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Synthesis of Mixed-ligand Complexes of Co^{II} and Cu^{II} with Glycyl-L-Tyrosine Anion and Cytidine

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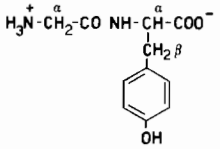
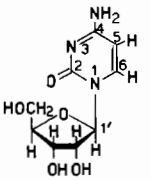
The study of dipeptide–metal–nucleoside complexes, as models for the enzyme–metal–nucleic interactions, is most interesting to understand the many enzymic and other catalytic processes requiring metal ions [1] We report here the synthesis and characterisation (by i r and ^{1}H n m r spectroscopies) of the hydrated complexes of glycyl-L-tyrosine (H_2GT)



of general formulae $M(H_nGT)X$, and of those of H_2GT and cytidine (Cyd)



of general formulae $M(GT)(Cyd)$ ($M = Co^{II}$, Cu^{II}, $X = H_2O$, $n = 0$, $X = Cl$, $n = 1$) as well as of the charged species $[Cu(HGT)Cyd]NO_3$

Experimental

$Cu(GT)H_2O$

The complex was prepared by addition of commercial H_2GT (0 238 g, 10^{-3} *M*) to an aqueous suspension of $Cu(OH)_2$ (1 25 10^{-3} *M*) at pH = 6 5, according to A R Manyak *et al* [2] The product was recrystallised by slow evaporation of an aqueous solution

$Co(GT)H_2O \cdot 2H_2O$

A water methanol (1 1) solution of H_2GT (0 238 g, 10^{-3} *M*) was added to a water solution of $Co(CH_3COO^-)_2 4H_2O$ (0 249 g, 10^{-3} *M*) The solution (pH = 5 9) was heated (60 °C) and stirred for 4 hr, under nitrogen, then evaporated to dryness The final product was redissolved in methanol and precipitated by diffusion of diethylether

$Cu(HGT)Cl \cdot 2H_2O$

An aqueous solution of H_2GT (0 238 g, 10^{-3} *M*) and of $CuCl_2 \cdot 2H_2O$ (0 215 g, 1 25 10^{-3} *M*) were stirred at room temperature and at pH = 3 65 for 2 hr The complex was then isolated by addition of ethanol diethylether (2 1)

$Co(HGT)Cl \cdot H_2O$

H_2GT (0 476 g, 2 10^{-3} *M*) and $CoCl_2 \cdot 6H_2O$ (0 475 g, 2 10^{-3} *M*) dissolved in water were stirred and heated (40 °C) for 9 hr After cooling, the solution (pH = 4 6) was filtered to remove the unreacted H_2GT The complex was isolated by addition of acetone

$Cu(GT)Cyd \cdot 2H_2O$

The complex was synthesised according to Szalda *et al* [3], from 0 084 g (0 34 10^{-3} *M*) of cytidine and 0 105 g (0 34 10^{-3} *M*) of $Cu(GT)H_2O$, and precipitated by addition of methanol diethylether (1 1)

$Co(GT)(Cyd) \cdot 6H_2O$

The same method as for $Cu(GT)(Cyd) \cdot 2H_2O$ was followed

$[Cu(HGT)Cyd]NO_3 \cdot 4H_2O$

A solution of the $[Cu(HGT)]^+$ cation was obtained by addition of $AgNO_3$ (0 043 g, 0 25 10^{-3} *M*) to an aqueous solution of $Cu(HGT)Cl \cdot 2H_2O$ (0 100 g, 0 25 10^{-3} *M*) The solution was filtered to remove the precipitated AgCl, and 0 062 g (0 25 10^{-3} *M*) of cytidine were added to the pale-green filtrate The resulting solution turned immediately blue and was heated at 60–70 °C for 2 hr, when the colour had become deep green The resulting complex was isolated by addition of acetone, and recrystallised by slow evaporation from an aqueous solution

The microanalytical results (C, H, N, see Table) agree satisfactorily with the proposed formulae The i r spectra (4000–400 cm^{-1}) were obtained from KBr pellets on a Beckman IR 12 spectrophotometer The ^{1}H n m r spectra were recorded on a FT Brucker WH 90 spectrometer, from DMSO-d_6 solutions with Me_4Si as internal standard

Results and Discussion

The presence of the ν and $\delta(^+NH_3)$, ν_{as} and $\nu_s(COO^-)$ vibration bands in the i r spectrum of the free dipeptide H_2GT indicates the gly–L–tyr to be zwitterionic In the isolated complexes, the bands due

TABLE I. Analytical and Spectral Data.

Compound		Colour	Analyses						ν(amide I)[a]	ν(C=O)[b]
			C%		H%		N%			
			Calcd	Found	Calcd	Found	Calcd	Found		
I	$Cu(GT)H_2O$	Blue	41.21	41.0	4.41	4.3	8 78	8.7	1600 S	
II	$Co(GT)H_2O \cdot 2H_2O$	Pink	37.62	37.6	5.15	5.1	8.15	8.4	1605 S	
III	$Cu(HGT)Cl \cdot 2H_2O$	Blue Green	35.39	35.3	4.58	4.3	7.51	7.3	1620 S	
IV	$Co(HGT)Cl \cdot 2H_2O$	Blue	37.88	38.0	4.31	4.5	8.01	7.9	1624 S	
V	$Cu(GT)Cyd \cdot 2H_2O$	Blue	41.48	41.4	4 65	4.7	11.95	11.8	1600 S, br	1655 S
VI	$Co(GT)Cyd \cdot 6H_2O$	Purple	36.95	36.7	4.92	4.6	10.50	10.1	1603 S, br	1658 S
VII	$[Cu(HGT)Cyd]NO_3 \cdot 4H_2O$	Green	35.39	35.2	4.86	4.5	11.62	12.0	1625 m	1710 S 1723 S

[a] H_2GT: $\nu(N^+H_3)$ = 3030 cm^{-1}; $\nu(COO^-)$ = 1603 cm^{-1}; amide I = 1660 cm^{-1}, S,
[b] Cyd: ν(C=O) = 1675 S; ν(C=N) = 1635 S. S = strong; br = broad; m = medium.

to the $^+NH_3$ group have disappeared, while those associated to COO^- are shifted to lower frequencies (1585 cm^{-1}). We suppose therefore that the gly–L–tyr is always bound to the metal through both its terminal groups $-NH_2$ ($\nu(NH_2)$ at 3200–3250 cm^{-1}) and $-COO^-$.

Whereas to the peptide bond –CONH–, two different binding ways are possible according to the reactional pH range. In the complexes I, II, V and VI prepared at pH = 6–7, the amide I band position at 1600–1605 cm^{-1} suggests, as shown by Martell and Kim [4], that the peptide bond is bound through the deprotonated nitrogen. On the contrary, at pH lower than 5.5 (in the case of complexes III, IV and VII), the peptide N is protonated and therefore less likely to bind to the metal ion. The corresponding shift of the ν (amide I) to 1620–1625 cm^{-1} suggests a co-ordination through the ketone group [4]. This is confirmed by the crystal structure of an analogous complex, chloroglycyl-glycinato copper II monohydrate, reported by Shiro *et al.* [5], where the peptide bond is indeed co-ordinated through the oxygen.

Our results are further confirmed by a titration study of gly-L-tyr in presence of Cu^{II} ions, with the loss of 2 protons (from $^+NH_3$ and –CONH–), at pH values higher than 6, as shown by Dobbie *et al.* [6].

The i.r. spectra of the mixed ligands complexes indicate the cytidine to be coordinated through N3, since the ν(C=N) bands have disappeared. Moreover, in the case of $Cu(GT)Cyd \cdot 2H_2O$ and $Co(GT)Cyd \cdot 6H_2O$ the ν(C=O) band is shifted from 1675 cm^{-1} (in the free cytidine) to 1655 and 1658 cm^{-1} respectively. This suggests the ketone group to interact with the metal ion. Szalda *et al.* [3] have indeed put into evidence such a Cu–O interaction by X-ray analysis of the analogous complex Cu^{II}(glycyl-glycine)Cyd, where the coordination geometry about the copper atom is approximately square pyramidal. However, no such interaction appears to take place in the charged species $[Cu(HGT)Cyd]NO_3 \cdot 4H_2O$, since the ν(C=O) band appears at a higher frequency (see Table). This may be explained by the competitive presence of the NO_3 anion near the metal ion.

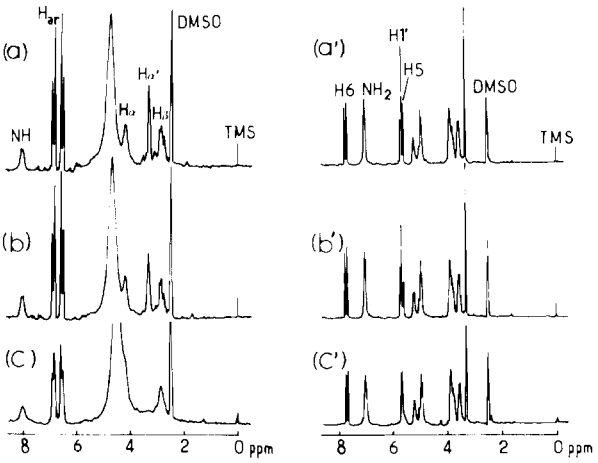


Figure. ^1H n.m.r. spectra in DMSO-d_6 solution of: (a) glycyl-L-tyrosine alone (0.1 *M*) and in presence of (b) 2.10^{-5} *M*, (c) 2.10^{-4} *M* $CuCl_2$; (a') cytidine alone (0.1 *M*) and in presence of (b') 2.10^{-5} *M*, (c') 10^{-4} *M* $Cu(GT)H_2O$.

The Cu^{II} and Co^{II} broadening effect on the p.m.r. lines of the free ligands was used to identify the nature of the different binding sites.

When adding progressively 2.10^{-4} mol of $CuCl_2$ to a solution of gly–L–tyr in DMSO at pH = 6 (see Figure), the $CH_{2\alpha}$ proton resonance is the first to

broaden and then to disappear The adjacent NH_2 group should therefore be the first coordination site When 10^{-3} mol of $CuCl_2$ are added, the peptide proton and the CHα signals are also affected, indicating that the peptide N and the terminal carbonyl group react in a second time [7]

In the case of the mixed ligand complexes, when adding the paramagnetic complex $Cu(GT)H_2O$ to the free cytidine, the H-5 doublet is the first to broaden and disappear while the H-6 resonance does not broaden appreciably and remains a doublet This indicates a greater vicinity of H-5 than H-6 to the coordination site and we suppose therefore the cytidine to have reacted with Cu^{II} and Co^{II} through N3 [8]

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