J. Chem. Research (S),
1997, 106–107†

# Binding of Copper(II) to Pilocarpine†

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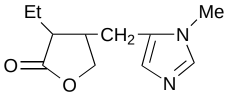
The speciation in the pilocarpine-Cu$^{II}$ system, as studied by potentiometric and spectroscopic techniques on aqueous solutions, is described.

Pilocarpine, (3*S*,4*S*)-3-ethyl-4,5-dihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]furan-2-(3*H*)-one **1**, an alkaloid obtained from the leaves of South American shrubs of the genus *Pilocarpus*, is an imidazole derivative exhibiting pharmacological activity.



**1**

The imidazole nucleus is an effective target for metal binding. Therefore, studies on the coordinating ability of this molecule have been performed. Namely, bis(pilocarpine)-halide complexes of Co$^{II}$, Ni$^{II}$, Cu$^{II}$ and Zn$^{II}$ have been characterized in the solid state and in solution and, particularly, $^1$H NMR and EPR spectroscopic analyses were successful in assigning the pyridine-type nitrogen of the imidazole ring as the donor atom of the ligand.[1] Potentiometric and spectrophotometric methods were also employed to follow complex formation processes with some metal ions and to determine the respective stability constants.[2]

The copper(II) complexes formed by this ligand were investigated by potentiometric and EPR methods and compared to those formed by the simple imidazole molecule. Noteworthy, reports on Cu$^{II}$ imidazole complex species have been devoted to the existence of the species involving 1:4 or greater ligand to metal molar ratios.[3,4] No spectral data are available, at least to the best of our knowledge, for complexes involving a lower number of coordinated ligands.

**Table 1** Complex formation constants (log $\beta$) for the Cu$^{II}$ complexes of pilocarpine and imidazole at $T = 25$ °C and $I = 0.10$ mol dm$^{-3}$ (KNO$_3$)

| Ligand | Species | log $\beta$ This work | log $\beta$ Ref. 2 | $g_\parallel$ | $A_\parallel$(Cu)/ $10^{-4}$ cm$^{-1}$ |
|---|---|---|---|---|---|
| Pilocarpine L | $[CuL]^{2+}$ | 4.29 (2) | 4.00 | 2.359 | 144 |
| | $[CuL_2]^{2+}$ | 7.91 (2) | 7.63 | 2.314 | 173 |
| | $[CuL_3]^{2+}$ | 11.16 (5) | 10.83 | 2.288 | 173 |
| | $[CuL_4]^{2+}$ | 13.97 (7) | 13.75 | 2.255 | 190 |
| | $[CuL_5]^{2+}$ | | 15.00 | | |
| | $[CuL_2H_{-1}]^+$ | 0.94 (2) | | | |
| | $[CuL_2H_{-2}]$ | −7.64 (2) | | | |
| Imidazole L′ | $[CuL']^{2+}$ | | 4.30 | 2.357 | 144 |
| | $[CuL'_2]^{2+}$ | | 7.85 | 2.314 | 173 |
| | $[CuL'_3]^{2+}$ | | 10.78 | 2.283 | 175 |
| | $[CuL'_4]^{2+}$ | | 12.95 | 2.259 | 191 |
| | $[CuL'_5]^{2+}$ | | 13.60 | | |

Over the measurable pH range pilocarpine **1** (L) exhibits a protonation process with log $K = 7.02(1)$ (*cf.* 6.97 in ref. 2) attributable to the pyridine-like nitrogen of the imidazole nucleus. Potentiometric and spectral data of the Cu$^{II}$ complexes are reported in Table 1. Calculations based on potentiometric data indicate the formation of six monomeric species (Fig. 1). The mononuclear complexes $[CuL]^{2+}$, $[CuL_2]^{2+}$, $[CuL_3]^{2+}$ and $[CuL_4]^{2+}$ exhibit formation constants in good agreement with those measured previously on the same system and on the Cu$^{II}$–imidazole system.[2,5] This confirms the fact that in these species the ligand adopts a monodentate binding mode and acts as a donor through the imidazole nitrogen. $[CuL_2H_{-1}]^+$ and $[CuL_2H_{-2}]$ are also formed in basic solution. In order to check the complexation scheme and to identify the species, EPR spectra were obtained as a function of pH and compared to those recorded on the system involving imidazole (L′). EPR spectra were successful in detecting the stepwise formation of four monomeric species in both the imidazole– and pilocarpine–Cu$^{II}$ complex systems and enabled us to assign distinctive spectral parameters. Notably, the spectral data of the two systems are very close to each other. On this basis, it can be concluded that the coordination through the nitrogen atom of imidazole nucleus can also be assumed for pilocarpine complexes. In these complexes, large distortions of the coordination sphere, in comparison to the imidazole species, can be ruled out, most likely because pilocarpine can assume a coplanar conformation which minimizes the extent of the interligand repulsion. The data are also concordant with previous results which, besides monodenate *N*-binding, supported the absence of folding in the molecule upon copper complexation.[6] It is also noteworthy to observe that in both complex systems an increase in the number of coordianted nitrogen atoms results in a stepwise decrease of the $g_\parallel$ value. Instead, the $A_\parallel$ values, whilst increasing on passing from $[CuL]^{2+}$ to $[CuL_2]^{2+}$ and from

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†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1997, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

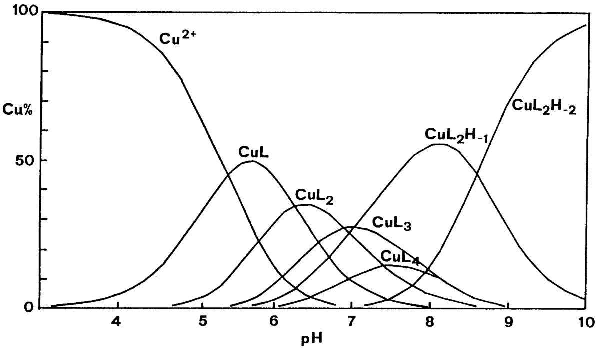

**Fig. 1** Distribution curves of the complexes formed in the $Cu^{II}$–pilocarpine system at metal ion to ligand molar ratio 1:4 $[Cu^{II}] = 0.001$ mol $dm^{-3}$

$[CuL_3]^{2+}$ to $[CuL_4]^{2+}$, are almost the same for $[CuL_2]^{2+}$ and $[CuL_3]^{2+}$. This could be explained by a change of stereochemistry at the metal ion taking place upon coordination of the third ligand molecule. It can be suggested that the geometry, which is tetragonal in $[CuL_2]^{2+}$ changes *e.g.* to five-coordinate in $[CuL_3]^{2+}$ and then to tetragonal in $[CuL_4]^{2+}$. Finally, the $[CuL_2H_{-1}]^+$ and $[CuL_2H_{-2}]$ complexes do not give distinct EPR spectra. Their formation results in a considerable decrease of signal intensity which suggests that the species are formed by deprotonation of coordinated water molecules, *e.g.* $[CuL_2(OH)]^+$ and $[CuL_2(OH)_2]$, and adopt hydroxo-bridged polymetallic arrangements.

## Experimental

The ligands were bought from Sigma. Anisotropic X-band EPR spectra (9.15 GHz) of frozen solutions were recorded at 140 K, using a Varian E-9 spectrometer after addition of ethylene glycol to ensure good glass formation. The concentration stability constants $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$ were calculated from pH titration curves at 25 °C using total volume of 2.0 $cm^3$. Alkali was added from 0.1 $cm^3$ micrometer syringe calibrated by both weight titration and titration of standard materials. Experimental details: ligand concentration, $2 \times 10^{-3}$ mol $dm^{-3}$; metal to ligand molar ratios, 1:1, 1:2, 1:3 and 1:4; ionic strength, 0.10 mol $dm^{-3}$ $KNO_3$; pH-metric titrations on a MOLSPIN pH-meter system using a micro combined glass–calomel electrode (Russell, CMAWL) calibrated in concentration using $HNO_3$;[7] number of titrations, 4; method of calculations, SUPERQUAD program.[8] The samples were titrated from pH 2.5 to 10.5. Standard deviation ($\sigma$ 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 in the equilibrium.

*Received, 7th October 1996; Accepted, 28th November 1996*
*Paper E/6/06851K*

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