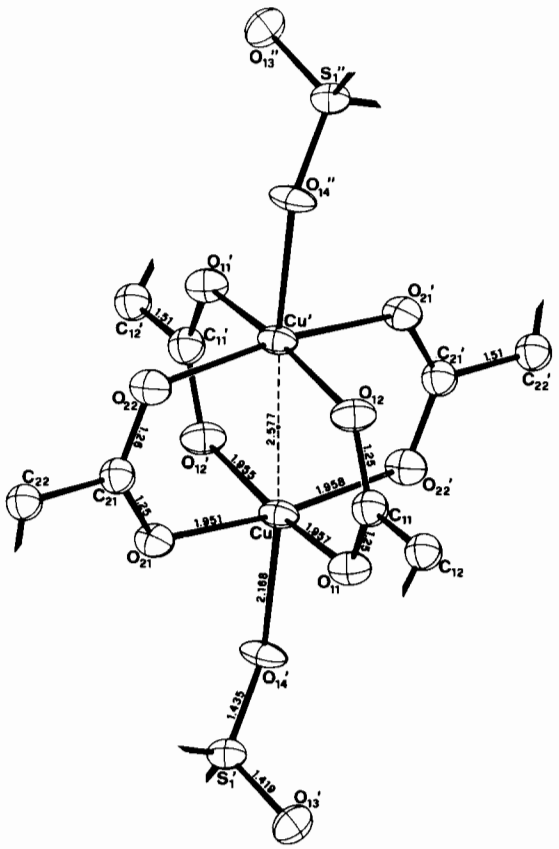

Fig. 1. Structure of $[Cu(TsglyH)_2]_n$.

structure, linked *via* Cu–O (sulphonic) bonds. Its magnetic and spectroscopic properties are interpreted on the basis of the crystal structure. The exchange integral (−2J) is 353 ± 4 cm^{-1}.

In ethanolic solution at metal ion concentrations similar to those normally found in biological systems, by polarographic measurements, for $C_{NaOH}/C_{Complex}$ ratios between 1 and 3 the $Cu(TsglyH)_2$ species is the only present, while for ratios greater than 4 the $Cu(Tsgly)_2^{2-}$ prevails.

The main conclusions from this work are:

In ethanolic media the N-tosylglycine parallels the behavior in aqueous solution, even if more complicated equilibria are present in this last solvent. The separation of only two solid compounds from ethanol with respect to the four from aqueous solution also confirm this.

These results, and those previously observed for the N-acetyl and N-benzoyl-amino acids [2], suggest as a general rule that the ethanolic media enhances the behavior as simple carboxylate ligands of these amino acids, favouring the separation of compounds which possess dimeric arrangement of the copper(II) acetate type with strong antiferromagnetic exchange.

Nevertheless, in the N-tosylglycinate–copper(II) ion system in ethanolic solution in the experimental conditions (*i.e.* at metal ion concentrations close to those of biological systems), there is no evidence for a binuclear species.

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U7

The Use of Metal Additive in Reversed Phase Liquid Chromatography: Structure–Retention Relationships

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The fact that biologically active molecules such as amino acids, nucleotides, nucleosides and their bases form complexes with metal cations is well known. A great deal of work is devoted to the elucidation of properties and structures of such complexes. To date most of the research in the field was done using spectroscopic and electrochemical methods. We wish to report here the use of liquid chromatography for investigating the properties of such complexes. The experimental system is rather simple, consisting of a reversed phase column and an aqueous mobile phase. The mobile phase contains the metal cations under study. To such a system we inject amino acids, nucleotides, nucleosides and their bases.

These solutes form complexes with the cations in the mobile phase. Since the nature of the complexes is different from that of the parent compounds, their chromatographic behavior changes. The change can be related to the properties and structures of the complexes.

The partition coefficients, and hence retention times, of all amino acids studied increased when the metal cations were introduced to the mobile phase. The increase in the retention is related to the stability of the complex. For example the retention time order is $t_R(Cu) > t_R(Ni) > t_R(Zn) > t_R(Co)$, where $t_R(M)$ indicates the retention time with metal cation M in the mobile phase. The magnitude of the amino acids–metal complex formation constant is $K_f(Cu) > K_f(Ni) > K_f(Zn) > K_f(Co)$. Since the chromatographic system is that of reversed phase, longer retention times can indicate greater hydrophobic interactions. In the present case the greater hydrophobicity is due to partial charge neutralization of the amino acid by the metal cations. The degree of neutralization is a function of the stability of the complex.

TABLE I.

	Adenine		AMP		Cytosine		CMP	
	log K_f	k′	log K_f	k′	log K_f	k′	log K_f	k′
No metal	–	4.60	–	2.69	–	0.46	–	0.36
Mg(II)	N.A.	4.1	1.97	2.44	N.A.	0.39	1.75	0.26
Ni(II)	1.47	4.87	2.84	2.57	N.A.	0.49	1.9	0.32
Cu(II)	2.68	9.39	3.18	3.48	2.0	0.46	N.A.	0.45
Ag(I)	N.A.	<20	N.A.	4.26		1.16	N.A.	1.13

With nucleotides, nucleosides and their bases the situation is much more complicated. The metal cations can complex with the phosphate moiety of the nucleotides and/or with the base part of the molecule. This fact is also reflected in the retention behavior of these solutes. The following table shows typical behavior (Table I).

In the table k′ is a measure of the partition coefficients and hence the retention times. Mg causes a decrease in the retention of the bases (as well as the monophosphate nucleotides) shown in the table: this metal is known to be bound to the phosphate group only. However, the extent of retention change is roughly the same for all solutes shown. Thus in the concentration range and pH used here, the effect of the Mg ion added to the mobile phase may be related to changes in the ionic strength. Similar arguments can be made when nickel ions are added to the mobile phase although the increase in the retention of adenine should be noted. The use of copper ions gives different results. Purines are known to complex Cu(II) better than pyrimidines. Indeed the retention of adenine and AMP increases significantly while that of cytosine and, to some extent CMP, does not change much. The presence of a heavy metal such as silver in the mobile phase increases the retention of all the solutes drastically. This is clear in view of the strong complexes formed by such metals.

Similar results found with other solutes and metal cations will be discussed. The chromatographic data correlates well with that known about the structures and properties of biologically important complexes. Thus, chromatographic methods can aid scientists in analyzing bioinorganic complexes.

U8

Use of Cyclic Peptides to Mimic the Active Sites of Metalloproteins: ^{13}C and ^{1}H NMR, ESR and Visible Spectra of Copper(II) Complexes with the Cyclo-(Gly–His–Gly–His–Gly–His–Gly) Peptide in Aqueous Solution

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Cyclic peptides have been used to model various aspects of protein conformation and active sites [1]. In such models the usual flexibility of peptide chains is substantially reduced through cyclization. Furthermore, it is generally assumed that specific amino acid residues such as Glu, Asp, His, Lys··· have side chains that can bind to transition-metal ions. We chose to synthesize cyclo-(Gly–His–Gly–His–Gly–His–Gly) (hereinafter denoted (G_4H_3)) because the histidine residue plays an important role in metal ion coordination of metalloproteins and frequently encountered *in vivo*. Complexation of (G_4H_3) with the transition metal ion Cu(II) in aqueous solution over a wide pH range and with different peptide/metal ratios has been studied using carbon-13 and proton NMR, ESR and visible spectroscopy. From analyses of the spectral data, it is concluded that Cu(II) binds at two metal-binding sites depending on the pH. At physiological pH, the binding of Cu(II) at the first site involves the three HisN(3) imidazole groups to give a 1:1 species in a tetracoordinated structure; whereas, at higher pH, the binding of Cu(II) at the second site uses four deprotonated peptide nitrogens.

The present results further confirm the concept of molecular design and the feasibility of designing peptide molecules mimicking the complicated metal-binding sites of biological macromolecules.