EPR, Ligand Field Spectra and Antimicrobial Activity of some *N*-Pyruvideneglycinatocopper(II) Complexes

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(Received July 7, 1987)

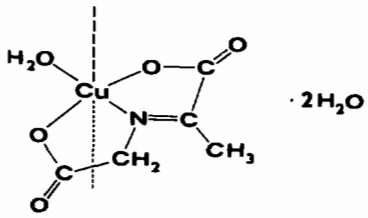
Abstract

N-Pyruvideneglycinatocopper(II) complexes were synthesized of composition Cu(pyrgly)(L)$(H_2O)_x$ with L = H_2O, $x = 2$ **(I)**; L = pyridine, $x = 2$ **(II)**; L = quinoline, $x = 2$ **(III)**; and L = aniline, $x = 1$ **(IV)**. The ligand field spectra provide evidence of a distorted octahedral Cu(II) coordination in **(I)** and **(III)**, whereas in **(II)** and **(IV)** the coordination polyhedron around Cu(II) is square-pyramidal. According to the results of EPR spectra, the neutral ligand L strongly influences the weak exchange interactions between Cu(II) ions in the solid state. The effect of the ligand L on the local and cooperative symmetry is discussed. The complexes show antimicrobial activity, being most active against fungi.

Introduction

Copper(II) complexes of tridentate Schiff bases derived from pyruvic and amino acids have been shown to be catalytic intermediates in non-enzymatic transamination reactions [1]. From the Cu(II)–glyoxalic acid–α-alanine system *N*-pyruvideneglycinatocopper(II) trihydrate **1** was isolated as a product of the transamination reaction. An identical complex was obtained from the Cu(II)–pyruvic acid–glycine system.

In all mononuclear Cu(II) complexes with analogous tridentate Schiff base ligands a square-pyramidal



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coordination of Cu(II) ions was found by X-ray structural analysis [2–7]. The O,N,O donor atoms of the tridentate ligand are bound in the base of the pyramid. The apex is occupied by a water molecule or an oxygen atom from the neighbouring complex molecule. In the remaining site in the plane various neutral ligands may be bound. As we have recently shown, these neutral ligands strongly influence mainly the cooperative symmetry in Cu(II) complexes with tridentate Schiff base ligands [8, 9].

This work was carried out to study *N*-pyruvideneglycinatocopper(II) adducts with some N-donor ligands. In particular a comparison of the results of EPR and ligand field spectral investigations yields valuable information about local and cooperative symmetry effects in Cu(II) compounds [10]. It is well known that some Cu(II) complexes with chelating ligands exhibit antimicrobial activity, the best known example being Cu(II) complexes with 8-quinolinol [11]. Therefore the antimicrobial efficiency of the prepared compounds was evaluated against selected strains of bacteria and fungi.

Experimental

N-Pyruvideneglycinatocopper(II) trihydrate, Cu(pyrgly)$(H_2O)_3$, was prepared according to ref. 1.

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0020-1693/88/$3.50

TABLE I. Ligand Field Bands (at 77 K), EPR Data (Molecular g Values) and Spin–Orbit Reduction Parameters of *N*-Pyruvideneglycinatocopper(II) Complexes

Compound[a]	Electronic spectra[b] $\times 10^{-3}$ (cm^{-1})			EPR data				Spin–orbit reduction parameters	
				g_x	g_y	g_z	$\bar{g}$[e]		
	$4\delta_1$	$\Delta_\parallel$	$\Delta_\perp$	$g_\perp$		$g_\parallel$		$\kappa_\perp$	$\kappa_\parallel$
Cu(pyrgly)$(H_2O)_3$[d]	10.0sh	13.0	15.5sh	2.058	2.088	2.333	2.160	0.81	0.80
Cu(pyrgly)(py)$(H_2O)_2$[c]		14.5	17.5sh	2.056		2.245	2.119	0.75	0.73
Cu(pyrgly)(quin)$(H_2O)_2$[c]	10.5sh	13.5	16.0sh	2.048	2.079	2.291	2.139	0.77	0.77
Cu(pyrgly)(an)(H_2O)[d]		15.0	17.0sh	2.044	2.074	2.267	2.128	0.76	0.77

[a]pyrgly = *N*-pyruvideneglycinato anion, py = pyridine, quin = quinoline, an = aniline. [b]±0.5. [c]Uncertainty in g values ±0.002. [d]Uncertainty in g values ± 0.01. [e]$\bar{g} = 1/3(g_x + g_y + g_z) = 1/3(2g_\perp + g_\parallel)$.

N-Pyruvideneglycinatopyridinecopper(II) Dihydrate

Cu(pyrgly)$(H_2O)_3$ (2.6 g, 1×10^{-2} mol) was dissolved in 1.6 ml (2×10^{-2} mol) of pyridine (py) and 20 ml of ethanol was added. This system was heated to 40 °C for 30 min. The resulting solution was allowed to cool. Dark blue crystals of Cu(pyrgly)(py)$(H_2O)_2$ were deposited and washed with ether. *Anal.* Calc. for $CuC_{10}H_{10}N_2O_4 \cdot 2H_2O$; C, 37.33; H, 4.38; N, 8.71. Found: C, 37.30; H, 4.28; N, 8.64%.

N-Pyruvideneglycinatoquinolinecopper(II) Dihydrate

Cu(pyrgly)$(H_2O)_3$ (2.6 g) was dissolved in 120 ml of dilute ethanol (ethanol:water = 1:1) at 50 °C. To this solution 13 ml of quinoline (quin) (1×10^{-1} mol) was added. The light blue product of composition Cu(pyrgly)(quin)$(H_2O)_2$ which crystallized from this system was deposited and washed with ether. *Anal.* Calc. for $CuC_{14}H_{12}N_2O_4 \cdot 2H_2O$: C, 45.23; H, 4.33; N, 7.53. Found: C, 45.23; H, 4.20; N, 7.46%.

N-Pyruvideneglycinatoanilinecopper(II) Monohydrate

Cu(pyrgly)$(H_2O)_3$ (2.6 g) was dissolved in 30 ml of hot water (50 °C) and 3.6 ml (4×10^{-2} mol) of aniline (an) dissolved in 30 ml of ethanol was added. The resulting solution was kept at 40 °C for about 20 min. After this time dark blue–green crystals of Cu(pyrgly)(an)(H_2O) crystallized. The product was collected and washed with ethanol. *Anal.* Calc. for $CuC_{11}H_{12}N_2O_4 \cdot H_2O$: C, 41.58; H, 4.44; N, 8.81. Found: C, 41.84; H, 4.60; N, 8.95%.

Spectra

The ligand field reflectance spectra of finely powdered samples were scanned in the range of 4000–28 000 cm^{-1} on a Zeiss DMR-21 spectrometer at room temperature and at 77 K using MgO as a standard.

The EPR powder spectra were recorded with a Varian E-15 spectrometer at Q-band frequency (34.4 GHz) at room temperature and at 130 K using DPPH as an internal standard. No significant changes were observed in the spectra scanned at both temperatures.

Antimicrobial Activity

The antimicrobial efficiency of the complexes was tested by their ability to inhibit the growth of microorganisms in cultivation media (Agar medium No. 2 for bacteria and Sabouraud's agar for fungi). The compounds were suspended in 0.1% of an aqueous solution of Tween 80 emulsifier and diluted in the medium. Cultivation media containing 1500, 1000, 500, 250, 125, 62, 31 and 16 μg/ml of the complexes were examined and the minimum inhibitory concentrations (MIC) were determined. Bacterial cultures were incubated for 24 h at 37 °C and the fungal ones for 5 days at room temperature. The tests were performed with the following microorganisms: (1) Gram-negative bacteria: *Escherichia coli* EC 377/79, *Enterobacter aerogenes, Pseudomonas aeruginosa* Ps 168/79, *Salmonella typhimurium* 122/100, *Shigella flexneri;* (2) Gram-positive bacteria: *Staphylococcus epidermis, Staphylococcus aureus* Mau 29/58, *Bacillus subtilis* 16/65, *Bacillus cereus, Streptococcus faecalis;* (3) Fungi: *Microsporum gypseum* 109/56, *Trichophyton terrestre* 61/62, *Aspergillus niger* CCM 330, *Epidermophyton floccosum* 580, *Penicillium expansum* CCM F516, *Candida albicans* 45/54, *Saccharomyces cerevisiae* ET XII.

Results and Discussion

In the electronic spectra of the complexes under investigation asymmetric ligand field bands centered at 13×10^3–15×10^3 cm^{-1} are observed at 77 K (Table I, Fig. 1). The broad shoulders, which are present in all compounds on the high energy side can be assigned to the $d_{x^2-y^2} \leftarrow d_{xz,yz}$ ($\Delta_\perp$) transition. In the spectra of Cu(pyrgly)(py)$(H_2O)_2$ and Cu(pyrgly)(an)(H_2O), the maximum of the ligand

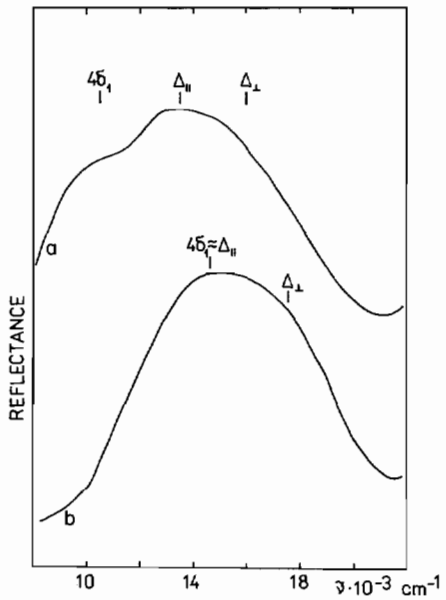

Fig. 1. Ligand field spectra at 77 K: (a) Cu(pyrgly)(quin)-$(H_2O)_2$; (b) Cu(pyrgly)(py)$(H_2O)_2$.

field band is composed of the $d_{x^2-y^2} \leftarrow d_{xy}$ ($\Delta_\parallel$) and $d_{x^2-y^2} \leftarrow d_{z^2}$ ($4\delta_1$) transitions. In Cu(pyrgly)-$(H_2O)_3$ and Cu(pyrgly)(quin)$(H_2O)_2$ the $4\delta_1$ transition is clearly resolved as a shoulder on the low energy side. Such assignment leads to quite reasonable values for the hypothetical octahedral splitting of $\Delta_0 = 9 \times 10^3 - 10 \times 10^3$ cm^{-1} ($\Delta_0 = \Delta_\perp - \delta_2 - 2\delta_1$, where $3\delta_2 = \Delta_\perp - \Delta_\parallel$ is the splitting of the cubic T_{2g} term) [10]. The d–d transitions of the Cu(pyrgly)$(H_2O)_3$ and Cu(pyrgly)(quin)$(H_2O)_2$ complexes are shifted to lower energies in comparison with the other two complexes. This effect is most pronounced on the energies of the $d_{x^2-y^2} \leftarrow d_{z^2}$ transition, which corresponds to the $4\delta_1$ splitting of the cubic E_g ground term of the Cu(II) ion by ligand fields of lower symmetry. The lower value of $4\delta_1$ in Cu(pyrgly)$(H_2O)_3$ and Cu(pyrgly)(quin)$(H_2O)_2$ indicates a stronger axial ligand field component. Therefore an octahedral coordination of Cu(II) ion can be assumed in these complexes. On the other hand, the ligand field spectra of Cu(pyrgly)(py)$(H_2O)_2$ and of Cu(pyrgly)(an)(H_2O) indicate a square-pyramidal coordination for the Cu(II) ion.

The Cu(pyrgly)(py)$(H_2O)_2$ complex shows an axial EPR spectrum (Table I, Fig. 2a). The value of the parameter $G = 4.5$ [$G = (g_\parallel - 2.002)/(g_\perp - 2.002)$] proves that the experimental values of the g parameters correspond to molecular ones [12]. The sharp EPR lines indicate weak exchange coupling between ferro-distortively ordered complex units in the structure [8]. Such ordering was found in the related *N*-pyruvidene-β-alaninatocopper(II) trihydrate by X-ray structural analysis [3]. The

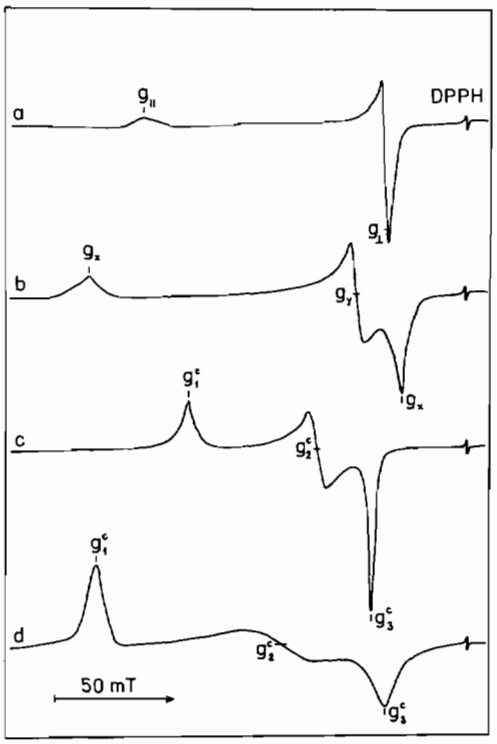

Fig. 2. EPR powder spectra at 34.4 GHz and 130 K: (a) Cu(pyrgly)(py)$(H_2O)_2$; (b) Cu(pyrgly)(quin)$(H_2O)_2$; (c) Cu(pyrgly)(an)(H_2O); (d) Cu(pyrgly)$(H_2O)_3$.

spin–orbit reduction parameters (Table I) evaluated from the equations

$$g_\perp = 2.0023 - 2\lambda_0 k_\perp^2/\Delta_\perp$$
$$g_\parallel = 2.0023 - 8\lambda_0 k_\parallel^2/\Delta_\parallel \quad (1)$$

(where $\lambda_0 = 830$ cm^{-1} is the spin–orbit coupling constant for the free Cu^{2+} ion) are reasonable for a mixed N,O-coordination of the Cu(II) ion.

The EPR spectrum of Cu(pyrgly)(quin)$(H_2O)_2$ is orthorhombic (Table I, Fig. 2b). Taking for simplicity $g_\perp \simeq (g_x + g_y)/2$, a value of $G = 4.7$ is obtained. This again indicates that the experimental g values are molecular. The increase in g values in comparison with Cu(pyrgly)(py)$(H_2O)_2$ corresponds to the decrease in the ligand field splitting energies. The introduction of a more bulky ligand such as quinoline instead of pyridine leads to some deviation from axial symmetry around the Cu(II). The broader EPR lines in comparison with Cu(pyrgly)(py)$(H_2O)_2$ indicate that no exchange interactions operate between crystallographically equivalent Cu(II) ions. Similar effects of the size of the neutral ligand on exchange interactions have been observed in *N*-salicylideneglycinatocopper(II) complexes [8]. However, a definitive decision as to whether the Cu(II) ions in these complexes are

exchange-coupled or not must await the results of EPR measurements on monocrystals.

The Cu(pyrgly)(an)(H_2O) and Cu(pyrgly)(H_2O)$_3$ complexes exhibit orthorhombic EPR patterns with exchange-coupled g parameters: $g_1^c = 2.207$, $g_2^c = 2.109$, $g_3^c = 2.069$ for Cu(pyrgly)(an)(H_2O); and $g_1^c = 2.282$, $g_2^c = 2.136$, $g_3^c = 2.061$ for Cu(pyrgly)(H_2O)$_3$ (Fig. 2c,2d). This is proved by the fact that no reasonable G values and spin–orbit reduction parameters can be calculated using these g values.

When the unit cell contains two sets of non-equivalent exchange-coupled axial molecules with the main axes canted by the angle 2γ ($90° < 2\gamma < 180°$), the equations for crystal g values are given by [10]

$$g_1^c = \sin^2 \gamma g_\parallel + \cos^2 \gamma g_\perp$$
$$g_2^c = \cos^2 \gamma g_\parallel + \sin^2 \gamma g_\perp \quad (2)$$
$$g_3^c = g_\perp$$

Using the above expressions, some unreasonable results are obtained: $g_\parallel = 2.247$, $g_\parallel = 2.069$ ($G = 3.7$, $k_\parallel = 0.74$, $k_\perp = 0.83$) for Cu(pyrgly)(an)(H_2O); and $g_\parallel = 2.357$, $g_\perp = 2.061$ ($G = 6.0$, $k_\parallel = 0.83$, $k_\perp = 0.74$) for Cu(pyrgly)(H_2O)$_3$. As found in ref. 8, it is to be expected that the complexes under investigation, in which three donor atoms in the plane are fixed by the tridentate ligand and only differences in the fourth ligand in the plane and in the axial ligand field are involved, would lead to consistent G parameters. Therefore the obtained G values (remarkably different from the usually found range of G = 4.4–4.8) indicate that the molecular g tensors of both complexes show some deviation from axial symmetry. In this case eqns. (2) are not fully valid.

For orthorhombic complex molecules no analytical equations for crystal g values are available [12]. Therefore the molecular g factors can only be estimated if we take some reasonable approximations, by which the average g value [$\bar{g} = 1/3(g_1^c + g_2^c + g_3^c) = 1/3(g_x + g_y + g_z)$] is not affected [8]. We assume that G reaches a value of 4.7 (the same as found for Cu(pyrgly)(quin)(H_2O)$_2$). Introducing the same anisotropy between g_x and g_y as in this complex, we obtain reasonable values of g factors and of spin–orbit reduction parameters (Table I). The uncertainty in the molecular g factors obtained in such a way is estimated to be not greater than ±0.01.

The Cu(pyrgly)(H_2O)$_3$ complex with an (O_3N) in-plane coordination shows higher spin–orbit reduction parameters than the complexes with (O_2N_2) coordination. These are, together with the lower values of the ligand field splitting, the reasons for the distinctly higher g values in this complex. Thus the estimated molecular g values are consistent with the ligand field spectra; they build a consistent set with the g parameters obtained directly from experiment.

All complexes studied show some antimicrobial effects. The Gram-negative bacteria were resistant against the compounds under investigation (MIC > 1500 μg/ml). Some better results were obtained on Gram-positive bacteria. The Cu(pyrgly)(quin)(H_2O)$_2$ complex shows MIC = 500 μg/ml against *Staphylococcus aureus* and Cu(pyrgly)(an)(H_2O) exhibits MIC = 500 μg/ml against *Bacillus subtilis*. The other compounds show MIC values of 1000 μg/ml or greater. The best results were obtained in the tests on fungi (Table II). Generally the complexes are mostly efficient against *Candida albicans*, *Microsporum gypseum* and *Trichophyton terrestre*. Cu(pyrgly)(an)(H_2O) shows remarkable selective activity (MIC = 16 μg/ml) against *Microsporum gypseum*.

Conclusions

The obtained data show in accordance with ref. 8 that the neutral ligands strongly influence the cooperative symmetry (ordering) in Cu(II) complexes with tridentate Schiff bases. However, these ligands exhibit a more distinct effect on the local symmetry of *N*-pyruvideneglycinatocopper(II) complexes, as in related *N*-salicylideneglycinatocopper(II) complexes [8]. Probably the *N*-salicylideneglycinato ligand, which forms complexes involving one six- and one five-membered chelate ring wherein

TABLE II. Antifungal Activity (MIC (μg/ml)) of *N*-Pyruvideneglycinatocopper(II) Complexes

Compound	Microbial species[a]						
	CAN	SAC	MSP	TRI	ASP	EPD	PEN
Cu(pyrgly)(H_2O)$_3$	500	>1500	1500	250	1000	500	500
Cu(pyrgly)(py)(H_2O)$_2$	500	>1500	>1500	1500	1500	1500	>1500
Cu(pyrgly)(quin)(H_2O)$_2$	1500	1000	500	1000	125	1000	>1500
Cu(pyrgly)(an)(H_2O)	500	>1500	16	250	1500	1500	1500

[a]CAN = *Candida albicans*, SAC = *Saccharomyces cerevisiae*, MSP = *Microsporum gypseum*, TRI = *Trichophyton terrestre*, ASP = *Aspergillus niger*, EPD = *Epidermophyton floccosum*, PEN = *Penicillium expansum*.

possibility of conjugation from the aromatic ring, stabilizes the local symmetry more effectively than the *N*-pyruvideneglycinato ligand with two five-membered aliphatic rings.

Acknowledgements

One of the authors (G.P.) gratefully acknowledges the award of a research grant for Philipps University in Marburg from 'Deutscher Akademischer Austauschdienst'.

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