosine).[19] In this case the N-terminal amino group is one carbon further from the amide nitrogen than that in Ala-His. In the case of carnosine the dimeric complex was bridged by two imidazoles and was symmetric. In the Cu^{II}–X-Ala-His (X = nps or aps) systems the assumption of the formation of an 'asymmetric' dimer is based mostly on the absorption spectra. However the 'symmetric' carnosine-like dimer cannot be excluded.

The sets of chemical species formed by carnosine and X-Ala-His are very similar to each other but complexes of the sulfonamide analogues are considerably more stable than those of carnosine (Table 2). The most effective ligand is aps-Ala-His the CuL complex of which is even more stable than that of Gly-His. The dinuclear complex of aps-Ala-His is more than two orders of magnitude more stable than that of carnosine and 1.2 log units more than that of nps-Ala-His (Table 2).

On increasing the pH above 9, OH^- competes for co-ordination to Cu^{II} leading to the formation of monomeric $CuH_{-2}L$ with 3N co-ordination completed by a fourth OH^- group. The d–d transition energy around 590 nm strongly supports the 3N co-ordination.[7] The involvement of both amide nitrogens is indicated by the well resolved CD spectra in which two charge-transfer bands are observed around 290 and 340 nm (Table 3). The latter band also contains the N (imidazole)$\rightarrow Cu^{II}$ charge-transfer transition.[27–29]

Conclusion

The major conclusions which can be drawn from these studies are as follows.

Copper(II) ions can easily deprotonate and bind to sulfonamide nitrogen without using an anchoring site. The sulfonyl group makes the sulfonamide proton much more acidic than that of a peptide amide. This excludes the possibility of binding *via* the *p*-amino nitrogen of the aps moiety.

The *para* substituent on the phenylsulfonyl moiety has a very distinct effect on the acidity of the sulfonamide proton. This may influence very dramatically the binding ability of sulfonamide ligands.

Phenylsulfonamide derivatives of oligopeptides are very efficient chelating agents for copper(II) ions competing even with the parent oligopeptides. The co-ordination equilibria (*e.g.* the binding modes) in the case of phenylsulfonylpeptides could be, however, distinctly different from those found for the parent peptide ligands.

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